Characterization, comparison, and cross-validation of *in vivo* alpha-synuclein models of parkinsonism

411.02

Terina. N. Martinez[1], Michael Sasner[2], Mark T. Herberth[3], Robert C. Switzer III[4], S.O. Ahmad[5], Kelvin C. Luk[6], Sylvie Ramboz[7], Andrea E. Kudwa[7], Deniz Kirik[8], Joe Flores[8], Ronald J. Mandel[9], Matthew P. Getz[9], Ryan Brown[9], Joshua C. Grieger[10], R. Jude Samulski[10], David Dismuke[10], Sonal S. Das[11], Mark A. Frasier[1], and Kuldip D. Dave[1]

[1]The Michael J. Fox Foundation for Parkinson' s Research, [2]The Jackson Laboratory, [3]WIL Research, [4]NeuroScience Associates, [5]Saint Louis University, [6]Universityof Pennsylvania Perelman School of Medicine, [7]PsychoGenics, Inc. [8]BRAINS Unit, Lund University, [9]McKnight Brain Inst., University of Florida,[10]Gene Therapy Center, University of North Carolina at Chapel Hill, [11]MRC Protein Phosphorylation & Ubiquitylation Unit, University of Dundee

INTRODUCTION

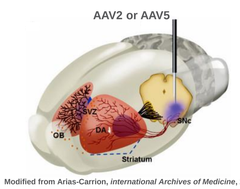
As part of its aggressive strategy to accelerate efforts to find a cure for Parkinson's disease (PD) and to improve therapies for PD patients, the Michael J. Fox Foundation for Parkinson's research (MJFF) endeavors to generate and rigorously characterize preclinical tools and animal models and provide them to the PD research community with minimal barriers. Here we highlight MJFF-generated alpha-synuclein preclinical tools and animal models and describe *in vivo* characterization and validation efforts with the objective of informing the PD research community of the utility of these tools for potential use in studies aimed at understanding alpha-synuclein pathology or to test potential therapeutics targeting alpha-synuclein.

AAV Viral Vectors Expressing Human Alpha-synuclein Transduce Nigral DA Neurons *in vivo*

AAV2 and AAV5 viral vectors expressing human WT alpha-synuclein or eGFP were constructed, produced, and titered by the UNC Vector Core. *In vivo* validation in rats was performed by the laboratories of Dr. D. Kirik (BRAINS Unit, Lund University) & Dr. R. Mandel, (The McKnight Brain Inst., University of Florida).

Study Design for *in vivo* Validation of Alpha-synuclein Expressing AAV Vectors in Rats

Vector	Injected Titer (% of Stock Titer)	Analysis	*N*
rAAV2-CBA-eGFP Stock Titer: $8.1x10^{12}$ vg/ml Production Date: 14/12/2011	100	Histology/Stereology 4 and 12 Weeks Post-Injection	16
rAAV2-CBA-α-Synuclein Stock Titer: $1.5x10^{13}$ vg/ml Production Date: 27/10/2011	100	Histology/Stereology 4 and 12 Weeks Post-Injection	16
rAAV5-CBA-eGFP Stock Titer: $9.5x10^{12}$ vg/ml Production Date: 9/2/2012	100	Histology/Stereology 4 and 12 Weeks Post-Injection	16
rAAV5-CBA-α-Synuclein Stock Titer: $1.0x10^{13}$ vg/ml Production Date: 16/11/2011	100	Histology/Stereology 4 and 12 Weeks Post-Injection	16



Modified from Arias-Carrion, *international Archives of Medicine*, 2008

Figure 1. TH Staining in SNc Following Stereotaxic Injection of AAV Vectors in Rats

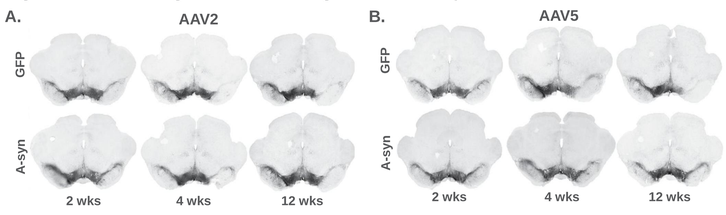


Figure 2. Analysis of TH+ Nigral Neurons, TH striatal density, and Striatal DA Content

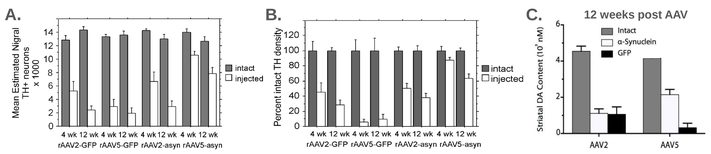
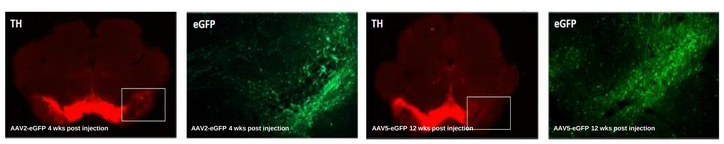


Figure 3. Loss of TH in Neurons Expressing GFP Does not Correlate with Cell Death



Viral vectors (2 uL of AAV2-Asyn-eGFP, AAV2-eGFP, AAV5-Asyn-eGFP or AAV5-eGFP all at 100% of stock titer) were stereotactically injected unilaterally into the right SNc of female Sprague Dawley rats. Immunohistochemistry analysis of TH staining indicated reduced numbers of TH-expressing DA neurons (Fig. 1A and 1B) and reduced striatal TH-intensity (Fig. 2A and 2B) at 2, 4, and 12 wks post AAV injection for both Asyn and eGFP vectors of both AAV2 and AAV5 serotype. Striatal DA content was also reduced at 12 wks post AAV2 and AAV5 injection (Fig. 2C). Notably, infrared analysis of native GFP expression revealed presence of AAV-eGFP transduced neurons without TH-expression (Fig. 3), indicating that TH loss does not correlate with frank neuron loss following eGFP AAV2 or AAV5 injection.

Comparative Study of Alpha-synuclein Transgenic Mouse Models

Numerous transgenic alpha-synuclein mouse models have been developed over the years. However, a lack of standardization and reproducibility of phenotype make it difficult to select appropriate preclinical mouse models to use in efficacy studies for potential alpha-synuclein therapeutics. Thus, MJFF endeavored to independently compare and cross-validate several alpha-synuclein mouse models using standardized outcome measures.

Table 1. Transgenic Mouse Models in the Alpha-synuclein Comparison Study

Model	Reported Phenotypes	Reference
Masliah (Thy-1 aSyn WT, line 61)	Human WT expression on murine thy1 promoter, deficit in pole test, no change in gait, abnormal ldopa response, olfactory deficits, decreased dopamine content at 14 months	Rockenstein et al., 2002 (*J. Neurosci Res.*)
Lee (B6;C3-Tg(Prnp-SNCA*A53T)83Vle/J)	Human A53T expression on mouse prion promoter, at 8 months of age, some homozygous mice develop progressive severe motor dysfunction, spinal cord pathology, mortality at older ages (>10 mos.)	Giasson et al., 2002 (*Neuron*)
Nussbaum (FVB;129S6 $Snca^{tm1Nbm}$ Tg(SNCA*A53T)1Nbm Tg(SNCA*A53T)2NbmlJ)	Two A53T transgenes inserted on knockout background, show normal brain function, but robust enteric nervous system phenotypes reported, no changes observed in nigral neurons, behaviors or dopaminergic system	Kuo et al., 2010 *(Human Molecular Genetics)*
Richfield (C57BL/6J-Tg(Th-SNCA*A30P*A53T)39EricJ)	Human A53T and A30P double-mutant on rat TH promoter, mainly expressed in nigrostriatal system, reduction of DA, DOPAC and HVA at older ages, progressive loss of DA neurons, increased microglial activation, motor activity decline with age	Richfield et al., 2002 (*Exp. Neurology*)
Elan (B6N.Cg-Tg(SNCA*E46K)3ElanJ)	Human E46K mutation under a BAC promoter, age-dependent loss of TH-fibers in striatum, decreased open-field activity, astrogliosis in striatum	Hilton et al., 2011 (SFN abstract)

Figure 4. Human Alpha-Synuclein Protein Expression in Transgenic Mouse Models

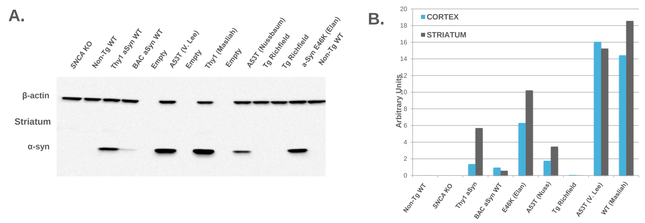


Figure 5. Behavior Analyses in Alpha-synuclein Transgenic Mouse Models

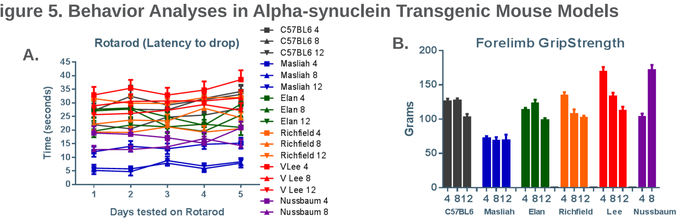


Figure 6. Striatal Neurochemistry for Neurotransmitters in Tg Mouse Brain

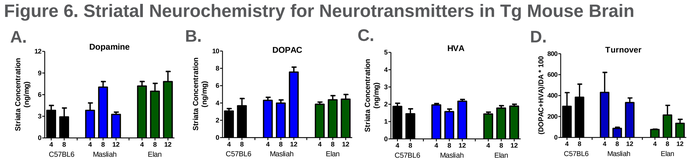


Figure 7. Immunohistochemistry: Human Alpha-synuclein in Tg Mouse Brain Tissue

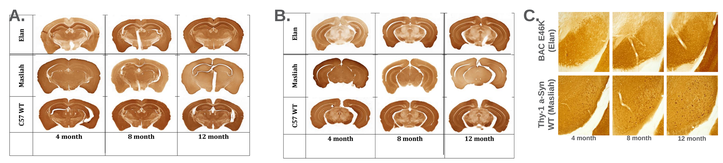
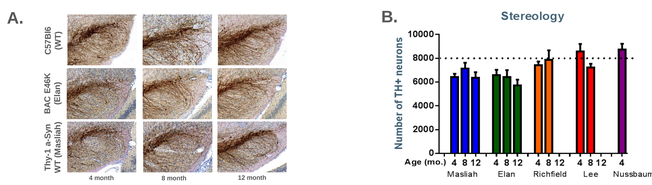


Figure 8. Dopamine Neuron Counts in SNc in Tg Mouse Brain

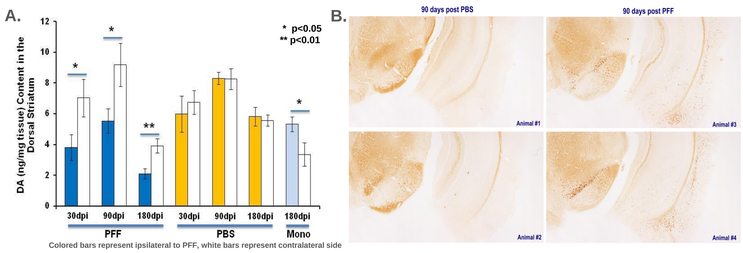


Five popular *SNCA* transgenic mouse models (Table 1.) were selected for this rigorous characterization and age-related phenotyping study at three different ages (4, 8, and 12 months old). The same CROs and outcome measures were used for all studies in all models. Colony aging and western blot analyses was done by The Jackson Laboratory. Behavioral and striatal neurochemistry analyses were performed by WIL Research. Histology, immunohistochemistry, and stereology analyses were conducted by NeuroScience Associates. The levels of human alpha-synuclein protein expression (Figures 4 & 7) and striatal neurotransmitters (Fig. 6) varied among the different models. None of the mouse models evaluated in this study exhibited progressive loss of nigral DA neurons at the ages examined (Fig. 8).

Replication of Alpha-syn Fibrils Propagation Study

Numerous studies including the Braak hypothesis of alpha-synuclein pathology[A] and transfer of alpha-synuclein in cellular and animal models[B] support a hypothesis of neurotoxic transfer of alpha-synucelin protein. Moreover, a single intracerebral injection of alpha-synuclein pre-formed fibrils (PFF) is reported to induce Lewy-like pathology in cells – that can spread from affected to unaffected regions – along with concomitant neurodegeneration and motor dysfunction[C]. To independently replicate these seminal findings, the MJFF partnered with PsychoGenics in collaboration with Drs. Kelvin Luk and Virginia Lee.

Figure 9. Synthetic PFF Inoculation Significantly Reduces Striatal DA Content and Increases Phospho-S129 Alpha-synuclein Immunoreactivity



Colored bars represent ipsilateral to PFF, white bars represent contralateral side

Figure 10. No Significant Effects on Behavior Following α-syn PFF Inoculation

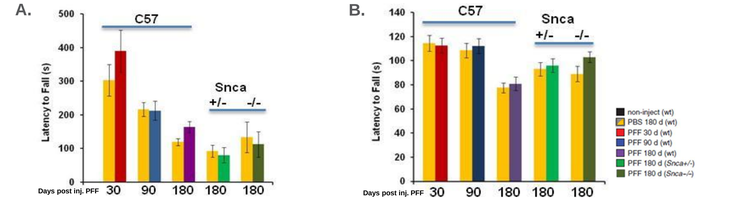
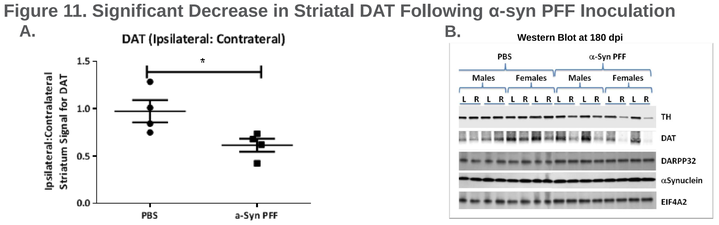


Figure 11. Significant Decrease in Striatal DAT Following α-syn PFF Inoculation



Summary of Replication Study Findings

Reported by Luk et al., 2012[C]	Replicated
↑ pS129 α-syn pathology	YES
↓ TH+ cells in SNpc (at 180 dpi)	In progress
↓ Striatal DA content	YES
↓ Striatal TH intensity	YES
↓ DAT in striatum	YES
Motor deficits (rotarod & wire hang)	NO

This cross-validation study aimed at reproducing the findings of Luk et al.[C], namely PD-like neurodegeneration and motor dysfunction following a single injection of synthetic α-syn PFF. Our current data largely confirmed the published findings, with reduction in DA concentration (approx. 40% Fig. 9A), DAT, and TH expression in the striatum (Fig. 11 A & B), and increased pS129 α-syn immunoreactivity (Fig. 9B). No changes in DARPP32 or total α-syn were observed (Fig. 11 B). Additional crucial aspects of this replication study are still underway at NeuroScience Associates, including histopathological analyses by IHC and stereological estimates of DA neurons. We did not however, observe motor deficits (Fig. 10 A & B) as reported in [C].

SUMMARY

Taken together, the data presented here can help inform the PD research community of the utility and reproducibility of various *in vivo* rodent models of alpha-synucleinopathy, thereby potentially informing selection of appropriate models in which to test prospective therapeutics targeting alpha-synuclein. More information on other available MJFF tools and resources (as well as new projects in development) can be found at the MJFF website.

References: [1] Braak, H. and Tredici, K., *Neurology*, 2008, PMID: 18474848; [2] Olanow, C.W., and Brundin, P., *Movement Disorders*, 2013, PMID: 23390095; [3] Luk, K.C. et al., *Science*, 2012, PMID: 23161999

Acknowledgements for contributions to these studies: Mike Olsen, PsychoGenics[7]; Afshin Ghavami, PsychoGenics[7]; Jan Kehr, Pronexus Analytical AB, Stockholm, Sweden