Systems/Circuits

Bidirectional Modulation of Recognition Memory

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Perirhinal cortex (PER) has a well established role in the familiarity-based recognition of individual items and objects. For example, animals and humans with perirhinal damage are unable to distinguish familiar from novel objects in recognition memory tasks. In the normal brain, perirhinal neurons respond to novelty and familiarity by increasing or decreasing firing rates. Recent work also implicates oscillatory activity in the low-beta and low-gamma frequency bands in sensory detection, perception, and recognition. Using optogenetic methods in a spontaneous object exploration (SOR) task, we altered recognition memory performance in rats. In the SOR task, normal rats preferentially explore novel images over familiar ones. We modulated exploratory behavior in this task by optically stimulating channelrhodopsin-expressing perirhinal neurons at various frequencies while rats looked at novel or familiar 2D images. Stimulation at 30–40 Hz during looking caused rats to treat a familiar image as if it were novel by increasing time looking at the image. Stimulation at 30–40 Hz was not effective in increasing exploration of novel images. Stimulation at 10–15 Hz caused animals to treat a novel image as familiar by decreasing time looking at the image, but did not affect looking times for images that were already familiar. We conclude that optical stimulation of PER at different frequencies can alter visual recognition memory bidirectionally.

Key words: **brain oscillations; familiarity; novelty; optogenetics; perirhinal; vision**

Significance Statement

Recognition of novelty and familiarity are important for learning, memory, and decision making. Perirhinal cortex (PER) has a well established role in the familiarity-based recognition of individual items and objects, but how novelty and familiarity are encoded and transmitted in the brain is not known. Perirhinal neurons respond to novelty and familiarity by changing firing rates, but recent work suggests that brain oscillations may also be important for recognition. In this study, we showed that stimulation of the PER could increase or decrease exploration of novel and familiar images depending on the frequency of stimulation. Our findings suggest that optical stimulation of PER at specific frequencies can predictably alter recognition memory.

Introduction

Recognition memory can be defined as judgment of the prior occurrence of an event (familiarity) accompanied by memory for the context in which the event occurred (recollection) (Mandler, 1980). Abundant evidence from animal and human studies shows that familiarity-based recognition is supported by the perirhinal cortex (PER) (Meunier et al., 1993; Mumby and Pinel, 1994; Brown and Xiang, 1998; Xiang and Brown, 1998; Brown and Aggleton, 2001; Winters et al., 2004; Gonsalves et al., 2005; Montaldi et al., 2006; Brown et al., 2010). PER neurons recorded in nonhuman primates exhibit decreases in firing rates as novel objects become familiar (Riches et al., 1991; Fahy et al., 1993; Brown and Xiang, 1998; Xiang and Brown, 1998; Hölscher et al., 2003; but see Thome et al., 2012). In rats, recording and imaging studies of the PER have identified visually responsive, repetition-sensitive neurons (Zhu et al., 1995; Wan et al., 1999; but see Burke et al., 2012). In addition to signaling novelty and familiarity, the PER also processes information about individual objects. PER firing rates exhibit behavioral correlates in object-guided memory and learning tasks (Lehky and Tanaka, 2007; Yanike et al., 2009) and PER neurons show selectivity for particular visual images (Naya et al., 2001; Sato and Nakamura, 2003), odors (Young et al., 1997), and 3D objects (Burke et al., 2012; Deshmukh et al., 2012).

The question of how the PER might code for novelty and familiarity while also coding for the identity of particular objects

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is currently under debate (Cowell et al., 2010; Lulham et al., 2011). One possibility is that relative novelty and object identity are both coded by firing rates. Another possibility is that one of these functions is coded by firing rates and the other by oscillatory or synchronized neuronal activity. Consistent with this idea, depth recordings in the human hippocampus showed that the power of brain oscillations in high-frequency bands, particularly low-gamma frequency bands, decreased as novel environments became familiar (Park et al., 2014). In rodents, exploration of novel objects was accompanied by increased power in the low-gamma frequency range (Lapray et al., 2009). Increased gamma power is also associated with increases of attention in humans (Herrmann and Knight, 2001; Debener et al., 2003; Fries, 2009) and exposure to novelty has been shown to increase attention (Lee et al., 2007; Marco-Pallarés et al., 2015). Depth recordings in humans also showed that the power of lower-frequency oscillations increased as novel environments become more familiar (Park et al., 2014). This is consistent with a prior rodent study in which spontaneous local field potentials (LFPs) in the PER showed a prominent oscillation in the 10–12 Hz (low-beta) frequency band in rats foraging in a familiar environment (Nerad and Bilkey, 2005). The 10–12 Hz oscillation disappeared when rats were transferred to a novel environment.

Here, we show that behavioral exploration of novel and familiar visual images can be modulated differentially by stimulating the PER at specific frequencies. Stimulation in the low-gamma frequency band increased exploration of familiar images and stimulation in the low-beta frequency band decreased exploration of novel images.

Materials and Methods

Subjects

Subjects were adult male Long–Evans rats weighing 250–300 g at the time of surgery. Rats were housed individually and maintained in a 12 h light/dark cycle. Animals were kept at 85–90% body weight of a naive animal and were allowed *ad libitum* access to water. All procedures were performed according to National Institutes of Health guidelines and were approved by Brown University's Institutional Animal Care and Use Committee.

Viral vectors

For viral transduction of the PER, pLenti-Synapsin-hChR2(H134R)-EYFP-WPRE plasmid with an enhanced channelrhodopsin-2 (ChR2)-EYFP fusion gene driven by a synapsin1 promoter packaged into a VSV-G pseudotyped lentiviral vector at the University of Pennsylvania Vector Core was used. Plasmid maps are available at www.optogenetics.org. Viral titers were $\approx 10^{10}$ IU/ml.

Surgery

Anesthesia was induced with 3% isoflurane and maintained with 2.5–1.5% isoflurane throughout the surgical procedure. The rat was then secured in a stereotaxic frame in the flat skull position. An incision was made to expose the underlying skull. After attachment of anchor screws, craniotomies were made at appropriate sites for viral vector infusions, lesions, and implantations of fibers or optrode, depending on the study.

For animals used in the spontaneous object recognition (SOR) task in Study A ($n = 8$) and Study B ($n = 11$), a 24 G guide cannula (Plastics One) was used to guide infusion of the virus and placement of the fiber into caudal PER. The cannula was fixed above cortex and secured to the skull with bone cement (DePuy) at an angle of 12–13° from vertical in a mediolateral plane 6.65 mm posterior to bregma and 5.1 mm lateral to the midline. Viral injections were made at a depth of 6 mm below skull through an infusion cannula connected to an infusion pump (Harvard Apparatus). The viral vector suspension was injected at a rate of 0.1 μl/min for a total volume of virus injected into one hemisphere of 1 μl. After the 10 min infusion and a 5 min waiting time, the infusion cannula was slowly removed and replaced by an optical fiber inserted into the guide cannula such that the tapered fiber tip was centered in the transduced region. The optical fiber was then cemented into place with bone cement (DePuy) and the wound was closed by sutures. For excitotoxic lesions of the caudal PER contralateral to the vector and fiber, NMDA (250 mM dissolved in 0.5 N NaOH; Tocris Bioscience) was delivered by a pulled glass micropipette (30–50 μm outside tip diameters) by iontopheresis (−6 μA, 7 s on and 7 s off for 9 min). Lesions were made at 4 locations: all 4 at 12–13° from vertical in a mediolateral plane and 5.1 mm lateral from the midline, 2 at 6.35, and 2 at 6.95 mm behind bregma at both 6.2 and 6.0 mm below the skull.

For animals used for *in vitro* recordings in Study C ($n = 6$), a viral vector injection was made unilaterally in caudal PER using the same coordinates as for Studies A and B. The viral vector suspension was pressure injected using a glass micropipette (30–50 μm outside tip diameter) at a rate of 0.1 μl/min, for a total 1 μl volume of virus injected into 1 hemisphere. After the infusion, the infusion cannula was removed slowly.

For animals used for *in vivo* recordings in Study D ($n = 6$), optrodes consisting of three tungsten FORMVAR-coated wires (25 μm diameter) (A-M Systems) connected to an Omnetics connecter (Plexon) and an optical fiber were implanted. The wires were epoxied (Optical Adhesive 81; Norland Products) diametrically opposite one other onto an optical fiber such that the tips extended 500–750 μm from the tip of the optical fiber. Before implantation of the optrode, 2 0.5 μl lentiviral injections were made into caudal PER via a glass micropipette (30–50 μm outside tip diameter) at a rate of 0.1 μl/min. The injections were directed at an angle of 12° from vertical in the mediolateral plane, 6.35 and 6.95 mm behind bregma, 5.1–5.35 mm from the midline, 6.2 and 6.0 mm below the skull. After completion of the final injection, the optrode was lowered into position at an angle of 12° from vertical in a mediolateral plane, 6.65 mm posteror to bregma, 5.1–5.35 mm lateral to the midline, and 6.1 mm below the skull. The optrode was cemented into place using bone cement (DePuy). Three rats received viral injections and three served as untransduced controls.

After completion of the surgical procedures, animals were maintained on a calibrated (37°C) heating pad until recovery from anesthesia before being returned to the vivarium. Rats were allowed to recover for at least 14 d to allow sufficient transduction of the viral vector.

Histology

ChR2 expression and the contralateral NMDA lesion were located between 6.6 and 7.1 mm posterior to bregma in caudal PER for all subjects in Studies A and B (Fig. 1*c–f*). For all subjects, the fiber tip was located at the center of the viral injection. Of the eight transduced rats used in Study A, the fiber implant was lost in one rat before any testing. In the remaining seven rats, median ChR2 expression in the rostrocaudal axis ranged from 480 to 1200 μm in diameter. Contralateral to ChR2 and the fiber, excitoxic damage from NMDA injections was observed in all layers throughout the caudal PER (Fig. 1*d*). Of the 11 rats prepared for Study B, three were eliminated because of a broken optical fiber, no virus expression, or the lack of a contralateral lesion, respectively. Location of the virus for the remaining eight animals was similar to the locations in Study A, with ChR2 expression ranging from 450 and 1170 μm in diameter. Contralateral to the ChR2-expressing site, damage was observed in all layers throughout caudal PER. There were cases in which damage extended slightly to the external capsule, postrhinal cortex, entorhinal cortex, or area Te_v, but these subjects were retained because damage was unilateral and it would not be expected to impair behavior (Hannesson et al., 2004; Barker and Warburton, 2011). Indeed, behavioral results reported below show that, in control (no stimulation) conditions in which a novel stimulus and a familiar stimulus were presented at choice, rats always showed normal discrimination, indicating that the unilateral lesion did not influence behavior. Of the 6 rats prepared for Study D, optical fibers were located in caudal PER between 5.64 and −6.96 mm caudal to bregma ($n = 6$). As in Studies A and B, viral vector expression in the virus animals ($n = 3$) was colocalized with the fiber. No signs of photo-induced cell damage were observed in the transduced regions in any study.

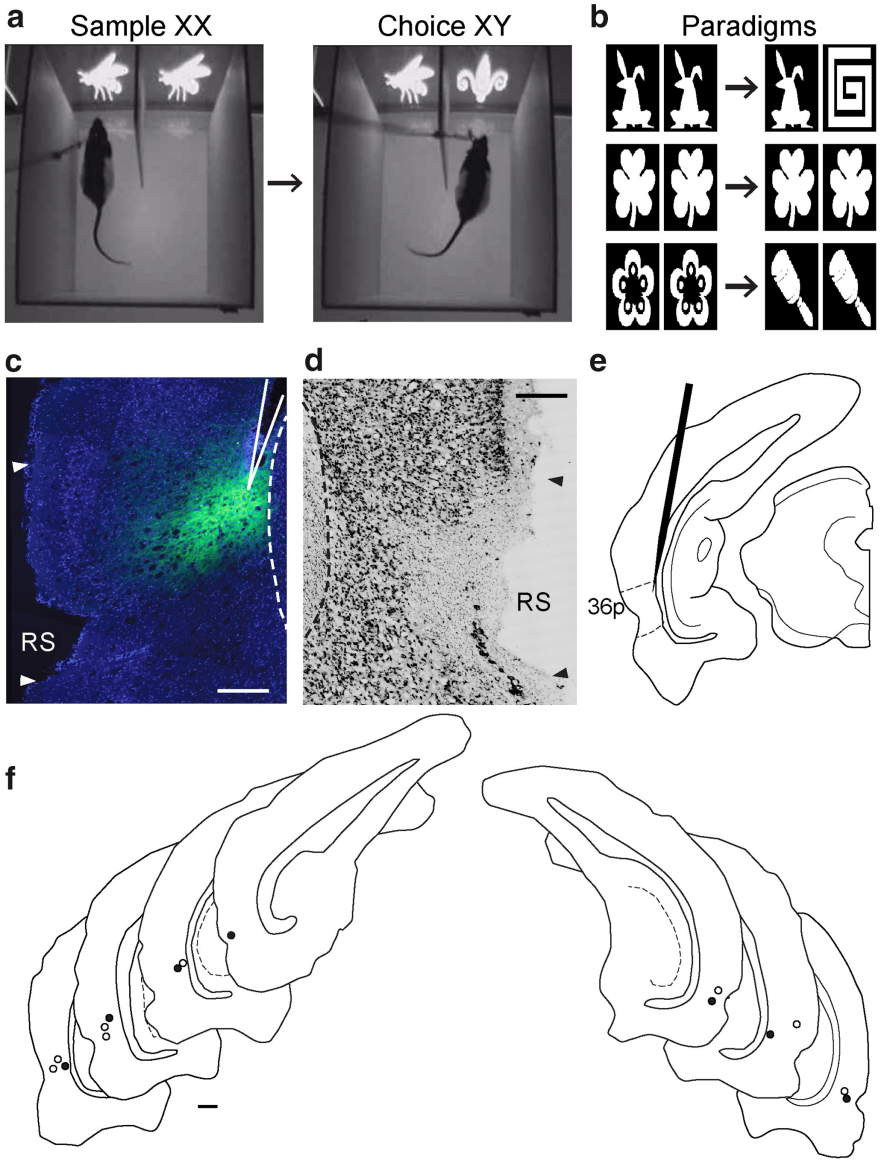


Figure 1. Experimental design and histology. ***a***, Rat performing the SOR task. X represents the familiar image, i.e., presented in the sample and choice periods. Y represents the novel image, i.e., presented only in the choice period. A 5 min delay separated the two periods. ***b***, Three experimental paradigms. In all paradigms, two identical images (XX) were always presented in the sample period. The standard configuration was XX⟶XY. In some experiments, the two sample images were both presented again at choice (XX⟶XX). In other experiments, two novel identical images were presented at choice (XX⟶YY). ***c***, Expression of ChR2-EYFP (green) in a 30-μm-thick coronal section in caudal PER in a representative subject. Neurons are labeled with DAPI (blue). Position of the fiber is indicated. Scale bars: ***c***, ***d***, 250 μm ***d***, Example of excitotoxic lesion for the same subject labeled for NeuN and counterstained for Nissl bodies. ***e***, Schematic of the location of the tapered optical fiber. ***f***, Contours showing locations of optical fiber tips. All fiber tips were located in caudal PER. The solid dots are for Study A and the open dots are for Study B. Scale bar, 500 μm. RS, Rhinal sulcus; 36p, caudal PER area 36.

SOR task

Materials and apparatus. The SOR task was performed in a rectangular enclosure (40 L × 30 W × 40 H cm) open on the top and bottom. The side and back walls were composed of white acrylic. The front wall was transparent acrylic. An opaque divider 40 cm in height extended 15 cm from the front wall to divide the front of the arena into two equal compartments. The divider also extended 5 cm on the outside of the front panel. The SOR box was placed on a floor projection maze (Jacobson et al., 2014) and a gray floor was projected. Rats were monitored by an overhead video camera. For Study A, images were back projected (Epson) onto a vertical rear projection screen situated 5 cm beyond the front wall (Fig. 1*a*). For Study B, a standard monitor positioned at the same location as the rear projection screen was used. Black and white clipart images were obtained online (Microsoft). The projected images varied in complexity and were ~10 × 15 cm when displayed on the screen or monitor. Two images were projected simultaneously onto the screen directly in front of the transparent wall. Rats show preferential exploration of novel 2D images comparable to when 3D objects are used (Forwood et al., 2007).

Habituation. Rats were habituated to the testing room and arena for 3 consecutive days before the first day of testing. On each day of habituation, rats were handled for 5 min before habituation to the apparatus. On days 1–3, each rat was allowed to explore the arena for 5 min before being returned to its home cage. On day 2, the implanted optical fiber was coupled to the laser patch cord during habituation. On day 3, the patch cord was connected and two images, not used for experiments, were presented during habituation.

Task procedures. The SOR task relies upon rats' innate preference for novelty and has been used extensively to study object recognition memory (Ennaceur and Delacour, 1988; Dix and Aggleton, 1999; Winters et al., 2008). In the present study, the task consisted of a sample and choice period separated by a 5 min delay in which the rat was returned to its home cage. In the sample period, 2 identical images were presented simultaneously in each compartment and the rat was allowed 15 s of active exploration of the images or 5 min in the arena, whichever was completed first. Active exploration of the images was scored for the first 3 min of the choice period.

For Studies A and B, three behavioral paradigms were used (Fig. 1*b*). In the standard XX → XY paradigm, two identical images were shown during the sample period and one of the two images was replaced by a novel image in the choice period. Two nonstandard paradigms were also used. In the first, the familiar images were simply presented again in the choice period (XX → XX). In the second, two identical novel images were presented in the choice period (XX → YY). With these three paradigms, optical stimulation could be used to interfere with preferential exploration of a novel image in the standard paradigm or to induce preferential exploration in the nonstandard paradigms.

Data analysis. Data acquisition, control over projection of the images, and control of the laser for stimulation were performed using Med Associates hardware and custom software written in Med State Notation (Med Associates). Two buttons on a button box interfaced with a SmartCTL Interface Module (DIG-716B; Med Associates) were used to signal active exploration. One button was used to record exploration of the image in the left compartment and the other to record exploration of the image in the right compartment. The first button press indicated the start of an exploration bout and the second button press indicated the end. During conditions in which optical stimulation was paired with looking, button presses resulted in custom software issuing TTL pulses to control laser stimulation trains at the appropriate frequency and pulse duration. Time stamps were saved in a data file. Information about exploration bouts and duration were extracted using MATLAB (The MathWorks).

Exploration of the left and right images was coded by an experimenter who was blind to the experimental conditions. Active exploration was defined as a period when the rat's nose was past the central divider, its nose was directed toward the image, and it was not rearing or grooming. Preferential exploration of one image over another during the choice period was assessed by computing a discrimination ratio (DR) as the difference between the time exploring the images divided by the total exploration time. The DR measure was used because it takes into account individual differences in total exploration time (Bussey et al., 1999). Pilot experiments validating the scoring procedure were performed with two scorers blind to the experimental conditions. Recorded choice exploration times and obtained DRs were highly correlated across scorers (Pearson's correlation coefficient = 0.83 and 0.86, respectively).

Behavioral analysis. Two cohorts of rats were used for the SOR experiments (Study A and Study B). The order of experiments for both studies is shown in Table 1. In each cohort, the hemisphere that was optically stimulated was counterbalanced. Otherwise, all experiments used a within-subject, crossover design such that subjects were run once in each condition. For example, half the subjects would be subjected to the test condition in the first run and the control condition in the second run, whereas the second half of subjects would be subjected to the control condition followed by the test condition. When appropriate, which images were used as novel and familiar and the position of the novel image (left or right) were counterbalanced. When images were identical, the side of stimulation was counterbalanced. At least 48 h separated each run. Rats were removed from data analysis if ferrules or optical fibers broke or if optical fibers became disconnected during the task.

One-sample Student's *t* tests were used to determine whether DRs differed significantly from zero. Our experiments were designed such that DRs would be positive if the results were in line with our predictions. Therefore, unless otherwise noted, tests for difference from zero were one-tailed. For differences in the DR, choice exploration times, exploratory bout number, and bout duration across conditions, repeated-measures ANOVA (rANOVA) with factors for condition (optogenetic (30 or 11 Hz) vs control) and image type (novel vs familiar) was used. Level of significance was $p = 0.05$. SEs were obtained from normalized means (Cousineau, 2005).

Optical stimulation

Caudal PER neurons were stimulated optogenetically at specific frequencies during the SOR task and control experiments. We chose caudal PER because it has the strongest connections with visual regions (Burwell and Amaral, 1998; Agster and Burwell, 2009). PER was transduced with the light-sensitive excitatory protein ChR2 for optical stimulation (Nagel et al., 2003; Boyden et al., 2005). For optical stimulation during *in vivo* experiments, we used multimode optical fibers (Polymicro Technologies; 200 μm core, 0.22 NA). The fibers were pulled to a taper before implantation using a laser-based pipette puller (P2000; Sutter Instruments). Tapering minimized damage to the tissue and ensured that the light was diffused throughout the transduced tissue. All pulled fibers were tested before implantation and only fibers delivering light with at least 45% efficiency were implanted. Light was delivered by a 473 nm laser diode (Opto Engine) through a laser patch cord (Doric Lenses). The laser was controlled by TTL pulses issued by custom software and hardware coupled to the behavioral control system (SuperPort 16 Output Module, DIG-26TTL; Med Associates).

Depending on the experiment, optical stimulation parameters ranged from 5 to 60 Hz and from 4 to 12 ms pulse width. Fiber output was ≈12 mW. The laser and optical cable were tested before every experiment and the implanted optical fiber was examined before cabling each rat. If fibers were broken, the rat was removed from subsequent experiments.

In control conditions in which no stimulation was paired with either image, a laser patch cord was connected and exploration triggered laser operation identical to the experimental condition, but the light path from the laser patch cord into the implanted optical fiber was blocked physically. To keep the experimenter blind to condition, two patch cords were provided, one blocked and one capable of passing light. The experimenter was instructed as to which patch cord to use for each subject, but was not informed which patch cord was blocked. It should be noted that the patch cords were equipped with an opaque sleeve that completely occluded the light from both the rat and the experimenter. In addition, rats showed no overt behavioral sign or any sort of orienting to the stimulation that could alert the experimenter to the stimulation condition.

Conditioned place preference task

To determine whether stimulation at 30 or 11 Hz could be appetitive or aversive, an experiment was conducted to determine whether optical stimulation could support conditioned place preference. Two mazes were used, a horseshoe maze and a V-shaped maze, placed on the floor projection maze. The mazes were open on the top and bottom and were constructed of white acrylic with walls 40 cm high. Different greyscale patterns were back projected onto the floors of the east and west zones. The arms of the mazes were divided into three zones: east, west, and center. The position of the rat was tracked by CinePlex (Plexon) and the behavioral program was controlled by Med Associates hardware and custom software, as in other experiments. The stimulation parameters for 30 and 11 Hz were determined by calculating the means of the bout duration (laser ON duration) and interval between exploration bouts (interbout interval) from Experiments 1–4 of Study A. For the 30 Hz condition, the laser ON duration and the interbout interval was 486 ms and 20.574 s, respectively; for the 11 Hz condition, the laser ON duration and the interbout interval was 460 ms and 21.344 s, respectively. The laser was controlled by Med Associates hardware and custom software.

Each animal was conditioned and tested for a place preference twice, once in each maze and once with each frequency. Order of conditioning (11 Hz and 30 Hz) and the side of the maze on which optical stimulation was presented were counterbalanced. The conditioning procedure consisted of 4 sessions performed across 4 consecutive days. Each session lasted 15 min.

In Session 1, rats were habituated to the maze. No optical stimulation was delivered and the rat was allowed to freely explore the whole maze. In Session 2, the rat was confined to one side of the maze and, in Session 3, the rat was confined to the other side. Optical stimulation was delivered in Session 2 or 3, counterbalanced for order (stimulation or no stimulation). In the test, Session 4, the partition was removed and rats were allowed to freely explore the east and west sides of the maze. The laser patch cord was attached to the implanted fiber in all four sessions, even though stimulation was presented only in Session 2 or Session 3. The maze floor was cleaned between subjects to remove odor cues.

For Session 4, the rat was placed in the center zone, and the session began when the rat exited this zone. The amount of time spent and number of entries into the east, center, and west zones were recorded. The experimenter was blind to stimulation condition. Results of the 11 Hz test and the 30 Hz test were analyzed separately. A two-tailed, paired *t* test was used to compare time spent exploring the stimulation-paired side of the maze with time spent exploring the unpaired side of the maze.

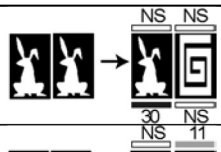
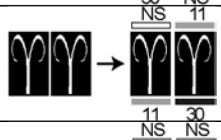
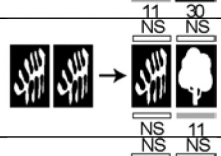
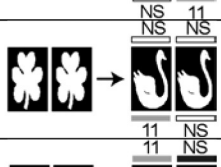
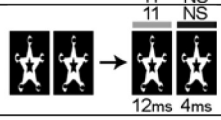
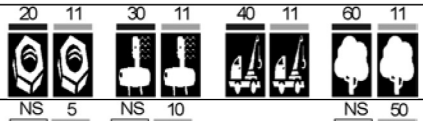
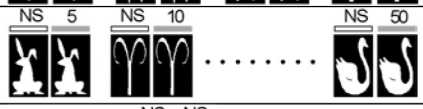
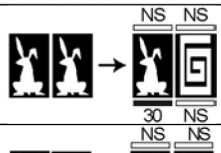
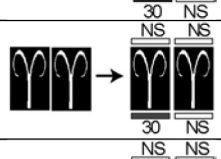
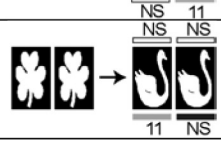
In vitro *electrophysiology*

Rats were deeply anesthetized with isoflurane before being decapitated. The brain was quickly removed and 30-μm-thick horizontal sections containing the injection site were collected. Immediately after slice preparation, the slices were incubated at 32°C for 30 min and at room temperature for at least an additional 30 min before recording in a submersion chamber at 32°C. The bathing solution (artificial CSF) used for recordings and brain slicing contained the following (in mM): 126 NaCl, 1.25 NaH_2PO_4, 26 $NaHCO_3$, 2 $CaCl_2$, and 10 dextrose saturated with 95% O_2/5% CO_2.

Whole-cell recordings were made with low-resistance (5–7 MΩ) electrodes and the intracellular solution had the following composition (in mM): 130 K-gluconate, 4 KCl, 0.2 EGTA, 10 HEPES, 4 ATP-Mg, 0.3 GTP-Tris, and 14 phosphocreatine-Tris. A MultiClamp 700B amplifier (Molecular Devices) was used for recordings. The series resistance was typically 6–20 MΩ. Recordings were not corrected for the liquid junction potential.

Blue light stimulation with a spectral peak ≈470 nm was produced by a light-emitting diode (LED; M470L2-C1; Thorlabs) powered by a Thorlabs LEDD1B LED driver mounted on a dual lamp house adapter posi-

Table 1. Description of studies and order of experiments

Study-Exp	Description	Images and conditions	Figure
A-1	30 Hz optical stimulation increases exploration of a familiar stimulus (n=7)		2a
A-2	30 Hz optical stimulation increases exploration of a familiar stimulus, but 11 Hz stimulation does not (n=6)		2c
A-3	11 Hz optical stimulation decreases exploration of a novel stimulus (n=6)		3a
A-4	11 Hz optical stimulation decreases exploration of a novel stimulus (n=6)		3c
A-5	Duration of pulse width is not responsible for effects (n=6)		7b
A-6	Stimulation does not affect activity (n=5)		7a
A-7	Novelty frequency screen: all familiar images (n=4)		4b
A-8	Familiarity frequency screen: all novel images (n=4)		4a
B-1	30 Hz optical stimulation increases exploration of a familiar image (n=8)		2b
B-2	30 Hz optical stimulation increases exploration of a familiar image (n=8)		2d
B-3	11 Hz optical stimulation decreases exploration of a novel image (n=8)		3b
B-4	11 Hz optical stimulation decreases exploration of a novel image (n=8)		3d
B-5	11 Hz optical stimulation does not drive place preference and appears not to be aversive (n=8)	11 Hz stimulation vs. no stimulation (NS)	5b, left
B-6	30 Hz optical stimulation does not drive place preference and appears not to be rewarding (n=8)	30 Hz stimulation vs. NS	5b, right
C	In vitro electrophysiology (n=29 cells from 6 rats)	10 Hz and 30 Hz stimulation	6a-e
D	In vivo electrophysiology (n=6)	NS, 11 Hz, and 30 Hz stimulation	6f-h

Order of experiments, final subject numbers, and other information for Studies A–D. A different cohort of rats was used for each study. Subject number varied per experiment due to loss of optical fiber connection, side bias, or other experimental error as documented in text. Note that there were multiple runs, so not all images are shown.

NS, No stimulation.

tioned in the epifluorescence port of an Olympus BX50WI microscope. Light was delivered through a LUMPlanFl 40×/0.80 W Olympus immersion objective and a FF655-Di01 (Semrock) dichroic mirror and produced a circular spot with a radius of 270 μm. The power output was ≈12.5 mW for the stimulation protocol.

In vivo *electrophysiology*

To characterize *in vivo* responses to 11 and 30 Hz stimulation, caudal PER was transduced with ChR2 as in other experiments and a custom-made electrode with integrated optical fiber (optrode) was implanted at the virus transduced location (virus, $n = 3$) or into untransduced PER (no virus, $n = 3$). Neuronal activity recorded from our optrodes was multiplied 20 times with an operational amplifier at the head stage (HST/8o50-G20-GR; Plexon). Signals were then passed through a differential preamplifier with a gain of 12.5 (64-channel OmniPlex Amp; Plexon). Wide-band activity (0–8 kHz) was recorded in awake animals at weeks 2 and 3 after viral injection during sessions in the SOR apparatus. Rats were allowed to explore, but no images were projected. Data were digitized at a sampling rate of 40 kHz. Recordings were obtained under 3 optical stimulation conditions: a no stimulation control, 3 s trains of 8 ms pulses of 11 Hz stimulation, and 3 s trains of 8 ms pulses of 30 Hz stimulation. In each session, the three conditions were presented three times. This was repeated 20 times for a total of 60 trials. The intertrial interval was 2 s. Data and time stamps for test conditions were collected and analyzed offline with custom MATLAB programs (The MathWorks) using Chronux (www.chronux.org), an open source MATLAB toolbox. Multiunit activity (MUA) was extracted by high-pass filtering the wide-band signal to >500 Hz. To quantify the effect of optical stimulation, the number of peaks crossing a voltage threshold during each 3 s trial was determined. Threshold was 3 SDs above the mean voltage of the signal. Data for all trials during weeks 2 and 3 were combined. Outlier trials (trials with peaks >3 SDs above the mean for that session) were replaced with the mean for that condition.

Results

30 Hz optical stimulation of PER simulates novelty

We first tested the hypothesis that 30 Hz optical stimulation of the PER could serve to simulate the effects of novelty on exploratory behavior (Fig. 2). In the first experiment of Study A ($n = 7$), we used the standard SOR task (XX → XY) to test whether pairing exploration of the familiar image with 30 Hz optical stimulation would result in increased exploration of that image (Fig. 2*a*). In the control (no stimulation) condition, as expected, rats preferentially explored the novel image (Y) over the familiar image (X) (DR > 0, $t_{(6)} = 3.6$, $p = 0.005$). In the experimental condition when the familiar image was paired with 30 Hz optical stimulation, rats did not preferentially explore the novel image (DR ≈ 0, $t_{(6)} = -2.1$, $p = 0.959$). Therefore, rats appeared to treat the familiar image paired with 30 Hz stimulation as if it were novel. The control DR was significantly higher than the experimental DR ($F_{(1,6)} = 10.2$, $p = 0.019$).

Novel object exploration is often accompanied by increased duration of exploratory (looking) bouts (Renner and Seltzer, 1991). As would be expected, exploratory bouts of rats in the control condition were longer for novel (600 ± 20 ms) than familiar images (560 ± 14 ms). In the experimental condition, exploratory bout duration was longer for familiar images paired with the 30 Hz novelty signal (614 ± 27 ms) than for unpaired novel images (507 ± 25 ms). This was confirmed by a condition by image type interaction ($F_{(1,7)} = 8.9$, $p = 0.021$). Therefore, bout durations provided additional evidence that 30 Hz optical stimulation modulated novelty exploration or attention to novelty.

We replicated these findings with a second cohort of rats (Study B, $n = 8$). Again, looking at a familiar image paired with 30 Hz optical stimulation resulted in increased exploration (Fig. 2*b*, Table 1). During the control condition, rats preferentially explored the novel image over the familiar image (DR > 0, $t_{(7)} = 2.4$, $p = 0.024$). In the experimental condition, the rats preferentially explored the familiar image that was paired with 30 Hz optical stimulation (DR < 0, $t_{(7)} = -2.5$, $p = 0.020$). The differ-

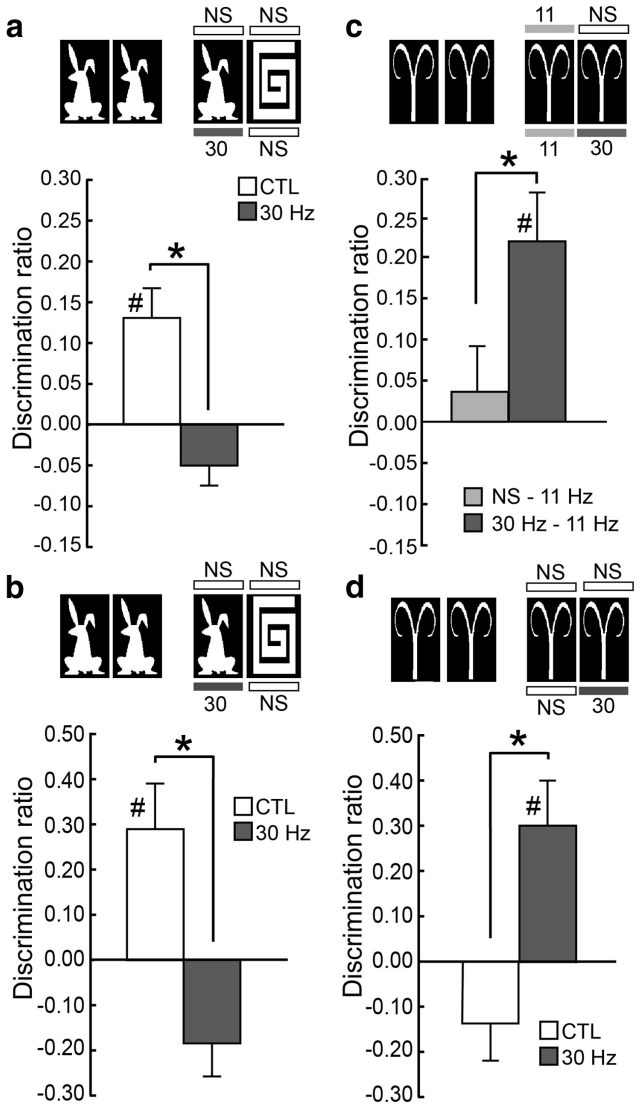


Figure 2. Optical stimulation at 30 Hz results in increased exploration of familiar images. Paradigms used are shown above the bar graphs. Stimulation was paired with exploration of images (looking) in the choice period. Control conditions (CTL) are above the images and experimental conditions (EXP) are below the images. An empty box indicates no stimulation (NS). For CTL conditions, the laser was connected and operated exactly as in the EXP condition, but light was physically blocked from entering the fiber. Side was always counterbalanced, but for illustration purposes, choice images on the left and right represent familiar/novel (***a***, ***b***) or familiar/familiar-paired images (***c***, ***d***), respectively. Therefore, in all panels, the image and stimulation condition in which greater exploration was expected is shown on the right such that DR = (R − L)/(R + L). ***a***, In CTL, as expected, rats ($n = 7$) preferentially explored the novel image. In EXP, when the familiar image was paired with 30 Hz optical stimulation, rats spent similar amounts of time exploring the paired familiar image and the novel image. The CTL DR was significantly higher than the EXP DR. ***b***, Experiment in ***a*** was replicated with a second cohort of rats ($n = 8$). In CTL, again, rats explored the novel image more than the familiar one. In EXP, when looking at a familiar image was paired with 30 Hz stimulation, rats explored the familiar image more as if it were novel. ***c***, In CTL, stimulation at 11 Hz does not decrease exploration of an already familiar stimulus. In EXP, stimulation at 30 Hz increased exploration of the paired familiar image ($n = 6$), showing that modulation of novelty exploratory behavior is frequency dependent. ***d***, Replication of ***c*** except that NS is compared with 30 Hz ($n = 8$). Data are means ± normalized SEM. *$p < 0.05$; #$p < 0.05$ (*t* test), significant difference from zero.

ence between DRs in the control and experimental conditions was, again, significant ($F_{(1,7)} = 8.0, p = 0.026$). Exploratory bout duration was numerically longer for the novel image (379 ± 11 ms) over the familiar image in the control condition (333 ± 23 ms). In the experimental condition, bout duration was numerically longer for the familiar image paired with 30 Hz (508 ± 67 ms) compared with the unpaired novel image (418 ± 67 ms).

We further addressed the hypothesis that 30 Hz stimulation of PER could simulate novelty by pairing stimulation with one of two identical familiar images. In this experiment, we also confirmed that the frequency of stimulation is important for driving exploration. We used a paradigm in which the same two identical familiar images were presented during the sample and the choice periods (XX → XX; Fig. 2*c*). In the control condition, exploration of one familiar image was paired with 11 Hz stimulation, an LFP frequency associated with exploration of familiarity (Nerad and Bilkey, 2005). Exploration of the other identical familiar image was not accompanied by optical stimulation. If 11 Hz simulates familiarity, then 11 Hz stimulation paired with a familiar image should be redundant. In the experimental condition, exploration of one familiar image was paired with 30 Hz stimulation and exploration of the other identical familiar image was paired with 11 Hz stimulation. In the control condition, exploration did not differ significantly between the 11 Hz and NS stimulation conditions (DR ≈ 0; $t_{(5)} = 0.7, p = 0.258$). In the experimental condition, however, rats explored the familiar image paired with 30 Hz significantly more than the familiar image paired with 11 Hz (DR > 0; $t_{(5)} = 3.7, p = 0.007$). Therefore, rats again treated a familiar image paired with 30 Hz optical stimulation as novel.

The mean exploratory bout duration during exploration was longer for familiar objects paired with 30 Hz stimulation (763 ± 66 ms) compared with familiar objects paired with 11 Hz in the experimental condition (598 ± 39 ms). In the control condition, bout duration was similar for the familiar image paired with 11 Hz (496 ± 15 ms) and the unpaired familiar image (528 ± 17 ms). This was confirmed by rANOVA showing a significant condition by image interaction ($F_{(1,6)} = 14.0, p = 0.019$).

We replicated these findings in a second cohort of animals ($n = 8$) with a slightly altered experimental design (Fig. 2*d*). There was no stimulation in the control condition. For the experimental condition, exploration of one of the identical familiar images was paired with 30 Hz stimulation and the other was not paired with stimulation. For rats in the no stimulation control condition, exploration of the images was not significantly different (DR ≈ 0; $t_{(7)} = -1.7, p = 0.065$). In the experimental condition, rats preferentially explored the familiar image paired with 30 Hz stimulation compared with the unpaired identical familiar image (DR > 0; $t_{(7)} = 2.4, p = 0.025$). The DR in the experimental condition differed significantly from the control condition ($F_{(1,7)} = 7.2, p = 0.031$). Exploratory bout durations were no different in the control condition (452 ± 59 and 357 ± 32 ms). For the experimental condition, exploratory bouts were numerically, though not significantly, longer when exploration of the familiar image was paired with 30 Hz stimulation (1170 ± 762 ms) compared with the unpaired image (302 ± 49 ms).

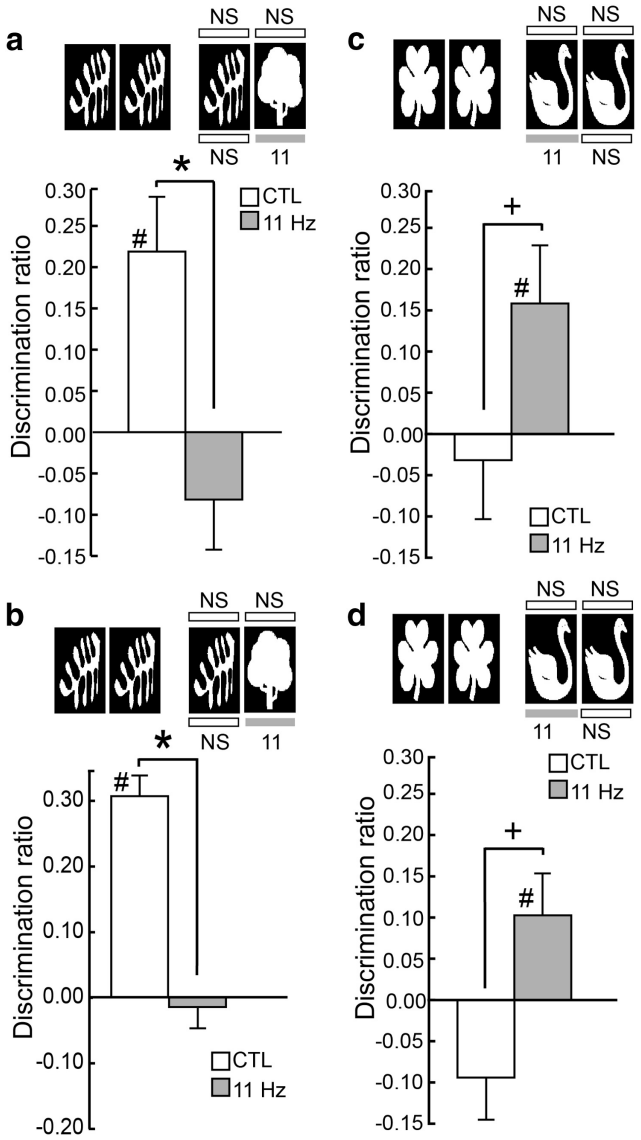


Figure 3. Optical stimulation at 11 Hz results in decreased exploration of novel images. As for Figure 2, paradigms are shown above the bar graphs. Stimulation was paired with exploration of image(s) in the choice period (the two right panels in each set of four). Control conditions (CTL) are above the images and experimental conditions (EXP) are below. An empty box indicates no stimulation (NS). For CTL, the laser was connected and operated exactly as in the experimental condition, but light was physically blocked from entering the fiber. For illustration, left and right choice images represent familiar/novel images (***a***, ***b***) or novel-paired/novel images (***c***, ***d***), respectively. Therefore, in all panels, the image and stimulation condition in which greater exploration was expected is shown on the right such that DR = (R − L)/(R + L). ***a***, In CTL, rats ($n = 7$) discriminated normally. In EXP, when optical stimulation at 11 Hz was paired with looking at the novel image, rats explored the familiar image and the novel paired image equally. ***b***, Replication of ***a*** ($n = 8$). ***c***, In CTL, rats ($n = 6$) explore the two novel images equally. In EXP, stimulation at 11 Hz decreased exploration of the paired novel image ($n = 6$). ***d***, Replications of ***c*** ($n = 8$). Data are means ± normalized SEM. *$p < 0.05$; +$p < 0.10$ (rANOVA); #$p < 0.05$ (*t* test), significant difference from zero.

11 Hz optical stimulation of PER simulates familiarity

We tested directly the hypothesis that 11 Hz optical stimulation of PER could simulate the effects of familiarity on exploratory behavior. In the standard SOR task (XX → XY; Fig. 3*a*), rats in the no stimulation control condition preferentially explored the novel image more than the familiar image during the choice period (DR > 0, $t_{(5)} = 3.3, p = 0.011$). When exploration of the novel image was paired with 11 Hz optical stimulation, however, rats explored the paired novel and familiar images for similar amounts of time, suggesting that the rats were treating the novel image paired with 11 Hz stimulation as if it were also familiar (DR ≈ 0; $t_{(5)} = -1.3, p = 0.882$). The DRs in the two conditions were significantly different ($F_{(1,5)} = 12.7, p = 0.016$). Bout durations in the control condition were numerically longer for the novel image over the familiar image (567 ± 126 over 399 ± 64).

Bout durations in the experimental condition were about the same for the unpaired novel image and for the novel image paired with 11 Hz (507 ± 37 vs 475 ± 12 ms, respectively).

These findings were replicated with a second cohort of animals (Study B). In Study A, variability was greater for the 11 Hz stimulation experiments compared with 30 Hz, so in these replications, subjects were run twice in each condition. For the XX → XY paradigm (Fig. 3*b*), 1 rat was removed due to an error in data collection ($n = 7$). Rats in the control condition preferentially explored the novel image over the familiar image (DR > 0; $t_{(6)} = 6.2$; $p = 0.0004$). In the experimental condition, rats did not explore the novel image paired with 11 Hz stimulation more than the unpaired familiar image (DR ≈ 0; $t_{(6)} = -0.2$; $p = 0.585$). Bout durations in the control condition were numerically higher for the novel image over the familiar image (739 ± 279 over 615 ± 297 ms). Bout durations in the experimental condition were, again, about the same for the unpaired familiar image and for the novel image paired with 11 Hz (387 ± 61 vs 384 ± 21 ms, respectively).

We further tested the hypothesis that familiarity is simulated by 11 Hz stimulation in a complementary experiment; in this case both images in the choice period were identical and novel (XX → YY; Fig. 3*c*). As expected, rats explored the novel images equally during choice in the no stimulation control condition (DR ≈ 0; $t_{(5)} = -0.4$, $p = 0.338$). In the experimental condition, we paired exploration of one of the novel images with 11 Hz optical stimulation. Pairing the novel image with 11 Hz stimulation reduced exploration compared with the identical unpaired novel image (DR > 0; $t_{(5)} = 2.2$, $p = 0.037$). The difference in DRs between the 11 Hz and control conditions was also marginally significant (rANOVA: $F_{(1,5)} = 4.1$, $p = 0.099$). Bout durations were similar for the two novel images in the control condition (513 ± 24 and 479 ± 18 ms). Bout duration was numerically longer for the unpaired novel image compared with the novel image paired with 11 Hz (499 ± 47 vs 445 ± 22 ms). Together, these findings suggest that animals treated the novel image as familiar when it was paired with 11 Hz optical stimulation.

These findings were replicated with a second cohort of rats (Study B, $n = 8$; Fig. 3*d*). Again, rats in the control condition explored two identical novel images equally during choice (DR ≈ 0; $t_{(7)} = -1.5$; $p = 0.911$). In the experimental condition, rats explored the novel image paired with 11 Hz stimulation less than the identical unpaired novel image (DR > 0, $t_{(7)} = 2.3$; $p = 0.026$). As before, bout durations were similar in the control condition (498 ± 36 and 492 ± 57 ms). In the experimental condition, bout duration was numerically longer for the unpaired over the 11 Hz paired novel image (584 ± 49 over 510 ± 28 ms).

High- and low-frequency stimulation have differential effects on exploratory behavior

The results described above imply that the frequency of stimulation is important for modulating exploration of novel and familiar images. To determine the optimal frequencies, we used a single-trial SOR task to test the efficacy of a range of optical stimulation frequencies. In the prior experiments, 11 Hz decreased exploration of novelty. Therefore, we examined the efficacy of frequencies ranging from 5 to 60 Hz for decreasing exploration of novelty. A new pair of identical novel images was used to test each frequency. Exploration of one novel image was not paired with stimulation (no stimulation, NS) and the identical novel image was paired with the "familiarity" test frequency (Stim). Therefore, the DR was calculated as DR = (NS − Stim)/(NS + Stim), such that DR > 0 indicates preferential exploration of the unpaired novel image over the identical novel image paired with optical stimulation (Fig. 4*a*). We found that pairing exploration of a novel image with 10 or 15 Hz stimulation significantly reduced exploration of that image compared with the unpaired novel image (DR > 0, $p < 0.035$). The other frequencies were ineffective in decreasing exploration of the novel image ($p > 0.05$).

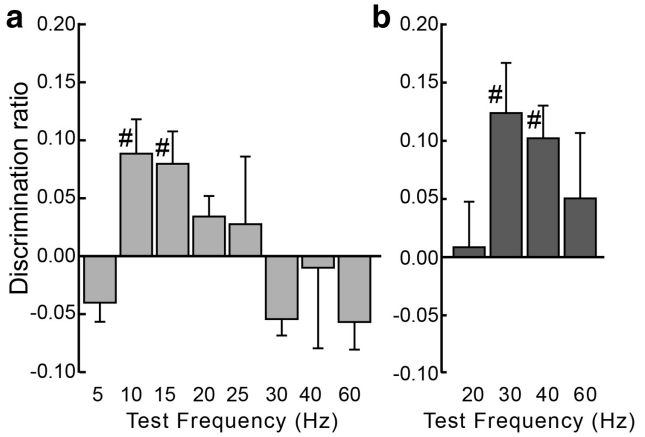


Figure 4. Screening for the most effective optical stimulation frequencies for decreasing exploration of novel images and increasing exploration of familiar images. ***a***, The 10 and 15 Hz frequency stimulation were effective for decreasing exploration of novelty. In each trial, both images were novel and identical; one was not paired with optical stimulation (NS) and the other was paired with stimulation (5–60 Hz). DR = (NS − Stim)/(NS + Stim) such that a positive DR indicates an effective frequency. ***b***, The 30 and 40 Hz frequency stimulation were effective for increasing exploration of familiar images. Both images used were familiar; one was paired with 10 Hz to ensure baseline familiarity and the other was paired with a range of stimulation frequencies (20–60 Hz). DR = (Stim − 10 Hz)/(Stim + 10 Hz) such that a positive DR indicated an effective frequency. Data are means ± SEM. #$p < 0.05$, one-tailed Student's *t* test ($n = 4$).

We also used the single-trial SOR paradigm to find optimal frequencies for increasing exploration of familiar images. In the prior experiments, 30 Hz increased exploration. Therefore, we used novelty test frequencies of 20, 30, 40, and 60 Hz (Fig. 4*b*). Familiar test images were images that had been used in earlier experiments. To ensure a baseline of familiarity, we compared each of the test frequencies (T) with 10 Hz stimulation (F) because this was the most effective frequency for simulating familiarity. Therefore, DR = (T − F)/(T + F) and a DR significantly greater than zero would indicate preferential exploration of the image paired with the novelty test stimulation frequency, that is, it would be effective in increasing exploration of a familiar stimulus. We found that pairing exploration of a familiar image with 30 or 40 Hz optical stimulation significantly increased exploration of that image compared with the unpaired identical familiar image (DR > 0; $p < 0.032$). The 20 and 60 Hz test frequencies were ineffective ($p > 0.05$; Fig. 4*b*).

Effects of optical stimulation cannot be explained by valence of stimuli

One possible explanation for our results is that PER stimulation at these frequencies is intrinsically aversive or rewarding. To test this, we used a conditioned place preference paradigm (Study B). After preconditioning in Session 1, rats were conditioned to one side of the maze in either Session 2 or Session 3 (Fig. 5*a*). In Session 4, rats were tested for a place preference. We compared time spent exploring the side of the maze that had been paired with the unpaired side of the maze using a two-tailed, paired *t*

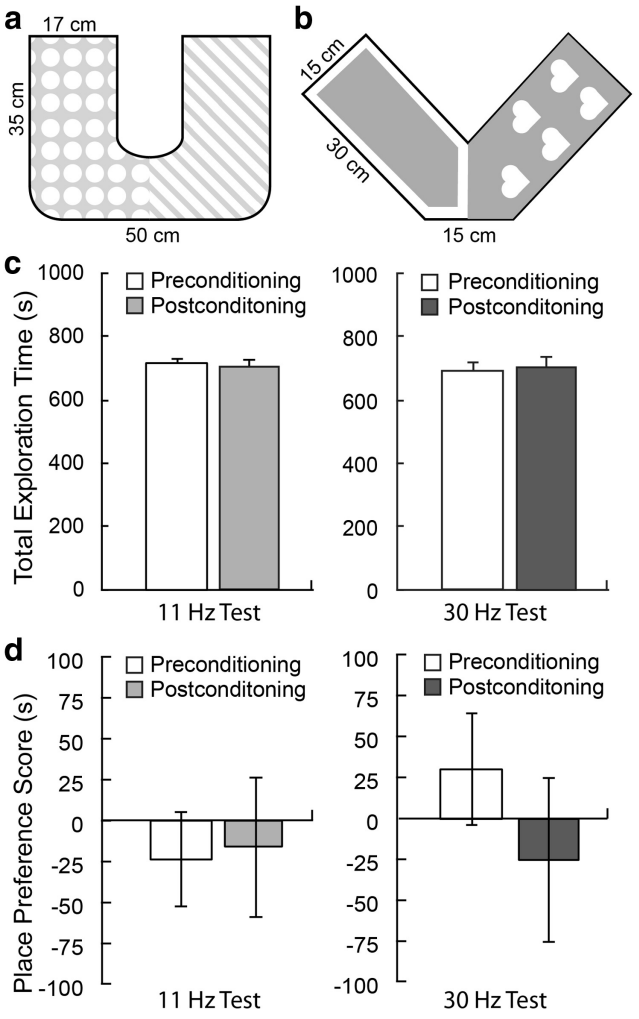


Figure 5. PER stimulation at 11 or 30 Hz does not result in place preference or avoidance. ***a***, ***b***, Schematic showing the floor patterns and dimensions of the horseshoe maze (***a***) and the V maze (***b***). ***c***, Total exploration time for the unpaired (No Stim) and paired (Stim) sides of the mazes during the postconditioning test for 11 and 30 Hz ($n = 7$). ***d***, Place preference scores for the rats during preconditioning and the postconditioning test for 11 and 30 Hz. Neither frequency resulted in a place preference or a place avoidance.

test. For the 11 Hz test (Fig. 5*b*, left), rats showed no preference for the paired or the unpaired side ($t_{(7)} = 0.43$, $p = 0.68$). Likewise, for the 30 Hz test (Fig. 5*b*, right), rats showed no preference for the paired or the unpaired side ($t_{(7)} = 0.46$, $p = 0.66$).

Cellular effects of optical stimulation: *in vitro* and *in vivo* studies

To characterize the cellular effects of our optical stimulation protocol, we recorded from neurons in slices of PER maintained *in vitro*. Slices were obtained from rats that had been injected with ChR2-EYFP-transducing lentiviral vector at least 2 weeks earlier ($n = 6$), the minimum incubation period of the lentiviral vectors before behavioral experiments. Imaging of EYFP fluorescence in the live slices indicated expression in layers 2 through 6. Whole-cell current-clamp or voltage-clamp recordings were made from physiologically identified pyramidal cells in layers 2 through 6 within ChR2-expressing regions of PER ($n = 29$). The sampled neurons had regular-spiking properties (Beierlein et al., 2003) with a mean input resistance of 127 ± 12 MΩ and resting membrane potentials of −72 ± 1.5 mV. Whole-field (~500 μm in diameter) light pulses (8 ms duration) were applied to stimulate the local network.

Of 29 neurons tested, 26 responded to light stimuli (Fig. 6); the three nonresponsive cells were recorded from regions with relatively weak fluorescence. In general, the robustness of light-evoked responses across neurons correlated well with the EYFP fluorescence in the region of the recording. ChR2-expressing and non-ChR2-expressing neurons (Fig. 6*a*,*b*) were identified by the latency of their light-evoked changes in membrane potential or current (Cruikshank et al., 2010). Twelve cells expressed ChR2 directly and generated large amplitude depolarizations (mean maxima of 72 mV) with very short latency (≤1 ms) (Fig. 6*c*). Four of these ChR2-expressing cells reliably generated action potentials in response to light stimuli (Fig. 6*a*); two of them spiked in response to every stimulus at frequencies from 10 Hz to 60 Hz. The other eight cells generated short-latency, variably sized depolarizations that did not reach spike threshold when tested at resting potential. Another 14 neurons within the ChR2-expressing regions of PER did not express ChR2 themselves, but responded to light with synaptic events at onset latencies >3 ms (Fig. 6*b*). Synaptic events can be triggered by ChR2 activation in both somatodendritic and axon terminal membranes (Cruikshank et al., 2010; Cruikshank et al., 2012). Responses of non-ChR2-expressing neurons usually included combinations of excitatory and inhibitory postsynaptic events, as deduced by the polarity of the long-latency responses when varying the cells' holding potentials between −80 and −20 mV under voltage-clamp conditions.

Most cells were also tested with trains of 10–40 Hz light pulses while steady, low-frequency firing was induced with continuous current injection. In seven ChR2-expressing neurons, light pulses could trigger and entrain spiking at rates higher than their prestimulus baseline rates. In seven other neurons, most of them non-ChR2 expressing, similar light trains induced mixed effects or a net inhibition of baseline spiking rates (Fig. 6*d*). Voltage clamping in these same neurons revealed light-induced IPSCs. In current-clamp conditions, after termination of 10–40 Hz light trains, the membrane of most cells hyperpolarized for durations of 0.5–10 s (depending on train length and frequency; Fig. 6*e*), during which baseline spiking ceased or slowed. Post-light-train afterhyperpolarizations (AHPs) were observed in both ChR2-expressing and non-ChR2-expressing neurons, and the AHPs did not depend on light-evoked action potentials in the recorded cells (Fig. 6*d*). When brief suprathreshold current pulses were substituted for light pulses in 10–40 Hz stimulus trains, neurons also generated AHPs that suppressed spiking. Light-induced AHPs were longer and more robust than current-induced AHPs. The reversal potentials of light-induced and current-induced AHPs pulses were similar, about −90 mV, suggesting that both are mediated by increased potassium conductance.

Overall, the data from ChR2-expressioning PER slices suggest that 10–40 Hz light trains rapidly excited the majority of pyramidal cells, as identified by morphology and physiological characteristics. This was accomplished either by direct ChR2 activation or indirectly via synaptic events mediated by local circuits. Action potentials in many cells were entrained by the periodic light stimuli. Most neurons, regardless of whether they expressed ChR2, received both excitatory and inhibitory synaptic inputs during stimulation and an interval of membrane hyperpolarization and spike suppression followed trains of light stimuli.

We were also interested in the *in vivo* effects of optical stimulation on PER neuronal activity and LFPs. Optrodes were im-

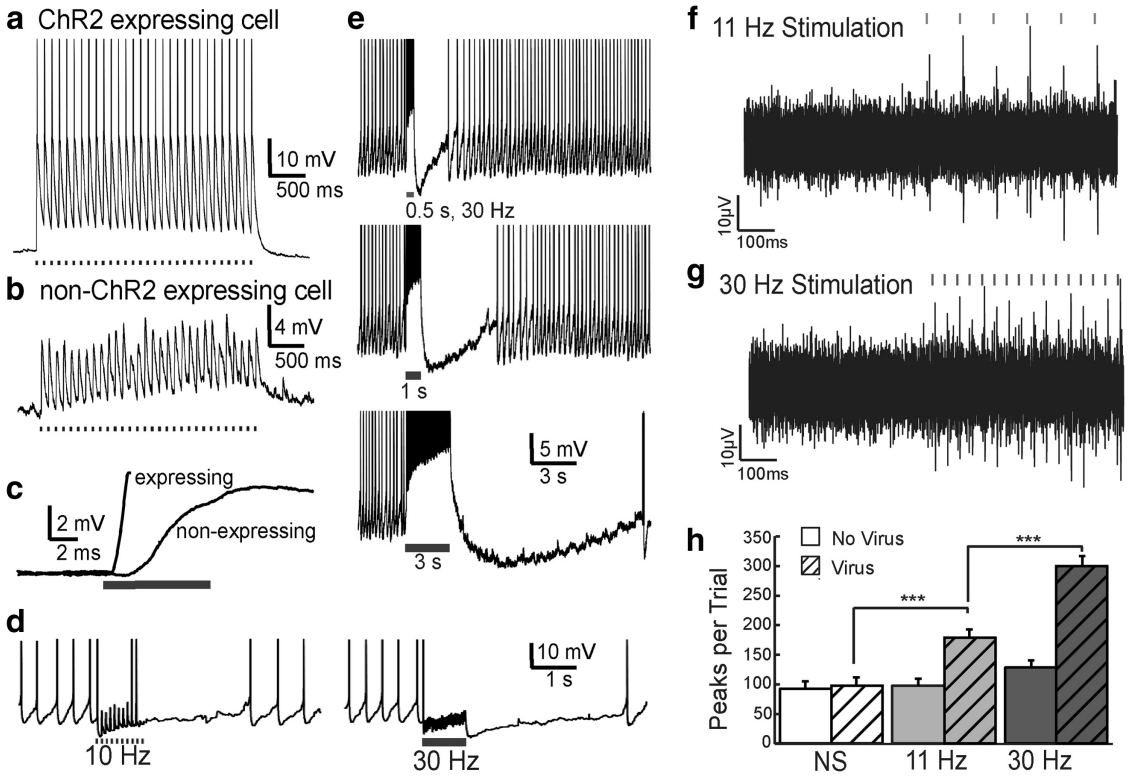


Figure 6. Responses of PER neurons to optical stimulation *in vitro* and *in vivo*. ***a***, *In vitro* responses were recorded from 29 cells. Expressing cells generated large amplitude depolarizations with short latencies; one-third of such cells, including this one, reliably generated action potentials. Light stimuli (8 ms pulses) are indicated by tick marks. ***b***, Example of synaptic responses to 10 Hz optical stimulation from a cell that did not express ChR2. ***c***, ChR2-expressing and non-ChR2-expressing neurons were identifiable by the latency of light-evoked changes in membrane potential or current. (***d***), When steady depolarizing current was used to activate low-frequency spiking in a non-ChR2-expressing neuron, trains of light pulses triggered mixed effects, including stimulus-entrained spikes (at 10 Hz), net inhibition of baseline spiking (at 30 Hz), and poststimulus hyperpolarization and spike suppression. ***e***, After termination of trains of light stimuli (10 – 40 Hz) the membrane of most neurons hyperpolarized for durations of 0.5–10 s (depending on train length and frequency). ***f–h***, *In vivo* activity during optical stimulation in PER. MUA 500 ms before and 500 ms during 11 Hz (***f***) and 30 Hz (***g***) stimulation in a virally transduced animal. ***h***, Spiking activity during stimulation trials (20 trials per condition per rat) shows a frequency-dependent increase in activity during optogenetic stimulation in the transduced group (virus, $n = 3$), but not in the no virus group ($n = 3$). $^{***}p < 0.001$. Data are means + SEM.

planted in caudal PER after transduction with ChR2 (virus, $n = 3$) and in normal control rats (no virus, $n = 3$). MUA was assessed under three optical stimulation conditions: a no stimulation control, stimulation at 11 Hz (8 ms pulses), and stimulation at 30 Hz (8 ms pulses). Stimulation trains were 3 s in duration with 2 s interstimulus intervals. During stimulation in the virus rats, LFP recordings showed that power increased at the stimulation frequency, but there was no long-lasting change in the LFP or MUA after the offset of stimulation (data not shown). Optical stimulation in the virus animals also induced spiking activity and MUA increased with increasing frequency of optical stimulation (Fig. 6*f–h*). Importantly, there was no change in MUA during stimulation of PER in the no virus condition, indicating that light artifact is not responsible for our effects. Moreover, there was no difference between virus and no virus animals in baseline MUA, indicating that viral infection does not affect baseline neuronal activity.

Control experiments

Locomotor activity

To examine the effect of optical stimulation at 30 and 11 Hz on locomotor activity, we calculated average running speed (pixels/mm) using a CinePlex V2 tracking system (Plexon) while rats ($n = 5$) freely explored the SOR arena. No images were presented during the trials. Testing consisted of 20 randomized trials of 3 s duration under no stimulation, 11 Hz stimulation, and 30 Hz stimulation conditions with a 2 s intertrial interval for a total of 60 trials. Light pulses were 8 ms in duration, as in the primary experiments. A one-way rANOVA was used for comparisons of speed under the stimulation conditions. As shown in Figure 7*a*, average speed (pixels/s) did not differ significantly across the three stimulation conditions ($F_{(2,8)} = 1.2$, $p = 0.359$). Therefore, optical stimulation of PER did not alter locomotor activity.

Nonspecific effects of amount of light

Another control experiment provided further evidence that exploratory behavior is modulated by frequency of stimulation and not total light delivered. In the main experiments, we used an 8 ms pulse duration, which has been used reliably to evoke a single spike in prior optogenetic studies (Boyden et al., 2005; Desai et al., 2011). In a control experiment, we examined the effect of total light delivery per bout. As in the main experiments, the experimenter was blind to stimulation condition. We used the XX → XX paradigm in which exploration of one familiar image is paired with 30 Hz and exploration of the other identical familiar image is paired with 11 Hz. When pulse widths were 8 ms for both 30 and 11 Hz stimulation, we saw significant discrimination (Fig. 2*b*, bar on the right, Fig. 7*b*, bar on the left). In this control experiment, pulse widths were 4 ms for the image paired with 30 Hz and 12 ms for the image paired with 11 Hz such that the amount of light energy delivered during looking was approximately equivalent for 30 Hz and 11 Hz. The DR was similar to the earlier experi-

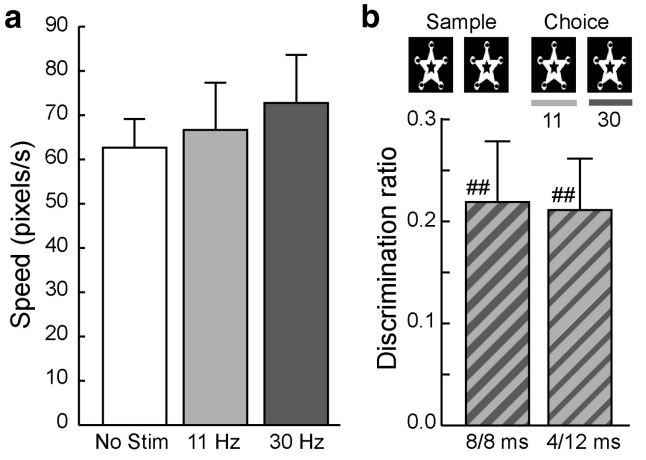


Figure 7. Control experiments for effects on locomotor activity and differences in amount of light delivered. ***a***, Optical stimulation at 11 or 30 Hz had no effect on locomotor activity ($n = 5$). ***b***, Controlling for total light between 11 and 30 Hz conditions by compensating with pulse widths did not affect differential exploration ($n = 6$ for each condition). For both experiments, the DR = (time exploring in 30 Hz condition − time exploring in the 11 Hz condition)/(time exploring in 30 Hz condition + time exploring in the 11 Hz condition). The bar on the left shows the DR when pulses are 8 ms for both conditions. The bar on the right shows the DR when 30 Hz pulses are 4 ms and 11 Hz pulses are 12 ms. Data are means ± normalized SEM. ##$p < 0.01$, significant difference from zero, $p < 0.05$ (*t* test).

ment, indicating that pulse width does not account for our findings (Fig. 7*b*, bar on the right). This was confirmed by *t* tests ($t_{(5)} = 3.7$, $p = 0.01$ for the 8/8 ms experiment and $t_{(5)} = 4.2$, $p < 0.01$ for the 4/12 ms experiment).

Rats ($n = 6$) preferentially explored the image paired with 30 Hz, 4 ms/pulse stimulation over the image paired with 11 Hz, 12 ms/pulse stimulation (DR > 0; $t_{(5)} = 4.2$, $p = 0.004$). The DR for this experiment did not differ significantly from that obtained when the pulse widths were equivalent at 8 ms (DR > 0; $t_{(5)} = 3.7$; $p = 0.007$; $F_{(1,5)} < 1$, $p = 0.36$). Furthermore, the total choice exploration time at the adjusted pulse widths (10.7 ± 1.7 s) did not differ significantly from that at the 8 ms pulse widths (13.9 ± 2.1 s; $F_{(1,5)} = 1.3$, $p = 0.30$). Exploration bout duration increased significantly with 30 Hz, 4 ms/pulse optical stimulation compared with 11 Hz, 12 ms/pulse ($F_{(1,5)} = 15.6$, $p = 0.01$), a result also observed when pulse widths were 8 ms. Therefore, differential behavioral effects observed with 30 and 11 Hz optical stimulation cannot be explained by differences in total light delivery.

Discussion

The ability to identify novelty and familiarity is important for memory, learning, and adaptive behavior. Identification of familiarity depends on the PER (for review, see Brown and Aggleton, 2001; Eichenbaum et al., 2007), but how novelty and familiarity are encoded and transmitted in the brain is not understood. Using optogenetic methods, we tested whether exploration of novelty and familiarity could be altered differentially by stimulation of the PER at different frequencies. We found that optical stimulation of the same ensemble of ChR2-expressing caudal PER neurons resulted in a striking bidirectional modulation of spontaneous exploratory behavior. Stimulation at 30–40 Hz increased exploration of familiar images. In contrast, stimulation at 10–15 Hz decreased exploration of novel images. Modulation of exploratory behavior was expressed primarily as changes in the duration of individual bouts of looking at the images. This is important because longer exploratory bouts are a signature of attention and of novelty exploration (Ennaceur et al., 2009).

Two findings are worth further discussion. Whereas stimulation at 30–40 Hz increased exploration of familiar images, stimulation at these frequencies had little or no effect on exploration of novel images. Likewise, stimulation at 10–15 Hz decreased exploration of novel images, but had little or no effect on exploration of familiar images. This suggests that the 30–40 Hz stimulation of caudal PER does simulate the effects of novelty on exploratory behavior and that the 10–15 Hz stimulation does simulate the effects of familiarity. This is consistent with an earlier example in which behavioral preferences were modified. Electrical stimulation at different frequencies in the chick hyperstriatum, the region important for imprinting, induced later preference for lights flashing at the frequency of the stimulation (McCabe et al., 1979).

Our *in vivo* and *in vitro* electrophysiology experiments yield insight into the mechanisms by which exploratory behavior was modulated. Our *in vitro* recordings showed that a substantial proportion of ChR2-expressing cells in caudal PER reliably generated action potentials with optical stimulation. Our *in vivo* electrophysiology experiments showed that stimulation at 11 Hz or 30 Hz increased MUA. Examination of individual traces revealed that the increase in spiking activity was largely entrained with optical stimulation. Although our study does not address mechanisms directly or rule out rate coding, synchronous firing in PER is a candidate mechanism for recognition memory. Indeed, in other regions, synchronous activity is implicated in plasticity (for review, see Martin and Morris, 2002; McBain and Kauer, 2009).

Prior studies report that neuronal firing rates in the PER decrease as novel items become familiar (Riches et al., 1991; Fahy et al., 1993; Brown and Xiang, 1998; Xiang and Brown, 1998; Hölscher et al., 2003). If firing rates were monotonically related to novelty exploration, then we would expect to see a monotonic relationship between frequency and exploration (Fig. 4). Instead, 30 and 40 Hz stimulation increased exploration of familiar images, whereas 20 and 60 Hz had no effect (Fig. 4*b*). In addition, 10 and 15 Hz stimulation decreased exploration of novel images, whereas 5 and 20 Hz had no effect (Fig. 4*a*). Interestingly, stimulation at 30–60 Hz during exploration of novel images had variable effects, suggesting that, if images are already novel, the effects of 30 and 40 Hz stimulation do not reliably increase exploration. Similarly, stimulation at 11 Hz during exploration of familiar images had no effect (Figs. 2*c*, 7*b*), suggesting that, if images are already familiar, 11 Hz stimulation does not further decrease exploration.

Neuroanatomical studies show that the PER is interconnected with other regions also known to be involved in processing novelty and familiarity (Burwell and Amaral, 1998; Agster and Burwell, 2009), including prefrontal cortex (PFC) (Dias and Honey, 2002; Matsumoto et al., 2007; Kishiyama et al., 2009). Given that the PFC has an identified role in executive function (Dalley et al., 2004), it may be in a position to guide exploratory behavior based on the current context. Medial PFC ensembles of neurons recorded in rats differentiate entire environmental contexts and changes in environmental contexts (Hyman et al., 2012). The PER is most heavily interconnected with the medial PFC and with a subarea of medial agranular PFC (Burwell and Amaral, 1998; Agster and Burwell, 2009). Both of these areas are implicated in attention. Damage to medial PFC impairs the ability to shift attention from one perceptual domain to another (Birrell and Brown, 2000) and damage to medial agranular PFC results in multimodal attentional neglect (King and Corwin, 1992, 1993;

Burcham et al., 1997; Reep and Corwin, 2009). Interestingly, the latency of PFC neurons to signal repetition is longer than that of PER neurons (Riches et al., 1991; Xiang and Brown, 1998; Xiang and Brown, 2004). Together, our findings suggest a model in which a bidirectional flow of visual information between PER and PFC coordinates exploration of novelty and familiarity. In this model, similar to one proposed recently (Preston and Eichenbaum, 2013), the PER would signal the presence of a novel or familiar item to the PFC. The PFC then would guide exploration of the item based on other information, for example, the relative familiarity of the context.

How might information about novelty and familiarity be transmitted between the PER and PFC? Computational and experimental studies have suggested two general coding mechanisms by which neurons can transmit information across brain regions-: an asynchronous (rate) code and a synchronous (temporal) code (for review, see Kumar et al., 2010). Although both rate and temporal codes certainly coexist (Singer, 2009; Kumar et al., 2010), propagation across brain regions may be more efficiently processed by temporal codes. Fries (2005) proposed the communication-through-coherence hypothesis, which states that neuronal populations communicate most efficiently when they are oscillating in phase. Although our data do not address this hypothesis directly, one possibility is that oscillations or synchronous spiking support transmission of information about novelty and familiarity between the PER and PFC.

To conclude, the PER has a well established role in distinguishing novel from familiar objects, but how novelty and familiarity are encoded and signaled to other brain regions is unknown. In the present study, we were able to modulate exploration of novelty and familiarity by stimulating the PER at different frequencies. We showed that stimulation of the PER at 30–40 Hz resulted in increased exploration of familiar images as if those images were novel. Stimulation of the PER at 10–15 Hz resulted in decreased exploration of novel images as if those images were familiar. We propose that, when PER neurons fire in phase at these frequencies, transmission of information about novel and familiar objects or images to and from other brain regions implicated in object recognition memory is enhanced.

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