



ALPHA SYNUCLEIN MOUSE COMPARISON STUDY



Phenotyping Data Results

NOTE FOR USE

- This comparison study was run as an opportunistic look at the phenotype of multiple aSyn mouse lines.
- The primary purpose of these data are to display common phenotypes of PD preclinical models and provide investigators with an idea of how the different lines broadly compare and perform in multiple outcome measures. This could aid in choosing a model for one's studies.
- The secondary purpose of these data are to provide investigators an idea of the phenotype of models previously unpublished or an idea of robustness of previously reported phenotypes.
- The mice used in this study were acquired at different times and were not necessarily analyzed in the same run. Data should be interpreted with caution. Based on this, we recommend:
 - Statistical comparisons *were* made across ages. It is advisable to look for consistent changes to determine if the statistical change is true/robust.
 - Statistical comparisons *were not* made between groups as the lines are on different backgrounds and ideal controls were not included for each group. General trends can still be inferred.



DESCRIPTION OF MICE

Genotype	Transgene/Promoter/Zygotity	Background	Previous Reports on Phenotype	JAX #
C57Bl/6 WT	N/A – Control	C57Bl/6J	N/A	N/A
Thy-1 aSyn	Human wildtype aSyn driven under the murine Thy-1 promoter (line 15). (Hemi)	C57Bl/6NJ	No Reports.	17682
Thy-1 aSyn (Masliah)	Human wildtype aSyn driven under the murine Thy-1 promoter. (Hemi)	C57Bl/6-DBA2	No motor impairment, no neuronal loss, no neurochemistry deficits.	N/A
BAC aSyn OE	Human wildtype aSyn driven under the human SNCA promoter on the BAC transgene. (Hemi)	C57Bl/6NJ	No Reports.	18442
A53T aSyn (Lee)	M83 Line. Human A53T aSyn driven under the mouse prion promoter. (Hom)	C57Bl/6xC3H	Motor impairment.	4479
A53T aSyn (Nussbaum)	Human A53T aSyn driven under the mouse prion promoter in a mouse aSyn KO model. (Hom)	FVB/N x 129S6/SvEvTac	Motor impairment, no neuronal loss, no neurochemistry deficits.	10799
A30P/A53T aSyn (Richfield)	Human A30P/A53T aSyn driven under the rat TH promoter. (Hemi)	C57Bl/6J	Neurochemistry deficits, locomotor deficits, no neuronal loss.	8239
BAC E46K aSyn (Elan)	Human E46K aSyn driven under the human SNCA promoter on the BAC transgene. (Hemi)	B6D2F1 x C57Bl/6NJ	No Reports.	17000
aSyn KO	Knockout of exons 1-4 of the mouse SNCA gene. (Hom)	C57Bl/6NJ	No Reports.	16123
aSyn KO (Hets)	Mouse aSyn KO (Het)	C57Bl/6NJ	No Reports.	16123



DESCRIPTION OF METHODS

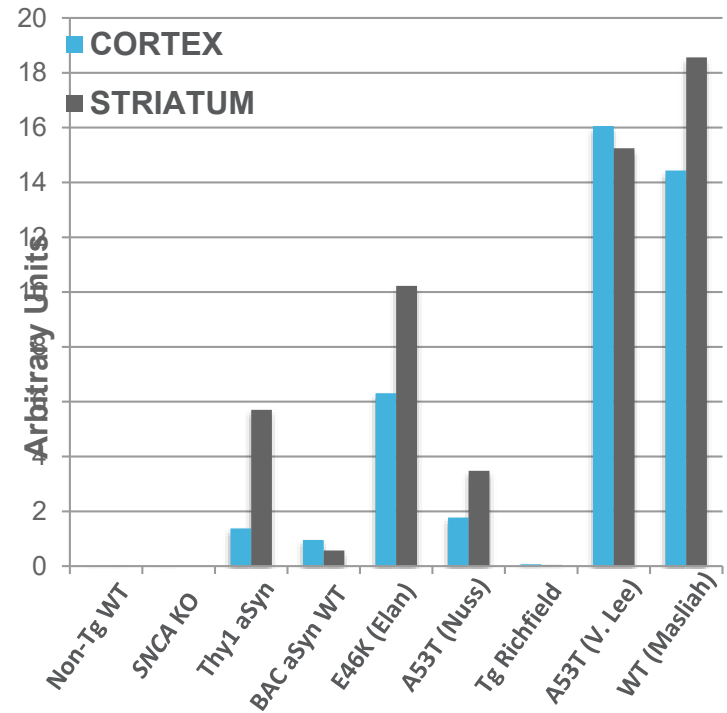
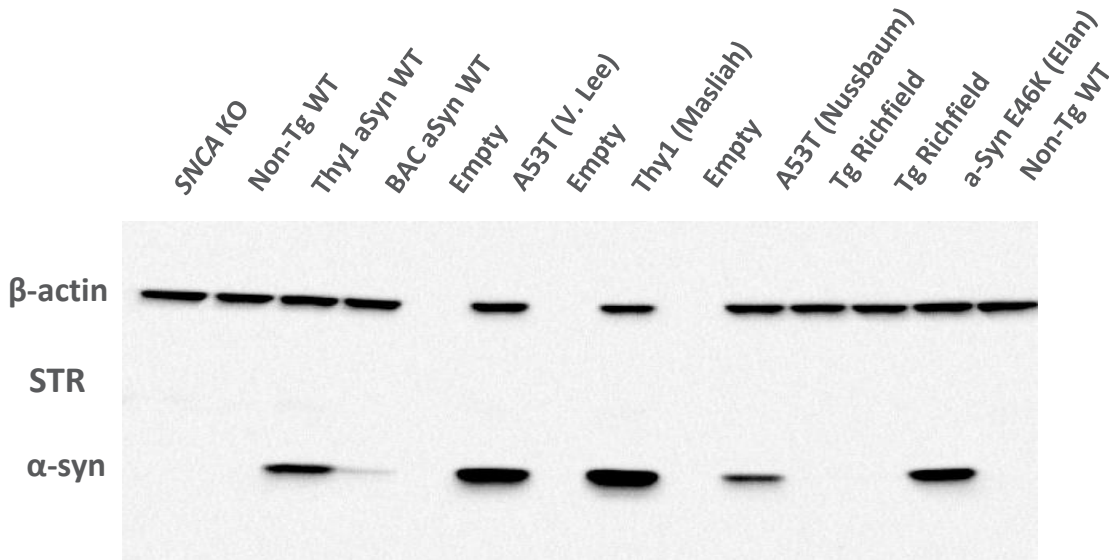
Outcome Measure	Description
aSyn Western Blot	A trial western blot run was performed for total human aSyn protein expression (n=1 mouse per genotype). Due to technical issues, the large run failed and data was never obtained for protein expression in all mice in various structures.
aSyn IHC	Immunostaining was performed for total human aSyn protein. Qualitative analysis of the staining was performed blinded. Readouts were absent, minimal, medium, or high. Details on the location of the stain relative to structures important in PD were included in some cases where staining was notably present/absent in the structures.
Body Weight	Individual body weights were recorded twice weekly, beginning the day of animal receipt and ending the day of euthanasia. Body weights graphed are averages of the recorded weight for each animal at the given age.
Rotarod	Animals were tested once daily for 5 days on an accelerating rotarod. Rotation speed was set to increase at a constant rate to 40 RPM over 60 seconds (testing period was 60 seconds in duration). As no statistically significant effect of testing day was observed for any group, averages for each animal were taken and graphed as a single point.
Forelimb and Hindlimb Grip Strength	The animal is allowed to grip a T-shaped grip bar with its forepaws and is pulled back gently along a platform until its grip is broken. As the backward locomotion continues, the animal's hindpaws reach a T-shaped rearlimb grip bar, which it is allowed to grasp and then forced to release by continued pulling. Digital force gauges record the maximum strength required to break grip. The average of three valid measurements is taken as and reported as an average score.
Hindlimb Footsplay	Heel pads of the hindfeet of each animal are painted with a non-toxic paint. The animals are gently dropped onto a Hindlimb Foot Splay test sheet for three trials, with the first designated as "practice". The closest distance between the inner edge of the ink blots made by each foot is measured. The measurements from the two test trials are used to calculate an average footsplay distance.
Stereology	Brains were embedded and sectioned at 40um in the coronal plane and stored in antigen preserve solution. Tyrosine hydroxylase staining was performed on every 6th free-floating section. Stereological counts were performed in the substantia nigra pars compacta in one hemisphere of each animal. Counter was blinded to animal age and genotype.
Statistics for Behavior	Statistics were not run between groups due to the caveats mentioned in the previous page. A one way ANOVA for the effect of age within genotype was run with Bonferroni <i>post hoc</i> tests for differences between ages. Outliers were removed uniformly across groups. All analyses and graphing were performed using GraphPad Prism software.



ASYN PROTEIN EXPRESSION

HUMAN ASYN PROTEIN EXPRESSION

(NOTE: DATA IS N=1; UNABLE TO COMPLETE STUDY)



QUALITATIVE PS129 ASYN PROTEIN (IHC)

Thy-1 aSyn

Block	Mouse ID	Pathology Report	Age
MFP39	818	absent	4
MFP39	820	Minimal - Mostly HPC, OB	4
MFP39	830	Minimal - Mostly HPC, OB	4
MFP47	847	minimal	4
MFP47	857	absent	4
MFP48	854	absent	4
MFP40	819	absent	4
MFP40	825	Minimal - Mostly HPC, OB	4
MFP40	846	Minimal - Mostly HPC, OB	4
MFP45	9953	absent	8
MFP47	3517	Minimal	8
MFP47	3536	Minimal to Medium	8
MFP47	9949	Minimal to Medium	8
MFP46	9942	Minimal to Medium	8
MFP48	3514	Medium	8
MFP48	3518	Minimal to Medium	8
MFP48	9947	Minimal	8
MFP48	9950	absent	8
MFP47	674	Minimal - Mostly HPC, OB	12
MFP47	687	absent	12
MFP47	9915	absent	12
MFP47	9940	absent	12
MFP48	685	Minimal to Medium	12
MFP48	688	absent	12
MFP48	9938	Minimal - Mostly HPC, OB	12
MFP45	9935	absent	12
MFP54	9948	Mostly Absent	12

Thy-1 aSyn (Masliah)

Block	Mouse ID	Pathology Report	Age
MFP33	10TW	Medium - no SN	4
MFP33	17TW	Medium - no SN	4
MFP33	22CW	Medium - no SN	4
MFP33	2TW	Medium - no SN	4
MFP33	4TW	Medium - no SN	4
MFP33	6TW	medium - no SN	4
MFP33	7TW	medium - no SN	4
MFP33	8TW	Medium - no SN	4
MFP33	CW1886	Medium - no SN	8
MFP33	CW1887	Medium - no SN; disfigured	8
MFP33	CW1888	Medium - no SN	8
MFP33	CW1890	Medium - no SN; disfigured	8
MFP33	CW1895	Medium - no SN; disfigured	8
MFP33	CW1897	Medium - no SN; disfigured	8
MFP33	CW1898	Medium - no SN; disfigured	8
MFP33	CW1907	Medium - no SN; disfigured	8
MFP33	1649CW	absent	12
MFP33	1651CW	Medium - no SN	12
MFP33	1666CW	Medium - no SN	12
MFP33	1671CW	minimal	12
MFP33	1704CW	absent	12
MFP33	1709CW	Medium - no SN	12
MFP33	1765CW	Medium - no SN	12
MFP33	1771CW	Medium - no SN; disfigured	12

QUALITATIVE PS129 ASYN PROTEIN (IHC)

BAC aSyn OE

Block	Mouse ID	Pathology Report	Age
MFP49	2040	Minimal - Mostly HPC, OB	4
MFP51	3696	Absent	4
MFP53	3691	Mostly Absent	4
MFP55	3715	Minimal - Mostly HPC, OB	4
MFP50	2037	absent	4
MFP50	2270	absent	4
MFP52	3702	Minimal - Mostly HPC, OB	4
MFP52	3716	Minimal - Mostly HPC, OB	4
MFP54	3881	Absent	4
MFP49	2173	Minimal - Mostly HPC, OB	8
MFP49	9562	Minimal - Mostly HPC, OB	8
MFP51	8520	Minimal - Mostly HPC, OB	8
MFP53	3974	Medium	8
MFP55	3973	Medium - Mostly HPC, OB	8
MFP50	3503	Minimal - Mostly HPC, OB	8
MFP52	8521	Minimal - Mostly HPC, OB	8
MFP54	9568	Medium - Mostly HPC, OB	8
MFP55	3971	Medium - Mostly HPC, OB	8
MFP53	3925	Minimal - Mostly HPC, OB	12
MFP53	5035	Minimal - Mostly HPC, OB	12
MFP53	5042	Minimal - Mostly HPC, OB	12
MFP55	3917	Absent	12
MFP55	3926	Minimal - Mostly HPC, OB	12
MFP55	3928	Minimal - Mostly HPC, OB	12
MFP54	3909	Minimal - Mostly HPC, OB	12
MFP54	3913	Mostly Absent	12
MFP54	3923	Mostly Absent	12

A53T aSyn (Lee)

Block	Mouse ID	Pathology Report	Age
MFP45	668	Medium - no SN	4
MFP47	666	Medium	4
MFP39	628	medium - no sn	4
MFP39	633	Medium - no SN	4
MFP39	644	absent	4
MFP46	650	Medium to High	4
MFP40	630	Minimal	4
MFP40	639	Minimal to Medium	4
MFP40	646	Minimal - Mostly HPC, OB	4
MFP39	7228	medium	8
MFP39	7709	medium	8
MFP39	7726	absent	8
MFP46	2098	absent	8
MFP48	7744	Medium	8
MFP40	7712	absent	8
MFP40	7727	absent	8
MFP40	7735	medium - no sn	8
MFP45	940	Medium - no SN	12
MFP45	6546	absent	12
MFP47	939	Medium	12
MFP47	949	Medium	12
MFP47	7342	Minimal to Medium	12
MFP46	945	Medium - no SN	12
MFP46	7340	Medium - no SN	12
MFP48	948	Medium to High	12
MFP48	955	High	12

QUALITATIVE PS129 ASYN PROTEIN (IHC)

A53T aSyn (Nussbaum)

Block	Mouse ID	Pathology Report	Age
MFP45	98507	Minimal - Mostly HPC, OB	4
MFP47	98504	Medium	4
MFP39	98496	Minimal - Mostly HPC, OB	4
MFP39	98498	minimal to medium	4
MFP39	98500	absent	4
MFP46	98505	Medium	4
MFP40	98497	minimal	4
MFP40	98499	minimal	4
MFP40	98501	Minimal - Mostly HPC, OB	4
MFP45	21139	Medium	8
MFP45	21146	Medium	8
MFP47	21144	Medium	8
MFP47	21150	Medium	8
MFP46	21140	Medium	8
MFP46	21148	Minimal	8
MFP48	21142	Medium	8
MFP48	21149	Medium	8
MFP48	21152	Minimal to Medium	8
MFP45	33909	Medium	12
MFP45	33913	Minimal	12
MFP47	33911	Minimal to Medium	12
MFP47	33915	Medium	12
MFP47	33917	Minimal	12
MFP46	33910	Minimal to Medium	12
MFP46	33914	absent	12
MFP48	33912	Minimal	12
MFP48	33916	Medium	12

A53T/A30P aSyn (Richfield)

Block	Mouse ID	Pathology Report	Age
MFP45	894	absent	4
MFP39	202	absent	4
MFP39	209	absent	4
MFP39	223	absent	4
MFP39	890	absent	4
MFP40	207	absent	4
MFP40	212	absent	4
MFP40	229	absent	4
MFP40	891	absent	4
MFP45	806	Minimal - Mostly HPC, OB	8
MFP45	817	absent	8
MFP39	8666	Minimal - Mostly HPC, OB	8
MFP39	8668	minimal	8
MFP46	609	Medium - no SN	8
MFP46	669	Medium - no SN	8
MFP46	820	Minimal - Mostly HPC, OB	8
MFP48	8673	absent	8
MFP40	8667	Minimal	8
MFP40	8669	Minimal	8
MFP45	8624	absent	12
MFP45	8643	Minimal - Mostly HPC, OB	12
MFP45	8739	Minimal - Mostly HPC, OB	12
MFP47	8635	Minimal - Mostly HPC, OB	12
MFP47	8730	Minimal - Mostly HPC, OB	12
MFP46	8627	absent	12
MFP46	8646	Minimal - Mostly HPC, OB	12
MFP48	8636	absent	12
MFP48	8736	absent	12

QUALITATIVE PS129 ASYN PROTEIN (IHC)

BAC E64K aSyn (Elan)

Block	Mouse ID	Pathology Report	Age
MFP32	5698	Minimal - Mostly HPC, OB, Amy	4
MFP32	5710	Minimal - Mostly HPC, OB, Amy	4
MFP32	5723	Minimal - Mostly HPC, OB	4
MFP32	5725	medium - no SN	4
MFP32	5748	Minimal - Mostly HPC, OB, Amy	4
MFP32	5753	minimal	4
MFP32	5763	Minimal - Mostly HPC, OB, Amy	4
MFP32	5765	minimal	4
MFP32	5470	Minimal - Mostly HPC, OB, Amy	8
MFP32	5473	Minimal - Mostly HPC, OB, Amy	8
MFP32	5476	Minimal - Mostly HPC, OB, Amy	8
MFP32	5483	Minimal - Mostly HPC, OB, Amy	8
MFP32	5488	Minimal - Mostly HPC, OB, Amy	8
MFP32	5491	Minimal - Mostly HPC, OB, Amy	8
MFP32	5513	minimal	8
MFP32	5536	absent	8
MFP32	5554	medium - no SN	8
MFP32	3660	Minimal - Mostly HPC, OB, Amy	12
MFP33	3689	medium	12
MFP32	3663	Minimal - Mostly HPC, OB, Amy	12
MFP32	3668	Minimal - Mostly HPC, OB, Amy	12
MFP32	3669	Minimal - Mostly HPC, OB, Amy	12
MFP32	3671	Minimal - Mostly HPC, OB, Amy	12
MFP32	3680	minimal	12
MFP32	3686	Minimal - Mostly HPC, OB, Amy	12
MFP32	3688	medium - no SN	12

aSyn KO and aSyn KO (Het)

Block	Mouse ID	Pathology Report	Genotype	Age
MFP49	1801	absent	αSyn KO	8
MFP49	1839	absent	αSyn KO	8
MFP49	1865	absent	αSyn KO	8
MFP49	4880	absent	αSyn KO	8
MFP45	1813	absent	αSyn KO	8
MFP46	1815	absent	αSyn KO	8
MFP50	1826	absent	αSyn KO	8
MFP50	1859	absent	αSyn KO	8
MFP50	1866	absent	αSyn KO	8
MFP49	601	absent	aSyn KO (hets)	8
MFP49	658	absent	aSyn KO (hets)	8
MFP45	606	Minimal - Mostly HPC, OB	aSyn KO (hets)	8
MFP45	637	absent	aSyn KO (hets)	8
MFP46	639	absent	aSyn KO (hets)	8
MFP46	662	absent	aSyn KO (hets)	8
MFP50	0605	absent	aSyn KO (hets)	8
MFP50	0659	absent	aSyn KO (hets)	8



BEHAVIOR DATA

BODY WEIGHT

ROTAROD TIME TO FALL

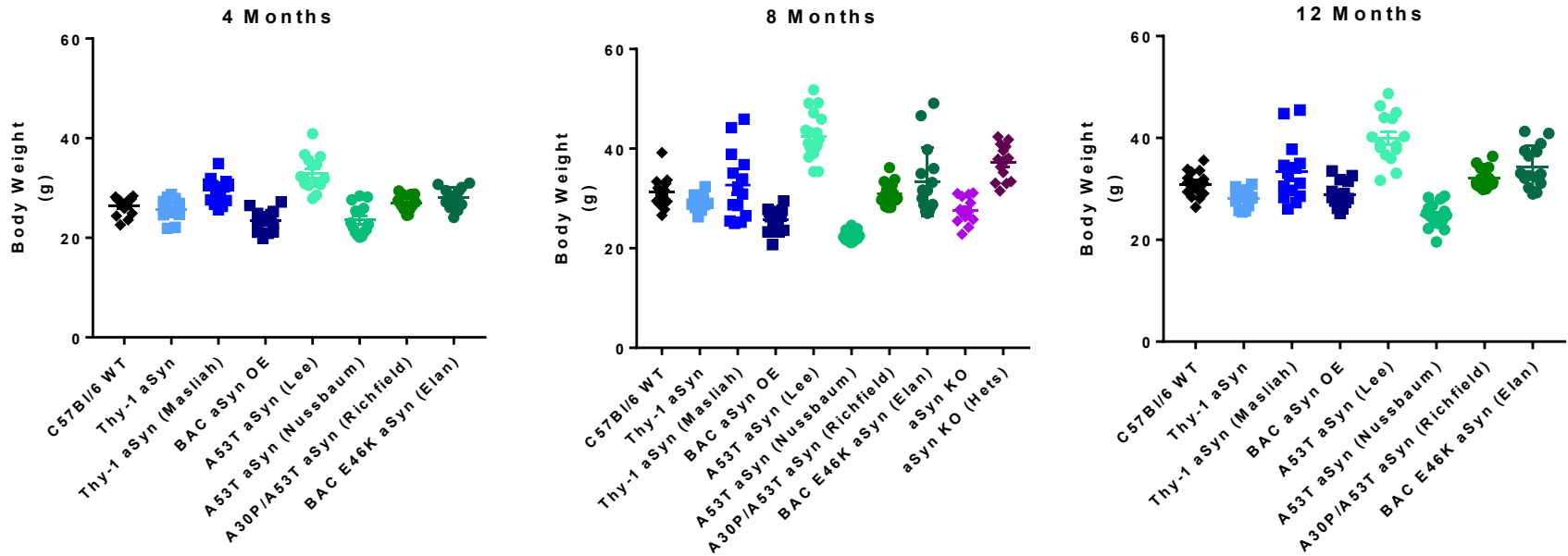
FORELIMB GRIP STRENGTH

HINDLIMB GRIP STRENGTH

HINDLIMB FOOTSPRAY

[n = 15 male mice per group]

BODY WEIGHT



Body Weight Change Across Age

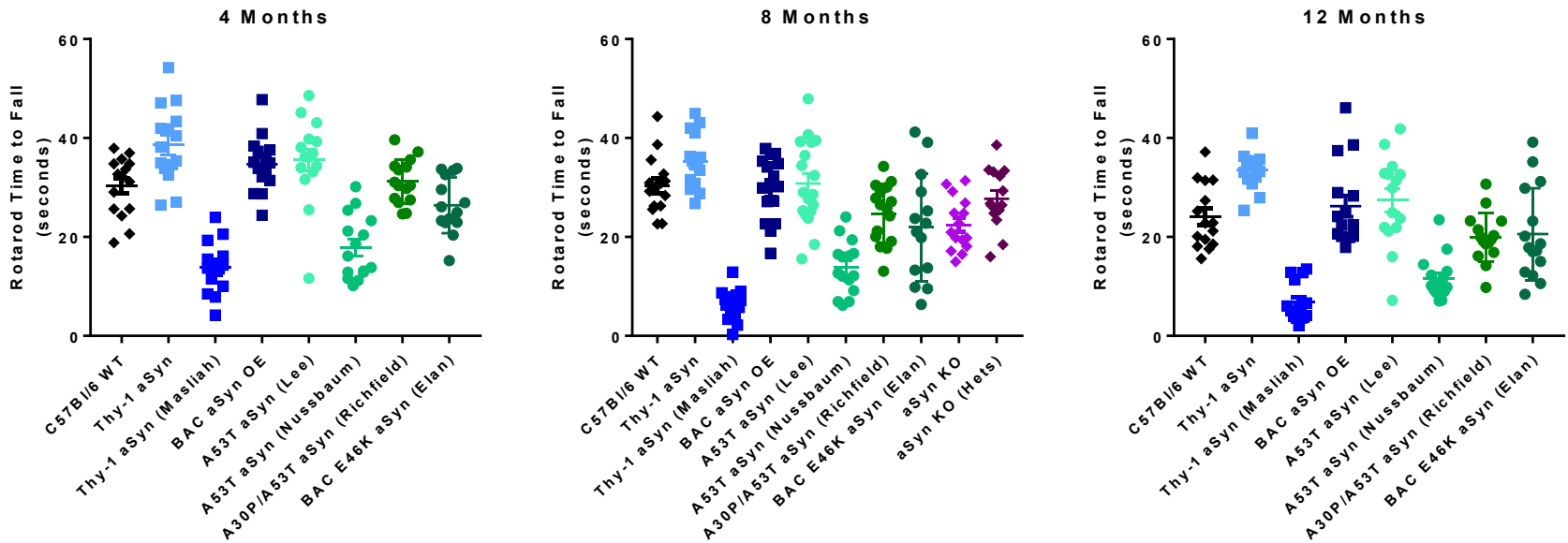
	C57Bl/6	Thy-1 aSyn	Thy-1 aSyn (Masliah)	BAC aSyn OE	A53T aSyn (Lee)	A53T aSyn (Nussbaum)	A30P/A53T aSyn (Richfield)	BAC E46K aSyn (Elan)
4-8 Mo	↑***	↑***	↑*	=	↑***	=	↑**	↑***
8-12 Mo	=	=	=	↑**	=	=	=	=
4-12 Mo	↑**	↑***	↑*	↑***	↑***	=	↑***	↑***

Significance: ***= p<0.001; **= p<0.01; *= p<0.05

[Statistics were not run between mouse strains. Statistics were run within strains across ages.]



ROTAROD TIME TO FALL



Rotarod Time to Fall Change Across Age

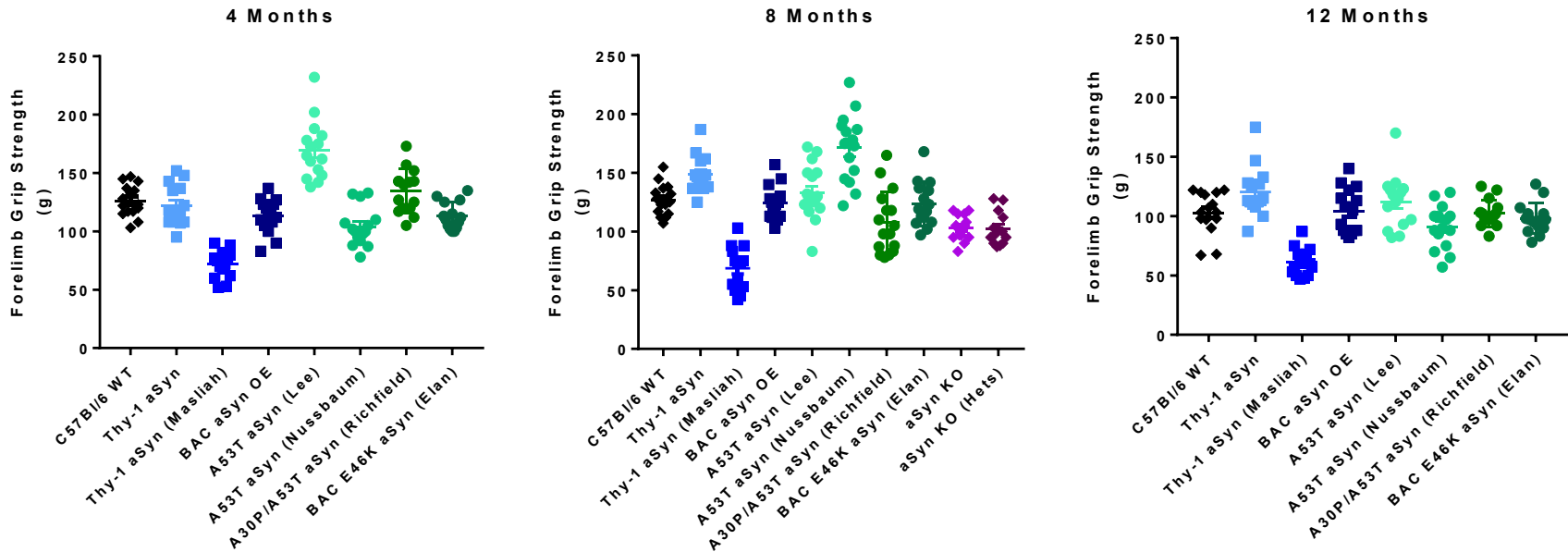
	C57Bl/6	Thy-1 aSyn	Thy-1 aSyn (Masliah)	BAC aSyn OE	A53T aSyn (Lee)	A53T aSyn (Nussbaum)	A30P/A53T aSyn (Richfield)	BAC E46K aSyn (Elan)
4-8 Mo	=	=	↓**	=	=	=	↓*	=
8-12 Mo	↓*	=	=	=	=	=	=	=
4-12 Mo	↓*	=	↓*	↓**	↓**	↓*	↓***	=

Significance: ***= p<0.001; **= p<0.01; *= p<0.05

[Statistics were not run between mouse strains. Statistics were run within strains across ages.]



FORELIMB GRIP STRENGTH



Forelimb Grip Strength Change Across Age

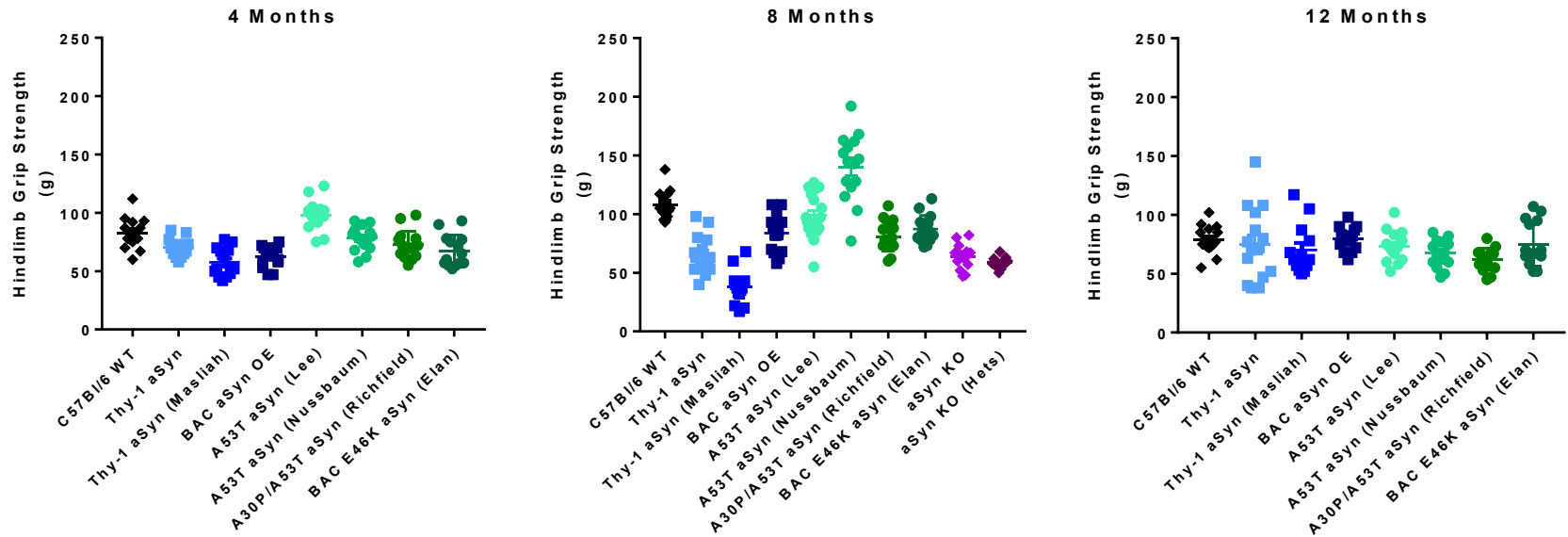
	C57Bl/6	Thy-1 aSyn	Thy-1 aSyn (Masliah)	BAC aSyn OE	A53T aSyn (Lee)	A53T aSyn (Nussbaum)	A30P/A53T aSyn (Richfield)	BAC E46K aSyn (Elan)
4-8 Mo	=	↑***	=	=	↓***	↑***	↓***	=
8-12 Mo	↓**	↓***	=	↓**	↓**	↓***	=	↓***
4-12 Mo	↓**	=	=	=	↓***	=	↓***	=

Significance: ***= p<0.001; **= p<0.01; *= p<0.05

[Statistics were not run between mouse strains. Statistics were run within strains across ages.]



HINDLIMB GRIP STRENGTH



Hindlimb Grip Strength Change Across Age

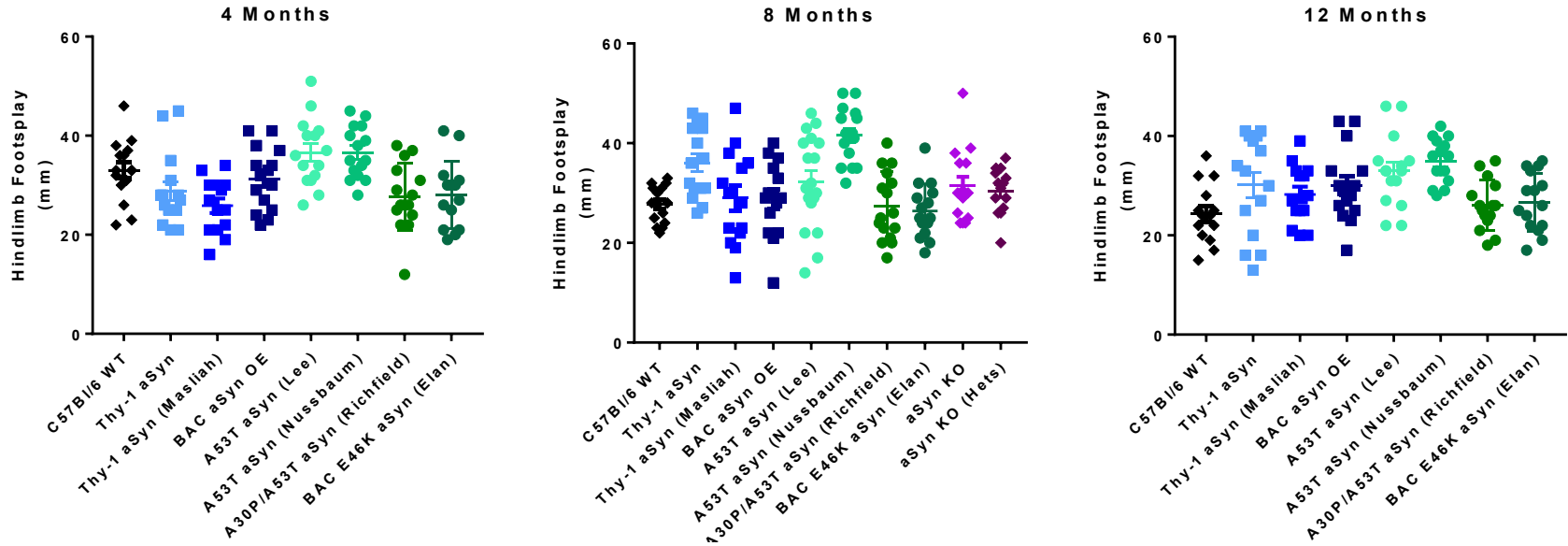
	C57Bl/6	Thy-1 aSyn	Thy-1 aSyn (Masliah)	BAC aSyn OE	A53T aSyn (Lee)	A53T aSyn (Nussbaum)	A30P/A53T aSyn (Richfield)	BAC E46K aSyn (Elan)
4-8 Mo	=	=	↓*	↑*	=	↑***	=	↑*
8-12 Mo	↓***	=	↑***	=	↓***	↓***	↓*	=
4-12 Mo	↓*	=	=	=	↓**	=	=	=

Significance: ***= p<0.001; **= p<0.01; *= p<0.05

[Statistics were not run between mouse strains. Statistics were run within strains across ages.]



HINDLIMB FOOTSPRAY



Hindlimb Footspray Change Across Age

	C57Bl/6	Thy-1 aSyn	Thy-1 aSyn (Masliah)	BAC aSyn OE	A53T aSyn (Lee)	A53T aSyn (Nussbaum)	A30P/A53T aSyn (Richfield)	BAC E46K aSyn (Elan)
4-8 Mo	=	↑**	=	=	=	=	=	=
8-12 Mo	=	=	=	=	=	↑*	=	=
4-12 Mo	↓***	=	=	=	=	=	=	=

Significance: ***= p<0.001; **= p<0.01; *= p<0.05

[Statistics were not run between mouse strains. Statistics were run within strains across ages.]



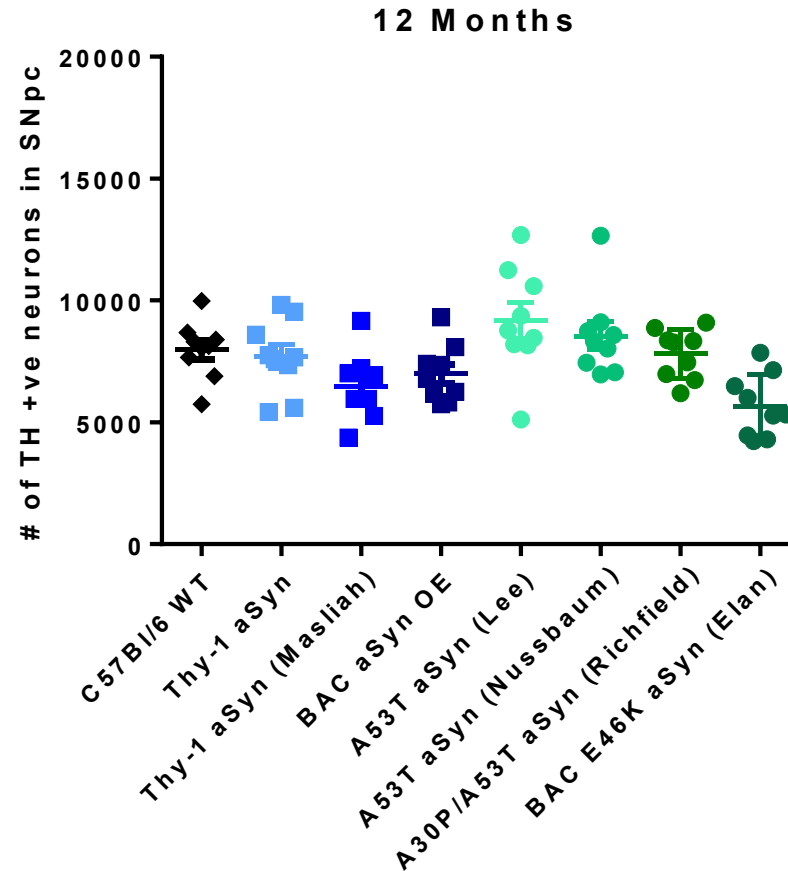


STEREOLOGY DATA

TH+ NEURONS IN THE SUBSTANTIA NIGRA PARS COMPACTA

[n = 9 male mice per group]

TH-POSITIVE CELLS IN SN



Statistics were not run between mouse strains.

