Webinar Q&A Report:

Single-Cell Electrophysiology and 2-Photon Imaging in Awake Mice with 2D-Locomotion Tracking

1. How long does it take to train a mouse to run within the Mobile HomeCage? Are there any critical factors which should be taken into account?

(N, Hájos): First, we habituate mice for 3 days in the Mobile HomeCage, usually twice a day for 10 minutes. Then we start the training, which usually takes 7–10 days. We find that mice learn faster if their whiskers can occasionally touch the wall of the Mobile HomeCage platform, so we think the mouse's ability to touch the wall with its whiskers is a critical factor.

2. How well did head-fixed mice learn in the Mobile HomeCage compared to either freely moving animals or other head-fixed setups?

(S, Stuart): For the task that I showed in my talk, where a mouse had to go to a specific target location and wait in order to get the reward, it took about 2 weeks of training to get them to do that in the MHC. In a freely moving animal, I would expect them to be able to do that in just a few sessions.

I think that's typical with any head-fixed setup, it takes longer for them to learn than you would expect in a freely moving animal. In a lot of the tasks that I've been running, I tend to follow similar training protocols as those that have been used in virtual reality systems, and in some cases, mice learn in fewer sessions by comparison. I think a key factor is that in the Mobile HomeCage, mice can rely both on the tactile as well as the visual cues in their environment.

One thing that we're looking into now is whether training animals in a task before any surgery or head fixation translates well enough such that mice require fewer training sessions in head fixation to be able to perform the same task in the Mobile HomeCage, which should help with training.

3. Can the Mobile HomeCage be used to study rats?

(J, Palacios): Due to the actual size of the MHC, it is suitable for rodents which weigh between 20 and 100 g. Below 20 g the animals wouldn't have enough strength to move the platform or would get exhausted easily. Above 100 g the platform would float with difficulty, preventing natural movement.

(S, Stuart): In addition, adult rats would require much greater air flow to float the maze underneath them, which then might contribute to unsuitable noise levels... also adult rats would not be suitable for the kind of headplate fixation that is used for the MHC (they're too strong and would probably result in headplate detachment).

4. How are behaviors such as grooming, sniffing and sleep affected in the Mobile HomeCage?

(S, Stuart): With sniffing, the angled clamp that we use provides a huge improvement in terms of allowing the animal to sniff and whisk the environment as it would if it would naturally. While head-fixed, mice are able to carry out face grooming, but because of the head fixation clamp they are unable to do a full body groom while they're in the Mobile HomeCage. What we've noticed is that on most occasions, mice will carry out a full body groom as soon as they're released from the clamp.

We haven't looked at sleep specifically in the MHC – all of our recording sessions are carried out during their active phase. For anyone wanting to look at sleep, an important consideration would be the rate of air flow underneath the cage. You would want the MHC platform to float easily enough so the mouse can run with minimal effort, but also stable enough underneath them so when they're not moving it's not wobbling about.

5. How long can you keep a whole cell recording while the animal is actively moving? And in head-fixed, behaving animals, are motion artefacts an issue?

(J, Palacios-Filardo): The length of the recording depends on several factors. First, you have the stability of the clamp, of course, which is quite good. However, stability also depends on the size of the craniotomy, since you might see brain movements due to breathing and heart pulsing. This can be minimized if you use a solid agar plug to stabilize the brain and the recording electrode. Secondly, stability will also depend on how much the mouse is moving and if those movements occur smoothly. This is why it is always recommended to train mice beforehand to avoid abrupt movements. Overall, I would say that a good 10 minutes can be expected from a trained mouse. But this is very variable, it might range from 5 to 20 minutes.

Regarding the motion artefacts, I would recommend having a very good reference electrode which is stable upon movement. In addition, if the mouse touches any metal lever or electricity conductive metal you might expect to see a small artefact. These artefacts are easy to remove because they have a very characteristic electrophysiological signature – unlike action potentials and other electrophysiological signals, they have similar amplitude in both direction of the recording trace.

6. What is the average time for recording single-unit activity with juxtacellular electrodes in head-fixed, behaving mice?

(N, Hájos): We found that the average time for a juxtacellular recording is about 10-15 minutes and depends on many factors like the size and the quality of the craniotomy and the tip of the glass pipette. In some occasions we have recorded neural spiking for more than 20 minutes while the mouse was grooming,

running and receiving external air puffs. So, if everything goes well, a significant amount of data can be collected during various behaviors.

7. How much can the MHC and tracking system be customized to make it suitable to reproduce a more complex environment?

(J, Palacios-Filardo): Up to now MHC is limited to circular mazes, but Neurotar can assist with a custom design. Regarding the tracking system, the MHC has 4 digital TTL pulses that are triggered when the animal stays in a certain zone of the arena. Then it is up to the user to play with those output pulses to trigger more elaborated responses such as sound cues, visual cues, reward or punishment, etc.

8. How long does the head-fixation procedure take, how quickly does a mouse adapt to feel unstressed in such apparatus after fixation and how many days of pre-training are necessary to avoid this stress?

(W, Sun): Following cranial surgery and recovery, the head fixation procedure itself is quite easy and can be done within one minute. Since no behavioral task is involved in our study and we did acute imaging just one day after operation, we did not perform any pre-training, but just had 3 sessions of habituation before recording. Each habituation session was 10 minutes and we did not observe animals to be stressed after the habituation.

9. Can you specify how image analysis was performed to quantify morphology of microglia and velocity of microglia approach to a photodamage site?

(W, Sun): In order to quantify the morphology of microglia in the surveilling state, we traced the processes using the Fiji plug-in Simple Neurite Tracer and further confirmed by manual check. The same cells were traced twice at time 0 and in 30 min to quantify the cell motility.
Regarding the damage-directed state, the so-called Sholl analysis—also implemented in Fiji—was used to calculate the velocity.

10. How easily can you expect to find CA1 pyramidal neurons with place cell activity?

(J, Palacios-Filardo): From our few single-cell recordings (and I say few because all together they are not more than 20 recordings), we found a wealth of neurons that showed a location preference to spike. However, we are planning to do acute recordings with silicon probes where we expect to record between 100 and 200 units per experiment and have a more definitive result.

11. How would you record peripheral nerves in awake rodents?

(J, Palacios): The MHC allows stability around the skull to access the brain, which allows for single cell electrophysiology or imaging across time. You could study peripheral nerves doing a chronic surgery to implant some kind of recording electrode or miniscope in the desired area. The MHC would not help much here, unless you could fix a region of the rodent, which may work for the spinal cord with some

modifications in the actual design. Not to mess around with cables, going wireless would be the best option.

12. How long do you wait after insertion of a silicon probe into the amygdala region before the recording? What is the stability of units on an acutely inserted probe?

(N, Hájos): After the insertion of a silicon probe, we wait about 30 minutes and often an hour. We have found that stabilization of the signal takes at least half an hour, so for that long you can't really record stable units. But if we are losing units, we can move the probe slowly deeper to search for and to find new units to record distinct neuronal population activity.

13. What are the molecular pathways by which anesthetics may affect microglia surveilling and damage-directed motility?

(A, Dityatev): Recently, Madry and colleagues have demonstrated that isoflurane and related gaseous anesthetics strongly suppressed the motility of microglial processes in acute brain slices. This suppression was mediated by anesthetics' blocking effect on TWIK-related Halothane-Inhibited K+ channel. Other mechanisms may involve modulation of NMDA-type glutamate receptors, TLR4 pathway, GABAA receptors, and isoflurane-induced elevation in TrkB signaling. Ketamine may potentially act via inhibition of NMDA receptors, as well as via BDNF, TLR3 and NO.

14. Would it be possible to visualize the neural circuitry activation in the spinal cord or sub-cortical nucleus during manual task behavior? What kind of experimental model (i.e. transgenic mice) would be most suitable for this?

(L, Khiroug): Yes, awake mice implanted with either a cranial window or a spinal cord window can be studied in the Mobile HomeCage while they are performing manual tasks such as reaching for food and grasping it. Unfortunately, I do not have a first-hand experience that would be required for recommending a specific mouse model for this.

15. Any comments on the quality and efficacy of 2-photon opsin stimulation in awake mice?

(L, Khiroug): In my opinion, two-photon activation of opsins may be more challenging in awake mice compared to anesthetized ones, because activity-dependent brain tissue displacements within a firmly immobilized skull are more pronounced during locomotion.

16. What is the best angle of approach for a silicon probe when combined with a 2-photon setup? How does one best to position the probe within the field of view?

(L, Khiroug): In my experience, the angles around 30-45 degrees work best for combining imaging with ephys and for visually guided insertion of a probe (either glass electrode or silicone probe) under a two-photon microscope water-immersion objective.

If you have additional questions for Neurotar regarding content from this webinar or wish to receive additional information about the Mobile HomeCage, please contact them by phone or email:

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