Results:

As a first step, I wanted to closely examine the patterns of neuronal activity that emerge when the mice get reward, during a simple task that combines (?) the use in place cells, as a marker for spatial memory. For this I used previously published data (Rubin & Geva et al., 2015) and some unpublished data that was collected by Nitzan Geva from the lab, which imaged, using previously described calcium imaging routine (Rubin et al., 2015; Ziv et al., 2013), hippocampal CA1 pyramidal cells in freely behaving mice that repeatedly explored one or two familiar environments (Figure 1). Each session consisted of five to eight 3-min trials in one environment, and one 3-min bucket trial 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. The unpublished data has the same structure per session, but contains only environment A. The imaging data was processed using commercial software (Mosaic, version 1.1.1b,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.

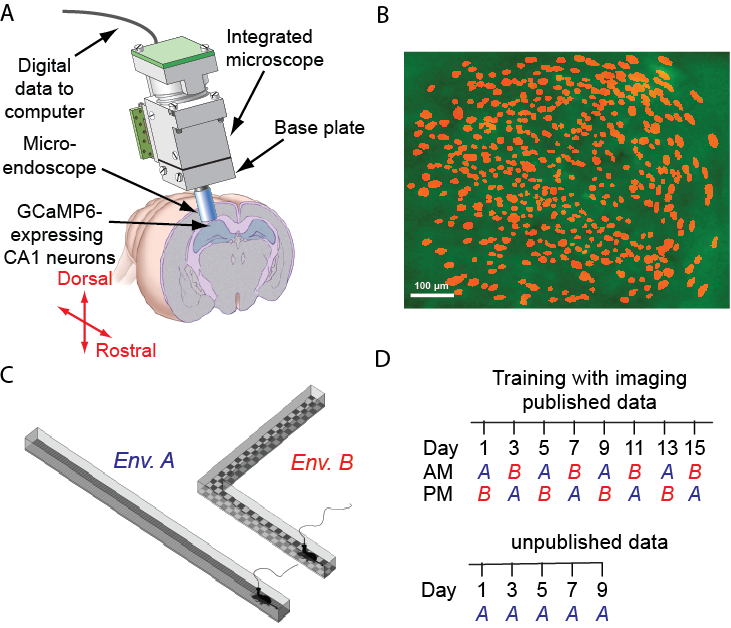


Figure : Experimental setup

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 Experiment timeline for the published data set (top) and unpublished data set (bottom)

In order to find a possible pattern of neuronal activation, to be used as a feedback for the memory based BMI, I looked at the neuronal activity at rest epochs, where the mice get a reward .previous work has shown that temporal spike sequences from hippocampal place cells recurred at rest epochs in reverse or forward order(Diba and Buzsáki, 2007; Pfeiffer and Foster, 2013). Inspired by that work, I wanted to see; to what extent the activity at the edges represents the activity during the run epochs. To do so, I divided each trial to segments of run and rest epochs, according to the mice place on the track; the frames on which the mice were up to 16 cm far from each of the edges of the track were defined as rest epochs, while rest of the frames were defined 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 before or the one that came after the rest epoch. Then I calculated for each neuron the conditional probability to be active at the rest epoch given the activity in 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) VFV J. 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.

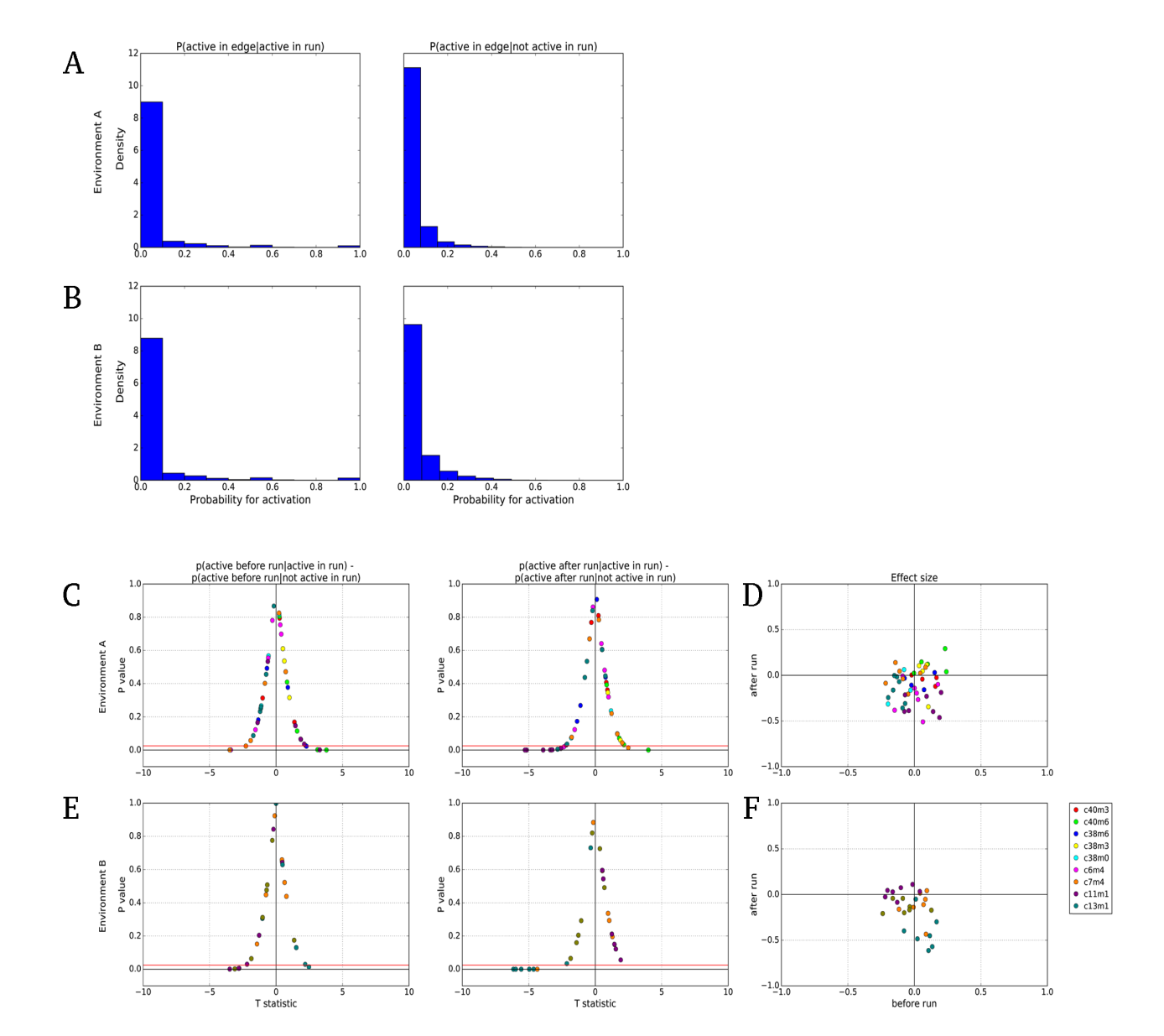


Figure : No significant activation in rest epochs given activation during run epochs

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 at a length of 200 ms. Time windows that had number of events above chance level (which I calculated by shuffling in time the activity for each session separately) 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.

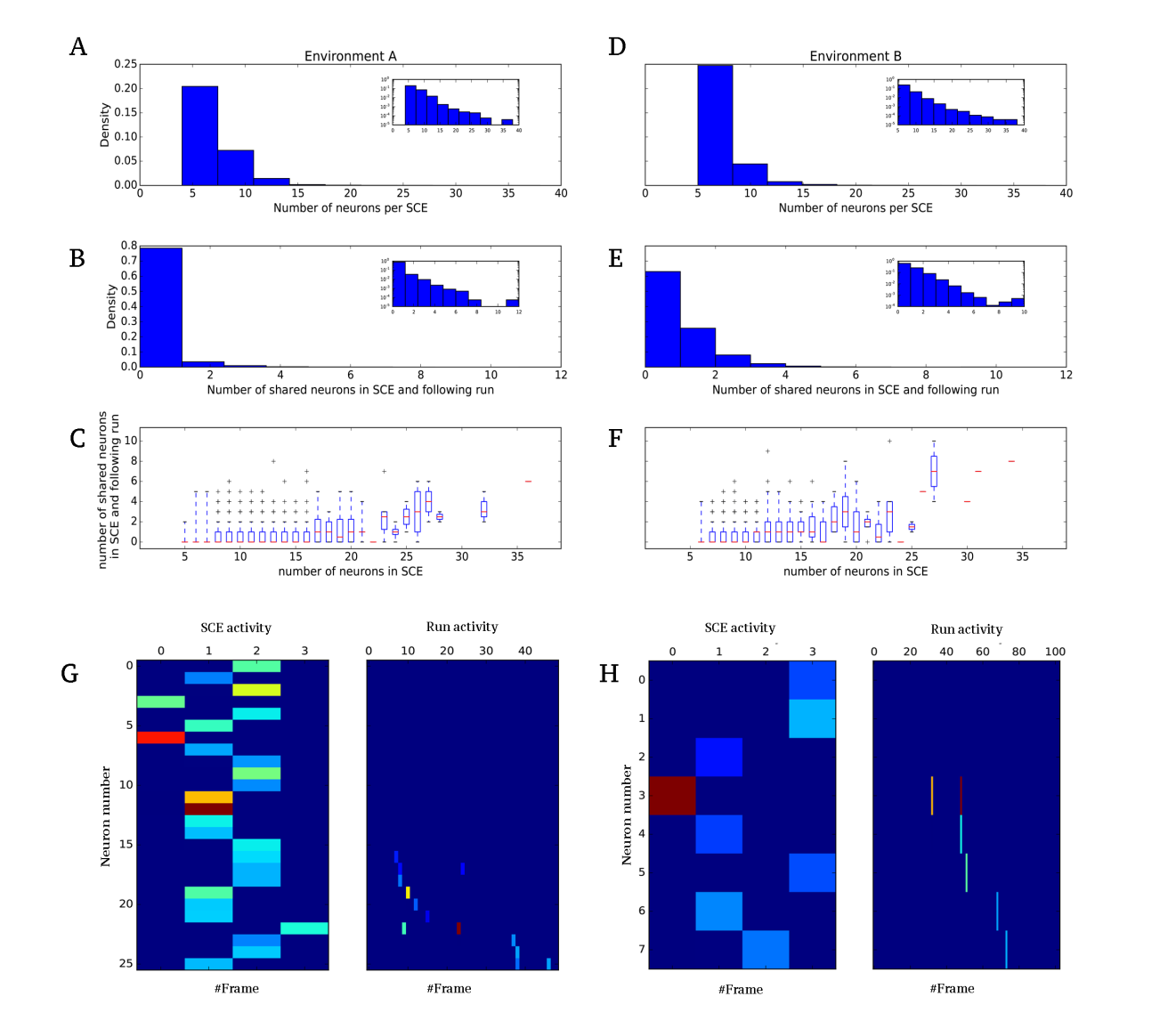


Figure : Neurons that participate in synchronous calcium events before running are unlikely to be activated in upcoming run epoch

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

The next step was to see if I can detect this edge activity on another environment which isn’t linked with the reward (off-context), in purpose of condition this activity with reward given by the memory based BMI. This could further prove that the BMI is indeed based on memory representation of the reward, since the mice would activate it off-context to get it. For this I used a memory-less maximum likelihood estimation decoder. First, the location of the track was binned into 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:



One can see in figure 3A,B 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.

I then tested the decoder on the bucket trials, which proximate to either of the environments. To see to what extent the bucket trial’s activity tells us about the environment it is proximate to, I calculated the fraction of frames which their estimation of environment matched to the proximate one (Fig 3C), it was significantly higher then chance level in both environments. In addition I calculated the distribution of the estimated bins in the bucket, compared to the natural distribution of their occupancy on the linear tracks (fig 3E-F). I found that the in both cases, one can see similar distribution of the bins in both environments. This means that I can condition this representation off-context with water reward given by the memory based BMI. Furthermore, the frames that were estimated as edge bins were frames with mean positive activity of one neuron in them (Fig 3C-D). I could therefore give up the use of maximum likelihood estimation on real-time decoding, and use much simpler approach to save feedback time.

With this information, as a first step, I conducted a pilot with Or Pinchasof and the help of Nitzan Geva from the lab. We used mice number 3 and 6 from cage 40, which participated in the linear track experiment of the unpublished data, from now on, we call this experiment “phase 0”. In the pilot, we used python based custom program (See methods), to track the online neuronal activity of the mice while they were running on the linear track. When they reached the edges, we automatically gave them water, only upon activation of at list two cells out of pre-chosen population. We chose manually the pre-chosen cells, by analyzing the activity of the entire population of cells that were active 3 days before the pilot started on a regular session on the linear track (five times of 3-minute trials with 3-minute trials of bucket before and after the session). Cells that approximately ninety percent of their calcium events were at the edges (n=26 for mouse 3 and n=47 for mouse 6). The mice ran on the track for five sessions, every other day, as in phase 0 (see Figure 1‎D, bottom panel). Mouse no. 6 got the reward almost every time he reached the edge (add figure) while mouse no. 3 got about a reward about half of the times. There was no significant change in the amount of reward across the sessions. Since there was no behavioral report for learning the conditioning (e.g rise in number of activations of the chosen cells across sessions), we further wanted to examine what did this conditioning do 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. 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. 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 …). { Add conclusions about the test }. The second test was ensemble correlation test. In this test I correlate between sessions the 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 …) { Add conclusions about the test }. 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 two sessions, the correlation was calculted between each bins’ activity vector and then averaged on all bins to get the PV correlation between the two sessions. This test gives the additional information about how much is the activity pattern on each bins is reserved across days. For each reference group of edge/ non-edge cells, I calculated the PV correlation, only between its chronological sessions. Meaning, for a group of cells that was chosen by the activity on the third session, I calculated the PV correlation between these couples of session: 3&4, 4&5, 3&5. 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 …) { Add conclusions about the test }.