MJFF KNOCKOUT RAT COMPARISON STUDY

Phenotyping Data Results

NOTE FOR USE

- This comparison study was run as an opportunistic look at the phenotype of multiple MJFF-generated knockout rat 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- The secondary purpose of these data are to provide investigators an idea of the phenotype of models previously unpublished or an idea of robustness of previously reported phenotypes.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 rats used in this study were acquired at different times and were not necessarily analyzed in the same run. Data should be interpreted with caution.
- Additional MJFF publications on these models can be found here:
  - Parkin, PINK1, DJ-1 KOs: Dave, K.D., De Silva, S., Sheth, N.P., *et al*., (2014). Phenotypic characterization of recessive gene knockout rat models of Parkinson's disease. *Neurobiol Dis,* 70: 190-203.
  - LRRK2 KO: Baptista, M.A.S., Dave, K.D., Frasier, M.A. *et al*. (2013). Loss of leucine-rich repeat kinase 2 (LRRK2) in rats leads to progressive abnormal phenotypes in peripheral organs. *Plos One,* 8(11): e80705.

DESCRIPTION OF RATS

Genotype	Description	Background	Horizon #
Non-Transgenic	N/A – Control	Long Evans	N/A
Parkin KO	Homozygous knockout of Parkin	Long Evans	TGRL4760
PINK1 KO	Homozygous knockout of PINK1	Long Evans	TGRL4690
DJ-1 KO	Homozygous knockout of DJ-1	Long Evans	TGRL4830
LRRK2 KO	Homozygous knockout of LRRK2	Long Evans	TGRL4620

DESCRIPTION OF METHODS

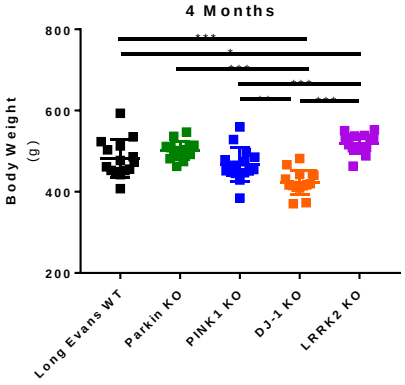
Outcome Measure	Description
Body Weight	Individual body weights were recorded twice weekly, beginning the day of animal receipt and ending the day of euthanasia. Body weights graphed are averages of the recorded weight for each animal at the given age.
Rotarod	Animals were tested once daily for 5 days 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.
Neurochemistry	Stratial samples were microdissected and processed for neurochemistry. Samples were analyzed for NE, DA, 5-HT, 5-HIAA, DOPAC, and HVA levels. Quality control samples were prepared in charcoal treated brain homogenates spiked with neurotransmitters at four concentration levels. Levels were detected via UPLC-MS/MS analysis.
Statistics for Behavior and Stereology	A one way ANOVA for the effects of genotype within age was performed with Bonferroni *post hoc* tests for differences between genotypes. A one way ANOVA was also performed for the effect of age within genotype with Bonferroni *post hoc* tests for differences between ages. A two way ANOVA was not performed as not all rats were analyzed at the same ages. Outliers were removed uniformly across groups. All analyses and graphing were performed using GraphPad Prism software.

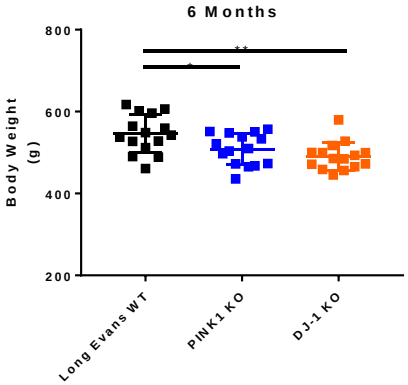
BEHAVIOR DATA

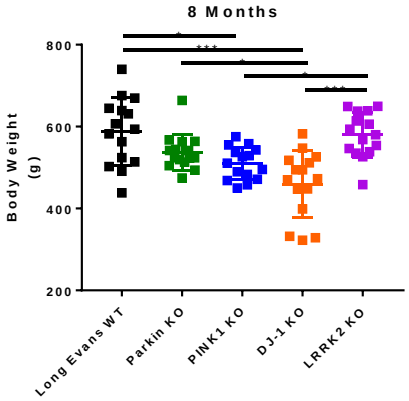
BODY WEIGHT
ROTAROD TIME TO FALL
FORELIMB GRIP STRENGTH
HINDLIMB GRIP STRENGTH
HINDLIMB FOOTSPLAY

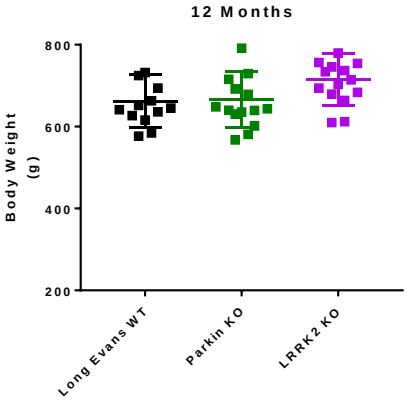
[n = 15 male rats per group]

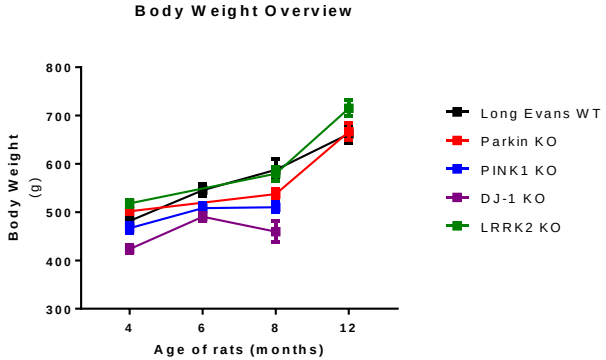
BODY WEIGHT









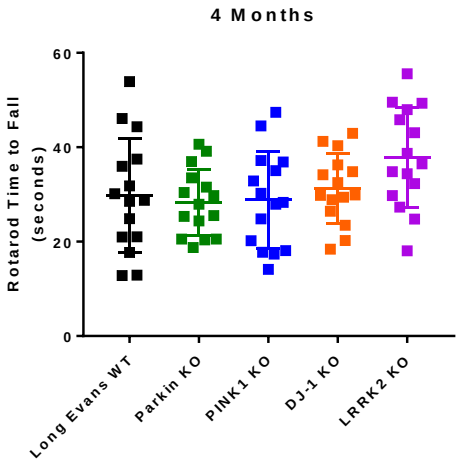


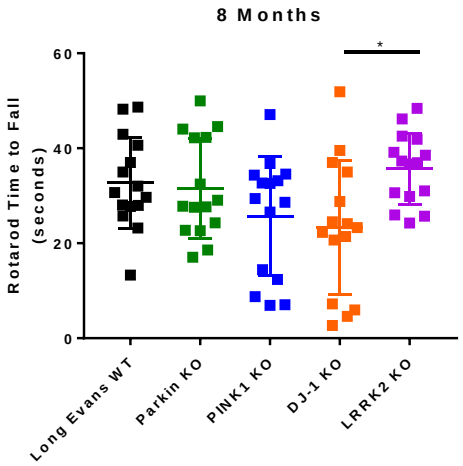
Body Weight Change Across Age

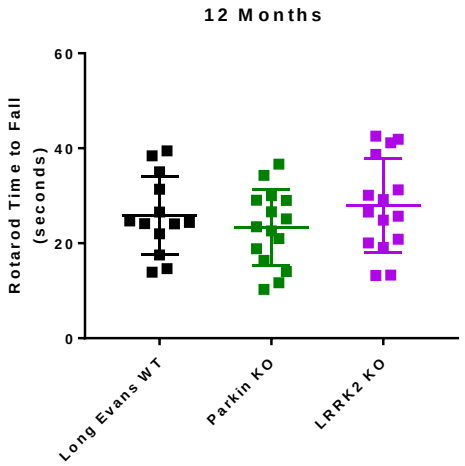
	Long Evans	Parkin KO	PINK1 KO	DJ-1 KO	LRRK2 KO
4-6 Mo	↑*	N/A	↑*	↑**	N/A
6-8 Mo	=	N/A	=	=	N/A
4-8 Mo	↑***	=	↑*	=	↑**
8-12 Mo	↑*	↑***	N/A	N/A	↑***
4-12 Mo	↑***	↑***	N/A	N/A	↑***

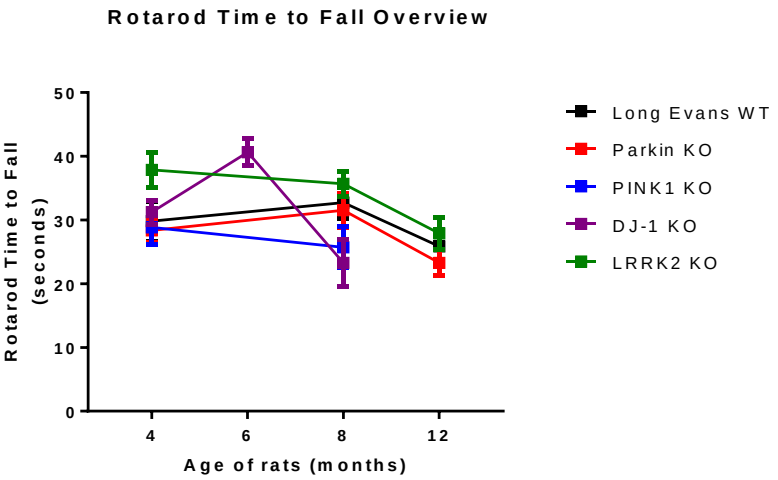
Significance: ***= p<0.001; **= p<0.01; *= p<0.05; (Orange = age overview)

ROTAROD TIME TO FALL







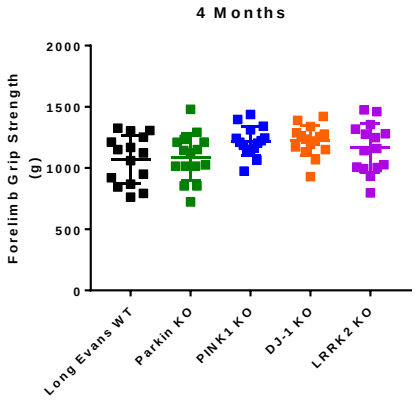


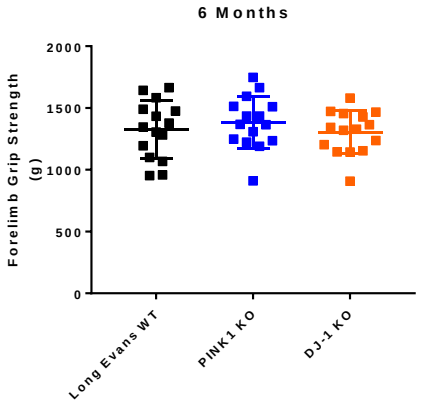
Rotarod Time to Fall Change Across Age

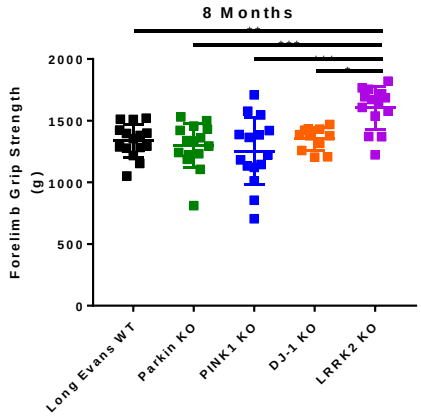
	Long Evans	Parkin KO	PINK1 KO	DJ-1 KO	LRRK2 KO
4-6 Mo	N/A	N/A	N/A	↑*	N/A
6-8 Mo	N/A	N/A	N/A	↓*	N/A
4-8 Mo	=	=	=	=	=
8-12 Mo	=	↓*	N/A	N/A	=
4-12 Mo	=	=	N/A	N/A	↓*

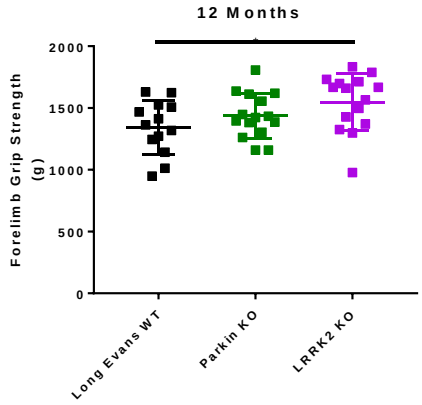
Significance: ***= p<0.001; **= p<0.01; *= p<0.05; (Orange = age overview)

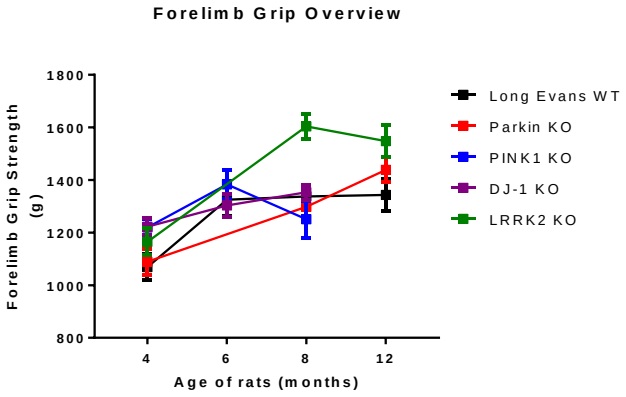
FORELIMB GRIP STRENGTH









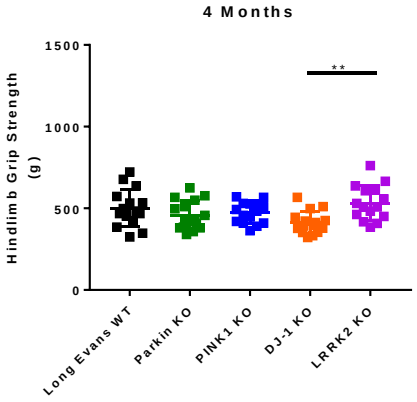


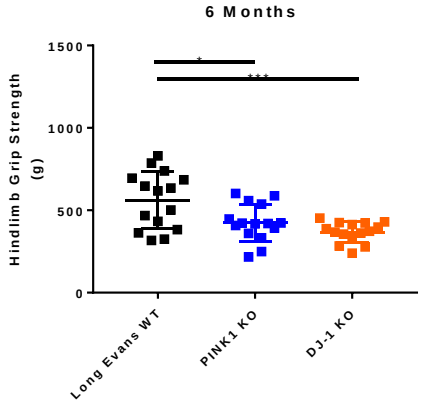
Forelimb Grip Strength Change Across Age

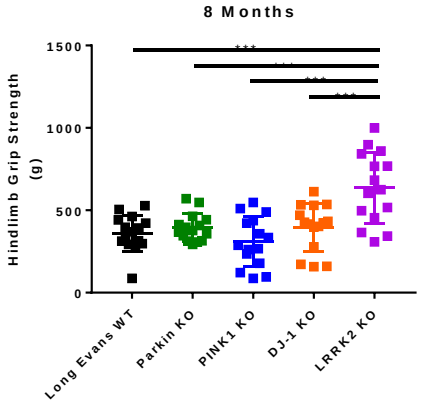
	Long Evans	Parkin KO	PINK1 KO	DJ-1 KO	LRRK2 KO
4-6 Mo	↑**	N/A	=	=	N/A
6-8 Mo	=	N/A	=	=	N/A
4-8 Mo	↑**	↑*	=	=	↑***
8-12 Mo	=	=	N/A	N/A	=
4-12 Mo	↑**	↑***	N/A	N/A	↑***

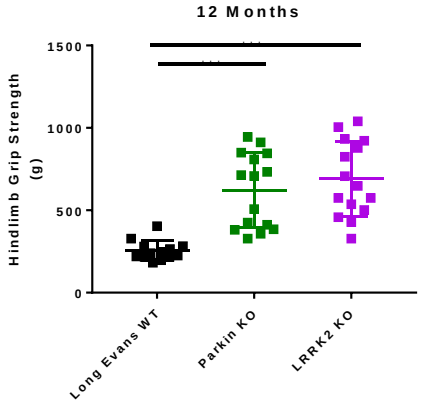
Significance: ***= p<0.001; **= p<0.01; *= p<0.05; (Orange = age overview)

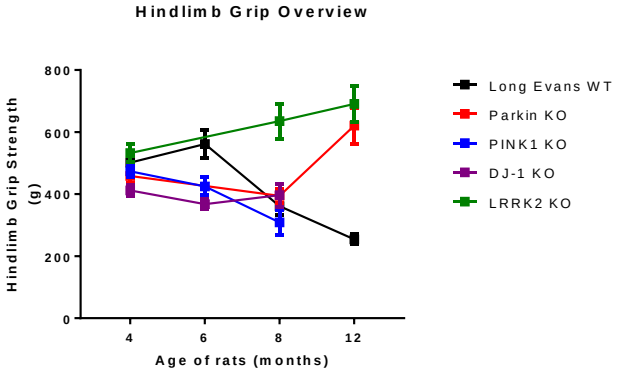
HINDLIMB GRIP STRENGTH









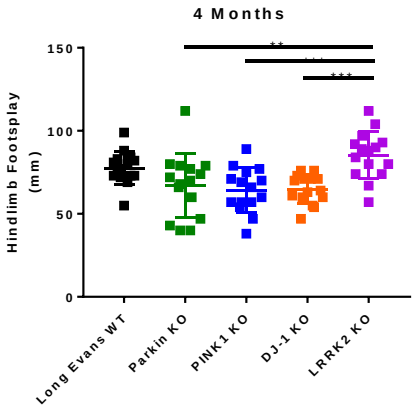


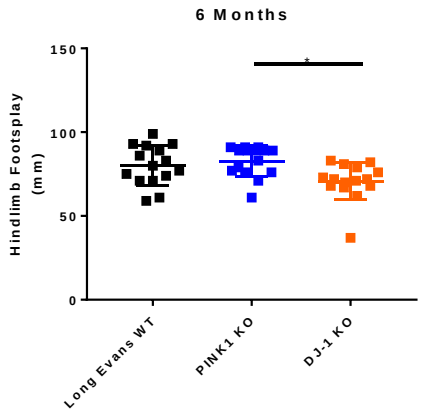
Hindlimb Grip Strength Change Across Age

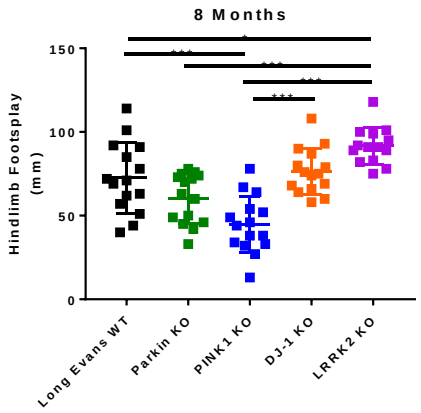
	Long Evans	Parkin KO	PINK1 KO	DJ-1 KO	LRRK2 KO
4-6 Mo	=	N/A	=	=	N/A
6-8 Mo	↓***	N/A	↓*	=	N/A
4-8 Mo	↓*	↑***	↓***	=	=
8-12 Mo	=	=	N/A	N/A	=
4-12 Mo	↓***	↑*	N/A	N/A	=

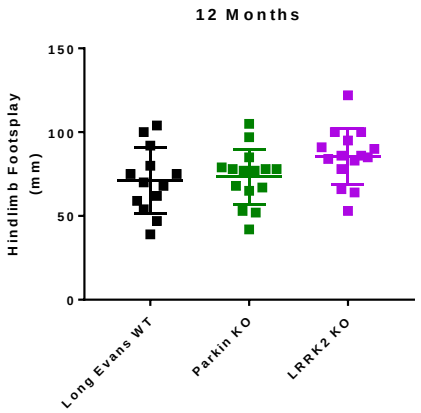
Significance: ***= p<0.001; **= p<0.01; *= p<0.05; (Orange = age overview)

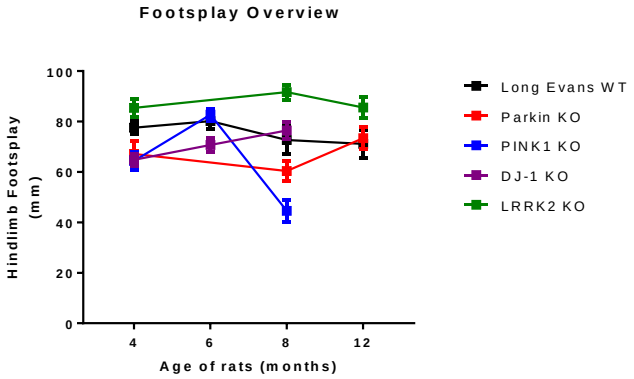
HINDLIMB FOOTSPLAY











Hindlimb Footsplay Change Across Age

	Long Evans	Parkin KO	PINK1 KO	DJ-1 KO	LRRK2 KO
4-6 Mo	=	N/A	↑**	=	N/A
6-8 Mo	=	N/A	↓***	=	N/A
4-8 Mo	=	=	↓***	↑*	=
8-12 Mo	=	=	N/A	N/A	=
4-12 Mo	=	=	N/A	N/A	=

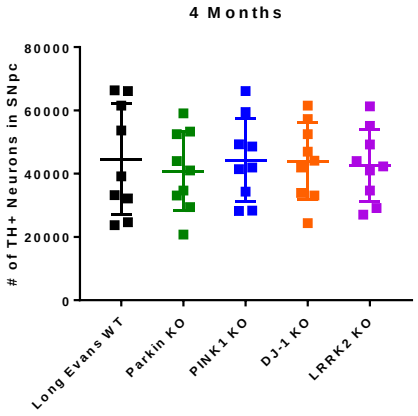
Significance: ***= p<0.001; **= p<0.01; *= p<0.05; (Orange = age overview)

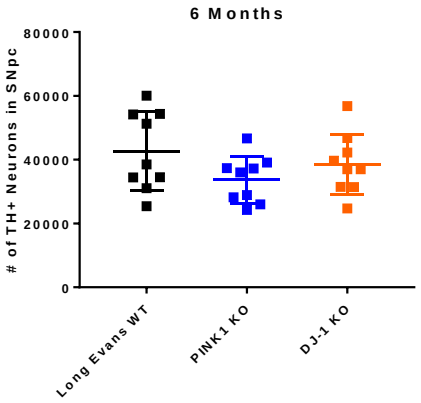
STEREOLOGY DATA

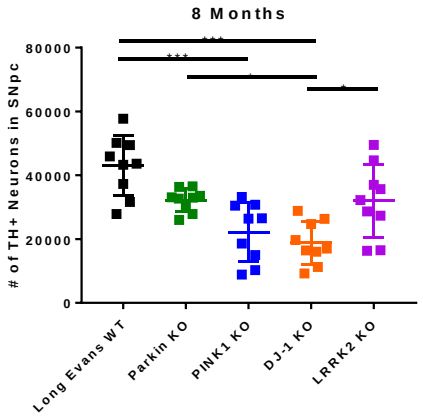
TH+ NEURONS IN THE SUBSTANTIA NIGRA PARS COMPACTA

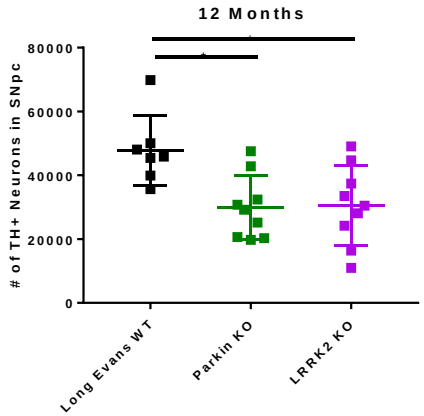
[n = 9 male rats per group]

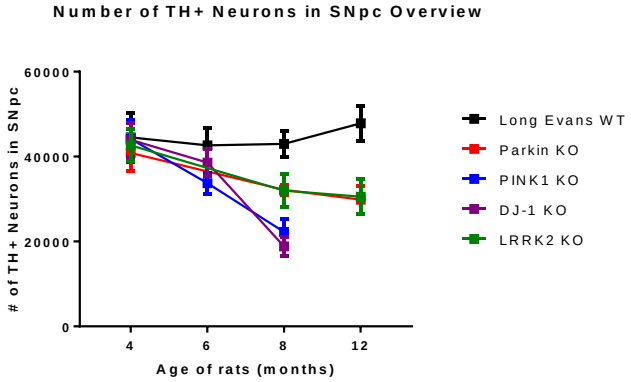
TH-POSITIVE CELLS IN SN









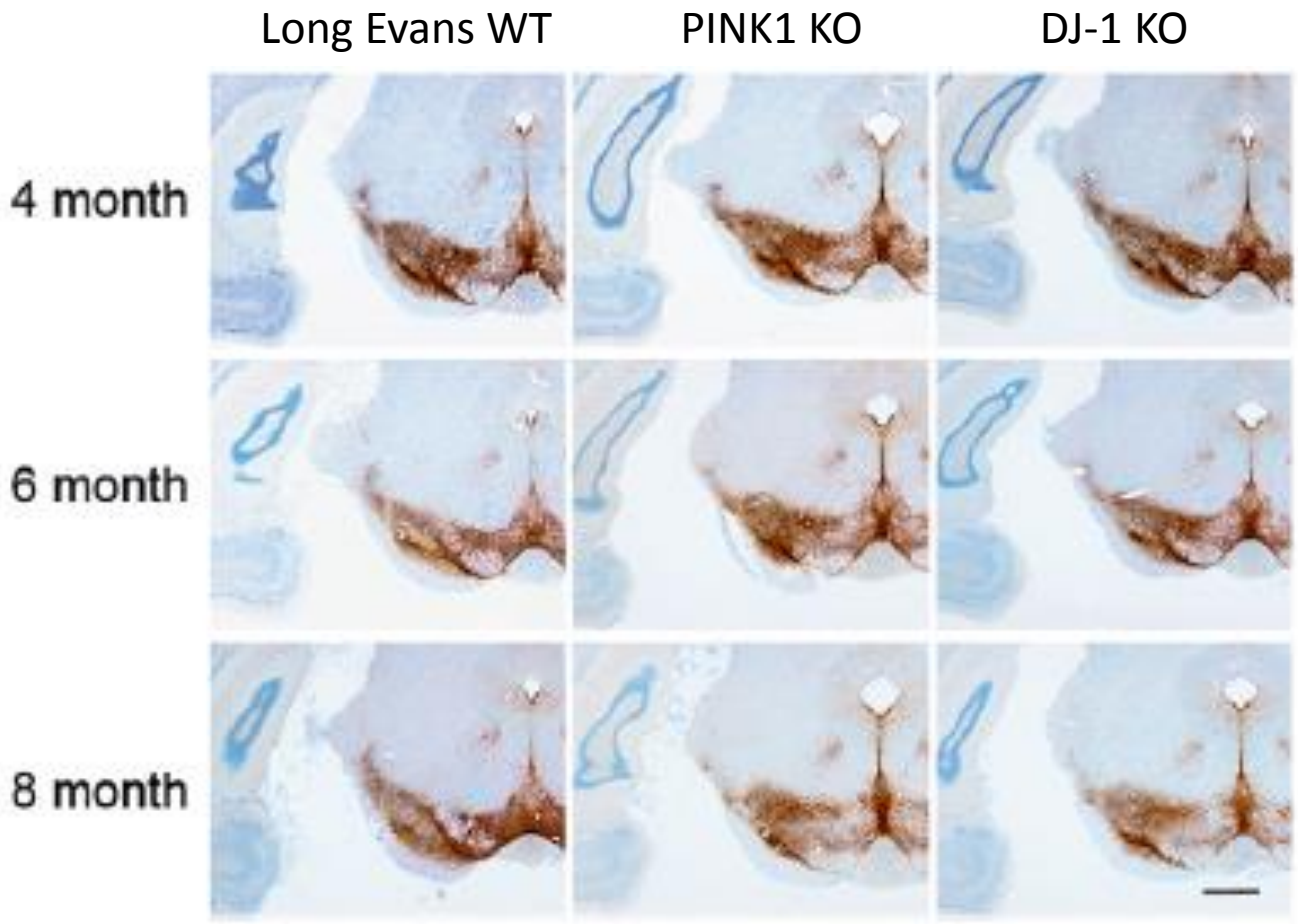


SNpc Dopamine Neuron Number Change Across Age

	Long Evans	Parkin KO	PINK1 KO	DJ-1 KO	LRRK2 KO
4-6 Mo	=	N/A	=	=	N/A
6-8 Mo	=	N/A	=	↓***	N/A
4-8 Mo	=	=	↓***	↓***	=
8-12 Mo	=	=	N/A	N/A	=
4-12 Mo	=	=	N/A	N/A	=

Significance: ***= p<0.001; **= p<0.01; *= p<0.05; (Orange = age overview)

TH-POSITIVE CELLS IN SN



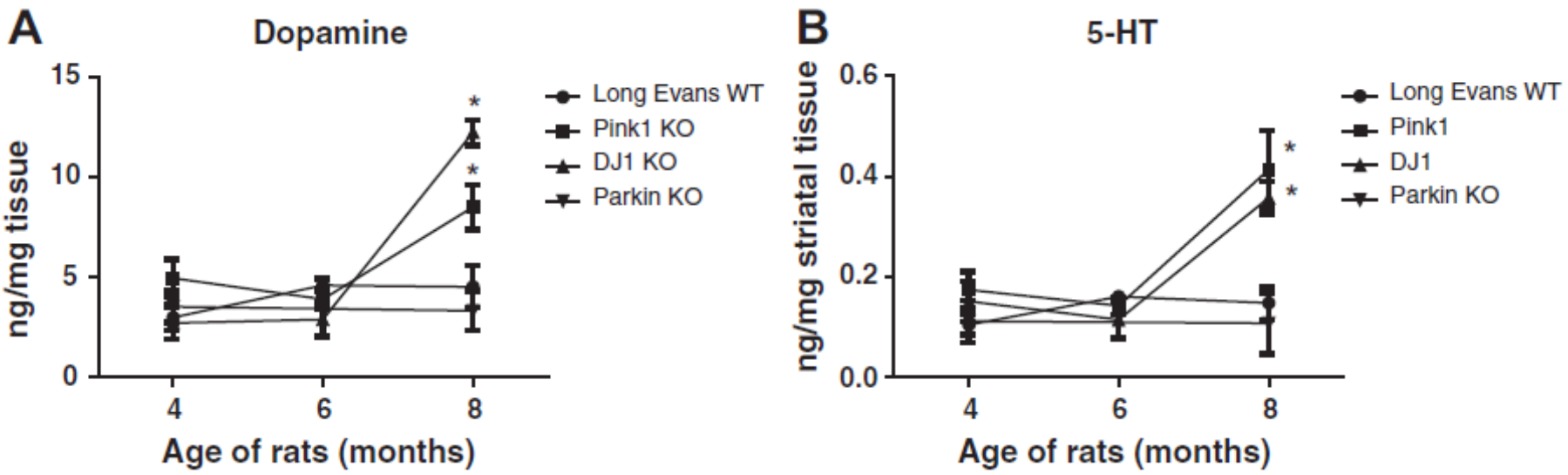
Dave, K.D., De Silva, S., Sheth, N.P., *et al*., (2014). Phenotypic characterization of recessive gene knockout rat models of Parkinson's disease. *Neurobiol Dis,* 70: 190-203.

NEUROCHEMISTRY

DOPAMINE
5-HT

[n = 6 male rats per group]

DOPAMINE AND 5-HT



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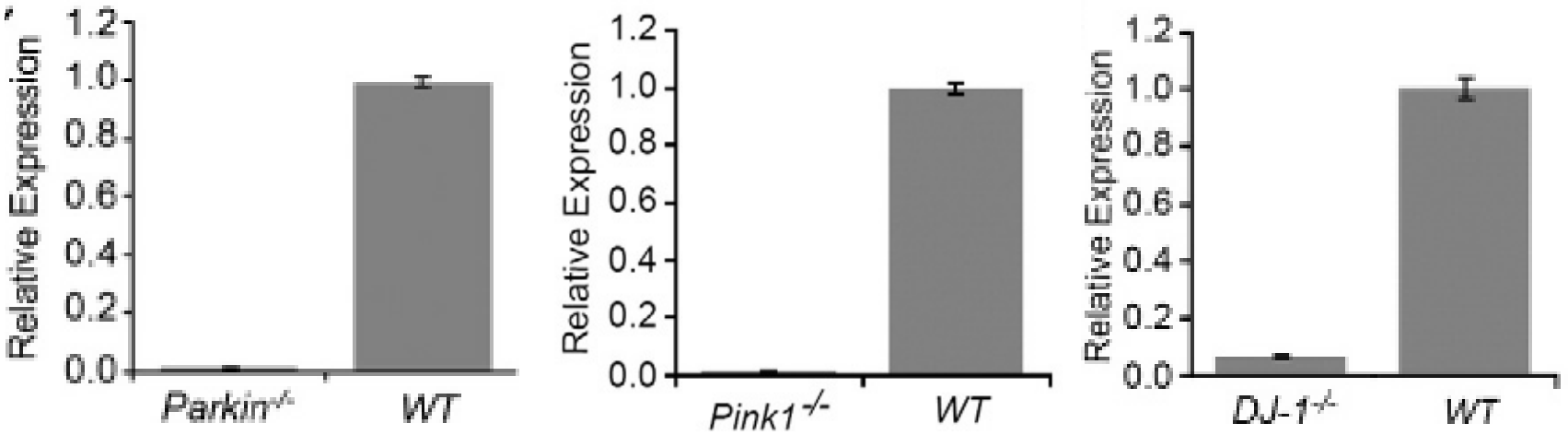
MRNA EXPRESSION

[n = 6 male rats per group]

MRNA EXPRESSION LEVELS



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