# Overall information

Throughout, M = male and F = female for adults. Larvae were not sexed.

L, followed by a number, indicates a larva of a particular stage (larval stages 1, 4, and 6 were used). VNC will stand for ventral nerve cord, and B for brain, for the larvae. For the adults, CB stands for central brain (everything except the optic lobes in the protocerebrum), while OL stands for optic lobes. Total is the sum of the CB + OL data for a single individual.

For Isotropic Fractionator (IF) data, three L6 larvae were squished and averaged together per data point; this was done to get enough brain matter for the method. L1 and L4 larvae could not be counted reliably using IF, thus immunohistochemistry was used. For Immunohistochemistry (IHC), which was completed on L1/L4 larvae, individual larvae were imaged and counted separately.

# Sheet: “Cell Numbers”

**Cell Numbers** provides the number of cells in the brain for L1 and L4 larvae (counted using IHC, reference “Raw counts for IHC”), as well as L6 larvae and adult male/female brains organized into columns (counted using IF, reference “Raw IF data” and “Raw Counts for IF”).

 + To view this data by individual, go to the **Raw IF data** and **Raw counts for IHC** sheets

To explain the column names:

L followed by a number indicates a larva of a particular stage (1, 4, or 6 were used). VNC stands for ventral nerve cord; B stands for brain. Thus each data point in these columns represents the mean number of cells in the brain, or VNC, of a larva of a particular larval stage.

Female and male columns provide the data for the adults (as larvae were not sexed). For the adults, CB stands for central brain (everything except the optic lobes in the protocerebrum), while OL stands for optic lobes. Total is the sum of the CB + OL data for a single individual. Thus, each data point in these columns represents the mean number of cells in the total brain, or optic lobes/central brain regions, of a male or female adult black soldier fly.

# Sheet: “Masses”

**Masses** provides the OL, CB, and total brain masses of the adult males and females, as well as body mass. These data are used to obtain relative brain:body mass. Each row represents the same individual fly. The raw data, tagged to an individual identifier, can be found within the spreadsheet “Raw IF data”.

Column names:

“Brain mass – M/F” = Mass of the total brain, in mg, of a single male or female adult BSF

“OL/CB mass M/F” = Mass, in mg, of the optic lobes (OL) or central brain (CB) of a single male or female adult BSF

“Body mass M/F” = Mass, in mg, of the entire body of a single male or female adult BSF

“Rel Br:Body mass M/F” = the relative total brain to body mass of a single male or female adult BSF. To calculate this value, divide column A by column G for females and column B by column H for males (e.g., divide brain mass columns by body mass columns for each sex).

# Sheet: “Log BrBd Mass”

**Log BrBd Mass** provides the log (base 10) masses for adult black soldier fly central brains, optic lobes, and total brains, as well as log (base 10) body mass. These data are generally used for allometric analyses – as demonstrated in the paper. Each row represents an individual – individuals starting with ‘F’ are females, while individuals starting with ‘M’ are males. The original masses, tagged to each individual, can be found within the spreadsheet “Raw IF data”.

Column names:
“Log body mass” = log (base 10) of the body mass (in mg) of each individual

“Log Br mass” = log (base 10) of the brain mass (in mg) of each individual

“Log OL mass” = log (base 10) of the mass of the optic lobes (in mg) of each individual

“Log CB mass” = log (base 10) of the mass of the central brain (in mg) of each individual)

# Sheet: “Raw IF data”

**Raw IF data** provides the original values for body and brain mass, and the summed totals for brain cell numbers (as obtained in the Raw counts for IF sheet) for all samples where isotropic fractionation was done.

The yellow and blue rows represent adult data. The white rows represent larval data. Each row is an individual for the adults. For the larvae, each row represents an individual for the body mass and head width data – however, three larvae were then summed together into a single sample for the brain cell counts and so only the ‘top’ larvae in each sample will have a mean brain cell number for the VNC and brain which represents the mean of the three individuals in that sample.

Column names:

“Life stage” – Lifestage of the individual/sample

“Age” – for adults only, this represents how many hours may have passed between eclosion and fixation of the sample in hours (0-24 or 0-96 hours).

“Sample #” – unique sample identification used for IF counts. Note that each adult individual has a unique ID, however for larvae, three larvae together have a single sample ID.

“HW\_mm” – head width in mm. N.d. = no data for that individual.

“HH\_mm” – head height in mm.

“Body/OL/CB/BrainMass\_mg” – body/optic lobe/central brain/total brain mass in mg. Body mass was taken for adults and larvae; brain masses were only taken for adults.

“OL cell #” – For adults, the mean number of cells in the optic lobes for that individual as counted using IF (raw counts used to determine this number found in the ‘Raw counts for IF’ sheet).

“OL cell den” – optic lobes cell density, obtained by dividing cell number column by optic lobe mass column.

“CB cell #” – For adults, the mean number of cells in the central brain for that individual as counted using IF (raw counts used to determine this number found in the ‘Raw counts for IF’ sheet).

“CB cell den” – central brain cell density, obtained by dividing cell number column by mass column.

“Total Cell #” – total number of cells in the CB + OL of the brains of adults; total number of cells in the brains of the larvae (note, this is already divided by three – so it’s not the number per sample, but the number per larvae within the sample).

“Total cell den” – total brain cell density for adults only, obtained by dividing cell number by brain mass.

“VNC cell number” – for larvae only, the number of cells in the ventral nerve cord (note, this is already divided by three – so it’s not the number per sample, but the number per larvae within the sample).

# Sheet: “Raw Counts for IF”

**Raw counts for IF** are the original data values for the isotropic fractionation counts for L6 and adult BSF. These values were used to obtain the mean numbers of cells per individual per brain region. Please recall that three L6 brains/VNCs were homogenized together, resulting in the final mean number of cells being divided by 3 for these samples (“BrainCellEstimation” column).

Column names:

“Vial ID” – The unique ID for each sample. For adults (samples starting with F/M, following BSF): F/M refers to the sex, the number following F/M refers to the sample ID, and CB/OL refers to central brain vs. optic lobes. For L6 larvae (samples starting with L6, following BSF), the letters after L6 refer to the sample ID, and CB refers to brain while VNC refers to ventral nerve cord.

“Grid Number” - As indicated in the methods, 12 grids of the homogenated brain tissue were counted using a hemocytometer – each grid had 16 squares within it, and the number of nuclei within each square was placed within columns C:R.

“1” – “16” – The number of nuclei within each square in the grid on the hemocytometer.

“box volume” – volume of homogenate in a single square of the hemocytometer.

“TVH” – Total volume of the homogenate solution made from the brain tissue of that individual.

“TVH/box” – Volume of homogenate solution divided by box volume (e.g., number of boxes that could be made out of the total volume of homogenized brain tissue solution).

“MeanAll” – Mean number of nuclei in a box for that grid (Mean Columns C through R).

“StdevAll” – Standard deviation of the number of nuclei in a box for that grid (STDEV columns C through R).

“MeanCellsAll” – Mean number of cells estimated to be in the total homogenate volume for that brain, based on that grid (Mean All multiplied by TVH/box).

“BrainCellEstimation” – Number in the first row for that individual is the mean of “MeanCellsAll” for all 12 grids counted for that individual. This represents the mean number of cells expected to be in the brain of that individual, based on 12 counts of homogenated volume. This is the final number used in all the other analyses for that individual.

“BCE\_STDEV” – Standard deviation of “Brain Cell Estimation” column.

# Sheet: “Raw Counts for IHC”

**Raw counts for IHC** contains the total number of nuclei counted in the VNC or CB of a L1 or L4 larva, by a single counter, using immunohistochemistry methods. Four people (counters) were used across the different samples – two different counters independently counted a sample, and their numbers were averaged to obtain a final count.

N/a means that particular counter did not count that sample.

Column names:

“ID” - The names of the samples can be found in column A (each sample is a row) where L1/L4 refers to the lifestage of the sample, 01/02 refers to whether it is the first or second larva of that lifestage, and VNC/B refers to the ventral nerve cord vs. brain.

“Counter 1/2/3/4” – Each column shows the values obtained by that individual’s count of the number of nuclei in that brain region of that sample.

“Average” – The average number of brain cells obtained by two counters in that brain region of that sample, taken by averaging columns B:E. This is the number used for the final analyses (e.g., in the sheet ‘cell numbers’).