

Task 1 - Object tracking

This task is solved with openCV library (version 3) with python 3.x + interface. First the sequence of images are converted to a video format (*rat_video.avi*) with the *img2vid.py* script. The algorithm for motion detection is based on background subtraction¹ and is implemented in the following steps:

- 1) Iterate over each video frame
- 2) Convert the frame to greyscale
- 2) Smooth the pixel values with gaussian filter
- 3) Compute the absolute difference between the first frame and the current frame
- 4) Find the pixels above threshold value
- 5) Dilate the thresholded pixels to fill in gaps
- 6) Fit contours to areas above threshold
- 7) Select only the largest contour

This algorithm is executed by the *motion_detector.py* script and is based on the example from <http://www.pyimagesearch.com/2015/05/25/basic-motion-detection-and-tracking-with-python-and-opencv/>

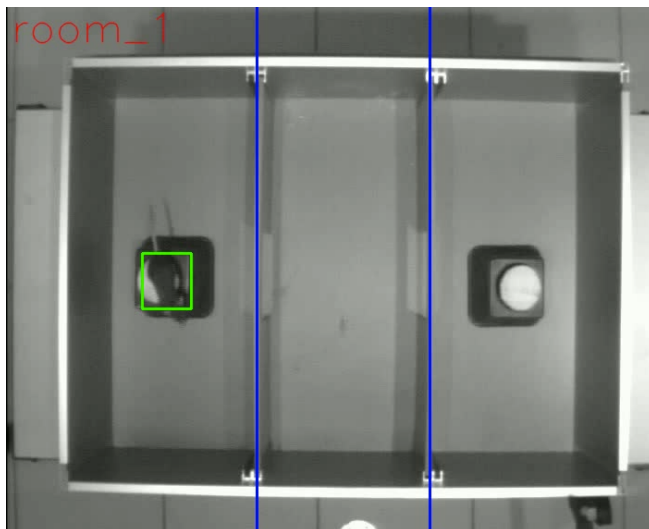


Fig1. Example frame from the *object_track.avi* video. Green square is the contour around a moving object (a rat), blue lines are the room borders and the red text denotes the occupied room.

The *motion_detector.py* script outputs a csv file (*rat_path.csv*) with the position of the rat at each frame.

¹ The first frame of the video has to be the background without the object to be detected

The *Rat_cage_analysis.py* script divides the path of the rat into slices between entering and leaving the rooms. The same script computes the following results, grouped by room number:

- 1) Total time spent in the room
- 2) Total distance traveled in the room
- 3) Distribution of visit durations
- 4) Distribution of path lengths

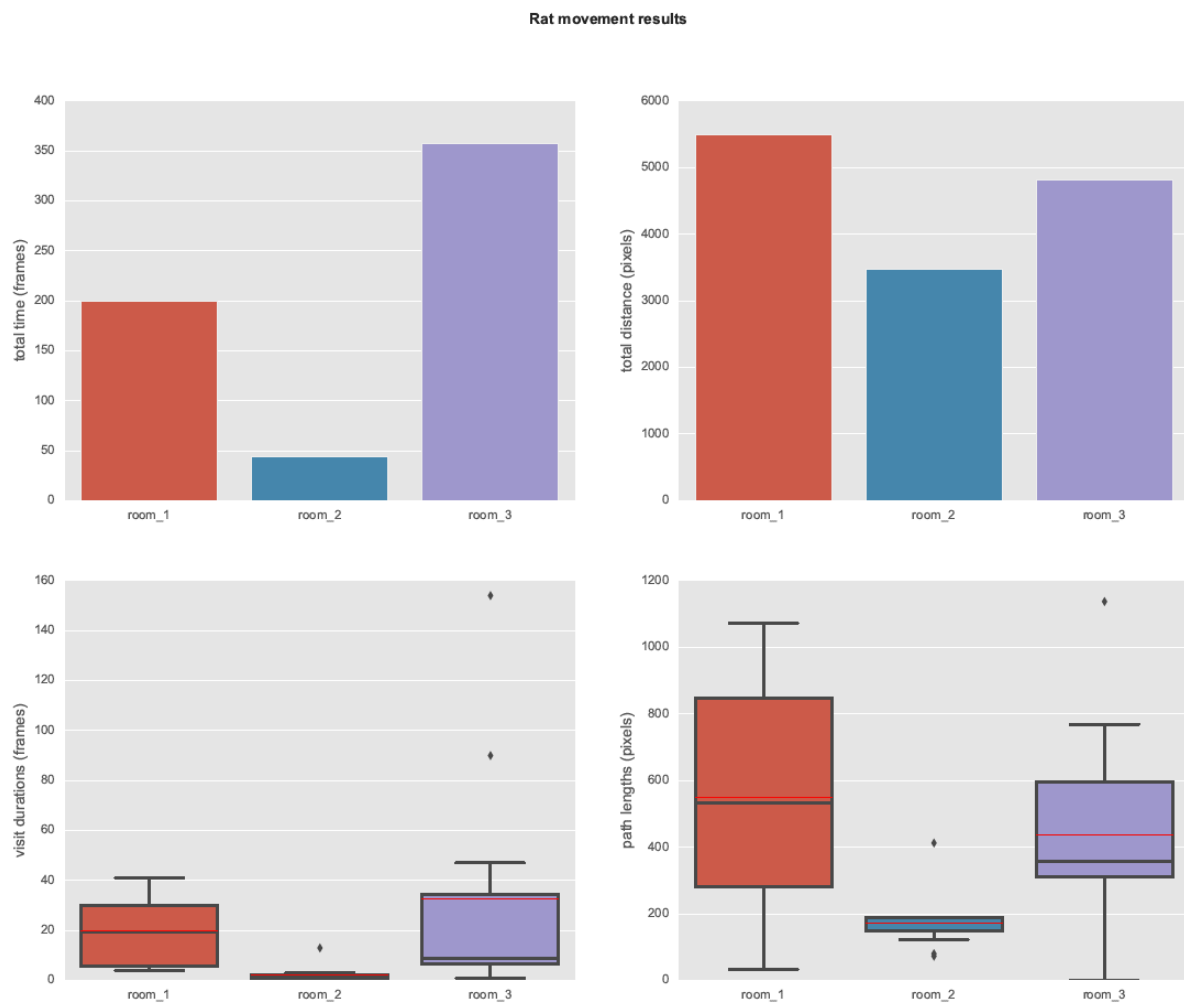


Fig2. Results of the Rat_cage_analysis.py script. Parameters calculated from rat visits to each room are: Total distance in the room, total time in the room, distribution of visit durations, distribution of

Based on the results in Fig2 we can conclude that the rat spent more time in room number 3 (the right room) but he traveled more in room number 1 (the left room). Necessarily the rat also had to move faster in room 1 in comparison to room 3. Room number 2 was mostly used to travel across to the other side of the cage and thus is not comparable with other rooms.

Task 2 - Rats behavior analysis

This task is solved in three steps:

- 1) *ParseData.py* script reads the data for a single mouse during a single phase and outputs a pandas library dataframe. The original epoch timestamps are converted to utc datetime format.
- 2) The *IndividualAnalysis.py* and *PairAnalysis.py* prepare the databases for analysis and save them in the `parsed_data` folder..
 - *IndividualAnalysis.py* outputs *indiv_times.csv* which is a dataframe containing durations of all individual visits to each room in each phase.
 - *PairAnalysis.py* outputs *pair_times.csv* which is a dataframe containing results computed for all possible mice combinations, i.e. mice pairs. Durations of all individual meetings in the same room are computed for all mice pair per room per phase.

3) *PlotResults.py* reads the results from the csv into the dataframe. Seaborn library² is used to display the different combinations of independent and dependent variables.

The result of the individual analysis is the average time spent in the room in a given phase (Fig3).

The results of the pair analysis are (Fig4):

- Top row, total meeting duration - how much time mice pair spent together
- Middle row, number of meetings - how many times they met
- Bottom row, average meeting duration

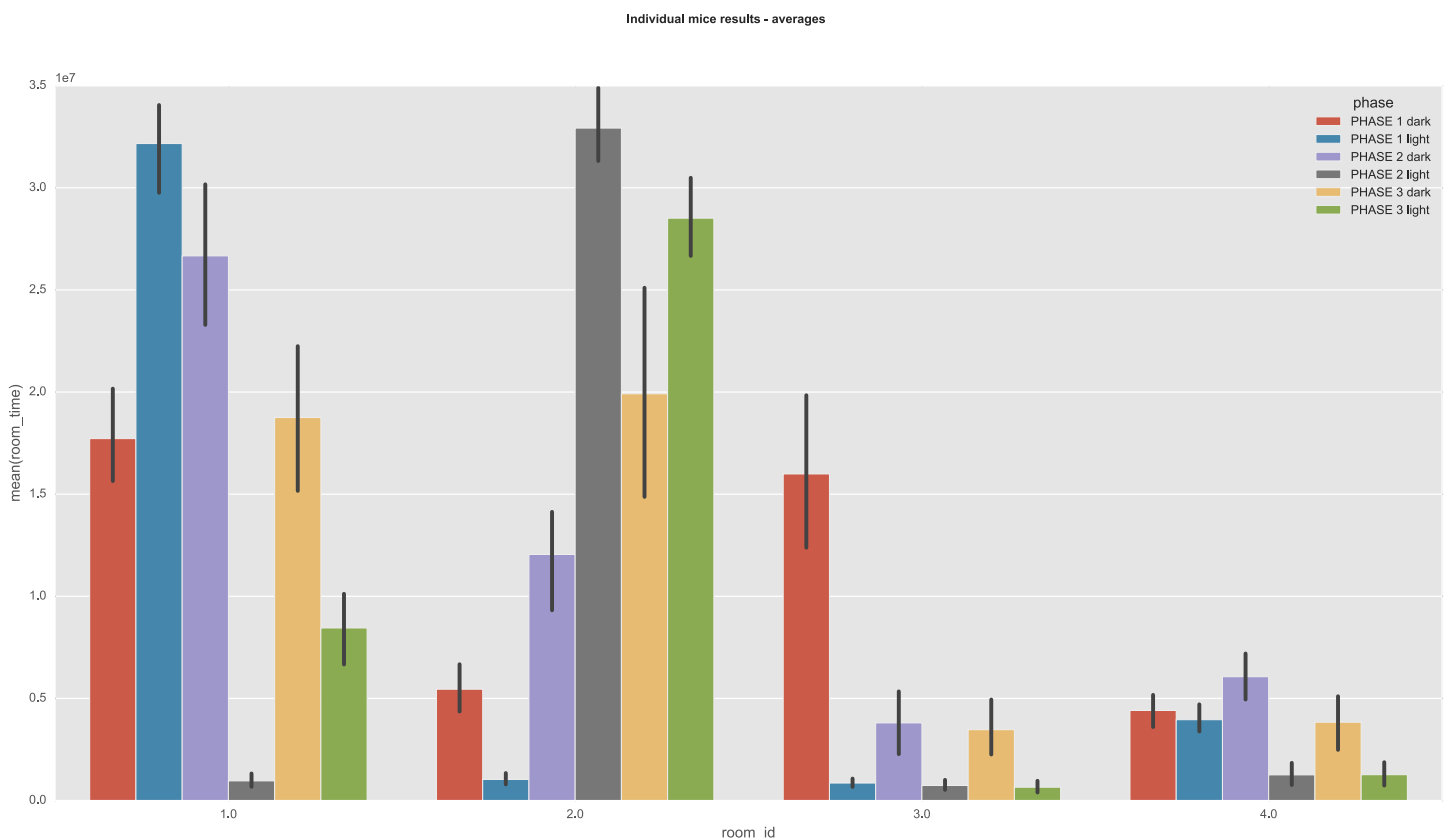


Fig3. Results of individual analysis. Average time a mouse spent in a room in a given phase. Error bars represent 95% confidence intervals.

² All error bars represent 95% confidence intervals of the population mean obtained by empirical bootstrapping.

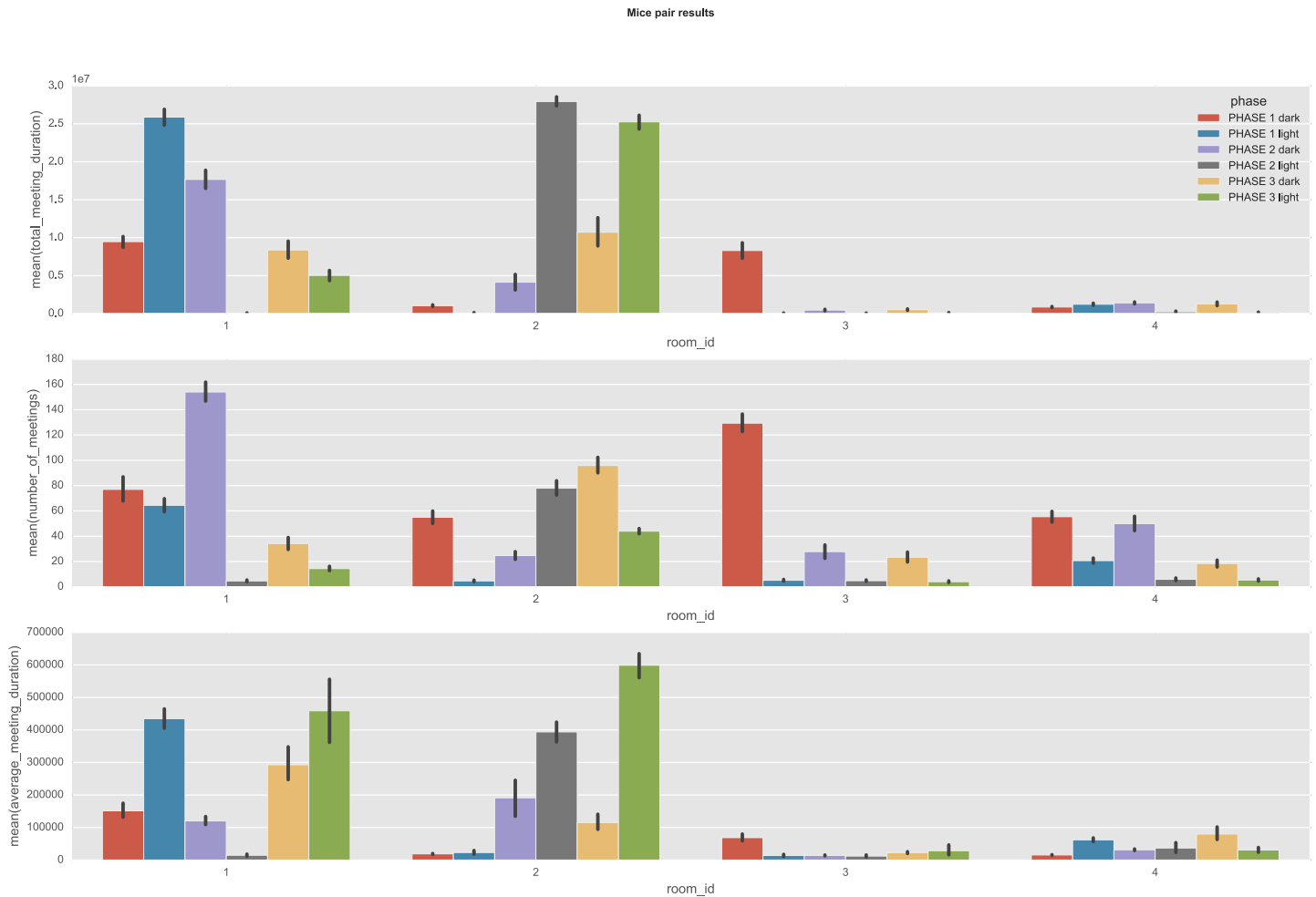


Fig4. Analysis of the behavior of mice pairs. Top row - total meeting duration, how much time mice pair spent together. Middle row - number of meetings, how many times the pairs have met. Bottom row -

Results in Fig4 show a similar tendency to one from Fig3, which is more meetings in room 1 in the first half of the experiment and then more meetings in room 2 in the second half. Another tendency which can be observed is that in a given room the meetings have different characteristics depending on a phase. For example, in room 1 during Phase 1 light there was a low number of meetings with high average duration. In contrast in Phase 2 dark in the same room, there were more meetings but with shorter duration. Perhaps the average meeting duration and average number of meetings can be used to predict the experimental phase in a given room (Fig5).

A support vector clustering method was used to predict the experimental phase. The number of meetings and average duration were used as features. The resulting decision boundaries for phase produced 90% accuracy in classifying the training data (Fig5).

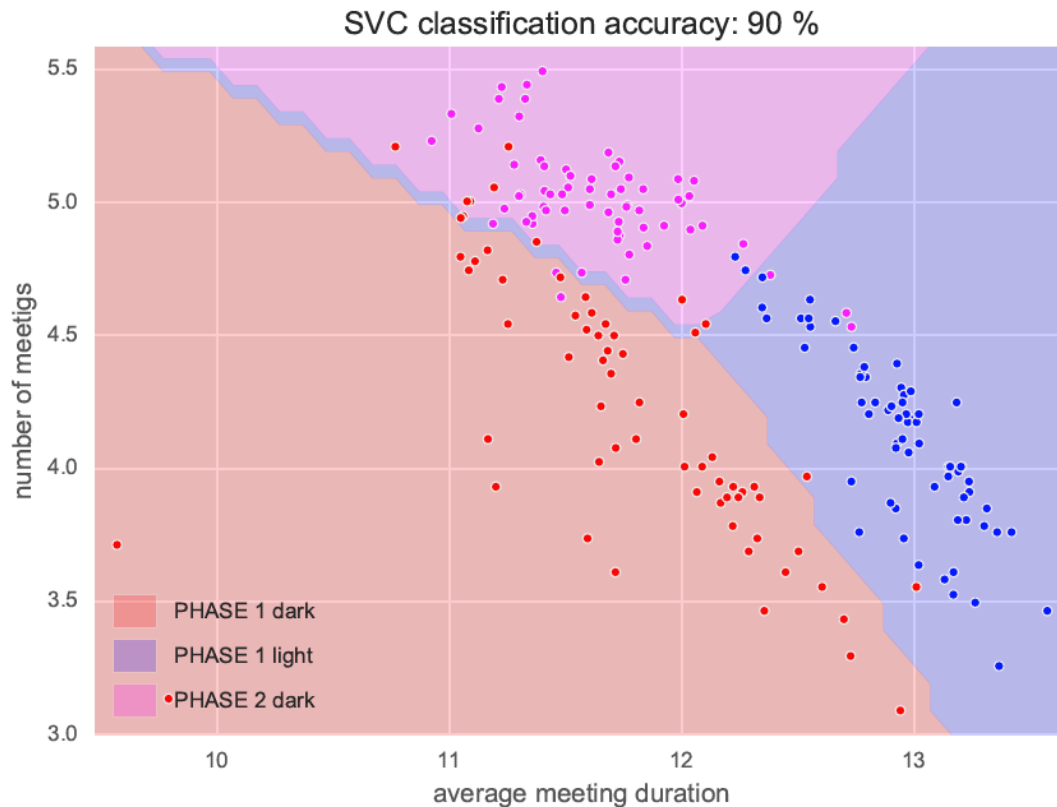


Fig5. Support vector clustering method for phase classification. Using the average meeting duration and number of meetings features phase in room 1 in training dataset could be predicted with 90% prediction accuracy.

Furthermore we can observe a negative linear trend between meeting duration and number of meetings in phase 1. It seems that the change between light and dark in phase 1 increased the average meeting duration but the correlation with number of meetings remained the same. However, such correlation might be trivial, since mice mice that have longer meetings have less time to have more meetings.

Supplementary figure *supp_indiv_split.pdf* shows total time spent in each room and phase for all mice individually, i.e. before averaging.

Supplementary figure *supp_rat_intersection.pdf* shows an example time fragment of history of visits to rooms for two mice, which was used to calculate when they were in the same room.

Task 3 - Cell imaging analysis

This task is solved in three steps. First a dataframe is created where size and intensity values are annotated with animal age and number. Second average size and intensity per animal is calculated. Third average size and intensity are correlated with age. The results are presented in Fig6.

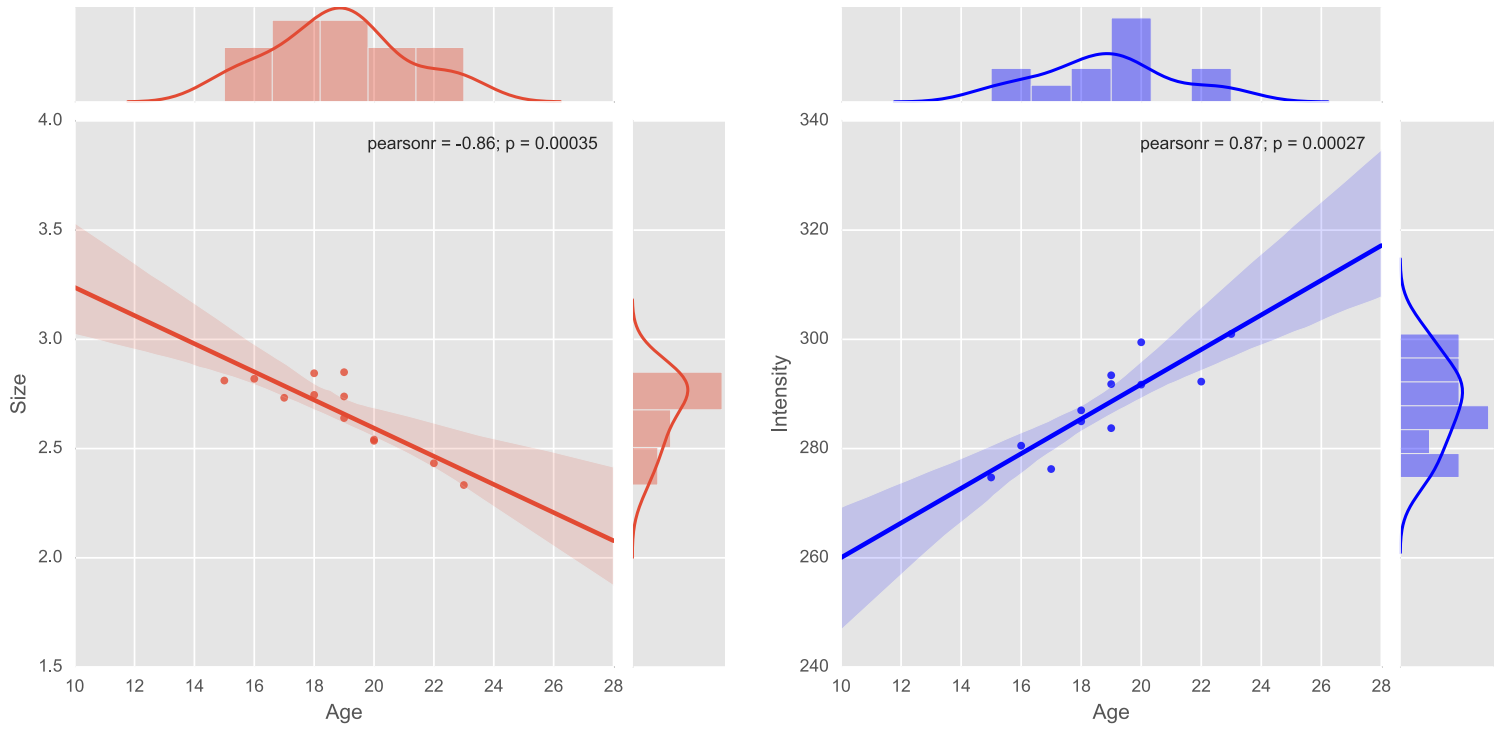


Fig6. Average size and intensity values of cells were correlated with animal age. These variables have respectively significant positive and negative correlation with age.

Supplementary figure *supp_size_int_boxes.pdf* shows distribution of size and intensity values per animal, before they were averaged.

