Whole-cell 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. This is extremely difficult in humans and only possible during procedures requiring the insertion of electrodes for the treatment of certain conditions such as epilepsy. Therefore, animal recordings are required. Extracellular recordings can detect the spiking activity of neurons but to measure the underlying subthreshold membrane dynamics requires intracellular recording. To achieve this in an awake freely moving animal is almost impossible due to mechanical instability. So to circumvent this problem methods to reduce mobility by head fixation have been developed to make recordings in awake mice. One popular 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.

Here we present a novel experimental system where head fixed mice explore a large (~40cm diameter) circular environment that is able to freely move in response to the animals' movements. At the same time we are able to record neuronal activity from neurons within the brain. We are working to optimize animal comfort in this environment by for example setting a more naturalistic angle for the head clamp to enable walking that is similar to that which occurs under freely moving conditions. The ultimate goal is to be able to study the neuronal responses during decision making tasks based on spatial memory, which will require mice to confidently explore the environment and be free from excessive stress. Therefore, we are trying several additional experimental alterations to reduce stress for the animals.

Our preliminary data show recordings from principle neurons in the hippocampus in a variety of configurations including extracellular, juxtacellular and whole-cell configurations. Our current experiments aim to record place fields to validate the exploratory behaviour of mice within our novel system.

Conclusions

- Animal well being increases behavioural performance. Mouse head's posture influences exploratory performance.
- In order to improve recording stability, mice need to be trained to move smoothly.
- This novel approach enables to record brain activity in different configurations.

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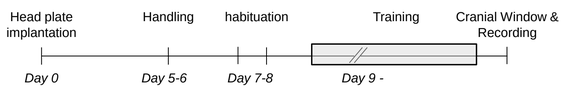
Experimental configuration

Experimental plan

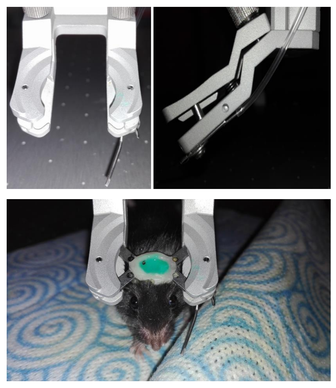


We use a flexible experimental plan depending on animal behavioural output. As a starting point, our standard protocol states 5 days recovery between head plate implantation and handling. Then, at least two 30-60 minutes session to habituate experimental environment. After that, if training is required,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

Experimental conditions

- A first surgery is made to implant the head plate, which is ~1.5 gr weight.
- Mice are kept in reverse circadian cycle after head plate implantation.
- Whenever possible, mice are housed in groups of 2-3 animals per cage.
- Mice cage is enriched with a wheel to reinforce their exploratory behaviour.
- In some experiments, mice are kept in water deprivation for no longer than 20 hours prior to training and rewarded with water when they are actively exploring.

Head fix clamp

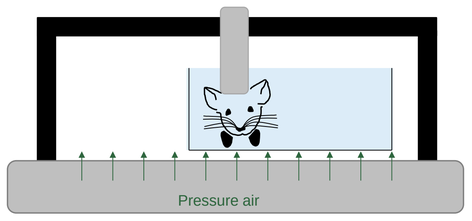


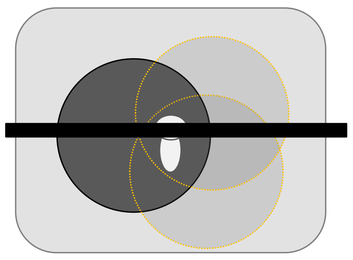
Mice head fixation system, which is 4.2 cm wide, enabling the animal to sense its surroundings with the whiskers. The fixation clamp has been tilted 35˚ to mimic mice head's exploratory posture.

Arena

The circular arena has 34 cm diameter and is made by carbon fibre with a total weight of ~20 gr. The arena has been decorated with stripes on the walls to facilitate mice orientation.

Floating cage

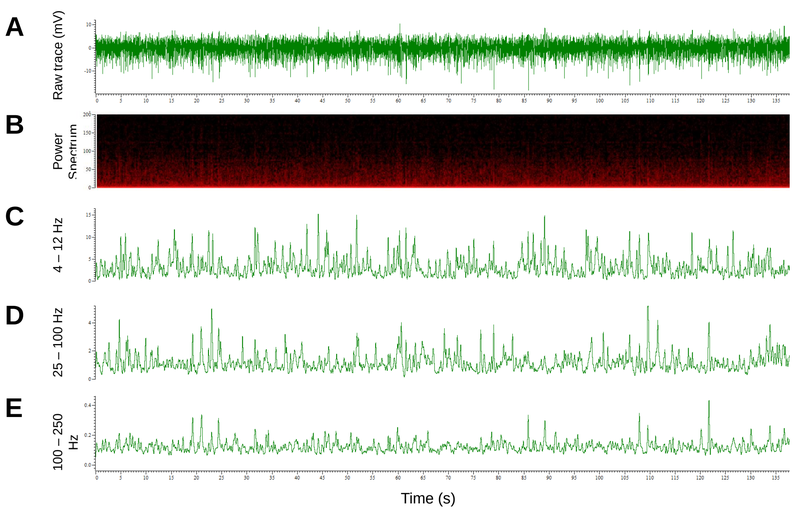




Mice are standing over a floating arena, which is moved by animal's free will, allowing them to freely explore the environment. The mice are in the same location related with an external observer, while the internal subject (the mouse) has the perception of being moving around the arena.

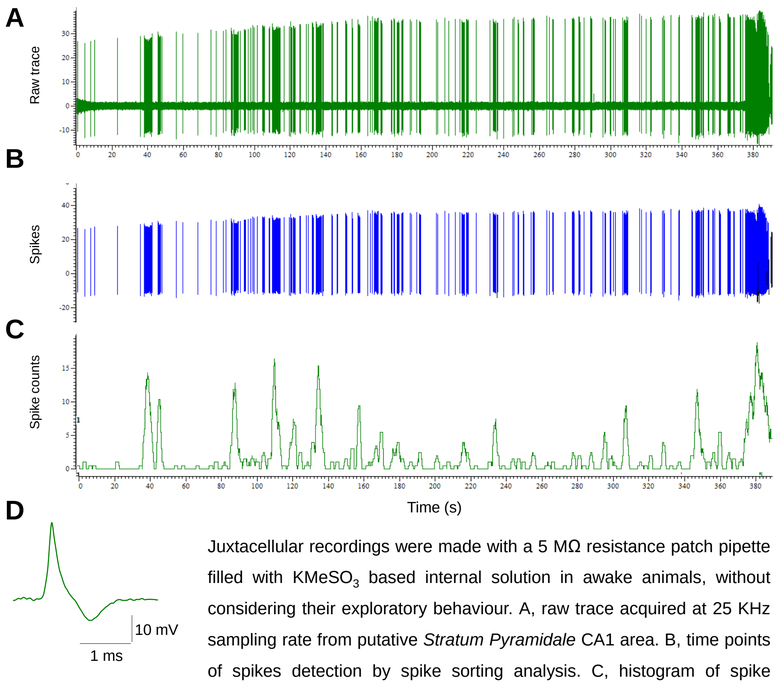
Conclusions

Extracellular recordings



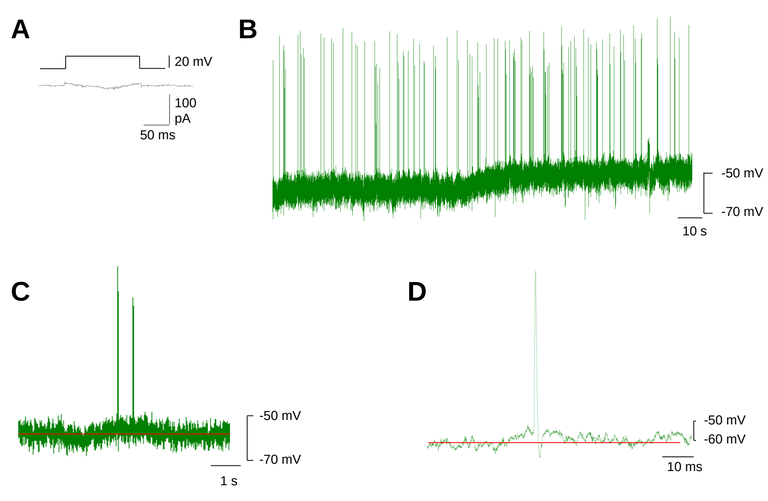
Extracellular recordings were made with a 5 MΩ resistance patch pipette filled with $KMeSO_3$ based internal solution in awake animals, without considering their exploratory behaviour. A, raw data recordings acquired at 25 KHz sample rate from putative hippocampal CA1 *Stratum Radiatum*. B, LFP spectral profile is shown in a spectrogram ranging 2-250 Hz. C-E, power calculation for frequencies range 4-12 Hz, 25-100 Hz and 100-250 Hz, respectively.

Juxtacellular recordings



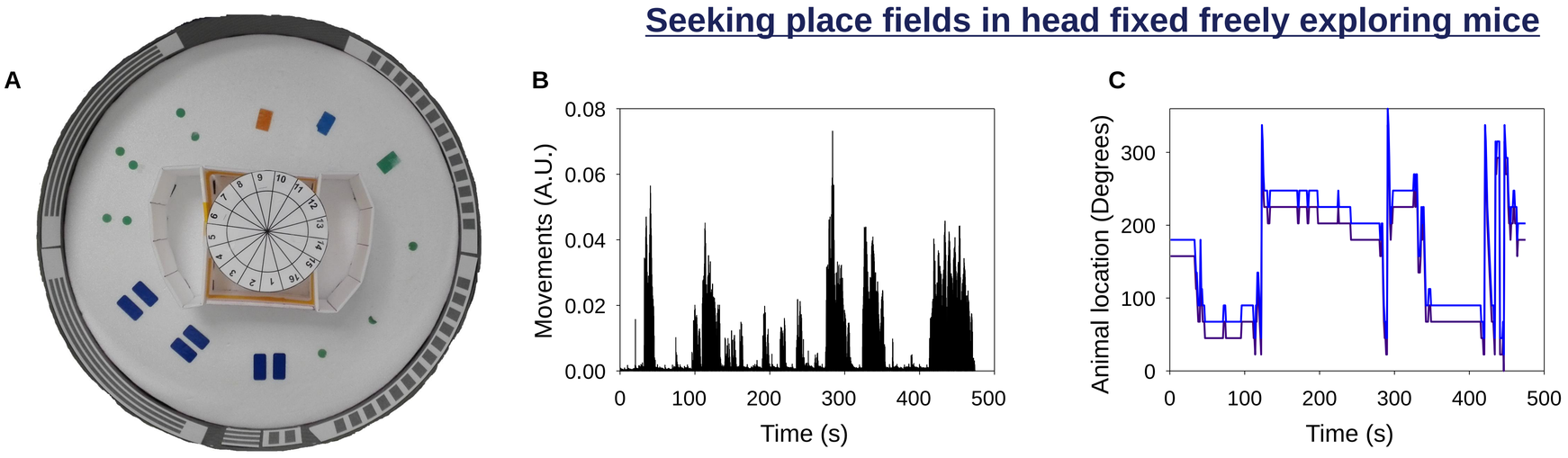
Juxtacellular recordings were made with a 5 MΩ resistance patch pipette filled with $KMeSO_3$ based internal solution in awake animals, without considering their exploratory behaviour. A, raw trace acquired at 25 KHz sampling rate from putative *Stratum Pyramidale* CA1 area. B, time points of spikes detection by spike sorting analysis. C, histogram of spike counts. D, average waveform of detected spikes.

Whole-Cell recordings



Whole cell recordings were made with a 5 MΩ resistance patch pipette filled with $KMeSO_3$ based internal solution, without considering their exploratory behaviour. A, Putative CA1 pyramidal cell was recorded with a series and input resistance of 61 ΩM and 159 ΩM resistance respectively. B, raw trace recorded at 25 KHz sampling rate showing a wealth of action potentials. C and D, magnification of action potentials where it is perceived a slight depolarization prior to action potential appearance, presumably by an increase of excitatory inputs.

Seeking place fields in head fixed freely exploring mice



Place cells recording preliminary experiments were made in a modified arena where a toroid like path is set to increase mice exploratory path length. A, picture of used arena decorated with visual cues on the walls and floor. On top of the cage a circle divided in sections helps to track animal position. B, plot indicating movement periods of a mouse; the plot quantifies the pixel decorrelation over time, where an increase indicates movement. C, plot indicating mouse position over the arena, where this has been divided in sections and quantified the angular degree of the position over time.