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Medial Prefrontal-Perirhinal Cortical Communication is Necessary for Flexible Response Selection

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Abstract

The ability to use information from the physical world to update behavioral strategies is critical for survival across species. The prefrontal cortex (PFC) supports behavioral flexibility; however, exactly how this brain structure interacts with sensory association cortical areas to facilitate the adaptation of response selection remains unknown. Given the role of the perirhinal cortex (PER) in higher-order perception and associative memory, the current study evaluated whether PFC-PER circuits are critical for the ability to perform biconditional object discriminations when the rule for selecting the rewarded object shifted depending on the animal's spatial location in a 2-arm maze. Following acquisition to criterion performance on an object-place paired association task, pharmacological blockade of communication between the PFC and PER significantly disrupted performance. Specifically, the PFC-PER disconnection caused rats to regress to a response bias of selecting an object on a particular side regardless of its identity. Importantly, the PFC-PER disconnection did not interfere with the capacity to perform object-only or location-only discriminations, which do not require the animal to update a response rule across trials. These findings are consistent with a critical role for PFC-PER circuits in rule shifting and the effective updating of a response rule across spatial locations.

Keywords

entorhinal cortex; executive functions; functional connectivity; hippocampus; memory

1. Introduction

The capacity to update one's actions based on environmental contingencies is critical for adaptive behaviors. Dysfunction in this type of cognitive flexibility is associated with

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schizophrenia (Enomoto, Tse, and Floresco, 2011), aging (Barense, Fox, and Baxter, 2002; Beas, Setlow, and Bizon, 2013; Moore, Killiany, Herndon, Rosene, and Moss, 2003), and other disease states (Buckner, 2004; Cunha, Gonçalves, Ometto, Dos Santos, Nicastri, Busatto, et al., 2013; Lavoie and Everett, 2001). Cognitive flexibility is supported by the dorsolateral prefrontal cortex in humans (Demakis, 2003; Owen, Roberts, Polkey, Sahakian, and Robbins, 1991) and other primates (Dias, Robbins, and Roberts, 1996a; b; Moore, Schettler, Killiany, Rosene, and Moss, 2009), and the homologous medial prefrontal cortex (mPFC) in rodents (Birrell and Brown, 2000; Bissonette and Powell, 2012; Floresco, Block, and Tse, 2008; Uylings, Groenewegen, and Kolb, 2003). Specifically, the ability to extinguish a cue-driven behavior, one measure of cognitive flexibility, is mediated by increased neuronal activity within the infralimbic region of the rodent mPFC (Burgos-Robles, Vidal-Gonzalez, Santini, and Quirk, 2007; Milad and Quirk, 2002; Quirk and Mueller, 2008), and age-related decreases in the excitability of these neurons have been linked to flexibility impairments in old animals (Kaczorowski, Davis, and Moyer, 2012).

While the prefrontal cortex, and mPFC in particular, is unequivocally involved in behavioral flexibility (Logue and Gould, 2014), this brain region does not act in isolation to update response selection. In support of this idea, damage to the hippocampus has been shown to impair performance on attentional set-shifting tasks of behavioral flexibility (Cholvin, Loureiro, Cassel, Cosquer, Geiger, De Sa Nogueira, et al., 2013; Malá, Andersen, Christensen, Felbinger, Hagstrøm, Meder, et al., 2015). Furthermore, both mPFC and hippocampal activity are associated with the inhibition of an incorrect response (Lee and Byeon, 2014), and functional connectivity between the frontal cortices and medial temporal lobe is involved in dynamic task switching (Clapp, Rubens, Sabharwal, and Gazzaley, 2011; Wais and Gazzaley, 2014). While these data support that communication between the mPFC and hippocampus is important for behavioral flexibility, the anatomical projections between these areas are relatively sparse (Beckstead, 1979; Sesack, Deutch, Roth, and Bunney, 1989; Vertes, 2002), suggesting that other cortical regions may be important for updating associations between sensory information and desirable outcomes.

A candidate brain region that could be critical for facilitating flexible response selection is the perirhinal cortex (PER), an area within the medial temporal lobe that receives direct input from all sensory modalities (Burwell and Amaral, 1998a; Suzuki and Amaral, 1994). The PER is involved in both memory (Buffalo, Ramus, Clark, Teng, Squire, and Zola, 1999; Suzuki, Zola-Morgan, Squire, and Amaral, 1993) and higher-order sensory perception (Barense, Gaffan, and Graham, 2007; Barense, Ngo, Hung, and Peterson, 2012; Bartko, Winters, Cowell, Saksida, and Bussey, 2007a; b). Moreover, the PER shares reciprocal connections with the mPFC (Agster and Burwell, 2009; Burwell and Amaral, 1998a; Delatour and Witter, 2002; McIntyre, Kelly, and Staines, 1996; Sesack et al., 1989) and the hippocampus (Naber, Witter, and Lopez da Silva, 1999; Witter, Naber, van Haeften, Machielsen, Rombouts, Barkhof, et al., 2000; Witter, Wouterlood, Naber, and Van Haeften, 2000). Furthermore, communication within the mPFC-PER-hippocampal circuit is necessary for an animal's ability to detect when the relationship between an object and its spatial location has changed (Barker, Bird, Alexander, and Warburton, 2007; Barker and Warburton, 2008; 2015). Thus, the PER is positioned to contribute stimulus-specific information as well as link activity patterns in the hippocampus to the mPFC in support of flexible behavior.

Consistent with this idea, mPFC activity enhances interactions between the PER and entorhinal cortex (Paz, Bauer, and Pare, 2007). As PER-entorhinal cortical interactions are believed to gate the flow of information into the hippocampus (de Curtis and Pare, 2004), mPFC modulation of rhinal cortical activity is likely critical for higher cognitive function.

Although the mPFC is necessary for an animal's ability to inhibit an incorrect response (Lee and Byeon, 2014; Lee and Solivan, 2008), and mPFC-PER communication is involved in an animal's ability to detect novel object-place associations (Barker et al., 2007; Barker and Warburton, 2008; 2015; Jo and Lee, 2010a; b), it is not known if communication between these brain areas is critical for flexible behavior. The objective of the current experiments was to examine whether mPFC-PER communication is necessary for performance on the object-place paired association (OPPA) task (Jo and Lee, 2010b), which tests an animal's ability to flexibly update which of two objects is rewarded based on an incrementally learned object-in-place rule that requires knowledge of both object identity and current spatial location. After rats acquired the biconditional association, the necessity of mPFC-PER communication was investigated by infusing the $GABA_A$ receptor agonist muscimol (MUS) into one hemisphere of the mPFC and the contralateral PER to reversibly disconnect these areas. This approach capitalizes on the fact that the mPFC and PER are densely connected within the same hemisphere, but not across hemispheres (Bedwell, Billett, Crofts, MacDonald, and Tinsley, 2015). Thus, unilateral mPFC and contralateral PER inactivation blocks communication between these areas. Importantly, because only one hemisphere of each region is inactivated, the PER and mPFC remain functional as independent entities.

2. Materials and Methods

2.1. Subjects and Handling

Twelve male Fischer 344 rats (NIA colony at Taconic; 6–13 mo. old) were single housed and kept on a reverse 12-hour light/dark cycle, with all testing occurring during the dark phase. After histological verification of cannula placement (see below), 9 rats were included in the current analyses. Upon arrival to the facility, rats acclimated to the colony room for 7 days. After acclimation, the rats were handled by the experimenters for several days before being placed on food restriction. Rats were restricted to 85% of their initial free-feeding body weight, with *ad libitum* access to water for the duration of the experiment. For all surgical and behavioral procedures, adequate measures were taken to minimize pain or discomfort to all animals. All protocols were in accordance with the *Guide for the Care and Use of Laboratory Animals* and the University of Florida Institutional Animal Care and Use Committee.

2.2. Habituation and Training

Rats were habituated to the OPPA arena (Figure 1A) for 10 minutes a day for 2 days prior to training. For all experiments, a two-arm maze constructed from wood and sealed with waterproof black paint was used (Figure 1A; Hernandez et al., 2015). The two arms of the maze were separated by black poster board with distinctive markings on each side to prevent the rat from seeing the opposite arm while providing environmental cues to differentiate the two arms. The arms radiated from a starting platform that was 48.3 cm in diameter. Each

arm was 84.0 cm long and had a rectangular choice platform (31.8 cm × 24.1 cm) attached at the end. Each choice platform contained two food wells (2.5 cm in diameter) that were recessed into the maze floor by 1.0 cm and were separated by 12.8 cm. The arms and choice platforms had 5.5 cm high walls. During habituation, food rewards (Froot Loops; Kellogg's, Battle Creek, Michigan) were scattered throughout the maze to encourage exploration. Once comfortable with foraging, rats were shaped to alternate arms by placing a single reward on the choice platform of the unoccupied arm. After the rat retrieved the reward, the opposite arm was baited. Once a rat consistently alternated 30 times in less than 20 minutes, they began training on the OPPA task.

2.3. Object-Place Paired-Association Task and Pre-Training

Rats were trained to use an object-in-place rule, in which the rewarded object of a pair was contingent on the spatial location (see, Hernandez, Maurer, Reasor, Turner, Barthle, Johnson, et al., 2015; Lee and Solivan, 2008) prior to surgery for cannula placement. Rats began the trial in either the left or right arm of the maze, randomly chosen. During pre-training, rats were required to traverse between the left and right arms for a total of 32 trials, regardless of how many correct choices were made. Arms were not blocked so rats had to correctly remember to alternate. A failure to alternate was recorded as a working memory error. The incidence of this type of error was low (≤1/testing session), and did not significantly vary across infusion conditions ($p > 0.05$ for all comparisons). Because rats freely alternated between the 2 arms to initiate a new trial, the inter-trial interval was based on the amount of time it took a rat to ambulate from one arm to the next and variable across trials.

The same two objects were presented in both arms of the maze, with a food reward hidden in the well beneath the correct object. For example, in the left arm, the "chicks" object was always the correct choice, regardless of whether it was presented on the left or right well (Figure 1B). In the right arm, the "chicks" object was the incorrect choice, and instead the "frog" object was always rewarded. The locations of objects within the arms pseudorandomly varied across trials in order to ensure that the left and right wells were equally rewarded. The rat was required to push the object off of the well it was covering to retrieve the food reward beneath the correct object. If the incorrect object was pushed or touched by the rat's nose, the objects and the food reward were removed from the arm and the rat was not allowed to make another choice in that arm.

During testing, as well as during all training and control sessions, the experimenter stood behind the choice platforms so that the next trial could be set up before the rat exited the current arm it was in. The barrier located between the arms ensured that the rat could not observe which well was baited. All rats completed 32 trials/day for 6 days a week until they achieved criterion performance of 26/32 correct trials 2 days in a row. In cases in which there was a delay between OPPA training and cannulation surgery, rats were tested 3 days per week to maintain OPPA performance.

2.4. Surgery

Each rat was stereotaxically implanted with cannulae bilaterally targeting the mPFC (infralimbic cortex) and PER under isoflurane anesthesia (1–3%). An incision was made to expose Bregma. Small holes were drilled for the placement of 22-gauge guide cannulae (Plastics One, Roanoke, VA; C313G-L20/SPC) at +3.2 mm AP and ±0.9 mm ML from Bregma and 3.8 mm ventral to the skull surface for mPFC, and −5.5 mm AP, ±6.6 mm ML from Bregma and 6.5 mm ventral to the skull surface for PER. The mPFC coordinates were determined based on a previous study showing a role for the infralimbic cortex in the conceptual set-shifting task of behavioral flexibility (Beas, McQuail, Bañuelos, Setlow, and Bizon, 2016). Anchoring screws (1/8″ length; 00–120) were placed in the skull and all hardware was secured with dental cement. Dust caps with dummy stylets were screwed into each cannula to prevent tubing from becoming obstructed. During surgery and post-operatively, the non-steroidal anti-inflammatory Meloxicam (Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO; 1.0 mg/Kg S.C.) was administered as an analgesic. All animals were given 7 days to recover before resuming behavioral testing.

2.5. Infusions

The $GABA_A$ receptor agonist muscimol (MUS) (0.5ug/0.5ul; Sigma-Aldrich, St. Louis, MO) was injected intracerebrally with a microinfusion pump (Harvard Apparatus; Holliston, MA) for the temporary inactivation of the PER and/or mPFC. Polyethylene tubing (Plastics One) was attached to a 10 μl syringe (Hamilton, Franklin, MA), backfilled with sterile water, and then loaded with 2 μl muscimol. An air bubble of 1–2 μl volume was maintained between the backfill and drug to prevent mixing, as well as ensure that the pump was infusing the correct volume of drug. Dust caps were removed and 28-gauge needles (Plastics One, 8IC313ISPCXC) were placed into the proper guide cannulae. Each injector needle protruded 1mm below the guide cannula into the brain, such that the depth of injection was 7.5 mm from the surface of the skull for the PER and 4.8 mm from the surface of the skull for the mPFC infusions. MUS was infused at a rate of 0.1 μl/min over 5 min. Upon infusion completion, needles were left in place for a minimum of 2 min to allow diffusion of the drug. Dust caps were reinserted and the animal was returned to their home cage for a 30-min period before testing began.

2.6. Behavioral Task Post Surgery

Figure 1C shows the order and timeline for all experimental procedures. Once rats recovered from surgery, they were retrained on the OPPA task until performance was at or above criterion (26/32 trials, ≥ 81.25% correct) for at least 2 consecutive days. A pseudorandomized infusion schedule was used for each rat, with a baseline testing session (no infusion) between each infusion day for a total of 15 test days. The infusion conditions were: 1) bilateral mPFC MUS, 2) bilateral PER MUS, 3) left ipsilateral mPFC-PER MUS, 4) right ipsilateral mPFC-PER MUS, 5) contralateral left mPFC-right PER MUS, 6) contralateral right mPFC-left PER MUS, 7) contralateral left mPFC-right PER vehicle control, 8) contralateral right mPFC-left PER vehicle control.

Upon completion of these 8 infusion conditions, rats were trained on 2 additional control tasks. First, rats were trained on an object-only control task (Figure 1B middle panel), during

which only one choice platform of the maze was used. In this task, rats traversed back and forth between a single choice platform and the other arm, choosing a single target rewarded object regardless of its position over the left or right well. Thus, in this task only object information was required to make a correct choice. Once the rat reached criterion performance of 26/32 trials on the object-only control task, a contralateral MUS infusion was administered 30 min prior to testing. After completion of this object discrimination control task, rats were then trained on a side-only task (Figure 1B bottom panel) in the opposite choice platform. In this control task, the correct choice of two identical objects was determined by their placement over a single well on a specific side (left versus right). For example, a rat had to learn to always select the object over the right well, and the left well never contained the reward. The rewarded well was counterbalanced across rats. Rats performed this task until a criterion of 26/32 trials was reached and a final contralateral MUS mPFC-PER infusion was administered 30 min prior to testing.

2.7. Histology and for Fluorescence In Situ Hybridization

At the conclusion of behavioral testing, rats were sacrificed and tissue collected to evaluate accurate placement of the guide cannula in the PFC and PER with either standard histological techniques (n = 7), or by labeling the expression of the activity-dependent immediate-early gene *Arc* (n = 5). For the 7 rats that underwent standard histology, a lethal dose of sodium pentobarbital (Vortech Pharmaceuticals, Dearborn, MI) was administered prior to transcardial perfusion with 4% paraformaldehyde. Brains were stored in 4% paraformaldehyde with 30% sucrose at 4°C for 72 hours and then sectioned at 40 μm on a cryostat (Microm HM550; Thermo Scientific, Waltham, MA), thaw-mounted on Superfrost Plus slides (Fisher Scientific, Waltham, MA) and nuclei were stained prior to cannulae placement confirmation with microscopy. Figure 1D shows the cannulae placement for all rats included in the current experiments. Based on the histology 3 rats were excluded from the analyses because the guide cannula could not be localized to the mPFC and PER.

In a second subset of rats (n=5), fluorescence *in situ* hybridization for the activity-dependent immediate-early gene *Arc* was used to provide a functional assay of MUS infusion specificity to the target regions. In these animals, MUS was infused unilaterally into the PFC and PER 30 min prior to sacrifice (left PFC-PER n = 2, right PFC-PER n = 2, control n = 1). Just prior to sacrifice, the 4 MUS infused rats performed the OPPA task for 10 minutes, completing an average of 46 trials (SD = 1.22). At the conclusion of the behavioral session, rats were deeply anesthetized with isoflurane (Abbott Laboratories, Chicago, IL) and euthanized by rapid decapitation. Tissue was extracted and flash frozen in chilled 2-methyl butane (Acros Organics, NJ). One additional rat was sacrificed directly from the home cage as a caged control. Tissue was stored at −80°C until it processing for fluorescence *in situ* hybridization.

Fluorescence *in situ* hybridization (FISH) for the immediate-early gene *Arc* was performed as previously described (e.g. Guzowski et al., 1999; Burke et al., 2012a). Tissue was sliced at 20 μm thickness on a cryostat (Microm HM550) and thaw-mounted on Superfrost Plus slides (Fisher Scientific). *In situ* hybridization for *Arc* mRNA was performed and z-stacks were collected by fluorescence microscopy (Keyence; Osaka, Osaka Prefecture, Japan) to

confirm that target regions were inactivated and adjacent structures did not have a significant blockage of activity-dependent *Arc* induction. Briefly, a commercial transcription kit and RNA labeling mix (Ambion REF #: 11277073910, Lot #: 10030660; Austin, TX) was used to generate a digoxigenin-labeled riboprobe using a plasmid template containing a 3.0 kb *Arc* cDNA. Tissue was incubated with the probe overnight and *Arc* positive cells were detected with anti-digoxigenin-HRP conjugate (Roche Applied Science Ref #: 11207733910, Lot #: 10520200; Penzberg, Germany). Cyanine-3 (Cy3 Direct FISH; PerkinElmer Life Sciences, Waltham, MA) was used to visualize labeled cells and nuclei were counterstained with DAPI (Thermo Scientific). Two images were taken per region from each hemisphere of all infused rats and the caged control for the PER, mPFC, lateral entorhinal cortex (LEC), area TE and anterior cingulate cortex (AC). This process was repeated on a second section of tissue from each rat. Two rats did not have tissue analyzed for the mPFC and AC, as the *Arc* signal was degraded. For these rats, cannula placement was confirmed with fluorescence microscopy.

Following FISH, z-stacks were taken at increments of 1 μm and the percentage of *Arc* positive cells was determined by experimenters blind to infusion condition using ImageJ software. In order to exclude nuclei that were cut off by the edges of the tissue, only those cells that were visible within the median 20% of the optical planes were included for counting. All nuclei were counted with the *Arc* channel off, so as to not bias the counter. When the total number of cells in the z-stack were identified, the *Arc* channel was turned on to classify cells as positive or negative for *Arc*. A cell was counted as *Arc* positive if the fluorescent label could be detected above threshold anywhere within or around the nucleus on at least 3 adjacent planes.

2.8. Statistical Analysis

To examine the effect of bilateral inactivation of the PER and mPFC on the percent of correct responses, bilateral infusions of MUS were compared to the vehicle control condition with a repeated measures analysis of variance (ANOVA) with 3 different drug conditions of: 1) vehicle control (n = 9), 2) bilateral MUS in PER (n = 9), and 3) bilateral MUS in mPFC (n = 7). Because the primary comparisons of interest were the rats' performances following inactivation of the PER or mPFC relative to vehicle controls, a planned simple contrast was used to compare each bilateral MUS condition to the control.

In order to examine the effects of contralateral mPFC and PER inactivation on OPPA task performance, a second repeated measures ANOVA was used to test the effects of 3 different infusion conditions on behavior: 1) vehicle control (n = 9), 2) contralateral mPFC-PER inactivation (n = 9), and 3) ipsilateral mPFC-PER inactivation (n = 9). For the conditions in which there were two infusions in different brain hemispheres, the mean was determined for each rat such that sample size (n) was the number of rats for each infusion type rather than the total number of infusions. This was done as to not arbitrarily inflate statistical power. In order to quantify the effect of blocking mPFC-PER communication on behavior, the comparisons of interest were contralateral mPFC-PER inactivation relative to the vehicle control and to the ipsilateral mPFC-PER inactivation. Therefore, a planned simple contrast was used to test whether there was a statistical difference between the contralateral mPFC-

PER MUS infusion relative to the vehicle control, and relative to the ipsilateral mPFC-PER MUS infusion. Table 1 summarizes the statistical tests used for the primary comparisons of infusion condition described above.

The same statistical model described above was also used to test the effect of inactivation condition on the side and object bias indices. Rats often display response biases during the acquisition of the OPPA task prior to learning the object-in-place rule (Hernandez et al., 2015; Jo and Lee, 2010a; b; Lee and Byeon, 2014). Thus, indices of a side bias (left vs. right well) and object bias (e.g. “chicks” vs “frog”) were calculated during initial training and for each infusion condition. Side bias was calculated as the absolute value of (total number left choices-total number right choices)/total number of trials. The object bias was calculated as the absolute value of (total number of object 1 choices-total number of object 2 choices)/ total number of choices.

Finally, for two-way comparisons of the effects of hemisphere on performance, and *Arc* expression in infused versus non-infused hemispheres, significance was tested with paired-samples T tests. All analyses were performed with the Statistical Package for the Social Sciences v23 (IBM, Armonk, NY), and statistical significance was considered at p values less than 0.05.

3. Results

3.1. Muscimol Selectively Blocked Activity-dependent Arc Expression in Perirhinal and Medial Prefrontal Cortices

To confirm the MUS infusion in the current study, the expression of the neural activity-dependent gene *Arc* was used to determine if MUS infusion blocked neuronal activity selectively in the PER and mPFC. Specifically, if MUS blocked PER/mPFC activity then the expression of *Arc* should be reduced in these brain areas compared to adjacent areas not targeted for inactivation: LEC, area TE and AC. To test this idea, 4 rats received ipsilateral infusions of MUS 30 minutes prior to performing the OPPA task, while the other hemisphere served as the non-infused control. These rats were tested on OPPA for 10 min and were then immediately sacrificed. In 2 of these rats, tissue was processed for the PER, LEC, area TE, mPFC, and AC. In the other 2 animals, *Arc* labeled tissue was not available for the mPFC and AC. Figure 2 shows representative images from the hemisphere that received MUS infusion and the control hemisphere for the mPFC (Figure 2A) and the PER (Figure 2B). Note the reduced *Arc* signal (red) in the MUS infused regions. Figure 2C shows the mean proportion of *Arc* positive cells in the PER, mPFC, area TE, LEC, and AC for the hemisphere infused with MUS, the control hemisphere, and the caged controls. Repeated-measures ANOVA with the within subjects factor of infusion hemisphere (muscimol versus control) and the between subjects factor of brain region (targeted for muscimol versus adjacent, non-targeted area) revealed a significant main effect of brain region ($F_{[1,14]} = 8.13$, $p < 0.02$), such that the targeted regions (PER and mPFC) had fewer *Arc* positive neurons compared to the non-targeted regions. Moreover, there was a trend towards a significant interaction effect of infusion hemisphere and target region ($F_{[1,14]} = 3.20$, $p = 0.09$). Post hoc analysis indicated that the percent of *Arc* positive cells in the mPFC and PER was significantly reduced in the hemisphere that received MUS infusions relative to the non-

infused hemisphere ($p < 0.04$; Tukey), which was not the case for adjacent regions not targeted for infusions ($p > 0.73$; Tukey).

The percent of *Arc* positive cells in the caged control rat was low (4.0%; SD = 3.53). For the mPFC and PER the levels of *Arc* expression after MUS infusion were not significantly different than those observed in the caged controls ($T_{[5]} = 0.51$, $p = 0.63$; one sample). In contrast, in the hemisphere that was not infused, *Arc* expression was significantly greater relative to the caged control ($T_{[5]} = 3.37$, $p < 0.02$; one sample). Moreover, in the adjacent brain regions that were not targeted for inactivation, *Arc* expression in the MUS infused hemisphere was significantly greater than the caged control ($T_{[9]} = 4.55$, $p < 0.01$; one sample). The observation that MUS blocked *Arc* expression is consistent with previous data (Kubik, Miyashita, Kubik-Zahorodna, and Guzowski, 2012). Moreover, the reduction in activity-dependent *Arc* expression observed in the mPFC and PER, but not LEC, area TE or AC, of MUS infused hemispheres indicates that neural activity in the target regions of the current experiments was selectively blocked. Although this was qualitatively the case for both the PER and mPFC, it was not possible to compare *Arc* expression across hemispheres for the individual regions due to lack of statistical power from the small sample sizes.

3.2. Pre-training and Initial Response Bias

During the initial training sessions on the OPPA task, rats exhibit a “side bias” for selecting the object over a well on a particular side, regardless of the object or the arm of the maze (Hernandez et al., 2015; Jo and Lee, 2010a; Lee and Byeon, 2014; Lee and Kim, 2010). This bias has to be suppressed, presumably through mPFC activity projecting back to sensorimotor areas (Lee and Byeon, 2014) before OPPA task performance shows an improvement. The mean side bias and percent correct responses as a function of days before reaching criterion performance are shown in Figures 3A and B, respectively. The rats in the current study began with a side bias and over the course of 20 days of testing, this bias decreased as indicated by a significant main effect of test day ($F_{[19,76]} = 12.80$, $p < 0.001$; repeated measures). Planned orthogonal contrasts comparing the side bias on each day to the mean of the preceding days indicated that the side bias did not significantly change across testing days until one day prior to reaching criterion performance ($p > 0.1$ for all comparisons; difference contrast). The day before criterion performance was achieved, however, the mean side bias significantly decreased from the preceding day ($p < 0.005$; difference contrast; Figure 3A). Importantly, the significant shift away from a side bias corresponded with rats’ improved performances on the OPPA task. In fact, although rats showed a significant main effect of testing day on performance ($F_{[19,76]} = 14.22$, $p < 0.001$), planned orthogonal contrasts comparing performance on each test day to the mean of the preceding test days did not detect a significant performance improvement across testing days ($p > 0.1$ for all comparisons; difference contrast) until one day prior to reaching criterion performance ($p < 0.001$; difference contrast; Figure 3A). Together these data suggest that the rats perseverated, using a maladaptive response strategy that prevented them from making incremental progress on the task over weeks of training, and that reductions in this side bias were associated with rats reaching criterion performance. Consistent with this idea, when the response bias was plotted against percent correct for all testing days, there was a significant negative correlation over all days of testing between the response bias and percent correct on

the OPPA task ($R^2_{[181]} = 0.71$, $p < 0.001$; Figure 3C). When a correlation value was calculated separately for each rat so that an animal only contributed 1 data point, the mean correlation was also significant ($R^2_{[8]} = 0.87$, $p < 0.01$). Together, these data indicate that rats must move away from using a non-adaptive side bias in order to acquire the biconditional response of flexibly choosing the correct object associated a given maze arm.

3.3. Bilateral Inactivation of the mPFC or PER Impaired Behavioral Flexibility

Before examining the effect of disconnecting the mPFC and PER, whether these regions are independently critical for normal performance on the OPPA task was tested. Rats were bilaterally infused with MUS into the mPFC or PER during separate testing sessions. Infusions involving the left mPFC cannula were excluded for 2 of the 9 rats due to this cannula being misplaced, but the PER infusion conditions were included for these animals. Mean performance was 90.23% correct (SD = 9.10) for vehicle control infusions. Bilateral MUS infusion into the mPFC decreased the percent of correct trials to 69.64% (SD = 16.43) and bilateral PER MUS infusions resulted in 63.67% correct (SD = 15.76; Figure 4). The overall main effect of bilateral infusion condition was statistically significant ($F_{[2,12]} = 10.25$, $p < 0.01$). Planned comparisons of performance across the different drug conditions indicated that the percent correct trials was significantly greater during the vehicle control relative to the bilateral mPFC inactivation ($p < 0.01$; simple contrast) and to the bilateral PER inactivation ($p < 0.01$; simple contrast). Importantly, the amount of time it took rats to complete the 32 trials within a test session did not significantly vary between the MUS and vehicle control conditions for either the mPFC ($T_{[8]} = 1.53$, $p = 0.16$) or PER ($T_{[8]} = 1.36$, $p = 0.21$) inactivation. This observation indicates that the MUS infusions did not cause any overt sensorimotor or motivational impairments that influenced performance.

When the effect of drug condition on the side bias and object bias indices was quantified, there was a significant main effect of infusion condition on an animal's tendency to choose one side over another (i.e., the side bias; $F_{[2,12]} = 9.99$, $p < 0.01$). Planned comparisons indicated that, relative to the vehicle control, the side bias was greater for both the bilateral mPFC MUS ($p < 0.02$; simple contrast) and PER MUS ($p < 0.01$; simple contrast) conditions (Figure 4B). In contrast, there was no main effect of infusion condition on the object bias ($F_{[2,12]} = 2.94$, $p = 0.09$; Figure 4C). This suggests when either the mPFC or PER is inactivated, rats regress to their initial strategy during training in which they select one side, regardless of the object or arm of the maze. Together, these results are consistent with previous studies (Lee and Solivan, 2008; Jo and Lee, 2010b) and suggest that the PER and mPFC may need to interact in order to suppress non-adaptive object selection and use the object-in-place rule for optimal performance.

3.4. mPFC-PER Disconnection Impaired Behavioral Flexibility Relative to Ipsilateral Inactivation

To investigate whether communication between the mPFC and PER is necessary for OPPA task performance, percent correct following contralateral MUS infusions was compared to ipsilateral MUS infusions and vehicle controls. Since inter-region projections are typically more extensive within same hemisphere compared to across hemispheres (Bedwell et al., 2015), this approach blocks communication between inactivated regions while leaving one

hemisphere of each brain region intact to support behavior. For the contralateral infusions, MUS or saline was infused simultaneously into the mPFC of one hemisphere and the PER of the contralateral hemisphere. Ipsilateral mPFC and PER infusions of MUS were used to measure the effect of unilateral inactivation when mPFC-PER communication was left intact. Figure 5A shows the percent correct trials following the different infusion conditions. During ipsilateral MUS infusions, rats maintained 77.29% correct (SD = 16.19), which was not significantly different from criterion ($T_{[8]} = 0.24$, $p = 0.81$). In contrast, contralateral MUS infusions resulted in a reduction of correct responses (59.79%; SD = 15.76). In fact, overall there was a significant main effect of infusion condition ($F_{[2,16]} = 23.62$, $p < 0.001$). Planned comparisons with a corrected α level of $p < 0.017$ (for 3 comparisons) were used to determine if there was a significant difference between different infusion conditions. The percent correct during contralateral mPFC-PER MUS infusions was significantly different when compared to the vehicle control ($p < 0.01$; simple contrast) as well as when compared with ipsilateral mPFC-PER MUS infusions ($p < 0.01$; simple contrast). There was a trend towards an effect of ipsilateral MUS inactivation relative to vehicle infusion that did not reach statistical significance ($p = 0.04$; simple contrast). This is consistent with several studies that have shown behavioral deficits following unilateral inactivation of higher-level association cortical areas (Poe, Teed, Insel, White, McNaughton, and Barnes, 2000; Tanninen, Morrissey, and Takehara-Nishiuchi, 2013; Wilson, Langston, Schlesiger, Wagner, Watanabe, and Ainge, 2013; Wilson, Watanabe, Milner, and Ainge, 2013). Importantly, the amount of time it took rats to complete 32 trials within a testing session did not significantly vary between any of the infusion conditions (contralateral, ipsilateral, or vehicle control; $p > 0.13$ for all comparisons), indicating that MUS infusions did not cause sensorimotor or motivational impairments. Together these data show that blocking mPFC-PER communication impaired the ability of rats to update the selection of the correct object in the different spatial locations, compared to control conditions.

Figures 5B and C show the side and object biases, respectively, for the different infusion conditions. There was a main effect of MUS infusion compared to vehicle controls on side bias ($F_{[2,16]} = 19.56$, $p < 0.01$). Planned comparisons indicated that the side bias was greater for the ipsilateral ($p < 0.03$) and contralateral ($p < 0.01$) conditions relative to saline. There was no main effect of MUS infusions relative to saline controls on object bias during the task for either the ipsilateral or contralateral conditions ($F_{[2,16]} = 0.464$, $p = 0.64$). Importantly, these data indicate that, similar to the bilateral mPFC and PER infusions, disconnecting these regions also resulted in an inability to flexibly update response selection, with rats regressing back to the side bias that is observed early in training.

3.5. Side of Infusion and Repeated Infusions Did Not Alter Behavioral Performance

In order to examine whether or not different hemispheres were similarly affected by inactivation, the lateralization of infusions was compared. This analysis showed there was no significant effect of left versus right hemisphere ($T_{[6]} = 0.04$, $p = 0.97$; Figure 6A). The two possible disconnection positions, left mPFC and contralateral PER and right mPFC and contralateral PER, were also found to not differ significantly ($T_{[6]} = 0.14$, $p = 0.89$; Figure 6B).

The potential effect of repeating the infusions over days was also assessed. Multiple infusions did not adversely affect behavior over the course of testing. Specifically, performance on the OPPA task between infusion days remained above criterion with an average percent correct of 91.95% (SD = 0.10). Moreover, performance on the first non-infusion day was not significantly different than performance on the last non-infusion day ($T_{[7]} = 0.06$, $p = 0.96$), demonstrating that rats were not experiencing adverse effects of multiple infusion conditions. Additionally, there was not a significant difference between vehicle infusion and non-infusion days ($T_{[7]} = 0.64$, $p = 0.54$).

3.6. Contralateral Inactivation Did Not Impair Performance on Control Conditions

Object-only and side-only control tasks were used to determine if communication between the mPFC and PER is needed for the individual elements of the OPPA task, namely the ability to discriminate between rewarded sides or objects, which does not require the animal to update their responses across trials and minimizes the working memory component across trials. There was no significant effect of contralateral MUS infusion on performance during the object-only ($T_{[6]} = 1.54$, $p = 0.18$) or side-only ($T_{[5]} = 0.35$, $p = 0.74$) control tasks when compared to the previous day of testing with no infusion (Figure 7). Importantly, these data suggest that the behavioral deficit resulting from the mPFC-PER disconnection was selective to a behavior that required the integration of object and place information in order to facilitate rule shifting between different target objects.

4. Discussion

The current study examined the extent to which an animal's ability to update the selection of a rewarded object when the correct choice is contingent on spatial location requires communication between medial prefrontal (mPFC) and perirhinal cortices (PER). Blocking neural activity with muscimol (MUS) infusions into either region bilaterally, resulted in significant impairments. This finding confirms previous reports that both regions are necessary for object-place paired association (OPPA) task performance (Jo and Lee, 2010a; b; Lee and Solivan, 2008). The novel insight from the current data is that inactivation of one mPFC hemisphere and the contralateral PER resulted in a decline in OPPA task performance when compared to ipsilateral mPFC-PER inactivations and a regression back to the side bias that is observed early in training. This observation supports the conclusion that mPFC-PER communication is required for rule switching and retrieval of the appropriate biconditional response based on the current spatial location. As rats are able to use both intra- and extra-maze cues to aid response selection, these data are also consistent with previous work showing that communication across the mPRC and PER is necessary for an animal's ability to use spatial or contextual information to guide behavior (Barker et al., 2007), even when the hippocampus remains intact. Conversely, animals' performance on object-only and place-only discrimination tasks, which do not require the association of an object with a location or behavioral flexibility across trials, remained normal after mPFC-PER disconnection. This indicates that mPFC-PER communication is not necessary when the correct response only depends on either stimulus or spatial information and does not vary across trials.

Medial PFC and PER inactivation was verified by visualizing expression of the activity-dependent immediate-early gene *Arc* in rats that were ipsilaterally infused with MUS 30 min prior to testing on the OPPA task. Similar to previous reports (Kubik et al., 2012), blockade of *Arc* expression only occurred in the MUS infused hemisphere, as shown by the presence of *Arc* predominantly in the non-infused hemisphere. Moreover, *Arc* expression was not significantly blocked in brain regions adjacent to the MUS infusion site. Interestingly, in the current experiment, the proportion of *Arc* positive cells observed following OPPA behavior in the non-infused PER hemisphere was only 11%. A previous study reported that over 20% of cells were activated following object exploration (Burke, Hartzell, Lister, Hoang, and Barnes, 2012a). There are several possibilities for this apparent discrepancy. In the previous experiment, rats were presented with 5 objects, as opposed to 2 used in the current study. These data suggest that encountering a greater number of stimuli leads to greater PER activity. An alternative, but not mutually exclusive, possibility is that ipsilateral inactivation led to lower neuronal activity levels overall, which is consistent with the modest deficit in performance following ipsilateral inactivation. These possibilities will need to be explored with future experiments.

The mPFC supports OPPA task performance through several possible and potentially related mechanisms. First, the role of the mPFC may be to govern interactions among medial temporal lobe structures. The PER communicates heavily with the entorhinal cortex, both of which send projections to the hippocampus (Burwell and Amaral, 1998b). During distinct stages of learning, this interaction is facilitated by the mPFC (Paz et al., 2007). It could be that during the OPPA task, the mPFC updates representations in the rhinal cortices, based on reward prediction, to gate the flow of information between the hippocampus and neocortex. Consistent with this hypothesis is the observation that disconnection lesions of the PFC and inferotemporal cortex in monkeys impair delayed nonmatching-to-sample performance (Browning, Baxter, and Gaffan, 2013) and object-in-place scene memory (Wilson, Gaffan, Mitchell, and Baxter, 2007). Additional evidence for a unified mPFC-PER network that supports performance on the OPPA task is that the theta rhythm in the mPFC and hippocampus becomes more synchronized after acquisition of the OPPA task rule (Kim, Delcasso, and Lee, 2011). Because there are limited direct projections from mPFC to dorsal hippocampus, this synchrony may require that information flows through the rhinal cortices, thus making the PER an integral part of this circuit. The PER, however, is not the only structure reciprocally connected with both the hippocampus and prefrontal cortex. In fact, the nucleus reuniens of the ventral midline thalamus in also anatomically poised to functionally link the prefrontal cortex to the hippocampus (McKenna and Vertes, 2004; Vertes, 2002; 2006; 2015; Vertes, Hoover, Do Valle, Sherman, and Rodriguez, 2006; Vertes, Linley, and Hoover, 2015). Moreover, inactivation of the nucleus reuniens leads to deficits in strategy switching (Cholvin et al., 2013), suggesting that this brain area may be critical for flexibility by modulating prefrontal-hippocampal interactions. (Cassel, Pereira de Vasconcelos, Loureiro, Cholvin, Dalrymple-Alford, and Vertes, 2013). The fact that either PER or nucleus reuniens lesions produce behavioral flexibility deficits indicates that, while both regions are necessary for prefrontal-hippocampal interactions, neither structure alone is sufficient.

The mPFC has also been shown to be involved in the flexible control of behavioral responses (Beas et al., 2013; Chadick, Zanto, and Gazzaley, 2014; Ridderinkhof, Ullsperger, Crone, and Nieuwenhuis, 2004), as well as working memory (Sloan, Good, and Dunnett, 2006), both of which may be involved in OPPA task performance. Previous studies using MUS to inactivate the mPFC have reported deficits in rats' abilities to inhibit incorrect responses (Izaki, Maruki, Hori, and Nomura, 2001). Additionally, blocking glutamatergic transmission in the mPFC enhances impulsivity and leads to compulsive perseveration in rats (Carli, Baviera, Invernizzi, and Balducci, 2006). Rats show a significant side bias during the acquisition of the OPPA task and inhibiting this perseveration is essential to being able to learn the object-in-place rule (Hernandez et al., 2015; Lee and Byeon, 2014). Although rats may not stop displaying a side bias, in the days after acquisition of the rule, they may show inhibitory behavior towards the object on the preferred side. In line with this idea, mPFC neurons show selective firing for trials requiring this inhibition before object selection (Lee and Byeon, 2014). Thus, the importance of communication between the mPFC and PER may be to ensure that actions with unwanted outcomes are inhibited, enabling subjects to make the choice with a more desirable outcome. Additionally, the OPPA task requires some active maintenance of previously visited locations in order to correctly alternate. In fact, because the order of left versus right arm trials was not randomized in the current study, rats could have successfully performed the task by alternating between object selection regardless of arm location. Future experiments will randomize left versus right arm trials and increase the inter-trial interval to determine the relative contribution of working memory versus flexibility in OPPA performance.

A final possibility is that the mPFC is necessary for the expression of memory at remote time points (Takehara-Nishiuchi, Nakao, Kawahara, Matsuki, and Kirino, 2006). It is theorized that the mPFC initially relies on the hippocampus to form memories, but later may independently use previous experience to guide adaptive responses (Takehara-Nishiuchi and McNaughton, 2008). In line with this idea, hippocampal-dependent memory consolidation causes changes in the synaptic density in the mPFC that support the retrieval of remote memories (Insel and Takehara-Nishiuchi, 2013; Restivo, Vetere, Bontempi, and Ammassari-Teule, 2009). Thus, disconnecting the mPFC and PER could prevent the PER from having access to consolidated object-place associations. If the mPFC selectively supports remote memories that are dependent on the hippocampus shortly after acquisition, it is possible that infusing MUS into the mPFC during an earlier time point in training, or before acquisition, would not result in a deficit. This idea would predict that rats over-trained for a month on the OPPA task would be able to complete the task without the hippocampus. While previous bilateral inactivation of the hippocampus during the OPPA task has shown a performance deficit (Jo and Lee, 2010a; Lee and Solivan, 2008), the time frame of hippocampal involvement has not been explicitly examined.

One critical component of OPPA task performance is the ability to identify the different objects. The dense connectivity of the PER with different sensory cortical areas enables it to link individual features of an object in order to identify it as a single entity (Murray and Bussey, 1999; Suzuki and Amaral, 1990; 1994). In fact, lesions to the PER cause impairments in object recognition, but not in spatial memory (Bussey, Muir, and Aggleton, 1999), and lesion data indicate the PER contributes to both object perception and memory

(Buckley and Gaffan, 1998b; Murray and Bussey, 1999). Furthermore, a portion of PER neurons are selectively activated by different objects (Burke, Maurer, Hartzell, Nematollahi, Uprety, Wallace, et al., 2012b; Deshmukh, Johnson, and Knierim, 2012), even when the environment in which they are presented is changed (Burke et al., 2012a). Although it is clear that the PER encodes object information, it is unlikely its contribution to the OPPA task is limited to object representations. Synaptic weight changes within the PER support associations between object pairs (Fujimichi, Naya, Koyano, Takeda, Takeuchi, and Miyashita, 2010; Higuchi and Miyashita, 1996; Murray and Richmond, 2001), and it is conceivable that this could extend across modalities. In fact, the PER is critical for linking visual to tactile information (Buckley and Gaffan, 1998a; Goulet and Murray, 2001; Jacklin, Cloke, Potvin, Garrett, and Winters, 2016; Parker and Gaffan, 1998; Reid, Jacklin, and Winters, 2014). A similar situation may occur when an object is associated with a place. While PER cells do not show spatial selectivity (Burke et al., 2012b; Burwell, Shapiro, O'Malley, and Eichenbaum, 1998), plasticity within PER may bias the retrieval of one object representation over another when the animal is in a specific location and this interaction could be modulated by projections from the mPFC (Paz et al., 2007). A critical issue not addressed by the current study is the necessity of mPFC-PER connectivity to acquire new object-place associations. Although not explicitly tested here, available data indicating that communication between these brain regions is necessary for an animal's ability to detect novel object-place associations (Barker et al., 2007; Barker and Warburton, 2008) suggest that this component of task performance would also be impaired by a mPFC-PER disconnection.

The mPFC-PER disconnection would disrupt any one of the aforementioned aspects of mPFC influence on the medial temporal lobe circuitry. Alternatively, it is possible that the deficit resulting from blocking communication across these regions is due to the inability to deal with proactive interference based on the rule used in the previous trial. This scenario, however, would predict that rats would default back to the correct choice on the previous trial and show an object bias. This behavioral outcome was not observed following the mPFC-PER disconnection, rather rats regressed to the side bias seen during pre-training.

It is likely that the role of the mPFC on the network is diverse and may encompass several aspects of learning and memory in regards to both behavioral flexibility and object-place associations. The observation that a contralateral deficit impaired performance on the OPPA task, which requires flexibility in selecting the correct object based on spatial location, but not the control tasks, is interesting in the context of aging and disease. The PER (Burke, Ryan, and Barnes, 2012c; Khan, Liu, Provenzano, Berman, Profaci, Sloan, et al., 2014; Reagh, Ho, Leal, Noche, Chun, Murray, et al., 2015; Ryan, Cardoza, Barense, Kawa, Wallentin-Flores, Arnold, et al., 2012) and mPFC (Griffith, Dubois, Fincher, Peebles, Bizon, and Murchison, 2014; Morrison and Baxter, 2012) are among the most vulnerable brain regions to age-related dysfunction. Even when these areas are compromised, however, aged animals are still able to perform object discriminations and differentiate the left from right side (Beas et al., 2013; Burke, Wallace, Hartzell, Nematollahi, Plange, and Barnes, 2011; Hernandez et al., 2015). Therefore, behaviors that require large-scale integration across different neural networks may be particularly sensitive to aging. Because individual brain regions may not age in the same manner, it is vital that potential treatments for cognitive

aging and dementia not only alleviate dysfunction in an individual area, but also maintain the balance in global network interactions.

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HIGHLIGHTS

- It is unknown whether prefrontal (PFC)-perirhinal cortical (PER) connectivity is necessary for flexible behavior.
- Rats were tested on a biconditional object-place paired association test of flexibility.
- Disconnection of the PFC and PER significantly disrupted performance.
- Disconnected rats regressed to a perseverative response of selecting objects on one side.
- These findings are consistent with a critical role for PFC-PER circuits in updating a response rule across spatial locations.

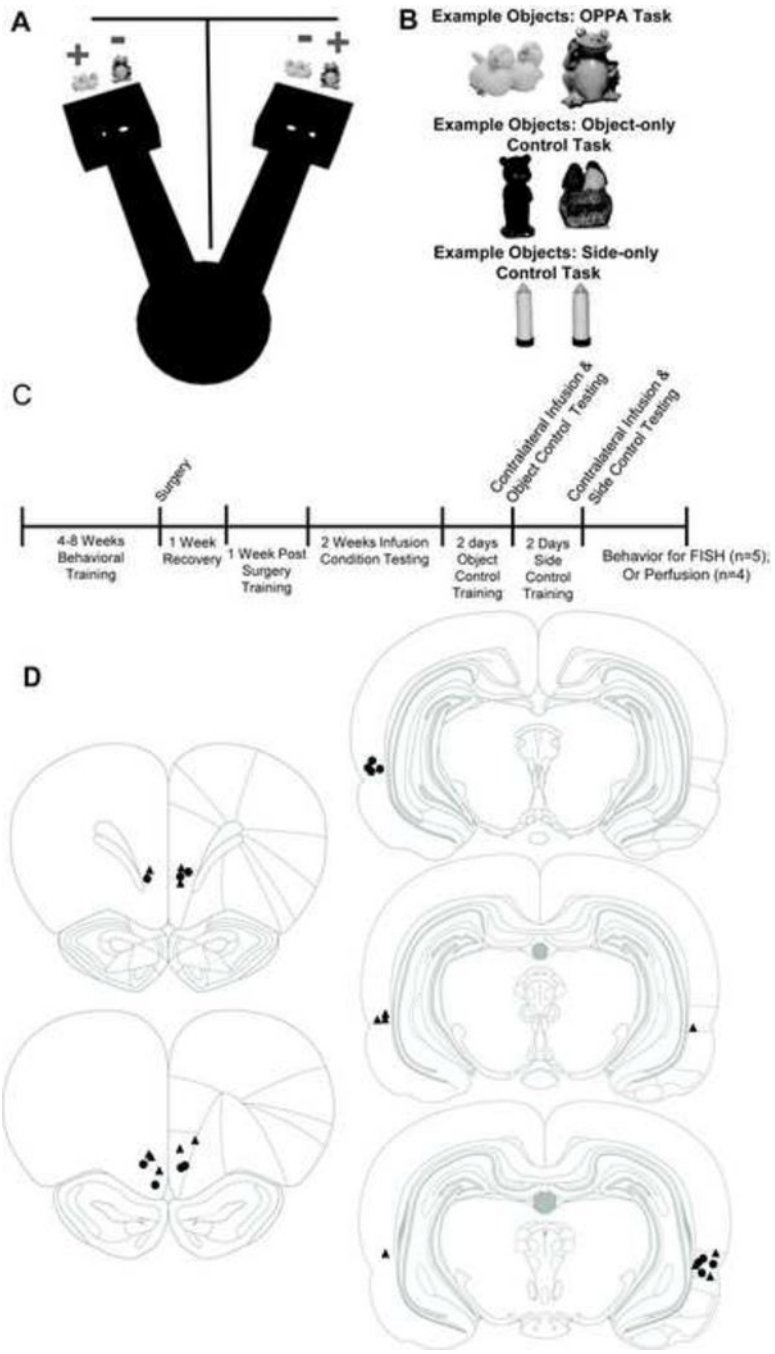

Figure 1. Experimental design and cannulae placement

A) The Object-Place Paired Association (OPPA) Task apparatus. Two objects were placed at the end of each arm, covering a food well with a hidden food reward underneath the correct object. The rat had to choose the correct choice in each arm while alternating back and forth. Arms were separated by black poster board with different markings on each side to prevent the rat from viewing the opposite arm and to differentiate between the two arm locations. Each object was correct in only one arm of the maze and the same objects were used within a test condition. The same apparatus was used for object-only and side-only control tasks

with one arm blocked off during each task. **B)** Representative objects used during the OPPA task (top), object only control task (middle), and the left versus right side discrimination control task (bottom). **C)** Timeline of experimental procedures. **D)** Bilateral cannulae placement in the mPFC and PER shown for each rat included in analyses. Triangles indicate the placement from animals in which tracts were verified with histology and circles represent placements from animals in which *Arc in situ* hybridization was used to verify selectivity of inactivation.

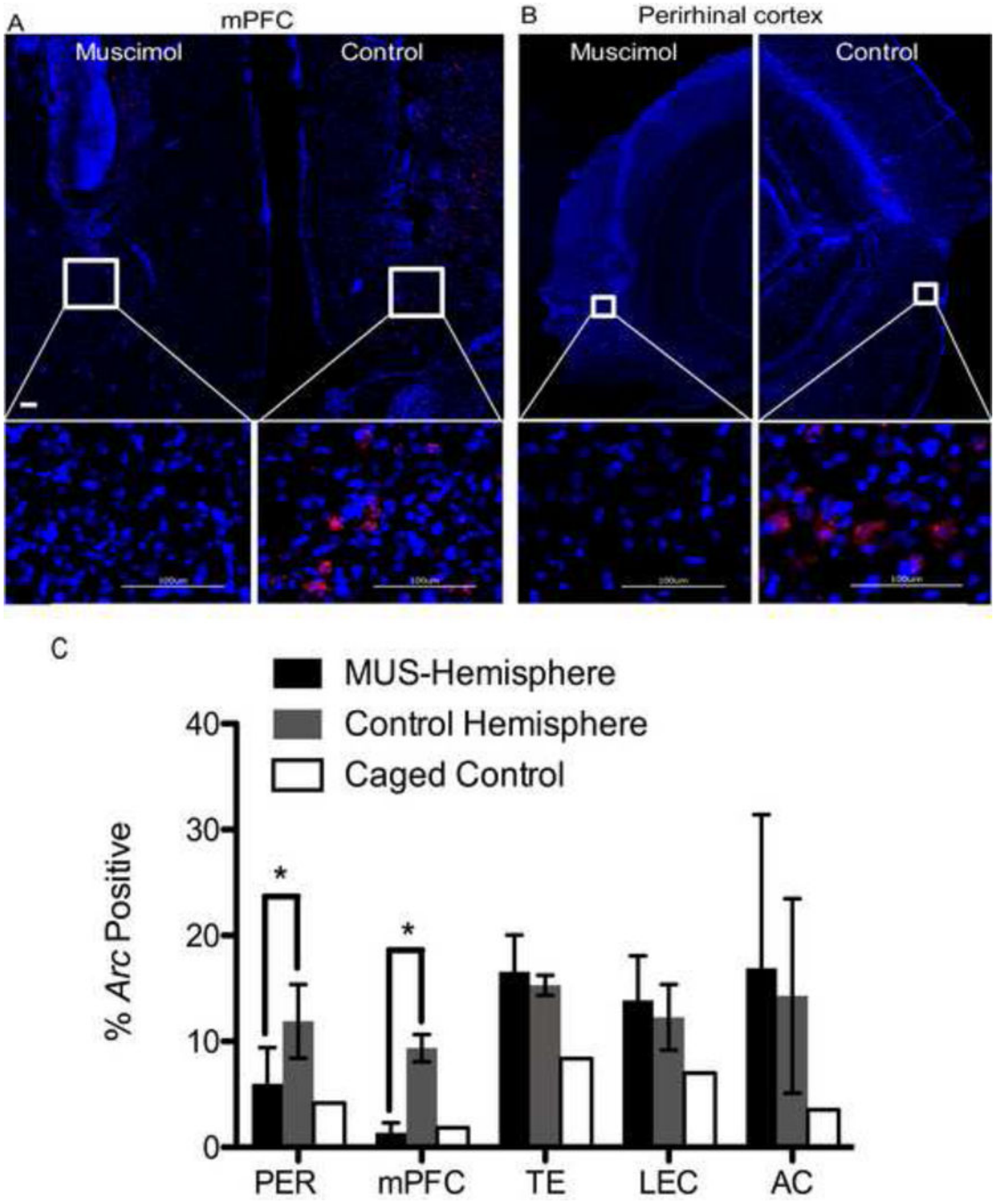

Figure 2. *Arc* expression following muscimol (MUS) infusion

A) A representative image of *Arc* expression in the mPFC in which the left hemisphere was inactivated with MUS and the right was intact (*top*). 40X images of the regions of interest outlined in top panel from MUS infused (left panel) and non-infused tissue (right panel) (*bottom*). **B)** A representative image of *Arc* expression the PER in which the right hemisphere was infused with MUS and the left as intact (*top*). 40X images of the regions of interest outlined in top panel from non-infused (left panel) and MUS infused tissue (right panel) (*bottom*). **C)** Percent of *Arc* positive cells in the perirhinal cortex (PER), medial

prefrontal cortex (mPFC), area TE, lateral entorhinal cortex (LEC), and anterior cingulate cortex (AC) in the MUS infused (black) and non-infused control (grey) hemispheres relative to caged controls (white). For the PER and mPFC, there was a significantly greater percent of *Arc* positive cells in the hemisphere that was not infused with MUS ($p < 0.05$), indicating that the MUS inhibited activity-dependent gene expression in the mPFC and PER. This was not the case for the adjacent brain regions (TE, LEC and AC) that were not targeted for infusions ($p > 0.05$ for all comparisons). Error bars are +/−1 Standard error of the mean (SEM).

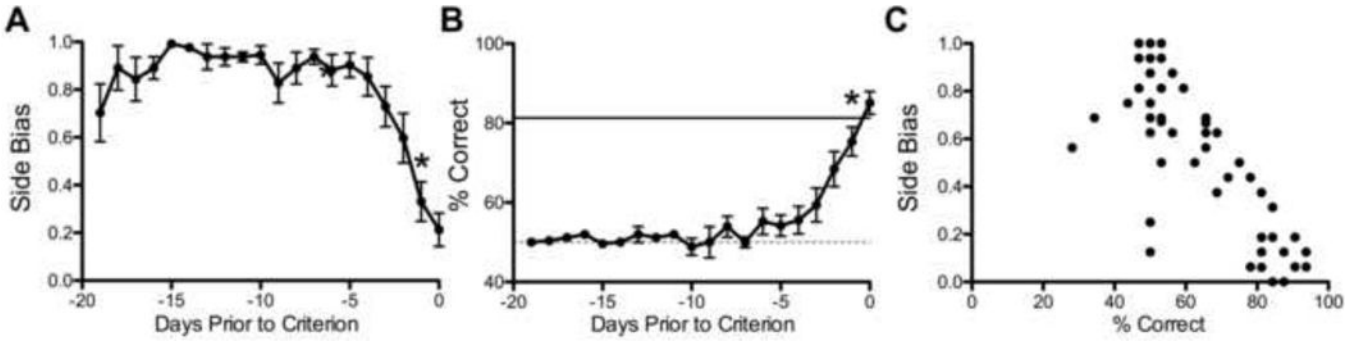

Figure 3. Side bias during pre-training

A) The mean side bias (Y axis) as a function of days before reaching criterion performance (X axis). During initial training, there was a response bias for a particular side. This bias did not significantly change across testing days until 1 day prior to hitting criterion performance (*$p < 0.005$). **B)** The percent correct responses (Y axis) as a function of days before reaching criterion performance (X axis). The mean percent correct did not significantly improve across testing days until one day prior to hitting criterion performance (*$p < 0.001$). **C)** There was a significant negative correlation over all days of testing between the side bias and percent correct on the OPPA task ($R^2_{[181]} = 0.71$, $p < 0.001$). Error bars are +/−1 SEM.

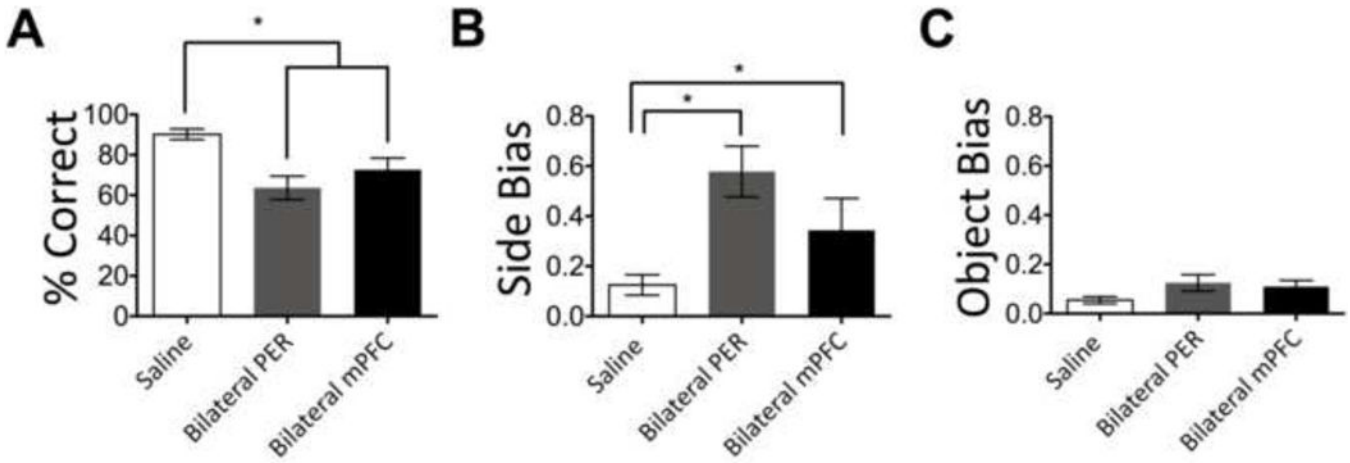

Figure 4. Effects of bilateral mPFC or PER inactivation on OPPA task performance

A) The Y axis shows percent correct for the bilateral MUS versus the vehicle control (saline) infusions (X axis). Bilateral mPFC and bilateral PER inactivation impaired performance relative to the vehicle control (*$p < 0.01$ for both comparisons). **B)** Side bias of rats' tendencies to choose one side over the other during the different infusion conditions. The side bias was significantly higher during both bilateral inactivation conditions relative to the vehicle control infusion (*$p < 0.01$ for both comparisons). **C)** Object bias during bilateral PER and mPFC MUS infusions and vehicle control infusions was not significantly different ($p = 0.09$). Error bars are +/−1 SEM.

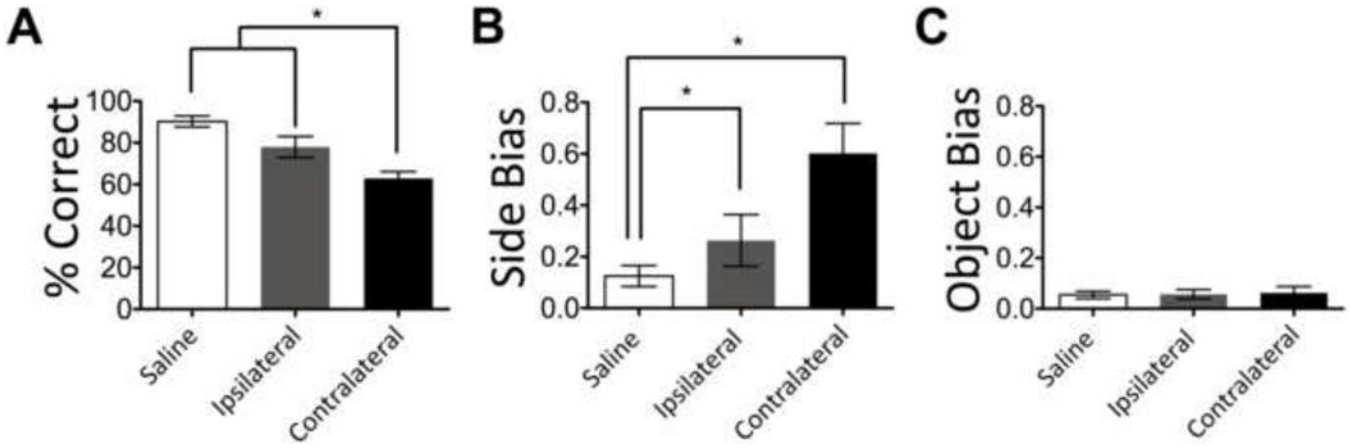

Figure 5. Effects of contralateral mPFC-PER MUS infusions on OPPA task performance

A) Percent correct (Y axis) for the disconnection versus ipsilateral and vehicle control infusion conditions. Contralateral infusions that disconnected the mPFC and PER impaired performance when compared to vehicle control or to ipsilateral inactivation ($p < 0.01$ for both comparisons). **B)** The side bias during ipsilateral and contralateral MUS infusions was significantly greater compared to contralateral vehicle control infusions ($p < 0.05$ for both comparisons). **C)** The object bias (Y axis) was not significantly different across infusion conditions (X axis; $p = 0.64$). Error bars are +/−1 SEM.

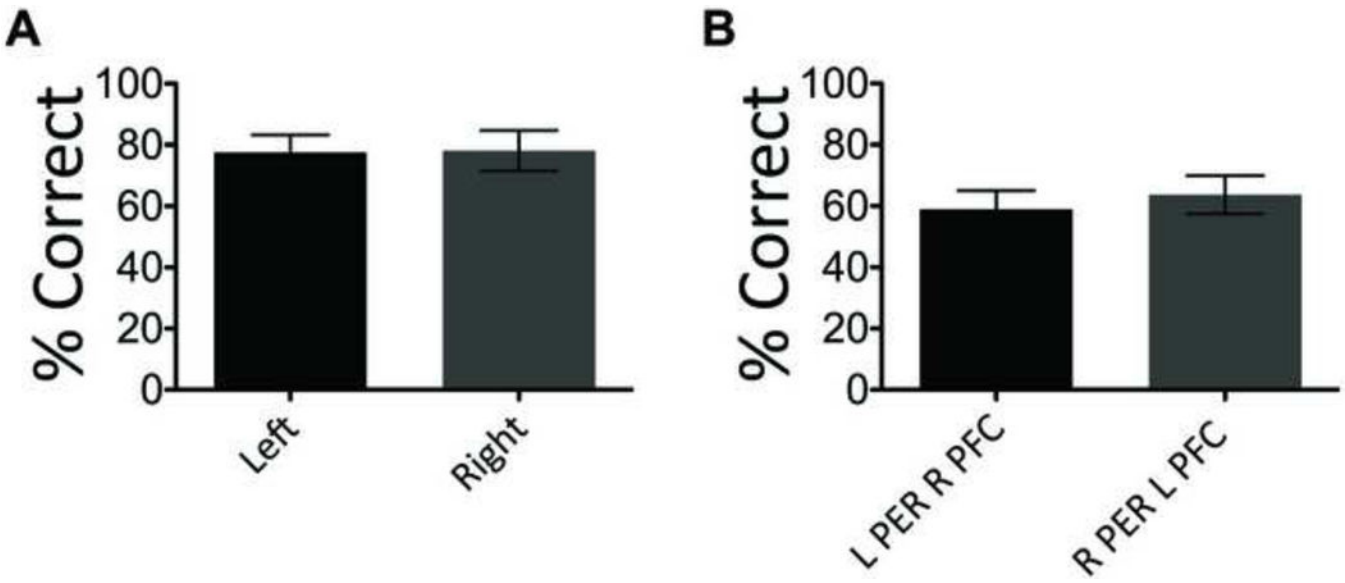

Figure 6. Contralateral and ipsilateral infusion conditions did not show lateralization effects

A) The Y axis shows percent correct on the OPPA task across the different hemispheres inactivated ipsilaterally (X axis). Left versus right hemisphere ipsilateral inactivation were not significantly different from each other ($p = 0.97$). **B)** Percent correct (Y axis) on the OPPA task during left hemisphere mPFC/right hemisphere PER inactivation versus right hemisphere mPFC/left hemisphere PER inactivation (X axis). There was not a significant lateralization effect of infusion side between the disconnection conditions ($p = 0.87$). Error bars are +/−1 SEM.

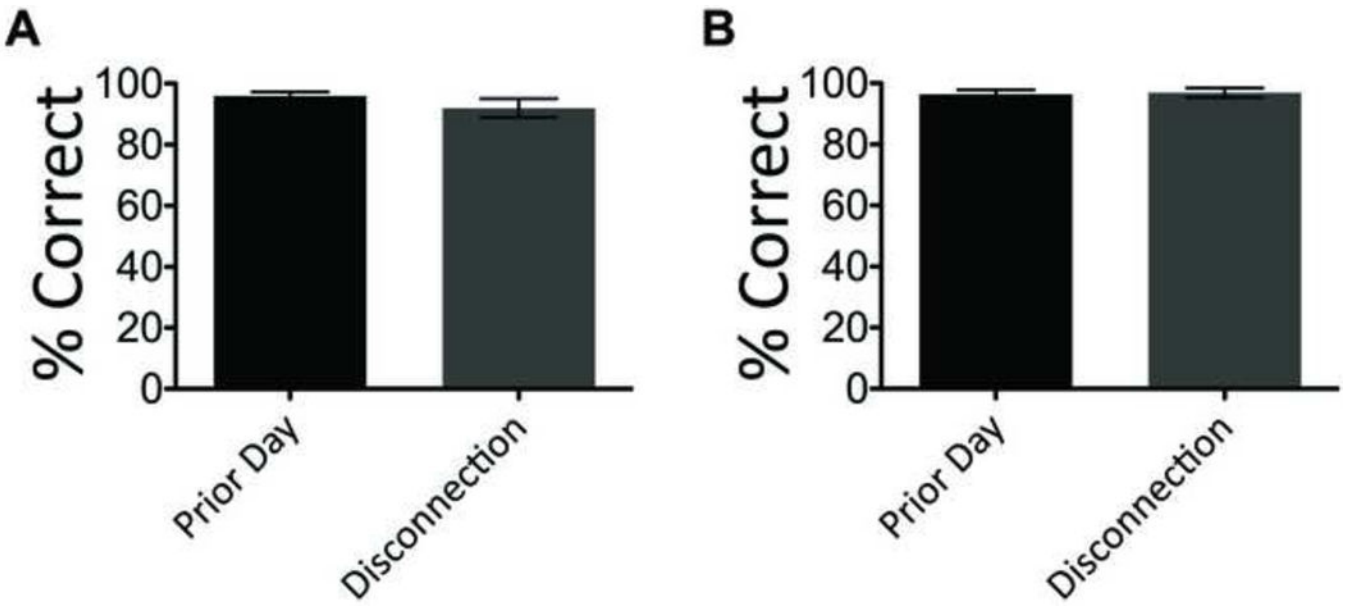

Figure 7. Contralateral MUS infusions did not affect performance on side only or object only control tasks

Percent correct during an object-only control task **(A)** and a side-only control task **(B)** was not significantly different between the contralateral MUS inactivation compared to the previous non-infused day ($p > 0.18$ for both comparisons). Error bars are +/−1 SEM.

Table 1

Summary of Infusion Condition Statistical Model

The effect of infusion condition was tested with repeated measures ANOVAs and planned contrasts.

Main Effect	Statistical Test	Comparison	n	Statistic	p value
Bilateral infusion (3 levels: PER, mPFC, control)	Repeated measures ANOVA $F[2,12] = 10.25$, $p < 0.01$	Vehicle control vs bilateral MUS in PER	9	Simple contrast	$p < 0.01$
		Vehicle control vs bilateral MUS in mPFC	7	Simple contrast	$p < 0.01$
Disconnection infusion (3 levels: contralateral mPFC-PER, ipsilateral mPFC-PER, control)	Repeated measures ANOVA $F[2,12] = 23.62$, $p < 0.001$	Contralateral mPFC-PER MUS vs Vehicle control	9	Simple contrast	$p < 0.01$
		Contralateral mPFC-PER MUS vs Ipsilateral mPFC-PER MUS	9	Simple contrast	$p < 0.01$