Phenotypic Characterization of a Genetically Modified Animal Model of Parkinson's Disease

Sylvie Ramboz, Kim Cirillo, Raynel Springer, Matt Mazzella, Daniel Havas, Kendall Walker, Javier A. Sanchez-Padilla, Geoffrey Tombaugh and Afshin Ghavami.

PsychoGenics, Tarrytown, NY

Introduction

Psycho*Genics* Inc has characterized a genetically modified α-synuclein mouse line expressing the human wild-type a-synuclein under the murine Thy-1 promoter (SCNA). This transgenic line has been reported to display pathological features of Parkinson's disease patients including impaired motor and cognitive deficits, a-synuclein aggregates, and accumulation of phosphorylated a-synuclein in striatum and substantia nigra pars compacta (SNc). For the past year, we have conducted a longitudinal phenotypic profiling of this model using a combination of behavior, in situ analysis of catecholamines, immunohistochemistry, and brain slice electrophysiology. Our data confirm previous published data and demonstrate earlier motor impairment onset. In addition using brain slice electrophysiology, we analyzed spontaneous miniature excitatory postsynaptic currents (mEPSCs) in spiny projection neurons (SPNs) in the dorsal striatum and confirmed a decrease in the frequency but not amplitude of mEPSCs in 6-month old mice.

Methods

Animals:
Breeding pairs were acquired from Masliah's laboratory from UCSD, California at the time of the acquisition. To generate experimental offspring, female Thy-1 alpha synuclein (Thy-1 asyn) mice (SCNA Het) were bred to male C57DBA wild type mice. Genotyping was performed as per Rockenstein's protocol at PsychoGenics. Offspring were assigned unique identification numbers (ear notch), implanted with unique RFID chips (DataMars) and housed in polycarbonate cages with filter tops in groups of 7-8 animals. All animals were examined, manipulated and weighed prior to initiation of the study to assure adequate health and suitability and to minimize non-specific stress associated with manipulation.
During the course of the study, 12/12 light/dark cycles will be maintained. The room temperature was maintained between 20 and 23°C with a relative humidity maintained around 50%. Chow and water were provided ad libitum for the duration of the study. Wet chow was placed on the cage floor and was changed daily. The tests were performed during the animal's light cycle phase unless otherwise specified.

Behavioral Assessments: Group size of 12 animals per treatment group and genotype.

- **Tapered Balance Beam test** consisted of a beam angled and elevated from the floor. At the opposite side of the balance beam ('end' portion) there was a goal box which rests on the aforementioned support stand. Following habituation to the testing room, mice were place on the 'starting' end of the balance beam. Mice received 2-8 trials per day, with an ITI of at least 60 sec, and returned to the home cage between trials. The number of trials and timing was optimized for each animal model tested on the apparatus, but the maximum number of trials per day didn't exceed 8 trials. Latency to traverse the beam (sec), and number of foot slips (left / right; fore / hind) were recorded. All tests were also recorded using a video camera for aid in scoring. For longitudinal studies that monitor disease onset and progression, mice were tested weekly, but weren't tested if they were unable to walk. Testing time points: 6 and 12 weeks of age.
- **Wire Hang:** The four limb hanging test (SOP: DMD_M.2.1.005) from the TREAT-NMD Neuromuscular network was employed. Briefly, mice were placed on top of a steel grid cage lid which was then inverted over a 35cm high circular Plexiglas cylinder. Mice were allowed to grip the steel grid for as long as possible with no maximum testing time cut-off. Mice were given three trials with an ITI of 2-3minutes Testing time point: 10 weeks of age.

Electrophysiology Assessments: Group size of 3 animals per genotype

- **Brain slice electrophysiology**: 26-28 week old male wild-type and SCNA Het mutant mice were sacrificed by decapitation under isoflurane anesthesia. The brains were rapidly removed and cooled in ice-cold oxygenated sucrose-ACSF (in mM): Sucrose 220; KCl 2.5; CaCl2 0.5, MgSO4 3, NaH2PO4 1.2, NaHCO3 26, glucose 5. Parahorizontal slices (300 micron) were prepared in ice-cold oxygenated ACSF with a vibratome (LeicaVT1000S), then warmed to 36oC for 30min, allowed to cool to room temperature, and transferred as needed to a submerged slice chamber mounted on the stage of an upright microscope, perfused at 2 ml/min with oxygenated normal ACSF (in mM): NaCl 125; KCl 2.5, CaCl2 2, MgSO4 1, NaH2PO4 1.2, NaHCO3 25, glucose 11. Whole-cell patch clamp recordings were made from visually identified SPNs using Nikon upright microscopes with IR-DIC optics, using pipettes (5-7MΩ) filled with internal solution containing (in mM): Cs-methansulfonate 110, EGTA 10, HEPES 10, TEA-Cl 10, NaCl 10, CaCl2 1, Mg-ATP 5, Na2GTP 0.5, Qx314-Cl 5, pH 7.3. Miniature excitatory postsynaptic currents (mEPSCs) were isolated by including 0.5μM TTX and 40μM picrotoxin in the bath solution, sampled at 2KHz and filtered at 1KHz, collected continuously for 5 minutes from each cell at a holding potential of -80mV. mEPSC recordings were analyzed using MiniAnalysis (Synaptosoft) to calculate frequency and amplitude of synaptic events.

Immunohistochemistry and pathology Assessments: Group size of 2 animals per treatment group and genotype

- **Immunohistochemistry:** Mice were transcardially perfused with room temperature 0.9% saline to remove blood cells. Brain was removed and split into left and right hemispheres. The right hemisphere was post-fixed in fresh 4% PFA/PBS for two hours at room temperature. Duration in 4% PFA/PBS didn't exceed 2 hours. Sample will then be transferred to 15% sucrose at 2-8°C. Hemispheres were sections at 10 micron thickness. Sections were labeled with Syn1 (tinted red; BD # 610787) and 15G7 (tinted green; Enzo # ALX-804-258-L001) immunohistochemistry in two 12 month old SCNA Het and an age- sex-matched WT littermate at three different sagittal levels. Primary antibody binding was visualized using highly cross adsorbed fluorescent secondaries Alexa Flour™ 555 and 647, labeled sections were imaged on a Zeiss Z1 slide scanner at 20 x magnification. Green autofluorescence (tinted blue) was imaged as well to determine autofluorescence ('AF'; most of it Lipofuscin).

Statistics Methods: Data was analyzed via multi-factorial analyses of variance (ANOVA) with a Tukey Post-Hoc. Statistical analysis on mEPSCs electrophysiological data was performed with non-parametric analysis using the Mann-Whitney two-tailed test built in GraphPad Prism software (version 7.0).

Summary - Discussion

In our previous longitudinal study, we have been able to reproduce published data like hyperactivity and lack of motor coordination however we demonstrated the emergence of this phenotype as early as 2 month of age (2015 SFN poster).

In the current study, we demonstrated a phenotype progression in motor coordination by an increased in latency to turn and in traverse speed of the tapered balance beam from 6 to 12 weeks age and also a reduction in hanging time in SCNA Het animals compared to their wild type littermates. We also confirmed the typical pathological pattern of alpha-synuclein expression and aggregation in 12 months old SCNA Het male mice, whereas it seems that the often published Syn1 antibody does not show the total pathological range of alpha-synuclein aggregates.

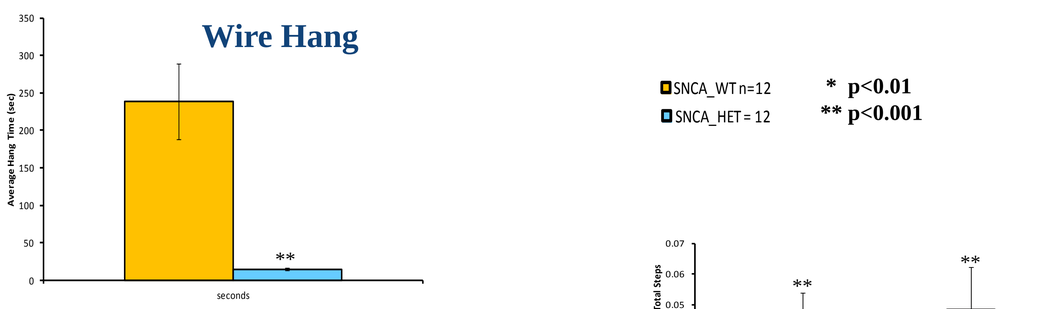
Finally, by utilizing brain slice electrophysiology, we were able to measure a reduction in the frequency of mEPSCs in striatal spiny projection neurons in 6 month old SCNA Het mice. This data together with unaltered paired-pulse ratio in corticostriatal synapses (data not shown) suggest that the reduction in glutamatergic transmission in SCNA Het mice is most likely related to a decrease in synapse number, and not due to alterations in the probability of transmitter release.

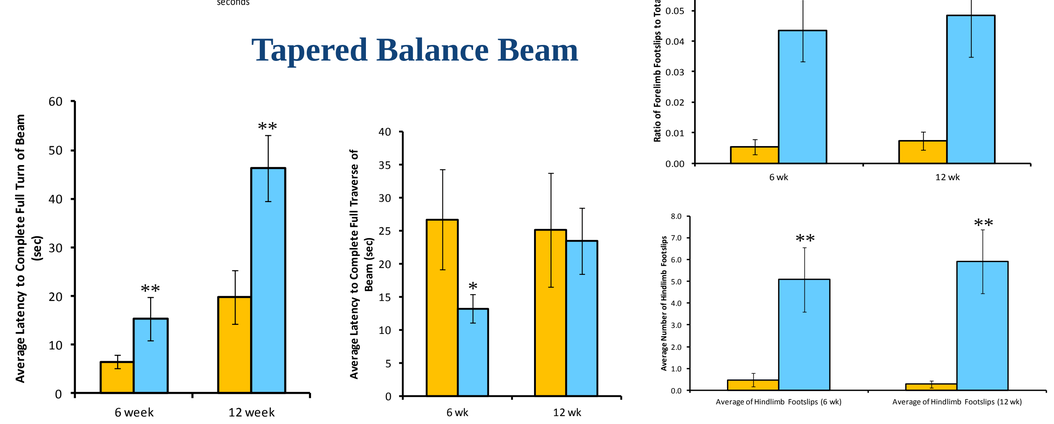
Reference:

•Wu N, Joshi PR, Cepeda C, Masliah E, Levine MS (2010) Alpha-synuclein overexpression in mice alters synaptic communication in the corticostriatal pathway. *Journal of neuroscience research* 88:1764-1776.
•Stephens B, Mueller AJ, Shering AF, Hood SH, Taggart P, Arbuthnott GW, Bell JE, Kilford L, Kingsbury AE, Daniel SE, Ingham CA (2005) Evidence of a breakdown of corticostriatal connections in Parkinson's disease. *Neuroscience* 132:741-754
•Villalba RM, Lee H, Smith Y (2009) Dopaminergic denervation and spine loss in the striatum of MPTP-treated monkeys. *Experimental neurology* 215:220-227
•Zaja-Milatovic S, Milatovic D, Schantz AM, Zhang J, Montine KS, Samii A, Deutch AY, Montine TJ (2005) Dendritic degeneration in neostriatal medium spiny neurons in Parkinson disease. *Neurology* 64:545-547
•Ingham CA, Hood SH, Arbuthnott GW (1989) Spine density on neostriatal neurones changes with 6-hydroxydopamine lesions and with age. *Brain research* 503:334-338

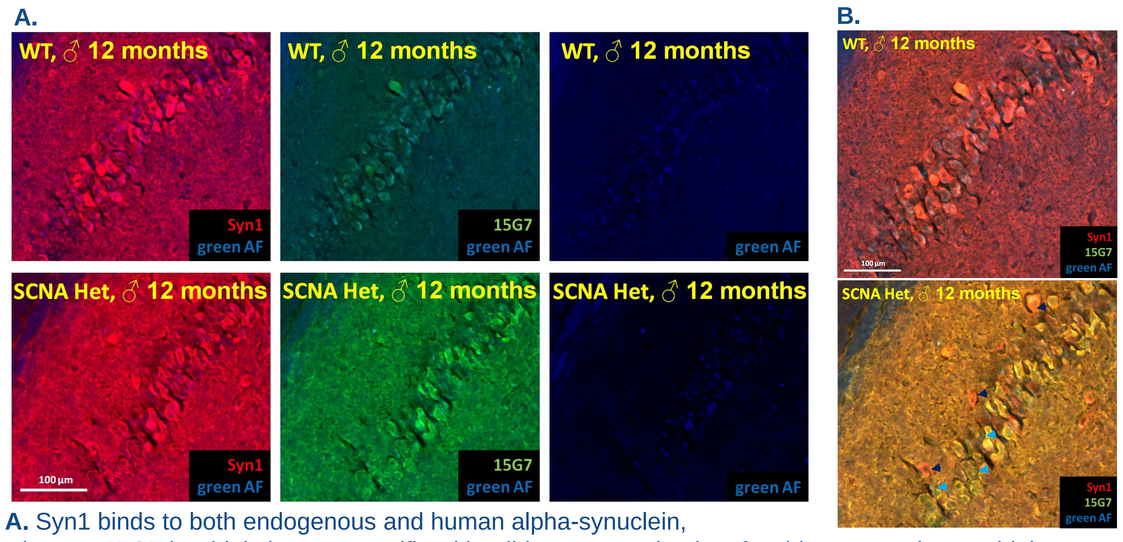
Behavioral Assessment

Reduced hanging time in SCNA Het animals compared to WT littermates in the Wire Hang test at 10 weeks of age.
A progressive increment in latency to turn and on average to traverse the beam in SCNA Het animals from 6 to 12 weeks of age. Number of footslips from the hindlimb or forelimb is higher in SCNA Het at the two testing time points.





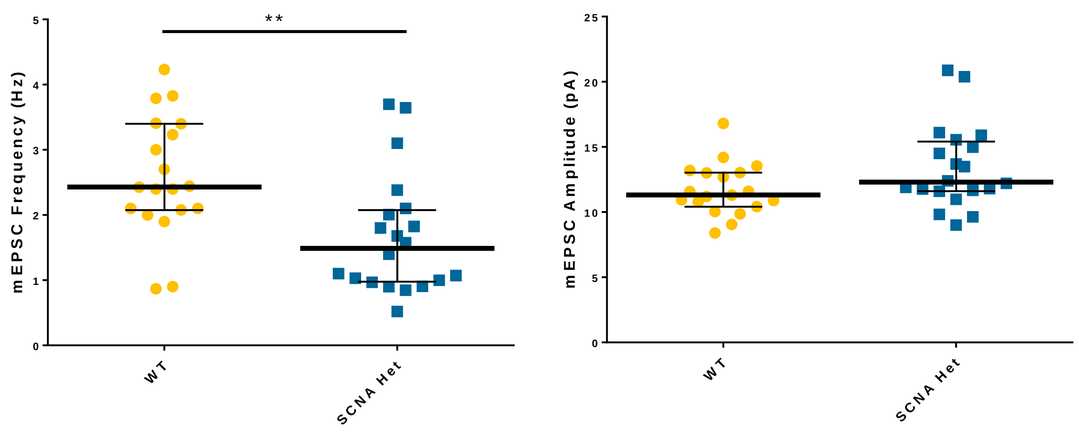
Immunhistochemistry Assessment



A. Syn1 binds to both endogenous and human alpha-synuclein, whereas 15G7 is widely human specific with mild cross reaction in a few hippocampal pyramidal neurons (upper middle). CA1/2 = cornu ammonis ½; cc = corpus callosum **B.** The two antibodies overlap in parts, especially some intracellular aggregates seem to be labeled by 15G7 only (light blue arrowheads), while some neurons are largely only positive for endogenous alpha-synuclein (dark blue arrowheads).

Electrophysiology Assessment

Reduced frequency of miniature excitatory postsynaptic currents (mEPSCs) in striatal spiny projection neurons (SPNs) of 6 month old SCNA Het mice.



A) Scatter dot plot displaying mEPSC frequency in SPNs from SCNA Het mice (median frequency: SCNA Het, 1.487 Hz; wild-type, 2.428 Hz; **p=0.0027). B) mEPSC amplitude in SPNs was not affected in SCNA Het mice (median amplitude: SCNA Het, 12.3 pA; wild-type, 11.3 pA; p=0.0612). Frequency and amplitude of mEPSC were collected from 19 wild-type SPNs and 20 SCNA Het SPNs (n=3 mice per genotype). Data bars depict median and interquartile range (IQR).

Acknowledgments:

Psycho*Genics* would like to thank Dr. Masliah and Edward Rockenstein for their constant scientific support and all our colleagues who helped in gen
experimental animals and putting these data together.