Day	Session 1 (~1.5h)	Session 2 (~1.5h)	Session 3 (~1.5h)	Session 4 (~1.5h)	Session 5 (~1.5h)
Task order	categorical perception task	2 recognition runs	2 recognition runs	2 recognition runs	8 recognition runs
		10 feedback runs	10 feedback runs	10 feedback runs	
	8 recognition runs	2 recognition runs	2 recognition runs	2 recognition runs	categorical perception task

Table 1: Multi-session study protocol for each participant.

Methods

Participants

A total of 20 healthy young adults (mean age = 23.55; age range = 19–28; 14 female) with normal or corrected-to-normal visual acuity participated in this study. There are 2 pilot subjects and 9 subjects who did not finish all five sessions for various reasons. They completed five fMRI sessions lasting 1.5 hours each and were paid $20/hour. Sessions were scheduled on separate days but as close together as possible, from a minimum of five days in a row to a total span of eight days.

Ethics Statement

All participants provided informed consent to a protocol approved by the Institutional Review Board at Yale University.

Data acquisition

Data were acquired using a 3T Siemens Prisma scanner with a 64-channel head coil at the Brain Imaging Center at Yale University. For recognition and feedback functional runs, an echo-planar imaging (EPI) sequence was used to collect BOLD data (TR=2 s; TE=30 ms; voxel size=3 mm isotropic; FA=90°; IPAT GRAPPA acceleration factor=2; distance factor=25%), yielding 36 axial slices. Each recognition run contained 145 volumes and each feedback run contained 176 volumes. Two field map volumes (TR=5 s; TE=80 ms; otherwise matching the EPI scans) were acquired in opposite phase encoding directions. For anatomical alignment and visualization, we collected a 3D T1-weighted magnetization-prepared rapid acquisition gradient echo (MPRAGE) scan (TR=2.5 s; TE=2.9 ms; voxel size=1 mm isotropic; FA=8°; 176 sagittal slices; IPAT GRAPPA acceleration factor=2), and a 3D T2-weighted fast spin echo scan with variable flip angle (TR=3.2 s; TE=565 ms; voxel size=1 mm isotropic; 176 sagittal slices; IPAT GRAPPA acceleration factor=2).

Real-time system

After image reconstruction, the DICOM files were streamed in real-time to Milgram, a high-performance cluster mounted on the Siemens console. The RT-Cloud software package [31] was used for preprocessing and analysis of each image, with the results transmitted to the task computer at the scanner over the network. This output was used to update the task on the next time point.

Data preprocessing

For real-time analyses, each new DICOM file was aligned with 3dvolreg [32] to a template volume acquired from the middle volume of the first recognition run in the current session. This alignment helps address potential movement artifacts. The BOLD activity of every voxel was subjected to z-scoring over time using the running mean and standard deviation from the prior TRs in the current run. The data were masked to include only voxels in the region of interest (ROI) used for feedback prior to classifier training and testing. For offline analyses, the functional data were field map corrected with the topup tool in FSL [33], registered to the middle volume of the current run with MCFLIRT [34], and z-scored based on the full-time series from that run. Z-scoring was applied across time for each voxel in order to normalize the mean and variance of each voxel's BOLD activity prior to classification, which ensures that baseline differences in the mean and scaling of BOLD activity across voxels do not confound or bias classifiers.

Study design

The study consisted of five sessions (Table 1). Session 1 contained eight recognition runs in which the presented, competitor, and control objects were shown repeatedly. These data were used to train classifier models that could distinguish between all pairs of objects. The trained models were then tested on the feedback runs in Sessions 2, 3, and 4 to measure the amount of evidence for the competitor object during the viewing of the presented object. Participants were encouraged to increase the activation level of the competitor through adaptive, closed-loop neurofeedback, inducing coactivation between the presented and competitor objects. Session 5 mirrored the first session with eight recognition runs, in order to assess representational change.

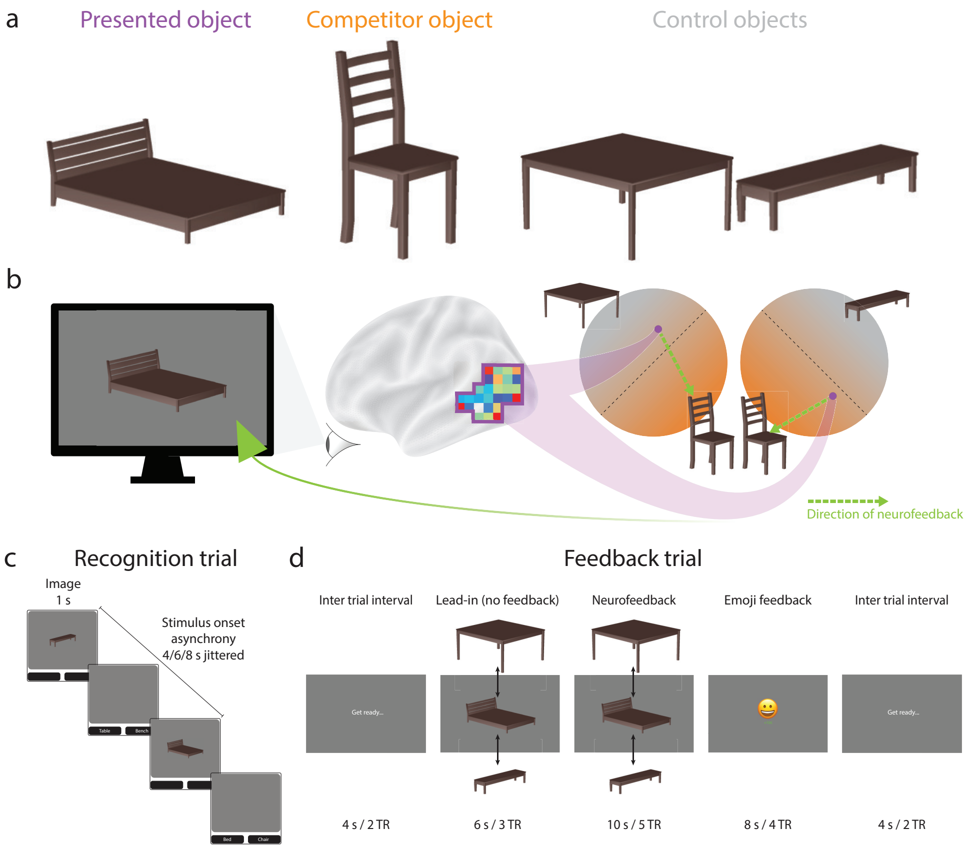

Figure 1: Study design. (a) Each participant received two objects for neurofeedback and the other two objects served as a baseline. (b) The presented object (e.g., bed) was shown on neurofeedback trials and began oscillating in size and shape. The goal of the participant was to make this wobbling stop, which they could achieve by activating the representation of the competitor object (e.g., chair) in their mind. Evidence for the competitor object was quantified based on a classifier trained to decode the competitor object relative to two control objects (e.g., table, bench). The amount of classifier evidence for the competitor needed to reduce the magnitude of wobbling was staircased to maximize coactivation between these objects. (c) Recognition trials showed one of the four objects at a time with no neurofeedback. These trials were used to train the classifier models and to measure neural snapshots of how the object is presented in the hippocampus. (d) Feedback trials were collected during real-time fMRI in order to induce coactivation. The main neurofeedback occurred during the object presentation (the amount of wobbling), though participants also received a valenced emoji based on their performance relative to their staircase. Real-time feedback is processed within the duration of a single TR immediately after the TR's DICOM file is uploaded to the high performance cluster, making the feedback signal almost instant. However, the inherent delay in hemodynamic responses means that feedback based on the initial TR of the wobbling image isn't available until the fourth TR. Therefore, during the lead-in phase, the first three TRs exhibit a fixed maximum level of wobbling. In the neurofeedback phase, the feedback signal is derived from TRs encompassing both the lead-in phase and selected TRs from the neurofeedback stage.

The assignment of objects to conditions was counterbalanced across participants in two batches. For the 10 participants in Batch 1, the presented object was bed, the competitor object was chair, and the control objects were table and bench (Fig. 1a). For the remaining 10 participants in Batch 2, the assignments were reversed with table as presented object, bench as the competitor object, and bed and chair as control objects.

We incorporated a built-in within-participant control mechanism. Specifically, neurofeedback training was conducted for one pair of objects (trained) while another pair of objects served as a no-neurofeedback baseline (untrained). Importantly, the assignment of which pair was trained and which was untrained was counterbalanced across participants. This within-participant control design allows each participant to serve as their own control, which is an effective approach to account for between-subject variability and internal biases. This methodology aligns with established research practices in the field [28], and ensures that the observed effects are specifically due to the neurofeedback intervention rather than other external variables.

Recognition runs

During each trial of the recognition runs (Fig. 1c), participants were presented with one of four rendered furniture objects (bed, bench, chair, table) in one of several potential viewing angles [35,36]. After 1 s, the object was removed from the screen and two furniture labels appeared below, from which participants had to choose which matched the object with an MRI-compatible button box. This response occurred during a jittered interval between trials of 4, 6, or 8 s. Each recognition run contained 48 trials, allowing for 12 repetitions per object and run. Three repetitions of each object appeared in each quarter of a run (no back-to-back repetitions), to ensure an even distribution of objects over time.

The data from the recognition runs in Session 1 were used to train six binary classifiers, corresponding to all combinations of the four objects. We used logistic regression classifiers with L2-norm regularization (penalty=1). Each of the six classifiers contrasted one pair of objects (e.g., chair vs. bench). The training data consisted of patterns of BOLD activity across voxels in a feedback ROI (described below) extracted 4 s after the onset of the object on each trial to account for the hemodynamic lag, labeled by the identity of the object.

Feedback runs

During each trial of the feedback runs (Fig. 1d), the presented object was shown dynamically on the screen, appearing to wobble in size and shape [29]. BOLD activity patterns were extracted from the feedback ROI and supplied as input to the classifiers. To determine the activation level of the competitor object (Fig. 1b), we averaged the output of the two classifiers trained to discriminate the competitor object from each of the control objects (i.e., competitor vs. control1; competitor vs. control2). Similarly, the activation level of the presented object was determined by averaging the output of the two classifiers trained to discriminate the presented object from each of the control objects (i.e., presented vs. control1; presented vs. control2). Note that we did not consider the output of the classifier directly pitting presented vs. competitor objects because we wanted separate estimates of the evidence for these objects. For example, if both were active, the classifier output may be at chance, but this result is also possible if neither is active. Instead, we rely on the control objects to provide a neutral baseline for both presented and competitor objects. We decided not to use the presented vs. competitor classifier because evidence would always favor the presented object being shown versus the competitor object being incepted. We feared a ceiling effect in which evidence for the presented object was at the noise ceiling, preventing us from obtaining meaningful variance in competitor evidence to be provided as neurofeedback. We felt that pitting the competitor object against the control objects would be a more equal footing, as none were available perceptually.

Participants received multiple forms of feedback to help motivate them to increase the activation level of the competitor object and foster its coactivation with the presented object, including visual feedback via wobbling, monetary feedback via an increase in their bonus compensation, and valenced feedback via an emoji. If participants successfully raised the activation level of the competitor above an adaptive threshold, the magnitude of wobbling decreased: wobbling began each feedback trial (consisting of 5 TRs) at level 13 (maximal), and reduced to level 9 after 1 TR above the threshold, level 5 after 2 TRs above the threshold, and level 1 (minimal) after 3-5 TRs above the threshold. Participants also received a monetary reward and an emoji after the trial based on the final number of above-threshold TRs: 0 TRs means no money and an unhappy face; 1 TR means no money and a neutral face; 2-3 TRs means 5 cents bonus and a smiling face; and 4-5 TRs means 10 cents bonus and a laughing face. The reduction in wobbling provides the most immediate feedback at the level of individual fMRI time points. The smileys and monetary rewards provide summative feedback at the end of each multi-timepoint trial. The combination of multiple forms of feedback was to provide a timely and accurate indication of performance and to engage participants in a motivating task. The morphing degree ranges from 1 to 100, where 1 represents one end of the morphing axis and 100 the opposite end. In the feedback morphing animation, four specific morphing degrees (13, 9, 5, 1) were set to reflect the subject's proficiency in activating the competitor activation. The sequence of images presented for these four morphing degrees can be found in the detailed files provided at `https://github.com/KailongPeng/real_time_paper/tree/main/morphingDegree` (this link).

Participants were informed about these types of feedback and that the feedback depended on their performance in the task. However, they were not instructed that the feedback was based on competitor activation, nor were they instructed on what mental strategy to use. Instead, they were instructed to explore different strategies that seemed to improve feedback. After the study, participants completed a debriefing questionnaire.

The threshold used for determining feedback was adjusted dynamically using an adaptive staircase procedure (Supplementary Table 1). The goal in using staircasing was to start participants at a difficulty level they could achieve at the beginning during their strategy exploration, but then to increase difficulty across runs and sessions such that they would be incentivized to activate the competitor object more and more strongly as they gained control of the feedback. When participants exhibited poor performance, the threshold was decreased, giving them an opportunity to improve and catch up. Conversely, the threshold was increased to create room for further improvement when participants demonstrated higher levels of control.

Feedback ROI

The BOLD activity patterns used to train and test the object classifiers were extracted from a data-driven region of interest (ROI). To define this feedback ROI, we used the neuroSketch dataset [36], in which the same four objects were shown multiple times to other participants. Each of the 300 parcels in the Schaefer atlas [37] classified as gray matter by Freesurfer [38] were retained for further analysis. Individual classifiers were trained for each parcel, and their test performance was quantified using a leave-one-run-out methodology. The performance from each parcel was averaged across all 25 participants in the neuroSketch dataset, resulting in a ranking of parcel performance. To identify the set of parcels that yielded the best performance, we built a mega ROI adding in the voxels from the top-N parcels and re-calculating test performance for each value of N using a leave-one-run-out approach. The mega ROI composed of the 78 highest-performing parcels yielded the best object classification performance in the neuroSketch dataset.

This mega ROI with 78 parcels served as the starting point for each participant in the current study, but was further refined per individual through a greedy approach. We first removed the voxels from one parcel (77 parcels remaining) and trained and tested a 4-way classifier on the recognition runs from Session 1 with leave-one-run-out cross-validation, and then iterated until all 78 parcels had been the one parcel left out; the iteration in which the remaining 77 parcels yielded the highest decoding performance was retained. Then we repeated the whole procedure, dropping one parcel (76 parcels remaining), iterating until all 77 remaining parcels had been left out, and then retaining the best set of 76 parcels. This process repeated until the voxels from only one parcel remained, yielding performance values for mega ROIs containing 1–78 parcels; the mega ROI with the best performance of all of these combinations was used as the feedback ROI for this participant. As a result, different participants had a different number of parcels in their mega ROI. However, there was good consistency in which parcels were selected, especially in the visual cortex (Fig. 2a).

Our design does not require a control ROI as we are not making any claim about where in the brain these training effects do vs. do not occur, hence the use of a large ROI that covers most task-related activations. Our conclusions pertain to the nature of the changes that occur within our ROI for trained vs. untrained objects.

Representational change ROIs

To examine how cortical coactivation related to representational change in the hippocampus, we segmented the hippocampus and its subfields anatomically for each participant using the automatic segmentation of hippocampal subfields (ASHS) software package [39] and a reference library of 51 manual segmentations [40,41]. These segmentations defined participant-specific ROIs for the bilateral hippocampus and subfields CA1, CA2/3, dentate gyrus, and subiculum. We also explored broader effects in the cortex by using Freesurfer [42] to create masks for V1, V2, lateral occipital (LO) cortex, inferior temporal (IT) cortex, fusiform gyrus (FG) and parahippocampal cortex (PHC).

Categorical perception task

To assess the impact of representational change on behavior, we conducted a categorical perception task [29,35] before and after neurofeedback training (in Sessions 1 before any fMRI data collection and 5 after all fMRI data collection, respectively). During this task, participants categorized images sampled from along a morph continuum between two object endpoints (e.g., bed to chair). Because the objects were rendered from 3-D models with a matching number of vertices, it was possible to smoothly morph between them. The morph percentage (of the second object) was sampled at 13 steps: 18 (i.e., 18% chair, 82% bed), 26, 34, 38, 42, 46, 50, 54, 58, 62, 66, 74, and 82. These morphs were shown 12 times each during both the pre-test and post-test, always from a trial-unique viewpoint. On each trial, participants were briefly presented (1 s) with the morph and asked to make a forced-choice judgment about which object they saw by clicking one of two buttons that appeared below the image. The assignment of labels to left vs. right buttons was randomized across trials. A logistic regression model was used to analyze the relationship between the morphing parameter and categorization responses:

$$p(x) = \frac{1}{1 + e^{-(x-\mu)/s}}$$

For the Session 1 categorical perception task, the slope and μ parameters were estimated. In Session 5, the μ value from Session 1 was fixed (to reduce noise and enhance model capability) and we estimated the slope. The change in slope indicates the type and degree of representational change between the two objects being discriminated. In particular, a decrease in slope (reduced discriminability) when comparing the presented and competitor objects would be consistent with integration of their representations, whereas an increase in slope (improved discriminability) would be consistent with differentiation. We thus defined a behavioral integration index as the Session 1 slope minus Session 5 slope (positive for integration, negative for differentiation). Importantly, these changes can be evaluated with respect to the analogous change observed for the untrained control objects.

Representational change analysis

We examined representational change in the hippocampus and elsewhere by comparing the overlap of neural patterns for the presented and competitor objects (using control objects 1 and 2 as a baseline) in Session 1 vs. Session 5. For Session 1, we built eight regularized logistic regression classifiers, each using seven of the recognition runs for training and the final recognition run for testing in a leave-one-run-out manner. The area under the ROC Curve (AUC) for each of the eight classifiers was averaged to derive an overall performance score. For Session 5, we tested the eight trained classifiers from Session 1 on the eight recognition runs and again averaged their AUCs to compute an overall score. Higher classification AUC indicates less neural overlap, and thus a decrease in AUC from Session 1 to Session 5 is consistent with integration and an increase in AUC is consistent with differentiation. We thus defined a neural integration index for each ROI as the Session 1 AUC minus Session 5 AUC (positive for integration, negative for differentiation).

Brain-behavior relationship

To the extent that categorical perception is a behavioral readout of neural overlap in one or more ROIs, the behavioral and neural integration scores should be positively correlated across participants. We quantified this association in each ROI by calculating the Pearson correlation coefficient.

Statistics

We used non-parametric statistics where possible to avoid assumptions of parametric tests. To estimate the sampling distribution of an effect, we performed bootstrap resampling at the group level. Namely, from the original sample of 20 participants, for each of 1,000 iterations we sampled 20 participants with replacement and averaged their values. The mean and 95% bounds of the resulting sampling distribution were used to generate the bar plot. For hypothesis testing, we determined the p-value as the proportion of iterations on which the average had the opposite sign from the original effect.

Data availability

Data and analysis code will be shared publicly upon acceptance and are available for the review process at: https://drive.google.com/drive/folders/17ZUIoqzzBoURJyPX0Wj0JlQ5ipl0WmmX (data) and https://github.com/KailongPeng/real_time_paper (code).