



MJFF LRRK2 MOUSE COMPARISON STUDY

Phenotyping Data Results

NOTE FOR USE

- This comparison study was run as an opportunistic look at the phenotype of multiple MJFF-generated LRRK2 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- The secondary purpose of these data are to provide investigators an idea of the phenotype of models previously unpublished or an idea of robustness of previously reported phenotypes.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.



DESCRIPTION OF MICE

Genotype	Transgene/Promoter/Zygoty	Background	JAX #
C57Bl/6 WT	N/A – Control	C57Bl/6J	N/A
LRRK2 WT	Human wildtype LRRK2 driven by the human LRRK2 promoter on the BAC transgene. (Hemi)	B6SJLF1/J	13725
LRRK2 G2019S	Human G2019S LRRK2 driven by the human LRRK2 promoter on the BAC transgene. (Hemi)	C57Bl/6J	18785
LRRK2 R1441G	Human R1441G LRRK2 driven by the human LRRK2 promoter on the BAC transgene. (Hemi)	C57Bl/6J	18786
LRRK2 KO	Knockout of exons 39-40 of the LRRK2 gene. (Hom)	C57Bl/6NJ	16121
LRRK1 KO	Knockout of exons 24-29 of the LRRK1 gene (Hemi)	C57Bl/6NJ	16120
LRRK1/2 KO	Knockout of exons 39-40 of the LRRK2 gene (Hom) crossed with the knockout of exons 24-29 of the LRRK1 gene (Hemi).	C57Bl/6NJ	16122



DESCRIPTION OF METHODS

Outcome Measure	Description
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/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.
LRRK2 Protein Levels*	Human LRRK2 protein was quantified using western blots and the Protein Simple WES system using the Antibodies Inc anti-LRRK2 antibody (N231B/34, Catalog #: 75-266) and Abcam anti-Vinculin antibody (Catalog #:ab155120) as the control in the striatum, and cortex. Absolute amounts of LRRK2 protein were detected and normalized to vinculin.
Statistics	A one way ANOVA for the effect of genotype within each age and the effect of age within each genotype were run with Bonferroni <i>post hoc</i> tests for differences between ages/genotypes. Outliers were removed uniformly across groups. All analyses and graphing were performed using GraphPad Prism software.

*= due to experimental issues, data will not be available



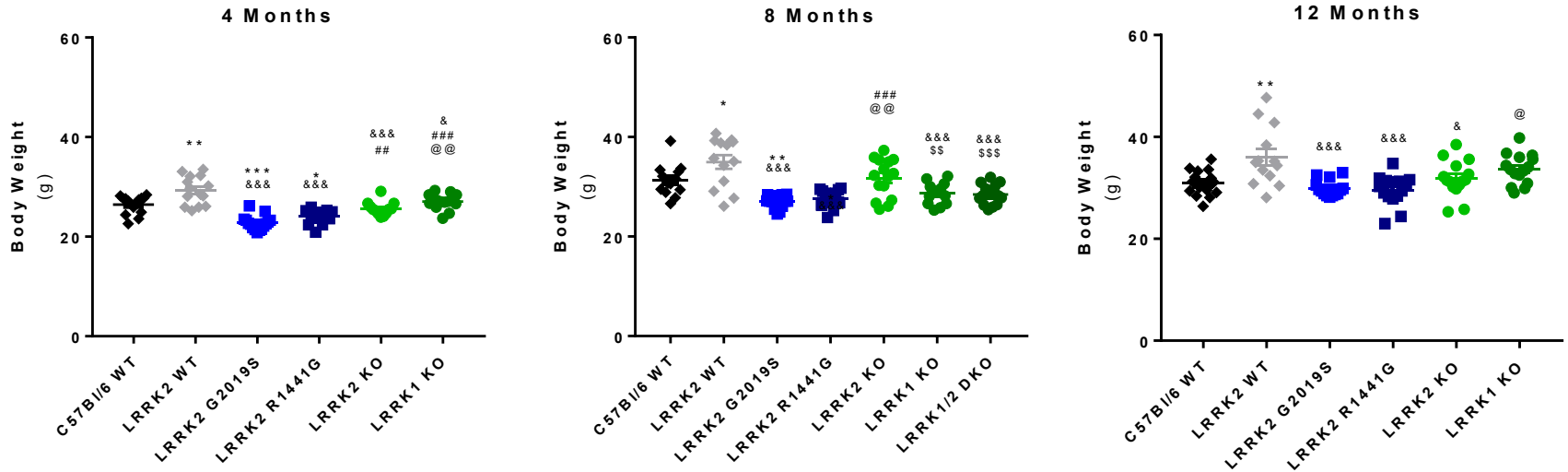


BEHAVIOR DATA

BODY WEIGHT
ROTAROD TIME TO FALL
FORELIMB GRIP STRENGTH
HINDLIMB GRIP STRENGTH
HINDLIMB FOOTSPRAY

[n = 15 mice per group]

BODY WEIGHT



Body Weight Change Across Age

	C57Bl/6	LRRK2 WT	LRRK2 G2019S	LRRK2 R1441G	LRRK2 KO	LRRK1 KO
4-8 Mo	↑***	↑***	↑***	↑**	↑***	=
8-12 Mo	=	=	↑*	=	=	↑***
4-12 Mo	↑***	↑***	↑***	↑***	↑***	↑***

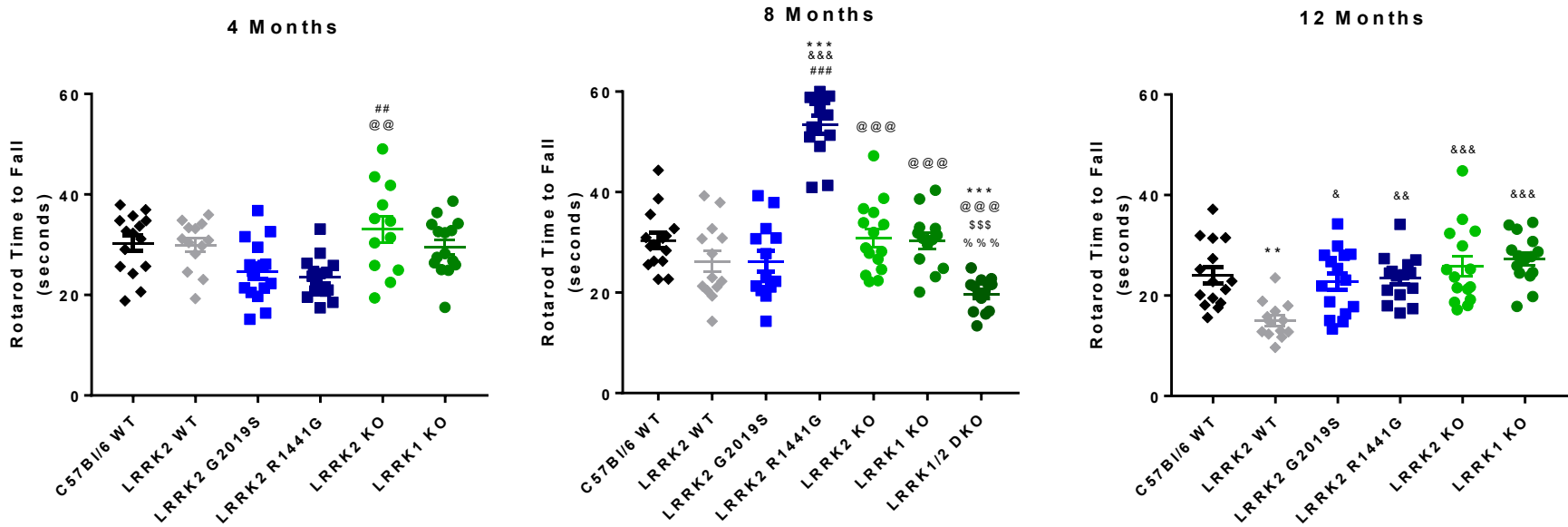
Significance: ***= p<0.001; **= p<0.01; *= p<0.05

Key to Statistical Comparisons within Age

- * significant vs C57Bl/6 WT
 - & significant vs LRRK2 WT
 - # significant vs LRRK2 G2019S
 - @ significant vs LRRK2 R1441G
 - \$ significant vs LRRK2 KO
 - % significant vs LRRK1 KO
- (1=p<0.05; 2=p<0.01; 3=p<0.001)



ROTAROD TIME TO FALL



Rotarod Time to Fall Change Across Age

	C57Bl/6	LRRK2 WT	LRRK2 G2019S	LRRK2 R1441G	LRRK2 KO	LRRK1 KO
4-8 Mo	=	=	=	↑***	=	=
8-12 Mo	↓*	↓***	=	↓***	=	=
4-12 Mo	↓*	↓***	=	=	↓**	=

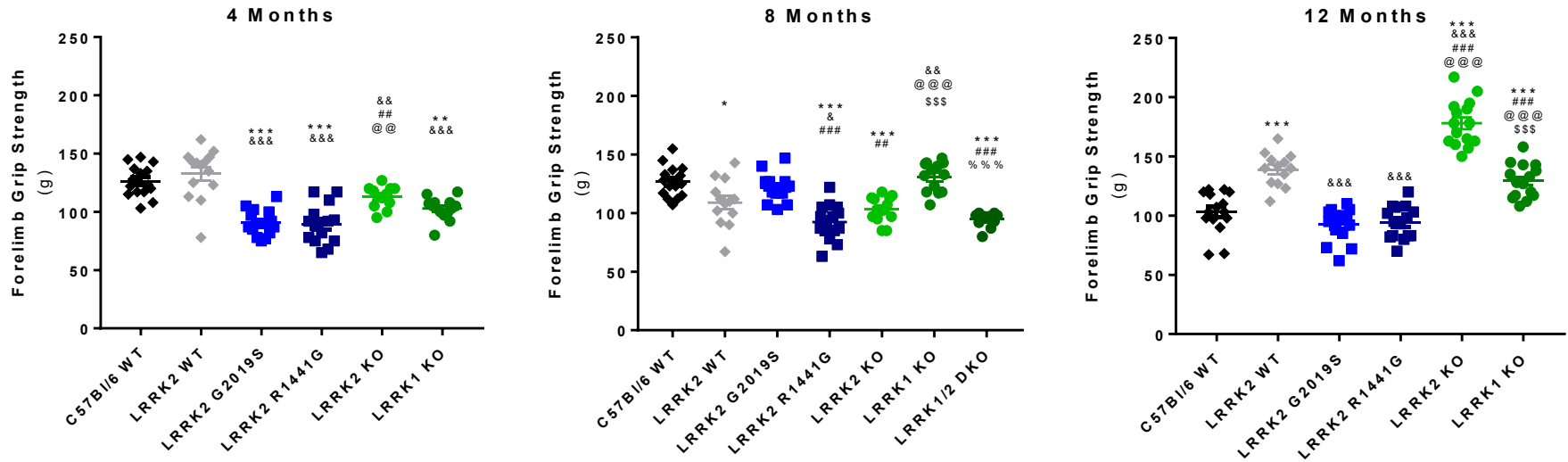
Significance: ***= p<0.001; **= p<0.01; *= p<0.05

Key to Statistical Comparisons within Age

- * significant vs C57Bl/6 WT
 - & significant vs LRRK2 WT
 - # significant vs LRRK2 G2019S
 - @ significant vs LRRK2 R1441G
 - \$ significant vs LRRK2 KO
 - % significant vs LRRK1 KO
- (1=p<0.05; 2=p<0.01; 3=p<0.001)



FORELIMB GRIP STRENGTH



Forelimb Grip Strength Change Across Age

	C57Bl/6	LRRK2 WT	LRRK2 G2019S	LRRK2 R1441G	LRRK2 KO	LRRK1 KO
4-8 Mo	=	↓***	↑***	=	=	↑***
8-12 Mo	↓***	↑***	↓***	=	↑***	=
4-12 Mo	↓***	=	=	=	↑***	↑***

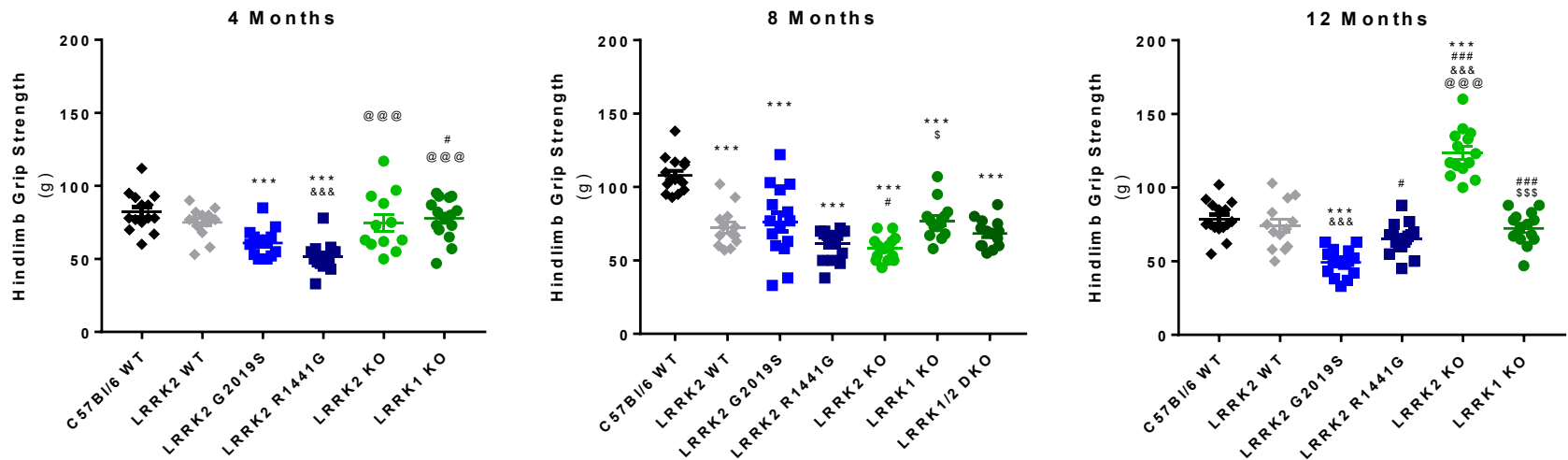
Significance: ***= p<0.001; **= p<0.01; *= p<0.05

Key to Statistical Comparisons within Age

- * significant vs C57Bl/6 WT
 - & significant vs LRRK2 WT
 - # significant vs LRRK2 G2019S
 - @ significant vs LRRK2 R1441G
 - \$ significant vs LRRK2 KO
 - % significant vs LRRK1 KO
- (1=p<0.05; 2=p<0.01; 3=p<0.001)



HINDLIMB GRIP STRENGTH



Hindlimb Grip Strength Change Across Age

	C57Bl/6	LRRK2 WT	LRRK2 G2019S	LRRK2 R1441G	LRRK2 KO	LRRK1 KO
4-8 Mo	↑***	=	↑**	=	↓**	=
8-12 Mo	↓***	=	↓***	=	↑***	=
4-12 Mo	=	=	=	↑*	↑***	=

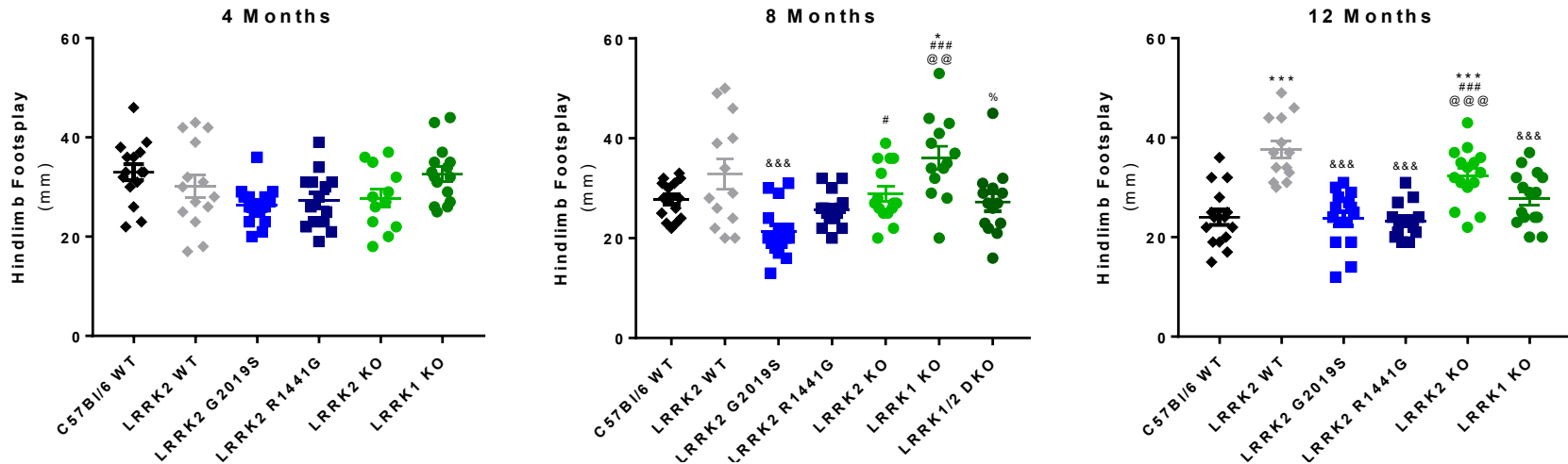
Significance: ***= p<0.001; **= p<0.01; *= p<0.05

Key to Statistical Comparisons within Age

- * significant vs C57Bl/6 WT
 - & significant vs LRRK2 WT
 - # significant vs LRRK2 G2019S
 - @ significant vs LRRK2 R1441G
 - \$ significant vs LRRK2 KO
 - % significant vs LRRK1 KO
- (1=p<0.05; 2=p<0.01; 3=p<0.001)



HINDLIMB FOOTSPRAY



Hindlimb Footspray Change Across Age

	C57Bl/6	LRRK2 WT	LRRK2 G2019S	LRRK2 R1441G	LRRK2 KO	LRRK1 KO
4-8 Mo	=	=	=	=	=	=
8-12 Mo	=	=	=	=	=	↓**
4-12 Mo	↓***	↑**	=	=	=	=

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

Key to Statistical Comparisons within Age

- * significant vs C57Bl/6 WT
 - & significant vs LRRK2 WT
 - # significant vs LRRK2 G2019S
 - @ significant vs LRRK2 R1441G
 - \$ significant vs LRRK2 KO
 - % significant vs LRRK1 KO
- (1=p<0.05; 2=p<0.01; 3=p<0.001)





STEREOLOGY DATA

TH+ NEURONS IN THE SUBSTANTIA NIGRA PARS COMPACTA

[n = 9 mice per group]

TH-POSITIVE CELLS IN SN

DUE TO EXPERIMENTAL ISSUES, DATA ARE NOT AVAILABLE.

TH-Positive Cell Count Change Across Age

	C57Bl/6	LRRK2 WT	LRRK2 G2019S	LRRK2 R1441G	LRRK2 KO	LRRK1 KO
4-8 Mo						
8-12 Mo						
4-12 Mo						
Significance: ***= p<0.001; **= p<0.01; *= p<0.05						

Key to Statistical Comparisons within Age

- * significant vs C57Bl/6 WT
- & significant vs LRRK2 WT
- # significant vs LRRK2 G2019S
- @ significant vs LRRK2 R1441G
- \$ significant vs LRRK2 KO
- % significant vs LRRK1 KO
- (1=p<0.05; 2=p<0.01; 3=p<0.001)





EXPRESSION DATA

HUMAN LRRK2 PROTEIN

[n = 3 mice per group]

LRRK2 PROTEIN

DUE TO EXPERIMENTAL ISSUES, DATA ARE NOT AVAILABLE.

Striatal (STR) and Cortical (CTX) LRRK2 Protein Change Across Age

	C57Bl/6		LRRK2 WT		LRRK2 G2019S		LRRK2 R1441G		LRRK2 KO		LRRK1 KO	
	STR	CTX	STR	CTX	STR	CTX	STR	CTX	STR	CTX	STR	CTX
4-8 Mo												
8-12 Mo												
4-12 Mo												
Significance: ***= p<0.001; **= p<0.01; *= p<0.05												

Key to Statistical Comparisons within Age

- * significant vs C57Bl/6 WT
 - & significant vs LRRK2 WT
 - # significant vs LRRK2 G2019S
 - @ significant vs LRRK2 R1441G
 - \$ significant vs LRRK2 KO
 - % significant vs LRRK1 KO
- (1=p<0.05; 2=p<0.01; 3=p<0.001)

