Supporting Information

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SI Text

Training and Data Acquisition. Male Long–Evans rats weighing 400–450 g were maintained at a minimum of 85% of normal body weight. Each rat was initially shaped to dig in 4-inch-tall flower pots filled with common playground sand for ¼ Froot Loops pieces. Subsequently, animals were trained on a simple discrimination between two flowerpots scented with different oil fragrances (aloe and clove) placed side by side simultaneously in the home cage. The left–right positions of the two stimuli were pseudorandomized although never in the same positions on >3 consecutive trials. The rat could dig in the aloe scented pot for a ¼ Froot Loop but digging in the clove scented pot resulted in a 5-s timeout. Rats achieved a performance criterion of 8 correct of 10 consecutive trials within a single session.

Subsequently, rats were exposed for 15 min to the environment where conditional discrimination testing would take place and they were allowed to forage for food scattered on the floor of the apparatus. The environment consisted of two 37 × 37-cm boxes connected by a central alley that allowed the rat to shuttle between them (Fig. 1*A*). The entrance to the central alley could be closed with dividers at each end of the alley to block the animal within either box. Each box differed substantially in contextual cues that included different flooring (wood vs. black paper) and different wallpaper (white paper vs. black paper). Rats were subsequently trained to alternate between the two contexts by traversing the central alleyway when the dividers were lifted. On each trial of the conditional discrimination task, the rat was allowed to enter a context, after which a divider would close, and the animal was permitted to explore the contextual cues for 40 s (Fig. 1*A*). Using another divider, the animal was then blocked within one side of the context briefly while two stimulus items were placed in different corners of the context, then the divider was removed, allowing the animal to approach the stimuli. Both items were common terra cotta flowerpots, each scented with a different odor (i.e., grapefruit and geranium) and filled with a different digging media (i.e., white foam and purple beads). The positions of the items were pseudorandomized such that they appeared in each position equally, but never in the same position on >3 consecutive trials. In context A, item X (i.e., grapefruit-white foam) would contain the Froot Loop reward, whereas in context B, item Y (i.e., geranium-purple beads) would contain the reward. Digging in the correct pot yielded the buried reward, but digging in the incorrect pot resulted in the removal of both pots and a 5-s timeout.

On day one, we trained the rat for as many trials as possible (usually 50–60 trials) in blocks of five trials within the same context, to allow corrections for that particular discrimination. On day two, trials alternated between the contexts, always permitting a 40-s exploratory period before item presentation. To ensure that the animal did not simply learn to alternate choices of items X and Y, trials were repeated within the same context every 10 trials on average. To ensure that the animal could not simply smell the buried reward, every 10 trials involved a probe trial in which neither pot contained a reward, and a reward was given only after digging in the correct pot. Initial training on this first conditional discrimination problem required 3–5 days of 80 trials per day until performance reached at least 70% in a 10-trial block within each context.

After reaching the performance criterion, rats were implanted with a recording headstage above the left dorsal hippocampus centered at +3.6 mm posterior and +2.9 mm lateral to bregma. The headstage contained 12–18 independently movable tetrodes aimed at CA3. Each tetrode was composed of four 12.5-μm nichrome wires with the tips plated with gold to bring the impedance to 200 kΩ at 1 kHz. Animals recovered for 7–10 days, after which the tetrodes were moved down slowly over the course of 1–2 weeks, until the tips reached the pyramidal cell layer of CA3 and the animal's performance had again met criterion level on the initial conditional discrimination problem. The locations of these tetrodes was estimated in vivo using driver turn counts to determine electrode depth and electrophysiological events, including the appearance of complex spikes, theta-modulated spiking, and the presence of theta and high-frequency ripples in the LFPs. Electrode location was confirmed by passing a 25-μA current for 20 s through each tetrode immediately before perfusion to create a lesion visible after histological processing with Nissle stain. Tetrodes within CA3 that were too noisy or that contained too much chewing artifact were excluded from the analysis. These traces generally did not show a theta peak in the power spectral density and exhibited an abnormally high power in the lower frequencies (0–5 Hz).

Once the tetrodes were in the desired locations, recordings were taken as the rats continued to perform the initial conditional discrimination problem. We defined overtraining sessions as sessions where the animal's performance had exceeded 80% for 3 consecutive prior testing days. After these overtraining sessions, the animal was introduced to a novel environment with the same configuration but with new flooring (rubber vs. sandpaper) and new wallpaper (vertical vs. horizontal stripes) defining each context. After 15 min of exposure to this environment, we began testing the animal on a second conditional discrimination problem using pots with new scented oils and digging media (patchouli-straws vs. mint-buttons). Rats learned this second conditional discrimination problem within a single recording session. On subsequent days, recordings were taken during overtraining on the second problem after the animal had again reached the criterion of 80% correct performance for 3 consecutive testing days.

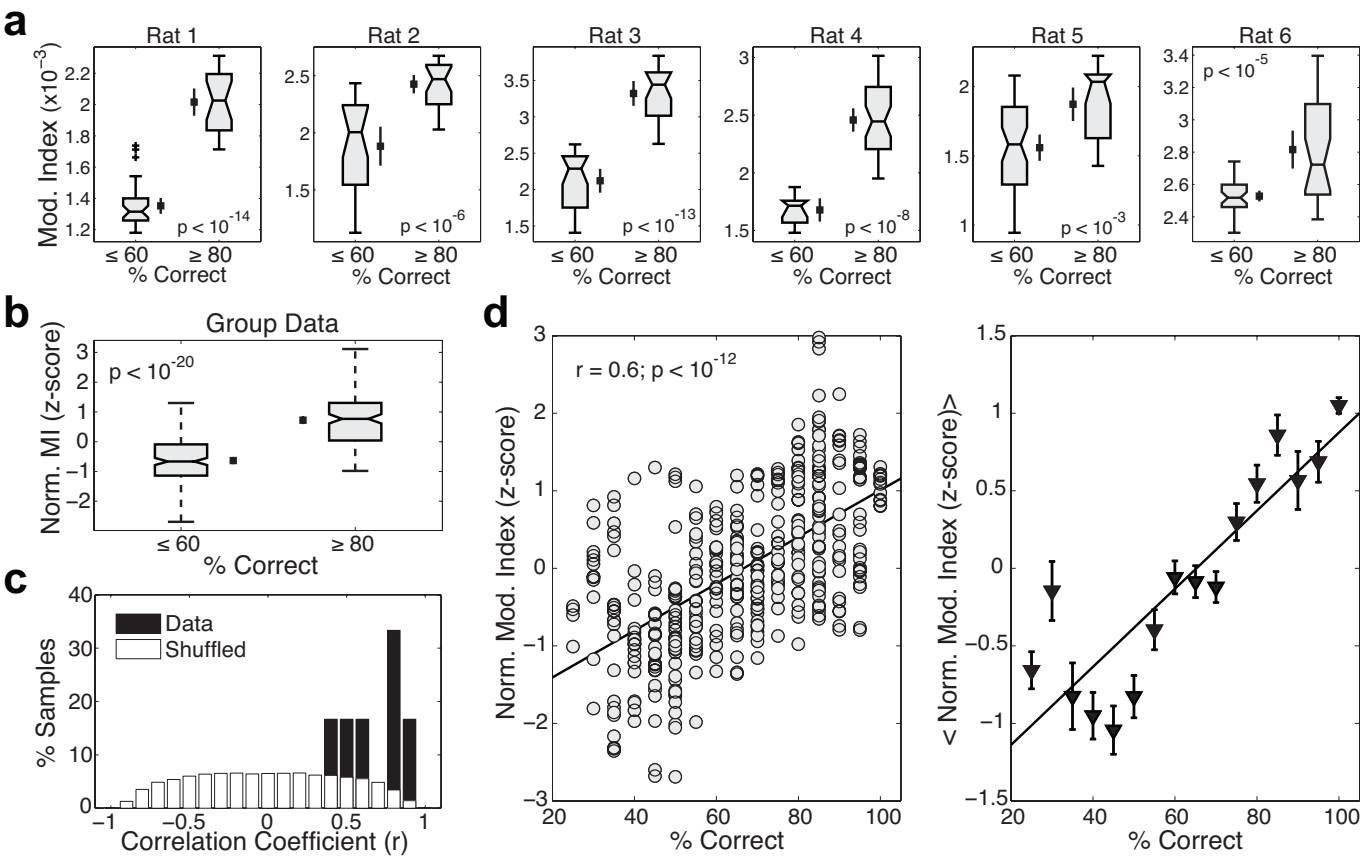

Fig. S1. Group data of the phenomenon described in Fig. 2. (*a*) Notched boxplots of the MI values computed for low- and high-performance trial windows for each of the six cases studied. The black squares and associated error bars denote the mean value and the 95% C.I. (*b*) Pooled normalized MI values for low- and high-performance trial windows. The *z*-score normalization was done to allow a combined analysis of all animals. (*c*) Correlation coefficient (*r*) values between the MI and learning curves for the six cases studied (black bars) and surrogate controls (white bars). The *r* values are statistically significant for each case studied (see Figs. S2 and S3), and the probability (*P* value) that they have been derived from the surrogate distribution is <0.001. (*d*) Linear correlation studies (as in Fig. 2) between the pool of normalized MI and learning curves.

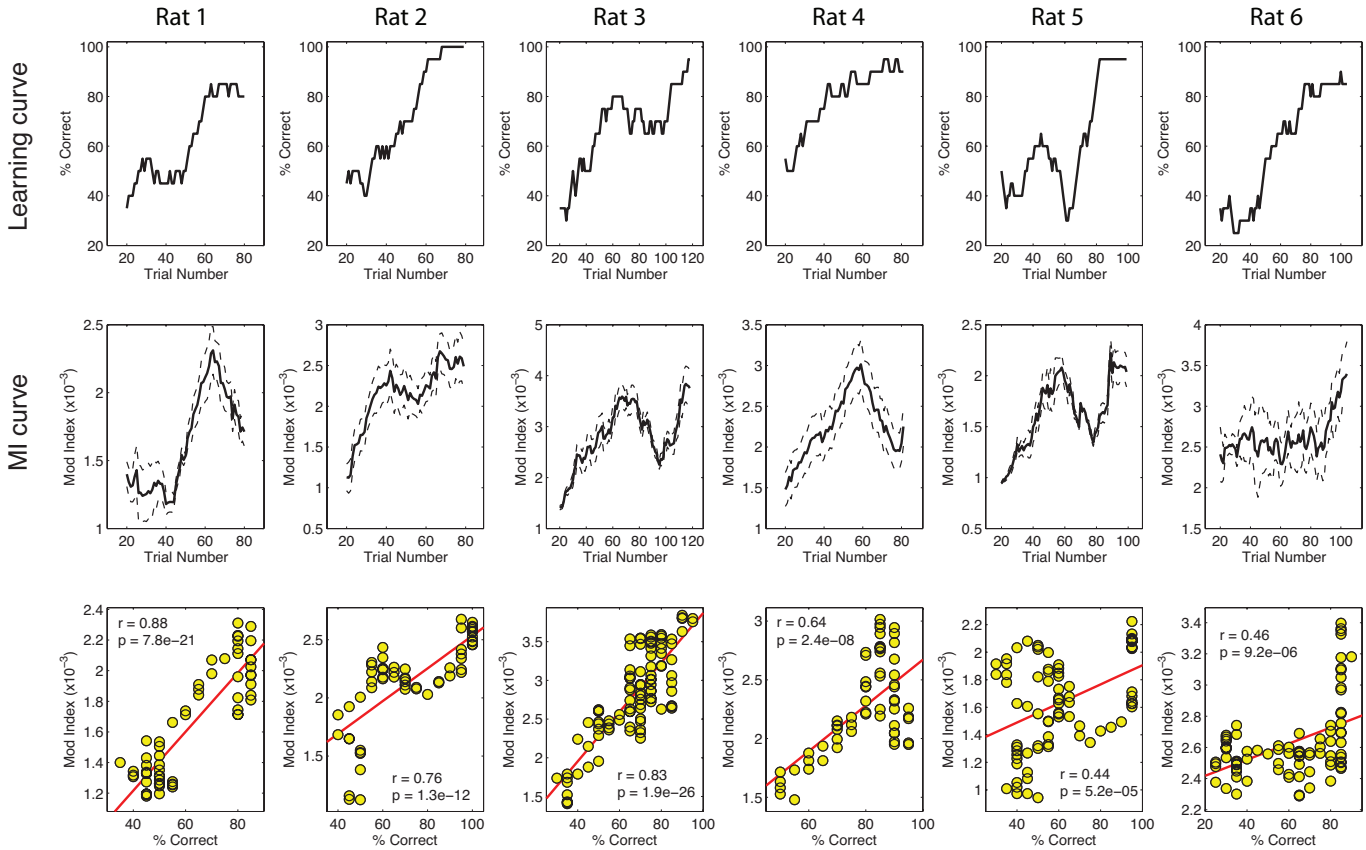

Fig. S2. Learning (*Top*) and MI (*Middle*) curves for each of the six cases studied are shown, along with their correlation analyses (*Bottom*).

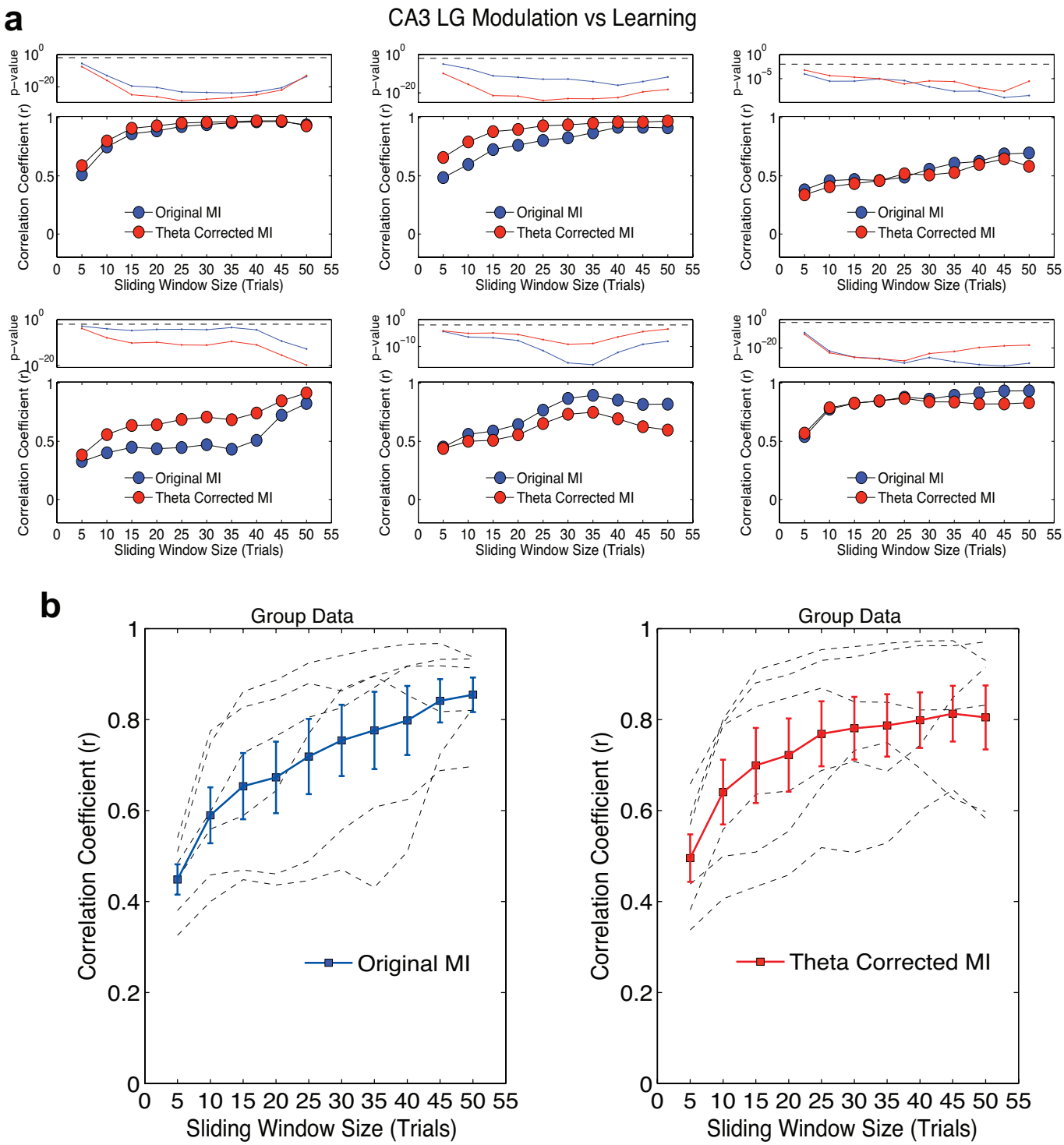

Fig. S3. Control analyses for theta power and sliding window size. (*a*) The correlation coefficient (*r*) between the MI and learning curve as a function of the number of trials (i.e., the sliding window size) used for the computation of these curves is shown for each of the six cases studied (blue). The correlation of task performance with the theta-corrected MI [defined as MI/(theta power)] is also shown (red). For each correlation coefficient value, the corresponding *P* value is shown in *Upper* (dashed line indicates the significance threshold). (*b*) Group data showing the mean correlation coefficient between the learning and the MI curves for different sliding window sizes (*Left*). The equivalent result for the theta-corrected MI is shown (*Right*). Error bars represent SEM. The results of each individual case are also reproduced in each panel (dashed lines).

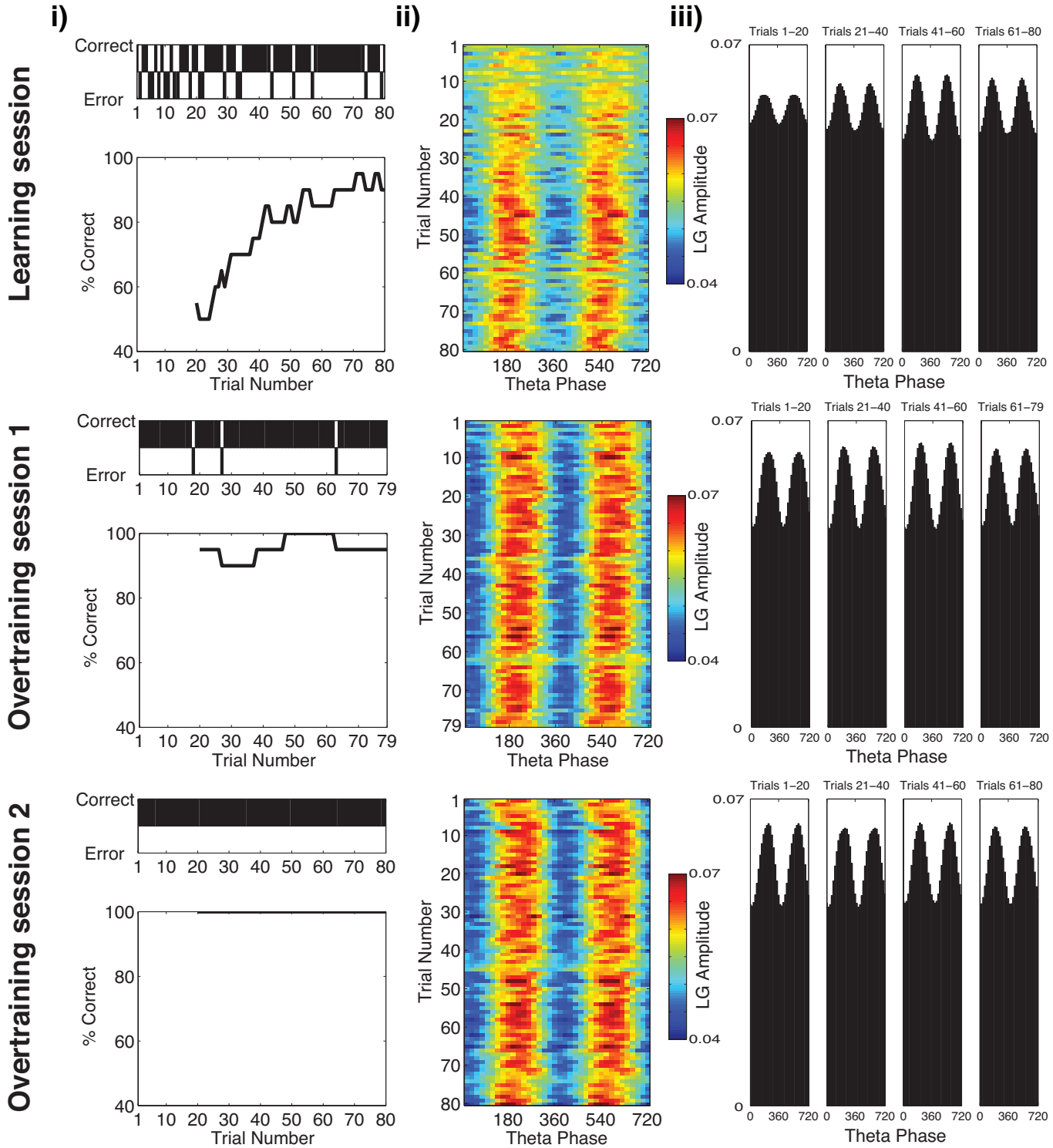

Fig. S4. Behavioral profile and theta–gamma coupling during learning (*Top*) and an extra overtraining session (*Bottom*) of the same animal depicted in Fig. 3*A* (*Middle*).

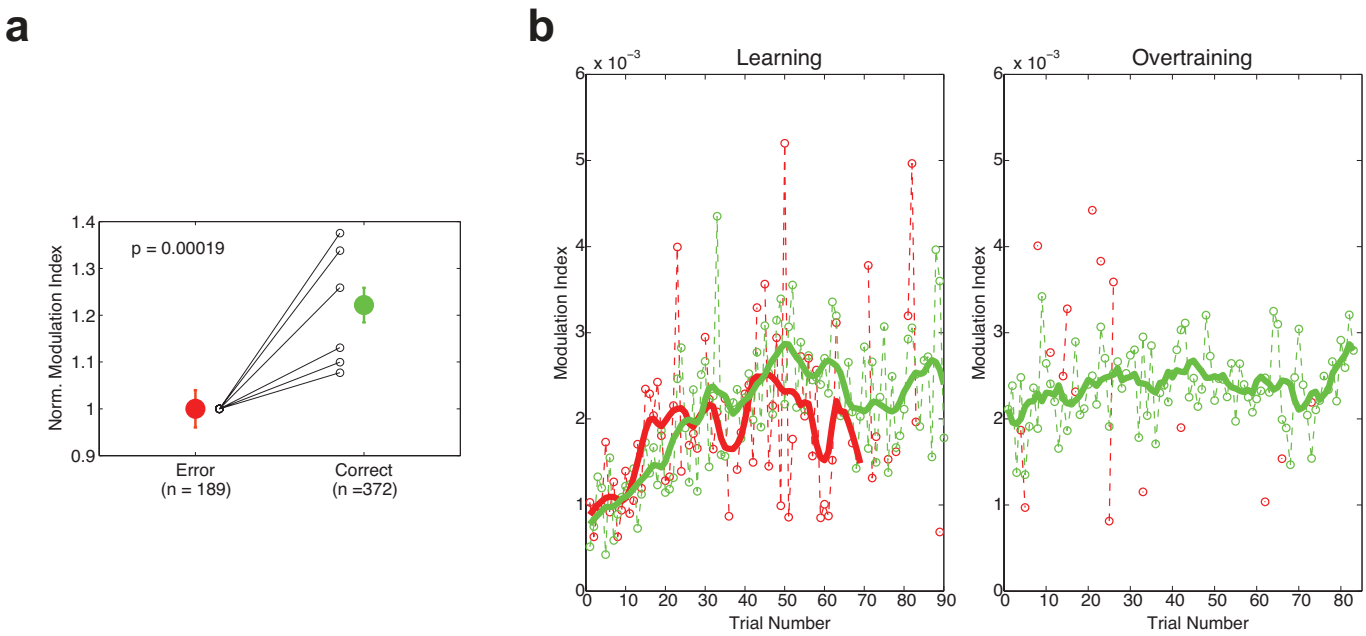

Fig. S5. Correct trials present higher theta–gamma coupling than error trials, but this difference can be accounted for by a higher prevalence of correct trials late in the learning session. (*a*) Normalized MI for correct (green) and error (red) trials. The normalization was done within each rat by dividing each MI value by the mean MI over error trials. Individual results for each rat are also shown (open circles). (*b*) Mean MI values among rats for each trial number during correct (green circles and dashed line) and error (red circles and dashed line) trials. The thick lines are smoothed curves derived from the data (10 points moving average). Notice that not all trial numbers presented error trials among rats, and therefore the error MI values are discontinuous.

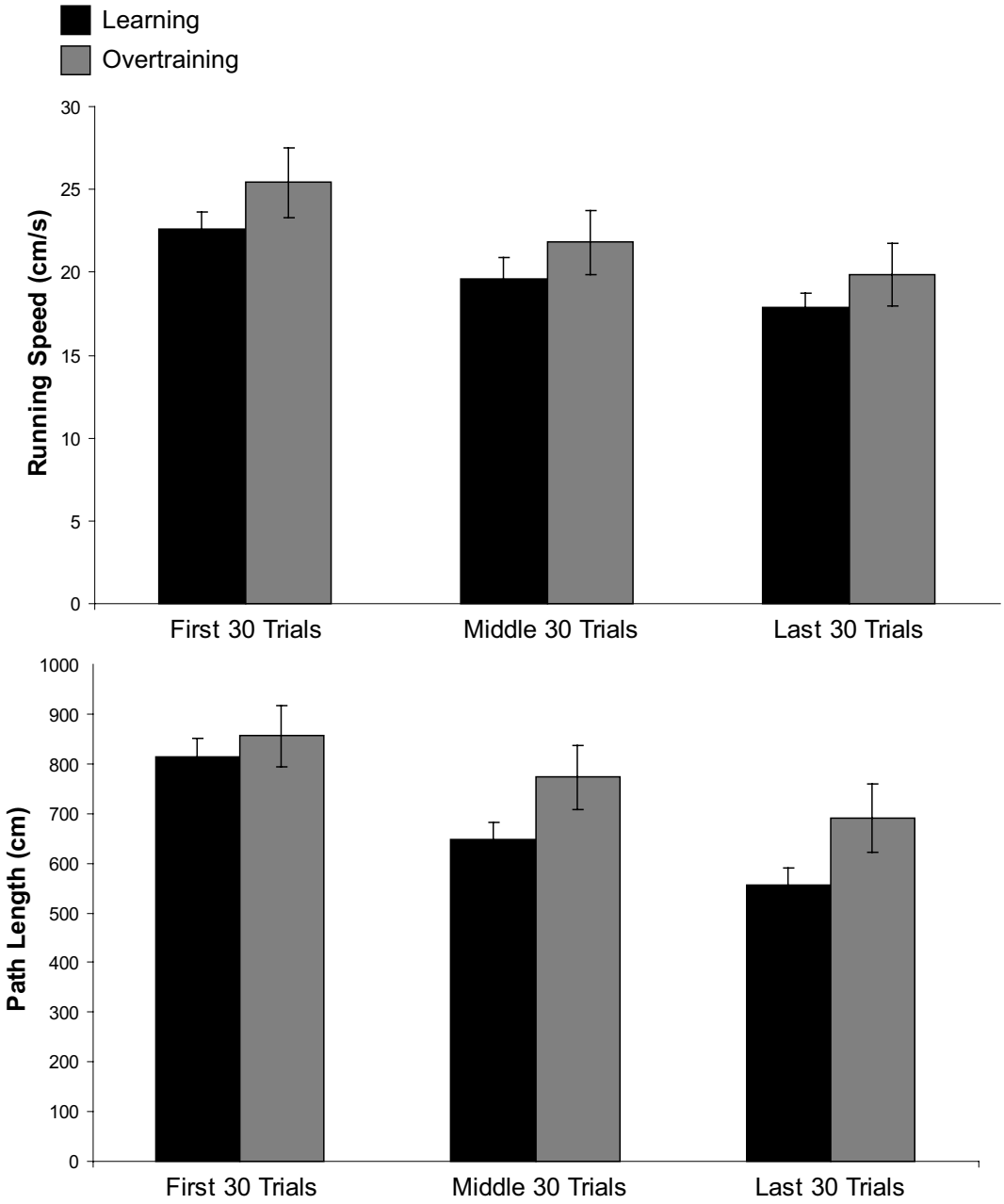

Fig. S6. Mean running speed (*Upper*) and path length (*Lower*) during the context exploration period of the task for learning and overtraining sessions.

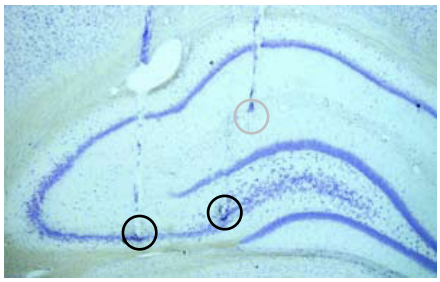

Fig. S7. Nissle-stained coronal slice with lesion marks made at tips of tetrodes. Two lesion marks circled in black were from tetrodes located within the CA3 region of hippocampus, while the lesion circled in gray was from a tetrode located in the hippocampal fissure. (Magnification: 4.63×.)

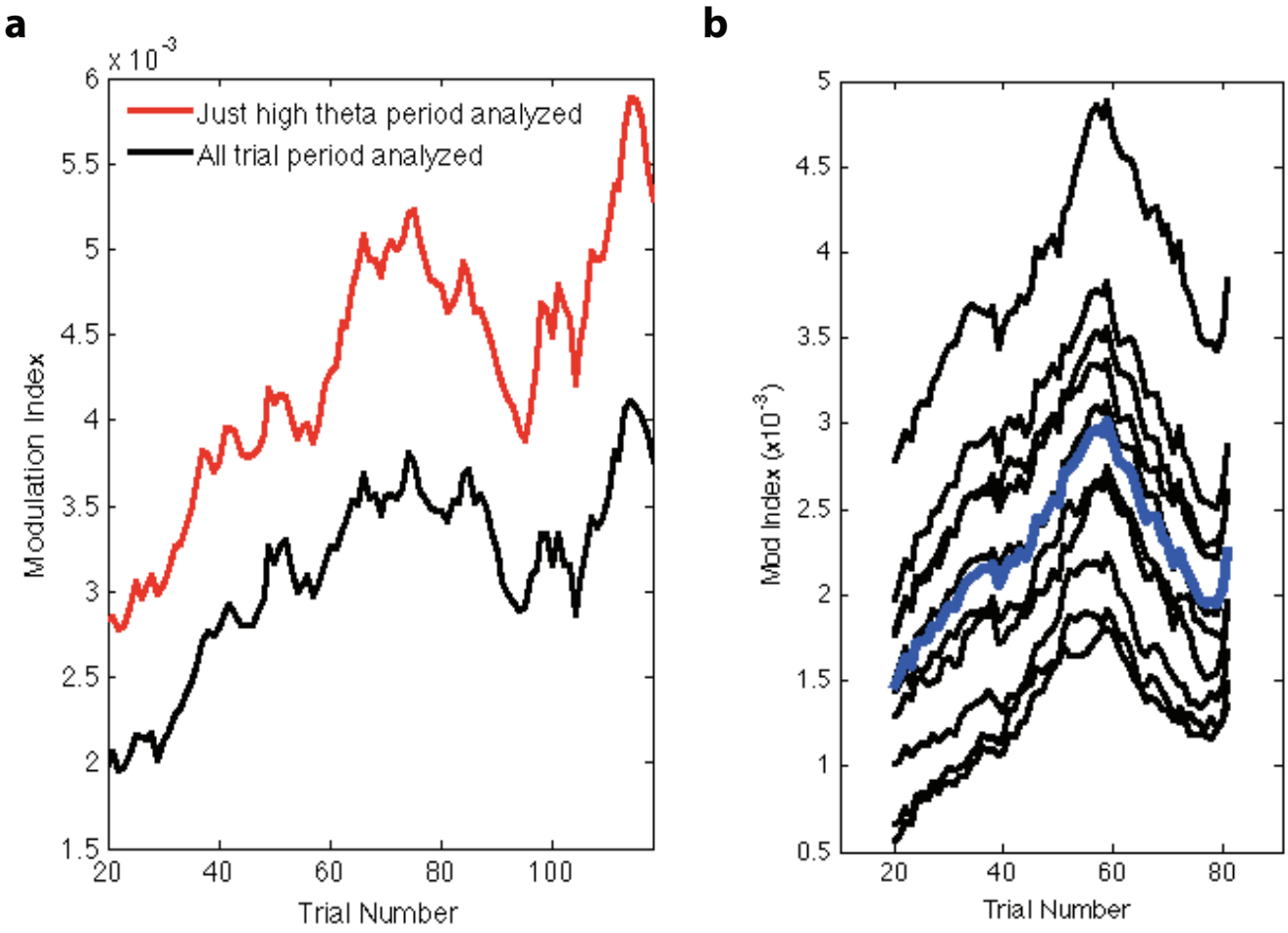

Fig. S8. (*a*) MI curve obtained for a representative electrode under two different analysis protocols. Restricting the analyzed period within trials to ''high-theta'' periods (red curve) provides higher MI values than when the whole trial period is analyzed (black curve). Notice that the difference between the curves is approximately constant and therefore both approaches lead to qualitatively similar results. (*b*) Examples of MI curves obtained for different electrodes (black traces) targeting the same region (CA3 pyramidal layer). The blue trace represents the mean over these electrodes.