Results:

The fact that place cell sequence reactivation or replays are a manifestation of a previous experience dependent internal representation, makes them a natural potential substrate for a memory based BMI. Thus, as a first step towards establishing a memory based BMI, I wanted to test the idea that replays could be detected with Ca2+ imaging in freely behaving mice. I therefore examined the patterns of neuronal activity that emerge during a spatial exploration task in which thirsty mice are trained to run back and forth along a linear track to collect water rewards at the ends of the track (Figure 1).For this analysis I used previously published data (Rubin & Geva et al., 2015) and some unpublished data that was collected by Nitzan Geva from the lab. The data is from an experiment in which mice repeatedly explored one or two familiar environments (Figure 1) while their hippocampal CA1 pyramidal cells were imaged using miniaturized fluorescence miroscopes (Rubin et al., 2015; Ziv et al., 2013)(Figure 1). Each session consisted of five to eight 3-min trials in one environment, and one 3-min trial in which mice were resting in a bucket (standard glass-sharps biohazard bin) before and after the session. To maximize the perceived differences between the environments, Geva et al constructed linear tracks (environments A and B), at a length of 96 cm that differed in shape, floor texture, surrounding proximal and distal visual cues, odor, and flavor of the water reward at the edges of the track. The bucket trials didn’t contain any reward. In the unpublished data mice were imaged only environment A, but the structure of the session was the same as in the published data. The imaging data was processed using commercial software (Mosaic, version 1.1.1b, Inscopix) and custom MATLAB routines into vectors of activity for each neuron, which specify the peak of every calcium event, and the location and velocity of the mice on the track in every frame.

During wakefulness, replays are detected mostly during idle times within the examined environment, or in another environment that the rodent visited shortly before or after the examined experience (Diba and Buzsáki, 2007; dragoi, george, tonegawa, 2011; Pfeiffer and Foster, 2013; Singer et al., 2013). I therefore focused my analysis on neuronal activity that took place during non-running epochs at the ends of linear track, close to the time in which the mice got their water reward, and on neuronal activity that took place during the bucket trails at the start and end of each session.

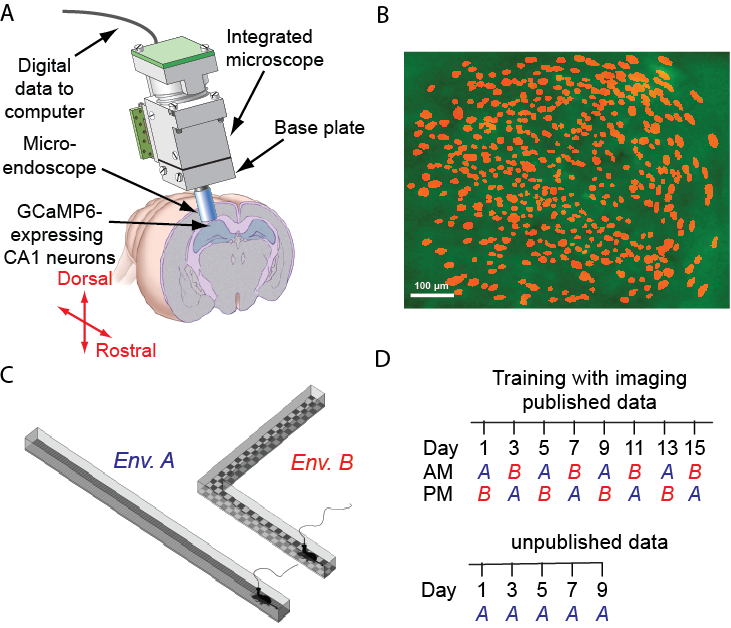


Figure 1: Experimental setup

1. A tiny microscope equipped with a microendoscope images cells expressing GCaMP3. The microscope’s base is fixed to the skull, for repeated imaging of the same cells
2. Shown are cells (red) identified by Ca2+ imaging in a behaving mouse, atop a mean fluorescence image (green) of CA1.
3. Environments A and B on which Mice trained to run back and forth and collect a liquid reward in. Before and after each session they rest inside a bucket with no reward.
4. Experiment timeline for the published data set (top) and unpublished data set (bottom)

To what extent does the activity at the edges of the track represent the activity during running epochs? To address this question, I divided each trial to segments of run and rest epochs, according to the position along the track. For consistency, I labeled the frames in which the mice were up to 16 cm away from each of the edges of the track as rest epochs, and the rest of the frames as run epochs. I then analyzed separately the epochs in which the mouse was at the edges with respect to either the running epoch that came immediately before or the one that came immediately after that rest epoch. Then, I calculated for each neuron the conditional probability to be active at the rest epoch given the activity during the run epoch (active\not active) for each 15-minute session. To test the difference between the two conditional probabilities, I conducted a matched T-test for each session, and calculated the effect size of the difference between them. As seen in Figure 2‎C and ‎E, for most of the sessions the activity during run epoch was not significantly associated with the activity during rest epoch before\after the run. Also, the effect size is smaller than 0.4 SD for both cases (Figure 2‎D, F) (environment A: n=X out of Y sessions from 9 mice. Environment B: n=X out of Y sessions from 4 mice). This analysis suggest that the activity at the edge is mainly unique to those bins, and may be related to the reward itself or to the representation of the edges of the track, rather than reflecting a forward or reverse replay activity.

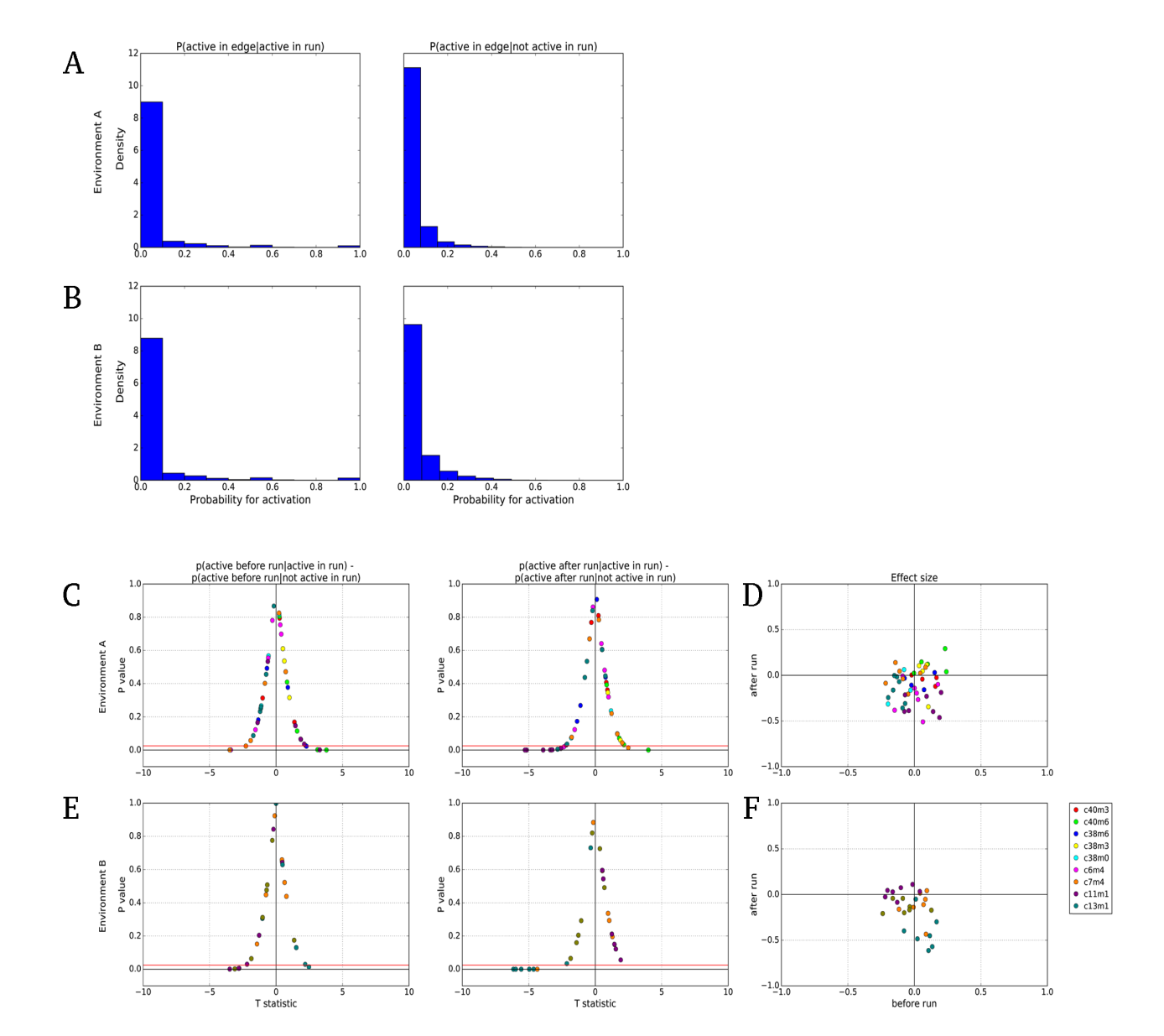


Figure 2: No significant activation in rest epochs given activation during run epochs

1. Environment A density of *P(cell active in rest | cell active in run)* (right) and *P(cell active in rest | cell* ***not*** *active in run)* (left) taken from n=48 sessions, from 9 mice
2. Same as A) for environment B taken from n=28 sessions, from 4 mice
3. Scatter plot of a matched t-test for the conditional probabilities:

*P(cell active in rest | cell active in run)* - *P(cell active in rest | cell* ***not*** *active in run)*

when rest can be before run(right), and after (left) in a linear track. Dots are the different color for each mouse. Red line is p=0.025 for two tailed matched t-test. Most sessions show no significant difference between the two conditional probabilities

1. Scatter plot of effect size of the difference between the conditional probabilities in C). X axis is for effect size of the difference:

*P(cell active rest* ***before*** *run | cell active in run)* - *P(cell active in rest* ***before*** *run | cell* ***not*** *active in run)*

Y axis is for effect size of the difference:

*P(cell active rest* ***after*** *run | cell active in run)* - *P(cell active in rest* ***after*** *run | cell* ***not*** *active in run)*

1. + F) same as C)+ D) respectively, for L-shape track.

Another approach to investigate the neuronal activity at rest epochs is based on synchronous calcium events (SCE), as described in previous calcium imaging experiments on head fixed mice using two photon microscopy (Malvache et al., 2016). These SCEs are significant peaks of synchronous neuronal activity during immobility periods. Malavache et al found that sequences that appeared when mice were running on a cue-less treadmill were reactivated during SCEs. I wanted to find whether SCEs in one photon data from freely behaving mice show the same pattern of activation. At each rest epoch I counted the number of events in a sliding time window of 200 ms. Time windows that had number of events above chance level (which I calculated for each session separately by shuffeling the events of each neuron in time) were labeled as SCE. I then looked for the shared neurons that were active both in SCE and the following run (Figure 3‎G, H as positive example) and found that each SCE had only few to none of these (Figure 3‎C for environment A and 3‎F for environment B). This further suggested that the majority of activity seen at the edges don’t carry information about the run epochs, and may be informative about the reward or the reward location. The reasons we don’t see higher overlap between cells that were active in SCEs and run epoch, in contrary to what was reported by Malvache et al. (2016), might be due to several differences between the experiments; their experiment was done on head fixed mice running on a dark treadmill, with no cues on it, and no reward. The ordered replays they report on, don’t accually represent place replay but rather sequential neuronal activation integrating traveled distance (Villette et al., 2015). In addition, two-photon microscopy has higher signal to noise ratio (SNR), which might lead to some changes in event detection.

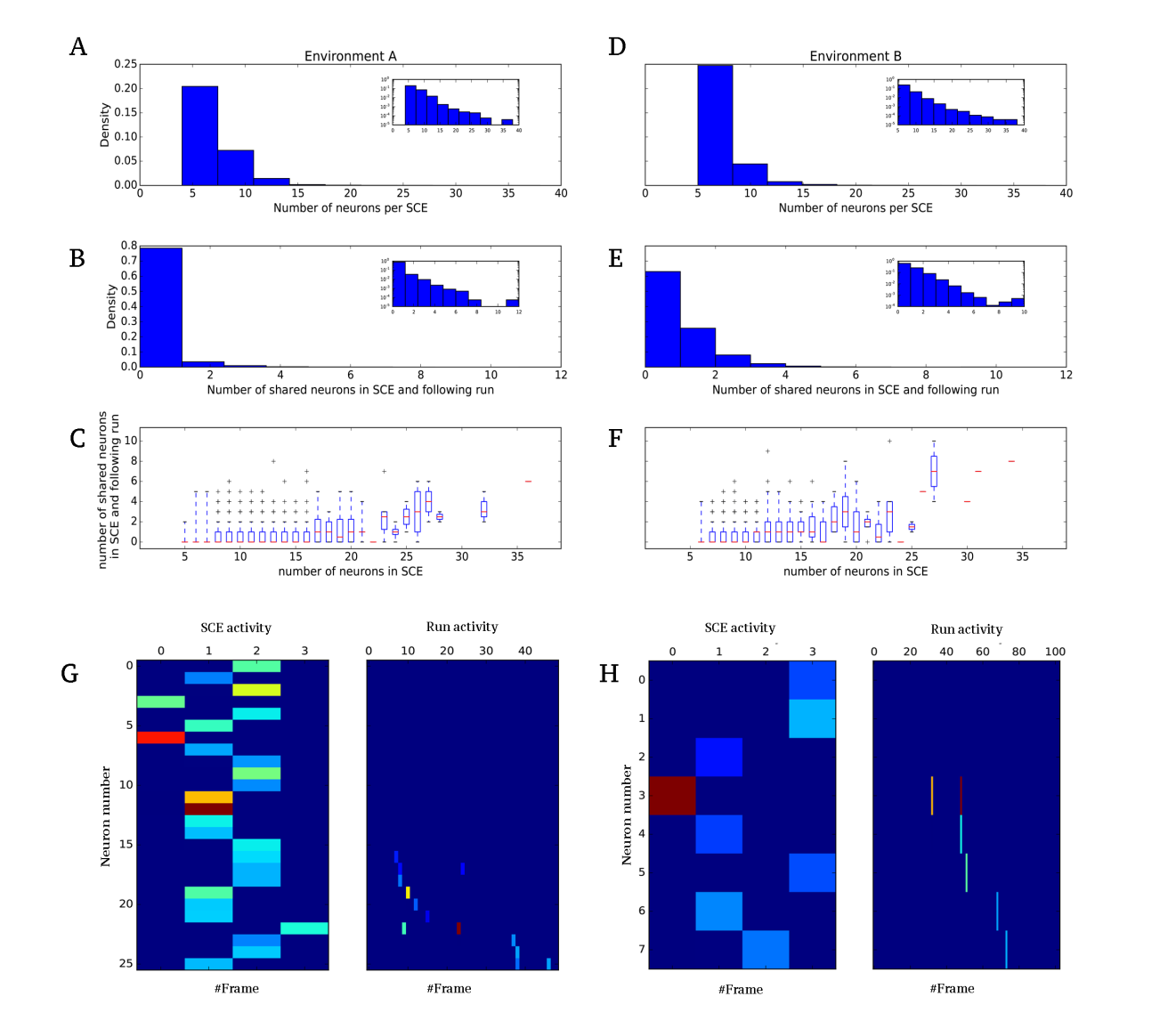


Figure 3: Neurons that participate in synchronous calcium events before running are unlikely to be activated in upcoming run epoch

1. Distribution of number of neurons per synchronous calcium event (SCE) in environment A, calculated for all neurons (not only place cells). Data pooled from n=9 mice running on a linear track. Inset show the same in log scale on y axis.
2. Distribution of number of neurons that participated in SCE and in the following run. Inset show the same in log scale on y axis.
3. Box plot of the number of neurons that were active in SCE and in the run epoch that followed.
4. Same as A) for environment B
5. Same as B) for environment B
6. Same as C) for environment B
7. +H) Examples of SCE and the following run activity. Color is the amplitude of the peak of the calcium event. In both examples, some of the neurons active in the SCE participate in the following run epoch

Next, I asked to determine if it is possible to detect patterns of activity that are associated with the linear track during off-context rest epochs in the buckets. I hypothesized that activity patterns that are associated with edges of the track – where the reward is given and where mice spend most of their time – will be more prevalent off-context than patterns that are associated with other, less salient and less occupied parts of the linear track. If this was the case, it could prove useful for a memory-based BMI, in which the mice could be trained to re-activate the representation of the reward (or reward location) in order to obtain water reward off-context. For this analysis I used a memory-less maximum likelihood estimation decoder. First, the location of the track was binned into twelve 8-cm bins, and the activity of place cells from all trials in both environments on the same day, was used to calculate the [joint probability function](https://en.wikipedia.org/wiki/Probability_density_function#Densities_associated_with_multiple_variables) for all bins observations, assuming that the neurons’ activity is independent:



Where  is the activity of the i'th neuron, either 1 for active neuron or 0 for not active. Is the bin number, which varies from 0 to 11, and  is the environment type; A or B. Then, for each frame in the test trial, I maximize the log-likelihood function to estimate the bin and environment of the activity vector:



Figure 3A,B shows the performance of the decoder on the linear tracks, where on each session one trial was left out from the calculation of the joint probability function and was tested later. To estimate chance level performance of the decoder, I shuffled1000 times the neurons identity. in all mice and on all trials the decoder’s performance was better than chance level.

Having established the decoder’s ability to accurately estimate position from neuronal activity during running epochs, I then tested the decoder’s performance on the bucket trials, which took place immediately before or after visits to either of the environments. To what extent is neuronal activity during the bucket trial informative about the identity of the environment in which the mice visited? I calculated the fraction of frames that were correctly decoded as “belonging” to the environment the mice visited before or after the bucket trail, and found it was significantly higher than chance level in both environments (Fig 3C). I also calculated the distribution of the decoded bins in the bucket, and compared it to the distribution of the mouse occupancy within these bins on the linear tracks. The distributions of the decoded bins and the mouse occupancy were similar (fig 3E-F). Surprisingly, the fraction of frames decoded as edge bins did not exceed the fraction expected from accounting for occupancy. However, the prevalence of frames that were decoded as edges suggests that it could still be useful for training a mouse to reactivate (off-context) neuronal activity patterns that are associated with the edges of the track in order to obtain water reward via the closed loop BMI. Furthermore, the frames that were estimated as edge bins were frames with mean positive activity of one neuron in them (Fig 3C-D). This sparse activity suggest that I could give up the use of maximum likelihood estimation for real-time decoding in the closed loop system, and instead use much simpler approach to save feedback time.

With this information, as a first step, I conducted a pilot experiment with Or Pinchasof and with the help of Nitzan Geva from the lab. We used two mice (40-3 and 40-6), which participated in the linear track experiment (which I will hereafter refer to as “phase 0”). In the pilot, we used python based custom program (See methods), to track in real time the neuronal activity in the hippocampus CA1 of the mice while they were running on the linear track. Prior to the pilot experiment (which I will hereafter refer to as “phase 1”), we manually chose a sub-population of cells that showed over ninety percent of her activation at the edges of the track in a pre-training session (see methods). In phase 1, mice would receive water at the edges of the linear track only upon activation of at least two out of the pre-chosen cells in a time window of X ms (n=26 cells for mouse 3 and n=47 for mouse 6). each session on the linear track was consisted of five 3-minute trials with 3-minute trials of bucket before and after the session. the mice ran on the track for five sessions, every other day, as in phase 0 (see Figure 1‎D, bottom panel). In most of the sessions (with exception of session no. 3, see figure), mouse no. 6 got the reward almost every time he reached the edge, while mouse no. 3 got about a reward about half of the times. The amount of reward across the sessions didn’t significantly change. We further wanted to examine what this conditioning did at network dynamics level. Did the cells that represent the edge, where the mice get the reward, changed over time in a different matter than the rest of the cells? To answer this, I divided the cells on each session into two groups; edge cells, which showed activity at edge bins (up to 10 cm from each edge) in more than ninety percent of their detectable events, and the rest of the cells, which I’ll call non-edge cells. For each phase I had five groups of edge and non-edge cells, each was chosen by its activity on a different session. In addition, I also looked at the dynamics of the chosen cells in phase 1 for reference. The dynamics was estimated on each of the groups separately, by three tests that were calculated on the events from the linear track trials, and two tests on the events from the bucket trials. We examine the results of the dynamics by looking whether the difference in dynamics between phase 0 and 1 is different for edge and non-edge cells.

The first test is the recurrence test; for each two sessions, I calculated the fraction of cells in a certain group that was active on both of them, with a minimum of five events. Then I averaged for all the couples with similar distance between them and between the edge and non-edge cells. This test allows us to examine the stability of a certain group across days – the higher the recurrence, the stable are the cells in that group. It was done both on the linear track (Figure …), and on the bucket trials. (Figure ..). For the bucket trials we see that the difference between the change in time of the recurrence of the edge and non-edge cells isn’t different between the phases for both mice. However for the track trials we can see that mouse no. 3 show a bit smaller difference between the recurrence of the edge and non-edge cells than its parallel in phase 0. We see similar, yet smaller effect for mouse no.6 in addition for higher recurrence probability for all population of cells in phase 1 compared to phase 0. The second test was ensemble correlation test. In this test I correlate for all pairs of sessions their activity vector, which contains the number of events each cell in the group had in a session. Then I average over couples of sessions with similar distance between them and between the edge and non-edge cells. This test allows us to look on the activity patterns, and the relations between the cells’ activity across days. This test was also done on both the linear track (Figure …) and the bucket trials (Figure …). We see no noticeable difference between edge and non-edge cells in both mice. The only difference is for mouse no. 6 who has higher ensemble correlation in phase 1 compared to phase 0 both in bucket and linear track .The third test is the population vector (PV) test. Now the activity vector was taken for each of the 9.6 cm bins separately. For each pair of sessions, the correlation was calculated between each bins’ activity vector and then averaged on all bins to get the PV correlation between a pair of sessions. This test gives the additional information about how much is the activity pattern on each bins is reserved across days. Then I average over couples of sessions with similar distance between them and between the edge and non-edge cells. This test was done on the linear track activity only (Figure …). Also here, we see no difference between the phases for mouse no. 3. Mouse no.6 show a bit higher PV correlation for non-edge cells compared to edge cells in phase 1 compared to phase 0, and overall higher PV in phase 1.

Another approach to examine the effect of the sub-population conditioning is to look at the density of the decoded bins in the bucket trials, similar to the analysis showed in figure 3E, now for each of the mice separately (figure …). The density of decoded bins in the bucket of mouse 3 show higher representation of the edge bins in phase 1 compared to phase 0. Mouse 6 doesn’t show any clear trend.

We next wanted to examine, as a proof of concept, whether the mice could activate the same set of chosen cells in a different environment, to get the same water reward. For this, we added to the bucket from phase 0 and 1, a pipe of water (see figure…), and conducted an experiment (phase 2) for 5 consecutive days, in which the mice had to activate at least two of the same chosen cells from phase 1 in a time window in order to get a water reward in the upgraded bucket. Upon receiving, a delay of 5 seconds was taken before they could get the reward again, even there was activation. Each day had one session that was consisted of five 3-minute trails in the upgraded bucket and linear trials before and after the session. The performance of the mice is shown in figure …. Mouse no. 3 shows stable amount of water rewards across the sessions, whereas mouse no. 6 shows a decrease in session no. 3 and is back to baseline in the next session. I conducted the same dynamics tests as in phase 1 for this experiment (figure …). The only test that shows a difference between the populations is the recurrence test, in which the edge cells have higher recurrence than the non-edge, for both mice.

Over all, there is no conclusive conclusion about the effect of the sub-population conditioning. This is due to several reasons; the amount of chosen cells was different between the mice, which led to a big difference in the amount of water reward the mice got. This to itself, might explain the results, since both of the mice were pre-trained on the linear track to receive water reward in both of the sides (phase 0), yet only mouse no. 6 continued to get it in most of the times in phase 1. This means that we can also interpret the results as a result of partial reinforcement for mouse no.3. In addition to this, the amount of water that the mice got in the upgraded bucket of phase 2 also differs, and we don’t have any control for the situation of dynamics over time in a bucket with similar amounts of reward.