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DeepLabCut Report

Jinxin HU* Adrien SMITH*

Abstract

Optogenetics is a new biological technique that was discovered in the beginning of the 21st century and that has become one of the biggest breakthroughs in neuroscience. In this research project, researchers were interested in the light-activation of head direction cells (HDs) in mice, using optogenetics: they injected a viral vector that targets specifically HDs in the anterior dorsal thalamic nucleus (ADn) to express light-sensitive ion channels (Channelrhodopsin-2, ChR2) in these cells. The HDs cells could therefore be activated by light, and the behaviour of the mice in a box was monitored by video. This project focused on the use of a deep learning software, DeepLabCut, to track the position of specific parts of the mouse in order to monitor the head direction during time, using a code on MatLab. We were finally able to determine how the specific activation of these HDs by light can change the behaviour of a mouse and therefore enlighten the role of HDs.

Keywords: DeepLabCut; Optogenetics; Head Direction Cells in Mice

Supplementary Material: Demonstrate Video 1 — Demonstrate Video 2— Downloadable code

*jinxin.hu@espci.fr, adrien.smith@espci.fr, *Élèves Ingénieurs Promotion 138, ESPCI Paris - PSL*

1. Introduction

Beforehand, researchers have injected ChR2 modified virus in the ADn of two mice and then have implanted an optic fiber (optrode) in order to control the activation of a group of HDs cells in the ADn. By video recording the movement of the two mice in an open field, we were able to track specific points on the mice by using DeepLabCut, a machine learning software based on deep neural networks. After having trained the neural network on a limited amount of frames and a low level of iterations, we were able to evaluate the network and compare the test and train error with a network that was previously trained using a large number of frames and iterations. Afterward, we accessed the direction of the head of the mouse through time (with or without light activation), using a code on MatLab to determine the angle of the head (neck to nose). We were then able to observe a clear difference of behavior between the time when the light was off and the time when it was on. This project gave us therefore an insight into what deep learning and optogenetics tools can bring to the research in neuroscience, and in particular to the study of head direction cells.

2. Experiment and Methods

2.1 Identification of the Behavior

In this project, we worked on two videos of the two mice with ChR2 modified HDs cells and with an implanted optrode. After a period of habituation, the movement of the mice in an open field was recorded for 15min, without light activation of the HDs (5 min), then with light (5 min), and finally without (5 min). We were then interested in the analysis of the two videos of each mouse. After having watched the videos the first time, we observed that the mice tended to rotate faster and more often, in a circular fashion, with several full rotations on itself (anticlockwise for the mouse n°1, or both clockwise and anticlockwise for mouse n°2). In

this case, because the cells that were targeted in this experiment with the modified ChR2 virus are specific to the direction of the head of the mouse, we focus our interest on the direction of the head, determined for example by the direction from neck to nose.

2.2 DeepLabCut Direction Track

We wanted to confirm this observation by tracking the head direction of the mouse across time. For this task, we used the software DeepLabCut that can track specific positions of the mouse by using a deep neural network. For the parameters, as shown in figure 1, we choose to select the tip of the nose and the middle of the neck of the mouse to be able to access the direction (ie. the angle) of the head. Due to a limited time, we trained our neural network on only 30 frames and with a small number of iterations (1000 maximum iteration limit).

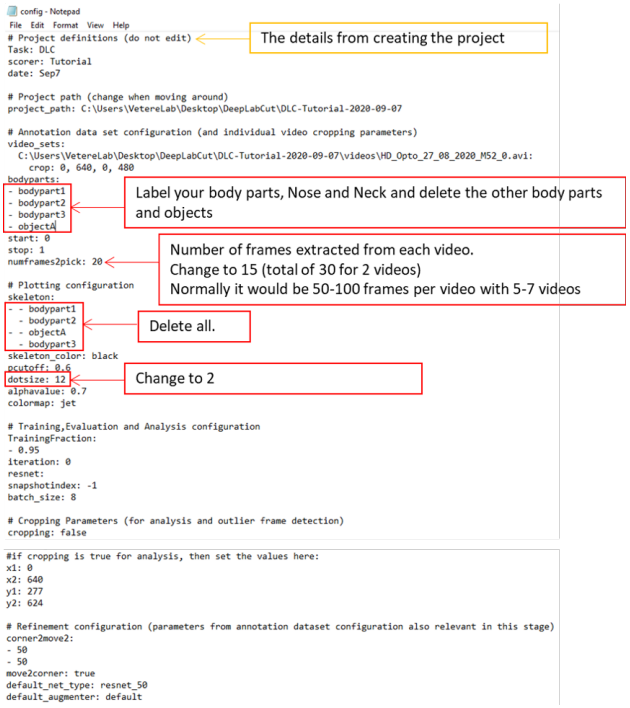

Figure 1. Parameters chosen in DeepLabCut

After 30 minutes of training, we tested our network on some of the frames we previously labeled (an example is shown in Fig 2). With P-cutoff, we obtained a training error of 4.12 pixels and a test error of 4.52 pixels. The test error is the average difference between the positions determined by the neural network and the positions labeled manually, on frames that were labeled but that were not used for the training. Therefore, it represents the mean accuracy of the network, compared with the labeling of a human. The training error is an indication of the accuracy of the manual labeling because it is the same error but calculated for the frames that were used for the network's training. The more training images we use, the smaller the test error will be. Therefore, in order to track accurately the direction of the head of the mice, the network was previously trained with 600 frames and 100,000,000 maximum iterations and gave a training error of 1.8 pixels and a test error of 1.37 pixels, which means that the error of the network is very similar to the error that a human can do while labeling the nose and the neck of the mouse. We watched the tracking results in the videos (bonus videos with each frame labeled for the nose and the neck), and the results will be discussed in the last section.

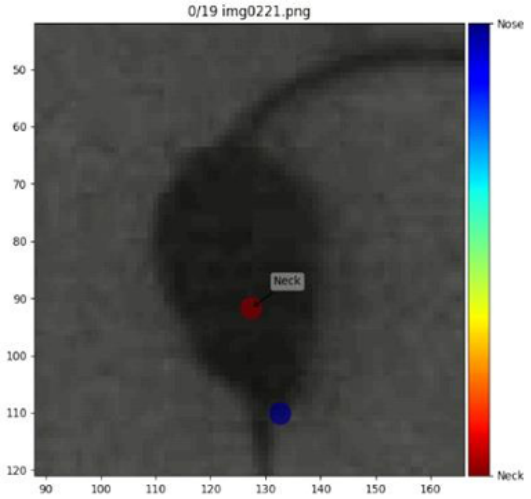

Figure 2. Instance of manual labelling

2.3 Results Analysis in MatLab

The output files of the tracking by DeepLabCut are 2 CSV files containing the x and y coordinates of both nose and neck labels for each frame of the video (27000 frames per video). The code first removes wrong data (labels outside of the box and data under a likelihood threshold of 0.9) and replaced them with the average of the previous and next frame. Afterwards, we wrote a function that computes the mouse's head (neck to nose line) angle, using the *atand* function of MatLab and converting the result into an angle between 0 and 360°.

```
angles=1:size(b,1);
%b is our original n (frames) by 4 ...
    (coordinates) matrix
angles=angles';
T=angles;
for i=1:size(angles)
   T(i)=(b(i,2)-b(i,4))/(b(i,1)-b(i,3));
end


for i=1:size(angles)
   if b(i,1)-b(i,3)>0 && b(i,2)-b(i,4)>0
```

```
        angles(i)=abs(atand(T(i)));
    end
    if b(i,1)-b(i,3)<0 && b(i,2)-b(i,4)>0
        angles(i)=180-abs(atand(T(i)));
    end
    if b(i,1)-b(i,3)<0 && b(i,2)-b(i,4)<0
        angles(i)=180+abs(atand(T(i)));
    end
    if b(i,1)-b(i,3)>0 && b(i,2)-b(i,4)<0
        angles(i)=360-abs(atand(T(i)));
    end
end
```

The data was then downsampled to 2000 frames to smooth out the curve, and the difference in angle between each consecutive frame was calculated. To correct the outliers that are due to the passing from a value close to 0° to a value close to 360°, we added the following code to fix this problem.

```
a_d1= a_d;
for i=1:length(angles)-1
    if a_d1(i,1) > 180
        a_d1(i,1) = a_d1(i,1) - 360;
    end
    if a_d1(i,1) < -180
        a_d1(i,1) = 360 + a_d1(i,1);
    end
end
figure(1)
hist(a_d1,180);
```

Then we plotted the histogram of the distribution of the difference in angles between consecutive frames, as shown in Figure 3, to confirm that the consecutive angles are all around 0 and that we removed all the outliers.

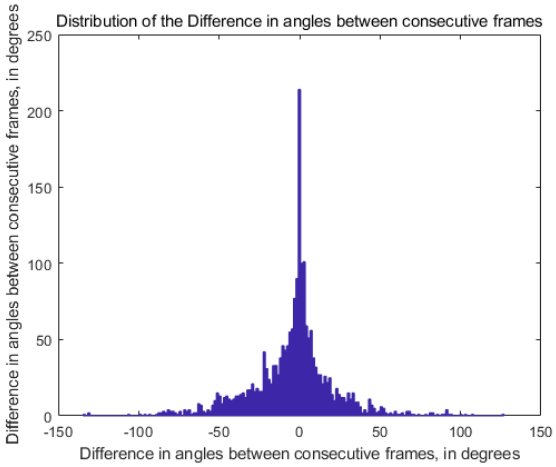

Figure 3. Histogram of the distribution of the difference in angles between consecutive frames

Then with the following algorithm, we can calculate the number of full rotations (positive or negative) that the two mice could do during the experiment and we took the beginning and end frames of these 360° positive and negative turns. We obtained that the mouse n°1 did no positive 360° turns but did multiple full negative turns (first turn starting at frame n°701, so a few frames after the activation of light, and the last turn starting at frame n°1374, a few frames after the extinction of light – the total downsampled number of frames is 2000), whereas the mouse n°2 did positive and negative turns during the period of the light activation.

```
sum_ang=0;
k=0; %counter for number of frames ...
    in this rotation
start_frame_pos = [];
end_frame_pos = [];

for i=1:length(a_d1)
    if a_d1(i)>0
        sum_ang=sum_ang+a_d1(i);
        k=k+1;

            if sum_ang > 360
                sum_ang = sum_ang-360;
                disp(sum_ang)
                start_frame_pos = ...
                    [start_frame_pos, ...
                    i-k+1];
                end_frame_pos = ...
                    [end_frame_pos, i];
                k=0;
            end

    else
        k=0;
        sum_ang=0;
    end
end

pos_count=length(start_frame_pos);
start_frame_pos=start_frame_pos';
end_frame_pos=end_frame_pos';
```

The principle of obtaining clockwise and counterclockwise turns is totally the same, so the code for obtaining counterclockwise turns is left out.

Using the algorithms above, all the data will be fully acquired. Therefore, we can plot the result in the next section.

3. Result and Discussion

3.1 Mouse 1 Analysis

By plotting the results of mouse 1, we can get the histogram to evaluate accuracy rate of our algorithm, shown in Figure 4 a). On Figure b) and c), we can observe the head direction of the mouse and the accumulated degrees turned by mouse 1 throughout experiment.

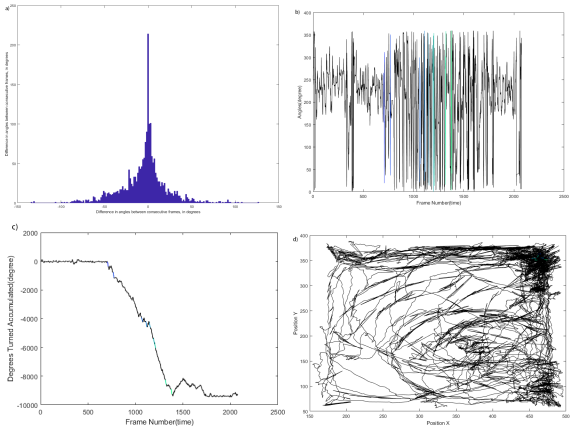

Figure 4. a) Histogram of the distribution of the difference in angles between consecutive frames for mouse 1. b) Direction of the mouse 1 head varies with time. c) Accumulated degrees turned by mouse 1 throughout experiment. d) Trace of the mouse 1 in the open field box.

Furthermore, by displaying different stages of this experiment, we can observe an obvious change in behaviour between OFF and ON periods, with light stimulation, in Figure 5 and 6. There is also an "afterglow effect" after the consistent stimulation in the second 5 minutes, which is clearly shown in Figure 6.

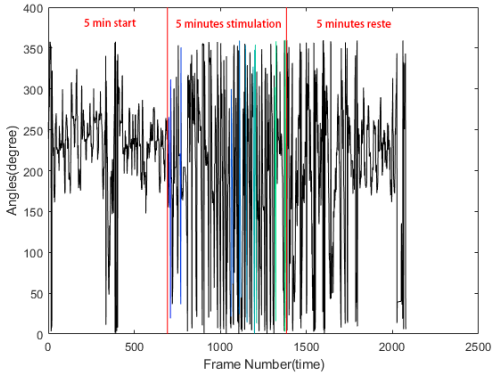

Figure 5. Direction of mouse 1 head varies with time

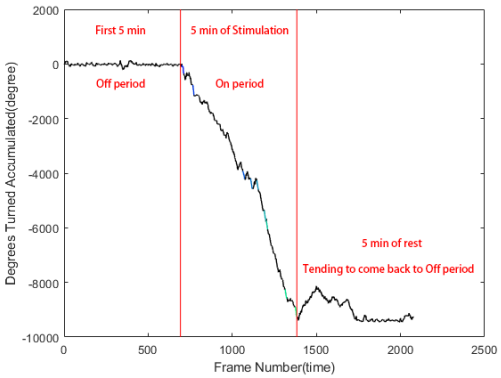

Figure 6. Accumulated degree turned by mouse 1 varies with time

Therefore, in this case, we can conclude that by stimulating HDs in the ADn, mouse 1 will turn mostly clockwise in the open field box. The hypothesis of HDs in mice ADn control head direction is consistent with this experiment result.

3.2 Mouse 2 Analysis

For mouse 2, the results are shown in Figure 7. These results are consistent with the original video and also with figure of the trace that mouse 2 is much more active than mouse 1. However we can not see a clear intense head direction change when optic stimulation is switched on. However, we can still notice from Figure 9, a change between ON and OFF periods with light stimulation. During OFF periods, mouse 2 is doing mostly random movements in different directions, with very few full rotations, whereas in the ON period, the mouse 2 is doing many 360° rotations. Additionally, mouse 2 is mainly turning clockwise while mouse 2 is turning counterclockwise throughout this experiment.

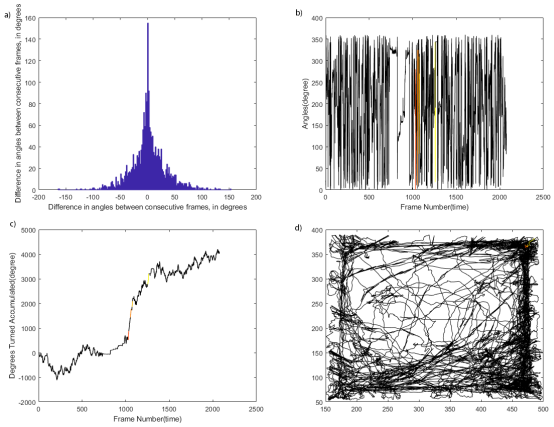

Figure 7. a) Histogram of the distribution of the difference in angles between consecutive frames for mouse 2. b) Direction of the mouse 2 head varies with time. c) Accumulated degrees turned by mouse 1 throughout experiment. d) Trace of the mouse 2 in the open field box.

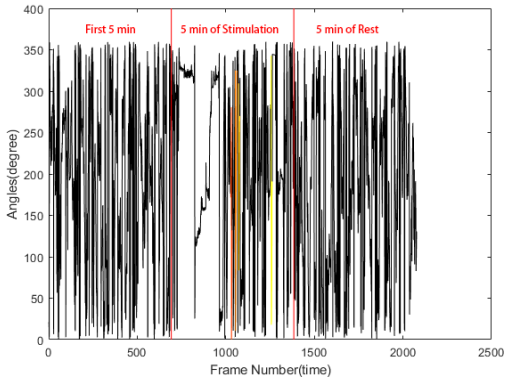

Figure 8. Direction of mouse 2 head varies with time

[Result comparison] In comparison with mouse 1, mouse 2 is more active in the resting state, which makes the evolution of head direction with time more

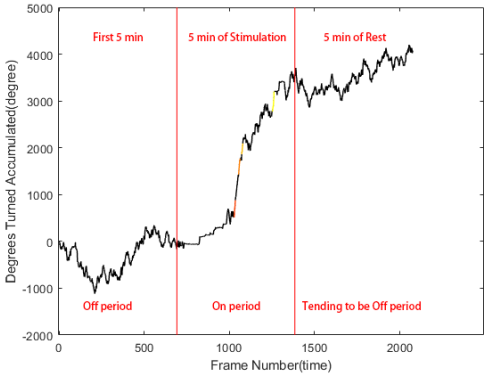

Figure 9. Accumulated degree turned by mouse 2 varies with time

difficult to interpret. However, we can still conclude that the optogenetic technique targeted HDs mostly for clockwise rotations, whereas for mouse 2, the stimulation was less efficient and the technique targeted mostly HDs for counterclockwise rotations.

3.3 Bonus Video Discussion

We observed that for a fast training on only 30 frames and limited iterations, the tracking was very poor and showed that labels were often displaced of several pixels from the target, and sometimes even on a totally different position of the mouse (on the tail or on the back of the body); whereas for a highly trained network (600 frames, 100,000,000 iterations), the positions of the labels were always on the target (the errors were not observable at first sight). As shown in Figure 10, there are multiple errors just like the following images, for example treating tail as nose; treating buttocks as nose, treating different body part as neck...

In a comprehensive observation of bonus video 1 & 2, the error rate for video 1 is much lower than video 2. So we can easily draw the conclusion that video 1 is trained by a network with 600 frames (100,000,000 iterations) and video 2 is trained with 30 frames (1000 iterations).

4. Conclusion

In this experiment, researchers have been able to target specifically HD cells in the ADn with a viral vector, in order to express ChR2 light-sensitive ion channels. By tracking the head direction of two mice in an open field, using DeepLabCut, we were able to confirm that the optogenetic technique has indeed targeted HD cells. In general, HD cells are firing action potentials mostly when the animal's head points in a specific direction. We can imagine that when the optrode is stimulating a group of HD cells, the mouse is disoriented and is therefore rotating on itself.

According to our results, during ON periods, the

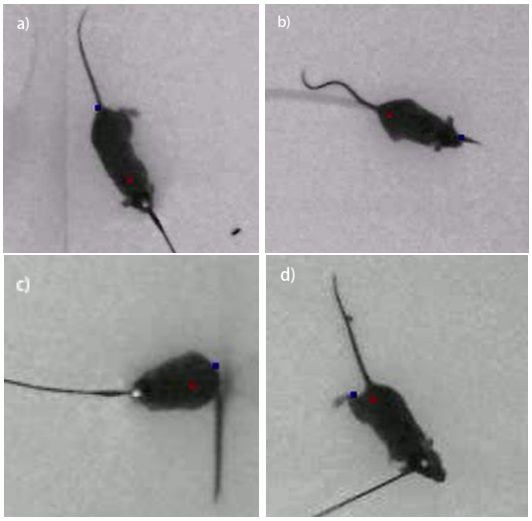

Figure 10. a) and b) Errors in bonus video 1. c) and d) Errors in bonus video 2.

mouse can rotate in one particular direction. For example, mouse 1 did multiple negative (clockwise) full rotations, and no positive (anticlockwise) turns. We can assume that this behavior can depend on the group of HD cells that were targeted optogenetically. In all cases, we were able to observe and quantify the change in behavior for these two mice, thanks to this deep learning tool.

DeepLabCut enabled us to use a recent machine learning program and to apply it in the context of optogenetics. We were, therefore, able to extract quantitative and objective results from the original videos, in order to confirm the results of this optogenetics experiment.

For better tracking of the different mouse parts by the deep learning program and to prevent the neural network from mistaking the optic fiber with the tail of the mouse, we could suggest making a clear distinction between the optic fiber implanted in the mouse brain and the tail. For example, making the optic fiber wider with a special shape different from mouse tails would probably decrease tracking errors. The recognition accuracy rate might be higher and the result analysis of the result would be more authentic. Last but not least, it would be interesting to conduct further researches inspired by this experiment, in order to study which group of cells can induce clockwise or counterclockwise turns with more fined targeted HD cell groups in mice ADn.

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