Apply ezTrack software for Alzheimer mice model in new object recognition test

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Abstract

In order to fully comprehend the pathophysiology of Alzheimer's disease (AD) and uncover prospective treatment strategies, it is necessary to conduct research on the condition using cutting-edge technologies and methodologies. In the context of AD research, this work intends to maximize the advantages of free and customizable software tools for video tracking and analysis, notably ezTrack. We want to circumvent the budgetary limitations associated with commercial software while improving the accessibility and repeatability of our research by utilizing open-source technologies. We want to investigate the behavioral patterns and cognitive deficits related to AD in preclinical models using ezTrack. The software will make it possible to precisely quantify mouse movement, object exploration, and memory performance thanks to its customizable tracking algorithms and data extraction capabilities. We seek to promote openness, promote cooperation, and support community-driven progress in the area of AD research by using ezTrack's open-source nature. Adding ezTrack to our research pipeline will also facilitate easy data analysis and workflow integration with current technologies in cost free way. We want to draw important conclusions from our experimental data, revealing the underlying mechanisms of AD pathology and identifying new treatment targets by fusing the power of ezTrack with other open-source technologies and statistical analytic software. This study highlights the need of using open-source, flexible software tools, like ezTrack, to research Alzheimer's disease.

Keywords: Alzheimer's disease; ezTrack; software; mice model.

Roadmap

Briefly explain the significance of Alzheimer's disease (AD) research and the need for advanced technologies.

Highlight the importance of video tracking software for precise measurement and analysis.

Limitations of Existing Software

Discuss popular video tracking software options (EthoVision, ANY-Maze, Noldus Observer, CleverSys TopScan).

Mention cost implications and limitations associated with commercial software.

Introducing ezTrack Software and present it as a free and customizable open-source tool.

Emphasize the benefits of ezTrack, including cost-effectiveness, customizability, collaboration opportunities, user support, and continuous development.

Outline the surgical procedure on Alzheimer's mice models.

Describe the NOR test protocol, including habituation, training, and testing phases.

Explain the calculation methods for exploration and discrimination measures.

Data Collection and Processing

Highlight the use of ezTrack and Python for data analysis

Emphasize the importance of open-source software in advancing AD research.

Contact Information

Provide author contact details and affiliations.

1- Introduction

Understanding learning and memory processes is key for deciphering the intricacies of neurological illnesses and advancing treatment strategies. Learning and memory processes play critical roles in the cognitive skills of animals, including mice. The Novel Object Recognition (NOR) test has become well-known among the different behavioral paradigms used to evaluate learning and memory because of its ease of use, dependability, and translational usefulness. The NOR test uses mice's natural interest for novel stimuli to measure how well they can distinguish between known and unfamiliar things. The effects of pharmaceutical interventions on learning and memory as well as cognitive deficits, neurodevelopmental disorders, neurodegenerative diseases, and other topics have all been studied using this test in preclinical research. The NOR test is based on the idea of spontaneous exploratory behavior, by regarding this fact that mice naturally have a tendency to examine new objects more thoroughly and for longer periods of time. Researchers can determine an animal's capacity for item recognition and memory by measuring how much time it spends exploring a novel thing compared to a familiar object. The NOR test is significant in that it permits evaluation of both short-term and long-term memory, allowing for the exploration of various facets of learning and memory processes.

Video tracking software is necessary for keeping track of and documenting the mouse movements during the NOR test. By analyzing video records and following the mice's movements, this software enables the extraction of useful behavioral information including exploration time, travel distance, and object interaction. EthoVision, ANY-Maze, Noldus Observer, and CleverSys TopScan are a few examples of well-liked video tracking software alternatives. These software and technologies collectively contribute to the accurate measurement, analysis, and interpretation of the NOR test results, providing valuable insights into learning and memory processes in mice. But it should be mentioned these are sometimes cost high as they exemplified there:

- EthoVision: Noldus Information Technology created the popular video tracking program EthoVision. Users are urged to get in touch with Noldus directly for a tailored quote since the price of EthoVision is normally not made public.
- ANY-Maze is a thorough video tracking and analysis program provided by Stoelting Co. ANY-Maze has a starting price of about $3,000 for a base kit, with additional modules and capabilities being offered at an additional cost.
- Noldus Observer: Noldus Observer is a flexible suite of tools for behavioral research that has the ability to track video. Noldus Observer's price is not made public, however interested consumers can get more information by getting in touch with the company.
- CleverSys TopScan is a video tracking program that was created especially for studying rodent behavior. CleverSys TopScan has a starting price of about $2,000, and there are extra modules and customizations available for additional fees.

It's crucial to remember that the aforementioned fees are only an estimate and may change depending on the version, any extra modules or features, academic or commercial license, and any ongoing support or maintenance agreements. It is advised to contact the software vendors

directly or visit their websites for full pricing information catered to your particular needs in order to acquire reliable and up-to-date pricing information.

We provide a thorough explanation of the NOR test technique for examining learning and memory in mice in this research. In here it will be offer free and simple platform which is called ezTrack software. It is available on GitHub, is an open-source video tracking tool developed by the Cai Lab. Here are some benefits of using ezTrack:

A. Cost: The fact that ezTrack is open-source and free for anyone to use is one of its primary advantages. This makes it possible for academics on a tight budget to use it without having to invest in pricey commercial video tracking software.
B. Customizability: Because ezTrack is open-source software, researchers have the freedom to adapt and alter the code to suit their particular requirements. This enables the tracking algorithms, data extraction, and analysis techniques to be modified to meet particular experimental needs.
C. Collaboration and Transparency: By allowing access to the source code, open-source software promotes collaboration and transparency in scientific research. The ezTrack algorithms and methodology may be examined and verified by researchers, encouraging scientific rigor and reproducibility. Additionally, the community-driven development and open-source philosophy promote collaboration, allowing researchers to add new features, problem fixes, and enhancements.
D. User Support and Community: Active user communities are common in open-source projects, which help and support other researchers. The learning and troubleshooting processes are improved by the ability for users to exchange experiences, ask questions, and receive advice from other ezTrack users.
E. Continuous Development: The community can provide continuing updates and contributions to open-source projects like ezTrack. This implies that the program can continue to develop, adding new functions and enhancements as time goes on.
F. Integrating and extending ezTrack with other software applications or workflows is simple thanks to its open-source nature. To improve their research capabilities, researchers can integrate ezTrack into their current data analysis pipelines or combine it with other open-source tools.

2- Aims

In this research our goal is to use “integratablity” and “customizability” options, in a cost free way, It just needed to be familiar with how to use iPython/Jupyter Notebook. It is just enough to follow up the steps which will be mentioned later.

3- Motivation to work on the OLS-7 project

- Budgetary restrictions: Since commercial software for linear regression analysis can be pricey, it is often out of reach for researchers, especially those on a restricted budget. Your goal was to create a project that would offer a reasonable substitute for expensive commercial software, enabling regression analysis without being constrained by budgetary considerations.

- Flexibility and customization: Existing software alternatives for linear regression analysis may not be flexible enough to meet certain research requirements. Your goal was to develop a project with adaptable features so that researchers could customize the analysis to their particular needs and experimental setups.
- Accessibility and repeatability: Open-source technologies have the benefit of being accessible and repeatable, making your project available to scholars all around the world. You wanted to encourage transparency, teamwork, and community-driven. advancement in the area of linear regression analysis by using open-source software.
- Offer a platform which maximize the result differences between the different lab settings.

4- Methods and materials

4.1. Few changes and comments on the ezTrack software

After downloading the related materials at **https://github.com/denisecailab/ezTrack,** first you should insert following section after the line 1057 inside the ***LocationTracking_Functions.py*** and then save it:

```
#Put grid lines
   line1= hv.VLine(1*w/2).opts(color='red', line_width=2)
   line2= hv.HLine(1*h/2).opts(color='red', line_width=2)
   if nobjects > 0:
      dmap = hv.DynamicMap(centers, streams=[poly_stream])
      return (image * poly * dmap * line1 * line2), poly_stream
   else:
      return (image),None
```

It might be interesting that you will see these red lines as depicted in the figure 1:

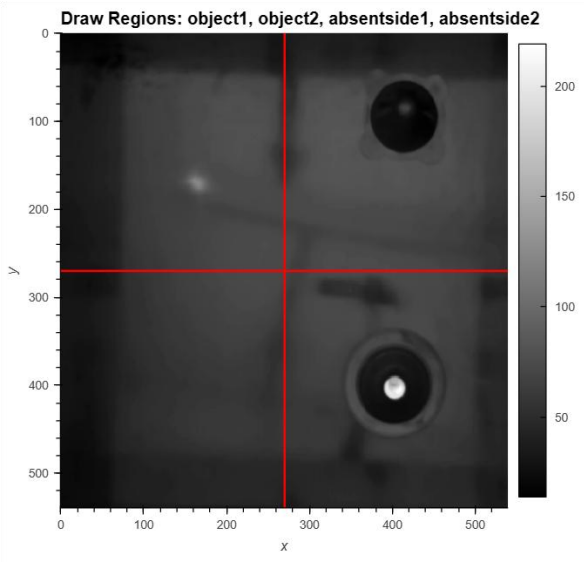

Figure 1: Illustrate easier seletion of object1, object2, absentside1, and absentside2.

Also in the Jupyter notebook in the cell as you see in the following Figure 2, which is named “2. User Sets Directory and File Information” you should add following code as it is with your directory name.

```
video_dict = {
  'dpath'      : "C:/Users/YOUR_USER/Downloads/ols7/ezTrack-master/LocationTracking/PracticeVideos/",
  'file'       : 'LocationTracking_Clip.mp4',
  'start'      : 0,
  'end'        : None,
  'region_names' : ['object1','object2','absentside1','absentside2'],
  'dsmpl'      : 1,
  'stretch'    : dict(width=1, height=1)}
```

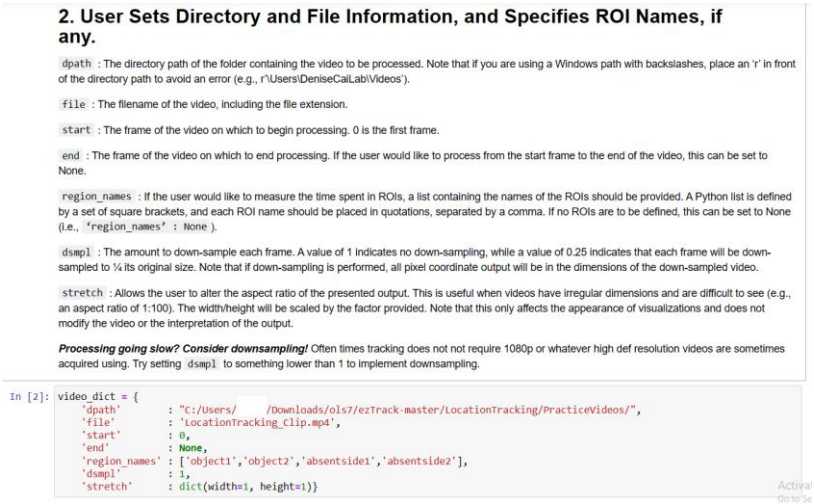

Figure 2

And I recommend using one by one each animal, because each experiment has its own settings. Also take into account that, when you define 4 pints you would do the same repeated action for object selection as it has been explained in the “Protocol for Novel Object Detection”. In this research The first introduced object always was set as a 'object1', as you can see in the Figure 3.

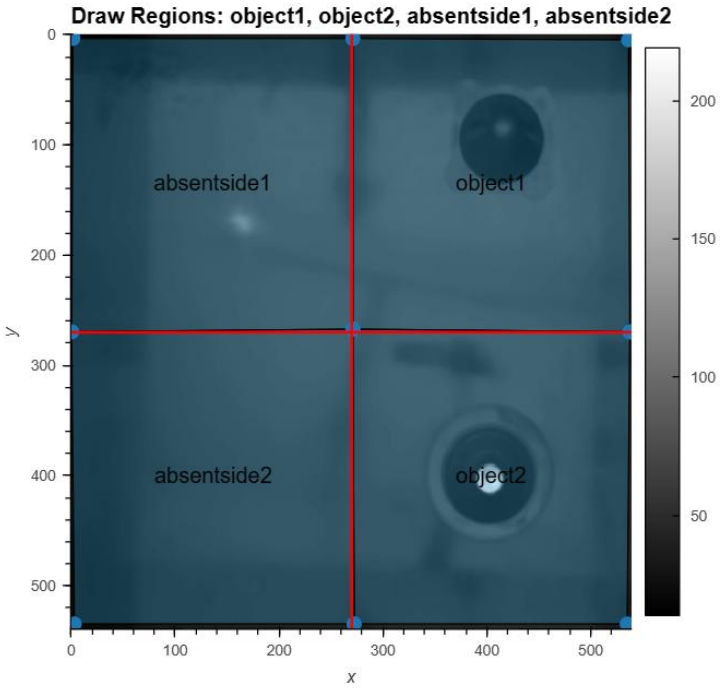

Figure 3: After the selection you see how to arrange the objects, this picture is for T24 (see protocol for further information).

4.2. Animals surgery

In PN25 animals separated from their mother. From PN25 kept in a regular condition with enough food and water until the PN90 so that mice underwent the implanting the mini-pump devices which charged with HEPES Buffer Solution or in combination with Amyloid-Beta feature of Alzheimer's (Aβ) with ALZET (model 1004 pump) company product. And in this research we applied the codes and consequent calculations based on the Alzheimer induced mice and its vehicle control group. So, in our current work, just the animal with surgery has been applied, as it can be seen in Figure 4.

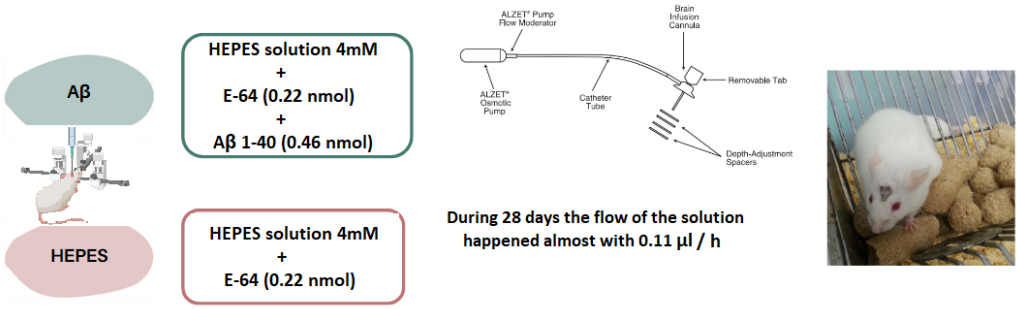

Figure 4: Plan of experiment design and solutions applied

4.3. Protocol for Novel Object Detection in Mice:

Day 1:

Pre-Experiment Preparation: Acclimate the mice to the testing room for at least 15 min before the start of the experiment.

T0: Habituation Phase and Training Phase:

- Place each mouse individually in the empty arena with two identical objects for 5 minutes to habituate them to the testing environment and objects. Record the total exploration time for a1 and a2.
- Clean the arena with a mild disinfectant solution between each mouse to remove any residual odors.

T1.5h: Introduce one new object (Object B) into the arena.

- Allow the mouse to freely explore the objects for a predetermined exploration time (e.g., 5 minutes).
- Record the total exploration time which spend for A and B.

Day 2:

T24h: Testing Phase:

- Place the familiar object (Object A) and object (Object C) into the arena.
- Allow the mouse to explore the objects for a predetermined exploration time (e.g., 5 minutes).
- Record the total exploration time (e2) spent by the mouse on both Object A and Object C.

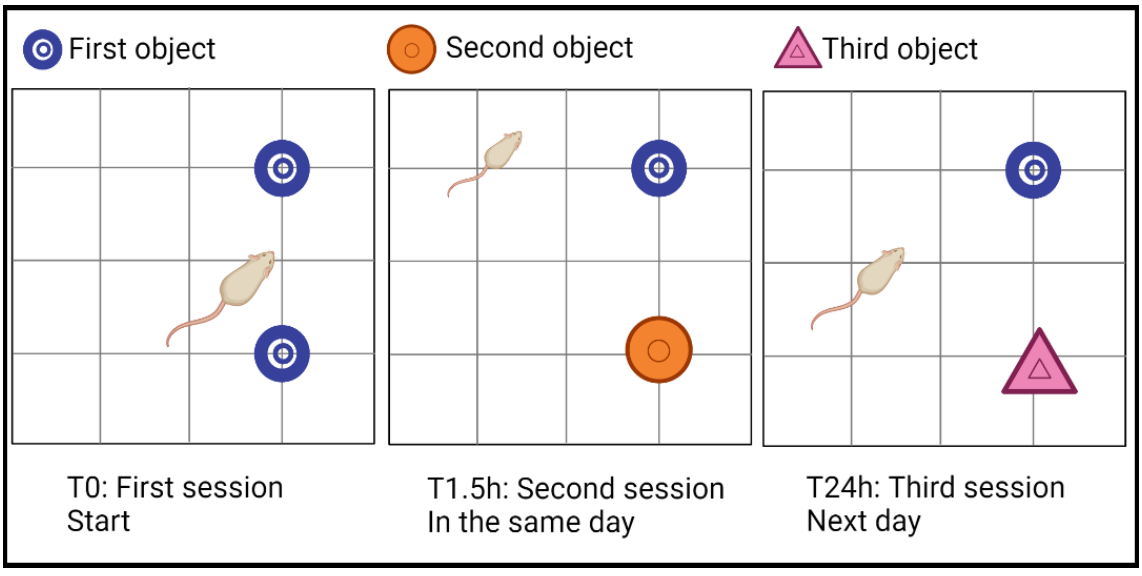

Figure 5: Illustrative format of performing the new object recognition for Alzheimer model mice.

3.3. Method of calculation

Different formulas for analysis and their interrelationships can be observed in Table 1 (some adaptation from reference: doi: 10.3791/55718).

Table 1: analysis and their interrelationships

Exploration	Discrimination
e1 = A1 + A2	**d1 = B - A**
e2 = A + B	**d2 = d1/e2**
	d3 = B/e2*100

To calculate e1, the total exploration time during training for two identical objects (A1 and A2), we use the equation e1 = A1 + A2.

For e2, the total exploration time during testing for a familiar object (A) and a novel object (B), we calculate e2 = A + B.

To determine d1, we simply subtract the time spent exploring the familiar object from the time spent exploring the novel object. The absolute discrimination measure (d1) disregards differences in exploration time among mice or treatment groups. However, in certain situations, it may be a more sensitive measure. We calculate d1 = B - A.

To obtain d2, we divide the time spent exploring the novel object minus the time spent exploring the familiar object by the total exploration time. This commonly used measure is known as the relative discrimination value or discrimination index (d2), which is not affected by variations in exploration time. Consequently, all values will fall within the range of -1 to +1. The formula for d2 is d2 = d1/e2.

Alternatively, the recognition or preference index (d3) can be calculated. It involves dividing the time spent exploring the novel object by the total time, resulting in values ranging from 0 to 1. It is often multiplied by 100 and expressed as a percentage. The formula for d3 is d3 = (b/e2) * 100.

For statistical analysis, the mean discrimination values for each group can be utilized to evaluate memory performance using a one-way ANOVA. To conduct further analysis, two-way post hoc comparisons can be made between the treated vs. vehicle condition groups.

3.4. Data collection and processing

With a camera Sony (HDR-CX440, full HD 1080, China) with 30 fps we recorded the animal behavior and then the resulted csv files through python program has been applied for CWT analysis. And the resulted csv files for each animal which have been derived from animal groups, collectively provides needed process which has been automated based on the

5- Result

As you can see in the Table 2, we have obtain this result, we did not incude the animals code arbitrarily and also anther control groups too. We just check it out the result for Vehicle (hepes solution, non Alzheimer model) and Vehcle+Aβ solution (Alzheimer model).

D2	D3	Time settings	1=vehicle(hepes) **0=Aβ containing**
.05	47.31	.00	1.00
.01	49.44	.00	1.00
.04	48.09	.00	1.00
-.11	55.31	.00	1.00
-.01	50.69	.00	1.00
.14	43.14	.00	1.00
-.06	52.84	.00	1.00
.13	43.47	.00	1.00
.12	43.98	.00	1.00
.02	49.06	.00	.00
.17	41.75	.00	.00
.15	42.32	.00	.00
-.14	56.88	.00	.00
-.05	52.56	.00	.00
.25	37.38	.00	.00
-.13	56.37	.00	.00
.23	38.27	1.50	1.00
-.04	52.13	1.50	1.00
.34	32.89	1.50	1.00
.36	32.06	1.50	1.00
.14	43.08	1.50	1.00
-.25	62.65	1.50	1.00
.36	31.78	1.50	1.00
-.07	53.51	1.50	1.00
.11	44.31	1.50	.00
.18	40.84	1.50	.00
.27	36.25	1.50	.00
.27	36.58	1.50	.00
-.07	53.28	1.50	.00
.35	32.43	1.50	.00
.18	41.03	1.50	.00
.64	18.04	1.50	.00
-.10	55.23	1.50	.00
-.18	58.88	1.00	1.00
-.16	57.99	1.00	1.00
.14	42.97	1.00	1.00
.35	32.46	1.00	1.00
-.04	52.06	1.00	1.00
-.20	59.94	1.00	1.00
.05	47.45	1.00	1.00
.01	49.44	1.00	1.00
.22	39.11	1.00	.00
-.17	58.60	1.00	.00
.10	44.95	1.00	.00
.35	32.28	1.00	.00
.19	40.29	1.00	.00
-.01	50.56	1.00	.00
.05	47.66	1.00	.00
.45	27.64	1.00	.00

It should be mentioned that by following code you would get your result which have obtained from mice videos, which basically are a name and csv type files.

```
import pandas as pd
import glob
import os
```

```
# chose the path to the directory containing the CSV files
path = '/Users/                                    /Downloads/t1.5/'

all_files = glob.glob(os.path.join(path, "*.csv"))
hepes_files = []
non_hepes_files = []   # Aβ containing( which belong Alzheimer induced animals)

# Iterate over each CSV file
for file in all_files:
   file_name = os.path.basename(file)
   if 'HEPES' in file_name: #our animal code names had hepes words in it & it could be change
      hepes_df = pd.read_csv(file)
      # Calculate cumulative sum of "Distance_cm" column
      hepes_df['Cumulative Distance_cm'] = hepes_df['Distance_cm'].cumsum()
      total_distance = hepes_df['Distance_cm'].sum()
      hepes_files.append((file_name, hepes_df, total_distance))
   else:
      non_hepes_df = pd.read_csv(file)
      # Calculate cumulative sum of "Distance_cm" column
      non_hepes_df['Cumulative Distance_cm'] = non_hepes_df['Distance_cm'].cumsum()
      total_distance = non_hepes_df['Distance_cm'].sum()
      non_hepes_files.append((file_name, non_hepes_df, total_distance))

# then count the true values for object1 and object2 in each HEPES file
hepes_counts = {}
for file_name, df, total_distance in hepes_files:
   true_count_object1 = df['object1'].eq(True).sum()
   true_count_object2 = df['object2'].eq(True).sum()
   differences= (true_count_object1 - true_count_object2)
   Exploration= (true_count_object1 + true_count_object2)
   d2= differences/Exploration
   d3= true_count_object2 = (df['object2'].eq(True).sum()/Exploration)*100
   hepes_counts[file_name] = {'object1': true_count_object1, 'object2': true_count_object2,
                        'total_distance': total_distance,
                        'differences': differences, 'Exploration': Exploration, 'd2': d2, 'd3': d3}

# repeat the count the true values for object1 and object2 in each non-HEPES file
non_hepes_counts = {}
for file_name, df, total_distance in non_hepes_files:
   true_count_object1 = df['object1'].eq(True).sum()
   true_count_object2 = df['object2'].eq(True).sum()
   differences= (true_count_object1 - true_count_object2)
   Exploration= (true_count_object1 + true_count_object2)
   d2= differences/Exploration
   d3= true_count_object2 = (df['object2'].eq(True).sum()/Exploration)*100
   non_hepes_counts[file_name]      =      {'object1':      true_count_object1,      'object2':
true_count_object2,
                        'total_distance': total_distance,
                        'differences': differences, 'Exploration': Exploration, 'd2': d2, 'd3': d3}

# make an Excel writer
```

```
excel_file = 't1.5.xlsx'
writer = pd.ExcelWriter(excel_file)

# save HEPES counts in a sheet
hepes_counts_df = pd.DataFrame(hepes_counts).T
hepes_counts_df.index.name = 'CSV File'
hepes_counts_df.to_excel(writer, sheet_name='HEPES Counts')

# save non-HEPES counts in a sheet
non_hepes_counts_df = pd.DataFrame(non_hepes_counts).T
non_hepes_counts_df.index.name = 'CSV File'
non_hepes_counts_df.to_excel(writer, sheet_name='Non-HEPES Counts')

# finally you should save and close the Excel writer
writer.save()
writer.close()
```

By assessing the result in the SPSS software you would get the several months active researching curiosities result and for us it was like Figure 6:

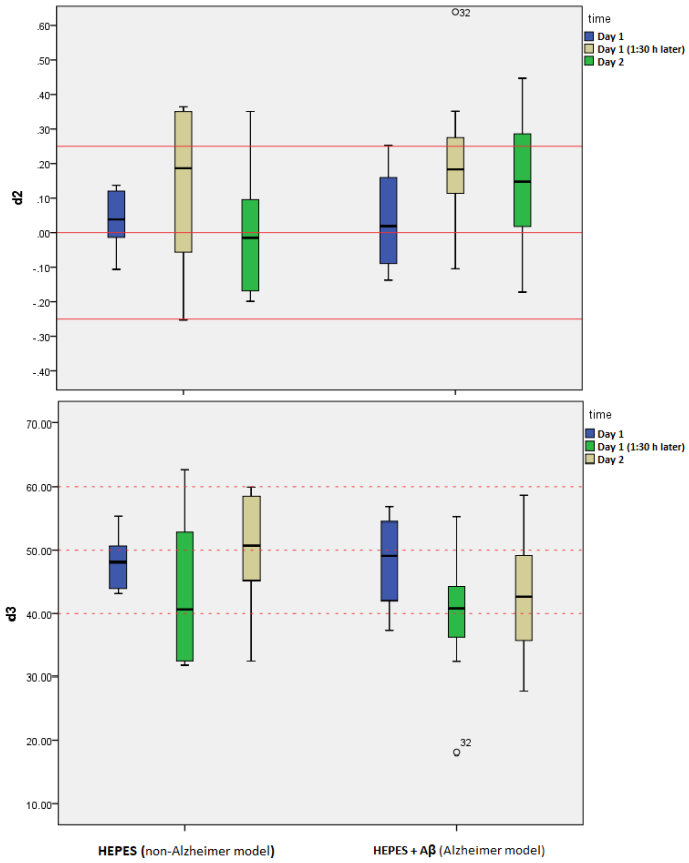

Figure 6

6- Conclusion

The development of the OLS project was driven by the motivation to offer a platform that maximizes the result differences between different lab settings. By addressing the limitations

of commercial software and promoting the use of open-source technology, the OLS project provides several key advantages for Alzheimer's disease (AD) research.

Firstly, cost savings are a significant advantage of using ez Track, as it offers a cost-effective alternative to expensive commercial software. This affordability enables researchers, particularly those with limited budgets, to conduct comprehensive and accurate linear regression analysis without financial constraints. By removing the barriers posed by high costs, the OLS project opens doors for researchers in various lab settings, enhancing collaboration and facilitating research advancements.

Secondly, the customizability of the is a crucial benefit. Unlike rigid commercial software, the OLS project allows researchers to tailor the analysis to their specific needs and experimental setups. This flexibility empowers researchers to optimize the analysis process and obtain more accurate and relevant results. By maximizing the result differences between different lab settings.

Furthermore, the OLS project promotes collaboration and cooperation within the research community. As open-source software, it enables researchers to access and modify the source code, facilitating transparency and scientific rigor. The open-source nature of the project encourages knowledge sharing, peer review, and the contribution of new features and enhancements by the community. This collaborative environment fosters advancements in AD research, as researchers can collectively work towards improved methodologies and analytical techniques.

Lastly, emphasizing the importance of open-source software in advancing AD research is crucial. Open-source tools like the OLS project promote openness, accessibility, and transparency in the scientific community. They facilitate the sharing of methodologies, data, and insights, enabling researchers worldwide to benefit from each other's work. Open-source software plays a vital role in accelerating progress, as it allows researchers to build upon existing knowledge and collaborate towards a deeper understanding of AD pathology and the identification of new treatment targets.

In conclusion, ez Track offers a cost-effective, customizable, and collaborative platform for linear regression analysis in AD research. Its advantages include cost savings, customizability, and collaboration opportunities, enabling researchers to maximize the result differences between different lab settings. The emphasis on open-source software highlights the importance of openness and community-driven progress in advancing AD research. By utilizing the OLS project and other open-source tools, researchers can enhance their research capabilities, promote cooperation, and contribute to the collective efforts in understanding and treating Alzheimer's disease.

7- Vision (after this project, next move!)

For the next round, my goal is to develop a freely available and user-friendly tool that empowers researchers to train small data for animal recognition which in ethologically have important meanings like:

a) Allo-grooming: Mice engaging in typical grooming behavior.
b) Bar-Mouthing: Mice nibbling or mouthing the bars of their enclosure.
c) Carrying: Mice transporting objects using their mouths.
d) Circling: Mice moving in circular patterns.
e) Drinking: Mice consuming liquid from a water source.
f) Feeding: Mice eating or foraging for food.
g) Grooming: Mice cleaning their fur or body parts.

Through fostering a collaborative community, my aim to facilitate multidisciplinary research, share expertise, and enhance scientific knowledge of animal behavior, conservation, and related topics. With our initiative, researchers worldwide will have the necessary tools to contribute to the advancement of animal detection techniques, benefiting both research and conservation efforts.

By offering a cost-effective, customizable, and collaborative platform for linear regression analysis, the project aims to break down financial barriers and enhance research capabilities. This vision is important as it allows researchers, particularly those with limited budgets, to conduct comprehensive and accurate analysis without constraints. Additionally, the emphasis on open-source software promotes transparency, collaboration, and the sharing of knowledge within the scientific community. Ultimately, this vision aims to contribute to a deeper understanding of Alzheimer's disease, improve methodologies, and identify new treatment targets, benefiting both researchers and the broader community in their efforts to combat this debilitating condition.

The vision for the development of a freely available and user-friendly tool for animal recognition stems from the importance of understanding animal behavior and its implications for various fields such as animal behavior research and conservation. In the next 5/10/20 years, the goal is to create a tool that empowers researchers worldwide to study and quantify important ethological behaviors in animals with more precise tools like key point detection in mice body (e.g. head, bogy and tail). By fostering a collaborative community and providing accessible tools, this vision aims to advance animal behavior research, contribute to scientific knowledge, and support conservation efforts. The vision holds significance as it enables researchers to accurately analyze and understand behaviors like grooming, carrying, feeding, and more, thereby benefiting researchers, the broader scientific community, and the preservation of animal species. I think I will apply and make more precise tools as far as I can.

8- Acknowledgements

the author was supported by a micro-grant from the OLS-7 and their committee selection especially Dr. Emmy Tsang. The author would like to thank her former mentor, Professor Alex C Manhães, Mr. Vitor D Pinheiro, and Miss Laura Balthazar for their support in this project.

9- How to collaborate with this work

For any inquiring doubt and question please make a contact with jafari.amir@posgraduacao.uerj.br and also video would be available based on the inquiring. I recommend you find another tests and develop this platform and make the related developed codes available on the github to facilitate the easy use. There are still remain some tests which provide some opportunities to arrange good proposal and send to OLS committee again and I would be pleased to help you in this regards. Probably one of the other options would be you could improve the code which changes the measurements based on the method we proposed in section 7.

Saturday, May 20, 2023

Amir Jafari

UERJ, Brazil