Supporting Online Material for

Fast-Forward Playback of Recent Memory Sequences in Prefrontal Cortex During Sleep

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SUPPORTING MATERIAL

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Methods

Subjects

Two male Brown Norway/Fisher 344 hybrid rats (7-9 months old at the time of surgery, 350-400 g) were housed in Plexiglas home cages, maintained on a reversed, 12:12 hr light-dark cycle. Training and recording sessions occurred during the dark portion of this cycle.

Apparatus

All behavior took place on a 1.3 m diameter circular arena, shown in Fig. S1A (1). Light emitting diodes (LEDs) were located at eight equally spaced locations around the perimeter. The LEDs were located 2 cm above the table surface and flashed at 2 Hz when lit.

The experiment was controlled by a microcontroller card and a standard PC computer. The computer also performed data acquisition. Custom software monitored the rat’s position and turned on lights, tones, and electrical brain stimulation...
as required. LEDs were driven directly from the microcontroller’s transistor-transistor logic (TTL) output. Electrical brain stimulation, delivered via a stimulus isolator, was also driven via microcontroller TTL output. Tones were delivered using the PC’s built-in audio controller. For every control event delivered by the microcontroller, a signal was delivered concurrently to the TTL input port on the recording apparatus (described below) for timestamping using the same clock used for neural and video data.

**Data Acquisition**

Neural recordings were obtained via a chronically implanted “hyperdrive” consisting of 12 independently manipulatable tetrodes. Each tetrode consisted of four, polyimide-coated, nichrome wires (diameter 14 μm) twisted together (2-4). Hyperdrive construction was as described in Gothard et al. (5) except that tetrodes were inserted into silica tubing (65 micron inner diameter, 125 micron outer diameter) for added rigidity, and secured with cyanoacrylate glue after insertion into the drive.

During recording sessions, the hyperdrive was connected to a unity-gain headstage that enabled low-noise transmission of neural data to the recording system. The headstage also contained an array of LED’s that could be detected by an overhead camera, enabling tracking of the position of the rat on the maze at 60 frames/sec. All data were recorded using a Neuralynx Cheetah recording system running in combination with the computer. Single unit data from each tetrode were amplified, filtered between 0.6 and 6 kHz and digitized at 32 kHz. Video spatial resolution was approximately 3 pixels/cm.
Surgery and Electrode Placement

NIH guidelines and IACUC approved protocols were followed for all surgical and behavioral procedures. Each rat was anesthetized with Isoflurane (1-1.5 % by volume in oxygen at a flow rate of 1.5 L/min), placed in a stereotaxic holder and injected with Penicillin G (30,000 units per hind leg i.m.). The skull was cleared of skin and fascia and craniotomies were opened for two stimulating electrodes targeting the Medial Forebrain Bundle, or MFB (rat 1:  - 3.25 mm AP, 1.65 mm ML bilaterally, 8.5 mm ventral from dura; rat 2:  -2.5 mm AP, 1.8 mm ML right, 8.5 mm ventral and -4.0 mm AP, 1.5 mm ML right, 8.2 mm ventral) and a hyperdrive. Each stimulating electrode consisted of two, Teflon-coated, stainless steel wires (coated diameter 0.0045") twisted together with approximately 0.5 mm of insulation removed from one tip. The hyperdrive was centered over the left mPFC at 3.0 (rat 1) or 2.9 (rat 2) mm AP, 1.3 mm ML, and angled at 9.5 degrees toward the midline. It should be noted that the bundle of cannulae holding the electrodes as they entered the brain, and therefore the electrodes themselves, extended about 1.4-2 mm in the anterior-posterior dimension so that cells were sampled from a relatively wide region of the medial frontal cortex. Medial-lateral extent varied between 0.5 and 1.4 mm, depending on the geometry of the cannula bundle. Rats were returned to ad libitum feeding and allowed to recover for 3-4 days after surgery.

Single units were recorded with respect to a reference electrode positioned deep in the mPFC (5000 μm from brain surface). The tetrodes were moved gradually from 2000 to roughly 4000 μm from the brain surface, keeping all electrodes at the same depth as nearly as possible. Typically, a full set of behavior (see below) was obtained at a given
depth and then the electrodes were moved down 80-120 μm to ensure a fresh group of
cells. After that, tetrodes were moved only as necessary to obtain good recordings
until the next ensemble turning event. This procedure was repeated until electrodes
reached a depth of 4000 μm (rat 1) or 3200 μm (rat 2). All tetrode positioning was
done after a given recording session, to allow the tetrodes at least 18 hours to stabilize.

After all recordings were complete, the tips of the recording electrodes were
marked by electrolytic lesions (5 μamp for 10 seconds, positive to electrode, negative
to ground). Histological sections confirmed that the electrodes in rat 1 were in the ACd
and PL regions with most electrodes in the deep layers. Electrode placement in rat 2
was inferred from stereotaxic coordinates and was not histologically confirmed.

**Reward**

Rat 1 was pretrained using manually delivered 45 mg food pellets. Rat 2 was
pretrained using liquid Ensure nutritional supplement. Following stimulation electrode
placement, MFB stimulation was used as reinforcing reward (for a review and training
techniques, see 6, 7, 8). All stimulation used two wires, though not necessarily the two
twisted wires in one stimulating electrode. The choice of electrodes was determined
empirically based on the rat’s response. Despite the bilaterally implanted stimulation
electrodes in Rat 1, all stimulation was delivered exclusively on right side of the brain,
contralateral to the hyperdrive. A range of stimulation parameters was explored using
an operant conditioning chamber equipped to deliver MFB stimulation when the rat
performed a nose poke. The final selected MFB stimulation consisted of a train of 400
μsec wide, 70 to 100 μA, biphasic current pulses, delivered at 150 Hz for 320-370 msec.
Behavioral Procedures

For pre-training purposes, all rats were food-deprived to 85% of their ad libitum weight. The rats were pre-trained to find reward at one of the eight, equally-spaced zones on the edge of the circular arena. A training session lasted 50-60 min, and was comprised of a randomly selected series of segments. The process of running each segment will subsequently be referred to as a “trial”. Each trial began with two, simultaneously presented cues: a non-directional 4 kHz tone that signaled the availability of reward somewhere in the arena, and the illumination of one blinking LED that marked the correct reward zone. Rats were trained to run to the vicinity of the correct reward zone (within 10 cm) whereupon the reward was delivered and the trial completed. The next trail began after a fixed delay from the onset of reward delivery (500 ms for stimulation-trained and 1000 ms for the food trained rat). This training continued until each rat made direct trajectories to reward locations.

Sequence training occurred following surgery. Sequence tasks were presented to each rat by cueing reward zones in a predetermined order. The trial structure and delay period were the same as during pre-training except that MFB stimulation was substituted for food reward. After a rat completed a sequence three times with guidance from LED cues (a “cued” block of sequences), a 5 sec delay was inserted between the non-spatial, audio cue and the illumination of the cue light, providing time for the rat to move to the next reward location without the aid of the visual cue. Given the typical running speed of a rat, the vast majority of cue-delay trials in well-learned
sequences were completed without the LED and are hence referred to as "non-cued" trials. After the rat completed the non-cued sequence three times, audio and visual cues were presented simultaneously, again, starting another cued block. Blocks of three, complete traversals of the sequence alternated between cued and non-cued throughout the duration of the recording session.

At the start of the experiment, the rat was brought into the recording room and placed in a towel-lined bowl in the center of the recording arena. The headstage was attached to the hyperdrive. The signal gain and threshold of each electrode were manually set via visual inspection, data recording was initiated, and the rat was allowed to rest for 20-40 min in the towel-lined bowl (pre-task sleep). Following this rest period, the rat was moved from the bowl to the arena. The task phase began when the first cue (consisting of a light and tone) was given, and lasted for 50 min. The rat was then returned to the towel-lined bowl and allowed to rest for another 20-40 min. Rats then ran another task phase and were given a final rest period (post-task sleep).

Two different sequences were used, each containing two repeated segments in the shape of a “V", followed by an alternating choice between two zones (see Fig. S1B). For each sequence, rats were trained until they reached asymptotic performance. Asymptotic performance was usually reached within three days, using two training sessions per day. Electrodes were then pushed down to acquire new cells and a new sequence was initiated. The new sequence was created by flipping and rotating the original sequence so as to create a sequence novel to the rat. The flip ensured that the order of turns was reversed while the rotation ensured that a different configuration of places was rewarded. Both rats were presented with a simple 6-element non-repeating
sequence for two days before starting each new repeat-element sequence. All data presented in the present paper was gathered on days when the rat had reached asymptotic performance.

Both rats were run daily for four months. A subset of these data with the highest sleep quality were used in the present analysis (Rat 1: 45 sessions, Rat 2: 21 sessions).

**Data Pre-processing**

Artifact arising from MFB stimulation was removed by deleting all spikes recorded during the stimulation and those occurring within 20 ms of stimulation termination (i.e., the black-out window was stimulus duration + 20 ms). Putative single neurons were isolated using a specialized software package that allows sorting based upon the relative action potential amplitudes on the different tetrode channels and other waveform parameters (9, 10). The end result was a collection of timestamps associated with each action potential from a given unit.

**Determination of Motionless Periods**

Position during each video frame was extracted by fitting a circle to the ring of LEDs on the headstage in each video frame. The positions were then smoothed by convolution of both x and y position data with a normalized Gaussian function with standard deviation of 120 video frames. After smoothing, the instantaneous velocity was found by taking the difference in position between subsequent video frames. An epoch during which the headstage velocity dropped below .78 pixels/second for more than 2 minutes was judged to
be a period of motionlessness. In practice, each 30 minute sleep period contained several motionless epochs with lengths varying between 2 and roughly 20 minutes.

**Explained Variance Measure of Reactivation Strength**

For each period (pre-task sleep, task, and post-task sleep), spike trains were binned into $T$ non-overlapping intervals of 40 ms, producing sequences of spike counts $x[t]$. The normalized correlation $C$ between each pair $(ij)$ of spike trains was computed using the equation:

$$C_{ij} = \frac{1/T \sum_{t=1}^{T} x_i[t] \cdot x_j[t] - \mu_i \cdot \mu_j}{\sigma_i \cdot \sigma_j},$$

derived from Pearson’s correlation coefficient, where $\mu$ is the mean and $\sigma$ is the standard deviation of $x[t]$. Thus the same population of cells produced three sets of firing-rate correlations, corresponding to pre-task sleep, task, and post-task sleep.

Any measure of reactivation must show that activity patterns induced during the task reappear during the subsequent sleep. In addition, activity patterns that existed during the first sleep session should be discounted because these were evidently not induced by the task experience. The explained variance (EV) measure is the square of the partial correlation of task and post-task sleep cell-pair correlations, partialing out any pre-existing correlations during pre-task sleep:
\[ EV = r^2_{r,s2|s1} = \left( \frac{r_{r,s2} - r_{r,s1} \cdot r_{s2,s1}}{\sqrt{1 - r^2_{r,s1}} \sqrt{1 - r^2_{s2,s1}}} \right)^2, \]

where \( T, S1 \) and \( S2 \) represent task, pre-task sleep, and post-task sleep periods (11). EV reflects the proportion of variability in the S2 sleep correlations that can be accounted for by task correlations after accounting for S1 sleep correlations. The EV reflects only reactivation seen immediately after a task; any pre-task sleep replay of task activity (e.g., from a previous session) would be eliminated.

EV during sleep was computed within a series of non-overlapping windows roughly corresponding to individual epochs of motionlessness. Epochs longer than 8 minutes were broken into smaller epochs of 5 minutes duration or less. Thus each sleep period yielded several EV values and the representative value for a given session was taken to be the maximum of these individual EV values.

**Analyses Based on Spike-Train Cross-Correlations**

**Cross-Correlations**

The cross-correlation (CC) between the spike trains from two cells, \( U \) and \( V \), with individual spike times \( u_i \) and \( v_j \), is given by

\[
CC[t] = \frac{\sum_{i=1}^{N} \sum_{j=1}^{N} \left\{ \begin{array}{ll} 1, & |u_i + c[t] - v_j| < b/2 \\ 0, & \text{otherwise} \end{array} \right\}}{b \cdot U},
\]

where \( b \) is the bin size, \( t \) is the current bin number, \( c[t] \) is the bin center time for bin \( t \), and \( N_u \) and \( N_v \) are the total number of spikes from cells \( U \) and \( V \), respectively. The bin size was 10 ms and bins centers (i.e., \( c[t] \)) typically ranged from -4 to 4 seconds. As with EV, cross-correlations during sleep periods were limited to spikes occurring during motionlessness.
epochs. The cross-correlation, as defined, is essentially a peri-event time histogram of the spikes from cell V around each of the spikes from cell U.

To estimate the variability of the cross-correlation between cells U and V, the cross-correlation was computed between two spike trains with the same spike rates as U and V, respectively, but with randomly assigned spike times. The cross-correlation values of these shuffled spike trains, computed over a range of 50 seconds, were used to obtain a mean and standard deviation which were, in turn, used to convert the original cross-correlation values between U and V into z-scores.

**Determination of Compression Rate**

The compression rate for a given session was determined by matching the peaks of the cross-correlations for individual cell pairs. Cross-correlations between all cell pairs were computed, as described above, for the second task period and the corresponding post-task sleep period. Cross-correlations were then smoothed by convolution with a normalized Gaussian curve with a standard deviation of 30 ms. To avoid shifting the mean during smoothing, Gaussian values were normalized so that the sum of values was one.

To find peaks, each smoothed cross-correlation function was scanned from left to right. For each sample point, if the current value, $c_i$, exceeded the running maximum value, $c_{max}$, the maximum value was updated. If $c_{max} - c_i > \delta$ for some positive value $\delta$, then the position of the last maximum was taken as a peak. The value of $\delta$ was set to 2.5 for the task and 2 for sleep. The program next searched for a trough using similar
criteria. A running minimum value, \( c_{\text{min}} \), was tracked. If \( c_{\text{min}} - c_i < \delta \), then the position of the last minimum was taken as a trough. The value of \( c_{\text{max}} \) was then reset and search continued for the next peak, starting from the current trough. (Algorithm and Matlab code by Eli Billauer, http://www.billauer.co.il/peakdet.html)

Two peaks were extracted from each cross-correlation: the positive peak nearest zero and the negative peak nearest zero. The ratio of the peak time during the task to time of the corresponding peak during sleep gave an estimate of the compression factor for a single cell pair (i.e., how much faster sleep replayed relative to task speed). For example, if the first positive peak during the task was at 4 seconds and the first positive peak during sleep was at time .5 seconds, this would correspond to a compression factor of 8.

The compression rate analysis was limited to cell pairs a) that had prominent peaks during the maze (i.e., a peak z-score of at least 5) and b) that showed a strong match between task and sleep cross-correlations after accounting for compression. In order to determine the task-sleep match strength, a compression rate was first estimated (as described above) for both positive and negative peaks. The average of these values was used to rescale the task cross-correlation linearly so that the peak locations corresponded with those during sleep. For example, if the original task cross-correlation ranged between -8 and +8 seconds and the estimated compression factor for the cell pair was 8, the entire function was compressed so that it ranged between -1 and +1 seconds. The match strength was then assessed by computing the Pearson’s correlation coefficient between all sleep and rescaled task cross-correlation values.
lying between -400 and +400 ms. To be included in the compression rate estimate, the correlation coefficient had to exceed .7.

To obtain the compression rate estimate for an entire session, the sleep peak time was first plotted against the maze peak time for all cell pairs meeting the aforementioned criteria. A form of robust regression was then used to extract the slope. The robust regression algorithm uses iterative reweighting, giving lower weight to points that do not fit the main regression line (robustfit function, Matlab statistics toolbox). The inverse of the slope of the regression line gives the rate of acceleration of sleep replay relative to rate of neural activity pattern evolution during the task.

**Determination of Temporal Extent of Replay**

The temporal extent of replay during sleep was assessed by comparing task and sleep cross-correlation similarity within a series of time bins. The task cross-correlation function was first compressed, using the session-specific compression factor described in the preceding section. The match between task and sleep cross-correlations was then quantified via the Pearson’s correlation coefficient between the cross-correlation values within a 200 ms window. These windows were equally spaced every 50 ms from -1500 to 1500 ms. Each cell pair with a task cross-correlation peak exceeding 10 z-scores was included. A mean cross-correlation similarity curve was computed for each of the 13 sessions showing strong EV. Because the directionality of the cross-correlation is arbitrary, this similarity curve was symmetric about zero; hence, bins with positive and negative times of the same absolute value were combined. The graph in Fig. 3B shows the grand mean across these 13 session and the associated standard error bars.
Template Matching

Templates

Each template was a matrix representing the firing rate of multiple cells over several time bins. Eight templates were generated, one for each segment of the sequence. A segment was defined as the time between arrival at one reward zone and arrival at the next reward zone, excluding times during which stimulation was delivered. Each row of the template comprised the spike counts from one cell within a series of 100 ms bins covering one segment, averaged over all repetitions of the sequence. Repetitions were first screened for segments that took inordinately long (i.e., segments during which the rat was off-task). For each segment, any repetitions during which the traversal time exceeded four times the distance of the quartile from the median were excluded. For the remaining repetitions, each segment was scaled so that traversal time equaled the median time. The spikes from these scaled repetitions were then averaged in 100 ms bins. Note that median times were different for the different segments, meaning that the number of bins varied between 15 and 27.

Template matching was based on a subset of cells that were active during all phases of the experiment and that exhibited task-related firing rate changes. Any cells with less than 50 spikes in any given period (pre-task sleep, task, or post-task sleep) were excluded. Two additional criteria were applied to determine whether a cell should be included in the template matching analysis. The first was a firing rate criterion. Cells in which the average spike count exceeded five spikes during a given template (i.e., the
sum of all spike counts within that cell’s row of the template exceeded five) were judged to have a sufficient spike count for that template. The second criterion was that the cell show strong task-related activity. Towards this end, each row of each template was first smoothed using a 5-point moving average. The coefficient of variation (standard deviation divided by the mean, computed on the 15-27 values in a given row) was then computed for each cell that exceeded the firing rate criterion. A cell was judged to have significant task-related firing in a given template if its coefficient of variation exceeded .39. To be included, a cell had to pass both the firing rate and coefficient of variation criteria on 4 of the 8 templates. These criteria lead to a significant reduction in the number of cells included in the template-matching analysis. In the data set presented in Fig. 4, 22 of 72 cells were included.

**Computation of Template Matches**

The spike activity of all included cells from a recording session is stored in an N x T spike-matrix, where rows correspond to N cells and columns to T discrete bins. The bin contents represent the number of spikes each cell fired during each time bin. As described above, the template matrix is an averaged firing pattern during each segment of the sequential task. A single template, X, is an N x M matrix, where M corresponds to the number of bins in the template. A target matrix Y, with the same dimensions as the template, is also selected from the N x T spike-matrix. In matrix form, both template matrix X and target matrix Y are represented as
The template matching method seeks to calculate the similarity of these two matrices (12). Based on our previous study on different measures of template match strength (13), we used the standardized Pearson correlation coefficient measure. In this measure, each row of the X and Y matrices is standardized to zero-mean and unit-variance by subtracting its row mean, $\bar{x}_c$ and $\bar{y}_c$, and dividing by the row standard deviation, $\sigma_{x,c}$ and $\sigma_{y,c}$, respectively. $\bar{x}_c$, $\bar{y}_c$, $\sigma_{x,c}$ and $\sigma_{y,c}$ are defined as

$$
\bar{x}_c = \frac{1}{M} \sum_{m=1}^{M} x_{cm}, \quad \bar{y}_c = \frac{1}{M} \sum_{m=1}^{M} y_{cm}, \quad \sigma_{x,c} = \sqrt{\frac{1}{M} \sum_{m=1}^{M} (x_{cm} - \bar{x}_c)^2}, \quad \sigma_{y,c} = \sqrt{\frac{1}{M} \sum_{m=1}^{M} (y_{cm} - \bar{y}_c)^2}.
$$

This normalization transforms the elements $x_{cm}$ and $y_{cm}$ to z-score variables $w_{cm}$ and $z_{cm}$ through

$$
w_{cm} = \frac{x_{cm} - \bar{x}_c}{\sigma_{x,c}}, \quad z_{cm} = \frac{y_{cm} - \bar{y}_c}{\sigma_{y,c}}.
$$
By construction, mean firing rate differences among different rows is fully suppressed with this normalization. Therefore, this measure is sensitive to the firing order relationships among different neurons. The standardized Pearson measure is then defined using the 2-dimensional Pearson correlation coefficient, COR, for the normalized W and Z matrices,

\[
COR = \frac{\sum_{c=1}^{N} \sum_{m=1}^{M} (w_{cm} - \bar{w})(z_{cm} - \bar{z})}{\sqrt{\sum_{c=1}^{N} \sum_{m=1}^{M} (w_{cm} - \bar{w})^2} \sqrt{\sum_{c=1}^{N} \sum_{m=1}^{M} (z_{cm} - \bar{z})^2}}
\]

**Computation of Template Match Z-Scores**

To assess the reliability of observed correlations between template and target matrices, the z-score for each match was computed using a sampling distribution estimated from shuffled templates. For this analysis, the column vectors of the template matrix were randomly shuffled, and 100 shuffled templates were generated for each template matrix. The template matching correlation was then calculated for each shuffled template, and a sampling distribution was generated for each time point. The observed correlation value at each time point was then converted into a z-score.
Computation of Template Matches with Compression

To assess whether the memory replay occurs faster or slower than the speed of firing patterns during behavior, a compression factor was introduced. We modified the bin width of the target matrix between 10 msec and 100 msec; 10 msec bin width corresponds to the case where the replay occurred 10 times faster than the original speed while 100 msec bin width corresponds to replay at the same speed. To quantify and compare the number of matches at different compression factors, the ratio of high peaks to all peaks was used. The z-score correlation values at each time point were first restricted to times when the rat was motionless, using the motion criteria described above (see “Determination of Motionless Periods”). For each compression factor, the locations of all peaks were then determined using the peak-finding algorithm described in the section entitled “Determination of Compression Rate”. The minimum difference criterion value, $\delta$, used by that algorithm was set to two. A ratio was then formed between the number of peaks exceeding a z-score of four and the total number of peaks. The z-score threshold value, four, was selected because it provided good discrimination between different compression factors, as shown in Fig. 4C.

Further Discussion

Variability of Reactivation Strength

Of 66 sessions analyzed, only a subset exhibited strong reactivation. Only 13 sessions had EV values exceeding a criterion value of 15%. Within these sessions, however, EV values reached as high as 34% (Table 1S). Thus there is a wide spread of EV values
across sessions. Furthermore, sessions run on adjacent days often exhibited widely
different EV values. There are many reasons for this variability, the most obvious is sleep
quality. If the rat fails to settle down, reactivation during the rest period will be very
weak.

In the present study, we partially controlled for sleep quality across sessions by using
motion tracking data to limit analyses to times when the rat was still. However, motionlessness
doesn’t guarantee quality sleep. Many other factors including arousal, anxiety, and time within the circadian cycle can influence the time spent in the various stages of sleep and, hence, the amount of reactivation measured.

Another source of variability may come from level of experience. Theory suggests that sessions with higher learning demands should exhibit higher reactivation. Differences in experience within a single session actually showed the opposite effect. The EV values after the second behavioral session, during which behavior was presumably better learned, showed stronger reactivation than the first behavioral session. This suggests that higher numbers of repetitions lead to higher likelihood of replay, but it does not discount the possibility that experience may also influence reactivation from session to session.

A definitive answer to the question of whether experience influences reactivation will require further experiments. Preliminary results indicate that learning induced by changing the sequence of reward zones does not affect reactivation strength. However, it is perhaps significant that, for both rats, the majority of sessions exhibiting strong EV were observed within 2-3 weeks of the start of the experiment. Unfortunately, electrode positions were also changed slightly over the course of the experiment,
leaving open the alternate hypothesis that specific brain regions exhibit stronger reactivation than others. Experiments designed to discriminate these two possibilities are ongoing.

**Possible Role of MFB in Reactivation**

Whether MFB stimulation played any role in mPFC reactivation reported in the present work remains unknown. The mPFC is one (of many) targets of the MFB (14). Although stimulation was always contralateral to the recording site, it is still possible that MFB stimulation lead to higher levels of mPFC activity than other forms of reward. Indeed, a significant subset of recorded cells fired during approach to reward sites or after reward receipt (note that spikes during stimulation were omitted from analyses). This strong activation might lead to enhanced plasticity, increasing the likelihood and strength of reactivation during off-line periods. However, it seems unlikely that increased task-related activity, in and of itself, could account for the acceleration of replay reported here. Behaviorally, both rats ran the sequence task faster than food-trained rats and learned novel sequences in fewer sessions, but the overall pattern of behavior was similar. Neural responses in the hippocampus, for which comparative data are available, are similar using MFB and food reward. Hippocampal cells express place fields using MFB reward just as they do using food (1). Hippocampal reactivation during sleep is also observed using either food (e.g., 11) or MFB stimulation (15). In our view, MFB may have strengthened an existing mechanism, making reactivation easier to detect, but is unlikely to have created a completely novel phenomenon.
**Fig. S1.** Behavioral Task. (A) The rat performed a spatial sequence task on a 1.3 m diameter circular platform. Attached to the rat’s head was a unity gain headstage equipped with light emitting diodes for tracking purposes, and signal cable. Surrounding the maze were 8 automated feeders, used for pre-training. A light emitting diode is located at the base of each feeder tower. (B) Examples of sequences used during the experiment. Rats ran the blue trajectories and then the red, alternating repeatedly. Red and blue sequences together comprised the entire sequence, consisting of 8 segments.
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<thead>
<tr>
<th>Rat</th>
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<th>EV (%)</th>
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**Table S1.** Explained variance and compression factor for each session included in temporal compression analysis. Each rat was trained daily; the session number, therefore, gives a rough indication of the number of days elapsed from the start of the experiment.
Supporting Material Bibliography