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Characteristic and intermingled neocortical circuits encode different visual object discriminations

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Abstract

Synaptic plasticity and neural network theories hypothesize that the essential information for advanced cognitive tasks is encoded in specific circuits and neurons within distributed neocortical networks. However, these circuits are incompletely characterized, and we do not know if a specific discrimination is encoded in characteristic circuits among multiple animals. Here, we determined the spatial distribution of active neurons for a circuit that encodes some of the essential information for a cognitive task. We genetically activated protein kinase C pathways in several hundred spatially-grouped glutamatergic and GABAergic neurons in rat postrhinal cortex, a multimodal associative area that is part of a distributed circuit that encodes visual object discriminations. We previously established that this intervention enhances accuracy for specific discriminations. Moreover, the genetically-modified, local circuit in POR cortex encodes some of the essential information, and this local circuit is preferentially activated during performance, as shown by activity-dependent gene imaging. Here, we mapped the positions of the active neurons, which revealed that two image sets are encoded in characteristic and different circuits. While characteristic circuits are known to process sensory information, in sensory areas, this is the first demonstration that characteristic circuits encode specific discriminations, in a multimodal associative area. Further, the circuits encoding the two image sets are intermingled, and likely overlapping, enabling efficient encoding. Consistent with reconsolidation theories, intermingled and overlapping encoding could facilitate formation of associations between related discriminations, including visually similar discriminations or discriminations learned at the same time or place.

Keywords

Visual discrimination learning; Neocortical circuits; Characteristic circuits; Overlapping encoding; Activity-dependent gene imaging

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Conflict of interest

AIG has equity in Alkermes Inc.

1. Introduction

Essential information for performing advanced cognitive tasks is encoded in distributed networks that span multiple forebrain areas, and, further, synaptic plasticity and neural network theories hypothesize that the essential information for specific discriminations is encoded in specific circuits [1–3]. [For this study, essential information is operationally defined as information that is required for performance; essential information is likely part of a memory trace.] Nonetheless, the critical circuits that encode specific discriminations remain incompletely characterized. We have limited knowledge about the size, neuron types, and spatial distribution of these critical circuits, or if a specific discrimination is encoded in a characteristic circuit, a similar circuit, among different animals, or in different circuits among multiple animals. Further, reconsolidation and spreading activation theories hypothesize that similar discriminations are encoded in intermingled or overlapping circuits, facilitating associations between specific memories [4]. But we have limited knowledge about the relative spatial locations of specific circuits that encode similar discriminations, although these locations may be critical for forming associations between similar discriminations. In this study, we used a genetically-modified circuit that encodes essential information for a cognitive task to examine the spatial distributions of the neurons that encode specific discriminations.

Visual object discrimination learning is encoded in a distributed rat neocortical network which spans a critical multimodal associative area, postrhinal (POR) cortex, that is required for this learning, as shown by lesioning studies [5–7]. POR cortex receives afferents from primary and secondary visual areas, and projects to multiple neocortical areas [8,9]. Of note, within a specific neocortical area, local circuits have a columnar organization, as initially established for primary visual cortex in classical work by Drs. Hubel and Wiesel [10], and subsequently extended to other areas involved in visual processing [11,12]. Thus, for the local circuits in POR cortex that encode specific visual discriminations, we examined the spatial distributions of the active neurons.

To enable studies on the encoding of specific visual discriminations in specific local circuits in POR cortex, we established a genetic intervention that targets some of the essential information for performing specific discriminations to the genetically-modified circuit in POR cortex. We showed that delivery of a constitutively active protein kinase C (PKC; via a virus vector) into several hundred spatially-grouped glutamatergic and GABAergic neurons in rat POR cortex activates PKC pathways, increases activation-dependent release of both glutamate and GABA, and supports faster, more accurate learning of new visual object discriminations [13,14]. In contrast, this intervention does not affect performance on control discriminations learned before gene transfer. Importantly, some of the essential information for performing specific discriminations is encoded in the genetically-modified, local circuit in POR cortex [7]: After gene transfer and learning, creation of small neurochemical lesions that ablate the genetically-modified circuit (~21% of POR cortex) selectively reduces performance for only discriminations learned after gene transfer. Given that this local circuit encodes essential information for specific discriminations, we examined the activity of this circuit during the learning, and found that this circuit is preferentially activated [7,13]: Using activity-dependent gene imaging, we showed that before learning, the genetically-modified

circuit exhibits minimal activity, establishing that the constitutively active PKC, alone, does not increase activity. Visual learning increases activity throughout POR cortex, and the genetically-modified circuit exhibits larger increases in activity, but only during performance of new discriminations learned after gene transfer. This critical local circuit contains ~500 neurons and is sparse-coded, with a coding density of ~3%.

As the local, genetically-modified circuit in POR cortex encodes essential information for specific discriminations and is preferentially activated during performance, here, we determined the spatial distributions of the neurons in this circuit, by mapping the positions of the active neurons, using activity-dependent gene imaging. Specific discriminations are encoded in characteristic circuits, and different discriminations are encoded in different circuits. This is the first demonstration that characteristic circuits encode specific discriminations in a multimodal associative area. Further, different discriminations can be encoded in intermingled, and likely overlapping, circuits, supporting efficient encoding. Consistent with reconsolidation theories, this proximal encoding of related discriminations could facilitate formation of associations between these discriminations.

2. Materials and methods

2.1. Vectors and packaging

These previously described vectors coexpress *Lac Z* and either a constitutively active PKC (Pkc vector, pINS-TH-NFHpkc \INS-TH-NFHIac), or an enzymatically inactive control (Pkc GG vector, pINS-TH-NFHpkc GG\INS-TH-NFHIac) [13,15]. pkc contains the flag tag fused to the 5' end of the catalytic domain of rat PKC β II (bp 994 to the 3' end) [16]. For Pkc GG, an absolutely conserved lys, required for phosphoryl transfer, was replaced with a gly residue [16,17]. Pkc exhibits a substrate specificity similar to rat brain PKC, and Pkc GG lacks protein kinase activity [16]. These vectors contain a neuron-specific promoter, composed of the chicken β -globin insulator (INS; 1.2 kb), an enhancer from the rat tyrosine hydroxylase promoter (TH; -0.5 kb to -6.8 kb), and a mouse neurofilament heavy gene promoter (NFH; 0.6 kb) [18]. The INS-TH-NFH promoter supports expression in forebrain neurons for 14 months, the longest time assayed, and time courses in the striatum and hippocampus showed expression in similar numbers of cells at times approximating the beginning or end of the visual testing [18,19]. Pkc mimics naturally occurring activation of PKCs; constitutively active PKCs are produced by calpain [20], and constitutively active PKM ζ is genetically-encoded, although it is not required for multiple learning tasks [21,22].

Helper virus-free Herpes Simplex Virus (HSV-1) vector packaging, purification, and titering were performed using standard procedures [23–25] (titers, $5.0 \pm 0.1 \times 10^6$ (mean $\pm$ s.e.m.) infectious vector particles/ml). For injections, titers of different stocks were matched by dilution. No HSV-1 (< 10 plaque forming units/ml) was observed in these stocks. This helper virus-free HSV-1 vector system causes minimal side effects [13,23,7].

2.2. Visual testing apparatus

Twelve operant chambers were used to perform the testing [26]. Each box (FIS units, Plas Labs) contained a computer-controlled apparatus, an overhead light, speaker, and a fan on the rear panel, which provided ventilation and masking white noise. Each operant chamber was a clear plastic rat cage (46 l × 20H × 24 D cm) with an infrared touchscreen (IRFP-10.4, Elotouch Systems) mounted in the center of one of the long sides and placed in front of a computer monitor. A liquid feeder (ENV-110 and ENV-201A, Med. Associates) was placed on the center of the wall opposite the monitor, and a lever (ENV-110, Med. Associates) was mounted below the feeder. Throughout the experiment, a blind code was used to identify the rats. For additional details, see [26] methods.

2.3. Simultaneous two object visual discrimination testing

These studies were approved by the LSUHSC New Orleans Institutional Animal Care and Use Committee (IACUC), and the VA Boston Healthcare System IACUC. The computer-controlled visual testing apparatus and procedure have been described [13,26]: Rats began each trial by pressing the lever. The lever press signaled two images to appear on the monitor. The left/right location of the images was counterbalanced over blocks of 20 trials and pseudo-randomly varied within each block with the constraint of three or fewer consecutive presentations on one specific side. When a rat pressed the touchscreen in the position corresponding to the correct stimulus, the feeder provided a 0.1 s milk reward, and after four consecutive correct responses, the reward amount was doubled until an error; also, following a correct response, the speaker emitted a 1 s sound. Immediately after an incorrect response, a large solid rectangle (23.5 × 10.1 cm) flashed three times on the monitor and then the overhead operant chamber light was turned off for 15 s. A correction procedure was employed; after every fourth incorrect response, that trial was repeated. Each session contained 120 discrimination trials or lasted 1 h, whichever occurred first. The rats were trained 7 days a week, 1 session per day. Three image sets were tested; each contained two images of the same size: ≡ vs. |||| vs. ||||, 7 alternating white and black bars, each one 1.6 (all lengths in cm) wide and 9.5 long; [] vs. +, [] 6.7 external side and 0.6 wide; + 6.4 long and 1.3 wide for each bar; / vs. \ 8.3 long and 1.0 wide with a 45° or 135° angle between the long sides [13,26]. Male Long Evans rats (initially ~6 weeks old; Charles River) were used in these studies. They were maintained on moderate food deprivation (8 gm/rat/day) during testing periods, but during the surgery period food was available *ad libitum*; water was available *ad libitum* throughout the experiment.

2.4. Gene transfer

Rats received vector stocks via stereotactic injections (3 µl/site, 2 sites, 1/hemisphere) into POR cortex; the coordinates were anterior-posterior (AP) −8.0, medial-lateral (ML) ± 6.0, and dorsal-ventral (DV) −5.2 [7,13,14]. For rats that received Pkc and testing on / vs. \, the injection sites were −7.5 AP, ML and DV as above [7]; this small difference in injection site does not alter accuracy on / vs. \ [7,13,14]. AP was relative to bregma, ML was relative to the sagittal suture, and DV was relative to the bregma-lambda plane [27]. All injections were performed using a micropump (Model 100, KD Scientific). Vector stocks were slowly

injected over a five-minute period, and after an additional five minutes, the needle (33 gauge) was gradually retracted.

Using these gene transfer conditions, POR cortex contains the vast majority of the transduced cells [13]. POR cortex contained several hundred, spatially-grouped, transduced cells; > 90 are neurons, 52 or 45% are glutamatergic or GABAergic, < 1% are cholinergic, and no catecholaminergic neurons are observed [13]. Only low numbers of transduced cells (1–4 cells) were observed in specific neocortical areas with large projections to POR cortex, including perirhinal cortex [13], and no transduced cells were observed in any of the subcortical areas that were examined, including the hippocampus, specific cholinergic basal forebrain areas, amygdala, and specific catecholaminergic mid-brain areas [13].

This intervention affects neuronal physiology and learning. Phosphorylation levels are increased for five PKC substrates with critical roles in synaptic plasticity, including a signal transduction protein (MARCKS), two receptors essential for glutamatergic neurotransmission and learning (AMPA GluR2 and NMDA NR1 subunits), and two proteins that have central roles in neurotransmitter release (GAP-43 and Dynamin) [13]. Further, Pkc supports activation-dependent increases in neurotransmitter release [13,16]. Importantly, both the learning rate and steady-state accuracy are increased for two image sets learned after gene transfer (□ vs. + or / vs. \), but performance is not altered for a control image set (≡ vs. ||| vs. ||) learned before gene transfer [13,14].

Some of the essential information for performing specific discriminations is encoded in the genetically-modified, local circuit in POR cortex [7]. After Pkc⁻ gene transfer and learning, creation of small neurochemical lesions that ablate the genetically-modified circuit (~ 21% of POR cortex) selectively reduces performance for only discriminations learned after gene transfer. In contrast, performance is not affected on a control discrimination learned before gene transfer. As another control, after gene transfer using Pkc^{GG}, learning, and lesioning, rats do not exhibit deficits on image sets learned either before or after gene transfer. Further, the deficit in performance is positively correlated with the size of the lesion, for Pkc⁻-rats and image sets learned after gene transfer, but not for Pkc⁻-rats and image sets learned before gene transfer, nor for Pkc^{GG}-rats and image sets learned either before or after gene transfer [7].

During learning, the genetically-modified circuit is preferentially activated, as shown by activity-dependent gene imaging (*c-fos*-immunoreactivity (IR), *Arc*-IR *Zif268*-IR) [7,13]. The observed changes likely reflect circuit activity, rather than coincidental induction of three immediate early genes by independent mechanisms unrelated to neuronal activity. Using stereology, *c-fos*-IR and *Arc*-IR densities were quantified in column-shaped volumes, extending from the surface of POR cortex to the white matter. Pkc⁻ alone did not induce neuronal activity; control rats that received Pkc⁻, but no visual testing, exhibited only very low levels of *c-fos*-IR [13]; this control also showed that the gene transfer procedures did not activate circuits. Visual learning increased activity throughout POR cortex. Importantly, the genetically-modified circuit exhibited larger increases in activity (2.7- or 2.5-fold, *c-fos*-IR or *Arc*-IR) during performance of new image sets learned after gene transfer [7,13]. Most of the transduced neurons were in an ~ 200 μm wide volume, consistent with the area of

increased *c-fos*-IR. The increased activity required learning a new image set ([vs. +), and was not observed in rats that received Pkc and retesting on a control image set learned before gene transfer [13]. Also, the increased activity proximal to the injection site required Pkc, and was not observed in control rats (Pkc GG, *Lac Z*, PBS) tested on a new image set ([vs. +). *c-fos*-IR cell counts showed that the critical circuit (defined by the lesion study [7]) contains ~500 neurons and is sparse-coded, with a coding density of ~3% [7]. Moreover, wild type (wt, no surgery) rats, after learning, contain similar circuits in POR cortex with increased activity relative to the remainder of POR cortex, but located in different positions in POR cortex among the rats.

Delivery of Pkc into a specific brain area and knockout mice harboring deletions in specific PKC genes differ in four critical properties, enzyme activity, affected neurons, subcellular location, and feedback control of gene expression. Of note, delivering Pkc into four different brain areas causes different changes in learning or behavior [13,16,28–30]. In contrast, PKC β or PKC γ knockout mice display deficits in fear conditioning [31] or specific learning paradigms [32], respectively. These different behavioral changes for Pkc -rats and specific PKC knockout mice are due to the differences between the interventions.

2.5. Experimental design

The design is summarized in Fig. 1a. The initial training to familiarize the rats with the apparatus was performed as previously described [26]. The rats were then trained on a control image set ($\equiv$ vs. $\parallel$ vs. $\llcorner$). Any differences in learning ability between the rats was controlled for by grouping the rats into sets of three based on the order in which they learned the initial discrimination and reached gene transfer: One rat received Pkc, one rat received no gene transfer/no surgery (wt rat), and one rat received the control vector (Pkc GG); within each set of three rats, the group for each rat was randomly chosen. After gene transfer (wt rats experienced a no-testing period similar in length to the gene transfer period), the rats were retested on the control image set learned before gene transfer, then trained on one new image set, either [vs. + or / vs. \, for 10 sessions, and sacrificed. Rats were sacrificed 30 min after the last session, when maximum *c-fos*-IR levels are observed [13].

Rats that met the learning criteria were analyzed for the positions of active neurons within POR cortex. Because rats that receive Pkc learn to a higher accuracy than control rats (wt or Pkc GG) [13,14], the criteria for inclusion used a modestly higher% correct for Pkc -rats than control rats: For rats that received Pkc, the learning criteria were 75% correct by session 5, 80% correct by session 6, and at least five sessions 75% correct. For rats that received either no surgery (wt rats) or Pkc GG, the criteria were 70% correct by session 6, and five sessions 70% correct. The rats reported in this study were obtained from 8 independent sets of rats tested over a > 24 month period; some rats in some sets were used for other experiments not reported here. Learning data analysis was performed by computer [13,26].

2.6. Histological analyses

Rats were perfused with phosphate buffered saline (PBS; 50 ml) and then 4% paraformaldehyde in PBS (200 ml). The brains were postfixed in 4% paraformaldehyde in

PBS (4 °C, 4 h), cryoprotected in 25% sucrose in PBS (4 °C, 2 days), and 25 µm coronal sections were prepared using a freezing microtome.

Immunohistochemistry was performed on free-floating sections. Sections were incubated in PBS containing 0.3% H₂O₂ (room temperature, 10 min) and then rinsed in PBS (5 min, 3 times). To permeabilize the sections, they were incubated in PBS, 2% normal goat serum, 0.2% Triton X-100 (buffer A; 37 °C, 30 min), and then incubated overnight at 4 °C in buffer A containing rabbit anti-*c-fos* (1:5000 dilution; Calbiochem). Sections were rinsed (PBS containing 0.2% Triton X-100, buffer B; room temperature, 3 times, 10 min each), and then incubated in buffer B containing biotinylated goat anti-rabbit IgG (1:200 dilution, Vector Laboratories; room temperature, 2 h). Sections were rinsed with buffer B (room temperature, 3 times, 10 min each), incubated with the ABC reagent (Vector Laboratories; room temperature, 1 h), and rinsed with PBS. IR was visualized with diaminobenzidine according to the manufacturer's instructions (Vector Laboratories).

Thirty-one rats were analyzed for this study. Fifty-nine hemispheres yielded *c-fos*-IR, and three hemispheres were discarded for technical reasons, such as poor perfusion or poor IR; one hemisphere was discarded from each of the Pkc / vs. \, wt [] vs. +, and wt / vs. \ groups. The numbers of hemispheres and rats in each group are detailed in the text.

The positions of the *c-fos*-IR positive neurons in POR cortex were determined using the StereoInvestigator program (MBF BioScience, Inc). For each section, under low power, the section was outlined, and then, under high power (60× magnification), POR cortex was outlined with reference to known landmarks [13,27,33]. The positions of virtually all the *c-fos*-IR positive neurons within the POR cortex contour were marked by systematically scanning through the contour, using the StereoInvestigator program.

For serial section reconstructions, the data were imported into the Neurolucida program (MBF BioScience, Inc), and the sections for each hemisphere were aligned.

A canonical POR cortex was constructed from three hemispheres. For each hemisphere, each of the sixty 25 µm coronal sections that contained POR cortex was stained with cresyl violet. For each section, the Neurolucida program was used to draw the section border under low power and the border of POR cortex under high power (60× magnification) [13,27,33]. Next, for each of the 60 sections, the three corresponding sections from each of the three hemispheres were superimposed, an average, canonical section was outlined, and the border of POR cortex contour was outlined. For each canonical section, the surface of POR cortex was marked at 10–15 points.

Using the StereoInvestigator program, each section containing *c-fos*-IR neuron positions was placed into its corresponding canonical POR cortex section. Each section was adjusted for best fit to the canonical section in the medial-lateral and dorsal-ventral dimensions. Next, the positions of the *c-fos*-IR neurons and the positions of the markers for the surface of POR cortex were exported into Excel files for further analyses.

The distance of each *c-fos*-IR neuron from the surface of POR cortex was calculated using macros in Excel. A cubic polynomial was used to model the surface of POR cortex, as the

least-squares best fit to the positions of the markers for the surface of POR cortex. The shortest distance between the polynomial and the position of each *c-fos*-IR neuron was calculated. These distances were placed into 50 μm wide bins, and, for each hemisphere, a graph of the number of *c-fos*-IR neurons in each bin was generated.

For each hemisphere, the ratio of the number of active neurons in the superficial to deeper layer was calculated. The boundary between the superficial and deeper layer was defined as 600 μm from the surface of POR cortex. For the superficial and deeper layers, we identified the four contiguous bins that together contained the largest number of active neurons, and included the bin with the highest value. The numbers of active neurons in these four bins was calculated for the superficial and deeper layers, and ratios were calculated. The inter-layer areas were defined as the other bins.

2.7. Statistical analyses

Statistical analyses were conducted using between groups or repeated measures ANOVAs (Sigmastat).

3. Results

3.1. The genetically-modified circuit contains a bilaminar pattern of active neurons

These experiments used our established design (Fig. 1A) [7,13,14]: Rats learned a control image set before gene transfer ($\equiv$ vs. $|||$ vs. $|||$). Next, gene transfer into POR cortex delivered either a constitutively active PKC (Pkc [13]) or a control vector (Pkc GG, a point mutation lacking enzyme activity); a comparison group was no surgery (wt) rats. After gene transfer/no surgery, rats were retested on the control image set learned before gene transfer, and then tested on one new, experimental image set, either $[]$ vs. $+$ or $/$ vs. $\backslash$. Following the last session (session 10), rats were sacrificed, and activity-dependent gene imaging was performed (*c-fos*-immunoreactivity (IR)). In rats that received Pkc , sections proximal to the injection site contained a bilaminar pattern of active neurons ($[]$ vs. $+$, Fig. 1B, high power in Fig. 1C. $/$ vs. $\backslash$, Fig. 1G, high power in Fig. 1H). One layer of active neurons was near the surface of POR cortex, in layers 2, 3, and the superficial part of layer 4; the second layer of active neurons was deeper, in the deeper part of layer 5 and in layer 6; the layers of POR cortex have been described [33]. In contrast, regions distant from the injection site, but within POR cortex, contained only low numbers of active neurons that were not arranged in a bilaminar pattern ($[]$ vs. $+$, Fig. 1D. $/$ vs. $\backslash$, Fig. 1I). The bilaminar pattern was confirmed by plotting the positions of all active neurons in serial section reconstructions that spanned the genetically-modified circuit ($[]$ vs. $+$, Fig. 1E. $/$ vs. $\backslash$, Fig. 1J).

The bilaminar pattern of active neurons was quantified by calculating the shortest distance from the surface of POR cortex to each active neuron. In each section, the surface of POR cortex was modeled using a polynomial, the position of each neuron was plotted, the shortest distance from the surface of POR cortex to each neuron was calculated, and the neuron positions were binned using 50 μm partitions (see methods). This quantitative method also revealed a bilaminar pattern of active neurons ($[]$ vs. $+$, Fig. 1F. $/$ vs. $\backslash$, Fig. 1K). Similar results were observed in additional rats ($[]$ vs. $+$, 12 hemispheres from 6 rats, Fig. 2. $/$ vs. $\backslash$,

13 hemispheres from 7 rats, Fig. 3). For all the rats in both the Pkc Δ/Δ vs. + and Pkc Δ/Δ vs. Δ groups, analyses of the shortest distance from the surface of POR cortex to each active neuron also revealed a bilaminar pattern (Fig. 4, top row).

For each hemisphere, the numbers of active neurons in each layer, and between the layers (inter-layer), were quantified. To give equal weight to each hemisphere, for each hemisphere, the numbers of active neurons was normalized to 100. Each layer was by defined as the bin containing the highest number of active neurons and the three contiguous bins that together yielded the highest number of active neurons; the inter-layer areas were defined as the other bins. The results showed that both the Pkc Δ/Δ vs. + and Pkc Δ/Δ vs. Δ groups contained two layers, and each layer contained statistically significantly higher numbers of active neurons than the inter-layer areas (Table 1. Δ/Δ vs. +: $F_{2,33} = 26.7$ $p < 0.001$ 1-way ANOVA; superficial layer vs. inter-layer $p < 0.001$, deeper layer vs. inter-layer $p < 0.01$, superficial layer vs. deeper layer $p < 0.001$. Δ/Δ vs. Δ : $F_{2,36} = 31.5$ $p < 0.001$; superficial layer vs. inter-layer $p < 0.001$, deeper layer vs. inter-layer $p < 0.001$, superficial layer vs. deeper layer $p > 0.05$).

3.2. In wt rats, the preferentially-activated circuit contains a bilaminar pattern of active neurons

We performed a similar analysis on wt rats. During visual learning, wt rats contain circuits in POR cortex with increased activity relative to the rest of POR cortex, but these circuits are located at different positions in POR cortex among the rats [7]. These preferentially-activated circuits exhibit similar properties as the genetically-modified circuits that encode essential information, specifically, similar numbers of active neurons and similar coding densities [7]. Thus, we hypothesized that the preferentially-activated circuits in wt rats would also contain a bilaminar pattern of active neurons. We examined wt rats, and found that specific sets of contiguous sections contained large numbers of active neurons, consistent with previous results [7], and these neurons were arranged in a bilaminar pattern (Δ/Δ vs. +, Fig. 5A. Δ/Δ vs. Δ , Fig. 5E). Control sections distant from the preferentially-activated circuits contained few active neurons (Δ/Δ vs. +, Fig. 5B. Δ/Δ vs. Δ , Fig. 5F). Serial section reconstructions of the regions containing the preferentially-activated circuits, also showed a bilaminar pattern of active neurons (Δ/Δ vs. +, Fig. 5C. Δ/Δ vs. Δ , Fig. 5G).

The bilaminar pattern of active neurons was quantified by calculating the shortest distance from the surface of POR cortex to each active neuron. This quantitative method also revealed a bilaminar pattern of active neurons (Δ/Δ vs. +, Fig. 5D. Δ/Δ vs. Δ , Fig. 5H). Similar results were observed in additional rats (Δ/Δ vs. +, 9 hemispheres from 5 rats in Fig. 6. Δ/Δ vs. Δ , 13 hemispheres from 7 rats, Fig. 7). For both the Δ/Δ vs. + and Δ/Δ vs. Δ groups, analyses of the shortest distance from the surface of POR cortex to each active neuron also showed a bilaminar pattern (Fig. 4, second row). Further, for each hemisphere, the numbers of active neurons in each layer, and between the layers (inter-layer), were quantified, as above. The results showed that both the Δ/Δ vs. + and Δ/Δ vs. Δ groups contained two layers that contained statistically significantly higher numbers of active neurons than the inter-layer areas (Table 1. Δ/Δ vs. +: $F_{2,24} = 12.0$ $p < 0.001$ 1-way ANOVA; superficial layer vs. inter-layer $p < 0.001$, deeper layer vs. inter-layer $p < 0.01$, superficial layer vs. deeper layer $p > 0.05$. Δ/Δ vs. Δ : $F_{2,36}$

= 30.5 $p < 0.001$; superficial layer vs. inter-layer $p < 0.001$, deeper layer vs. inter-layer $p < 0.002$, superficial layer vs. deeper layer $p < 0.001$).

3.3. In rats that received the control vector, the genetically-modified circuit contains few active neurons that are not arranged in a bilaminar pattern

For controls, we performed the same analysis on rats that received the control vector (Pkc⁻ GG), which lacks enzyme activity. Pkc⁻ GG-rats do not exhibit enhanced learning for image sets learned after gene transfer, nor do they exhibit increased activity in the genetically-modified circuit during this learning, nor do they encode essential information for these image sets in the genetically-modified circuit [7,13,14]. Thus, we hypothesized that the genetically-modified circuits in Pkc⁻ GG-rats would not contain a bilaminar pattern of active neurons. We examined rats that received Pkc⁻ GG, and found that specific sections proximal to injection sites contained only low numbers of active neurons ([vs. +, Fig. 8A. / vs. \, Fig. 8D), consistent with previous results [13], and these active neurons were not arranged in a bilaminar pattern. Serial section reconstructions of the regions containing the genetically-modified circuits, and analyses of the distances from the surface of POR cortex to the active neurons, confirmed the absence of a bilaminar pattern ([vs. +, Fig. 8B,C, Fig. 4 hemispheres from 2 rats in Fig. 9A. / vs. \, Fig. 8E,F, 8 hemispheres from 4 rats in Fig. 9B). For the hemispheres that received the control vector, Pkc⁻ GG, the average numbers of active neurons was <30% of the average numbers of active neurons in the hemispheres that received the experimental vector, Pkc⁺. Further, five of the twelve hemispheres that received the control vector each contained less than 65 active neurons. For the hemispheres that received the control vector, Pkc⁻ GG, the active neurons were not arranged in a bilaminar pattern (Fig. 4, bottom row). Atypically, one of the four rats that received Pkc⁻ GG and testing on / vs. \ contained significant numbers of active neurons proximal to the transduced neurons, which accounted for 79% of the active neurons in the Pkc⁻ GG-/ vs. \ group. Among control rats, the preferentially-activated circuit is located in different positions in POR cortex [7,13]; thus, we hypothesize that in this one Pkc⁻ GG-/ vs. \ rat, the preferentially-activated circuit overlapped by chance with the transduced neurons.

3.4. The two image sets are encoded in characteristic and different circuits

As detailed above, for each hemisphere in each group, we calculated the shortest distance from the surface of POR cortex to each active neuron. The results (Fig. 4, Table 1) showed that both image sets exhibited a bilaminar pattern of active neurons, the encoding is bilaminar. Of note, the bilaminar patterns were different for the two discriminations (Fig. 4): In the Pkc⁺ and wt groups, for the [vs. + discrimination, the superficial layer contained more active neurons than the deeper layer, but for the other discrimination, / vs. \, the superficial and deeper layers contained more similar numbers of neurons. To confirm that the bilaminar patterns in the Pkc⁺ and wt groups differed between the two discriminations, we quantified the differences. To give equal weight to each hemisphere, we used the data in which each hemisphere was normalized to 100 active neurons. For each image set, the Pkc⁺ and wt groups were similar ([vs. + or / vs. \ $p > 0.05$), and were combined. The distribution of distances from the surface of POR cortex to the active neurons was significantly different between the image sets (image set, $F_{3,1547} = 3.00$ $p < 0.035$; bin, $F_{32,1518} = 30.0$ $p < 0.001$; 2-way ANOVAs). Alternatively, to analyze the data without normalizing between

hemispheres, the ratio of the numbers of active neurons in the superficial layer to deep layer was calculated for each hemisphere (Table 2, see methods). Again, for each image set, the *Pkc* and wt groups were similar ($\square$ vs. + or/ vs. $\backslash$ $p > 0.05$), and were combined. Importantly, the results showed that $\square$ vs. + supported a larger ratio of active neurons in the superficial to deeper layer than / vs. $\backslash$ ($F_{1,45} = 4.49$ $p < 0.04$, 1-way ANOVA).

The ratios of the numbers of active neurons in the superficial to deep layer in Table 2 differ from the ratios that can be calculated using the data in Table 1. Tables 1 and 2 contain different calculations. For Table 2, the ratio was calculated for each hemisphere, and then the average for each group was calculated. In contrast, for Table 1, for each group, the average number of active neurons in each layer was calculated. Calculating the ratios from the average number of active neurons in each layer (Table 1) is not as informative as calculating the ratio for each individual hemisphere (Table 2).

4. Discussion

In this study, we mapped the positions of neurons in POR cortex that encode some of the essential information for specific visual object discriminations. The results showed that two visual object discriminations are encoded in local circuits in POR cortex that contain bilaminar patterns of neurons. Further, the two discriminations are encoded in characteristic and different circuits, which can be intermingled and overlapping.

The local circuits in POR cortex that encode essential information are activated during the learning of new visual discriminations, contain ~500 neurons, are sparse coded, and contain both glutamatergic and GABAergic neurons. The genetically-modified local circuit in POR cortex encodes some of the essential information for performance; following gene transfer, learning, and lesioning the genetically-modified circuit, *Pkc* -rats, but not control *Pkc* GG-rats, display deficits in performance selectively for image sets learned after gene transfer [7]. This local circuit is preferentially activated during learning of new image sets, as shown by imaging three activity-dependent genes (*c-fos*, *Arc*, *Zif268*) [7,13]. The critical local circuits contain an average of ~500 neurons, as shown by cell counts of the activity-dependent gene imaging [7]. The circuits are sparse coded, with a coding density of ~3%, as shown by two approaches: First, costaining for active neurons and all neurons in the circuit, and, second, a calculation based on the number of active neurons in the circuit, the volume of the circuit, the number of neurons in POR cortex, and the volume of POR cortex (assuming a uniform distribution of neurons in POR cortex) [7]. The circuit contains both glutamatergic and GABAergic neurons, as shown by costaining for transduced cells and neuron type-specific markers [13]. Correlatively, activity-dependent gene imaging detects activity in both glutamatergic and GABAergic neurons; in particular, following light or sensory stimulation, activity-dependent gene imaging visualizes activity in both neuron types in cat primary visual cortex [34–39]. Correlatively, physiological activation of circuits activates both neuron types. Although we have limited information about the wiring diagram for POR cortex, specific neurons are co-activated during a specific condition, learning a new image set, and they encode essential information: These neurons form a local circuit, consistent with the extensive connections between neurons in a neocortical column [40].

Within the local circuit, an identified subset of the neurons, the active transduced neurons, represent a significant fraction of the circuit. The critical local circuits contain an average of ~500 neurons [7], and one to several hundred neurons are transduced [13], but only some of the transduced neurons are part of the circuit: During learning of a new image set, learning-associated signaling pathways are activated in ~50% (CaMKII) or ~80% (MAP kinase and CREB) of the transduced neurons [14]. Thus, a majority of the transduced neurons are part of the circuit, and likely play an important role in encoding, see below.

Each image set is encoded in a characteristic and different pattern of active neurons among the rats, a bilaminar pattern with a specific ratio for the numbers of neurons in the superficial and deeper layers. The superficial layer of active neurons is near the surface of POR cortex, in layers 2, 3, and the superficial part of layer 4; and the deeper layer of active neurons is in the deeper part of layer 5 and in layer 6. As detailed above, these neurons are coactivated during learning and are part of a local circuit that encodes essential information for performing a specific discrimination.

These characteristic patterns of active neurons suggest that a specific image set may be encoded in similar circuits among the rats: Supporting this hypothesis, specific neocortical layers contain specific excitatory and inhibitory neuron subtypes, which receive characteristic feed forward and feedback projections, and form characteristic local and long range connections [41,42]. Thus, the different ratios of active neurons in the superficial and deeper layers may reflect the specific circuits that encode each image set, and the specific computations required to recognize each image set. Also supporting this hypothesis, animals typically attend to specific features of an image; in particular, rats tend to recognize specific image features near the bottom of images [43,44]. Thus, specific image features could be encoded in characteristic circuits among rats, producing the characteristic and different patterns of active neurons we observe for specific image sets. The present analysis for characteristic patterns of active neurons encoding specific image sets was performed in one dimension, the distances from the surface of POR cortex to the active neurons; a more sophisticated analysis of the neuron positions, in three dimensions, might yield additional features for each characteristic pattern. Nonetheless, this is the first report that a multimodal neocortical associative area encodes a specific discrimination in a characteristic circuit.

Neocortical sensory areas encode specific sensory information in characteristic local circuits. For example, in primary visual cortex, the same image, presented in the same place in the visual field, is processed by similar circuits among animals, due to the topographic organization of orientation and ocular dominance columns [10]. Further, inferior temporal cortex contains columns that recognize specific classes of images, independent of location in the visual field [12]. The topographic functional organization of neocortical sensory areas enables encoding of the same stimulus in similar circuits among animals [10]. In contrast, POR cortex, a multimodal associative area, appears to lack a topographic functional organization: For rats that received gene transfer, circuits located in different positions in POR cortex likely encoded the essential information, due to both inherent variation in the injection sites among the rats and because two different injection sites, in anterior or posterior POR cortex, support similar learning rates and accuracies [7]. Further, in wt rats, the preferentially-activated circuits are located in different positions in POR cortex among

the rats. Thus, the encoding process, and the specific image features that are encoded, appear to support encoding the same image in similar circuits among rats, located at different positions in POR cortex.

Multiple discriminations can be encoded in intermingled circuits that are likely also overlapping. The two image sets studied here can be encoded in proximal circuits; after gene transfer and learning of both image sets, small lesions that ablated the genetically-modified circuit caused deficits in performance for both image sets [7]. Further, there is likely significant overlap in encoding these two image sets: The critical circuits contain ~500 neurons, one to several hundred neurons are transduced [13], and during performance of one image set, three learning-associated signaling pathways (CaMKII, MAP kinase, CREB) are activated in a majority (50–80%) of the transduced neurons [14]. Thus, assuming similar neuron activation probabilities for each image set, then at least 25% of the transduced neurons are activated by both image sets, representing overlap in encoding. Overlap in encoding different discriminations is required for efficient use of available circuitry for encoding multiple discriminations. Of note, we showed that pigeons can maximally learn 800–1,200 images, and baboons can minimally learn 3500 to 5000 items [45]. Complementary, the two image sets can be encoded in intermingled circuits that likely contain large non-overlapping components; both image sets are sparse coded, with coding densities of ~3% [7], yielding a probability for coding overlap of $< 0.1\%$ ($0.03^2 = 0.0009$) for the neurons in the circuit that are not transduced. Of note, sparse coding, with significant differences between the specific circuits that encode different discriminations, is required for content-addressable memories in neural network theory [3].

Intermingled and overlapping encoding for different discriminations may enable formation of associations between similar discriminations. Repeated recall and reconsolidation is required for specific learning tasks and for forming associations between specific discriminations [4]. However, our knowledge is incomplete about the neural circuits and plasticity mechanisms that support these processes: For example, during reconsolidation of fear memories, specific circuits are activated, which are characterized at the level of specific brain areas [46], and during reconsolidation of spatial tasks, particular circuits containing specific hippocampal place cells are activated [47], but little is known about the relationship between circuits encoding different spatial tasks. In the present system, acquisition of visual object discriminations required repeated recall and reconsolidation; acquisition required ~5 sessions of ~120 trials a session [13,14]. Further, two discriminations that were learned concurrently were encoded in intermingled and overlapping circuits, which suggests possible circuit and plasticity mechanisms for forming associations between similar discriminations: With intermingled encoding, activation of one circuit might activate some neurons in a second circuit, and plasticity mechanisms could subsequently increase such coactivation by strengthening existing synapses and/or forming new connections. Further, overlapping circuits ensures that activation of one circuit will activate some neurons in the second circuit, and, again, plasticity mechanisms could then increase the coactivation. Thus, recall and reconsolidation of discriminations encoded in intermingled and/or overlapping circuits provides potential mechanisms for forming associations between the discriminations. These processes could support forming associations between visually similar discriminations or discriminations learned at the same time or place.

5. Conclusions

We genetically activated PKC pathways in several hundred spatially-grouped glutamatergic and GABAergic neurons in rat POR cortex, part of a distributed circuit that encodes visual object discriminations, and we mapped the positions of the active neurons. The results showed that two image sets are encoded in a bilaminar manner, in characteristic and different circuits. One layer of active neurons is near the surface of POR cortex, in layers 2, 3, and the superficial part of layer 4; the second layer of active neurons is deeper, in the deeper part of layer 5 and in layer 6. While characteristic circuits are known to process sensory information in sensory areas, this is the first demonstration that characteristic circuits encode specific discriminations, in a multimodal associative area. Further, the circuits encoding the two image sets are intermingled, and likely overlapping, enabling efficient encoding. Consistent with reconsolidation theories, intermingled and overlapping encoding could facilitate formation of associations between related discriminations, including visually similar discriminations or discriminations learned at the same time or place.

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References

1. Squire, LR., Kandel, ER. Memory. 2. Roberts & Company Publishers; Greenwood Village, CO, US: 2008.
2. Dudai, Y. The Neurobiology of Memory. Oxford Univ. Press, Oxford; England: 1989.
3. Rumelhart, DE., McClelland, JL., Group, PR. Parallel Distributed Processing. MIT Press; Cambridge MA: 1986.
4. Dudai Y. The neurobiology of consolidations or how stable is the engram? Annu. Rev. Psychol. 2004; 55:51–86. [PubMed: 14744210]
5. Mumby DG, Pinel JP. Rhinal cortex lesions and object recognition in rats. Behav. Neurosci. 1994; 108:11–18. [PubMed: 8192836]
6. Murray EA, Bussey TJ, Saksida LM. Visual perception and memory: a new view of medial temporal lobe function in primates and rodents. Annu. Rev. Neurosci. 2007; 30:99–122. [PubMed: 17417938]
7. Zhang G, Cao H, Kong L, O'Brien J, Baughns A, Jan M, Zhao H, Wang X, Lu X, Cook RG, Geller AI. Identified circuit in rat postrhinal cortex encodes essential information for performing specific visual shape discriminations. Proc. Natl. Acad. Sci. U.S.A. 2010; 107:14478–14483. [PubMed: 20660720]
8. Burwell RD, Amaral DG. Cortical afferents of the perirhinal postrhinal, and entorhinal cortices of the rat. J. Comp. Neurol. 1998; 398:179–205. [PubMed: 9700566]
9. Agster KL, Burwell RD. Cortical efferents of the perirhinal, postrhinal, and entorhinal cortices of the rat. Hippocampus. 2009; 19:1159–1186. [PubMed: 19360714]
10. Hubel DH, Wiesel TN. Ferrier lecture. Functional architecture of macaque monkey visual cortex. Proc. Royal Soc. Lond. - Ser. B. 1977; 198:1–59.
11. Felleman DJ, Van Essen DC. Distributed hierarchical processing in the primate cerebral cortex. Cereb. Cortex. 1991; 1:1–47. [PubMed: 1822724]
12. Fujita I, Tanaka K, Ito M, Cheng K. Columns for visual features of objects in monkey inferotemporal cortex. Nature. 1992; 360:343–346. [PubMed: 1448150]

13. Zhang G, Wang X, Kong L, Lu X, Lee B, Liu M, Sun M, Franklin C, Cook RG, Geller AI. Genetic enhancement of visual learning by activation of protein kinase C pathways in small groups of rat cortical neurons. *J. Neurosci.* 2005; 25:8468–8481. [PubMed: 16162929]
14. Zhang G, Zhao H, Choi EM, Svestka M, Wang X, Cook RG, Geller AI. CaMKII MAPK and CREB are coactivated in identified neurons in a cortical circuit required for performing visual shape discriminations. *Hippocampus.* 2012; 22:2276–2289. [PubMed: 22736516]
15. Wang X, Zhang G, Sun M, Geller AI. General strategy for constructing large HSV-1 plasmid vectors that co-express multiple genes. *Biotechniques.* 2001; 31:204–212. [PubMed: 11464513]
16. Song S, Wang Y, Bak SY, During MJ, Bryan J, Ashe O, Ullrey DB, Trask LE, Grant FD, O'Malley KL, Riedel H, Goldstein DS, Neve KA, LaHoste GJ, Marshall JF, Haycock JW, Neve RL, Geller AI. Modulation of rat rotational behavior by direct gene transfer of constitutively active protein kinase C into nigrostriatal neurons. *J. Neurosci.* 1998; 18:4119–4132. [PubMed: 9592092]
17. Hanks SK, Quinn AM, Hunter T. The protein kinase family: conserved features and deduced phylogeny of the catalytic domains. *Science.* 1988; 241:42–52. [PubMed: 3291115]
18. Zhang G, Wang X, Yang T, Sun M, Zhang W, Wang Y, Geller AI. A tyrosine hydroxylase–neurofilament chimeric promoter enhances long-term expression in rat forebrain neurons from helper virus-free HSV-1 vectors. *Molec. Brain. Res.* 2000; 84:17–31. [PubMed: 11113528]
19. Sun M, Kong L, Wang X, Holmes C, Gao Q, Zhang W, Pfeilschifter J, Goldstein DS, Geller AI. Coexpression of tyrosine hydroxylase GTP cyclohydrolase I, aromatic amino acid decarboxylase, and vesicular monoamine transporter 2 from a helper virus-free HSV-1 vector supports high-level, long-term biochemical and behavioral correction of a rat model of Parkinson's disease. *Hum. Gene Ther.* 2004; 15:1177–1196. [PubMed: 15684695]
20. Kikkawa U, Kishimoto A, Nishizuka Y. The protein kinase C family: heterogeneity and its implications. *Annu. Rev. Biochem.* 1989; 58:31–44. [PubMed: 2549852]
21. Volk LJ, Bachman JL, Johnson R, Yu Y, Huganir RL. PKM-zeta, is not required for hippocampal synaptic plasticity learning and memory. *Nature.* 2013; 493:420–423. [PubMed: 23283174]
22. Lee AM, Kanter BR, Wang D, Lim JP, Zou ME, Qiu C, McMahon T, Dadgar J, Fischbach-Weiss SC, Messing RO. *Prkcz* null mice show normal learning and memory. *Nature.* 2013; 493:416–419. [PubMed: 23283171]
23. Fraefel C, Song S, Lim F, Lang P, Yu L, Wang Y, Wild P, Geller AI. Helper virus-free transfer of herpes simplex virus type 1 plasmid vectors into neural cells. *J. Virol.* 1996; 70:7190–7197. [PubMed: 8794366]
24. Sun M, Zhang GR, Yang T, Yu L, Geller AI. Improved titers for helper virus-free herpes simplex virus type 1 plasmid vectors by optimization of the packaging protocol and addition of noninfectious herpes simplex virus-related particles (previral DNA replication enveloped particles) to the packaging procedure. *Hum. Gene Ther.* 1999; 10:2005–2011. [PubMed: 10466634]
25. Lim F, Hartley D, Starr P, Lang P, Song S, Yu L, Wang Y, Geller AI. Generation of high-titer defective HSV-1 vectors using an IE 2 deletion mutant and quantitative study of expression in cultured cortical cells. *Biotechniques.* 1996; 20:460–469. [PubMed: 8679207]
26. Cook RG, Geller AI, Zhang G, Gowda R. Touchscreen enhanced visual learning in rats. *Behav. Res. Methods Instrum. Comput.* 2004; 36:101–106. [PubMed: 15190705]
27. Paxinos, G., Watson, C. *The Rat Brain in Stereotaxic Coordinates.* Academic Press; Sidney: 1986.
28. Neill JC, Sarkisian MR, Wang Y, Liu Z, Yu L, Tandon P, Zhang G, Holmes GL, Geller AI. Enhanced auditory reversal learning by genetic activation of protein kinase C in small groups of rat hippocampal neurons. *Molec. Brain Res.* 2001; 93:127–136. [PubMed: 11589990]
29. Zhang G, Liu M, Cao H, Kong L, Wang X, Cook RG, Geller AI. Improved spatial learning in aged rats by genetic activation of protein kinase C in small groups of rat hippocampal neurons. *Hippocampus.* 2009; 19:413–423. [PubMed: 18942114]
30. Oh JD, Geller AI, Zhang G, Chase TN. Gene transfer of constitutively active protein kinase C into striatal neurons accelerates onset of levodopa-induced motor response alterations in parkinsonian rats. *Brain Res.* 2003; 971:18–30. [PubMed: 12691833]
31. Weeber EJ, Atkins CM, Selcher JC, Varga AW, Mirnikjoo B, Paylor R, Leitges M, Sweatt JD. A role for the beta isoform of protein kinase C in fear conditioning. *J. Neurosci.* 2000; 20:5906–5914. [PubMed: 10934237]

32. Abeliovich A, Paylor R, Chen C, Kim JJ, Wehner JM, Tonegawa S. PKC gamma mutant mice exhibit mild deficits in spatial and contextual learning. *Cell*. 1993; 75:1263–1271. [PubMed: 8269510]
33. Burwell RD. Borders and cytoarchitecture of the perirhinal and postrhinal cortices in the rat. *J. Comp. Neurol.* 2001; 437:17–41. [PubMed: 11477594]
34. Berretta S, Pantazopoulos H, Caldera M, Pantazopoulos P, Pare D. Infralimbic cortex activation increases c-Fos expression in intercalated neurons of the amygdala. *NeuroScience*. 2005; 132:943–953. [PubMed: 15857700]
35. Bertini G, Peng ZC, Fabene PF, Grassi-Zucconi G, Bentivoglio M. Fos induction in cortical interneurons during spontaneous wakefulness of rats in a familiar or enriched environment. *Brain Res. Bull.* 2002; 57:631–638. [PubMed: 11927366]
36. Miller CA, Marshall JF. Altered prelimbic cortex output during cue-elicited drug seeking. *J. Neurosci.* 2004; 24:6889–6897. [PubMed: 15295023]
37. Staiger JF, Masanek C, Bisler S, Schleicher A, Zschratte W, Zilles K. Excitatory and inhibitory neurons express c-Fos in barrel-related columns after exploration of a novel environment. *NeuroScience*. 2002; 109:687–699. [PubMed: 11927151]
38. Van der Gucht E, Clerens S, Cromphout K, Vandesande F, Arckens L. Differential expression of c-fos in subtypes of GABAergic cells following sensory stimulation in the cat primary visual cortex. *Eur. J. Neurosci.* 2002; 16:1620–1626. [PubMed: 12405976]
39. Van der Gucht E, Clerens S, Jacobs S, Arckens L. Light-induced Fos expression in phosphate-activated glutaminase- and neurofilament protein-immunoreactive neurons in cat primary visual cortex. *Brain Res.* 2005; 1035:60–66. [PubMed: 15713277]
40. Douglas RJ, Martin KA. Neuronal circuits of the neocortex. *Annu. Rev. Neurosci.* 2004; 27:419–451. [PubMed: 15217339]
41. Harris KD, Shepherd GM. The neocortical circuit: themes and variations. *Nat. Neurosci.* 2015; 18:170–181. [PubMed: 25622573]
42. Allene C, Lourenco J, Bacci A. The neuronal identity bias behind neocortical GABAergic plasticity. *Trends Neurosci.* 2015; 38:524–534. [PubMed: 26318208]
43. Minini L, Jeffery KJ. Do rats use shape to solve shape discriminations? *Learn. Mem.* 2006; 13:287–297. [PubMed: 16705141]
44. Lashley KS. The mechanism of vision: XV Preliminary studies of the rat's capacity for detail vision. *J. Gen. Psychol.* 18; 1938:123–193.
45. Fagot J, Cook RG. Evidence for large long-term memory capacities in baboons and pigeons and its implications for learning and the evolution of cognition. *Proc. Natl. Acad. Sci. U. S. A.* 2006; 103:17564–17567. [PubMed: 17088563]
46. Baldi E, Bucherelli C. Brain sites involved in fear memory reconsolidation and extinction of rodents. *Neurosci. Biobehav. Rev.* 2015; 53:160–190. [PubMed: 25887284]
47. Atherton LA, Dupret D, Mellor JR. Memory trace replay: the shaping of memory consolidation by neuromodulation. *Trends Neurosci.* 2015; 38:560–570. [PubMed: 26275935]

Fig. 1.

In rats that received Pkc⁺, during performance of an image set learned after gene transfer, the genetically-modified circuits contain bilaminar patterns of active neurons. (A) A time line showing the experimental design. Rats were trained on a control image set, gene transfer was performed, rats were retested on the control image set, trained on one new image set for 10 sessions, and sacrificed. Activity-dependent gene imaging (c-fos-IR) was performed. (B–F) Top row, [] vs. +. (G–K) bottom row, \ vs. /. (B, C, G, H) Near an injection site, low power (B, G), or high power (C, H); arrows, c-fos-IR cells. (D, I) POR cortex, away from an injection site. (E, J) Serial section reconstructions showing the positions of the active neurons in the genetically-modified circuits. (F, K) Distances from the surface of POR cortex to the active neurons. Scale bars: (B, D, G, I) 100 μm; (C, H) 25 μm; (E, J) 1 mm.

Fig. 2.

The distributions of distances from the surface of POR cortex to active neurons for individual hemispheres in rats that received PkcΔ followed by learning (□) vs. +. For each hemisphere, serial section reconstructions showing the positions of the active neurons in the genetically-modified circuits are on the left, and the distances from the surface of POR cortex to the active neurons are on the right. One rat is shown in each row.

Fig. 3.

The distributions of distances from the surface of POR cortex to active neurons for individual hemispheres in rats that received Pkc Δ followed by learning / vs. \. For each hemisphere, serial section reconstructions showing the positions of the active neurons in the genetically-modified circuits are on the left, and the distances from the surface of POR cortex to the active neurons are on the right. One rat is shown in each row. For one rat (bottom row), there were technical difficulties with the histology for the right hemisphere.

Fig. 4.

Specific image set are encoded in bilaminar patterns of active neurons. For each image set and condition, the distribution of the distances from the surface of POR cortex to the active neurons (numbers of hemispheres and rats per group are in the text).

Fig. 5.

In wt rats, during learning, preferentially-activated circuits in POR cortex contain bilaminar patterns of active neurons. (A–D) Top row, □ vs. +. (E–H) bottom row, / vs. \. (A, E) Sections containing preferentially-activated circuits, low power; arrows, *c-fos*-IR cells. (B, F) POR cortex, sections distant from the preferentially-activated circuits. (C, G) Serial section reconstructions showing the positions of the active neurons in the preferentially-activated circuits. (D, H) Distributions of the distances from the surface of POR cortex to the active neurons. Scale bars: (A, B, E, F) 100 μm; (C, G) 1 mm.

WT, □ vs. +

Fig. 6.

The distributions of distances from the surface of POR cortex to active neurons for individual hemispheres in wt rats that learned □ vs. +. For each hemisphere, serial section reconstructions showing the positions of the active neurons in the preferentially-activated circuits are on the left, and the distances from the surface of POR cortex to the active neurons are on the right. One rat is shown in each row. For one rat (bottom row), there were technical difficulties with the histology for the right hemisphere.

Fig. 7.

The distributions of distances from the surface of POR cortex to active neurons for individual hemispheres in wt rats that learned / vs. \. For each hemisphere, serial section reconstructions showing the positions of the active neurons in the preferentially-activated circuits are on the left, and the distances from the surface of POR cortex to the active neurons are on the right. One rat is shown in each row. For one rat (bottom row), there were technical difficulties with the histology for the left hemisphere.

Fig. 8.

In rats that received the control vector, Pkc GG, during performance of an image set learned after gene transfer, the genetically-modified circuits contain few active neurons that are not arranged in bilaminar patterns. (A–C) Top row, $\square$ vs. +. (D–F) bottom row, / vs. \. (A, D) Near an injection site, low power; arrows, *c-fos*-IR cells. (B, E) Serial section reconstructions showing the positions of the few active neurons in the genetically-modified circuits. (C, F) Distances from the surface of POR cortex to the active neurons. Scale bars: (A, D) 100 μ m; (B, E) 1 mm.

Fig. 9.

The distributions of distances from the surface of POR cortex to active neurons for individual hemispheres in rats that received $Pkc\Delta GG$ followed by learning an image set. For each hemisphere, serial section reconstructions showing the positions of the active neurons in the genetically-modified circuits are on the left, and the distances from the surface of POR cortex to the active neurons are on the right. One rat is shown in each row. (A) $\square$ vs. +; (B) / vs. \.

Table 1

Numbers of active neurons in the superficial or deep layer, and outside of these layers (inter-layer), for Pkc or no surgery rats that learned either [] vs. + or / vs. \.

Condition	Density per bin, %		
	superficial layer	deep layer	inter-layer
Pkc [] vs. +	8.7 ± 1.0	4.6 ± 0.6	2.0 ± 0.1
Pkc / vs. \	7.1 ± 0.7	5.9 ± 0.4	2.0 ± 0.1
WT [] vs. +	7.6 ± 1.1	5.3 ± 0.9	2.0 ± 0.1
WT/ vs. \	7.9 ± 0.8	4.7 ± 0.4	2.1 ± 0.1

For each section, the surface of POR cortex was modeled using a polynomial and the position of each neuron was plotted, the shortest distance from the surface to each neuron was calculated, and the neuron positions were binned using 50 µm partitions. To give equal weight to each hemisphere, for each hemisphere, the total number of active neurons was normalized to 100. Each layer was by defined as the bin containing the highest number of active neurons and the three contiguous bins that together yielded the highest number of active neurons. The inter-layer areas were defined as the other bins (see methods). All values, mean ± s.e.m.; Pkc , [] vs. + n = 12 hemispheres, / vs. \ n = 13 hemispheres; WT, [] vs. + n = 9 hemispheres, / vs. \ n = 13 hemispheres.

Table 2

Ratios of numbers of active neurons in the superficial and deeper layers for the different conditions and each image set.

Ratio of active neurons, superficial/deeper layer		
Condition	[] vs. +	/ vs. \
Pkc	3.7 ± 1.4	1.4 ± 0.2
WT	2.8 ± 1.1	1.7 ± 0.3
Pkc & WT	3.3 ± 0.9	1.5 ± 0.2

For each hemisphere, the ratio of the number of active neurons in the superficial and deep layer was calculated (see methods). All values, mean $\pm$ s.e.m.; numbers of hemispheres and rats in each group are in Table 1.