**Explanation of rearing data collection**

A subs-set of mice were evaluated for changes in exploratory and rearing behavior following exposure to functionalized and non-functionalized soot. *[explanation of how soot was injected into the nose every other day].*

Data for tracking animal rearing and distance traveled was collected using an Intel® RealSense™ Depth Camera D435. Animals were individually placed into an empty 13.5" x 12.5" x 5.6" (L x W x H) plastic tub where they were allowed to roam freely for 20 minutes. The tub was cleaned between successive animals and all experiments took place between the hours of 9:00 AM and 4:00 PM. Each animal was allowed to explore once, and was then perfused within the following 2 days for histological analysis. The experimenter collecting the data was blind to each animal’s treatment (water, Soot2040, Soot2040F) and the animals were randomly shuffled during data collection so that identical treatments didn’t all occur in succession.

The camera provides a true-color RGB image stream as well as an infrared stream used to calculate the animal’s distance from the camera. Data was acquired at ~15 Hz using a MATLAB wrapper to stream and save the image stacks for subsequent image processing. A side-by-side comparison of the original RGB camera and unprocessed depth camera can be viewed in the supplemental video (tbd – currently PresentationMotionVideo\_RGBvsOriginalDepth.avi). The unprocessed depth stream is highly noisy, with several holes in the image due to the scattering and imperfect collection of the infrared light off the imaging surface. Each infrared depth stack was run through a series of image processing steps to fill the holes in the image as well as remove the background to only leave the pixels associated with the mouse. An example of the final processed depth stack can be seen in supplemental video (tbd - currently PresentationMotionVideo\_RGBvsProcDepth). This background subtraction allows us to use the remaining pixels for height and motion tracking. A detailed explanation of the image processing can be found in the README of the analysis code, available at <https://github.com/KL-Turner/Norwood_Turner_Drew_Manuscript2020> - this is private and will change to a public repo on the drewlab github upon submission. I can share the README and repository with you if you’d like and will have it ok’d by Patrick.

To determine when the animal reared, we focused on how the mean of the lowest 20% of pixel values changed over time, which corresponds to the highest points on the mouse (the distance decreases as it stands up and approaches the camera). These pixel values typically represent the animal’s head as it moves throughout the bin, and serves as a good indicator for when the mouse stands up. A rearing event was defined as the mouse’s head surpassing 3 cm above baseline which corresponds to the mouse head height while exploring horizontal to the bottom of the bin. This threshold captures when the mouse stands up as a positive rearing event, but doesn’t count smaller elevations in height such as the mouse sitting and cleaning itself. Based on the sampling rate of the camera, we can estimate the number and duration of rearing events as the number of times the mouse’s head passes this threshold. To track the distance the animal traveled during the duration of the experiment, the distance between the centroid of the mouse was calculated between each successive camera frame. This distance between frames is then summed over the course of the 20 minutes. An example that visualizes how the mouse height/distance is calculated can be viewed in supplemental video (tbd – currently PresentationMotionVideo\_Results.avi – this needs to be rerun since the height of the new animal I used as an example surpassed the yaxis height for the previous example I used).

A generalized linear mixed-effects model (MATLAB function *fitglme*) was used to evaluate the differences in rearing events, rearing duration, and distance traveled. Each treatment (water, Soot2040, Soot2040F) is a fixed-effect with the mouse’s ID and sex (Male/Female) being a random-effect. After correcting for multiple comparisons using the 97.5% confidence interval, we found no significant difference between the water control and either soot treatment for any of the observed behaviors (rearing events, rearing duration, distance traveled). The water control group consisted of 10 mice (6M, 4F), Soot2040 group 8 mice (4M, 4F), and Soot2040F group 9 mice (5M, 4F).