

# The use of fMRI and DTI to characterize the PTEN-induced putative kinase 1 (Pink1) knockout rat model of Parkinson's disease

T. M. BARCHET<sup>1</sup>, K. GAMBER<sup>2</sup>, P. SIMMONS<sup>2</sup>, M. NEDELMAN<sup>3</sup>, P. KULKARNI<sup>1</sup>, C. F. FERRIS<sup>1</sup>;  
<sup>1</sup>Northeastern Univ., Boston, MA; <sup>2</sup>SAGE Labs, Saint Louis, MO; <sup>3</sup>Ekam Imaging, Boston, MA



## ABSTRACT

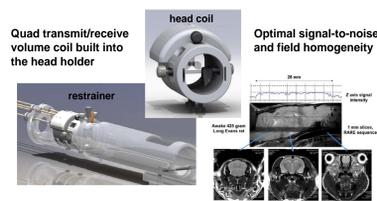
The hallmark symptoms of Parkinson's disease (PD) are the gradual loss of motor function and changes in affective behavior. Unfortunately, there is often no indication that a patient will develop the disease until they become symptomatic. Can subtle changes in behavior and neurodegeneration be detected noninvasively, prior to any disease phenotype? To address this question, we studied the Pink1-KO rat model of PD (SAGE Labs, St. Louis, MO) with magnetic resonance imaging (MRI) to evaluate disease progression. Because of the reported loss of olfactory sensitivity in PD patients and increased symptoms of anxiety and depression we presented Pink1-KO rats and wild-type controls with the odor of almond, a stimulus to elicit the innate reward response (Kulkarni et al. Behav. Brain Res, 230:201, 2012). This odor presentation was conducted during the imaging session while animals were fully awake. Pink1-KO rats showed a significant reduction in activation of the limbic cortex as compared to wild-type controls. Diffusion tensor imaging (DTI) with quantitative anisotropy was performed to detect differences in brain microarchitecture between Pink1-KO and wild-type controls. To do so we used a novel method of analysis in which different indices of anisotropy (IA), e.g. ADC, FA,  $\lambda_1$ , RA from over 20,000 voxels were registered into a 3D segmented, annotated rat atlas covering 152 brain areas. Statistical comparisons between brains of Pink1-KO and wild-type were examined at 16-18 wks of age and again several months later for all brain regions present in the 3D segmented MRI atlas. There was a progressive change in IA values in Pink1-KO as compared to wild-type particularly in the limbic cortex (anterior cingulate, prelimbic and infralimbic areas) dorsal striatum, and hippocampus. These results support the use of non-invasive MRI to identify potential biomarkers of early disease progression in a transgenic rat model of PD and open up the possibility for assessing the efficacy of new therapeutics on brain function and structure to compliment the more traditional behavioral assays.

## Materials and Methods

### Acclimating Rats for the Imaging

All animals were lightly anesthetized and placed into a copy of the restraining system used during awake imaging. When fully conscious, the animals were placed into a dark mock scanner tube with a recording of a standard MRI pulse sequence playing in the background. The reduction in autonomic and somatic response measures of arousal and stress improve the signal resolution and MR image quality. (King et al. 2005 J Neurosci Meth.)

### Imaging Technology



### Study Protocol

Adult, male Pink1 KO and age matched Long Evans Rats were used in this study. Experiments were conducted using a Bruker Biospec 7.0T/20-cm USR horizontal magnet (Bruker, Billerica, Massachusetts) and a 20-G/cm magnetic field gradient insert (ID = 12 cm) capable of a 120- $\mu$ s rise time (Bruker). Radiofrequency signals were sent and received with the quad-coil electronics built into the animal restrainer. On test days, animals (n=8) were placed in the restrainer, and positioned in the magnet. Following Scans were collected for each subject.

**Anatomical scans** : At the beginning of each imaging session, a high-resolution anatomical data set was collected using the RARE pulse sequence (20 slice; 1 mm; field of vision [FOV] 3.0 cm; 256 x 256; repetition time [TR] 2.5 sec; echo time [TE] 12.4 msec; NEX 6; 6.5-minute acquisition time).

**Functional Scan**: Functional data were acquired using a multi-slice half Fourier acquisition, single shot, turbo spin echo sequence. A single scanning session acquired 20 slices, 1mm thick, every 6.0 seconds (FOV 3.0 cm, matrix size 96 x 96, ETL 36, NEX 1) repeated 90 times for a total time of 9 minutes. Odor was presented through nose cone at 4 min into the imaging session, and odor was removed 7 minutes into the scan as a washout period

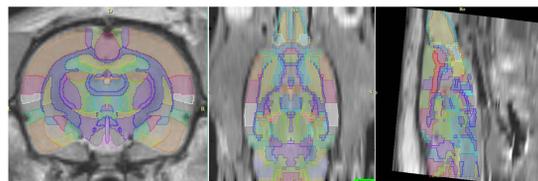
**Diffusion Tensor Imaging**: DTI images were acquired using 3D EPI pulse sequence with (TE = 19 ms, 8 segments, TR =500 msec). The data was collected in 10 direction with one B0 image and B value=1000. The data was processed using MedNRIA ( V1.9.4).

**Locomotor Assessment**: At 12 weeks of age, rats were placed in an open field and were recorded on video. Automated behavioral tracking software (EthoVision XT, Noldus Information Technology) was used to evaluate the basic locomotor data.

**Tyrosine Hydroxylase Staining** provided by SAGE and Dave, K. D., et al. "MJFF Animal Models 2: Phenotypic characterization of the autosomal recessive (Parkin, Pink-1 and DJ-1) gene knockout rat models of PD." Presented at 2012 SfN Annual Meeting.

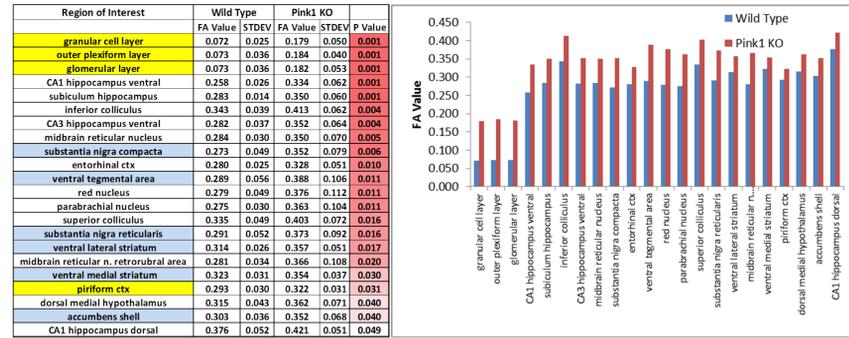
### Data Analysis

Each subject was registered to a 3D segmented and annotated rat brain atlas (Ekam Imaging Inc). The alignment process was facilitated by an interactive graphic user interface. The affine registration involved translation, rotation, and scaling in all 3 dimensions independently. The matrices that transformed the subject's anatomy to the atlas space were used to embed each slice within the atlas. All transformed pixel locations of the anatomy images were tagged with the segmented atlas regions, creating a fully segmented representation of each subject. Parameter values for each ROI was computed based on each segmented map.

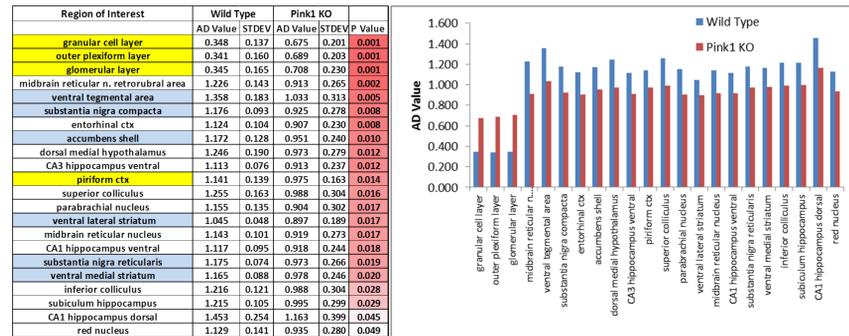


## MAIN RESULTS

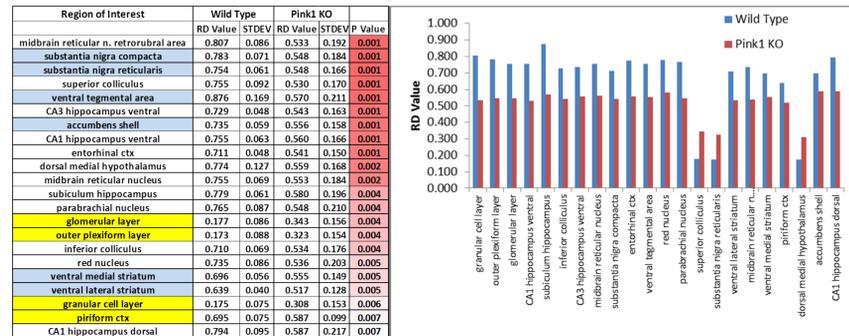
### Region-Specific FA Differences



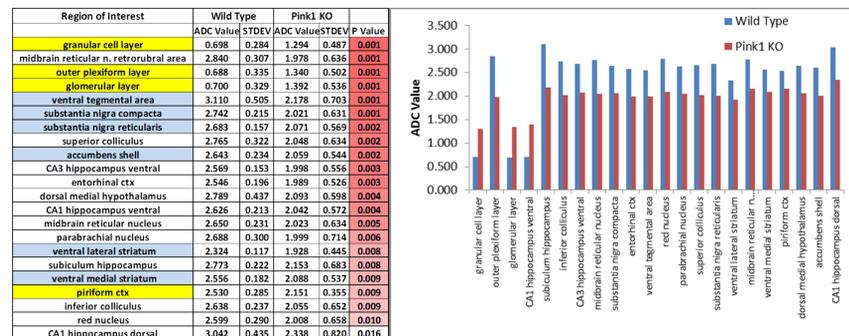
### Region-Specific AD Differences



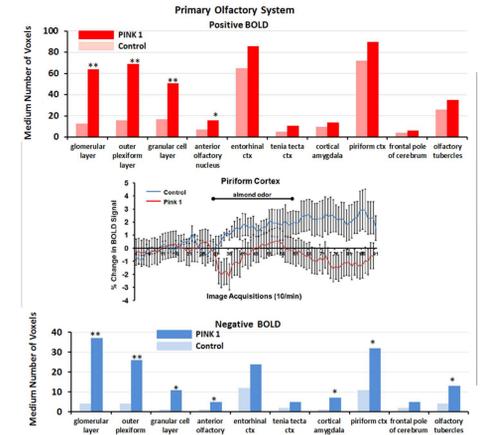
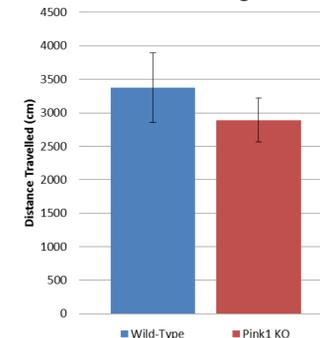
### Region-Specific RD Differences



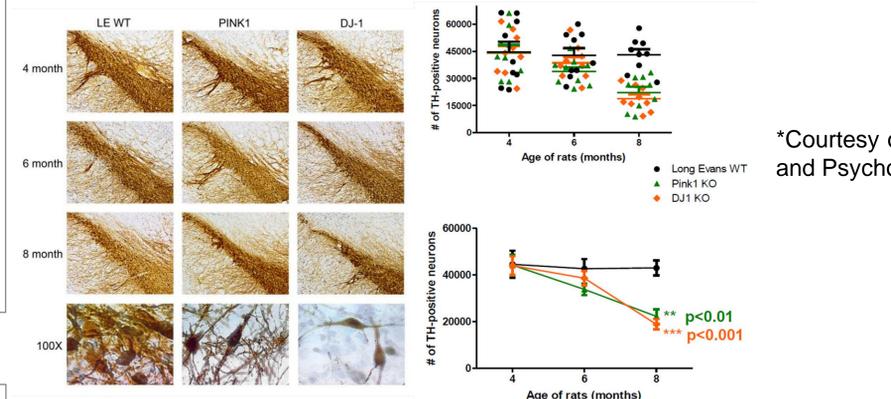
### Region-Specific ADC Differences



### Locomotor Differences at 12 Weeks of Age



### TH+ Nissl Cells in Substantia Nigra Pars Compacta



\*Courtesy of SAGE and PsychoGenics

## SUMMARY AND CONCLUSIONS

At 12 weeks of age in the Pink1 KO model of PD, prior to significant loss of TH+/Nissl cells in the substantia nigra pars compacta, there are signs of neurodegeneration that can be detected through automated behavioral tracking, functional MRI, and DTI/quantitative anisotropy.

The locomotor changes seen through automated tracking were subtle, and it is important to note that there were no gross motor/behavioral deficits at 12 weeks of age. This is consistent with the literature.

Decline in olfactory signaling was detectable at 12 weeks of age through functional magnetic resonance imaging.

DTI tractography showed no obvious disruption in white matter tracts in the rat Pink1 KO model of PD, but evaluation of the anisotropy data showed significant changes in water diffusivity in CNS regions associated with PD pathogenesis.

## ACKNOWLEDGEMENTS

The data for tyrosine hydroxylase was provided by SAGE and presented by Dave, K. D., et al. "MJFF Animal Models 2: Phenotypic characterization of the autosomal recessive (Parkin, Pink-1 and DJ-1) gene knockout rat models of PD." at 2012 SfN Annual Meeting.