Detailed Methods:

Animals. Male (n=12) and female (n=12) Sprague-Dawley rats (H1a: (SD) CVF, approximate body weight of 200 – 230 g at arrival), were obtained from Hilltop Lab Animals, Inc. (Scottdale, PA). All animals were free of viral pathogens, parasites, mycoplasm, *Heliobacter* and cilia-associated respiratory bacillus. Upon arrival, animals were acclimated in AAALAC International accredited animal facilities at NIOSH for one week. They were housed in ventilated micro-isolator units supplied with HEPA-filtered laminar flow air (Lab Products OneCage; Seaford, DE), Teklad Sanichip and Shepherd Specialty Paper's Alpha-Dri cellulose, tap water and autoclaved Teklad rodent diet (Harlan Teklad; Madison, WI) *ad libitum*. Rats were housed in same sex pairs, and under a controlled light cycle (12 h light/12 h dark) and temperature (22 – 25 °C) conditions.

One week following acclimation, animals were assigned to control or WBV conditions. The exposure and all other procedures performed were approved by the Institutional Animal Care and Use Committee and were in compliance with the Public Health Service Policy on Humane Care and Use of Laboratory Animals and the NIH Guide for the Care and Use of Laboratory Animals.

Equipment and characterization of the exposure. Platforms (L 20.32 cm x W 15.24 cm x thickness 1 cm) were printed on a 3D printer (Fusion3 F410) using a poly lactic acid polymer. Flat bottomed restrainers were purchased (Brain Tree Scientific, Brain Tree, MA; dimensions inside the restrainer L 22 cm x W 8.26 cm x height at center 6.35 cm) and the NIOSH machine shop removed the bottom and added an extension to the side of each restrainer so that it could be attached to the platform. Platforms were attached to the shakers, and a restrainer was attached to each platform. An 353B15 accelerometer (PCB Piezotonic, DePew, NY) was attached to the middle of the platform, next to the restrainer. The restrainer, platform and shaker were all placed

in a sound attenuating chamber. Vibration to the platform was generated by a shaker (Ling Dynamics, Royston, UK). . To ensure there were no resonances of the platform, a laser vibrometer was used to measure both the response of the restrainer and of the platform between 10 and 100 Hz at 0.3 g. The platform and restrainer were stable up to 100 Hz. Control animals were placed in Broome Style restrainers and housed in sound attenuating chambers during the restraint-only (control) exposure

Exposure. After acclimation to the facilities, animals were acclimated to restraint for 5 d. Acclimation to restraint consisted of putting animals in a Broom Style restrainer, or into a restrainer mounted onto a platform and shaker, for gradually longer lengths of time until the total time in the restrainer was 4 h. The restrainers were large enough so that animals could move and turn around, but they could not rear up onto their hind legs, thus ensuring that the animals paws and torso were in contact with the vibrating platform or stable bottom of the restrainer, throughout the exposure. After acclimation to restraint (and the demonstration of the presence of normal reproductive cycles in females), exposures were started. Animals were placed into their restrainers and rats in the WBV group were exposed to vibration at 31.5 Hz and an amplitude of 0.3 g for 4 h/day for 10 consecutive days. This frequency was chosen because 30 Hz was the resonant frequency of lumbar region of the spine in female rats. This frequency was also in the range of the resonant frequency of the lumbar spine region (between 20-30 Hz) in male rats. Control animals were placed into a non-vibrating restrainer for the length of the exposure (e.g. 4 h) each day. Pre-exposure body weights were collected on days 1, 5 and 10 of the experiment. Body weights were also collected immediately following the exposure on days 5 and 10 to determine if the exposure induced a transient reduction in body weight. Following each exposure, animals were returned to their home cage in the animal facility.

Monitoring reproductive (estrous) cycles in female. Estrous cycles were monitored in females by performing vaginal lavage and then recording the primary cell type in the lavage fluid. We began performing vaginal lavage while animals were being acclimated to restraint. Each day, prior to placing an animal in the restrainer, vaginal lavage was performed using the methods described in (1, 2). The lavage fluid was placed on a clean slide that was divided into 12 sections using a wax pencil. The fluid from an individual animal was placed in a single square on the slide. After all samples were collected, the slide was dried on a slide warmer and stained with Toluidine blue. Samples were examined using a Leica microscope (Deerfield, IL) at 20 x magnification. Day of the estrous cycle was determined as described in (2); diestrus day 1 and 2: primarily leukocytes, proestrus: primarily nucleated cells, and estrous: primarily cornified cells. Cycles were monitored for 10 consecutive days prior to beginning the exposure. All females displayed 2–4-day cycles prior to beginning the exposure. Estrous cycles were also monitored while animals were being exposed. Lavage fluid was collected each day, prior to exposing the animals to control of WBV conditions.

Tissue collection. The day after the last exposure, rats were euthanized by injection of sodium pentobarbital (100-300 mg/kg i.p.) and exsanguinated by cardiac puncture. Blood samples were collected, allowed to clot and centrifuged (1300 rcf for 15 min at 4°C). Serum was pipetted into tubes and stored at -20°C until assayed for various hormones by ELISA. Other tissues were collected to measure vibration-induced changes in oxidative stress and RNA concentrations. The tissues, including the pituitary, the left ovary and testes, uterus, and prostate were dissected, placed in cryotubes and stored at -80°C until analyzed. The right ovary and testis were collected, frozen in Tissue-Tek OTC compound (Fisher Scientific) and stored at -80ºC they were sectioned for histological analyses.

Oxidative stress: Tissues were homogenized in 1 ml of 0.1 M phosphate buffered saline (0.1 M PBS) containing protease inhibitors (ULTRA cOMPLETE protease inhibitor, ThermoFisher,) using a bead beater. The samples were then centrifuged at 1300 rcf for 15 min at 4ºC. The supernatant was removed and stored for protein analyses. The pellet was reconstituted in 500 µl of PBS, vortexed and stored on ice. ROS was measured using, 2´7´-dichlorofluorescien diacetate (DCFH-DA; Sigma-Aldrich). Duplicates of the supernatant from each pellet (10 µl) were pipetted into a 96-well plate. DCFH-DA was diluted 1:20 in PBS (final concentration 1 mM) and 50 µl was added per well. Plates were incubated in the dark for 45 min and then fluorescence was measured at 490 – 540 nM using a Synergy H1 All in One microplate reader and Gen 5 Software package (Biotek; Winooski, VT). Background measures (wells with dye plus PBS) were subtracted from each sample, and fluorescence/µg tissue was analyzed as described below.

Western Analyses. Westerns were performed in a manner similar to that described in (3). Samples were prepared by adding 1:1 volume of Laemelli's solution to each sample, heating at 100-110ºF for 5 min and then cooling on ice. A molecular weight ladder (Bio-rad, Irvine CA) and 10 µl (10 µg) of was added to a well of a pre-casted 12% acrylamide gel (BioRad, Irvine CA). Samples were run at 200 V for approximately 1h or until the dye front reached the bottom of the gel. Separated proteins were then electrophoretically transferred from the gel to a nitrocellulose membrane using a mini-gel transfer system (Biorad). Membranes were rinsed in 0.1 M Tris buffered saline (TBS) and incubated in Blocking Reagent (BioRad) for 5 min at rt. After blocking, membranes were incubated in primary antibody diluted in blocking reagent at 4ºC overnight with agitation. The following morning, blots were rinsed in 0.1 M Tris buffered saline (TBS) 3 x 5 minutes and then incubated in the appropriately labelled infra-red antibody

(anti-mouse or anti-rabbit; 1:5000, LiCor, Lincoln, NB) in TBS-0.3% Triton-X 100) for 1 h at rt with agitation. Blots were then rinsed, stored in the dark at 4ºC until dry, scanned using a LiCor Scanner and Odyssey Software, and the band area and density were analyzed using ImageJ (National Institutes of Health; Bethesda, MD).

ELISAs. ELISAs were used to measure circulating estradiol (E-Lab Sciences, Houston Tx), follicle stimulating hormone and prolactin (ALPCO, Salem, NH) in serum collected from females. In males, ELISAS for pituitary hormones were also performed, but instead of measuring estradiol, testosterone concentrations (E-Lab Sciences) were measured. Each ELISA was performed as described in the manufacturer's protocol. All samples were run in duplicate, and the coefficients of variation were calculated for each assay. Coefficients of variation were 12% or less for samples with either high or low concentrations of each of the hormones measured.

Tissue preparation for histology and immunohistochemistry. One ovary and one testis from each animal was frozen on dry ice, and sections (20 µm) were cut on a cryostat and thaw mounted onto charged slides (Fisherbrand Superfrost Plus, Pittsburgh, PA). To ensure that each slide contained sections throughout the range of the dissected tissue, the first section was placed on slide one, the second on slide two and so on until sections had been placed on 20 slides. Then the process was repeated until there were 4-6 sections on each slide. Using this procedure, each section on a single slide was approximately 200 µm apart. Sections with slides were air dried, placed in slide boxes and stored at -80°C. One set of sections was also processed using hematoxylin and eosin (H&E) stain and the others were used for immunohistological identification of specific proteins.

H&E and immunohistochemical staining. Harris H&E staining was performed on one a single slide from each animal (4, 5). Slides were thawed and fixed in 4% paraformaldehyde in 0.1 M phosphate buffered saline prior to staining. In the ovaries, the number of developing follicles was counted in each section of the ovary at a total magnification of 100X using a Leica DMLB microscope (Leica, Deerfield, IL) . The average number of follicles/section was calculated and used for analyses. In males, the number of Sertoli cells, Leydig cells and spermatids were counted in each section using a stereological technique similar to that described in (6). A field was randomly chosen and a 1 mm square grid with 100 μm divisions was placed over the field of view. The number of spermatids, Sertoli cells and Leydig cells that were within the boundaries of the grid were counted. This procedure was repeated 4 times in tissue sections from each animal. The average number of spermatids, Sertoli cells and Leydig cells was calculated for each animal and these averages were used for analyses.

Immunohistochemistry for nitrotyrosine, Erα and β receptors and LHr was performed in a single set of sections from the ovary, and Ar and LHr staining was performed in a single set of sections from the testes. All primary antibodies were from Santa Cruz Biotechnology (Santa Cruz, CA) and were used at a dilution of 1:200. Immunohistochemistry was performed using methods described in (7). Briefly, sections were fixed in 4% paraformaldehyde in 0.1 M PBS, rinsed, incubated in 0.3% hydrogen peroxide in methanol to block endogenous peroxidase activity, rinsed and incubated in primary antibody diluted in 0.1M PBS, 0.3% Triton-X 100 and 10% normal serum (goat or donkey; Santa Cruz Biotechnology) overnight at 4ºC. The following day sections were rinsed, incubated in w Cy3-labeled second antibody (Jackson ImmunoResearch Laboratories, West Grove, PA) diluted 1:800 in 0.1 M PBS, 0.3% Tx for 1 h at rt, rinsed, air dried, and cover-slipped with Prolong-Gold with DAPI (Thermofisher). Sections were viewed at

using an Olympus BX63 fluorescent microscope and photomicrographs made at a magnification of 200 x using DP73 camera and cellSense version 510 (Fisher Scientific, Indianapolis IN). The area labeled and the intensity of the immunolabeling were quantified in each image using NIH Image. Average labeling are staining intensity for each animal was calculated by averaging the values from each animal. These average values were used in the analyses described below.

Quantitative reverse transcriptase polymerase chain reaction (pRT-PCR). qRT-PCR was performed to determine if exposure to WBV resulted in changes in transcript levels in the pituitary, ovaries, uterus, testes and prostates of exposed animals using methods described in (5, 8, 9). RNA was isolated from each tissue using RNAeasy lipid Miniprep kits (cat # 74804; Qiagen, Valencia, CA), and first strand cDNA was synthesized from 1 μg of total RNA using a Reverse Transcription System (Invitrogen; Carlsbad, CA). Melt curves were run for each transcript using each tissue. Samples that did not show a single defined melt peak in the 80 ºC range were not included in the data set. To determine if the treatment resulted in a change in transcript levels, fold changes from the same day controls were calculated. This was done by calculating the average response for the control group and then subtracting the individual CT values for each sample from the average of the controls. Transcript levels for anti-oxidant enzymes, cytokines, factors involved in regulating cell cycle, and steroid hormone and anterior pituitary hormone receptors were measured in each tissue.

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