# Control of Perirhinal-dependent Novelty Exploration in Rats by Optogenetic Modulation of Prefrontal Cortex

By
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Thesis

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*Abstract of* ***Control of Perirhinal-dependent Novelty Exploration in Rats by Optogenetic Modulation of Prefrontal Cortex****, by Alexia E. Ioannou, ScM. Biomedical Engineering, Brown University, May 2019*

The perirhinal cortex (PER) has a well-established role in identification and recognition of objects. Our lab previously provided evidence that neuronal activity in PER may signal novelty and familiarity through synchronous activity at specific frequencies, with novelty signaled at 30 Hz. Medial prefrontal cortex (mPFC) is also known to be involved in processing information about novelty. Secondary motor cortex (MOs) in the rodent prefrontal cortex plays an important role in processing sensory information in order to guide appropriate goal-directed behavior. MOs and mPFC have strong anatomical connections to PER. Based on this evidence, we suggest that PER interacts directly with mPFC and MOs to provide the necessary cognitive control over novelty exploration. In this study we employed optogenetic techniques to address the hypotheses that PER-PFC interaction is necessary for novelty guided exploration and that these interactions are modulated by synchronous neuronal activity in the low gamma (30-40 Hz) frequency domain. We tested a model in which information about novelty is transmitted from PER to the infralimbic (IL) area of mPFC and subsequent exploratory behavior is modulated by an MOs projection to PER. Rats were bilaterally injected with viral vectors containing channelrhodopsin (ChR2) and implanted with optical fibers in either MOs or IL. During a spontaneous object recognition (SOR) task, rats underwent optical stimulation of 30 Hz, 8 Hz, 20 Hz, and a no-stimulation control. When rats were presented with one novel and one familiar image and exploration of the familiar image was paired with 30 Hz stimulation of either MOs or IL, the rats increased exploration of the familiar image, as if it were also novel. When rats were presented with two identical familiar images, rats treated the familiar image paired with 30 Hz stimulation of MOs as novel, but this did not happen with 30 Hz stimulation of IL. This suggests that novelty is signaled by synchronous activity in a particular frequency band, and that the function of the signal differs for MOs and IL. Our data provides evidence that novelty is communicated through a new type of temporal code in a circuit that includes PER, MOs, and IL.

# INTRODUCTION

## Recognition memory and the brain regions involved

Recognition memory relies on the identification of a prior occurrence of an event together with recalling the context in which this event occurred (Mandler, 1980). Evidence from multiple studies shows that recognition memory is supported by the perirhinal cortex (PER) (Meunier et al., 1993; Brown and Xiang, 1998; Xiang and Brown, 1998; Brown and Aggelton, 2001). PER neurons recorded in rodents can be both visually responsive and repetition-sensitive (Zhu et al., 1995; Wan et al., 1999). PER neurons recorded in nonhuman primates show increased firing rates when a novel image is presented; firing rates decrease with repeat appearances as the image becomes familiar (Brown and Xiang, 1998; Fahy et al., 1993). A previous study from our lab (Ho et al., 2015) found that exploration of novel images could be modulated by stimulation of PER at specific frequencies. PER stimulation in the low gamma frequency band (30-40 Hz) upregulated exploration of familiar images, as if they were novel. Importantly, low gamma stimulation had no impact on exploration of novel images. These findings provide evidence for a new type of neuronal signal or temporal code such that specific frequencies signal specific information. For example, 30-40 Hz might signal “novelty” or perhaps “ok to explore” as this frequency of stimulation increased exploration of familiar images, mimicking exploration seen in animals exploring novel images. The notion that PER oscillations in the low gamma frequency band provide a new type of temporal code raises the question of where the signal originates and what are the target brain regions?

A strong candidate for interacting with PER in the service of novelty exploration is prefrontal cortex (PFC). Indeed, experimental evidence suggests PFC, particularly mPFC, is involved in novelty-guided behavior (Dixon and Christoff, 2014; Rangel-Gomez and Meeter, 2016). Experimental lesion studies show that medial prefrontal cortex (mPFC) is involved in processing information about novelty (Dias and Honey, 2002; Kishiyama et al., 2009; Matsumoto et al., 2007). We also know that PER and PFC are strongly and reciprocally connected (Burwell and Amaral, 1998; Hwang et al., 2018; Suzuki and

Amaral, 1994). Disconnecting mPFC and PER in rats impaired recency discriminations and spatial memory (Barker et al., 2007). Disconnecting the PER and PFC in monkeys resulted in impairments, suggesting that interaction between PER and PFC is important to process novel information during visual object recognition memory (Parker et al. 1998a, Parker et al., 1998b).

What region in the mPFC is of the most interest for our study? Analyzing anatomy data regarding prefrontal connections to the PER showed that PER areas 35 and 36 project to prefrontal regions, most strongly the infralimbic (IL) and prelimbic (PL) regions. IL is the most densely labeled and inputs are stronger from rostral IL than from caudal levels (Hwang et al., 2018). Infusion of a protein synthesis inhibitor or an NMDA receptor antagonist into IL and PL of rats immediately after the sample phase of an object recognition task caused a discrimination impairment 24 hours later (Akirav & Maroun, 2006). Infusing the GABA agonist, muscimol, into the IL in one hemisphere and the PER in the contralateral hemisphere reversibly disconnects these areas and impaired rats' ability to learn rule switching. These results provide evidence that mPFC-PER communication is required for flexible and adaptive behavior based on current spatial location (Hernandez et al., 2017). They also suggest that the IL may be the more relevant structure for modulating exploratory behavior.

What other prefrontal region might be involved? Anatomical studies have also shown that based on comparison of prefrontal cortex in rats and primates, the rostral portion of the rodent secondary motor cortex (MOs) can be considered the functional homolog of the primate dorsolateral PFC (DLPFC), with the caudal portion of MOs being part of a zone homologous to primate frontal eye fields (Uylings and van Eden, 1990). MOs, also called medial agranular cortex, shoulder cortex, and sometimes rat frontal eye fields, plays an important role in guiding goal-directed behavior by processing sensory information (Sul et al., 2011). In rodents, damage to the MOs impairs motor learning, but not execution of motor responses (Kawai et al, 2015), and damage to the MOs also disrupts motor responses driven by sensory input (Erlich et al., 2011). Analysis of the PER-MOs connections shows that PER area 36

projections to MOs exhibit a bimodal topography such that one pathway terminates in rostral to mid-rostrocaudal MOs and the other terminates in caudal MOs (Hwang et al., 2018). This anatomy study provided additional evidence that rostral MOs in rodents is a homolog to primate DLPFC. Both DLPFC in primates and MOs in rodents receive input from sensory areas and project to motor related areas (Conde et al., 1995; Hoover & Vertes, 2011; Hwang et al., 2018; Reep et al., 1987; Saleem et al., 2014). Moreover, like rostral MOs in rodents, DLPFC in primates is thought to be involved in executive control functions (reviewed in Petrides, 2005).

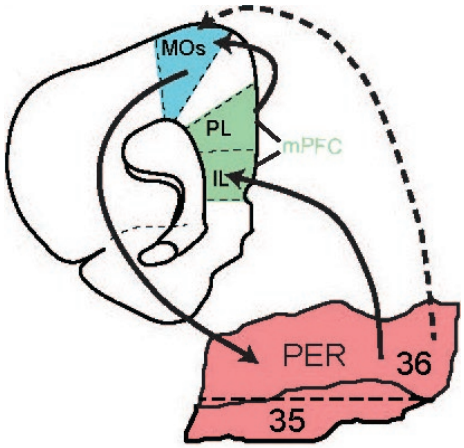



**Figure 1: Proposed circuit for PER-PFC interactions, black lines. Dashed line represents alternative pathway.**

Based on neuroanatomical studies, lesions studies, and preliminary data from our lab, we hypothesize that information about novelty is communicated between the PER and PFC through a temporal code comprised of coherent brain oscillations in the low gamma frequency domain. We propose a model in which information about novelty is transmitted from PER to mPFC area IL and subsequent exploratory behavior is modulated by an MOs projection to PER (Figure 1). If our model is correct, then low gamma stimulation in either the IL or the MOs should modulate exploratory behavior. We used optogenetic techniques to test the ability to alter novelty exploration by stimulating MOs and IL at particular frequencies. We predicted that stimulation in either area at 30 Hz would increase exploration of familiar images, simulating novelty. An alternative prediction was that these regions are

involved in novelty-guided behavior, but do not use the low gamma temporal code. In that case, any impact of optogenetic stimulation on prefrontal regions would not be frequency dependent.

## Optogenetics

Francis Crick proposed an optical method that could excite or inhibit specific neuron types without having an effect on other neuronal populations in 1999 (Crick, 1999; Zhao, 2017). From there, optogenetics began to evolve into what it is today. Optogenetics uses genetically encoded light-sensitive opsins to target specific neurons or proteins and allows for imaging, tracking, or control of these targets in living animals (Boyden et al., 2005; Deisseroth, 2006). Optogenetics has many advantages including cell-type specificity, reversible and repeatable stimulation, modulation of neuronal firing with a timescale of just milliseconds, and the ability to do this modulation in awake and freely moving animals. Optogenetics can be integrated with electrophysiological recordings and allows for anatomical tracing using fluorescent reporter proteins that are fused to opsins (Gradinaru et al., 2007).

Channelrhodopsin 2 (ChR2) was discovered in 2003 as a photosensitive protein that can be transgenically expressed in neurons (Nagel et al., 2003). ChR2 is an ion channel directly gated by light, such that when excited by blue light (~470 nm wavelength), ChR2 channels open and depolarise the membrane (Boyden et al., 2005). It has a rapid on-rate (Nagel et al., 2003) when it is stimulated with 470 nm light. ChR2 requires less than 1 $mW/mm^2$ light intensity to reach the threshold allowing for low-intensity optical neuromodulation (Dawydow et al., 2014).

Although optogenetics has the advantages that were previously mentioned, there are a few limitations. ChR2 has a fairly high level of desensitisation, and can reduce the current by almost 80%. However, this desensitised result fully recovers after about 25 seconds in the dark, therefore it is reversible (Nagel et al., 2003; Lin et al. 2009). Intracellular aggregates can form when ChR2 is expressed at high levels; this can reduce the effectiveness of membrane depolarisation. Low expression can also

reduce the effectiveness of membrane depolarisation. However, overexpression can also be toxic or adversely alter the properties of the membrane (Lin et al., 2009; Tsunoda and Hegemann, 2009).

For our experiments we used ChR2/H134R. This variant of ChR2 has some advantages. It shows a reduction in desensitisation, and a slight increase in light sensitivity when compared to ChR2. These are advantages that lead to an increase in photocurrents. It also has a slower channel closing which may not be considered an advantage as it makes ChR2/H134R less temporally precise than ChR2 (Nagel et al., 2005; Lin et al., 2009). We used this ChR2-H134R tagged by a fluorescent marker YFP to identify the cells that were transduced by the virus; this is done without affecting photosensitivity (Zhang et al., 2006). Light was delivered to the brain by a blue LED that was coupled to a patchcord which attached to a thin optical fiber that was implanted into the MOs or the IL of the rats. The fiber was aimed at the opsin expressing cells and due to the tapered tip, light should have dispersed in the shape of a cone to activate surrounding transduced cells.

## Specific stimulation frequencies

There is currently debate about how novelty, familiarity, and object identity are being encoded in the brain. It is possible that firing rates code for these functions. A rate code would simply be a change in firing rate with novelty. It is also possible that oscillatory or synchronized neuronal activity codes for these functions. There may even be a mixture of firing rates and oscillatory neuronal activity to code for novelty, familiarity, and object identity. Recordings in the human hippocampus have shown that low gamma power decreases as novel environments become familiar, and low frequency oscillation power increases as novel environments become familiar (Park et al., 2014). Similarly, low gamma power in rodents increased during exploration of novel objects (Lapray et al., 2009), and spontaneous local field potentials (LFPs) in the PER oscillated in the 10-12 Hz (low beta) band when rats foraged in a familiar environment (Nerad and Bilkey, 2005). With these prior studies, and the Ho et al. study in 2015

providing evidence of the importance of the low gamma (30-40 Hz) frequency band in novelty guided exploration, we decided to focus our study on 30 Hz stimulation of the MOs and IL.

Theta frequency (6-12 Hz) and theta oscillations are present during working memory tasks (Jensen & Tesche, 2002; Lee et al., 2014; Sarnthein et al., 1998), encoding new information (Klimesch, 1999; Lee et al., 2014), and memory retrieval (Bastiaansen et al., 2005; Guderian & Duzel, 2005; Klimesch et al., 2001; Lee et al., 2014). Therefore, theta oscillations and changes occur during novelty processing, especially in the right prefrontal cortex of humans (Mecklinger et al., 1997; Opitz et al., 1999). The importance of theta frequency oscillations in prefrontal cortex in humans during recognition memory interested us in the effect of theta frequency stimulation on novelty exploration in rats. In our experiments in the IL and MOs, we stimulate at theta frequency (8 Hz) during the exploration of familiar images and assess the effects on novelty guided exploration.

To confirm the frequency dependent nature of exploratory behaviour, we also tested at 20 Hz. We chose 20 Hz as it was not in the theta, low gamma, or low beta range. Based on preliminary data collected in our lab we saw no significant decrease in exploration of novel images, and no significant increase in exploration of familiar images when rats were stimulated at 20 Hz during exploration. This preliminary data also showed that LFPs of rats stimulated at 20 Hz were similar to rats receiving no stimulation.

We chose to use a spontaneous object recognition task (SOR) to assess behavior of rats. The SOR task relies on the innate preference of rats to explore novel objects over familiar objects. This task truly tests novel object recognition as it doesn't require any rule learning or other type of reference memory. It also does not utilize any kind of positive or negative reinforcements, which makes it repeatable (Ennaceur and Delacour, 1988). On any experiment day, the images that are used are always novel and never before seen by the animals prior to testing.

Here we provide evidence that PER-PFC interaction is important for novelty-guided exploration. We also provide evidence that PER-PFC interaction in the service of novelty-guided exploration engages a temporal code at 30 Hz. To our knowledge this is the first evidence that a temporal code carries specific information in the brain.

# MATERIALS & METHODS

## Animal procedures

Subjects were twenty-six adult male Long-Evans rats. Procedures were approved by the Brown University Institutional Animal Care and Use Committees (IACUC) and performed in accordance with the animal welfare guidelines of the National Institutes of Health.

Rats were pair housed before surgery and single housed after surgery in a 12-hour light/dark cycle. All animals were enriched with toys. In addition to toys, six animals had modest social enrichment as there was a porous barrier between cages allowing rats to smell and hear each other. All animals had *ad libitum* access to water.

## Viral injections

All animals were injected with a viral vector incorporating the channelrhodopsin (ChR2) gene driven by a Synapsin1 neuron specific promotor. Eight animals were injected with a lentiviral ChR2, VSVG.HIV.SIN.Syn.ChR2(H134R)-eYFP.WPRE(Addgene20945) from the University of Pennsylvania vector core. Eighteen animals were injected with an adeno-associated viral vector, AAV2-syn-ChR2(H134R)-EYFP-WPRE-PA from the University of North Carolina vector core.

## Surgeries

Animals were placed in an induction box and anesthesia was induced with 3% isoflurane. Once anesthetized, the animals were secured in a stereotaxic frame in a flat skull position with isoflurane through a nosecone maintained at 2.5 – 1.5% for the entire surgical procedure. The rat skull was exposed through an incision and anchor screws were attached to the skull. Craniotomies were made

bilaterally over the target sites in MOs for the first experiment and IL for the second experiment. A 24 G guide cannula (Plastics One) was implanted at stereotaxically determined sites in the bilateral target regions. The guide cannulae were fixed above cortex and secured to the skull with bone cement (DePuy).

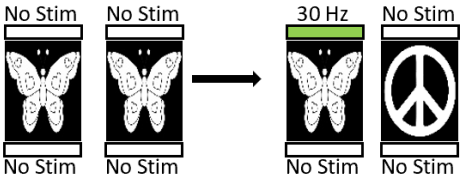
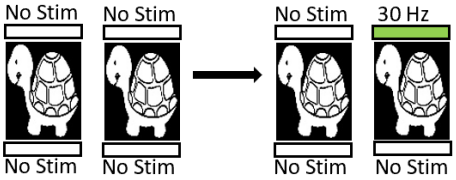
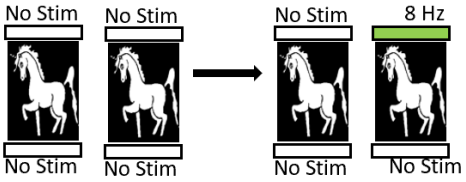
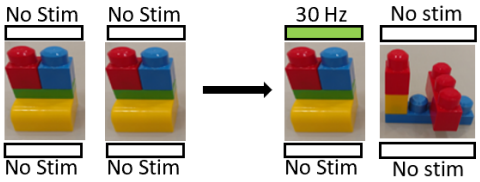
Viral injections were made with an infusion cannula connected to an infusion pump (Harvard Apparatus) and delivered by a glass micropipette pulled to 30-50 µm outer diameter. The viral vector was infused at a rate of 0.1 µl/min for a total volume of 1.5 µl of virus to be injected into one hemisphere. The infusion took a total of 15 minutes and was followed by a 5-minute waiting time. Then, the infusion cannula was removed slowly and replaced by an optical fiber inserted into the guide cannula such that the tapered fiber tip was centered in region that was just transduced with virus. The virus infusion and fiber implantation were repeated again in the other hemisphere. The optical fibers were cemented into place with bone cement (DePuy) and the incision was closed by sutures. 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 animal colony room. Rats recovered for at least 14 days to allow transduction of the viral vector before testing.

## Optical Stimulation

IL and MOs regions were bilaterally transduced with the light-sensitive excitatory protein ChR2 for optical stimulation (Nagel et al., 2003; Boyden et al., 2005) as outlined in the surgical procedures. The implanted fibers used for optical stimulation were multimode optical fibers (Polymicro Technologies; 200 µm core, 0.22 NA) that were pulled to a taper before implantation. Fibers were pulled using a laser-based pipette puller (P2000; Sutter Instruments). Tapering the fibers minimized damage to the brain tissue during implantation, and improved light diffusion throughout the transduced tissue. All pulled fibers were tested before implantation and only fibers delivering light with at least 1 mW/mm2 efficiency were implanted (Lin et al., 2009). During a spontaneous object recognition (SOR) task, a patch

cord (Doric Lenses, .37 NA) was connected to the implanted optic fiber. The patch cord was attached to a blue LED (wavelength = 465 nm) delivering light at either 30 Hz, 8 Hz, or 20 Hz frequencies, depending on the experiment's stimulation parameters. The LED was controlled by TTL pulses issued by Radiant software and hardware coupled to a 4-channel driver system by Plexon (PlexBright 4-channel driver Output Module, DIG-26TTL; Med Associates). Control of the LED for stimulation was performed using Med Associates hardware, custom software written in Med State Notation (Med Associates), and Radiant Software (Plexon, PlexBright). The LED and optical cable were tested before every experiment and the implanted optical fiber was examined before attaching the cable to each rat. Broken fibers were recorded and a note was made as to the date and experiment in which the fiber broke. If one fiber was broken, animals were still run unilaterally. We continued to run animals unilaterally since the damage was unilateral and it would not be expected to impair behavior (Hannesson et al., 2004). If both fibers were broken, the rat was removed from subsequent experiments. In control conditions in which no stimulation was paired with either image, the LED patch cords were connected but the software was programmed so no LED turned on during exploration. 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.

**Table 1: Order of Experiments**

| Paradigm | Description | Example Images | | Figure |
|---|---|---|---|---|
| | | Sample | Test | |
| AA-$A_{30}$B | During test phase, one novel image is shown and one familiar image is shown. During exploration of familiar image, the rat is stimulated with 30 Hz stimulation. (n=26, $n_{mos}$=13, $n_{IL}$=13) |   | | 3a, 7a |
| AA-$A_{30}$A | During test phase, two familiar images are shown. During exploration of one of the familiar images (counter balanced by week and run), the rat is stimulated with 30 Hz stimulation. (n=25, $n_{mos}$=12, $n_{IL}$=13) |   | | 3b, 7b |
| AA-$A_{8}$A | During the test phase, two familiar images are shown. During exploration of one of the familiar images (counter balanced by week and run), the rat is stimulated with 8 Hz stimulation. (n=23, $n_{mos}$=10, $n_{IL}$=13) |   | | 4, 8 |
| AA-$A_{20}$A | During the test phase, two familiar images are shown. During exploration of one of the familiar images (counter balanced by week and run), the rat is stimulated with 20 Hz stimulation. (n=11, $n_{mos}$=5, $n_{IL}$=6) |   | | 5, 9 |
| AA-$A_{30}$B, 3D | During test phase, one object built from large LEGOs is shown and one familiar object built from large LEGOs is shown. During exploration of the familiar object, the rat is stimulated with 30 Hz stimulation. (n=15, $n_{mos}$=6, $n_{IL}$=9) |   | | 3c, 7c |

## SOR task

### Materials and apparatus

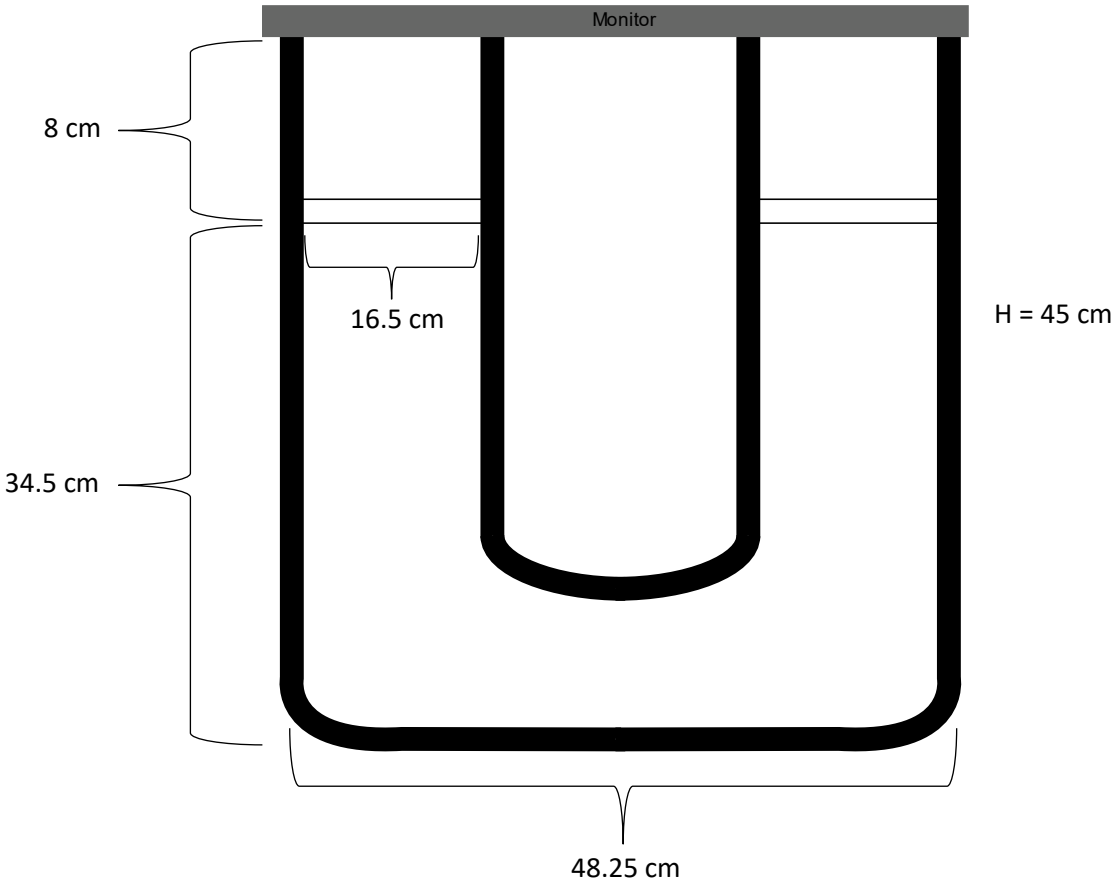



**Figure 2: Horseshoe Maze Dimensions**

The apparatus in which the SOR Task was performed was a horseshoe shaped enclosure (figure 2) that was open on the top and the bottom. The side, middle, and back walls were composed of white acrylic, with the front walls composed of transparent acrylic. The horseshoe maze was placed on a floor projection maze (Jacobson et al., 2014) and a gray floor was projected. A standard monitor was positioned in front of the transparent acrylic front walls of the maze. Black and white clipart images were obtained online (Microsoft) and projected to the monitor based on the idea that rats show preferential exploration of novel 2D images comparable to 3D objects (Forwood et al., 2007). Two images were projected simultaneously onto the monitor, with one image centered in front of each transparent front wall, 8 cm away. The projected images were 10-15 cm when displayed on the monitor

and were different for each run. Rats were monitored by an overhead video camera. 3D objects (large LEGO structures) were also used as a control task and the data is included in the data section. We compared this data to the same task using 2D images and found no significant difference in results.

## Habituation

Rats were habituated to the horseshoe shaped maze for at least 3 consecutive days before the first day of testing. On day 1, rats were placed in the maze with the lights on and no images projected to the monitor. Rats were allowed to explore for 5 minutes before being returned to their home cage. On day 2, the optical fiber ferrules were coupled to the patch cord during habituation. There were still no images being projected to the monitor on this day. Rats were allowed to explore for 5 minutes before being returned to their home cage. On day 3, the patch cord was connected and two images, not used for experiments, were presented during habituation. Rats were allowed to explore for 5 minutes before being returned to their home cage.

## Task procedures

We allowed for at least 14 days between surgery and testing to give time for transduction of the viral vector. After the 14 days, testing began; the rats were run in a series of SOR tasks during which there was a sample period and a test period, separated by a 5-minute delay period. Rats have a propensity to explore novel objects, so the SOR task relies upon this innate preference for novelty. This task has been used extensively to study object recognition memory (Ennaceur and Delacour, 1988; Dix and Aggleton, 1999; Winters et al., 2008). The task used in this study has a sample period in which two identical images are projected to the monitor in front of the maze. The rats were allowed to explore these images until a total of 15 seconds of exploration of the images is reached, or the rat was in the apparatus for 5 minutes, whichever came first. Then, the rats were returned to their home cage for a 5-minute delay period that separated the sample period from the testing period. After 5 minutes passed in the home cage, rats were returned to the horseshoe maze for the testing period. During this testing period two images were presented simultaneously in three different paradigms. There was a standard XX-$X_{stim}$Y

paradigm, in which two identical images were shown during the sample period and one of the two images was replaced by a novel image during the test period. During this paradigm, the familiar image was coupled with stimulation. There was a nonstandard XX-$X_{stim}$X paradigm, in which the familiar images were simply presented again during the test period. One of the familiar images presented during the test period was coupled with stimulation. There was also a control paradigm in which one of the two paradigms previously stated were presented during the test period but there was no stimulation coupled with either of the images in the test period. With these three paradigms, optical stimulation could be used to interfere with preferential exploration of a novel image. Stimulated images, sides, and whether the rat was run as a control or stimulated animal was counterbalanced by run and week to rule out any side bias in the results.

## Data analysis

Control over the projection of the images onto the monitor was performed using Med Associates hardware and custom software written in Med State Notation (Med Associates). Data was acquired using Med Associates hardware, custom software, and a button box. 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 on the left side of the horseshoe maze and the other recorded exploration of the image on the right side of the horseshoe maze. The button was pressed down to begin recording an exploration bout, and pressed down again to indicate the end of an exploration bout and stop the recording. During test conditions in which optical stimulation was paired with exploration of one of the images, the button that corresponded to that image exploration also resulted in Radiant software issuing TTL pulses that controlled LED stimulation trains at the appropriate frequency. During control conditions, the time spent exploring was recorded but no stimulation trains were sent via Radiant. Time stamps were saved in a data file after each sample phase and test phase reached 15 seconds of exploration recorded or 5 minutes of the rat being in the

maze. The information about exploration and bout duration were extracted using MATLAB (MathWorks). An experimenter who was blind to experimental conditions pushed the buttons on the button box to code for exploration while they watched the rats explore the horseshoe maze via an overhead camera above the maze. Active exploration was defined as a period when the rat's nose was directed toward an image, and it was not rearing or grooming. In some cases, rats seem to "zone out" and face an image but not actively explore the image. In this case, active exploration is defined by whisker or head movements, if whisker or head movements did not occur this was not considered active exploration. Preferential exploration of one image over the other during the test period was assessed by computing a discrimination ratio (DR) which was the difference between the time exploring the images divided by the total exploration time ($\frac{novel-familiar}{novel+familiar}$ for the standard SOR paradigm or $\frac{stim-no\ stim}{stim+no\ stim}$ for the non-standard SOR paradigm). The DR measure is used because it takes into account individual rats' differences in total exploration time (Bussey et al., 1999).

## Behavioral analysis

The order of experiments for the studies is shown in Table 1. For each rat, the image that was paired to optical stimulation was counterbalanced on different sides for each run. We also used a within-subject design such that subjects were run as their own controls. For example, half the subjects would be run with optical stimulation in the test condition during the first run and then they would be run as a control in the test condition during the second run. The second half of the subjects would be run as a control in the test condition during the first run, and then run with optical stimulation in the test condition during the second run. The position of the novel image (left or right) was counterbalanced, as well as which images were used as novel and familiar. If images were identical, the side of stimulation was counterbalanced. At least 48 hours separated each run. Rats were removed from data analysis if both ferrules or optical fibers broke. One tailed t-tests were used to determine whether DRs differed significantly from zero or DRs between stimulation and control during the test condition differed

significantly. For differences in the DR, choice exploration times, exploratory bout number, and bout duration across conditions, repeated measures ANOVA (rANOVA) with factors for condition (stim vs control) and image type (novel vs familiar) was used. Level of significance was p = 0.05. SEMs were obtained from normalized means (Cousineau, 2017).

## Histology

**Table 2: Target coordinates for virus injection and fiber location**

| | MOs | IL |
|---|---|---|
| AP | +2.8 | +3.0 |
| ML | +/- 1.7 | +/- 1.2 |
| DV | -1.2 (cortex) | -4.3 (cortex) |
| Angle | 10° | 10° |

At the end of behavioral testing, rats were anesthetized with 4.5% isoflurane and then injected with 1 µL beauthanasia solution to induce deep anesthesia. Subjects were then transcardially perfused with normal saline (8 min) followed by 4% paraformaldehyde (20 min) in 0.1 M sodium PB, pH 7.4. The brain was extracted and then incubated in a 0.1 M formalin for at least 3 days followed by a 30% sucrose solution for at least 3 days. The brain was then cut in the coronal plane into 40 µm sections on a microtome in two adjacent series, one for fluorescence and one for nissl. The sections were mounted onto positively charged slides using GMS (gelatin dissolved in 0.1 M PB) solution. One series was stained for fluorescent DAPI and the other for Nissl.

These were analyzed under the microscope to confirm virus expression and location as well as fiber location. ChR2 expression was located in the MOs coordinates for MOs implanted animals or the IL coordinates for IL implanted animals. For all subjects, the fiber tip was located at the center of the viral injection at the intended target coordinates (table 2).

# RESULTS

To answer the question of whether PER-PFC interaction is necessary for novelty guided exploration and whether it is frequency dependent, we optogenetically stimulated candidate areas in the PFC in rats performing a SOR task. We predicted that 30 Hz stimulation would result in increased exploration of familiar images, as if they were novel. Because oscillations in the theta frequency band are implicated in recognition memory (Lee et al., 2014), we also tested the efficacy of 8 Hz stimulation. Finally, in an earlier study, we found that 20 Hz PER stimulation had no impact, so we included 20 Hz stimulation as a control to confirm that modulation of exploration is frequency specific. We stimulated at 30 Hz, 8 Hz, and 20 Hz in two different experiments, first stimulating in the MOs, and then stimulating in the IL. The results for the test phase of each experiment are described below.

## Experiment 1: Stimulating MOs at different frequencies during exploration

### 30 Hz stimulation to the MOs simulates novelty during exploration

Based on the paper published from our lab in 2015 (Ho et al. 2015), 30 Hz stimulation in the PER was shown to simulate effects of novelty on exploratory behavior. We decided to test this in the MOs, since we know this area is connected to the PER and we hypothesized that MOs is communicating novelty to the PER (Figure 1). To examine whether coupling exploration of a familiar image with 30 Hz stimulation would simulate novelty exploration by increasing exploration time of that image, we began with an SOR task using the standard SOR paradigm AA-$A_{30}$B (n = 13).

In the standard SOR paradigm AA-$A_{30}$B, when rats were run as a control, no stimulation was delivered during exploration of either the novel or familiar image. In the control condition, as expected, rats showed preferential exploration for the novel image over the familiar image (Figure 3a; DR>0, $t_{(12)}$=6.06, p = 0.000). During the test condition of this paradigm, rats received a 30 Hz optical stimulation during exploration of the familiar image. In this stimulation condition, rats did not show a preference to explore the novel image (Figure 3a; DR≈0, $t_{(12)}$=-0.781, p = 0.450). This is in line with what was seen in

the PER, and shows that rats appear to treat familiar images as if they are novel when exploration is paired with 30 Hz MOs stimulation. The control DR was significantly higher than the MOS 30 Hz stimulation run DR (Figure 3a; $F_{(1,12)} = 15.306$, $p = 0.002$).

To confirm that 2D image exploration in the SOR task is similar to 3D object exploration in the SOR task we performed the AA-$A_{30}$B task with 3D objects (n = 6). The monitor was removed from in front of the horseshoe maze and two large LEGO structures were put in its place. During the sample phase, two of the same large LEGO structures were placed in front of the clear acrylic front walls of the maze (one on either side). During the test phase, one of these structures was removed and replaced with a different large LEGO structure that was novel to the rat. During the sample phase, no stimulation was delivered during exploration of either object. During the test phase, when the rat explored the familiar object, 30 Hz stimulation was delivered by a blue LED. During exploration of the novel object, no stimulation was delivered. During the control runs, no stimulation was delivered during exploration of either the novel or the familiar object.

As expected, results of this experiment were similar to the 2D task. During the control condition, rats showed preferential exploration of the novel object over the familiar object (Figure 3c; DR>0, $t_{(5)}=3.701$, $p = 0.014$). When stimulation was coupled with exploration of the familiar object during the test phase, exploration of the familiar object increased, and there was no longer preferential exploration for the novel object (Figure 3c; DR≈0, $t_{(5)}=0.575$, $p = 0.590$). The control DR was not significantly higher than the stimulation run DR but it did trend toward significant (Figure 3c; $F_{(1,5)} = 5.093$, $p = 0.074$). We compared the results of this experiment to the same experiment ran with 2D images and found no significant difference between them (Control: $t_{(5)} = -0.348$, $p = 0.742$; Stim: $t_{(5)} = -0.730$, $p = 0.498$).

To further explore the novelty simulation effects of 30 Hz stimulation on exploratory behavior, we next employed the non-standard paradigm AA-$A_{30}$A (n = 13). During the test phase of this paradigm,

rats were presented with two identical familiar images, the same images that were seen during the sample phase. During the control condition, no stimulation was delivered during exploration of either image. As was expected, rats did not discriminate between the two identical familiar images (Figure 3b; DR≈0, $t_{(11)}$=0.412, p = 0.689). When exploration of one of the familiar images during the test phase was coupled with 30 Hz MOs stimulation, rats explored the paired image more, as if it were novel, showing discrimination between the two images (Figure 3b; DR>0, $t_{(11)}$=3.515, p = 0.005). This apparent simulation of novelty is similar to what was seen in the AA-$A_{30}$B paradigm. When we compared the control DR to the DR when stimulation was coupled with exploration, there was an almost significant difference between these DRs in the animals that were implanted in the MOs (Figure 3b; $F_{(1,11)}$ = 4.725, p = m.052).

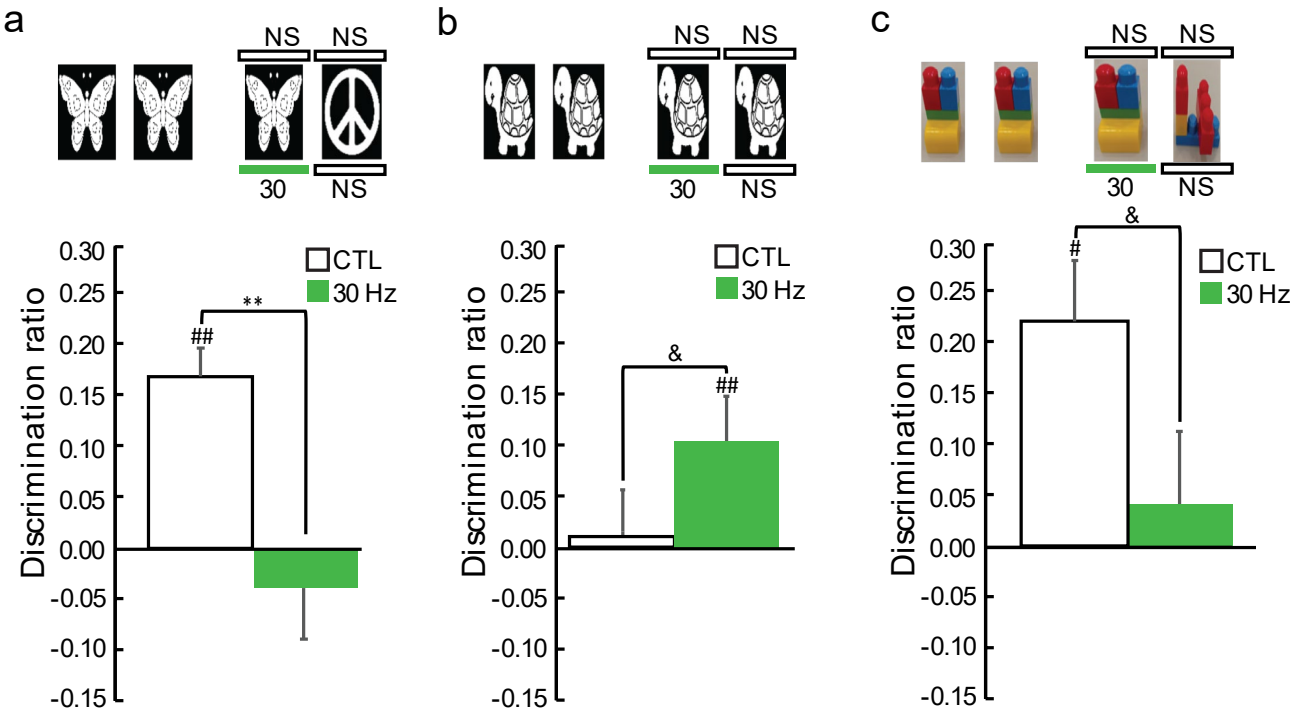



**Figure 3: 30 Hz stimulation to the MOs simulates novelty during exploration.**

**a.** AA-A30B paradigm. Familiar image is paired with 30 Hz stimulation, novel image does not receive stimulation. Control rats discriminated between the novel and familiar image that was presented. Stimulated rats did not discriminate between the familiar object that was paired with 30 Hz stimulation or the novel image, similar to what is observed when non stimulated rats explore two novel images. **b.** AA-A30A paradigm. Two familiar images presented during test phase. One of the two familiar images is paired with 30 Hz stimulation. Control rats did not discriminate between

the two identical familiar images. Stimulated rats explored the stimulated image more than the no stim image as if the stimulated image was novel. **c.** AA-A30B, 3D paradigm. 3D objects were presented instead of 2D images. Exploration of the familiar object is paired with 30 Hz stimulation, novel object exploration does not receive stimulation. Control rats discriminated between the novel and familiar object presented. Stimulated rats did not discriminate between the familiar object that was paired with 30 Hz stimulation or the novel object, similar to what is observed when non stimulated rats explore two novel images.
DR vs. zero: ## $p < 0.01$ , # $p < 0.05$ ; Control vs. Stim: ** $p < 0.01$ * $p < 0.05$ ; & $p < 0.075$

## Effect of theta frequency (8 Hz) stimulation in MOs on exploratory behavior (AA-$A_8$A)

We next examined the effects of 8 Hz (theta frequency) MOs stimulation on the exploration of a familiar image. Similar to the previous paradigm, two identical familiar (same as during the sample phase) images were projected to the monitor. Exploration of one of these images was coupled with 8 Hz stimulation (n = 10). Side of the stimulation was counterbalanced by run and week and the opposite image to the stimulated image did not receive stimulation. During the control phase where no stimulation was delivered during exploration, rats did not discriminate between the two identical images (Figure 4; DR≈0, $t_{(9)}$=-0.612, p = 0.556). When exploration was coupled with 8 Hz stimulation to the MOs, exploration time increased on the stimulated side trending toward significant (figure 4; DR>0, $t_{(9)}$=2.193, p = 0.056). The DR during the condition in which stimulation was coupled with exploration was not significantly different than the DR during the control condition ($F_{(1,9)}$ = 3.379, p = 0.099).

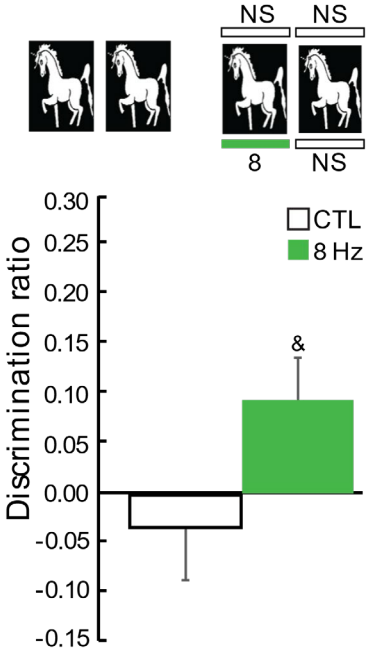



**Figure 4: Theta (8 Hz) stimulation to the MOs**
Two familiar images presented during test phase. One of the two familiar images was paired with 8 Hz stimulation. Control rats did not discriminate between the two identical familiar images. Stimulated rats explored the stimulated image more than the no stim image but not significantly more.
DR vs. zero: & $p<0.075$

## The effect of 20 Hz stimulation in MOs on exploratory behavior: Behavioral response to stimulation is frequency dependent

Next, we examined the effects of 20 Hz stimulation on exploratory behavior (n = 5), to determine whether exploration changes seen previously were stimulation dependent or specifically frequency dependent. In this experiment, similarly to the previous experiments, we used a paradigm in which rats were presented with two familiar images during the test phase (AA-$A_{20}$A). Results of control runs showed no significant discrimination between the two identical images, as expected (Figure 5; DR≈0, $t_{(4)}$=-0.592, p = 0.586). When exploration of one of the images during the test phase was coupled with 20 Hz stimulation, there was no significant discrimination between the two images observed in either brain region (Figure 5; DR≈0, $t_{(4)}$=-0.302, p = 0.777). When comparing the control runs where there was no stimulation coupled with exploration, to the runs where there was 20 Hz stimulation being coupled with exploration of one of the familiar images, there was no significant difference between the DRs of either condition ($F_{(1,4)}$ = 0.088, p = 0.781). This supports the hypothesis that exploration changes are not just stimulation dependent but are frequency specific.

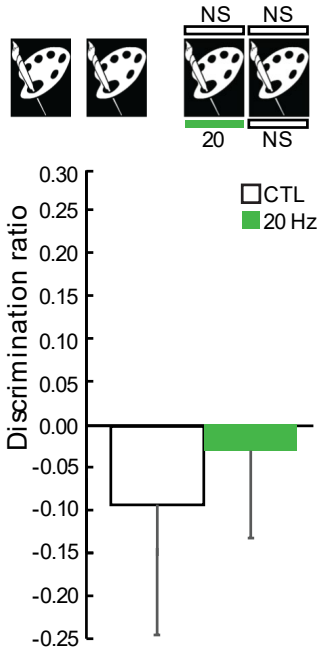



**Figure 5: 20 Hz stimulation to the MOs**
Two familiar images presented during test phase. One of the two familiar images was paired with 20 Hz stimulation. There was no significant difference between control rats and stimulated rats. Neither control rats nor stimulated rats discriminated between the two identical images.

## MOs Histology

Figure 6 is a fluorescent image taken at 4x magnification of sections that show actual MOs virus and fiber placement. Virus expression varied among the animals however all animals had cells that were transduced with the virus. Transduced cells were visible at 10x magnification or lower. The fiber was centered in the transduced area of the virus. Virus spread was variable between animals. In the MOs most animals showed strong virus expression, with an average AP spread of 1.2 mm (minimum .48 mm, maximum 2.4 mm) as seen under a microscope at 10x magnification. Borders were drawn around a section to confirm that virus and fiber were both located inside the confines of the MOs (figure 7).

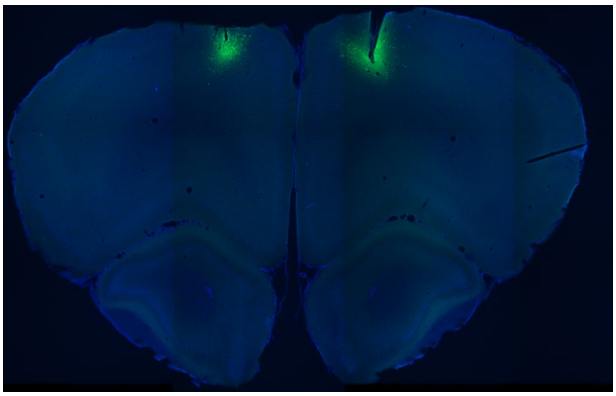


**Figure 6: Fluorescent image taken at 4x magnification of MOs virus and fiber location**
A coronal section through the prefrontal cortex. The green area is the fluorescent tagged virus allowing for visualization of transduced cells. Virus transduced in MOs. Target coordinates for the injection were AP +2.8, ML +/- 1.7, DV -1.2.

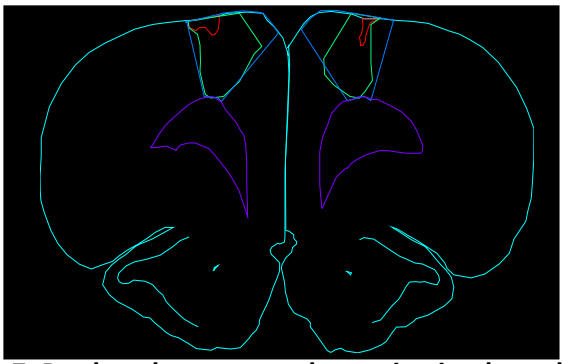


**Figure 7: Borders drawn around a section implanted in MOs**
A coronal section through the prefrontal cortex was analyzed and borders were drawn around it to confirm placement of fiber and virus hit the target areas. The cerulean border is the brain section outline, the green border is drawn around the fluorescent virus, the red border is surrounding the fiber track. The blue border surrounds the MOs drawn based on *The Rat Brain in Stereotaxic Coordinates,* Paxinos and Watson, 1982. The purple

outline surrounds the corpus callosum. The fiber and virus were both located inside the MOs border.

In some cases, the fiber implant was lost as stated in the optical stimulation section. These cases were analyzed and unilateral and bilateral data was compared. No significant difference was found between the unilateral and bilateral data therefore unilateral data was kept in. ChR2 expression and spread was analyzed. The transduced regions were observed for any photo-induced cell damage but none was observed.

## Experiment 2: Stimulating IL at different frequencies during exploration

### 30 Hz stimulation to the IL simulates novelty during exploration… or does it?

Based on the PER paper, and IL's connection to the PER (Fig. 1), we stimulated the IL at different frequencies to observe the effect on novelty driven exploration. To examine whether coupling exploration of a familiar image with 30 Hz stimulation to the IL would simulate novelty exploration by increasing exploration time of that image, we again began with an SOR task using the standard SOR paradigm AA-$A_{30}$B (n = 13).

In the control condition, as expected, rats showed preferential exploration for the novel image over the familiar image (Figure 8a; IL: DR > 0, $t_{(12)}$=2.49, p = 0.028). During the test phase of this paradigm, rats received a 30 Hz optical stimulation during exploration of the familiar image (A). In this stimulation condition, rats did not show a preference to explore the novel image (Figure 8a; IL: DR≈0, $t_{(12)}$=0.939, p = 0.366). This is in line with what was seen in the PER and in the MOs, and shows that rats appear to treat familiar images as if they are novel when exploration of the familiar image is paired with 30 Hz stimulation. However, the control DR was not significantly higher than the stimulation run DR during this experiment (IL: $F_{(1,12)}$ = 2.567, p = 0.135).

To confirm that 2D image exploration in the SOR task is similar to 3D object exploration in the SOR task we performed the AA-A30B task with 3D objects (n = 8). As expected, results of this experiment

were similar to the 2D task. During the control condition, rats showed preferential exploration of the novel object over the familiar object (Figure 8c; IL: DR>0, $t_{(7)}$=3.554, p = 0.009). When stimulation was coupled with exploration of the familiar object during the test phase, exploration of the familiar object increased, and there was no longer preferential exploration for the novel object (figure 8c; IL: DR≈0, $t_{(7)}$=0.722, p = 0.494). The control DR was not significantly higher than the stimulation run DR but it was trending toward significant (figure 8c; $F_{(1,7)}$ = 5.406, p = 0.053). We compared the results of this experiment to 2D results and found no significant difference between them (Control: $t_{(7)}$ = -1.022, p = 0.341; Stim: $t_{(7)}$ = 0.261, p = 0.801).

To further explore the novelty simulation effects of 30 Hz stimulation on exploratory behavior, we employed the paradigm AA-$A_{30}$A ($n_{IL}$ = 13). During the test phase of this paradigm, rats were presented with two identical familiar images, the same images that were seen during the sample phase. During the control condition, no stimulation was delivered during exploration of either image. As was expected, rats did not discriminate between the two identical familiar images (figure 8b; DR≈0, $t_{(12)}$=0.317, p = 0.756). Interestingly however, rats implanted in the IL did not show discrimination between the two images when 30 Hz stimulation was coupled with exploration of one of the images (figure 8b; IL: DR≈0, $t_{(12)}$=0.921, p = 0.375). There was no significant difference between the DR of the control runs or the DR of the stimulated runs in the rats that were implanted in the IL (IL: $F_{(1,12)}$ = 0.055, p = 0.818). This contradicts what was seen in the AA-$A_{30}$B paradigm where this 30 Hz stimulation appears to simulate novelty.

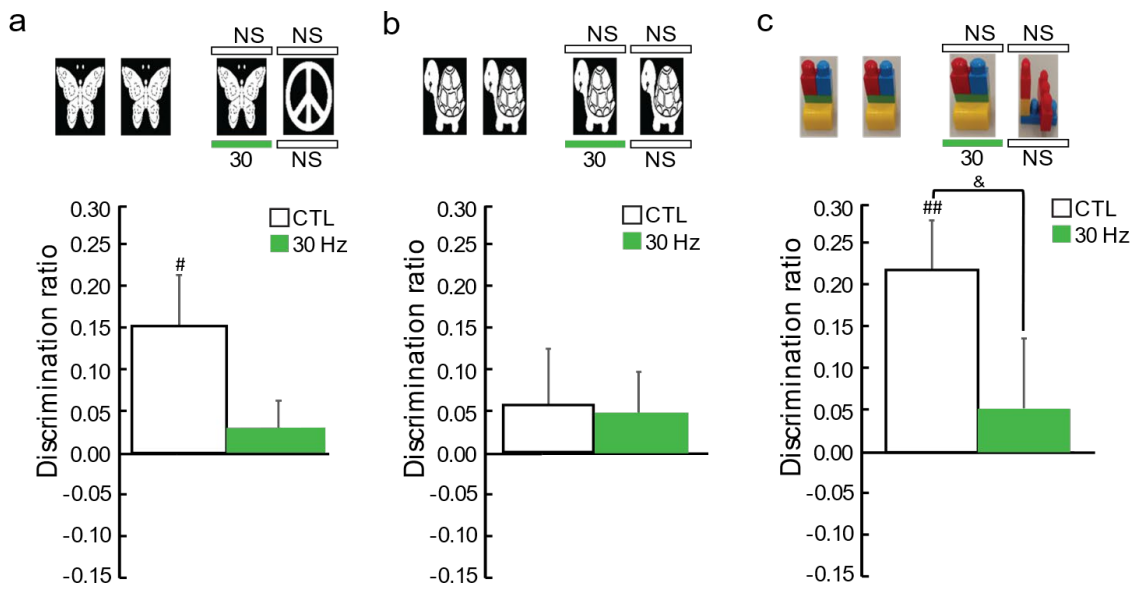



**Figure 8: 30 Hz stimulation to the IL**

**a.** AA-$A_{30}$B paradigm. Familiar image is paired with 30 Hz stimulation, novel image does not receive stimulation. Control rats discriminated between the novel and familiar image that was presented. Stimulated rats did not discriminate between the familiar object that was paired with 30 Hz stimulation or the novel image, similar to control rats presented with two novel images. **b.** AA-$A_{30}$A paradigm. Two familiar images presented during test phase. One of the two familiar images is paired with 30 Hz stimulation. Control and stimulated rats explored in similar ways, not discriminating between the two identical images. **c.** AA-$A_{30}$B, 3D paradigm. 3D objects were presented instead of 2D images. Exploration of the familiar object is paired with 30 Hz stimulation; novel object exploration does not receive stimulation. Control rats discriminated between the novel and familiar object presented. Stimulated rats did not discriminate between the familiar object that was paired with 30 Hz stimulation or the novel object, similar to control rats presented with two novel images. DR vs. zero: ## $p < .01$ , # $p < 0.05$; Control Vs Stim: & $p<0$ .075

## Effect of theta frequency (8 Hz) stimulation in IL on exploratory behavior (AA-$A_8$A)

We next examined the effects of 8 Hz (theta frequency) stimulation on the exploration of a familiar image. Two identical familiar (same as during the sample phase) images were projected to the monitor. Exploration of one of these images was coupled with 8 Hz stimulation (IL = 13). Side of the stimulation was counterbalanced by run and week and the opposite image to the stimulated image did not receive stimulation. During the control phase, rats did not discriminate between the two identical images (Figure 9; IL: DR≈0, $t_{(12)}$=-0.560, p = 0.586). When 8 Hz stimulation to the IL was coupled with exploration, no significant effect on exploration time or DR was observed (figure 9; DR≈0, $t_{(12)}$=0.351, p = 0.732). The DR during the condition in which stimulation was coupled with exploration was not significantly different than the DR during the control condition (IL: $F_{(1,12)}$ = 0.240, p = 0.633).

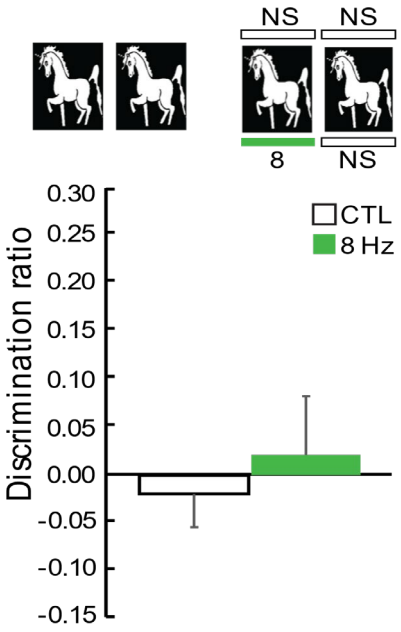



**Figure 9: Theta (8 Hz) stimulation to the IL**
Two familiar images presented during test phase. One of the two familiar images was paired with 8 Hz stimulation. Neither control nor stimulated rats discriminated between the two identical familiar images.

## The effect of 20 Hz stimulation to IL on exploratory behavior: frequency of stimulation is important

We examined the effects of 20 Hz stimulation on exploratory behavior (IL = 6), to determine whether exploration changes were stimulation dependent or specific frequency dependent. Using a paradigm in which rats were presented with two familiar images during the test phase (AA-$A_{20}$A), results

of control runs showed no significant discrimination between the two identical images, as expected (figure 10; DR≈0, $t_{(5)}$=0.606, p = 0.571). When exploration of one of the images during the test phase was coupled with 20 Hz stimulation, there was no significant discrimination between the two images observed (figure 10; DR≈0, $t_{(5)}$=0.969, p = 0.377). When comparing the control runs, to the runs where there was 20 Hz stimulation coupled with exploration of one of the familiar images, 20 Hz stimulation produced similar results to the control condition with no stimulation($F_{(1,5)}$ = 0.003, p = 0.960). This again supports the hypothesis that exploration changes are not just stimulation dependent but are frequency specific.

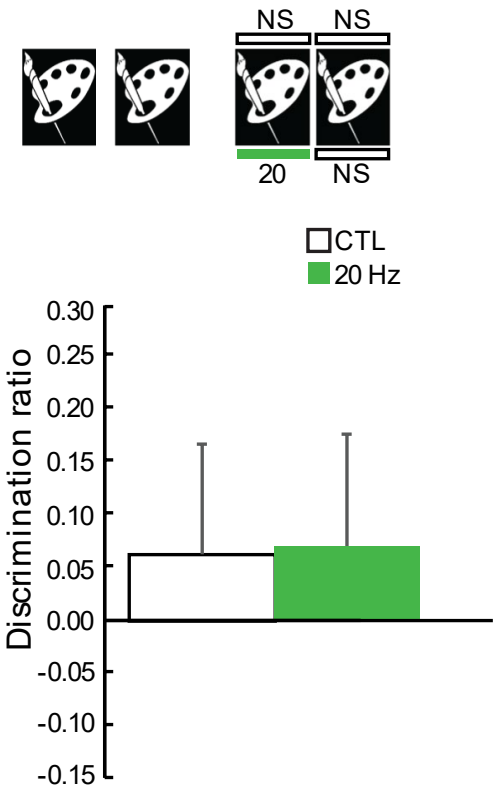



**Figure 10: 20 Hz stimulation to the IL Confirms Frequency Specific Modulation of Exploration**
Two familiar images presented during test phase. One of the two familiar images was paired with 20 Hz stimulation. Neither control nor stimulated rats discriminated between the two identical familiar images.

## IL Histology

Figure 11 is a fluorescent image taken at 4x magnification to show IL virus and fiber placement. Virus spread was variable between animals and virus expression in the IL very variable. Cells transduced by virus were hard to see in some animals, but all animals had at least some transduced cells visible

under 10x magnification. The approximate average AP spread in the IL was 1.3 mm (minimum .48 mm, maximum 1.92 mm) as seen under a microscope at 10x magnification. Borders were drawn around a section to confirm that virus and fiber were both located inside the confines of the IL (figure 12).

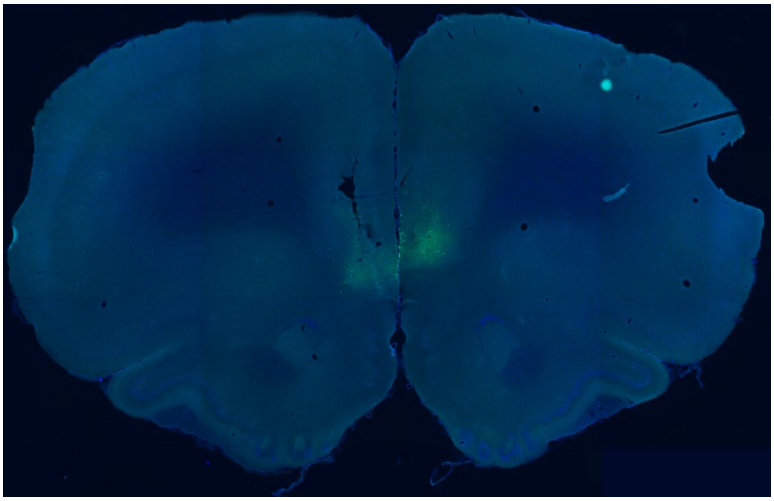


**Figure 11: Fluorescent image taken at 4x magnification of IL virus and fiber location**
A coronal section through the prefrontal cortex. The green area is the fluorescent tagged virus allowing for visualization of transduced cells. Virus transduced in IL. Target coordinates for the injection were AP +3.0, ML +/- 1.2, DV -4.3.

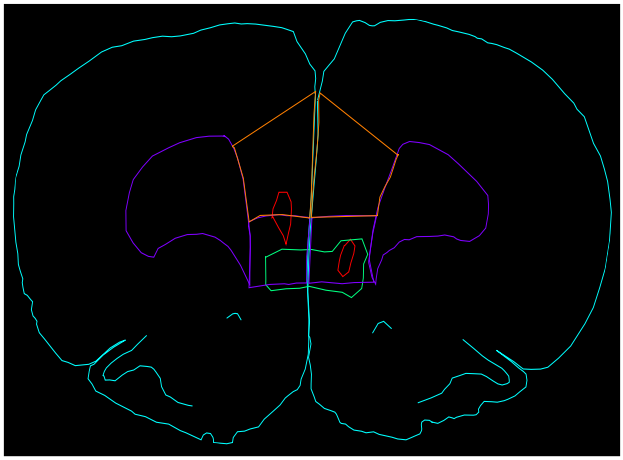


**Figure 12: Borders drawn around a section implanted in MOs**
A coronal section through the prefrontal cortex was analyzed and borders were drawn around it to confirm placement of fiber and virus hit the target areas. The cerulean border is the brain section outline, the green border is drawn around the fluorescent virus, the red border is surrounding the fiber track. The orange border surrounds the PL drawn based on *The Rat Brain in Stereotaxic Coordinates,* Paxinos and Watson, 1982. The purple outline surrounds IL drawn based on the rat atlas. The other purple border surrounds the corpus callosum. The fiber and virus were both located inside the IL border.

In some cases, the fiber tip and virus spread were located in the PL rather than the IL. IL vs PL data were analyzed and compared and no significant difference was found between the two. ChR2 expression and spread was analyzed. The transduced regions were observed for any photo-induced cell damage but none was observed.

## DISCUSSION

In 2015 Ho et al. published a study in which exploration of novel and familiar images was modulated using optogenetic techniques to stimulate the PER at different frequencies. Stimulation of the PER at 30-40 Hz resulted in an increased exploration of familiar images as if rats were perceiving these images as novel. It was then proposed that when PER neurons fire in phase at 30 Hz, information about novelty is transmitted to and from brain regions implicated in object recognition memory. Neuroanatomy studies show that PER is connected to the PFC (Dias and Honey, 2002; Matsumoto et al., 2007; Kishiyama et al., 2009; Hwang et al., 2018), with the strongest connections to the mPFC and MOs (Burwell and Amaral, 1998; Agster and Burwell, 2009; Hwang et al., 2018). Damaging mPFC impairs the ability to shift attention between perceptual domains (Birrell and Brown, 2000), and damaging MOs results in multimodal attentional neglect (King and Corwin, 1992; Burcham et al., 1997); suggesting that both of these regions are involved in attention.

Based on these anatomy studies and the previous data, we hypothesized that information about novelty is communicated between the PER and PFC through a temporal code comprising coherent brain oscillations in the low gamma frequency domain. We proposed a model in which information about novelty is transmitted from PER to mPFC area IL, and subsequent exploratory behavior is modulated by an MOs projection to PER. We used optogenetic techniques to test the ability to modulate novelty exploration by stimulating MOs and IL at different frequencies. We expected that if our model is correct, stimulation in either area at 30 Hz would increase exploration of familiar images, simulating novelty.

When stimulating the MOs at 30 Hz, we saw an increase in exploration of familiar images, as if the images were novel. This was the result when rats were presented with one novel image and one familiar image paired with 30 Hz MOs stimulation; in this condition, rats no longer discriminated between the novel and familiar image, apparently treating both images as if they were novel. When rats were presented with two identical familiar images, one of which was paired with 30 Hz MOs stimulation, rats explored the paired familiar image more than the unpaired familiar image, as though it was novel. This supports the idea that the MOs is important for modulating exploratory behavior, and that 30 Hz is the frequency supporting information transfer. As there was no significant difference in total exploration times between control animals and stimulated animals, this suggests that the optogenetic stimulation did not have an effect on the locomotor activity.

Results of stimulating IL at 30 Hz were inconsistent. When rats were presented with one novel image and one familiar image paired with 30 Hz IL stimulation, they explored the paired familiar image as much as the novel image. However, when rats were presented with two identical familiar images, one of which was paired with 30 Hz stimulation, there was no preference for the paired image. There was no significant difference in total exploration times between the non-stimulated controls and the stimulated animals confirming that the optogenetic stimulation did not have an effect on the locomotor activity.

Novelty is important for learning, memory, and adaptation. Many animals, including rats, monkeys and humans, have the innate preference to explore novel objects. Novelty and familiarity are important for adaptive behavior, as it is sometimes necessary to suppress that activity when exploration is not optimal; e.g. a hungry animal may avoid exploring a novel item when navigating toward a food source. Evidence indicates that such cognitive control relies on PFC (reviewed in Lehky and Tanaka, 2016). Experiments have confirmed that mPFC-PER communication is required for flexible and adaptive behavior (Hernandez et al., 2017). Whereas PER is an area that is prominently viewed as having a role in recognition memory (Brown and Aggleton, 2001; Warburton et al., 2010; Ho et al., 2015; Meunier et al.,

1993; Mumby and Pinel, 1994; Winters et al., 2004), PFC has a role in executive function (Dalley et al., 2004). MOs is most commonly regarded as an association area, that links sensory inputs and motor outputs (reviewed in Barthas and Kwan 2017; Reep et al, 1987). The suspected primate homolog, DLPFC, is important for novelty processing (Daffner et al., 2000; Knight and Scabini, 1998). IL is part of the mPFC which is considered one of the most important structures for processing spatial information (Delatour, 2000), and contextual information (Barker et al., 2007; Spanswick et al., 2012). In addition, mPFC ensembles of neurons recorded in rats differentiate environmental contexts and changes (Hyman et al., 2012). Due to the differences in these areas, the response to stimulation and novelty is different.

Novelty includes two different components: context novelty and feature novelty (Ranganath and Rainer, 2003). Matsumoto et al., 2007 recorded activity of single cells from the lateral and medial regions of the PFC during tasks in which either context novelty was detected or feature novelty was detected. It was discovered that both lateral and medial PFC contribute to detecting context novelty but it appears that only lateral PFC contributes to the detection of feature novelty (Matsumoto et al., 2007). Based on our belief that MOs is truly a homolog for the primate DLPFC, our results support this discovery as MOs seemed to be consistently responsive to stimulation in response to feature novelty (or the simulation of feature novelty). IL on the other hand responded to stimulation when one novel object and one familiar object were presented but not when two identical familiar objects were presented. This suggests that when one novel object and one familiar object are presented, IL may be interpreting this as a novel context. Since IL has not been proven to be involved in detecting feature novelty, when the two identical familiar objects are presented and one is stimulated, there is no response. IL is only responding to stimulation in an environment that could be interpreted as a novel context. Novelty is likely extremely important in MOs as it can signal danger, and animals would need to be able to respond to the novel stimulus with an appropriate motor output i.e. running away. IL on the other hand is

processing information from multiple areas and spatial cues are seemingly more important than simple novelty in this area.

Multiple lesion studies have gathered evidence that would suggest the mPFC has little to do with novelty-familiarity discriminations (Ennaceur et al., 1997; Hannesson et al., 2004a, Hannesson et al., 2004b). Barker et al., 2007 finds that ventromedial PFC is not required for novelty-familiarity discrimination. These studies don't completely support our results, as we did find that we were able to modulate exploratory behavior through 30 Hz stimulation, even though it was inconsistent. This suggests that this 30 Hz stimulation disrupts novelty guided exploration yet supports that IL is still important in this type of recognition memory. Several studies have shown that recognition memory deficits can occur after PFC damage (Kesner et al., 1996; Ragozzino et al. 2002). These studies more closely support our studies. Though simple novel object preference tasks are not disrupted by IL lesions, rodents with mPFC damage experience deficits in object/context mismatch tasks suggesting a critical role in the mPFC for contextual image processing (Barker et al., 2007; Spanswick et al., 2012). Based on these studies and our results, mPFC may not be directly responsible for novelty detection but it is important for recognition memory surrounding novel associations and contexts. That the rats don't discriminate between two identical images during stimulation in this region suggests that the rats implanted in IL are recognizing the familiar context (same maze, identical images, everything is the same as the sample phase), but when one of the images is novel and one of the images is familiar, this may be interpreted as a novel context.

Our results confirm that IL and MOs are both important areas in communicating novelty, though they communicate novelty in different ways. IL communicates context novelty whereas MOs communicates image or object (feature) novelty. Our results further confirm that 30 Hz is an important frequency to communicate this novelty, as stimulation at 30 Hz during exploration of familiar images appears to simulate novelty.

Based on the frequency dependent aspect of the research done in the PER, we further explored other frequencies to see if the same temporal code is relevant in MOs and IL. Theta oscillations and an increase in theta activity has been observed in the right prefrontal cortex during recognition (Guderian & Duzel, 2005; Klimesch et al., 2001; Lee et al., 2014) thus we explored the effect of theta frequency stimulation during exploration of familiar images in the IL and the MOs. We did not see any significant changes in exploration when stimulating at theta frequency in the IL during exploration. However, we did see some changes in exploration when stimulating at MOs at 8 Hz. The increase in exploration that we see in the MOs is not significant but is trending toward significance. As theta is often implicated in attention, the stimulation could be causing greater attention to the object that is being stimulated causing slightly more exploration than in the controls. We analyzed number of bouts and bout duration because increased duration of exploration in bouts often accompanies novel object exploration (Renner & Seltzer, 1991) and longer bout durations can mean greater attention. However, these measures were variable as some rats had more bouts with a shorter duration and some rats had less bouts of longer duration to show preference for novelty exploration. What we observed during stimulation of 8 Hz suggests that theta frequency is an important frequency band, especially in the MOs, but is not necessarily communicating novelty.

As another stimulation frequency we stimulated at 20 Hz. In our previous study (Ho et al., 2015) and preliminary data we saw no changes in exploration between control animals receiving no stimulation and stimulated animals receiving 20 Hz stimulation. Our results for these two experiments align with what we observed previously; 20 Hz stimulation during exploration did not result in any changes in exploration. This further confirms our hypothesis that novelty is being communicated by oscillations in specific frequency bands of 30-40 Hz.

To conclude, our results indicate that MOs and IL are important regions in novelty guided exploration. Our hypothesis that novelty is communicated between the PER and PFC through a temporal

code comprising coherent brain oscillations in the low gamma frequency domain was further confirmed during our study in MOs, however the inconsistent results in IL confirm the need for further testing. Our original hypothesis was that stimulation in either IL or MOs at 30 Hz would simulate novelty, and we only found this consistently true in MOs. A more context dependent task with stimulation during explorations of familiar contexts would be useful in elucidating if IL is communicating novelty in this same temporal code as PER and MOs appear to communicate within. Currently, our results suggest an alternative hypothesis that IL is involved in novelty-guided behavior but does not use the low gamma temporal code. Optogenetic stimulation to the IL may simply be disrupting exploratory behavior.

These results help to advance our understanding of the neural mechanisms and circuitry involved in novelty recognition. Combined, these results along with Ho et al., 2015 reveal a possible new function of temporal coding, where 30 Hz frequency enhances the transmission of novelty information across PER-PFC connections. This evidence can provide better understanding of how brain oscillations modulate cognitive processes and transmit abstract information.

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