In vivo electrophysiological recording of hippocampal cells in head fixed but freely exploring mice

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Summary

To fully understand the neuronal network mechanisms that underpin behaviour, it is necessary to record from individual neurons in the brain. Whilst extracellular recordings can detect the spiking activity of neurons, measuring the underlying subthreshold membrane dynamics requires intracellular recording. To achieve this in an awake, freely moving animal is almost impossible due to mechanical instability, therefore methods to reduce mobility by head-fixation have been developed in awake mice. A common technique involves head-fixed mice running on a treadmill in a virtual reality environment, but in these conditions naturalistic stimuli are limited, in particular the use of the whiskers which are a principle sensory input in mice.

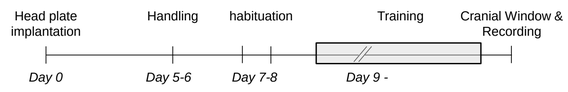
More recently however, a novel experimental system has been developed in which a head-fixed mouse can explore a large, air-lifted environment that is able to freely move around the animal in response to its movements. The Mobile HomeCage™ therefore offers the potential to perform high-precision neurophysiological recordings from individual neurons in the brain whilst the animal performs a behavioural task.

Our main aim is to be able to study neuronal responses in the hippocampus during decision-making tasks based on spatial memory, which will rely on the mice being able to freely explore in a minimally stressful environment. In order to assess the ability of mice to navigate while head-fixed in the Mobile HomeCage™ system, we trained them in a cued goal-localisation task. We show that these animals are able to effectively learn the location of a cued reward within the environment, and we present our preliminary data showing recordings from CA1 dorsal hippocampal neurons (whole-cell and cell-attached configurations) and discuss the neuronal firing correlates to the behavioral performance.

We obtained several cell-attached recordings from dorsal hippocampus principal neurons and analyzed their firing frequency at key points during the task. Furthermore, whole-cell recordings allowed us to evaluate submembrane potential dynamics, which showed different kinetics previous to the action potential happening between single and burst occurring first action potential (Grienberger et al., 2017). These differences will set a new line of study to unravel the synaptic correlates with behavioral activity.

1. Experimental configuration

General experimental timeline



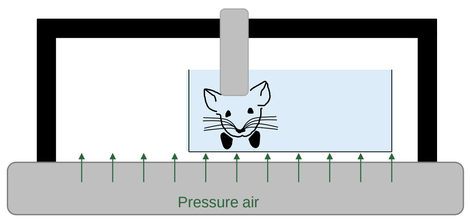
We use a flexible experimental plan depending on animal behavioural output. As a starting point, our standard protocol states 5 days recovery between head plate implantation and handling. Then, at least two 30-60 minutes session to habituate experimental environment. After that, if training is required, animal performance is monitored until desired level is reach. Finally, a cranial window is performed to enable recording of hippocampal activity.

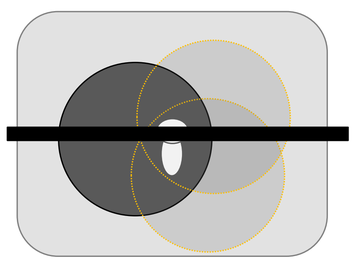
Head-fixation clamp

The mouse head-fixation system, which is 4.2 cm wide, enables the animal to sense its surroundings with the whiskers.

The fixation clamp has been tilted 35° to mimic normal exploratory posture, and we have designed a 'visor' to reduce the impact on the mouse of bright light from the microscope during recordings.

Floating Mobile HomeCage™

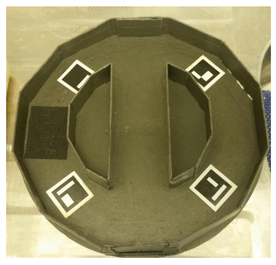




The mouse stands over a floating arena, which can be voluntarily moved by the animal, allowing them to freely explore the environment. The mouse is fixed in the same location relative to an external observer, whilst maintaining the perception of moving around the arena.

The circular arena is 34 cm diameter and is made from acrylic foam with a total weight of ~15 gr. The arena has been decorated with stripes on the walls to facilitate animal orientation.

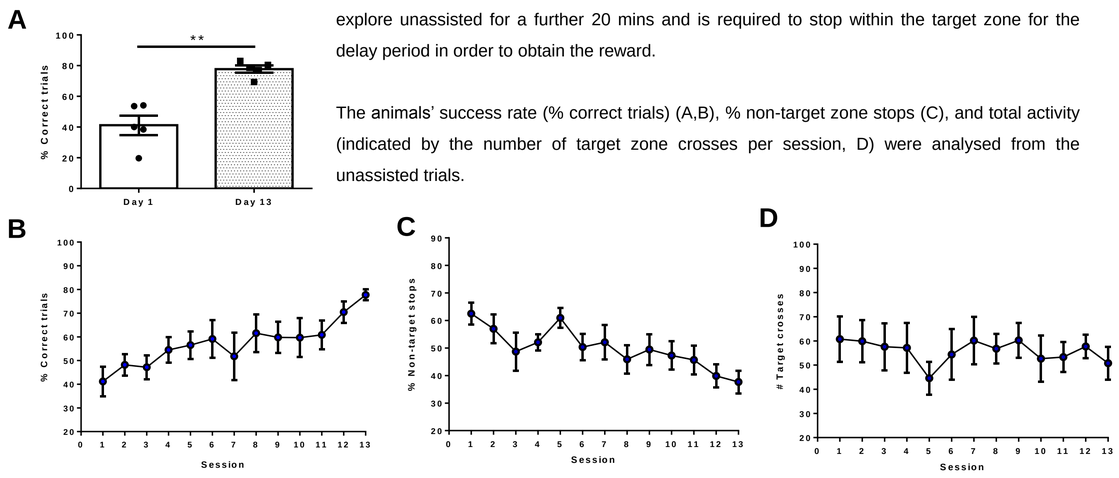
2. Cued goal-localisation task



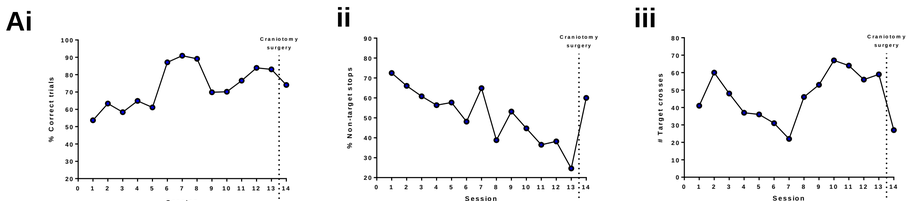
Mice undergo daily training sessions where they are head-fixed within the Mobile HomeCage™ and required to navigate around the maze to locate a sandpaper 'target zone'.

In the first 5 mins the mice are assisted by the experimenter to stay in the target zone for a delay period of 2s before receiving a sucrose reward via a lick-port. The animal is then allowed to explore unassisted for a further 20 mins and is required to stop within the target zone for the delay period in order to obtain the reward.

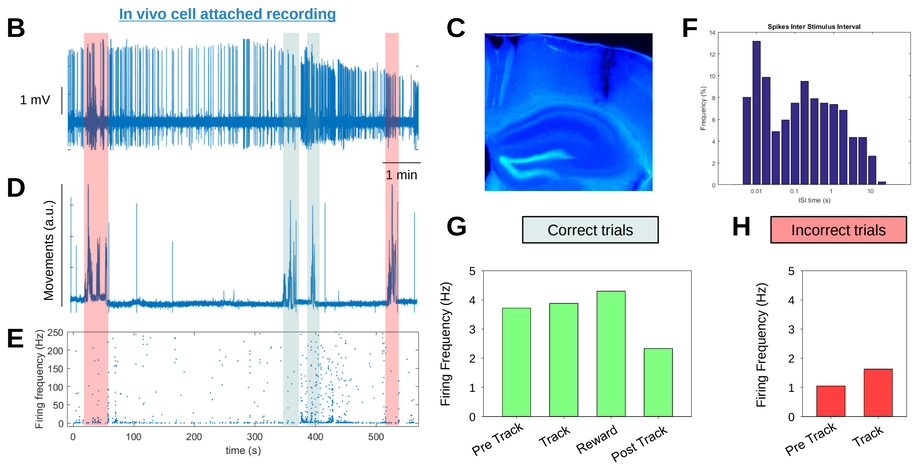
The animals' success rate (% correct trials) (A,B), % non-target zone stops (C), and total activity (indicated by the number of target zone crosses per session, D) were analysed from the unassisted trials.



3. Spiking correlates of mice behaviour



(Ai-iii) Behavioural performance in the cued goal-localisation task across training (sessions 1-13) and after craniotomy surgery (14) in one recorded mouse.

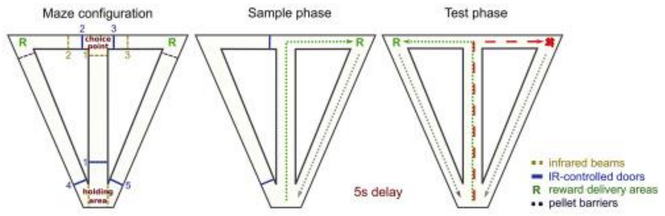


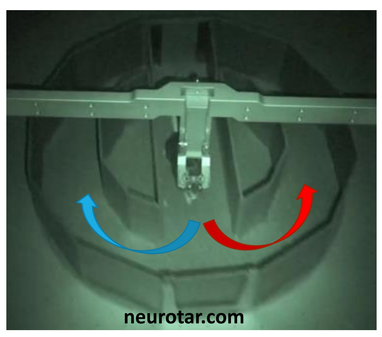
In vivo cell-attached recordings were obtained with a K^+ based (K^+ Glucanate$^-$) internal solution from putative CA1 area of dorsal hippocampus (B-C). Animal movement was analysed to assess performance in the cued goal localisation task (D), (green or red indicates correct and incorrect trials respectively), to measure the actual firing frequency in each grouped epoch (E). Inter-spike interval quantification showed a bimodal distribution which resembles place cell firing rate activity (F). Correct and incorrect trials were evaluated separately and the firing rates at different stages within the trials were quantified (G-H).

4. Future behaviour - Adapting a T-maze spatial working memory task for the Mobile HomeCage

The hippocampus is required for successful learning of spatial working memory tasks, such as this delayed non-match to place test using a T-maze (Teles-Grilo Ruivo et al., 2017).

In this task animals make a forced turn and are given a reward (sample phase) and, after a short delay, must make a choice turn and receive a reward when the alternate arm is chosen (test phase)

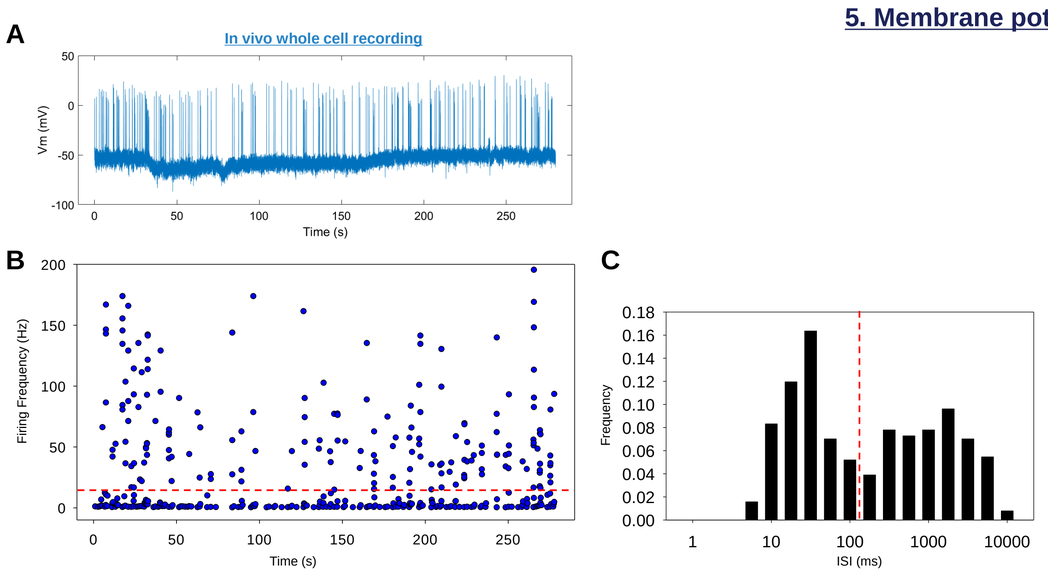


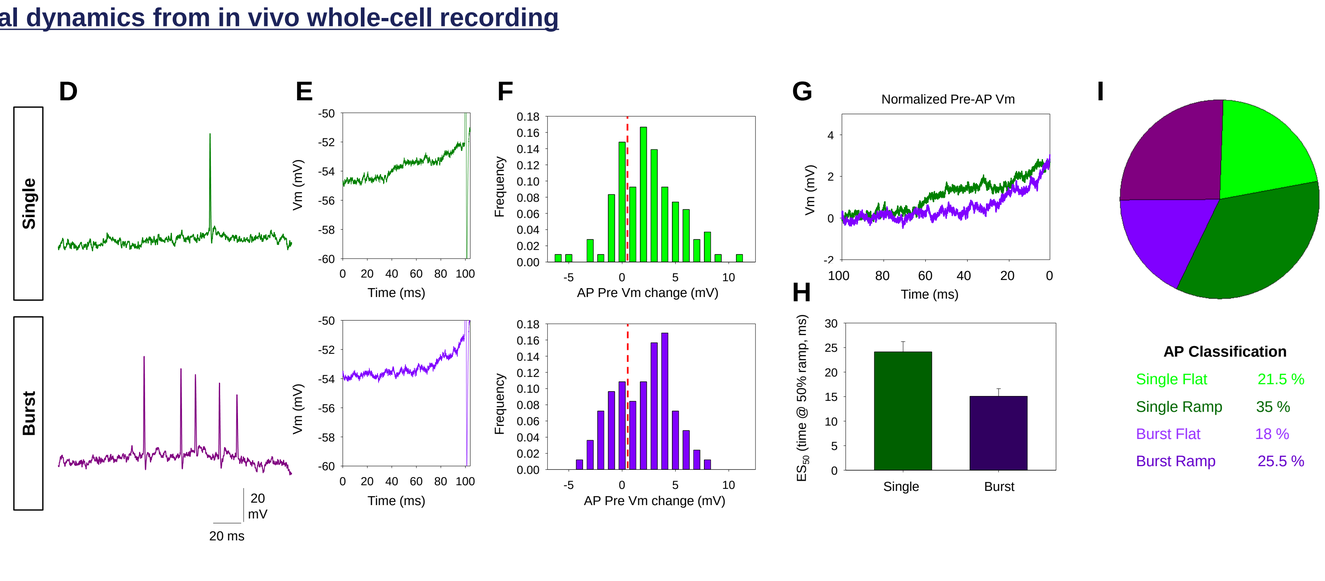


This task can potentially be modified for use in the Mobile HomeCage system. Our aim is to train the animals to make an initial, cued turn (left or right in response to one of two auditory tones), and after a short wait in the 'holding zone', make a free, un-cued choice.

In an alternative version of this task, it may also be possible to investigate the role of the hippocampus in choice uncertainty. In this assay the animal would be trained to make left or right turns indicated by high and low frequency cues, and then the animals response to an ambiguous, intermediate cue could be assessed in a number of probe trials.

5. Membrane potential dynamics from in vivo whole-cell recording





In vivo whole-cell recording was obtained with a K+ based (K+ Glucanate-) internal solution from putative CA1 area of dorsal hippocampus. The lack of animal movement allowed a stable recording over 5 minutes (A). Irregular spike firing rate was observed across the recording (B), showing a bimodal distribution for the inter-spike intervals (C). A dotted red line in B-C separates the single or burst occurring action potentials.

Action potentials were divided into single or within a burst occurring, where the sub-membrane potential dynamic was evaluated before occurrence of the first action potential (D) and averaged across groups (E). Most of the action potentials (see I) were observed to be preceded by a small ramp in the membrane potential which triggered the spike. Quantification of the amplitude of that ramp exhibited similar results for both groups (F). Interestingly, single and burst ramps differed in their dynamic (G), showing a slower ramp preceding a single occurring action potential, which takes more time to develop half of its ramp compared to the first action potential forming a burst (H).

Conclusions

- We show that head-fixed mice are able to effectively learn the location of a cued reward within the Mobile HomeCage™ system. We propose that this setup has advantages over virtual reality tasks by providing the animal with both tactile and visual cues with which to navigate.
- This initial behavioural study provides the basis for future experiments investigating the neural and molecular mechanisms underlying spatial navigation and working memory in head-fixed mice.
- Our whole-cell recording data suggests that single or burst occurring action potentials may arise following different synaptic activity. Thus, single action potentials would occur after less coordinated synaptic activity than action potentials forming a burst.

References:

- Grienberger C, Milstein AD, Bittner KC, Romani S, Magee JC. (2017) Nature Neuroscience 417-426.
- Teles-Grilo Ruivo LM, Baker KL, Conway MW, Kinsley PJ, Gilmour G, Phillips KG, Isaac JTR, Lowry JP, Mellor JR. (2017) Cell Report 905-917.