

FlyGroomA Instructions v1.2

10/8/2024, Eric Hoopfer

Video tutorials:

- [Building a param.csv file](#)
- [Processing text files with FlyGroomA](#)
- [Analyzing data with FlyGroomA](#)

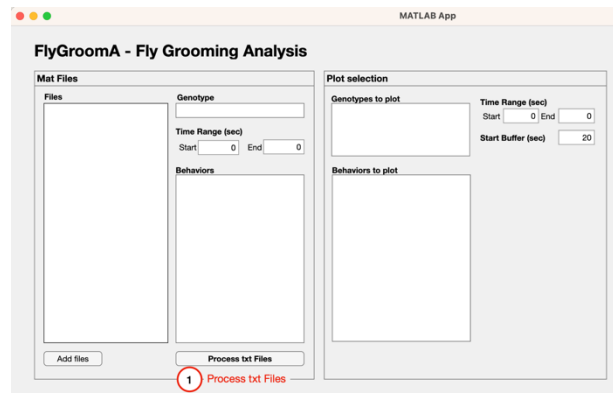
Part 1 – Download, Install and Run

1. Git hub address: <https://github.com/hoopfere/FlyGroomA>
2. Click on “<> Code” button and select “Download Zip”
3. Install Matlab Runtime: Open the “for_redistribution” folder and double-click on “MyAppInstaller_mcr”
4. Install FlyGroomA to your Program Files folder.
5. Install MATLAB Runtime to Program Files folder.
6. Installation can take several minutes.
7. After installation, open FlyGroomA app from the Applications menu.

Part 2 – Processing text files

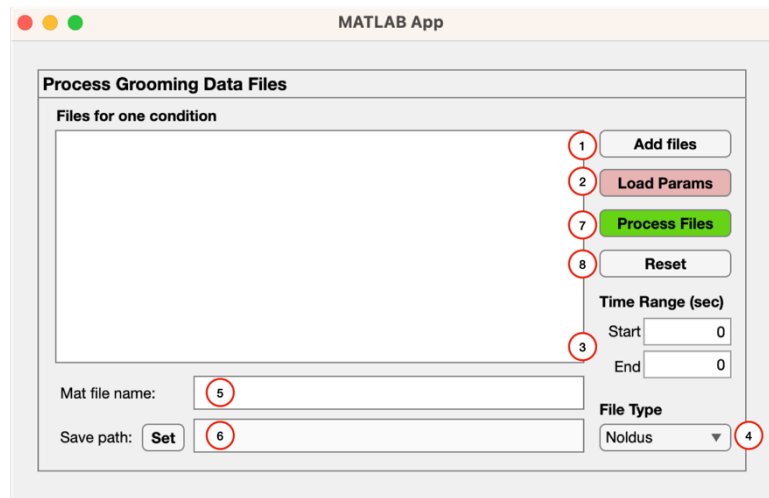
The first step in the analysis is to convert the txt files from your manual grooming program into mat files.

Click on the “Process txt Files” button in the lower right of the “Mat Files” tab. This will open the “Process Grooming Data Files” window.



1. Add files: select text files that correspond to experimental replicates from one experimental condition (e.g., the text files that correspond to separate flies of the same genotype run with the same experimental conditions).
2. Load Params: Select the params.csv file to use in processing

Process text files



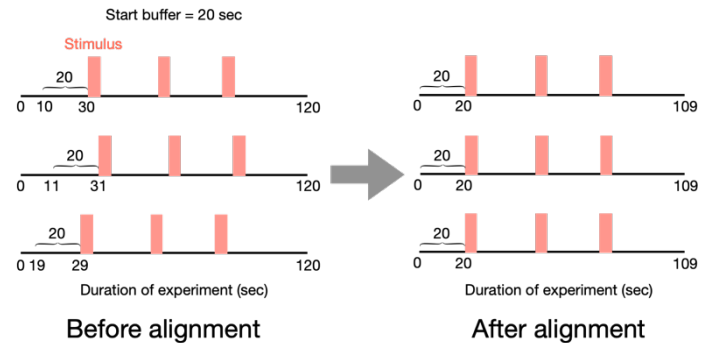
Notes about param.csv files:

- Do not change the text of the column headers. This will break the code.
 - Each behavior should be assigned to only one class. To include a behavior in different classes, you will need to create separate params.csv for each instance.
 - The name of the behaviors must exactly match the names used in the text files, including capitalization.
 - Avoid the following characters: spaces, / \ . +
 - The name use used to indicate the stimulus must belong to “class_stim”
3. Set the start and end times of the experiments. The duration should not exceed the minimum duration of any experiment. For example, if you have experiments that have durations of 120, 115, 130 second, you should set the end to 115 seconds.
 4. Select the filetype of the text files: Noldus or VCode.
 5. Type in name for mat file
 6. Select folder where you want to save mat file.
 7. Press “**Process Files**” button to create a mat file.
 - a. A window will appear notifying you when the mat file is created.
 - b. A warning may occur if the program finds behaviors in your text files that are not in the params.csv file. **This behavior will not be added to the mat file!** This error likely occurs because the name in the params file doesn’t exactly match what was used in the text files. Correcting the name in the params file should fix this issue.
 8. **Reset** will reset the following: text files, time range, mat file name. The params.csv file, save path and filetype will remain the same.
 9. When you are finished, close the window and return to the main FlyGroomA window.

Part 2 – Analyzing grooming

A. Add files and specify what to plot

1. Add mat files to analyze. Click on a file will display behaviors in the mat file.
2. Specify time range for plots.
3. Specify the start buffer. This specifies how many seconds before the first stimulus to start the analysis for each file. This is used to align different experiments to the same point in time, which is the start of the first stimulus.
4. Select which genotypes to plot.
5. Select which behaviors to plot.



Add files and specify what to plot

1 Add files

2 Specify time range for plots

3 Specify start buffer

4 Highlight genotypes to plot

5 Highlight behaviors to plot

B. Boxplots

1. **Grouping**: determines how data is grouped on plots
 - a. Behavior: Each plot shows one behavior for each genotype
 - b. Genotype: Each plot shows multiple behaviors for one genotype
2. **Control**: Specify which groups to use as the control in statistical analyses. When a control is specified, posthoc comparisons will be done between the control and other groups (e.g., con vs genotype1, con vs genotype2). When no control is

specified, comparisons will be done between all groups (e.g., con vs genotype1, con vs genotype2, genotype 1 vs genotype 2).

3. **Metric:** Select all metrics to plot.
 - a. Bout Frequency: number of bouts per minute
 - b. Bout Duration: duration of bouts in seconds
 - c. Percent time: % time performing behavior
 - d. Total time: Cumulative time performing behavior.
4. **Statistics:** Select parametric or non-parametric statistics.
 - a. Parametric statistics:
 - i. Comparison of two groups: 2-tailed T-test
 - ii. Comparison of more than two groups: ANOVA + posthoc pairwise 2-tailed T-tests with Bonferroni correction for multiple comparisons
 - b. Non-parametric statistics:
 - i. Comparison of two groups: Mann-Whitney U-test (2 tailed)
 - ii. Comparison of more than two groups: Kruskal-Wallis + posthoc pairwise Mann-Whitney U-tests with Bonferroni correction for multiple comparisons.
 - c. Statistical results are included in Excel file (see below).
5. **Export data to Excel:** Writes out raw data and statistics for each plot into an excel file. Subplots will be put on separate sheets. The Excel file name will be Figure_num_Metric_YearMonthDayHourMin.xls. For example, "Figure_2_Dur_2404051222.xlsx" was from Figure 2 plot of Duration made on 4/5/24 at 12:22PM.
6. **Specify where to save excel file.**
7. **Plot!**

Boxplots

FlyGroomA - Fly Grooming Analysis

Mat Files

Files: 26B12-24C08.mat, 77C10-26A02.mat, CON.mat

Genotype: 26B12-24C08

Time Range (sec): Start 0 End 120

Behaviors

abdominal_cleaning, antennal_cleaning, b_1st_and_2nd_leg_rub, b_2nd_and_3rd_leg_rub, back_leg_grooming_attempt, back_leg_rubbing, backing_up, body_cleaning, eye, first_leg_notum, front_leg_rubbing, front_proximal_leg_rub, genital_cleaning

Plot selection

Genotypes to plot: 26B12-24C08, 77C10-26A02, CON

Behaviors to plot: abdominal_cleaning, antennal_cleaning, b_1st_and_2nd_leg_rub, b_2nd_and_3rd_leg_rub, back_leg_grooming_attempt, back_leg_rubbing, backing_up, body_cleaning, class_abdominal_cleaning, class_back_leg_rubbing, class_front_leg_rubbing, class_head_cleaning, class_other

Time Range (sec): Start 0 End 120

Start Buffer (sec): 20

Boxplots | Histograms | Ethograms

1. Grouping: Behavior

2. Control: None

3. Metric: ☐ Bout Frequency, ☐ Bout Duration, ☐ Percent Time, ☐ Total Time

4. Statistics: None

5. ☐ Export data to Excel

6. Save Path: Set

7. Plot

C. Histograms

1. **Plot stimulus:** When checked this will add a shaded bar to regions of histogram that correspond to stimulus on. The stimuli will appear in the table below.
2. **Color of stimulus:** Specify this as Red, Green, Blue values between 0-1. The adjacent circle will turn that color.
3. **Transparency of stimulus:** Value must be between 0 (completely transparent) to 1 (completely opaque). Default = 0.25.
4. **Frame rate & Smoothing:**
 - a. Make sure the frame rate matches that of videos if different from 30 frames per second (fps).
 - b. **Smoothing:** The data in the histogram is smoothed with a running mean. This specifies the window of time over which to average the data. The default is 1 second, meaning the histogram will show the average fraction of flies performing a behavior in 1-second intervals.

5. Plot!

Histogram

MATLAB App

FlyGroomA - Fly Grooming Analysis

Mat Files
Files
26B12-24C08.mat
77C10-26A02.mat
CON.mat

Genotype
26B12-24C08

Time Range (sec)
Start 0 End 120

Behaviors
abdominal_cleaning
antennal_cleaning
b_1st_and_2nd_leg_rub
b_2nd_and_3rd_leg_rub
back_leg_grooming_attempt
back_leg_rubbing
backing_up
body_cleaning
eye
first_leg_notum
front_leg_rubbing
front_proximal_leg_rub
genital_cleaning

Add files Process txt Files

Plot selection
Genotypes to plot
26B12-24C08
77C10-26A02
CON

Time Range (sec)
Start 0 End 120

Start Buffer (sec) 20

Behaviors to plot
abdominal_cleaning
antennal_cleaning
b_1st_and_2nd_leg_rub
b_2nd_and_3rd_leg_rub
back_leg_grooming_attempt
back_leg_rubbing
backing_up
body_cleaning
class_abdominal_cleaning
class_back_leg_rubbing
class_front_leg_rubbing
class_head_cleaning
class_other

Boxplots **Histograms** **Ethograms**
Stimulus
☒ Plot Stimulus
Stimulus times (sec)

Start	End	Durat
20	25	
55	60	
90	95	

Stimulus Color
R G B T
1 0.5 0.5 0.25

Frame Rate (fps) 30
Smoothing (sec) 1

Plot

D. Ethograms

1. Ethogram type: Currently, only plots “Compact” which creates a raster plot of selected behaviors. Each line is a separate fly. When multiple genotypes are selected, one ethogram per genotype is created.
2. Plot Stimulus: Check to add a shaded bar indicating stimulation times. Color can be specified by RGB.
3. Plot!

Ethograms

MATLAB App

FlyGroomA - Fly Grooming Analysis

Mat Files

Files

- 26B12-24C08.mat
- 77C10-26A02.mat
- CON.mat

Genotype

26B12-24C08

Time Range (sec)

Start: 0 End: 120

Behaviors

- abdominal_cleaning
- antennal_cleaning
- b_1st_and_2nd_leg_rub
- b_2nd_and_3rd_leg_rub
- back_leg_grooming_attempt
- back_leg_rubbing
- backing_up
- body_cleaning
- eye
- first_leg_notum
- front_leg_rubbing
- front_proximal_leg_rub
- genital_cleaning

Add files

Process txt Files

Plot selection

Genotypes to plot

- 26B12-24C08
- 77C10-26A02
- CON

Time Range (sec)

Start: 0 End: 120

Start Buffer (sec)

20

Behaviors to plot

- abdominal_cleaning
- antennal_cleaning
- b_1st_and_2nd_leg_rub
- b_2nd_and_3rd_leg_rub
- back_leg_grooming_attempt
- back_leg_rubbing
- backing_up
- body_cleaning
- class_abdominal_cleaning
- class_back_leg_rubbing
- class_front_leg_rubbing
- class_head_cleaning
- class_other

Boxplots Histograms Ethograms

Ethogram type: Compact 1

☒ Plot Stimulus

Stimulus Color

R	G	B
1	0.5	0.5

2

3 Plot