

Evolution of Novelty Responses During Few-Shot Memory Formation in Mice

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Memory
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Abstract

This project investigates how the body's response to a new and potentially stressful experience changes as an animal encounters it repeatedly. To address this question, we study changes in pupil area in mice performing a virtual navigation task. During the task, mice can encounter a virtual trap, providing a controlled way to study their response to the same salient event over multiple experiences.

The project aims to improve our understanding of few-shot memory formation, which refers to the ability to form a memory after only a limited number of experiences. By studying how the pupil response changes between encounters, we aim to explore whether this physiological response could provide an indication of how an initially novel experience is processed as it becomes familiar.

The analysis provides preliminary evidence of a pupil response associated with virtual trap events in individual sessions, although this effect was not consistently observed across animals. Further research is needed to determine whether this response is robust and how it relates to memory formation.

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1. Introduction

This report summarises the work carried out during a two-month research internship in the Neurobiology of Memory Laboratory at EPFL, as part of the Laidlaw Scholars Leadership and Research Program. The lab investigates the neural mechanisms underlying memory formation, with a particular interest in how experiences are processed and stored in the brain. The project presented in this report focused on the physiological responses associated with few-shot memory formation in mice.

Few-shot memory refers to the ability to form a memory after only a limited number of experiences. This ability is particularly relevant when an animal encounters a new or unexpected event, as the brain must rapidly process the experience and determine whether it should be remembered. The hippocampus is known to play an important role in the formation of episodic and spatial memories, and studying the physiological responses associated with novel experiences can provide insight into the processes involved in memory formation.

One way of investigating these responses is through changes in pupil area. Although pupil area is primarily associated with the regulation of the amount of light entering the eye, it also changes in relation to physiological and behavioural states. Pupil dynamics can therefore provide a non-invasive physiological readout of an animal's response to an event. Studying how this response changes when the same event is experienced repeatedly may provide an additional way to investigate how an initially novel experience is processed as it becomes familiar.

In this project, mice performed a virtual navigation task in which they could encounter salient virtual events. These events provided a controlled and repeatable way of studying the animal's physiological response to an experience that could initially be novel or unexpected.

The main question addressed by this project was: **how do novelty-related pupil responses evolve during few-shot memory formation?** More specifically, the project aimed to investigate whether salient events were associated with measurable changes in pupil area and whether these responses could provide an indication of how the physiological reaction to an experience changes over repeated encounters.

To address this question, the project involved processing pupil recordings as well as extracting and analysing pupil data. The following sections first introduce the theoretical background underlying the project, before describing the methodology used to process and analyse the data. The results and their analysis are then presented, followed by a discussion of future directions.

2. Theoretical Background

2.1 The Hippocampus, Memory and Novel Experiences

The hippocampus is a brain structure strongly associated with memory formation. This role was first highlighted by studies of patients with hippocampal damage, which showed that the hippocampus is important for forming new memories. Research in rodents later provided another important perspective through the discovery of place cells: neurons whose activity is associated with particular locations in an environment [1].

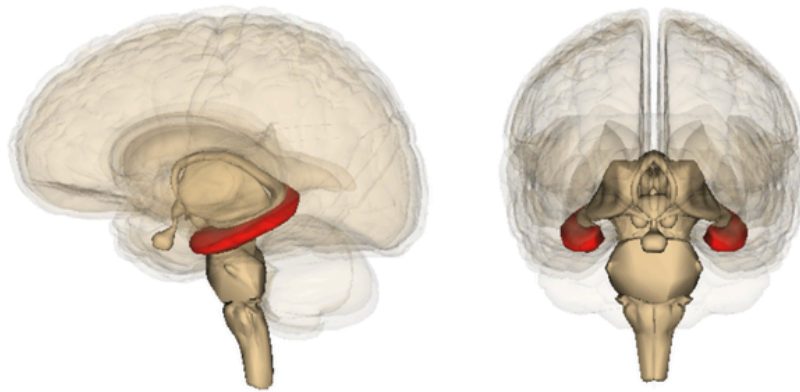


Figure 2.1 Visualisation of the hippocampus in the brain. *Source: commons.wikimedia.org*

These findings contributed to the idea that the hippocampus provides a spatial framework in which different elements of an experience can be associated. Place cells can combine information from different sources, including self-motion, environmental boundaries, objects and head direction, to represent the spatial context of an experience [1]. However, the role of the hippocampus is not limited to spatial navigation. Research on memory and the so-called cognitive map has highlighted its broader involvement in organising relationships between different elements of an experience [2].

The hippocampus is therefore relevant when an animal encounters and learns about an environment or event. Repeated experiences can provide information that allows an animal to update how an event is represented. In the present project, the virtual environment provides a controlled spatial context in which the same salient event can be encountered repeatedly. This makes it possible to investigate whether the physiological response to the event changes with experience, while considering the role of the hippocampus in memory.

2.2 Pupil Responses as a Physiological Measure of Salience

The pupil is primarily responsible for regulating the amount of light entering the eye. However, pupil diameter also changes with behavioural and physiological states.

In mice, pupil dynamics have been associated with arousal, movement and changes in brain state, making pupillometry a useful physiological measurement during behavioural experiments [3].

Importantly, pupil responses can also change as animals learn and adapt their behaviour. In a tactile decision-making task in mice, different behavioural responses were associated with distinct patterns of pupil dilation and constriction. These patterns also changed as the animals learned the task, with some responses becoming larger or occurring earlier during later stages of learning [3]. This suggests that pupil dynamics can provide information about changes in behavioural state during learning, although they are influenced by several processes and cannot be interpreted independently of behaviour.

Pupil responses have also been investigated in relation to salient or potentially stressful events. A study in mice examining fear responses found that aversive stimulation was associated with pupil dilation, supporting the use of pupil measurements as an additional measure of the response to salient events [4]. However, because pupil area is affected by multiple factors, a change in pupil diameter cannot by itself identify the specific process underlying the response.

In the present project, pupil area is therefore used as a physiological measure of the mouse's response to the virtual trap. The aim is not to use pupil area as a direct measure of memory, but to investigate whether this response changes as the same event is experienced repeatedly. Such changes could provide an additional indication of how the animal's response to a previously novel event evolves with experience.

3. Methodology

3.1 Experimental Setup and Data Collection

The experiments analysed in this project involved mice navigating through a virtual environment. Each experimental session consisted of multiple trials, during which the mouse remained in the same virtual environment and moved forward on a running wheel. Several types of data were recorded simultaneously throughout the sessions, including behavioural measurements such as running speed, pupil recordings, and, when available, hippocampal imaging data. These recordings were made prior to the start of this project and were used as the basis for the subsequent data processing and analysis.

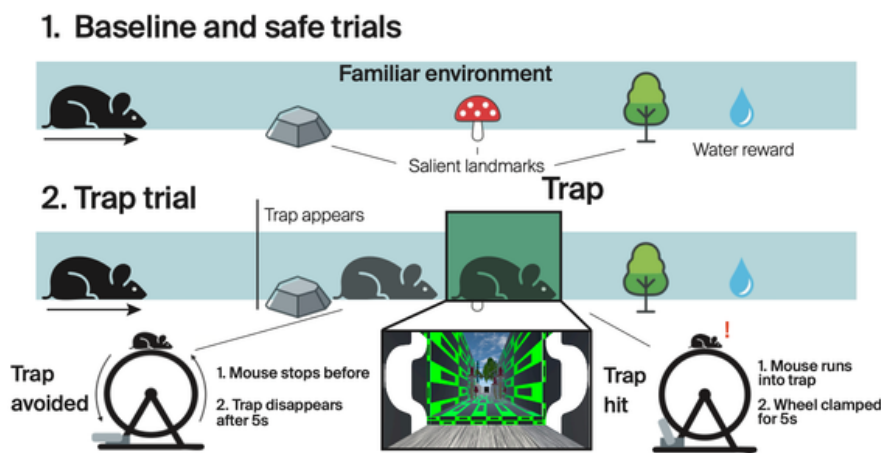


Figure 3.1 Virtual environment explored by the mice during the experimental session. Schematic by Daniel Molinuevo Gomez.

A key event in some trials was the presentation of a virtual trap. The trap consisted of a visual cue appearing at a specific place in the virtual environment, followed by a sudden stop of the running wheel. This interruption of the mouse's movement was designed to create a salient and potentially stressful event. Because the trap could occur in some trials but not in others, the pupil and behavioural responses associated with its presentation could be compared with responses during trials without a trap.

A control mouse was also used to distinguish the response to the visual cue from the response to the complete trap event. In the control condition, the visual cue could appear during a trial, but was not followed by a stop of the running wheel. These trials specific to the control mouse were referred to as trap trials when the visual cue was present, whereas no-trap trials were trials in which neither the visual cue nor the wheel stop occurred. Comparing this condition to other sessions made it possible to investigate whether changes in pupil area were specifically associated with the complete trap event, rather than with the animal moving through the environment, seeing the visual cue or just changes in light. The repeated structure of the trials provided a controlled setting in which physiological and behavioural responses could be examined across different experiences.

Behavioural information was already available for the experimental sessions, whereas the pupil information had to be extracted from the corresponding video recordings. A central part of the project was therefore to process the pupil recordings and integrate them with the existing behavioural data, and with hippocampal imaging data when available, into a common dataset for subsequent analysis.

3.2 Pupil Detection and Feature Extraction

Pupil information was extracted from the raw behavioural videos using DeepLabCut, a machine-learning-based tool for tracking predefined points in images. The pupil was represented by several anatomical keypoints as can be seen on the picture below. These keypoints were used to estimate properties of the pupil over time.

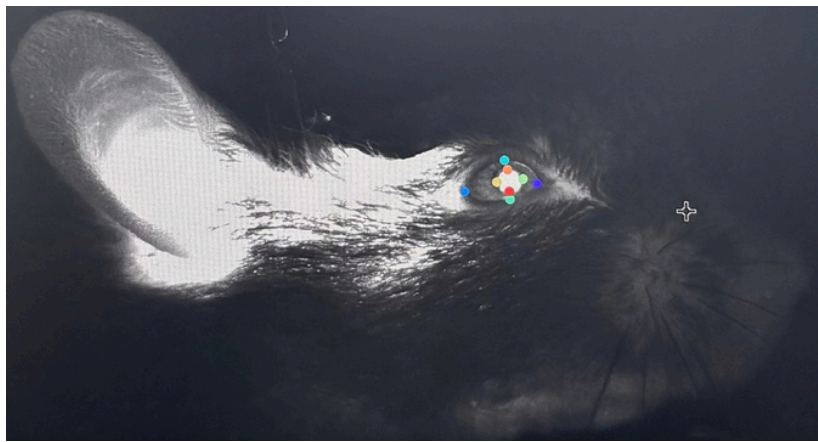


Figure 3.2 Manual labelling of pupil keypoints using DeepLabCut. Example frame from a behavioural video showing the anatomical keypoints manually labelled around the eye and pupil during the training of the DeepLabCut model. Screenshot taken by the author.

The existing DeepLabCut model was first applied to the available sessions. Its output included the estimated position of the keypoints as well as a confidence value for each detection. These confidence values were used to assess whether the extracted pupil measurements were sufficiently reliable for further analysis.

To improve detection in more challenging recordings, an additional DeepLabCut model, referred to as Shuffle 6, was trained using manually labelled data, as illustrated in Figure 3.2. Six sessions were selected from mice for which the pre-existing model showed low confidence. These sessions were chosen because they contained challenging conditions, such as a small pupil or poor illumination, which reduced the accuracy of the original model. The labelled data from these sessions were added to the dataset previously used to train the original model. The resulting model improved pupil detection in several of the selected sessions and allowed a larger proportion of the available recordings to be processed.

The extracted pupil data included measurements such as pupil area and pupil position, together with the model confidence. Changes in pupil area were subsequently used as the main measure of pupil response. Other extracted information, including pupil position and confidence, was retained to assess the quality of the measurements and to support the identification of potentially unreliable periods.

3.3 Data Processing and Standardisation

After pupil detection, the extracted data had to be aligned with the existing behavioural data. Accurate temporal alignment was essential because pupil measurements needed to be linked to the animal's behaviour and to specific events occurring during each trial. The different data streams were therefore organised according to their respective timestamps before being combined.

The processed data were organised into Neurodata Without Borders (NWB) files. NWB is a standardised format designed to store and organise neuroscience data. A file was created for each processed session, combining behavioural measurements, pupil data, and hippocampal imaging data when available. This provided a common structure for the different sessions and facilitated their use in subsequent analyses.

The organisation of the dataset presented several technical challenges. Sessions were not always organised or named in exactly the same way, and some recordings required additional preparation before they could be processed. In particular, some of the larger videos needed to be modified before being analysed with the Shuffle 6 model. A predefined pattern of frames had to be removed so that the resulting video remained correctly aligned with the associated timestamps. These cases required additional checks before continuing with the standard processing workflow.

3.4 Automated Analysis Workflow

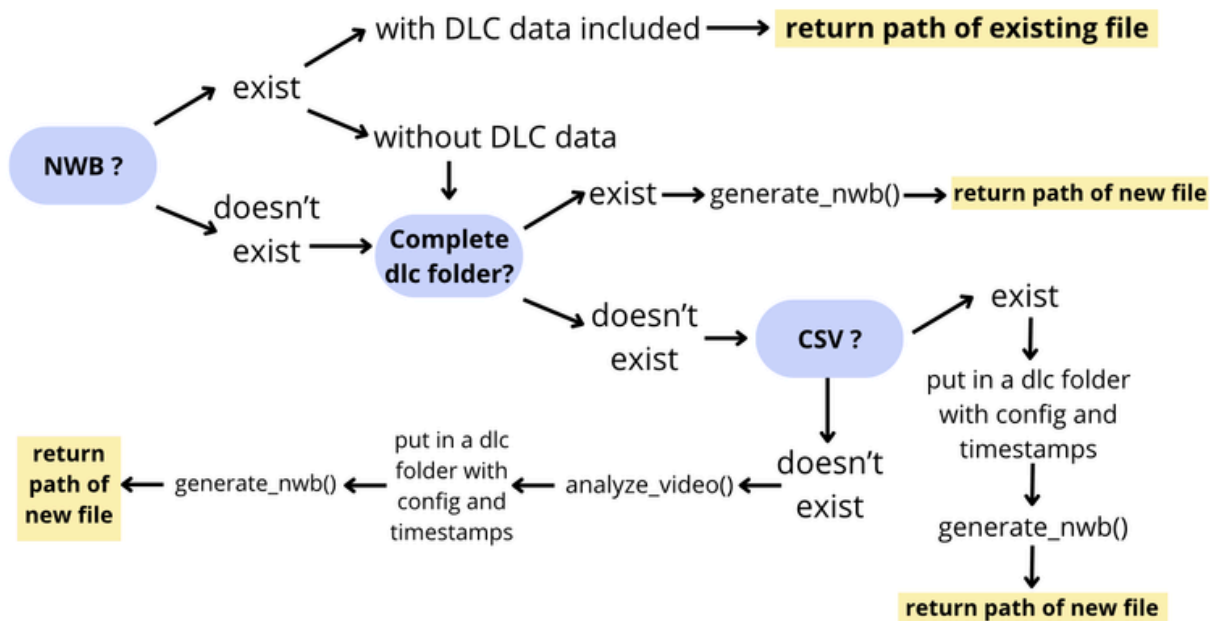
Processing the data involved several successive steps that would have been time-consuming to perform manually for every session. A Python-based workflow was therefore developed to connect these steps and make the processing more consistent across the dataset.

The workflow starts with the preparation of the video for pupil analysis. It then runs the DeepLabCut model and processes the resulting pupil data. The extracted measurements are organised and checked before being incorporated into an NWB file together with the available behavioural and imaging data. Then, the code extracts the data in the NWB file in order to generate various plots, used to visualise and compare results across sessions.



Figure 3.3 Data processing using the automated pipeline. Diagram created with BioRender.com

Several checks were also implemented to avoid repeating steps that had already been completed. For example, if an intermediate file was already available, the corresponding processing step could be skipped. This reduced unnecessary computation and made it easier to continue the analysis when a session had already been partially processed. The different checks performed by the workflow, along with the corresponding actions taken by the code, are illustrated below.



Last step: use the nwb path file to generate plots

Figure 3.4 Hierarchical file-system validation procedure for NWB file retrieval and generation. Diagram created with Canva.com

In addition, the different scripts and files used for this workflow were organised in a dedicated GitLab repository. This made the code easier to maintain and allowed the same processing procedure to be applied to different sessions.

However, the workflow was not fully automated for every dataset. Differences in file organisation, video format, timestamps, and recording quality meant that some sessions still required manual intervention.

The pipeline was therefore designed to automate the standard processing steps while allowing specific cases to be handled separately when necessary. This approach reduced repetitive work and made the overall data-processing procedure more reproducible.

The overall methodology used in this project is summarised in Figures 3.5 and 3.6. The first figure presents the main stages of the project, from the initial recordings to the extraction and processing of pupil data. The second figure provides a more detailed overview of the different aspects of the project.

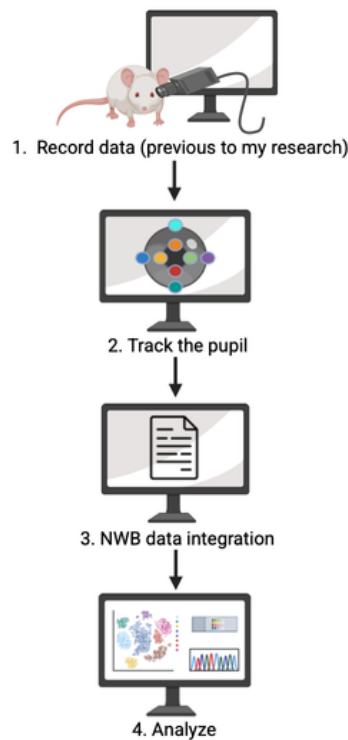


Figure 3.5 General workflow.
Created with BioRender.com.

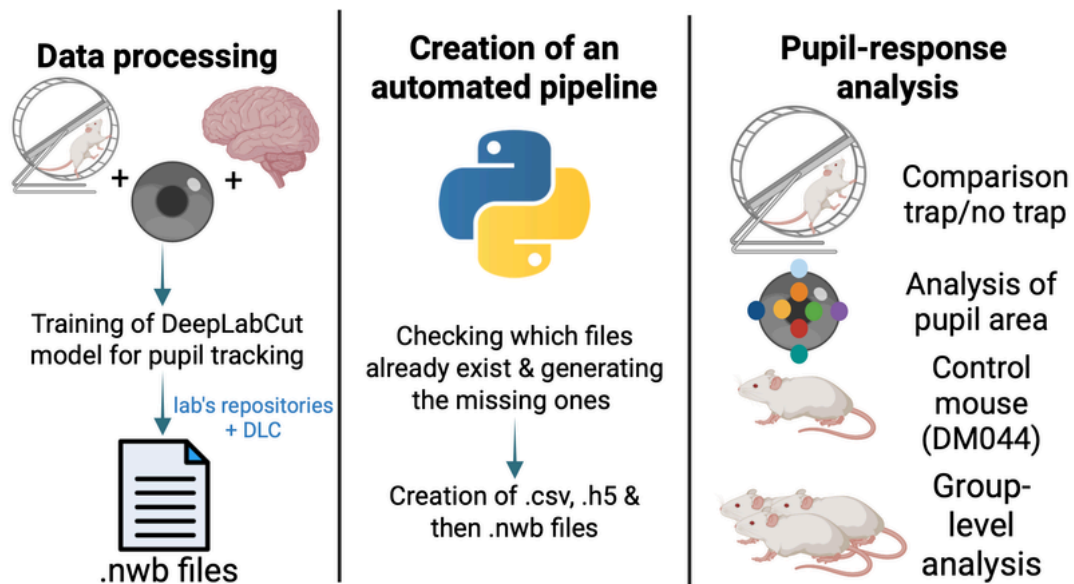


Figure 3.6 Experimental data processing and pupil-response analysis methods. Created with BioRender.com

This workflow provided a structured approach for transforming the raw recordings into organised datasets that could be used for the subsequent analysis of pupil and behavioural responses.

4. Results & Analysis

4.1 Individual Session Analysis

The analysis first focused on individual sessions to investigate whether virtual trap events were associated with changes in pupil area. Multiple sessions were analysed individually, and one session from mouse DM033 is presented here as a representative example. Several behavioural and pupil measurements were examined for this session. Among these analyses, pupil area was plotted as a function of the mouse's position in the virtual environment to examine the response around the position of the virtual trap.

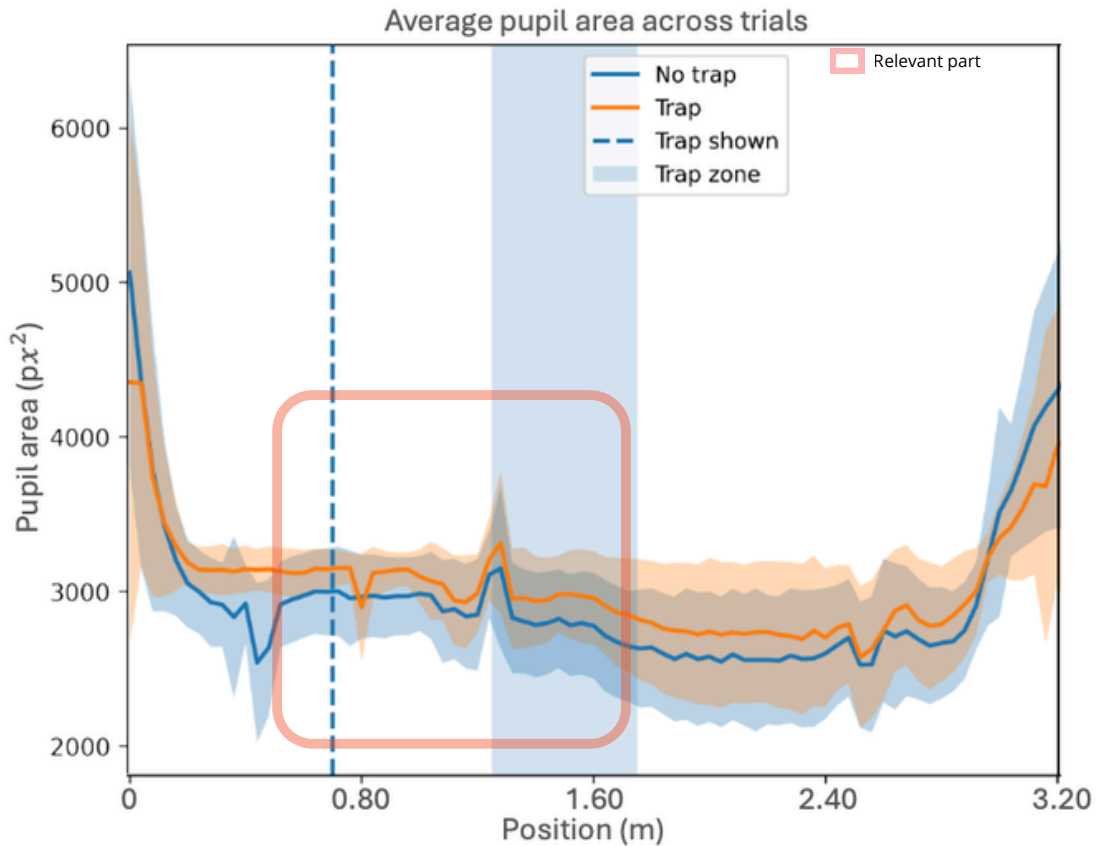


Figure 4.1 Evolution of pupil area for the mouse DM033 for trials with or without trap.

A decrease in pupil area of approximately 200 px² was observed after the mouse reached the position where the visual cue indicating the virtual trap became visible. This could indicate a transient pupil constriction due to the trap event. No similar change was observed in the no-trap trials, suggesting that the response may be associated either with the trap memory or with the visualisation of the cue, rather than with the mouse simply moving through the environment.

Other measurements, including running speed, licking behaviour, and pupil detection confidence, were also examined to assess the animal's behaviour and the quality of the pupil tracking.

For instance, the pupil detection confidence was also examined to assess the reliability of the tracking throughout the session. Brief drops in confidence to -1 correspond to moments when the mouse blinked, during which the pupil could not be detected. Overall, the confidence plot helps verify that the pupil measurements are reliable during the periods analysed.

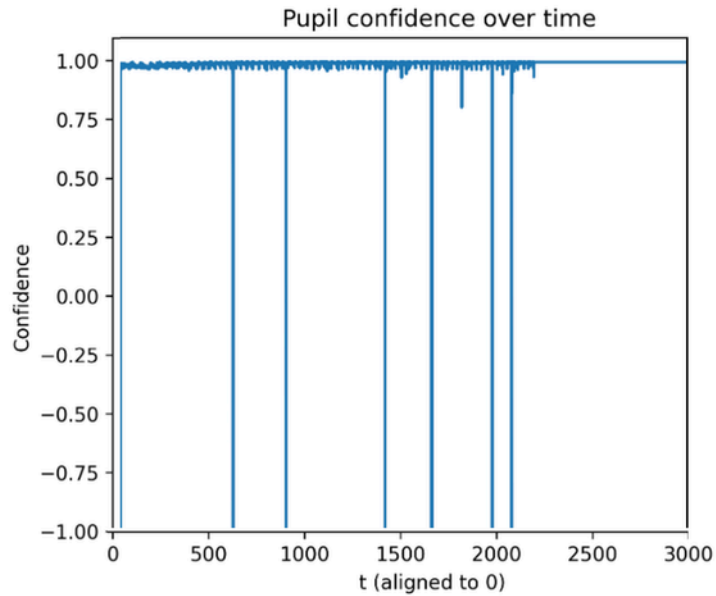


Figure 4.2 Evolution of pupil confidence over time for mouse DM033.

These results suggest a possible pupil response to the virtual trap in individual sessions, but further comparison was needed to determine whether the response was specifically related to the trap event. The control condition was therefore analysed to examine the effect of the visual cue without the subsequent stop of the running wheel.

4.2 Control Condition

The control session from mouse DM044 was analysed to compare the pupil response with the response observed during the complete trap event.

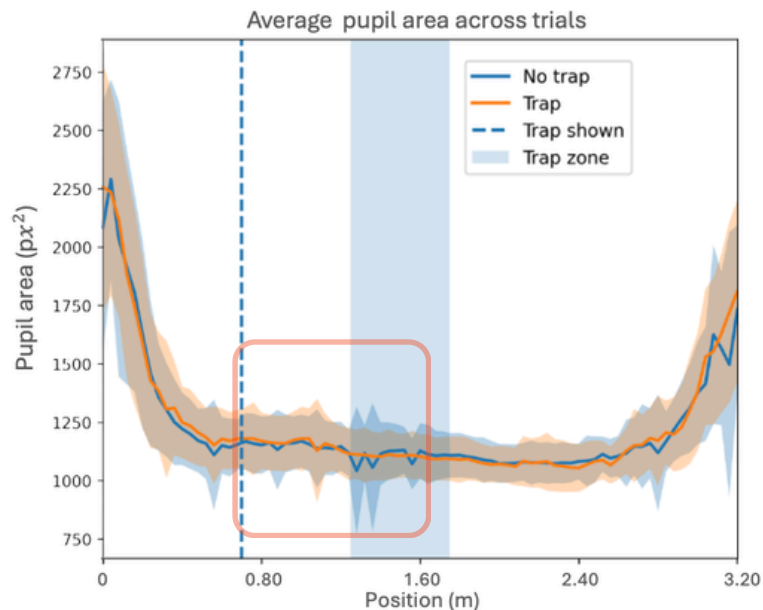


Figure 4.3 Evolution of pupil area for a mouse with an inactive trap (control).

In this session, the pupil area remained relatively stable around the position of the visual cue. This contrasts with the decrease in pupil area observed after the trap visualisation in DM033. The absence of a similar response in the control condition suggests that the pupil response may be related to the trap event rather than to the visual cue alone.

This observation is particularly relevant to the question of memory formation. If the pupil response is associated with the salient or potentially stressful experience produced by the trap, its evolution across repeated encounters could reflect how the animal processes and remembers this event. However, these data do not allow us to establish a direct link between the pupil response and memory. Further comparisons between initial and repeated trap encounters would be needed to investigate whether the response changes as the event becomes familiar. In addition, linking these results to the hippocampal data is needed to get solid conclusions.

4.3 Group-Level Analysis

The analysis was then extended to seven mice to determine whether the pupil response observed in individual sessions was also present at the group level. Trials were aligned according to the position of the virtual trap, and pupil area was averaged across trials and animals.

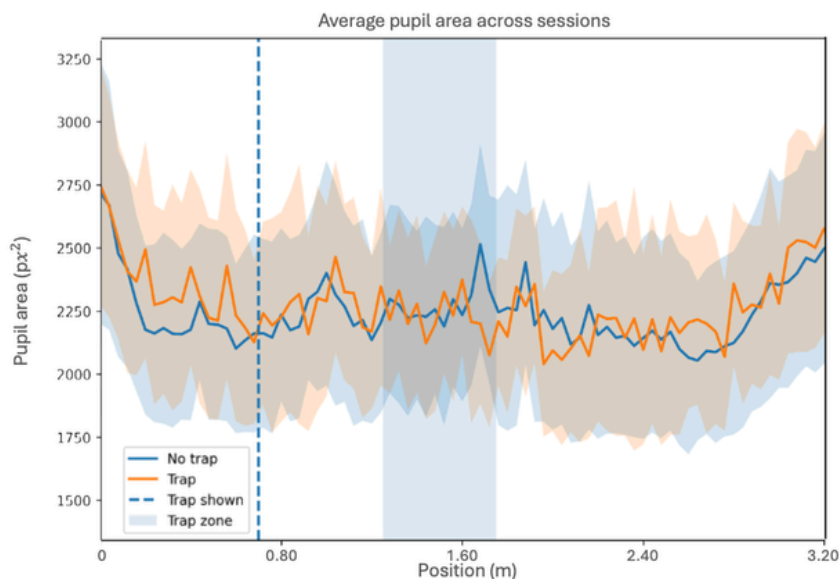


Figure 4.4 Group-level analysis. Evolution of pupil areas averaged for 7 mice.

No clear effect was observed at the group level. However, the sessions differed in several experimental conditions, which may have contributed to the variability between animals. In particular, no normalisation of pupil area was performed before combining the data. Differences in the distance between the mouse and the camera, as well as variations in illumination between recordings, could therefore affect the measured pupil area and make direct comparisons between animals more difficult.

The group-level analysis therefore does not exclude a trap-associated pupil response, but indicates that it was not consistent enough across the analysed animals to produce a clear overall effect.

4.4 Limitations and Conclusion

The analysis also has some limitations. For instance, using figure 4.5 below, we can see that across trials, the pupil measurements showed relatively consistent and smooth patterns, with changes in pupil area visible around the trap trials. However, clear perturbations were also observed at the beginning and end of the trials, corresponding to changes in illumination.

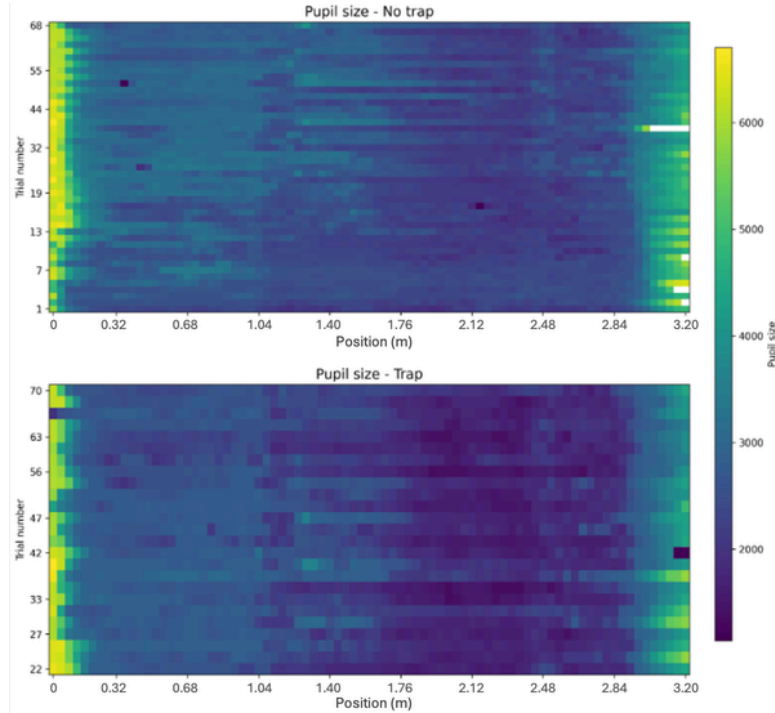


Figure 4.5 Evolution of pupil area by trials with and without traps for a mouse with an active trap.

These illumination changes represent an important limitation because light directly affects pupil area and may contribute to changes observed in the recordings. Other factors, such as arousal, attention, movement, and differences in camera distance, may also contribute to variability between sessions. In addition, no pupil-area normalisation was performed before comparing animals, making direct comparisons more difficult.

Importantly, this means that the current analysis cannot establish for sure that the observed pupil response reflects memory formation. Comparing the response to the first encounter with the virtual trap with responses during subsequent encounters would provide a more direct way to investigate whether the physiological response changes as the event becomes familiar. Combining these analyses with the available hippocampal recordings could further help determine whether changes in pupil responses are associated with the neural processes involved in memory formation.

Overall, the individual session analyses provide preliminary evidence of a pupil response associated with the virtual trap. The comparison with the control condition further suggests that this response may be related to the complete trap event rather than to the visual cue alone. However, the absence of a clear group-level effect prevents any strong conclusion from being drawn from the current dataset and further analysis is needed.

5. Future Directions

Several interesting steps could be taken to further investigate the relationship between pupil responses and few-shot memory formation.

- First, the analysis should be extended to a larger number of sessions. This would help determine whether the pupil response observed in some individual sessions is reproducible and whether it follows a consistent pattern across animals.
- A more systematic comparison between the first and subsequent encounters with the virtual trap would also be particularly informative. The first encounter represents a novel experience, whereas later encounters occur after the animal has already experienced the event. Examining how the pupil response changes across these encounters could therefore provide insight into how the physiological response evolves as the event becomes familiar.
- The analysis could also be improved by normalising pupil area between recordings, making the code more robust to variations in sessions and accounting for differences in illumination.
- Finally, combining pupil measurements with the available hippocampal recordings could provide a more complete view of the processes underlying the observed response. By aligning pupil changes with hippocampal activity around the trap event, it may be possible to investigate whether these changes in the physiological response are accompanied by changes in neural activity. This could help clarify whether the observed pupil dynamics are related to the neural processes involved in forming and updating memories of salient experiences.

6. Conclusion

This project investigated how pupil responses to salient events evolve during few-shot memory formation in mice, using a virtual navigation task in which animals encountered virtual traps. The project combined the processing of behavioural videos with the development of an automated workflow to extract and analyse pupil data across experimental sessions.

Although the analysis remains preliminary, the results from individual sessions suggest that virtual trap events can be associated with a measurable change in pupil area. This response was not consistently observed across animals at the group level, and the influence of illumination represents an important factor that needs to be addressed before drawing stronger conclusions.

The next phase of the research will involve analysing a larger number of sessions, improving the robustness of the data-processing workflow, and comparing pupil responses between initial and repeated encounters with the virtual trap. Combining these physiological responses with the available hippocampal recordings could also help investigate whether changes in pupil responses are related to the neural processes involved in forming memories of salient experiences.

Ultimately, this work provides a foundation for using pupil dynamics as a window into how an animal responds to, and potentially remembers, experiences that matter.

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