**Anesthetic effects on the progression of Parkinson's disease in the rat DJ-1 model**

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## Supplemental Material and Methods

### Animals

Rats were from randomly selected from liters by SAGE labs before shipping. The animals were housed in the University of Pennsylvania Animal Care Facility with 2 animals per standard rat cage on a 12-h light-dark cycle, and given plexiglass cylinders for enrichment, and food and water ad libitum. Microchip transponders IMI-1000 (BioMedic Data Systems (BMDS), Seaford, DE, USA) were implanted subcutaneously for animal identification around 9 weeks of age and at this time one animal from each cage was randomly assigned to either the control or the exposed group. The experimental unit was defined as an individual rat. Two groups of rats were studied, an acute group (AG) with n=6 each for group and a long-term group (LTG), with n=18 controls and n=16 exposed (2 rats in the exposure group in the LTG died unrelated to the procedures). *A priori*, the exclusion criteria for the behavioral assays was an animal's completion of the task. All testing occurred during the light cycle.

### Behavioral testing

The investigators were blinded to the animal's group and the order of testing was randomized by assigning each animal a letter of the alphabet and the testing proceeded in that order. Furthermore, to reduce experimenter bias [1, 2], both a male and a female

investigator conducted all of the behavioral tests together or the female's lab coat was placed near the apparatus to negate the reported stress to animals if only a male investigator was present[19]. In addition, to reduce animal stress/anxiety from handling during the behavioral testing, all animals were handled 2-3 times per week, from their arrival at 6-8 weeks of age until euthanasia.

***Horizontal Ladder Rung Task.***

The animals were first habituated to the testing room for 1 h in their home cages. The investigators were blinded to the animals' experimental group. The ladder rung apparatus, consisting of 2 long pieces (108cm long x 20 cm high) of plexiglass separated by metal rungs (0.3 cm diameter, rung holes separated by 1 cm), was placed between a clean cage with fresh bedding and a home cage. The training trial began by placing the rat in the clean cage and then gently placing the rat at the start of the ladder rung, with the rungs all equally spaced, and allowing the rat to walk across the rungs to their home cage, containing their cagemate. All rats were tested with the same 4 ladder rung configurations each time, with at least 30 min between trials. The apparatus was cleaned with a 50% ethanol solution after each behavioral testing. The rats were video recorded in slow-motion with an iphone mounted to a moveable tripod. Two investigators each analyzed half of the blinded videos. A higher score is indicative of impaired performance.

***Accelerating Rotarod.***

The investigators were blinded to the animals' experimental group. At each time point, the animals were habituated to the testing room for 1 h in their home cages. After acclimation, the animals underwent a 60s training trial with the rotarod (IITC Series 8;

IITC Life Sciences Inc., Woodland Hills, CA, USA) fitted with a large drum for rats and set at a constant speed of 8 rpm, with animals being placed back onto the rotating rod after falling off until the entire 60s had elapsed. This training trial was followed by three test trials with the rod speed gradually accelerating from 4 to 40 RPM over a 5 min

period, with at least 30 min between trials. The apparatus was cleaned after each trial with a 50% ethanol solution. These animals appeared to be stressed by the task, as higher than average defecation was noted. For each trial, the rats were given 3 chances to remain on the rotarod for more than 10s. If they refused to go on the rotarod or did not remain on the rotarod for at least 10 s, the data for that trial was not included. Our initial criterion for repeated measures 2-way ANOVA analysis was for all animals to complete 3 trials at each time point. Due to incomplete testing, the criteria had to be changed so that each animal had to complete 2 out of 3 trials at each time point to be included. If an animal completed 3 trials at one time point, the best 2 times were used. Any animals that completed none or only 1 of the 3 trials at any time point were excluded from the analysis. For the AG, 6/6 control rats and 4/6 exposed rats and for the LTG,13/18 controls and14/16 isoflurane exposed rats completed 2 trails at each time point for the analyses. Lower time spent on the rotarod is indicative of poor performance.

***Novel Object Recognition.***

The NOR test was conducted over the course of three days, and each day, rats were habituated to the testing room for 1 h in their home cages. The investigators were blinded to the animals' experimental group. On Day 1, rats were habituated to the testing container (45-gallon plastic Sterilite® latch storage bin (Sterilite Corp, Townsend,

MA) with a custom white Plexiglas base) with no objects in 10-minute intervals. On Day 2, rats were first allowed to explore the bin with two familiar objects (250 ml Corning cell culture flasks, Fisher Scientific, Waltham, MA USA**,** 08757503) for 10 minutes then, 2.5 hours later, one of the familiar objects was replaced with a novel object (100 ml pyrex glass media storage bottle, Fisher Scientific, Waltham, MA USA**,** 064141A), and rats were allowed to explore for 10 minutes. Objects were filled with water and secured to the floor with Velcro to stabilize. On Day 3, the pyrex glass media storage bottle was replaced with a 50ml falcon conical centrifuge tube (Fisher Scientific, Waltham, MA USA**,** 1443222), and the same procedure was repeated. On each day, the plastic bin and novel objects were cleaned with 50% ethanol solution after each rat's trial. Using multiples of the same objects allowed for ample time for any ethanol smell to dissipate. Given a rat's natural tendency to explore new objects, differences in time spent at a novel object were analyzed between control and exposed animals.

**Immunohistochemistry**

The brains were sectioned at 8 μm and 5-10 slides per animal, spaced at 10-20 slides apart, were deparaffinized in xylenes and graded alcohols, followed by antigen retrieval in an antigen unmasking solution (Vector laboratories, Burlingame, CA, USA, H-3300) in a pressure cooker, before immersing in hydrogen peroxide to reduce non-specific binding. The washed slides were blocked using the Vectastain blocking serum for 1h (Vector laboratories, Burlingame, CA, USA, PK-6101) and then incubated overnight with the primary antibody (anti-tyrosine hydroxylase, Pel-Freez Biologicals, Rogers, AR, USA, P40101-150 or anti-Iba-1: Fujifilm WakoChemicals, Richmond, VA , USA, 019-

19741) The next day, the washed slides were incubated in biotinylated secondary antibodies (Vector, PK-6101) for 1h, followed by incubation in avidin-biotin complex (Vector laboratories, Burlingame, CA, USA, PK-2200) for 1h before finally in 3,3'-diaminobenzidine (Vector laboratories, Burlingame, CA, USA,, SK4100), dehydration and cover slipped with permount mounting medium (Fisher Scientific, Waltham, MA, USA, SP15100). Slides, blinded to the investigator, were examined on an Olympus microscope (model BX41) at 4X, and images were captured using iVision software v4.5.5 (BioVision Technologies, Exton, PA, USA) and the number of cells per unit area was quantified using Image J (National Institutes of Health and the Laboratory for Optical and Computational Instrumentation, Bethesda, MD).

**References**

1. Sorge RE, Martin LJ, Isbester KA, et al. Olfactory exposure to males, including men, causes stress and related analgesia in rodents. *Nat Methods*. 2014;11:629-32.

2. Chapman CD, Benedict C, Schioth HB. Experimenter gender and replicability in science. *Sci Adv*. 2018;4:e1701427.