Conversion of object identity to object-general semantic value in the primate temporal cortex

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At the final stage of the ventral visual stream, perirhinal neurons encode the identity of memorized objects through learning. However, it remains elusive whether and how object perceives alone, or concomitantly a nonphysical attribute of the objects ("learned"), are decoded from perirhinal activities. By combining monkey psychophysics with optogenetic and electrical stimulations, we found a focal spot of memory neurons where both stimulations led monkeys to preferentially judge presented objects as "already seen." In an adjacent fringe area, where neurons did not exhibit selective responses to the learned objects, electrical stimulation induced the opposite behavioral bias toward "never seen before," whereas optogenetic stimulation still induced bias toward "already seen." These results suggest that mnemonic judgment of objects emerges via the decoding of their nonphysical attributes encoded by perirhinal neurons.

We daily encounter hundreds of visual objects and instantaneously evaluate whether they are safe or dangerous, friendly or hostile, delicious or toxic, or never seen before. In primates, visual input is analyzed along the ventral stream into constituent features to extract the visual identity of the object by which the representation in long-term memory is reactivated (1–5). The perirhinal cortex, the final stage of the stream, plays crucial roles in visual object discrimination (6) as well as in visual memory (4, 7, 8). Perirhinal neurons represent multiple attributes of objects, including complex combinations of visual features, linking of dissimilar objects, and object-reward association (4, 6, 7, 9). Notably, through learning, nearby perirhinal neurons often respond to physically dissimilar memorized objects (7, 10) (Fig. 1A).

By contrast, in early sensory areas such as the primary visual (V1) and middle temporal (MT) areas, principal neurons with selective responses to a similar physical attribute of objects (e.g., orientation or motion direction) are spatially clustered as a functional column (11, 12). Microstimulation of the clustered neurons has revealed that the physical attribute is decoded to drive sensory percepts associated with the attribute (13, 14). The apparent differences in the coding scheme between early visual areas and the perirhinal cortex raise the issue of what attributes the perirhinal neurons convey. Does the perirhinal cortex merely progressively relay physical attributes of object identity, such that only the sensory percept of objects is decoded for behavior? Or are nonphysical attributes that are encoded in perirhinal neurons also decoded under an appropriate behavioral context? Here, we addressed these two alternatives within a visual recognition paradigm, focusing on "learned" (a nonphysical attribute). We predict that if the first alternative is correct, then the experimentally excited cluster of neurons coding physically dissimilar objects would cause a subject to judge a presented object as novel, or the subject would not be able to make a judgment because intermingled physical attributes of memorized objects are activated; if the second alternative is correct, a subject would judge a presented object as seen before. To test these predictions, we optogenetically enhanced the output of perirhinal neurons (15–17), uncovering the causal link between the mnemonic coding of perirhinal neurons and the behavioral impact of their activities.

Monkeys were trained to discriminate whether a briefly presented (50 ms) visual cue was one of the previously memorized (OLD) objects or a trial-unique (NEW) object presented about once in a month (i.e., once in every 12,000 trials) (Fig. 1, B and C). To evaluate behavioral changes near the psychophysical threshold for OLD/NEW judgment, we obtained a series of perceived NEW/OLD valence of objects by masking cue objects with different levels of pixel-based random-dot dynamic noises (18, 19) (Fig. 1C). Single-unit recordings (Fig. 1, D to F) revealed that the response amplitudes of perirhinal neurons degraded as the OLD valence levels decreased (P = 2.84 × 10−10, Kruskal-Wallis test; Fig. 1F, top). However, their object selectivity was maintained across different valence levels (P = 0.691, Kruskal-Wallis test; Fig. 1F, middle and bottom), suggesting the recruitment of these neurons for object recognition near the psychophysical threshold.

We expressed Channelrhodopsin-2 (ChR2) in a population of excitatory neurons in the perirhinal cortex under the calcium/calmodulin-dependent protein kinase IIa (CaMKIIa) promoter (20–22) by injecting an adenovirus vector (AAV5-CaMKIIa-ChrR2-EYFP, where EYFP denotes enhanced yellow fluorescent protein) (fig. S1, A to D). In vivo fiber optic measurement (23) confirmed ChR2 expression around the location of virus injection (Fig. 1, G and H); subsequent histological analyses confirmed that ChR2 was expressed predominantly in excitatory neurons (Fig. 1, I to O, and fig. S1E). Using this in vivo measurement, we confirmed the expression of ChR2 in the respective behaving monkeys (fig. S2). Neurons were effectively excited by repetitive short-pulse optogenetic stimulation with 473-nm light optimal for ChR2 (24), with more than 50% efficacy of firing at 200-Hz stimulation (fig. S3).

Optogenetic stimulation during the task, relative to sham illumination, biased monkeys’ choices toward OLD objects across different NEW/OLD valence levels (Fig. 2. A to E), as indicated by significant leftward shifts of psychometric curves (13, 19). In 36 of 73 experimental sessions, optogenetic stimulation induced significant bias toward OLD choices (positive equi-NEW/OLD valence; P = 1.33 × 10−67 in χ² test against false positive rate of 5%), and there were no cases of significant bias toward NEW choices. As a result, the distribution of equi-NEW/OLD valence was significantly biased toward the positive direction (P = 2.97 × 10−12, Wilcoxon signed-rank test; Fig. 2E). This behavioral bias toward OLD choices was observed in the respective monkeys (fig. S4, A and B) (19). No behavioral changes were induced by stimulation with 594-nm light that was ineffective for ChR2 excitation (24) (P = 0.129, Wilcoxon signed-rank test; Fig. 2, F to H), nor by 473-nm light stimulation to neurons expressing only EYFP but not ChR2 (P = 0.938, Wilcoxon signed-rank test; fig. S5).

We further quantified the stimulation-induced bias toward OLD choices. The slope of psychometric curves that reflected the performance of monkeys’ discrimination was slightly flattened by stimulation (P = 0.00136, Wilcoxon signed-rank test; significant slope change in 9 of 73 experimental sessions, P = 0.00407 in χ² test against false positive rate of 5%; fig. S4, A and B). However, the effect size of slope flattening was significantly smaller than that of leftward shifts (P = 2.60 × 10−10, χ² test for numbers of experimental sessions with significant horizontal shift and slope change; fig. S4, A and B) (19). The optogenetic stimulation indeed induced behavioral bias toward OLD choices for judgments of both NEW and OLD objects (NEW, P = 8.59 × 10−11; OLD, P = 3.37 × 10−5; Wilcoxon signed-rank test with Bonferroni method; fig. S4C). Moreover, the stimulation-induced bias toward OLD choice did not accompany the increase in the total rewarded rate (fig. S4D), indicating that the behavioral change toward OLD choice occurred without any incentive for rewards (see fig. S4, E to H, for the time-series analysis of stimulation effects) (19).

We next examined whether and how these stimulation-induced behavioral changes were related to neuronal responsiveness to learned objects at the stimulation sites. First, we confirmed that the nearby perirhinal neurons with high

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Note: To confirm the expression of ChR2, we performed in vivo fiber optics measurement (23).
stimulus selectivity responded to learned objects distinct from each other (fig. S6). Then, we evaluated the neuronal representation at each stimulation site by calculating the density of OLD object-responsive neurons ($D_{\text{OLD}}$) and NEW object-responsive neurons ($D_{\text{NEW}}$) for each site. The optogenetic stimulation–induced bias did not depend on the relative dominance of $D_{\text{OLD}}$ and $D_{\text{NEW}}$ at the stimulation site (regression, $Y = -1.82X + 2.88$; Fig. 3A) (19).

We then tested the effect of conventional electrical stimulation. In contrast to the optogenetic stimulation, the electrical stimulation induced biases toward NEW choices as well as OLD choices (fig. S7). The induced choice bias was

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**Fig. 1. Optogenetic manipulation in macaque monkeys.** (A) Object coding in the ventral stream. In early visual cortices, nearby neurons selectively respond to objects with similar orientation. By contrast, in the perirhinal cortex, nearby neurons selectively respond to physically dissimilar complex objects. D, dorsal; V, ventral; A, anterior; P, posterior. (B) Behavioral task. Monkeys judged whether a cue object was one of the memorized OLD objects or a trial-unique NEW object. In half of the trials, the target perirhinal cortex, nearby neurons selectively respond to physically dissimilar complex objects (fig. S6). Then, we evaluated the neuronal representation at each stimulation site by calculating the density of OLD object-responsive neurons ($D_{\text{OLD}}$) and NEW object-responsive neurons ($D_{\text{NEW}}$) for each site. The optogenetic stimulation–induced bias did not depend on the relative dominance of $D_{\text{OLD}}$ and $D_{\text{NEW}}$ at the stimulation site (regression, $Y = -1.82X + 2.88$; Fig. 3A) (19).

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Fig. 2. Optogenetic stimulation of perirhinal neurons biases monkeys’ recognition judgment. (A and B) Schematic of optogenetic stimulation experiments. (A) Two optical channels were used for optogenetic stimulation as well as sham illumination to prevent the monkeys from using external cues to discriminate between stimulated and nonstimulated trials. (B) The order of stimulated and nonstimulated (sham-illuminated) trials was randomized. (C and D) Representative psychometric curves from two monkeys stimulated with 473-nm light optimal for exciting ChR2. Positive values show that the optogenetic stimulation biases monkeys’ judgments toward the OLD choices. Experimental sessions showing a significant horizontal shift (P < 0.05) are indicated by colored bars (†). Visual NEW/OLD valence (%). **S**timulation with 594-nm light was less effective than stimulation with 473-nm light (**Fig. 3A**). The amplitudes of neuronal responses to OLD objects (Max response) and DOLD were higher in the anterior region (**Fig. 4B** and fig. S10B; see fig. S10A for spatial distribution of behavioral effects). An unsupervised cluster analysis with four parameters (DOLD, DNEW, Max response, and the longitudinal coordinate) revealed two clusters that were mainly segregated along the longitudinal axis (fig. S11, A and B) (**19**). Electrical stimulation exerted the opposite behavioral effects along the axis: Behavioral biases toward OLD and NEW choices were induced by stimulation of the anterior and posterior subregion, respectively (**Fig. 4C**, lower panels, P = 0.0453 for anterior and P = 2.79 × 10⁻⁶ for posterior subregion, Wilcoxon signed-rank test with Bonferroni correction).
Fig. 3. Relationship between neuronal activity and stimulation-induced behavioral bias. (A) Main panel: Scatterplot of the behavioral effects of optogenetic and electrical stimulations as a function of normalized difference in the density of OLD object–responsive neurons \( D_{\text{OLD}} \) to that of NEW object–responsive neurons \( D_{\text{NEW}} \) in the anterior and posterior subregions (Fig. 4C), significantly different in the posterior subregion but not in the anterior subregion (Fig. 4C, \( P = 3.39 \times 10^{-6} \) for posterior, \( P = 1.00 \) for anterior; Wilcoxon rank sum test with Bonferroni method; see also fig. S13 and S14). Neither optogenetic nor electrical stimulations showed significant behavioral effects when applied at the anterior and posterior peripheral ends (Fig. 4D). Notably, neuronal responses to OLD objects were higher in the anterior focal spot, and stimulus selectivity to OLD objects abruptly decreased at the adjacent fringe region.

Our results show that optogenetic activation of projection neurons in the perirhinal cortex induced a systematic increase in monkeys’ OLD choices, irrespective of the local object-feature coding of neurons at the stimulation site. These results suggest that activation of perirhinal projection neurons produces an object-general “learned” signal. It is unlikely that nonphysiological effects of the optogenetic stimulation could explain the observed effects. First, we used optogenetic stimulation parameters comparable to those used in previous studies (19, 26, 27). Second, our findings did not result from nonselective energy transfer or heating (Fig. 2, F to H, and fig. S5). Third, using these parameters, we observed a long-tailed distribution of behavioral effects on one side with the peak at near-zero (Fig. 2E); this skewed distribution has frequently been observed in previous electrical stimulation studies that examined behavioral effects near the threshold for effective versus ineffective stimulation (13). Our electrical stimulation parameters were comparable to those used in previous studies in the inferior temporal cortex (18, 28) but stronger than those used in early visual areas (13, 14), presumably reflecting an increase in the effective current amplitude for inducing behavioral effects along the ventral visual stream (29) and/or inhibitory-dominant circuits of the perirhinal cortex (30, 31).

Our findings also shed light on a hypothesis for the mechanism underlying object recognition in the perirhinal cortex: When encountering a “learned” object, a group of perirhinal neurons are activated and produce an object-general “learned” signal by referring to the specific memory of that object. It is unlikely that nonphysiological effects of the optogenetic stimulation could explain the observed effects. First, we used optogenetic stimulation parameters comparable to those used in previous studies (19, 26, 27). Second, our findings did not result from nonselective energy transfer or heating (Fig. 2, F to H, and fig. S5). Third, using these parameters, we observed a long-tailed distribution of behavioral effects on one side with the peak at near-zero (Fig. 2E); this skewed distribution has frequently been observed in previous electrical stimulation studies that examined behavioral effects near the threshold for effective versus ineffective stimulation (13). Our electrical stimulation parameters were comparable to those used in previous studies in the inferior temporal cortex (18, 28) but stronger than those used in early visual areas (13, 14), presumably reflecting an increase in the effective current amplitude for inducing behavioral effects along the ventral visual stream (29) and/or inhibitory-dominant circuits of the perirhinal cortex (30, 31).

method; \( P = 9.64 \times 10^{-4} \) for difference in behavioral effects between anterior and posterior subregions, Wilcoxon rank sum test with Bonferroni method (see also fig. S11, C and D, for the respective monkeys; see also fig. S12, C to F, for the time-series analysis of stimulation effects) (19). In sharp contrast, optogenetic stimulation showed the behavioral bias toward OLD choices consistently in both the anterior and posterior subregions (Fig. 4C; upper panels, \( P = 1.51 \times 10^{-4} \) for anterior and \( P = 8.46 \times 10^{-6} \) for posterior subregions, Wilcoxon signed-rank test with Bonferroni method); the difference between the anterior and posterior subregions was not statistically significant (\( P = 1.00 \), Wilcoxon rank sum test with Bonferroni method; see also fig. S11, E to J). Accordingly, the behavioral effects of optogenetic and electrical stimulations were significantly different in the posterior subregion but not in the anterior subregion (Fig. 4C, \( P = 3.39 \times 10^{-6} \) for posterior, \( P = 1.00 \) for anterior; Wilcoxon rank sum test with Bonferroni method; see also figs. S13 and S14). Neither optogenetic nor electrical stimulations showed significant behavioral effects when applied at the anterior and posterior peripheral ends (Fig. 4D). Notably, neuronal responses to OLD objects were higher in the anterior focal spot, and stimulus selectivity to OLD objects abruptly decreased at the adjacent fringe region.

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an architeconic principle of the brain by which the nonphysical attributes of object information are processed for semantic reasoning.

REFERENCES AND NOTES

19. See supplementary materials.

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Supplementary Materials

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Materials and Methods

Supplementary Text

Figs. 52 to 53

References (22–98)

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Faulty remembrance of objects past
The primate brain analyzes visual input along the ventral processing stream to extract the identity of an object. The final stage of this stream, the perirhinal cortex, plays a crucial role in object recognition. Tamura et al. systematically biased the judgments of monkeys in an old-new object recognition task by using either optogenetic or electrical stimulation. The monkeys judged an encountered object as familiar when the stimulation site was in a hotspot where memory neurons were clustered. However, at the hotspot's fringe region, where neurons lost selective responses to the learned objects, electrical microstimulation led the monkeys to mistakenly judge an object as never seen before. Science, this issue p. 687