PARAHIPPOCAMPAL-ORBITOFRONTAL INTERACTION AT THE INTERFACE BETWEEN LATENT LEARNING AND GOAL-DIRECTED BEHAVIOR

Xiangyuan Peng

THESIS

A dissertation submitted in partial fulfillment of the requirements for the Degree of Doctor of Philosophy in the Department of Cognitive, Linguistics, and Psychological Sciences at Brown University, October 2023

Advisor:	Rebecca D. Burwell, Ph.D.
Dissertation Committee:	David Badre, Ph.D.
	Michael J. Frank, Ph.D.
	Geoffrey Schoenbaum, M.D., Ph.D.
	Travis P. Todd, Ph.D.

© Copyright 2023 by Xiangyuan Peng

This dissertation by Xiangyuan Peng is accepted in its present form by the Department of Cognitive, Linguistics, and Psychological Sciences as satisfying the dissertation requirements for the degree of Doctor of Philosophy.

Date: 08/30/2023

Rebecca D. Burwell, Ph.D., Advisor

Recommended to the Graduate Council

Date: 08/30/2023

David Badre, Ph.D., Reader

Date: 08/30/2023

Michael J. Frank, Ph.D., Reader

Date: 08/30/2023

Geoffrey Schoenbaum, M.D., Ph.D., Reader

Date: 08/30/2023

Travis P. Todd, Ph.D., Reader

Approved by the Graduate Council

Date: _____

Thomas A. Lewis, Ph.D., Dean of the Graduate School

Curriculum Vitae

XIANGYUAN PENG

EDUCATION

Graduate:

Doctor of Philosophy, Psychology — August 2023
Brown University, Providence, RI, USA
Advisor: Dr. Rebecca Burwell

Undergraduate:

Bachelor of Science, Psychology, first class — 2015
University of Prince Edward Island, Charlottetown, PE, Canada

FELLOWSHIPS

Brown University, Graduate Dissertation Fellowship — 2022 – 2023

PUBLICATIONS

Peng X, & Burwell, R. (2021). Beyond the hippocampus: The role of parahippocampal-prefrontal communication in context-modulated behavior. Neurobiology of Learning and Memory. 185:107520. doi: 10.1016/j.nlm.2021.107520. Epub 2021 Sep 16. PMID: 34537379

Peng X, & Burwell, R. (2023). Chemogenetic suppression of the perirhinal or postrhinal cortex impairs model-based inference. Manuscript in preparation.

Peng X, & Burwell, R. (2023). Perirhinal and postrhinal interactions with the orbitofrontal cortex are necessary for model-based inference. Manuscript in preparation.

CONFERENCE PRESENTATIONS

Peng X, & Burwell, R. *Chemogenetic suppression of the perirhinal or postrhinal cortex impairs associative chaining, but not first-order conditioning*. Data presented at: Park City Winter Conference on the Neurobiology of Learning and Memory; Park City, UT, 2023.

Peng X, Poeta, D, & Burwell, R. *Chemogenetic suppression of the perirhinal or postrhinal cortex impairs Pavlovian appetitive sensory preconditioning.* Poster presented at: Society for Neuroscience; San Diego, CA, 2022.

Peng X, Poeta, D, Slusarewicz, M, & Burwell, R. *Stimuli generalization during appetitive Pavlovian sensory preconditioning*. Poster presented at: Society for Neuroscience; Online, 2021.

Peng X, Poeta, D, & Burwell, R. *Perirhinal and postrhinal cortices contribute to the configural process of contextual learning*. Poster presented at: Society for Neuroscience Global Connectome; Online, 2021.

RESEARCH EXPERIENCE

Graduate Student Researcher, Brown University 2019 – present
Burwell Laboratory of Memory and Attention
Advisor: Dr. Rebecca Burwell

Graduate Student Researcher, Brown University 2016 – 2018
Colwill Laboratory of Learning and Memory
Advisor: Dr. Ruth Colwill

Research Assistant, University of Prince Edward Island 2015 – 2016
Zebrafish Behavioral Laboratory
Project: Automated behavioral analysis of social approaching behavior in zebrafish
Advisor: Dr. Catherine Ryan

TEACHING EXPERIENCE

Graduate Teaching Assistant, Brown University 2020
Course: Cognitive Neuroscience
Advisor: Dr. Dima Amso

Graduate Teaching Assistant, Brown University 2019
Course: Children's thinking: Nature of Cognitive Development
Advisor: Dr. David Sobel

Graduate Teaching Assistant, Brown University 2018
Course: Learning and Conditioning
Advisor: Dr. Ruth Colwill

Graduate Teaching Assistant, Brown University 2017
Course: Animal Behavior Laboratory
Advisor: Dr. Ruth Colwill

Acknowledgements

I would like to thank my parents for their years of love and support. When things in life are not going smoothly, they have always been there for me to make things better.

I would like to thank Devon Poeta for teaching me virtually everything I know about rodent behavioral and surgical procedures, and other skills that I will rely on for the rest of my scientific career.

I would also like to thank other members of the Burwell lab, Taylor Wise, and the undergraduate research assistants, including Isabella Pilkington, Rayna Simons, and Yihuan Dong for their assistance on my research projects.

I would like to thank Dr. Victoria Templer at Providence College for providing feedback on my practice presentation and for letting me use the microscope at her lab for fluorescence imaging.

I would like to thank my friends, fellow graduate students, and anyone who has ever provided me with company, joy, and help that has made this trip a lot easier.

I would like to thank my committee members, Dr. Geoffrey Schoenbaum, Dr. Travis Todd, Dr. Michael Frank, and Dr. David Badre for their guidance and feedback throughout the process of this research.

Finally, I would like to thank my advisor Dr. Rebecca Burwell for her knowledge, professional expertise, and general guidance as a highly accomplished scientist and academic. She has always been here to support and guide me as I traveled through this difficult journey. I will always keep in mind her kindness and optimism as a researcher and as a mentor, and I hope I can pass them along to others in my own future career. Without her, this journey would not have been possible.

Preface

The perirhinal cortex (PER) and the postrhinal cortex (POR) reside in the parahippocampal region of the medial temporal lobe. The anatomical connectivity and functional contribution of the PER and POR (homologous to the primate perirhinal and parahippocampal cortices) are well-preserved across species. Both of them receive polymodal and unimodal associational sensory input and project to areas including the entorhinal cortex and the hippocampus for spatial contextual learning, recognition, and episodic memory. The PER and POR also have reciprocal connections with a variety of areas in the prefrontal cortex. However, it remains an open question whether or how the PER and POR directly interact with the prefrontal cortex for memory and decision-making.

To answer this question, I started with a review of the literature on the functions of PER and POR, with a focus on context-modulated behavior (Chapter 1). Despite having notable functional and anatomical differences, the PER and POR are often collectively required when the expression of behavior depends on the spatial context. Our thinking is that the PER and POR directly share item, pattern, object, and spatial information through highly robust reciprocal connections in the service of representing context. The orbitofrontal cortex (OFC), known for supporting -based decision-making, is also involved in context-modulated behavior. Therefore, I proposed that the orbitofrontal cortex likely receives contextual information from the PER and POR when it is required for adaptive decision-making.

One important characteristic of context learning is that it can take place in the absence of biologically significant events such as rewards or punishments; therefore, it can be considered a form of latent learning. Evidence from behavioral and electrophysiological studies indeed supports the idea that PER and POR encode the spatial context automatically, and unlike the hippocampus, they are critically involved in retrieving remote as well as recent memories for context. Therefore, I chose the Pavlovian appetitive sensory preconditioning paradigm to evaluate the functional significance of the OFC-PER and OFC-POR connections. This paradigm was chosen because it relies on the interaction between latent learning and goal-directed behavior, in which the PER/POR and OFC are each known for their crucial involvement.

In the first experiment, described in Chapter 2, I induced temporary suppressions during the test phases of sensory preconditioning in either the PER or POR in rats that received bilateral injections of a DREADD (Designer Receptor Exclusively Activated by Designer Drugs) virus. When compared to the sham-surgery rats, rats with either PER or POR inactivation showed impaired sensory preconditioning, which suggested that both areas were crucially involved for retrieving latently acquired stimulus-stimulus associations for goal-directed behavior. In Chapter 3, I described a follow-up experiment in which a cross-inactivation method was used to create temporary disconnections between the OFC and PER or POR. The same DREADD virus was injected into the OFC in one hemisphere and the PER or POR in the opposite hemisphere (i.e., contralaterally), or into the OFC and PER or POR within the same hemisphere (i.e., ipsilaterally). The same sensory preconditioning procedure was used to test the effects of disrupting the OFC-

PER or OFC-POR interactions at tests, during which the OFC was shown to contribute critically. I predicted that deficits would be seen only in rats with contralateral inactivation. Unexpectedly, though, deficits were detected in rats with ipsilateral inactivation OFC-PER or OFC-POR, and in rats with contralateral OFC-PER inactivation, whereas rats under contralateral OFC-POR inactivation performed normally. This pattern of results pointed to the functional significance of the inter-hemispheric (cross-lateral)pathways between the OFC and each of the PER and POR (which were spared in the contralateral groups but disrupted in the ipsilateral groups), as well as the intra-hemispheric (ipsilateral) OFC-PER pathways.

Finally, in Chapter 4, I concluded that experiments in this thesis provided evidence for 1) the involvement of the PER and POR in latent learning of associations between transient events, and 2) that these associations can be used for goal-directed behavior via the PER and POR interacting directly with the OFC. These findings are significant because they complement existing knowledge about paths of communication between the medial temporal lobe and the prefrontal region. The PER and POR may be part of a network that centers around the OFC to support representing associative structures as cognitive maps and use them for value inference. Future directions were discussed in regard to how the specific functions of OFC-PER and OFC-POR pathways might be investigated.

Table of Contents

List of Tables

Chapter 1

Chapter 2

Chapter 3

List of Figures

Chapter 1

Chapter 2

Chapter 3

CHAPTER 1

BEYOND THE HIPPOCAMPUS:

THE ROLE OF PARAHIPPOCAMPAL-PREFRONTAL COMMUNICATION

IN CONTEXT-MODULATED BEHAVIOR

Peng, X., & Burwell, R. D. (2021). Beyond the hippocampus: The role of parahippocampal-prefrontal communication in context-modulated behavior. Neurobiology of learning and memory, 185, 107520. https://doi.org/10.1016/j.nlm.2021.107520

Abstract

Multiple paradigms indicate that the physical environment can influence spontaneous and learned behavior. In rodents, context-dependent behavior is putatively supported by the prefrontal cortex and the medial temporal lobe. A preponderance of the literature has targeted the role of the hippocampus. In addition to the hippocampus proper, the medial temporal lobe also comprises parahippocampal areas, including the perirhinal and postrhinal cortices. These parahippocampal areas directly connect with multiple regions in the prefrontal cortex. The function of these connections, however, is not well understood. This article first reviews the involvement of the perirhinal, postrhinal, and prefrontal cortices in context-dependent behavior in rodents. Then, based on functional and anatomical evidence, we suggest that perirhinal and postrhinal contributions to context-dependent behavior go beyond supporting context representation in the hippocampus. Specifically, we propose that the perirhinal and postrhinal cortices act as a contextual-support network that directly provides contextual and spatial information to the prefrontal cortex. In turn, the perirhinal and postrhinal cortices modulate prefrontal input to the hippocampus in the service of context-guided behavior.

KEYWORDS: perirhinal cortex, postrhinal cortex, prefrontal cortex, hippocampus, context memory, occasion setting

Overview of the parahippocampal region and context-dependent behavior

Since the case of HM, research on the neural bases of memory has focused on structures in the medial temporal lobe. The medial temporal lobe includes the hippocampal formation (dentate gyrus, hippocampus proper, subiculum) and the parahippocampal region. The parahippocampal region includes the perirhinal cortex (PER), the parahippocampal cortex, the lateral and medial areas of the entorhinal cortex (LEA and MEA), the presubiculum, and the parasubiculum (Burwell & Witter, 2002; Scharfman et al., 2000). The homologies between primate and rodent for the medial temporal lobe are well established, and complete since the definition of the rodent postrhinal cortex (POR), homolog of the primate parahippocampal cortex (Burwell & Witter, 2002; Burwell et al., 1995). This review will primarily focus on rodent studies, with occasional single-cell recording studies in monkeys. It should be noted, however, that patterns of anatomical connection and functions of the areas discussed are largely well-preserved across rodents, non-human primates, and humans.

The PER is located near the rhinal fissure and comprises the ventrally located area 35 and the dorsally located area 36 (Burwell, 2001). The patterns of cortical and subcortical connections differ substantially for areas 35 and 36, though the functional difference between them is not well-known. The POR lies dorsal to the extension of the rhinal fissure, bordering the caudal boundary of the PER. The PER and POR provide most of the cortical input to the hippocampus, both directly to CA1 and subiculum and indirectly through sub-areas of the entorhinal cortex (Figure 1.1). Reciprocal connections also exist between the PER and POR, with the heavier projection originating from the POR

(Burwell & Amaral, 1998b). The PER and POR receive both polymodal associational and unimodal associational input. The PER receives sensory associational information of all modalities, and the POR receives most input from cortical areas devoted to visuo-spatial processing (Burwell & Amaral, 1998a). Whereas PER efferents terminate mostly in frontal and temporal regions, the POR projects more heavily to caudal cortical regions including visual, visuo-spatial, and, to a lesser extent, auditory cortices (Agster & Burwell, 2009).

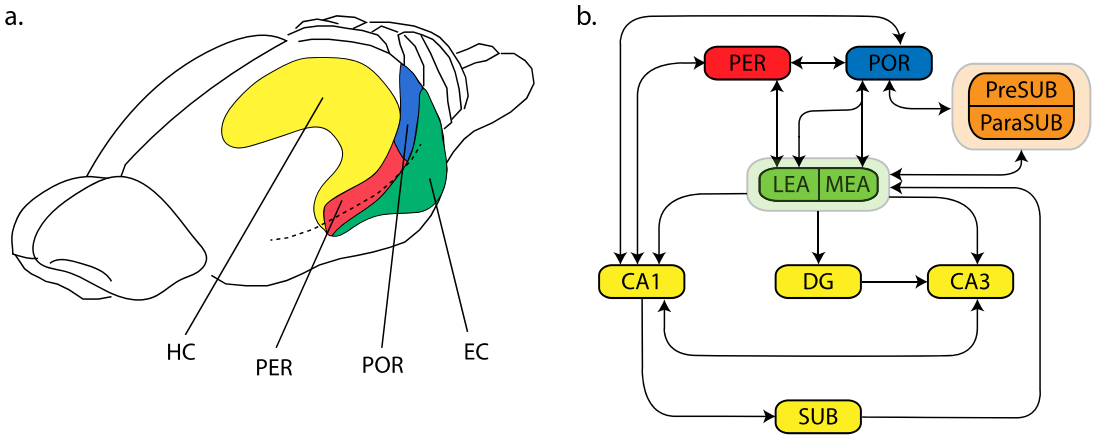

Figure 1.1. The perirhinal and postrhinal cortices in rodents. A) The perirhinal cortex (PER, red) and the postrhinal cortex (POR, blue). B) A schematic showing simplified connections within the medial temporal lobe. Entorhinal cortex is shown in green, the hippocampal formation in yellow, and the pre- and parasubiculum are in orange. Abbreviations: LEA: lateral entorhinal area. MEA: medial entorhinal area. DG: dentate gyrus. SUB: subiculum. PreSUB: presubiculum. ParaSUB: parasubiculum. CA1 and CA3: subfields of the hippocampus.

Functions of the PER and POR were described by the "Binding of items and contexts" (BIC) model (Diana et al., 2007; Eichenbaum et al., 2012; Eichenbaum et al., 2007) such that information about items and objects (i.e. "what") is processed by the PER and lateral entorhinal area, while contextual information (i.e. "where") is processed by the POR and medial entorhinal area. The hippocampus binds item information from the PER with context information form the POR for event memory (Figure 1.2). This model also

proposes that medial temporal lobe structures contribute to different processes of recognition memory. Familiarity-based recognition relies on an intact PER, and it does not require involvement of the POR or hippocampus. In contrast, recollection-based recognition involves recalling the item together with the context in which it occurred, requiring contribution from the PER, POR and hippocampus.

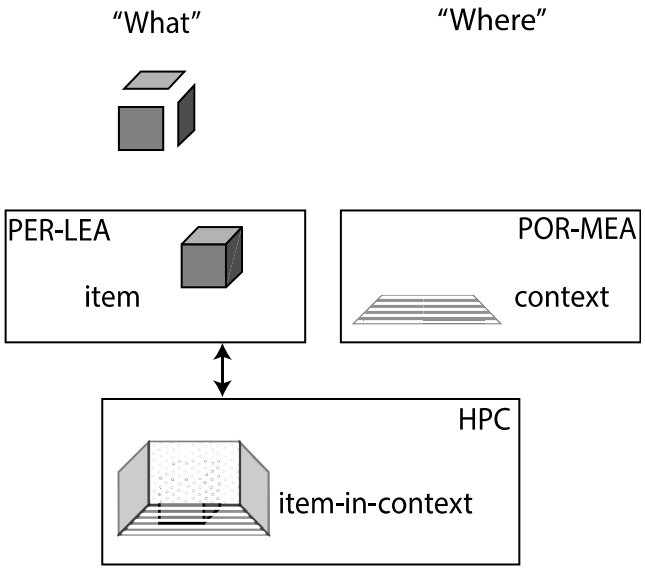

Figure 1.1. The "binding of items and contexts" (BIC) model (Eichenbaum et al., 2007). This is a model of one aspect of relational memory, the association of an item with a particular context. Item could be, for example, an object, odor, pattern, or prior experience of a stimulus. Abbreviations: HPC: hippocampus. LEA: lateral entorhinal area. MEA: medial entorhinal area. PER: perirhinal cortex. POR: postrhinal cortex.

One important function of the medial temporal lobe is processing context. In a broad sense, context encompasses a wide range of internal and external stimuli that are not directly and imminently related to an action or its goal. One important aspect of context is the external physical environment. Understanding how the physical environment controls behavior is a longstanding theme in psychology and neuroscience. Even though there is no clear-cut distinction between contextual and non-contextual stimuli, context differs from discrete signals or cues in that it often consists of sensory information from multiple

modalities that are temporally continuous and spatially diffuse. For example, in an experimental setting, events (such as electric shocks) may be preceded by an LED light or an auditory tone, which are localized unimodal stimuli with clear temporal onsets and termination. In contrast, the context of the experimental environment typically remains constant throughout each session, and it often contains multi-modal physical elements, including visual features, background noise, texture of the floor, odors, etc.

Context guides behavior either via direct associations or by indirectly modulating associations between other stimuli. The Rescorla-Wagner model proposes that context can be incorporated into stimulus-stimulus or stimulus-response associations in the same way as an explicit, discrete stimulus; hence, context itself can acquire either positive or negative associative strength and directly elicit behavioral changes through its associations (Rescorla & Wagner, 1972; Wagner & Rescorla, 1972). Alternatively, context may control behavior indirectly through modulating lower-order associative relations in a hierarchical organization; in other words, it "sets the occasion" in which certain associations apply while others do not (Fraser & Holland, 2019; Trask, Thrailkill, et al., 2017). For example, the same cue may signal the arrival of food pellets in one context and an impending electric shock in another. The context acts as an "occasion-setter" that disambiguates the meaning of the cue and allows the subject to respond accordingly.

Though its role in context learning is not as well-studied as the medial temporal lobe, the prefrontal cortex is also frequently implicated in context-guided behavior. The prefrontal cortex is responsible for executive functions in primates and rodents (Dalley et al., 2004;

Logue & Gould, 2014; Sharpe et al., 2019). Even though the precise homologies are still subject to ongoing debate, the prefrontal regions in rodents have been proposed based on both anatomical characteristics and functional similarity to the primate prefrontal cortex (Dalley et al., 2004; Hwang et al., 2018). Here, discussion of the rodent prefrontal cortices will include the medially located prelimbic (PL) and infralimbic (IL) cortices, orbitofrontal cortex (OFC), and secondary motor cortex (MOs, also called the medial precentral cortex, medial agranular cortex, dorsomedial prefrontal cortex, second frontal area, and frontal orienting field). The PL and IL in rodents are often collectively referred to as the medial prefrontal cortex (mPFC). Some studies explicitly target the PL or IL, whereas others investigate the mPFC as a whole and involve both the PL or IL via lesioning or inactivation. In the former case, the specific area will be used when discussing the finding, whereas in the latter case, the more general term of mPFC will be used. All these prefrontal areas are reciprocally connected with the PER and POR to various degrees (Agster & Burwell, 2009; Burwell & Amaral, 1998a; Hwang et al., 2018). In the following sections, we will examine studies showing the involvement of the PER/POR and prefrontal areas in rodent context-dependent behavioral paradigms. Then we will review anatomical evidence on the connections between the PER/POR and prefrontal cortices. Finally, we propose possible roles of these connections as part of the cortico-cortical network supporting context-dependent behavior.

The Roles of PER/POR and PFC in context-modulated behavior

Conditioning, Extinction, and Renewal

Classic associative learning paradigms have provided valuable insight into the neural circuits of learning and memory, including the effects of context on conditioned responses. Existing evidence indicates crucial roles for the PER and POR in associating fear with the environmental context. Pre-training or post-training damage to the PER or POR leads to reduced freezing to the context in both signaled and un-signaled Pavlovian fear conditioning (Bucci et al., 2000; Corodimas & LeDoux, 1995; Kholodar-Smith et al., 2008; Lindquist et al., 2004). PER or POR damage also produces deficits in context discrimination after one of the contexts is paired with shocks (Bucci et al., 2002). Furthermore, PER or POR lesions conducted even 100 days after training still lead to freezing deficits during the context test, indicating ongoing roles for the PER and POR in processing remote contextual fear memory (Burwell et al., 2004). In comparison, the hippocampus is typically necessary for recalling recent but not remote contextual fear memory (Maren et al., 1997; Winocur et al., 2013). These deficits produced by PER or POR lesions cannot be explained by impairment in associating simple stimuli, because lesioning either area has no effect on tone-elicited freezing (Bucci et al., 2000; Lindquist et al., 2004), or eye-blink responses in delayed eyeblink conditioning (Suter et al., 2013). Interestingly, disconnecting the PER and POR via unilateral cross-lesioning does not impair contextual fear conditioning, whereas it does impair another contextual memory task that will be discussed later, context-guided object recognition (Heimer-McGinn et al., 2017).

Even though lesioning either area impairs contextual fear conditioning, contributions from the PER and POR are likely to be different. Consistent with the BIC model, the PER likely processes and integrates multi-modal or complex sensory information about environmental elements, whereas the POR processes the spatial layout of the environment based on input from other visuospatial areas. In one series of experiments, a combination of auditory cue, context, and time of day ("what-where-when") jointly determined whether or not electric shocks were delivered (Iordanova et al., 2009). For example, a tone signaled shock only if it was presented in a chamber with dotted walls in the morning. Permanent lesions of the PER reduce freezing under the "what-where-when" and "what-where", but not the "what-when" combinations, suggesting that the PER critically contributes to processing the spatial environment but not temporal information of events hours apart (Iordanova et al., 2009). Other studies support the role of PER in processing complex stimuli in associative learning, particularly when stimuli are composed of temporally discontinuous units (Kent & Brown, 2012). For example, lesioning the PER leads to impaired fear conditioning to a complex auditory stimulus, while leaving conventional tone-signaled conditioning intact (Kholodar-Smith et al., 2008; Lindquist et al., 2004). Similarly, inactivating the PER during extinction reduces freezing to a discontinuous visual CS during both extinction and retrieval (Potter et al., 2020). It should be noted that suppressing the PER during extinction also decreases freezing to a continuous visual cue during the session, which indicates that PER is part of the preferred circuit regardless of the nature of the CS, and other structures can fully compensate for the loss of PER during conditioning unless the CS is discontinuous (Potter et al., 2020). In comparison, the POR is involved in processing visuospatial

information of the context, possibly through interaction with the retrosplenial cortex. Neurons projecting from the retrosplenial cortex to POR show increased c-fos level after fear conditioning, and disconnecting these two cortices diminishes conditioned freezing during context testing (Robinson et al., 2012).

Among prefrontal areas, the PL and IL in the mPFC are frequently shown to be involved in context-dependent associative learning paradigms. Their specific contributions are still under debate and likely vary between Pavlovian and instrumental conditioning as compared with appetitive and aversive conditioning. However, existing evidence suggests that the PL is generally needed for acquiring and expressing excitatory associations, whereas IL involvement is more frequently seen during acquisition of inhibitory associations (e.g. in extinction). In fear conditioning, the PL is not necessary for acquiring tone-shock association when no trace interval exists between the tone and the shock (Corcoran & Quirk, 2007; Gilmartin & Helmstetter, 2010; Zelikowsky et al., 2013). Whether the PL is critical for the acquisition of contextual fear depends on whether the shocks are explicitly signaled by a discrete cue. In un-signaled (or "foreground") contextual fear conditioning, inactivating the PL does not lead to reduced fear responses during subsequent re-exposure to the context (Corcoran & Quirk, 2007; Santos et al., 2017). In comparison, during signaled (or "background") contextual fear conditioning, context-shock association is blocked if the PL is temporarily suppressed (Gilmartin & Helmstetter, 2010; Gilmartin et al., 2013), but not if it is lesioned pre-training (Zelikowsky et al., 2013). PL functioning is also required when there is a short gap between context exposure and shock delivery (Santos et al., 2017). Together, lesion and inactivation studies suggest that the PL possibly maintains the contextual

representation when the context-shock association cannot be established at the exact moment of shock delivery, because either context exposure and shocks are temporally discontinuous (Santos et al., 2017) or there are more potent cues (e.g. tones) that overshadow the context for fear association (Gilmartin & Helmstetter, 2010; Gilmartin et al., 2013). Increased activity in the PL is indeed observed after contextual fear conditioning and after testing, suggesting that some neurons in the PL encode the context while others process the context-shock association (Zelikowsky et al., 2014). Similarly, the level of early-growth-response gene 1 (*Egr-1*) after training elevates in animals who have been pre-exposed to the training context, compared to those with pre-exposure to a different context, presumably because the former utilizes the PL to retrieve a representation of the context formed during pre-exposure to establish the context-shock association (Chakraborty et al., 2016). It should be noted, though, that some studies show that pre-training or post-training lesion of the PL does not affect discrimination between a shock context and a no-shock context (Kim et al., 2013; Zelikowsky et al., 2013). Instead, contextual discrimination deficits are present after pre-training damage to the IL, another area in the mPFC (Zelikowsky et al., 2013). The role of the IL in context-dependent fear is less well studied, though evidence suggests that it may serve as part of the circuit as well. For example, similar to the PL, *Egr-1* level in the IL is also increased after shock training with context pre-exposure (Chakraborty et al., 2016).

Context-induced renewal refers to the phenomenon in which extinguished conditioned responses re-appear after changes in context (Bouton & Bolles, 1979). The key component in any renewal procedure is re-exposure to a context distinct from the one in which extinction takes place; any switch in context causes return of the previously

extinguished response (Trask, Thrailkill, et al., 2017). In the commonly used "ABA" renewal procedure, the subject undergoes conditioning in context A, extinction in context B, and finally re-testing in context A. Context renewal has been observed for both Pavlovian and instrumental conditioning; however, their underlying mechanisms may be different. The dominant view on Pavlovian context renewal is that the extinction context acts as a negative occasion-setter that signals the negative contingency between the conditioned stimulus (CS) and the unconditioned stimulus (US) (Bouton, 2004; Trask, Thrailkill, et al., 2017). Since this occasion setting property is limited to the extinction context, re-testing in any other context prevents the retrieval of extinction memory and the CR re-appears because of the original excitatory CS-US association. In contrast, the context directly inhibits the CR during extinction of instrumental responses, and context renewal is due to the absence of such inhibitory association between the non-extinction context and the CR (Trask, Thrailkill, et al., 2017).

As with other types of contextual learning, both the medial temporal lobe and prefrontal cortices are involved in context-induced renewal. Among medial temporal lobe structures, existing research indicates a crucial role of the hippocampus in contextual fear renewal (Ji & Maren, 2007). Since the PER and POR are critically involved in processing contextual fear, however, it can be expected that they are also required for context renewal as well; future research is needed to confirm this. Within the prefrontal region, the orbitofrontal cortex (OFC) and the mPFC have been implicated as part of the context-renewal circuit. Inactivating the OFC attenuates context renewal of alcohol-seeking behavior and increased *fos* expression in the OFC is observed after the renewal session (Bianchi et al., 2018). Temporarily inactivating the lateral—but not medial—OFC

impairs context renewal of cocaine-seeking (Lasseter et al., 2009), and the connection between lateral OFC and basolateral amygdala is necessary for this effect (Lasseter et al., 2011). The functional differentiation between the medial and lateral portions of the OFC in context renewal is interesting, as will be discussed in the following sections, because the lateral OFC is more heavily connected with the PER and POR than the medial OFC (Hwang et al., 2018), and certain forms of spatial mapping have been observed in the lateral OFC (Farovik et al., 2015; Feierstein et al., 2006).

The mPFC also contributes to context renewal. Suppressing either the PL or IL diminishes renewal of a food-seeking response in its original acquisition context, i.e. in ABA renewal (Eddy et al., 2016), but PL inactivation has no effect when the renewal test is conducted in a third, novel context, i.e. in ABC renewal (Trask, Shipman, et al., 2017). This suggests that the PL particularly supports instrumental responding in the context where initial acquisition occurs, possibly through retrieving context-dependent action-outcome associations (Trask, Shipman, et al., 2017). The PL plays an important role in Pavlovian context renewal as well, even though it does not involve the same associative structures as instrumental conditioning. Pre-training PL damage causes similar levels of freezing when animals are tested in extinction and renewal context, abolishing the context renewal effect (Zelikowsky et al., 2013). In another study, pre-training lesions or inactivation of the PL diminished context renewal without affecting fear response during extinction (Sharpe & Killcross, 2015). Moreover, the absence of renewal is accompanied by deficits in orienting responses to the CS. Therefore, the impairment is at least partially due to attentional deficits towards the CS during re-testing. Since this procedure purposefully limits direct fear conditioning to the context (e.g. by using extended context

pre-exposure and long inter-stimulus intervals), it promotes the use of contextual cues as an occasion-setter over direct associations with the shock. Overall, these studies provide evidence that the PL is able to utilize context information to activate lower-order stimulus-stimulus associations.

To summarize, evidence from several commonly used associative learning procedures shows that the PER, POR, mPFC, and OFC are typically not required for delayed conditioning to simple cues, whereas they play crucial roles when responses are elicited or modulated by the spatial context. Specifically, as proposed by the BIC model (Diana et al., 2007; Eichenbaum et al., 2012; Eichenbaum et al., 2007), the PER and POR process non-spatial and spatial sensory input from various cortical and subcortical sources to efficiently construct context representations. Under certain circumstances, these representations may require maintenance or retrieval by the mPFC for associative learning. Moreover, when stimulus-stimulus or response-outcome relationships change after initial training (e.g. via context-specific extinction), the mPFC and OFC may use the context to re-structure existing associations and allow subjects to respond according to updated stimulus contingencies. The PL and IL in the mPFC, though differing in their specific contribution, both are capable of utilize context information to guide behavior.

Object recognition and discrimination

Object recognition and discrimination provide another set of rodent assays in which behaviors can be guided by their physical context. These assays can be categorized into two groups. One group is collectively referred to as context-dependent spontaneous

object exploration (SOR) tasks. These tasks take advantage of the rodent's natural tendency to explore novel objects and do not require reward deliveries or lengthy training sessions. The other group comprises biconditional discrimination tasks in which rodents are trained to discriminate objects to obtain rewards. In this second group, whether an object is associated with a reward is determined by object identity in combination with its spatial environment; in other words, the object-reward contingencies reverse when the objects are presented in a different context. Successfully obtaining rewards in such biconditional discrimination tasks requires the ability to not only discriminate objects but also acquire and retrieve context-specific response rules and then respond flexibly in different contexts.

Context-dependent SOR tasks include the contextual SOR and the object-in-place (OIP) task, whereas the standard SOR is often employed as a control task. In contextual SOR (Figure 1.3B), the animal undergoes two sampling phases where it is placed in two different physical contexts to explore two pairs of identical objects, then during the test phase it is put into one of the sample contexts with one object from each pair. Normal rats preferentially explore the object previously not appearing in the test context; in other words, more time is spent exploring the novel object-context pairing. Since this procedure involves multiple sampling phases, it is important to counter-balance the order in which objects and contexts are explored, either across subjects or by conducting multiple trials. Otherwise, results during the test phase may be confounded by the recency effect, in which case the rat explores an object more because it is the least recently encountered or it explores an object less because it is the most recent (Tam et al., 2015). In the OIP task (Figure 1.3C), the subject explores multiple different objects

during sampling, but during testing two of the objects switch places while others are not moved; innate preference is directed towards the objects appearing in novel locations. Whereas the standard SOR can be accomplished through simply encoding each object's familiarity, performance in contextual SOR and OIP depends on the recollection of associations between objects and the location or spatial context in which they occur (Barker & Warburton, 2011).

The PER is well-established as an important area for recognition memory (Eichenbaum et al., 2007; Suzuki & Naya, 2014; E. Warburton & M. W. Brown, 2015). Neurons in the monkey PER respond to specific objects and their activity decreases with repeated exposure, signaling increased familiarity (Xiang & Brown, 1998). Lesioning or inactivating the PER typically disrupts performance in standard object recognition tasks (Barker et al., 2007; Hannesson et al., 2004; Norman & Eacott, 2004, 2005; Winters & Bussey, 2005), though there have been exceptions (Jo & Lee, 2010). There are multiple possible explanations for the inconsistency. First, spared recognition memory may be observed if PER lesions are incomplete. It has been shown that the degree of SOR deficits are positively correlated with lesion sizes in the PER, particularly in its caudal portion (Albasser et al., 2009). Since it can be reasonably assumed that visual memory is one of the primary aspects of memory assessed in most object recognition procedures (typically conducted in the light), this is consistent with the fact that unimodal visual input mostly targets caudal area 36 of PER, whereas rostral PER mostly receives auditory, somatosensory and tactile information (Burwell & Agster, 2008; Burwell & Amaral, 1998a). Second, it has been proposed that the PER may serve a perceptual function in addition to its role in mnemonic processes (Bussey et al., 2002, 2005). The

idea is that the PER combines various elements of an object into one configural representation, therefore it is particularly important for discriminating objects that cannot be easily distinguished based on individual perceptual elements, e.g. when they have highly overlapping features. Therefore, intact object recognition memory after PER damage may also be due to the relatively low perceptual feature overlap between objects. Despite the null results of recognition test after PER lesions, the majority of evidence is in accordance with the view that the PER plays a fundamental role in object recognition.

It is perhaps not surprising, then, that the PER critically contributes to procedures involving associating objects with their location or context in addition to memorizing objects themselves. Bilateral lesions of the PER reduce preferential exploration of objects whose locations have been switched in the OIP task (Barker et al., 2007). Another study utilized a biconditional object discrimination task (Jo & Lee, 2010). In a multi-arm maze, food is hidden under object A and not B in one arm while it is under object B and not A in another arm on other trials. Therefore, successful discrimination requires associating objects with the context (arm) where they are rewarded. Post-training lesion of the PER disrupts the learned object-context associations before subjects gradually regain the pre-surgical level of accuracy. However, subjects are unable to discriminate new object-context pairings involving new objects, suggesting that the PER is necessary for the initial encoding of novel object-context associations. Simple object discrimination was not affected by PER lesions (Jo & Lee, 2010).These results were confirmed by another study using a similar procedure. Subjects with PER damage performed significantly worse compared to pre-lesion levels and revert to a side bias, preferentially selecting

objects on a particular side, regardless of object identity and spatial context (Hernandez et al., 2017).

Like the PER, the POR is also required for contextual object recognition tasks. POR damage impairs the ability to form object-context and object-place pairings without negatively affecting standard SOR (Norman & Eacott, 2005). This is consistent with single-cell recording evidence that some POR neurons selectively respond to object-place conjunctions, i.e. when an object appears in a certain location but not when the same object appears in other locations or other objects appear in the same location (Furtak et al., 2012). This object-place encoding likely directly depends on PER-POR interaction, because animals with PER-POR disconnection are significantly impaired in both two-dimensional and three-dimensional versions of the contextual SOR task (Heimer-McGinn et al., 2017). Object location conjunctive coding emerges in the hippocampus as animals learn to associate particular stimuli to places where they have differential consequences (Komorowski et al., 2009). Interestingly, object location conjunctive coding appears in the POR before it appears in the hippocampus (Estela, 2020). This is consistent with our proposal that object-location conjunctive coding in the hippocampus signifies associative learning, whereas object-location conjunctive coding in the postrhinal is a signature of representations of local contexts including the spatial layout of items and features of the context.

There is also evidence that the POR monitors the local environment and helps direct attention towards motivationally relevant events by communicating with cortices known for visuospatial attention, such as the posterior parietal cortex (Agster & Burwell, 2009;

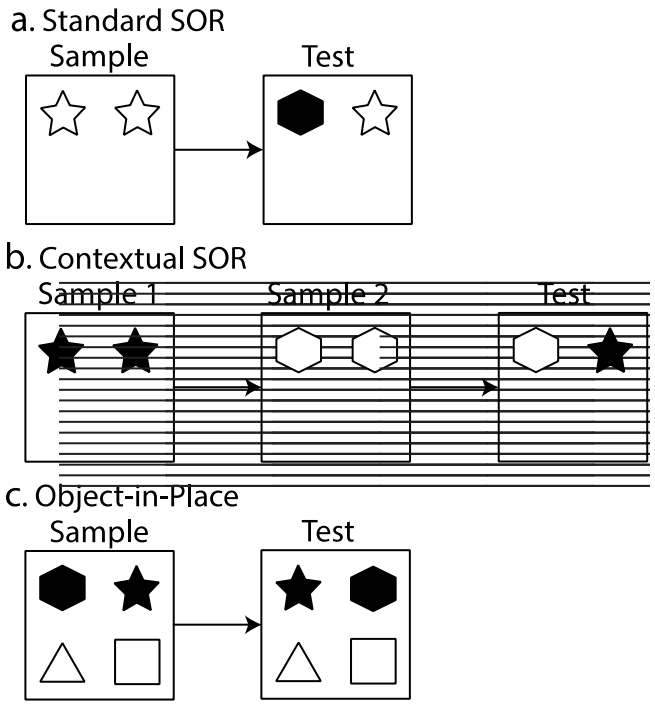

Figure 1.3. Variants of the spontaneous object recognition task. A) Standard SOR task: healthy animals are expected to spend more time exploring the triangle over the hexagon during test. B) Contextual SOR: the subject should show preference for the triangle if tested in the dotted context and for the hexagon if tested in the checkered context. The order in which objects and contexts are presented should be counter-balanced (Heimer-McGinn et al., 2017). Therefore, in contextual SOR, subjects as a group have an equal level of familiarity and recency with each object and context at testing, but one of the object-context pairing is novel. C) Object-in-place task: preferential exploration is expected for the square and the hexagon over the triangle and the circle.

Burwell & Amaral, 1998a). For example, POR lesions in rodents impair attentional orienting towards a light signal paired with food delivery (Bucci & Burwell, 2004), and spatial firing correlates in the POR remap when local and distal cues are moved (Burwell & Hafeman, 2003). In another study, neurons in the POR and surrounding visual association cortices selectively responded to food cues (Burgess et al., 2016). This preference disappeared after satiation, suggesting that visual association areas are updated when the motivational value of visual cues in the environment changes. It is further shown that different ensembles of POR neurons encode cue identity and motivational value (Ramesh et al., 2018), and ensembles encoding both the cue and the reward undergo preferential reactivation after training that strengthens their connectivity through hippocampal-related consolidation process (Sugden et al., 2020). Collectively, these results provide evidence for considerable item and cue encoding in the POR and

that the POR is involved in monitoring cues in the environment and updating changes in cue motivational value and spatial location.

Prefrontal cortex in rodents is not required to detect and explore novel objects (Barker et al., 2007; Ennaceur et al., 1997; Hannesson et al., 2004). However, the mPFC is necessary for biconditional object discrimination tasks (Hernandez et al., 2017; Lee & Solivan, 2008) and for discriminating object-place and object-context pairings in OIP tasks (Barker et al., 2007; Cross et al., 2013). The core factor shared by these tasks is the requirement to integrate object appearance with its spatial attributes, i.e. location or context. The fact that the loss of mPFC functioning impairs these tasks points to the idea that the mPFC critically contributes to object recognition only if it involves binding and retrieving the object together with the context of its prior appearance, i.e. via recollection-based recognition, not when the decision is based on familiarity judgement alone. Another reason why the mPFC supports biconditional discrimination may be related to its well-known role of enabling flexible decision-making. Since the object-reward contingency varies across contexts, the reward cannot be obtained above chance level by always selecting the same object or any object on the same side across all contexts. Evidence supporting this idea comes from the control condition in which rewards are associated with locations instead of objects ("location-in-place", e.g. rewards are always on the left side inside context A, regardless of what object is on the left). Under this condition, mPFC inactivation initially disrupts performance but it quickly rebounds to pre-inactivation level, possibly due to subjects switching towards an egocentric strategy to obtain rewards (Lee & Solivan, 2008). The need for mPFC for flexible action selection, however, does not apply to OIP, because it does not involve acquiring and

applying complex response rules that necessitate flexible action selection across trials. Finally, it has been proposed that although primarily a spatial-object memory tasks, the OIP in fact contains a significant temporal element due to the need to accurately and separately represent memories of objects and their layout into distinct instances (Aggleton & Nelson, 2020). Although this idea has yet been fully investigated, it does fit well with a known function of the mPFC to reduce interference when retrieving past memories, particularly through coordinating with the hippocampus (Eichenbaum, 2017).

In summary, context-dependent object recognition depends on the functional integrities of the PER and POR as well as the mPFC. Together with previously mentioned context-dependent tasks, the results of these studies are summarized in Table 1. A cortico-hippocampal-cortical network proposes that the PER is specifically involved in processing and storing object information, whereas the hippocampus is responsible for providing spatial information regarding where the object appears, and finally the mPFC integrates them to form object-place associations (E. C. Warburton & M. W. Brown, 2015). However, this model does not take into account the direct contribution of the POR. The fact that PER-POR disconnection impairs contextual SOR (Heimer-McGinn et al., 2017) and observations of object-location conjunctive coding in the POR (Furtak et al., 2012) suggest that the parahippocampal region itself does contain certain forms of contextual representation. Blocking PER-POR communication likely reduces the fidelity of context representations in the parahippocampal region as well as in the hippocampus, leading to disrupted performance in contextual object recognition, even though less-detailed "gist-like" representations may still exist that are sufficient for less demanding contextual tasks, such as contextual fear conditioning (Heimer-McGinn et al., 2017).

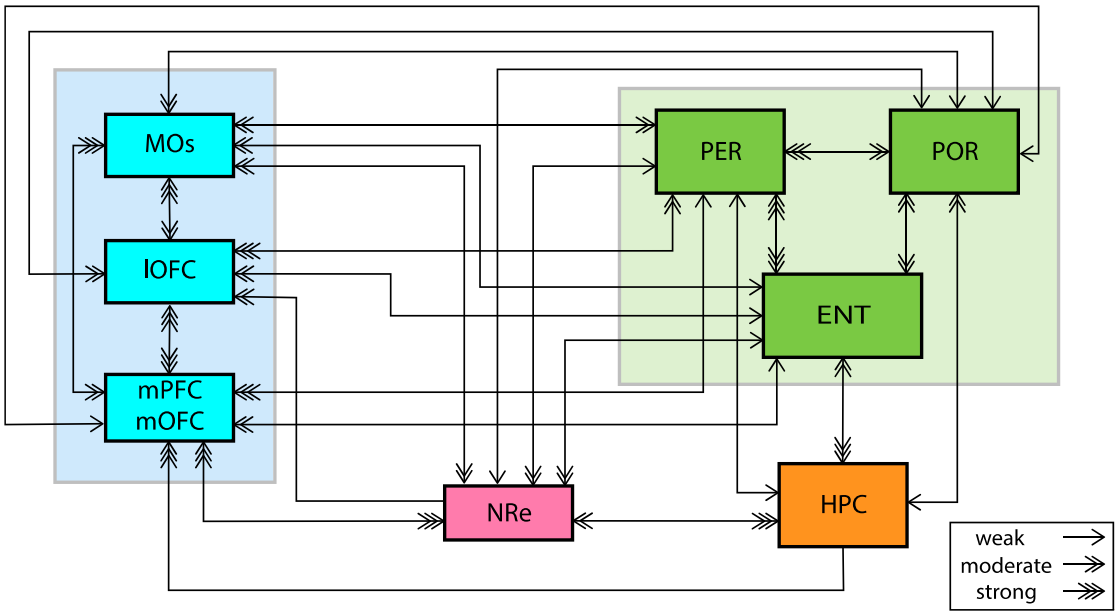

Figure 1.4. Anatomical connections between the prefrontal cortices, the hippocampus, and parahippocampal cortices. Abbreviations: ENT: entorhinal cortex. HPC: hippocampus. MOs: secondary motor cortex. NRe: nucleus reuniens. lOFC: the lateral, ventral, and ventrolateral portions of the orbitofrontal cortex. mOFC: medial orbitofrontal cortex. PER: perirhinal cortex. mPFC: medial prefrontal cortex. POR: postrhinal cortex. The shape of arrows indicates the strength of the connection. For parahippocampal cortices, the strength of an afferent connection is determined by the ratio of the number of retrograde-labeled cells in the origin over the number of all labeled cells. For other connections, the strength reflects the density of labeling. In articles where data is split into multiple sub-regions, the results across sub-regions are averaged (Agster & Burwell, 2009; Agster et al., 2016; Barreiros et al., 2021; Burwell & Amaral, 1998a; Delatour & Witter, 2002; Hwang et al., 2018; Jay & Witter, 1991; Kerr et al., 2007; Kondo & Witter, 2014; McKenna & Vertes, 2004; Murphy & Deutch, 2018; Reep et al., 1987; Reep et al., 1990; Reep et al., 1984; Tomás Pereira et al., 2016; Vertes et al., 2006).

In the rodent brain, there are both direct and indirect pathways by which prefrontal and parahippocampal areas communicate. Even though direct parahippocampal connections with prefrontal cortices are relatively weak compared to its connections with association and sensory cortices or within the parahippocampal region (Agster & Burwell, 2009; Burwell & Amaral, 1998a), direct reciprocal connections do exist between the PER/POR and multiple prefrontal areas, including the medially located PL and IL cortices, multiple subregions of the OFC, and the secondary motor cortex (MOs). Indirect prefrontal-parahippocampal communication is possibly mediated via the nucleus reuniens of the

Manipulation	OR	cxtSOR	OiP	BOD	cxtFC	FCs	FCc	CR
PER								
pre-training	✓✓✓×	✓	✓		✓✓✓✓	×××	✓✓	
					✓	×		
post-training				✓[1]	✓✓✓	✓[2]		
inactivation	✓✓			✓		✓	✓	
POR								
pre-training		✓			✓✓	××		
post-training					✓✓			
mPFC								
pre-training	×××		✓✓					✓✓
inactivation	×			✓✓	✓✓××	×××		✓✓
								✓×[3]
OFC								
inactivation								✓✓
Disconnection								
PER-mPFC			✓	✓				
PER-POR	×	✓			×			

Summary of rodent studies involving the PER, POR, and mPFC in common contextual and non-contextual tasks after pre-training lesion, post-training lesion, or temporary inactivation. ✓ : deficit. × : no deficit. CR: context renewal. OR: object recognition (including spontaneous object recognition and trained object discrimination. cxtSOR: contextual spontaneous object recognition. OiP: object-in-place. BOD: biconditional object discrimination. cxtFC: contextual fear conditioning. FCs: signaled fear conditioning where the CS is a simple stimulus, e.g. pure tone. FCc: signaled fear conditioning where the CS is complex or discontinuous. [1]Although the surgery group recovered to normal levels of performance after initially dropping post-surgery, they were severely impaired when learning new object-place associations (Jo & Lee, 2010). [2]There was significant damage to the auditory association cortex dorsal to the PER, which might explain reduced fear response to the auditory CS (Corodimas & LeDoux, 1995). [3]Deficits were observed in ABA renewal but not ABC renewal (Trask, Shipman, et al., 2017).

thalamus, which is reciprocally connected with the PER/POR (Agster et al., 2016; Tomás Pereira et al., 2016). Importantly, the pattern of reciprocal prefrontal-parahippocampal pathways differs from that between prefrontal cortices and the hippocampus. This section will mainly focus on direct prefrontal afferents and parahippocampal efferents based on Agster and Burwell (2009), Burwell and Amaral, (1998a), and Hwang, Willis, and Burwell (2018). Results reported by these studies are mostly consistent with others (Delatour & Witter, 2002; Kondo & Witter, 2014; Reep et al., 1987; Reep et al., 1990). As a comparison, the anatomical connections between prefrontal cortices and the hippocampus will be briefly reviewed as well (Figure 1.4).

Anatomical connections between prefrontal cortices and the perirhinal/postrhinal cortex

Both area 35 and 36 of the PER project to the entire rostrocaudal extent of both PL and IL. Area 36 projects to the PL and IL more strongly than area 35, with heavier projection originating from the rostral to mid-rostrocaudal sites of area 36. Area 35 sends strong projection to the mid-rostrocaudal portion of the PL. Both areas send axons to the medial, lateral, and ventrolateral portions of the OFC, though area 36 has more efferents compared to area 35. These projections originate more from the rostral and mid-rostrocaudal levels, and they arrive at rostral levels of all orbitofrontal subareas. Area 35 and 36 also project to the MOs, though relatively weaker than to other PFC areas. The projections originate from the entire rostrocaudal extent of both areas, with the rostral regions preferentially targeting rostral MOs. In terms of afferent connections, prefrontal areas project more strongly to area 35 than area 36. The orbitofrontal regions combined provide around half of the prefrontal output to the two PER areas, with the majority arising from the lateral or ventrolateral OFC. The MOs provides most of the remaining prefrontal output to the PER. Lastly, for medial prefrontal regions, the IL provides stronger input to the PER than the PL, and for both regions, input to the PER is stronger from the rostral levels.

The POR exhibits a different pattern of prefrontal connection compared to area 35 or 36 of the PER. Overall, the POR projects to prefrontal areas less strongly than the PER. The strongest projections originate from the caudal POR and terminate in rostral levels of both ventrolateral OFC and MOs. The entire rostrocaudal extent of POR projects to the

MOs. The POR projection to the IL and PL are weak overall. The MOs provides the most input to the POR among all prefrontal regions; stronger projection originates from the mid-rostrocaudal and the most caudal levels of the MOs. The orbital regions provide the second most prefrontal output, mostly from the ventrolateral portion. Output from mPFC to the POR is small.

In comparison, connectivity between the hippocampus and prefrontal areas is limited in two ways. First, hippocampal efferents terminate in the medial but not lateral or the most dorsal portions of prefrontal cortices (Hoover & Vertes, 2007; Jay & Witter, 1991; Vertes et al., 2007). The areas to which the hippocampus does not project, i.e. the lateral and ventrolateral OFC and the MOs, not only receive efferents from the PER and POR but also account for most of the prefrontal input to these two cortices. Second, whereas both direct and indirect pathways exist between the prefrontal and parahippocampal regions, the mPFC lacks direct projection to the hippocampus and the connection is relayed via the nucleus reuniens instead (Vertes et al., 2007).

Parahippocampal-prefrontal communication supports context-dependent behavior

Widespread reciprocal connectivity and mutual involvement in multiple context-guided tasks suggest that parahippocampal areas, such as the PER and POR, may directly provide contextual information to the prefrontal cortices. Despite the functional evidence and well-described connections, existing research has focused more on the hippocampus as the primary source of contextual input to prefrontal areas. For example, connections

between the mPFC and the hippocampus were shown to be necessary for context-dependent renewal following fear conditioning (Orsini et al., 2011; Wang et al., 2016). Another series of studies provided evidence that dorsal hippocampus and mPFC are involved in working memory versions of a biconditional discrimination task in which texture and the visual appearance of the floor in a maze guides spatial responses (Hallock et al., 2013; Hallock & Griffin, 2013). Moreover, inactivation of midline thalamic nuclei that provide an indirect pathway between the hippocampus and medial prefrontal cortex also impaired performance on the task (Urban et al., 2014).

Although prefrontal-hippocampal interaction indeed plays essential roles in some forms of contextual memory, such interactions, alone, seem insufficient to support all aspects of context-dependent behavior. Based on anatomical and functional evidence, we propose a more distributed prefrontal to medial temporal lobe circuit that also involves interaction and coordination between the parahippocampal and the prefrontal regions (Figure 1.5). Typical examples of context-dependent behavior that this model supports include paradigms reviewed in previous sections. However, it should also account for any behavior that requires encoding, storing, and retrieving memory for diffuse external stimuli (as opposed to simple cues or signals). Another example of a procedure that this model may support is the conditioned place preference for modeling substance abuse (Bardo & Bevins, 2000). Moreover, it should be noted that although the proposed model is based on research that operationally defines "context" as the spatial environment, there has been evidence that many of these regions are also involved for processing the temporal aspects of context. For example, the hippocampus, mPFC, and the PER are all required in tasks where subjects need to encode and retrieve the order in which events

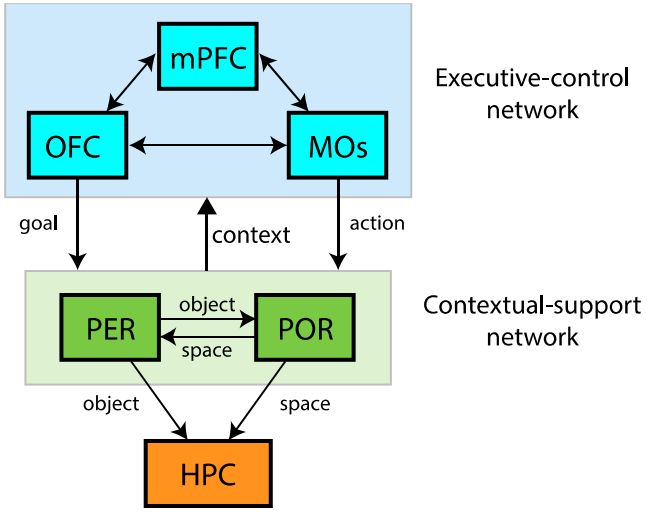

Figure 1.5. Schematics of information flow between the PFC, PER-POR and HPC. Some areas or connections are not included for simplicity, such as the entorhinal cortex and the nucleus reuniens.

occur (Allen et al., 2020; Barker et al., 2007; Hannesson et al., 2004). Thus, although this review focuses on the spatial context, the same network may also be responsible for processing a broader range of information that constitutes the "what-where-when" aspects of episodic-like memory.

In this model (Figure 1.5), the PER and POR form a cortical contextual-support network that provide context information to both the hippocampus and the prefrontal cortices for executive control. Instead of emerging in the hippocampus, basic context representations are already present at the PER-POR level, most likely in the POR where object-location conjunctive coding has been observed. These representations can be passed onto the hippocampus either directly to the CA1 or indirectly through the entorhinal cortex, finally reaching the hippocampus to support associative learning, some forms of contextual learning, and episodic memory. Although context representations are necessary for a number of hippocampal dependent functions, they can also be utilized directly by prefrontal cortices to support context guided behavior. Contextual information facilitates

cue-outcome and reward value encoding in the OFC, while also enabling efficient updating in response to varying contextual demands. In return, goal-related input from the OFC arrives at the PER and POR and it may modify place-field firing patterns in the hippocampus. Contextual input can also be utilized by the MOs to accurately encode similar actions or actions in sequence, which supports efficient action selection, planning, and execution. Though the mPFC does receive direct projections from the hippocampus and projects back via the nucleus reuniens, the PER and POR may provide additional contextual input that the mPFC uses to modulate other associations via occasion-setting mechanisms. Consistent with this view, context-activated ensembles in the mPFC show activity patterns distinct from those in the hippocampus: ensembles in the mPFC are more differentiated between successive exposure to the same context, whereas hippocampal representations are separated more in terms of distinct locations within the same environment (Hyman et al., 2012). This suggests that the mPFC relies on sources in addition to the hippocampus to maintain different forms of context representations that are suitable for coping with varying task demands.

Prefrontal-perirhinal connections are known to play important roles in object recognition and discrimination tasks with contextual demands but not simple object recognition (Barker et al., 2007; Barker & Warburton, 2009; Hernandez et al., 2017). These studies either involve creating permanent lesions or employ procedures that cannot be separated into distinct phases of acquisition and recognition, making the specific function of these connections difficult to pin down. It is possible that the mPFC facilitates retrieval of memories containing information about familiar objects as well as the context in which they occurred, therefore it is required for recollection-based recognition to retrieve

object-context pairings; the mPFC is not critically involved for simple object recognition, which may be solved by the PER and familiarity-based process alone. Alternatively, the mPFC may utilize contextual input to modulate retrieval of object information in the PER during recognition, eventually affecting neuronal firing pattern in the hippocampus (Navawongse & Eichenbaum, 2013; Place et al., 2016). To explore these possibilities, future research may employ techniques that silence the mPFC-PER pathway in a direction-specific manner. It has already been demonstrated that information flows of opposite directions take place between the mPFC and hippocampus during the sampling and retrieving phases of a context-guided object recognition task (Place et al., 2016). Beyond object recognition, mPFC-PER connectivity may also underlie the context renewal effect. We might speculate that disconnecting these areas will reduce conditioned responding during re-exposure to the acquisition context, and this effect may be strongest when temporary disconnection occurs during re-exposure, compared to during extinction.

In addition to mPFC connections, the PER and POR also have substantial connectivity with the OFC, particularly its lateral and ventrolateral portion (Delatour & Witter, 2002; Hwang et al., 2018; Kondo & Witter, 2014). The PER and POR are well positioned to provide contextual input to support the OFC in goal-oriented decision-making, and they may rely on projections from the OFC for cue-outcome associations and reward values. The OFC is proposed to house a task space for goal-oriented tasks as well as monitoring and updating the current location in that space (Bradfield & Hart, 2020; Wilson et al., 2014). The lateral and ventrolateral OFC contains detailed spatial mapping of goals, actions, and other task-related context information (Farovik et al., 2015; Feierstein et al., 2006). These types of mapping loosely resemble the “cognitive map” in the hippocampus

(Wikenheiser & Schoenbaum, 2016). Since the lateral and ventrolateral portion of the OFC receive no direct projection from the hippocampus (Jay & Witter, 1991), the parahippocampal region is a prime source for providing both spatial and non-spatial information that is represented by the OFC.

Jointly, PER and POR may provide information to the OFC about external environmental stimuli to be integrated with internal cues to aid goal-directed behavior. Specifically, the POR may communicate with the OFC to support and update spatial mapping containing reward locations and other task-relevant information. Inhibiting orbitofrontal neurons that receive projections from the POR indeed leads to deficits in detecting object location change (Qi et al., 2019). The PER is capable of efficiently combining and monitoring reward features and transmitting them to the OFC. Since the values of all relevant features of a reward need to be computed and integrated before optimal decisions are reached, having accurate input from the PER may be useful not only for maintaining choice strategies but also for flexibly adjusting behavior if reward properties change. A recent study supports this speculative role of the PER in maintaining reward representation: bilateral PER inactivation leads to decreased choice stability over two rewards with different magnitudes and less successful exploitation during a dynamic delay discounting task (Kreher et al., 2019). In turn, since the OFC has been shown to project outcome signals and influence other cortical areas (Banerjee et al., 2020; Liu et al., 2020), it may modify firing patterns of PER or POR neurons regarding outcome expectancy in goal-directed behavior, eventually altering spatial mapping in the hippocampus (Hok et al., 2007). In mice, the POR and its surrounding regions contain ensembles selectively encoding the identity or the motivational value of visual cues

(Burgess et al., 2016; Ramesh et al., 2018), and hippocampal-induced reactivation produces learning-related changes in their connectivity (Sugden et al., 2020). Future research is required to show that such mapping in the POR indeed relies upon input from the OFC, perhaps during spatial reward tasks in which spatial correlates have been detected in the POR (Burwell & Hafeman, 2003). After the POR establishes spatial mapping containing information about reward locations, partial or complete re-mapping may occur if reward location moves or reward value changes, e.g. via devaluation. Such re-mapping among POR neurons may directly result from value update signals originating from the OFC, in which case re-mapping will not occur if OFC input has been blocked.

So far, no study has directly examined the involvement of the secondary motor cortex (MOs) in classic context-dependent tasks; however, since the MOs is among the prefrontal areas most heavily connected to both the PER and POR (Hwang et al., 2018; Reep et al., 1987; Reep et al., 1990), it may be expected to contribute significantly to some aspects of context-dependent behavior. The MOs is characterized by expansive cortico-cortical connections with the primary motor cortex, the somatosensory, visual, auditory, retrosplenial and parahippocampal cortices (Reep et al., 1987; Reep et al., 1990), and it is proposed to be involved in selecting, planning, and executing voluntary actions based on sensory input (Barthas & Kwan, 2017). The primate analogue of the rodent MOs is not well established; possible candidates include the supplementary motor area (Reep et al., 1987; Reep et al., 1990) and the dorsolateral prefrontal cortex (Hwang et al., 2018). MOs neurons encode and discriminate upcoming choice actions during action planning and persist throughout its execution (Olson et al., 2020). The choice

signals occur in the MOs earlier than the primary motor cortex or other brain areas (Sul et al., 2011). In addition, the presence of value signals in the MOs suggests that it may be involved in certain decision-making processes as well (Sul et al., 2011). Both single-unit and population coding of rewarded choices exist in the MOs during an auditory discrimination task with probabilistic outcomes, though it does not encode the magnitude of rewards (Siniscalchi et al., 2019). Although it has been proposed that areas such as the retrosplenial and posterior parietal cortices are the primary sources of spatial information to the MOs (Olson et al., 2020), the anatomical evidence suggests that the MOs may rely on the PER and POR for contextual input as well. Encoding similar actions differently based on their context may facilitate efficient action selection according to variable situational demand. Contextual information may even be required to encode different instances of the same action separately, particularly when the outcome is determined by a sequence of actions, such as navigating a maze that contains multiple left and right turns (Olson et al., 2020).

To conclude, although hippocampal-mPFC communication is critical for some forms of context-dependent behavior, there is substantial evidence for cooperation between prefrontal areas and other medial temporal lobe structures. On the one hand, due to their mutual connectivity with multiple prefrontal areas, parahippocampal areas such as the PER and POR may provide context information directly to the prefrontal cortex in addition to supporting context associations in the hippocampus. On the other hand, prefrontal areas may send important information about values and actions back to the PER and POR and eventually to the hippocampus, serving as another route of prefrontal-hippocampal interaction. Examining the functional connections between prefrontal areas

and parahippocampal cortices, particularly the PER and POR, will contribute importantly to our understanding of learning and memory in general.

References

Aggleton, J. P., & Nelson, A. J. D. (2020). Distributed interactive brain circuits for object-in-place memory: A place for time? *Brain Neurosci Adv*, *4*, 2398212820933471. https://doi.org/10.1177/2398212820933471

Agster, K. L., & Burwell, R. D. (2009). Cortical efferents of the perirhinal, postrhinal, and entorhinal cortices of the rat. *Hippocampus*, *19*(12), 1159-1186. https://doi.org/10.1002/hipo.20578

Agster, K. L., Tomás Pereira, I., Saddoris, M. P., & Burwell, R. D. (2016). Subcortical connections of the perirhinal, postrhinal, and entorhinal cortices of the rat. II. efferents. *Hippocampus*, *26*(9), 1213-1230. https://doi.org/10.1002/hipo.22600

Albasser, M. M., Davies, M., Futter, J. E., & Aggleton, J. P. (2009). Magnitude of the object recognition deficit associated with perirhinal cortex damage in rats: Effects of varying the lesion extent and the duration of the sample period. *Behav Neurosci*, *123*(1), 115-124. https://doi.org/10.1037/a0013829

Allen, L. M., Lesyshyn, R. A., O'Dell, S. J., Allen, T. A., & Fortin, N. J. (2020). The hippocampus, prefrontal cortex, and perirhinal cortex are critical to incidental order memory. *Behav Brain Res*, *379*, 112215. https://doi.org/10.1016/j.bbr.2019.112215

Banerjee, A., Parente, G., Teutsch, J., Lewis, C., Voigt, F. F., & Helmchen, F. (2020). Value-guided remapping of sensory cortex by lateral orbitofrontal cortex. *Nature*, *585*(7824), 245-250. https://doi.org/10.1038/s41586-020-2704-z

Bardo, M. T., & Bevins, R. A. (2000). Conditioned place preference: what does it add to our preclinical understanding of drug reward? *Psychopharmacology (Berl)*, *153*(1), 31-43. https://doi.org/10.1007/s002130000569

Barker, G. R., Bird, F., Alexander, V., & Warburton, E. C. (2007). Recognition memory for objects, place, and temporal order: a disconnection analysis of the role of the medial prefrontal cortex and perirhinal cortex. *Journal of Neuroscience*, *27*(11), 2948-2957. https://doi.org/10.1523/JNEUROSCI.5289-06.2007

Barker, G. R., & Warburton, E. C. (2009). Critical role of the cholinergic system for object-in-place associative recognition memory. *Learning & Memory*, *16*(1), 8-11. https://doi.org/10.1101/lm.1121309

Barker, G. R., & Warburton, E. C. (2011). When is the hippocampus involved in recognition memory? *Journal of Neuroscience*, *31*(29), 10721-10731. https://doi.org/10.1523/JNEUROSCI.6413-10.2011

Barreiros, I. V., Panayi, M. C., & Walton, M. E. (2021). Organization of Afferents along the Anterior–posterior and Medial–lateral Axes of the Rat Orbitofrontal Cortex. *Neuroscience*, *460*, 53-68.

Barthas, F., & Kwan, A. C. (2017). Secondary motor cortex: where 'sensory'meets 'motor'in the rodent frontal cortex. *Trends in neurosciences*, *40*(3), 181-193. https://doi.org/10.1016/j.tins.2016.11.006

Bianchi, P. C., de Oliveira, P. E. C., Palombo, P., Leão, R. M., Cogo-Moreira, H., da Silva Planeta, C., & Cruz, F. C. (2018). Functional inactivation of the orbitofrontal cortex disrupts context-induced reinstatement of alcohol seeking in rats. *Drug and alcohol dependence*, *186*, 102-112. https://doi.org/10.1016/j.drugalcdep.2017.12.045

Bouton, M. E. (2004). Context and behavioral processes in extinction. *Learning & Memory*, *11*(5), 485-494. https://doi.org/10.1101/lm.78804

Bouton, M. E., & Bolles, R. C. (1979). Contextual control of the extinction of conditioned fear. *Learning and motivation*, *10*(4), 445-466. https://doi.org/10.1037//0097-7403.5.4.368

Bradfield, L. A., & Hart, G. (2020). Rodent medial and lateral orbitofrontal cortices represent unique components of cognitive maps of task space. *Neuroscience & Biobehavioral Reviews*, *108*, 287-294. https://doi.org/10.1016/j.neubiorev.2019.11.009

Bucci, D. J., & Burwell, R. D. (2004). Deficits in Attentional Orienting Following Damage to the Perirhinal or Postrhinal Cortices [Article]. *Behavioral neuroscience*, *118*(5), 1117-1122. https://doi.org/10.1037/0735-7044.118.5.1117

Bucci, D. J., Phillips, R. G., & Burwell, R. D. (2000). Contributions of postrhinal and perirhinal cortex to contextual information processing. *Behavioral neuroscience*, *114*(5), 882. https://doi.org/10.1037//0735-7044.114.5.882

Bucci, D. J., Saddoris, M. P., & Burwell, R. D. (2002). Contextual fear discrimination is impaired by damage to the postrhinal or perirhinal cortex. *Behavioral neuroscience*, *116*(3), 479-488. https://doi.org/10.1037/0735-7044.116.3.479

Burgess, C. R., Ramesh, R. N., Sugden, A. U., Levandowski, K. M., Minnig, M. A., Fenselau, H., Lowell, B. B., & Andermann, M. L. (2016). Hunger-dependent enhancement of food cue responses in mouse postrhinal cortex and lateral amygdala. *Neuron*, *91*(5), 1154-1169. https://doi.org/10.1016/j.neuron.2016.07.032

Burwell, R., & Agster, K. (2008). Anatomy of the hippocampus and the declarative memory system. https://doi.org/10.1016/B978-012370509-9.00117-0

Burwell, R. D. (2001). Borders and cytoarchitecture of the perirhinal and postrhinal cortices in the rat. *Journal of Comparative Neurology*, *437*(1), 17-41. https://doi.org/10.1002/cne.1267

Burwell, R. D., & Amaral, D. G. (1998a). Cortical afferents of the perirhinal, postrhinal, and entorhinal cortices of the rat. *Journal of Comparative Neurology*, *398*(2), 179-205. https://doi.org/10.1002/(sici)1096-9861(19980824)398:2<179::aid-cne3>3.0.co;2-y

Burwell, R. D., & Amaral, D. G. (1998b). Perirhinal and postrhinal cortices of the rat: interconnectivity and connections with the entorhinal cortex. *Journal of Comparative Neurology*, *391*(3), 293-321. https://doi.org/10.1002/(sici)1096-9861(19980216)391:3<293::aid-cne2>3.0.co;2-x

Burwell, R. D., Bucci, D. J., Sanborn, M. R., & Jutras, M. J. (2004). Perirhinal and postrhinal contributions to remote memory for context. In (Vol. 24, pp. 11023-11028).

Burwell, R. D., & Hafeman, D. M. (2003). Positional firing properties of postrhinal cortex neurons. *Neuroscience*, *119*(2), 577-588. https://doi.org/10.1016/s0306-4522(03)00160-x

Burwell, R. D., & Witter, M. P. (2002). *Basic anatomy of the parahippocampal region in monkeys and rats* [Chapter]. Oxford University Press. https://doi.org/10.1093/acprof:oso/9780198509172.003.0003

Burwell, R. D., Witter, M. P., & Amaral, D. G. (1995). Perirhinal and postrhinal cortices of the rat: a review of the neuroanatomical literature and comparison with findings from the monkey brain. *Hippocampus*, *5*(5), 390-408. https://doi.org/10.1002/hipo.450050503

Bussey, T. J., Saksida, L. M., & Murray, E. A. (2002). Perirhinal cortex resolves feature ambiguity in complex visual discriminations. *European Journal of Neuroscience*, *15*(2), 365-374. https://doi.org/10.1046/j.0953-816x.2001.01851.x

Bussey, T. J., Saksida, L. M., & Murray, E. A. (2005). The perceptual-mnemonic/feature conjunction model of perirhinal cortex function. *The Quarterly Journal of Experimental Psychology Section B*, *58*(3-4), 269-282. https://doi.org/10.1080/02724990544000004

Chakraborty, T., Asok, A., Stanton, M., & Rosen, J. (2016). Variants of contextual fear conditioning induce differential patterns of Egr-1 activity within the young adult prefrontal cortex. *Behavioural brain research*, *302*, 122-130. https://doi.org/10.1016/j.bbr.2016.01.018

Corcoran, K. A., & Quirk, G. J. (2007). Activity in prelimbic cortex is necessary for the expression of learned, but not innate, fears. *Journal of Neuroscience*, *27*(4), 840-844. https://doi.org/10.1523/JNEUROSCI.5327-06.2007

Corodimas, K. P., & LeDoux, J. E. (1995). Disruptive effects of posttraining perirhinal cortex lesions on conditioned fear: contributions of contextual cues. *Behavioral neuroscience*, *109*(4), 613. https://doi.org/10.1037//0735-7044.109.4.613

Cross, L., Brown, M. W., Aggleton, J. P., & Warburton, E. C. (2013). The medial dorsal thalamic nucleus and the medial prefrontal cortex of the rat function together to support associative recognition and recency but not item recognition. *Learning & Memory*, *20*(1), 41-50. https://doi.org/10.1101/lm.028266.112

Dalley, J. W., Cardinal, R. N., & Robbins, T. W. (2004). Prefrontal executive and cognitive functions in rodents: neural and neurochemical substrates. *Neuroscience & Biobehavioral Reviews*, *28*(7), 771-784. https://doi.org/10.1016/j.neubiorev.2004.09.006

Delatour, B., & Witter, M. (2002). Projections from the parahippocampal region to the prefrontal cortex in the rat: evidence of multiple pathways. *European Journal of Neuroscience*, *15*(8), 1400-1407. https://doi.org/10.1046/j.1460-9568.2002.01973.x

Diana, R. A., Yonelinas, A. P., & Ranganath, C. (2007). Imaging recollection and familiarity in the medial temporal lobe: a three-component model. *Trends in cognitive sciences*, *11*(9), 379-386. https://doi.org/10.1016/j.tics.2007.08.001

Eddy, M. C., Todd, T. P., Bouton, M. E., & Green, J. T. (2016). Medial prefrontal cortex involvement in the expression of extinction and ABA renewal of instrumental behavior for a food reinforcer. *Neurobiology of learning and memory*, *128*, 33-39. https://doi.org/10.1016/j.nlm.2015.12.003

Eichenbaum, H. (2017). Prefrontal-hippocampal interactions in episodic memory. *Nat Rev Neurosci*, *18*(9), 547-558. https://doi.org/10.1038/nrn.2017.74

Eichenbaum, H., Sauvage, M., Fortin, N., Komorowski, R., & Lipton, P. (2012). Towards a functional organization of episodic memory in the medial temporal lobe. *Neuroscience & Biobehavioral Reviews*, *36*(7), 1597-1608. https://doi.org/10.1016/j.neubiorev.2011.07.006

Eichenbaum, H., Yonelinas, A. P., & Ranganath, C. (2007). The medial temporal lobe and recognition memory. *Annu. Rev. Neurosci.*, *30*, 123-152. https://doi.org/10.1146/annurev.neuro.30.051606.094328

Ennaceur, A., Neave, N., & Aggleton, J. P. (1997). Spontaneous object recognition and object location memory in rats: the effects of lesions in the cingulate cortices, the medial prefrontal cortex, the cingulum bundle and the fornix. *Experimental brain research*, *113*(3), 509-519. https://doi.org/10.1007/pl00005603

Estela, V. J. (2020). *Neural correlates of early- and late-stage learning in the hippocampus and postrhinal cortex* [Neural correlates of early- and late-stage learning in the hippocampus and postrhinal cortex, Brown University]. Providence, RI.

Farovik, A., Place, R. J., McKenzie, S., Porter, B., Munro, C. E., & Eichenbaum, H. (2015). Orbitofrontal cortex encodes memories within value-based schemas and represents contexts that guide memory retrieval. *Journal of Neuroscience*, *35*(21), 8333-8344. https://doi.org/10.1523/JNEUROSCI.0134-15.2015

Feierstein, C. E., Quirk, M. C., Uchida, N., Sosulski, D. L., & Mainen, Z. F. (2006). Representation of spatial goals in rat orbitofrontal cortex. *Neuron*, *51*(4), 495-507. https://doi.org/10.1016/j.neuron.2006.06.032

Fraser, K. M., & Holland, P. C. (2019). Occasion Setting. In (Vol. 133, pp. 145-175).

Furtak, S. C., Ahmed, O. J., & Burwell, R. D. (2012). Single neuron activity and theta modulation in postrhinal cortex during visual object discrimination. *Neuron*, *76*(5), 976-988. https://doi.org/10.1016/j.neuron.2012.10.039

Gilmartin, M. R., & Helmstetter, F. J. (2010). Trace and contextual fear conditioning require neural activity and NMDA receptor-dependent transmission in the medial prefrontal cortex. *Learning & Memory*, *17*(6), 289-296. https://doi.org/10.1101/lm.1597410

Gilmartin, M. R., Kwapis, J. L., & Helmstetter, F. J. (2013). NR2A-and NR2B-containing NMDA receptors in the prelimbic medial prefrontal cortex differentially mediate trace, delay, and contextual fear conditioning. *Learning & Memory*, *20*(6), 290-294. https://doi.org/10.1101/lm.030510.113

Hallock, H. L., Arreola, A. C., Shaw, C. L., & Griffin, A. L. (2013). Dissociable roles of the dorsal striatum and dorsal hippocampus in conditional discrimination and spatial alternation T-maze tasks. *Neurobiol Learn Mem*, *100*, 108-116. https://doi.org/10.1016/j.nlm.2012.12.009

Hallock, H. L., & Griffin, A. L. (2013). Dynamic coding of dorsal hippocampal neurons between tasks that differ in structure and memory demand. *Hippocampus*, *23*(2), 169-186. https://doi.org/10.1002/hipo.22079

Hannesson, D., Vacca, G., Howland, J., & Phillips, A. (2004). Medial prefrontal cortex is involved in spatial temporal order memory but not spatial recognition memory in tests relying on spontaneous exploration in rats. *Behavioural brain research*, *153*(1), 273-285. https://doi.org/10.1016/j.bbr.2003.12.004

Heimer-McGinn, V. R., Poeta, D. L., Aghi, K., Udawatta, M., & Burwell, R. D. (2017). Disconnection of the perirhinal and postrhinal cortices impairs recognition of objects in context but not contextual fear conditioning. *Journal of Neuroscience*, *37*(18), 4819-4829. https://doi.org/10.1523/JNEUROSCI.0254-17.2017

Hernandez, A. R., Reasor, J. E., Truckenbrod, L. M., Lubke, K. N., Johnson, S. A., Bizon, J. L., Maurer, A. P., & Burke, S. N. (2017). Medial prefrontal-perirhinal cortical communication is necessary for flexible response selection. *Neurobiology of learning and memory*, *137*, 36-47. https://doi.org/10.1016/j.nlm.2016.10.012

Ho, J. W., & Burwell, R. D. (2014). Perirhinal and postrhinal functional inputs to the hippocampus. In *Space, time and memory in the hippocampal formation* (pp. 55-81). Springer. https://doi.org/https://doi.org/10.1007/978-3-7091-1292-2_3

Hok, V., Lenck-Santini, P.-P., Roux, S., Save, E., Muller, R. U., & Poucet, B. (2007). Goal-related activity in hippocampal place cells. *Journal of Neuroscience*, *27*(3), 472-482. https://doi.org/10.1523/JNEUROSCI.2864-06.2007

Hoover, W. B., & Vertes, R. P. (2007). Anatomical analysis of afferent projections to the medial prefrontal cortex in the rat. *Brain Structure and Function*, *212*(2), 149-179. https://doi.org/10.1007/s00429-007-0150-4

Hwang, E., Willis, B. S., & Burwell, R. D. (2018). Prefrontal connections of the perirhinal and postrhinal cortices in the rat. *Behavioural brain research*, *354*, 8-21. https://doi.org/10.1016/j.bbr.2017.07.032

Hyman, J. M., Ma, L., Balaguer-Ballester, E., Durstewitz, D., & Seamans, J. K. (2012). Contextual encoding by ensembles of medial prefrontal cortex neurons. *Proceedings of the National Academy of Sciences*, *109*(13), 5086-5091. https://doi.org/10.1073/pnas.1114415109

Iordanova, M. D., Burnett, D. J., Aggleton, J. P., Good, M., & Honey, R. C. (2009). The role of the hippocampus in mnemonic integration and retrieval: complementary evidence from lesion and inactivation studies. *European Journal of Neuroscience*.

Jay, T. M., & Witter, M. P. (1991). Distribution of hippocampal CA1 and subicular efferents in the prefrontal cortex of the rat studied by means of anterograde transport of Phaseolus vulgaris-leucoagglutinin. *Journal of Comparative Neurology*, *313*(4), 574-586. https://doi.org/10.1002/cne.903130404

Ji, J., & Maren, S. (2007). Hippocampal involvement in contextual modulation of fear extinction. *Hippocampus*, *17*(9), 749-758. https://doi.org/10.1002/hipo.20331

Jo, Y. S., & Lee, I. (2010). Perirhinal cortex is necessary for acquiring, but not for retrieving object–place paired association. *Learning & Memory*, *17*(2), 97-103. https://doi.org/10.1101/lm.1620410

Kent, B. A., & Brown, T. H. (2012). Dual functions of perirhinal cortex in fear conditioning. *Hippocampus*, *22*(10), 2068-2079. https://doi.org/10.1002/hipo.22058

Kerr, K. M., Agster, K. L., Furtak, S. C., & Burwell, R. D. (2007). Functional neuroanatomy of the parahippocampal region: the lateral and medial entorhinal areas. *Hippocampus*, *17*(9), 697-708. https://doi.org/10.1002/hipo.20315

Kholodar-Smith, D., Allen, T., & Brown, T. (2008). Fear conditioning to discontinuous auditory cues requires perirhinal cortical function. *Behavioral neuroscience*, *122*(5), 1178. https://doi.org/10.1037/a0012902

Kim, E. J., Kim, N., Kim, H. T., & Choi, J.-S. (2013). The prelimbic cortex is critical for context-dependent fear expression. *Frontiers in behavioral neuroscience*, *7*, 73. https://doi.org/10.3389/fnbeh.2013.00073

Komorowski, R. W., Manns, J. R., & Eichenbaum, H. (2009). Robust conjunctive item-place coding by hippocampal neurons parallels learning what happens where. *J Neurosci*, *29*(31), 9918-9929. https://doi.org/10.1523/JNEUROSCI.1378-09.2009

Kondo, H., & Witter, M. P. (2014). Topographic organization of orbitofrontal projections to the parahippocampal region in rats. *Journal of Comparative Neurology*, *522*(4), 772-793. https://doi.org/10.1002/cne.23442

Kreher, M. A., Johnson, S. A., Mizell, J. M., Chetram, D. K., Guenther, D. T., Lovett, S. D., Setlow, B., Bizon, J. L., Burke, S. N., & Maurer, A. P. (2019). The perirhinal cortex supports spatial intertemporal choice stability. *Neurobiol Learn Mem*, *162*, 36-46. https://doi.org/10.1016/j.nlm.2019.05.002

Lasseter, H. C., Ramirez, D. R., Xie, X., & Fuchs, R. A. (2009). Involvement of the lateral orbitofrontal cortex in drug context-induced reinstatement of cocaine-seeking behavior in rats. *European Journal of Neuroscience*, *30*(7), 1370-1381. https://doi.org/10.1111/j.1460-9568.2009.06906.x

Lasseter, H. C., Wells, A. M., Xie, X., & Fuchs, R. A. (2011). Interaction of the basolateral amygdala and orbitofrontal cortex is critical for drug context-induced reinstatement of cocaine-seeking behavior in rats. *Neuropsychopharmacology*, *36*(3), 711-720. https://doi.org/10.1038/npp.2010.209

Lee, I., & Solivan, F. (2008). The roles of the medial prefrontal cortex and hippocampus in a spatial paired-association task. *Learning & Memory*, *15*(5), 357-367. https://doi.org/10.1101/lm.902708

Lindquist, D. H., Jarrard, L. E., & Brown, T. H. (2004). Perirhinal cortex supports delay fear conditioning to rat ultrasonic social signals. *Journal of Neuroscience*, *24*(14), 3610-3617. https://doi.org/10.1523/JNEUROSCI.4839-03.2004

Liu, D., Deng, J., Zhang, Z., Zhang, Z. Y., Sun, Y. G., Yang, T., & Yao, H. (2020). Orbitofrontal control of visual cortex gain promotes visual associative learning. *Nat Commun*, *11*(1), 2784. https://doi.org/10.1038/s41467-020-16609-7

Logue, S. F., & Gould, T. J. (2014). The neural and genetic basis of executive function: attention, cognitive flexibility, and response inhibition. *Pharmacology Biochemistry and Behavior*, *123*, 45-54. https://doi.org/10.1016/j.pbb.2013.08.007

Maren, S., Aharonov, G., & Fanselow, M. S. (1997). Neurotoxic lesions of the dorsal hippocampus and Pavlovian fear conditioning in rats. *Behavioural brain research*, *88*(2), 261-274. https://doi.org/10.1016/s0166-4328(97)00088-0

McKenna, J. T., & Vertes, R. P. (2004). Afferent projections to nucleus reuniens of the thalamus. *J Comp Neurol*, *480*(2), 115-142. https://doi.org/10.1002/cne.20342

Murphy, M. J. M., & Deutch, A. Y. (2018). Organization of afferents to the orbitofrontal cortex in the rat. *J Comp Neurol*, *526*(9), 1498-1526. https://doi.org/10.1002/cne.24424

Navawongse, R., & Eichenbaum, H. (2013). Distinct pathways for rule-based retrieval and spatial mapping of memory representations in hippocampal neurons. *Journal of Neuroscience*, *33*(3), 1002-1013. https://doi.org/10.1523/JNEUROSCI.3891-12.2013

Norman, G., & Eacott, M. (2004). Impaired object recognition with increasing levels of feature ambiguity in rats with perirhinal cortex lesions. *Behavioural brain research*, *148*(1-2), 79-91. https://doi.org/10.1016/s0166-4328(03)00176-1

Norman, G., & Eacott, M. (2005). Dissociable effects of lesions to the perirhinal cortex and the postrhinal cortex on memory for context and objects in rats. *Behavioral neuroscience*, *119*(2), 557. https://doi.org/10.1037/0735-7044.119.2.557

Olson, J. M., Li, J. K., Montgomery, S. E., & Nitz, D. A. (2020). Secondary motor cortex transforms spatial information into planned action during navigation. *Current Biology*, *30*(10), 1845-1854. e1844. https://doi.org/10.1016/j.cub.2020.03.016

Orsini, C. A., Kim, J. H., Knapska, E., & Maren, S. (2011). Hippocampal and prefrontal projections to the basal amygdala mediate contextual regulation of fear after extinction. *J Neurosci*, *31*(47), 17269-17277. https://doi.org/10.1523/JNEUROSCI.4095-11.2011

Place, R., Farovik, A., Brockmann, M., & Eichenbaum, H. (2016). Bidirectional prefrontal-hippocampal interactions support context-guided memory. *Nature neuroscience*, *19*(8), 992. https://doi.org/10.1038/nn.4327

Potter, N. M., Calub, C. A., & Furtak, S. C. (2020). Perirhinal cortex inactivation produces retrieval deficits in fear extinction to a discontinuous visual stimulus. *Behavioral neuroscience*, *134*(2), 144. https://doi.org/10.1037/bne0000351

Qi, X., Du, Z. J., Zhu, L., Liu, X., Xu, H., Zhou, Z., Zhong, C., Li, S., Wang, L., & Zhang, Z. (2019). The Glutamatergic Postrhinal Cortex–Ventrolateral Orbitofrontal Cortex Pathway Regulates Spatial Memory Retrieval. *Neuroscience bulletin*, *35*(3), 447-460. https://doi.org/10.1007/s12264-018-0325-4

Ramesh, R. N., Burgess, C. R., Sugden, A. U., Gyetvan, M., & Andermann, M. L. (2018). Intermingled ensembles in visual association cortex encode stimulus identity or predicted outcome. *Neuron*, *100*(4), 900-915. e909. https://doi.org/10.1016/j.neuron.2018.09.024

Reep, R., Corwin, J., Hashimoto, A., & Watson, R. (1987). Efferent connections of the rostral portion of medial agranular cortex in rats. *Brain research bulletin*, *19*(2), 203-221. https://doi.org/10.1016/0361-9230(87)90086-4

Reep, R., Goodwin, G., & Corwin, J. (1990). Topographic organization in the corticocortical connections of medial agranular cortex in rats. *Journal of Comparative Neurology*, *294*(2), 262-280. https://doi.org/10.1002/cne.902940210

Reep, R. L., Corwin, J. V., Hashimoto, A., & Watson, R. T. (1984). Afferent connections of medial precentral cortex in the rat. *Neuroscience letters*, *44*(3), 247-252. https://doi.org/10.1016/0304-3940(84)90030-2

Rescorla, R. A., & Wagner, A. (1972). A theory of Pavlovian conditioning : Variations in the effectiveness of reinforcement and nonreinforcement. *Classical conditioning II: Current research and theory*, 64-99.

Robinson, S., Poorman, C. E., Marder, T. J., & Bucci, D. J. (2012). Identification of functional circuitry between retrosplenial and postrhinal cortices during fear conditioning. *Journal of Neuroscience*, *32*(35), 12076-12086. https://doi.org/10.1523/JNEUROSCI.2814-12.2012

Santos, T. B., Kramer-Soares, J. C., Favaro, V. M., & Oliveira, M. G. M. (2017). Involvement of the prelimbic cortex in contextual fear conditioning with temporal and spatial discontinuity. *Neurobiology of learning and memory*, *144*, 1-10. https://doi.org/10.1016/j.nlm.2017.05.003

Scharfman, H. E., Witter, M. P., & Schwarcz, R. E. (2000). *The parahippocampal region: Implications for neurological and psychiatric diseases*. New York Academy of Sciences.

Sharpe, M., & Killcross, S. (2015). The prelimbic cortex uses contextual cues to modulate responding towards predictive stimuli during fear renewal. *Neurobiology of learning and memory*, *118*, 20-29. https://doi.org/10.1016/j.nlm.2014.11.005

Sharpe, M. J., Stalnaker, T., Schuck, N. W., Killcross, S., Schoenbaum, G., & Niv, Y. (2019). An integrated model of action selection: distinct modes of cortical control of striatal decision making. *Annual review of psychology*, *70*, 53-76. https://doi.org/10.1146/annurev-psych-010418-102824

Siniscalchi, M. J., Wang, H., & Kwan, A. C. (2019). Enhanced population coding for rewarded choices in the medial frontal cortex of the mouse. *Cerebral Cortex*, *29*(10), 4090-4106. https://doi.org/10.1093/cercor/bhy292

Sugden, A. U., Zaremba, J. D., Sugden, L. A., McGuire, K. L., Lutas, A., Ramesh, R. N., Alturkistani, O., Lensjø, K. K., Burgess, C. R., & Andermann, M. L. (2020). Cortical reactivations of recent sensory experiences predict bidirectional network changes during learning. *Nature neuroscience*, *23*(8), 981-991. https://doi.org/10.1038/s41593-020-0651-5

Sul, J. H., Jo, S., Lee, D., & Jung, M. W. (2011). Role of rodent secondary motor cortex in value-based action selection. *Nature neuroscience*, *14*(9), 1202. https://doi.org/10.1038/nn.2881

Suter, E. E., Weiss, C., & Disterhoft, J. F. (2013). Perirhinal and postrhinal, but not lateral entorhinal, cortices are essential for acquisition of trace eyeblink conditioning. *Learning & Memory*, *20*(2), 80-84. https://doi.org/10.1101/lm.028894.112

Suzuki, W. A., & Naya, Y. (2014). The perirhinal cortex. *Annual review of neuroscience*, *37*, 39-53. https://doi.org/10.1146/annurev-neuro-071013-014207

Tam, S. K., Bonardi, C., & Robinson, J. (2015). Relative recency influences object-in-context memory. *Behav Brain Res*, *281*, 250-257. https://doi.org/10.1016/j.bbr.2014.12.024

Tomás Pereira, I., Agster, K. L., & Burwell, R. D. (2016). Subcortical connections of the perirhinal, postrhinal, and entorhinal cortices of the rat. I. afferents. *Hippocampus*, *26*(9), 1189-1212. https://doi.org/10.1002/hipo.22603

Trask, S., Shipman, M. L., Green, J. T., & Bouton, M. E. (2017). Inactivation of the prelimbic cortex attenuates context-dependent operant responding. *Journal of Neuroscience*, *37*(9), 2317-2324. https://doi.org/10.1523/JNEUROSCI.3361-16.2017

Trask, S., Thrailkill, E. A., & Bouton, M. E. (2017). Occasion setting, inhibition, and the contextual control of extinction in Pavlovian and instrumental (operant) learning. *Behavioural processes*, *137*, 64-72. https://doi.org/10.1016/j.beproc.2016.10.003

Urban, K. R., Layfield, D. M., & Griffin, A. L. (2014). Transient inactivation of the medial prefrontal cortex impairs performance on a working memory-dependent conditional discrimination task. *Behav Neurosci*, *128*(6), 639-643. https://doi.org/10.1037/bne0000020

Vertes, R. P., Hoover, W. B., Do Valle, A. C., Sherman, A., & Rodriguez, J. J. (2006). Efferent projections of reuniens and rhomboid nuclei of the thalamus in the rat. *J Comp Neurol*, *499*(5), 768-796. https://doi.org/10.1002/cne.21135

Vertes, R. P., Hoover, W. B., Szigeti-Buck, K., & Leranth, C. (2007). Nucleus reuniens of the midline thalamus: link between the medial prefrontal cortex and the hippocampus. *Brain research bulletin*, *71*(6), 601-609. https://doi.org/10.1016/j.brainresbull.2006.12.002

Wagner, A., & Rescorla, R. (1972). Inhibition in Pavlovian conditioning: Application of a theory. *Inhibition and learning*, 301-336.

Wang, Q., Jin, J., & Maren, S. (2016). Renewal of extinguished fear activates ventral hippocampal neurons projecting to the prelimbic and infralimbic cortices in rats. *Neurobiology of learning and memory*, *134*, 38-43. https://doi.org/10.1016/j.nlm.2016.04.002

Warburton, E., & Brown, M. W. (2015). Neural circuitry for rat recognition memory. *Behavioural brain research*, *285*, 131-139. https://doi.org/10.1016/j.bbr.2014.09.050

Warburton, E. C., & Brown, M. W. (2015). Neural circuitry for rat recognition memory. *Behav Brain Res*, *285*, 131-139. https://doi.org/10.1016/j.bbr.2014.09.050

Wikenheiser, A. M., & Schoenbaum, G. (2016). Over the river, through the woods: cognitive maps in the hippocampus and orbitofrontal cortex. *Nature Reviews Neuroscience*, *17*(8), 513-523. https://doi.org/10.1038/nrn.2016.56

Wilson, R. C., Takahashi, Y. K., Schoenbaum, G., & Niv, Y. (2014). Orbitofrontal cortex as a cognitive map of task space. *Neuron*, *81*(2), 267-279. https://doi.org/10.1016/j.neuron.2013.11.005

Winocur, G., Sekeres, M. J., Binns, M. A., & Moscovitch, M. (2013). Hippocampal lesions produce both nongraded and temporally graded retrograde amnesia in the same rat. *Hippocampus*, *23*(5), 330-341. https://doi.org/10.1002/hipo.22093

Winters, B. D., & Bussey, T. J. (2005). Transient inactivation of perirhinal cortex disrupts encoding, retrieval, and consolidation of object recognition memory. *Journal of Neuroscience*, *25*(1), 52-61. https://doi.org/10.1523/JNEUROSCI.3827-04.2005

Xiang, J.-Z., & Brown, M. (1998). Differential neuronal encoding of novelty, familiarity and recency in regions of the anterior temporal lobe. *Neuropharmacology*, *37*(4-5), 657-676. https://doi.org/10.1016/s0028-3908(98)00030-6

Zelikowsky, M., Bissiere, S., Hast, T. A., Bennett, R. Z., Abdipranoto, A., Vissel, B., & Fanselow, M. S. (2013). Prefrontal microcircuit underlies contextual learning after hippocampal loss. *Proceedings of the National Academy of Sciences*, *110*(24), 9938-9943. https://doi.org/10.1073/pnas.1301691110

Zelikowsky, M., Hersman, S., Chawla, M. K., Barnes, C. A., & Fanselow, M. S. (2014). Neuronal ensembles in amygdala, hippocampus, and prefrontal cortex track differential components of contextual fear. *Journal of Neuroscience*, *34*(25), 8462-8466. https://doi.org/10.1523/JNEUROSCI.3624-13.2014

CHAPTER 2

THE ROLES OF THE PERIRHINAL AND POSTRHINAL CORTICES IN THE RETRIEVAL OF LATENTLY-ACQUIRED PRECONDITIONING ASSOCIATIONS

Abstract

The perirhinal and postrhinal cortices are important structures in the parahippocampal region of the medial temporal lobe. They receive and process sensory information from cortical and subcortical sources, and support hippocampal functions via direct connectivity and indirectly via the entorhinal cortex. Previous studies using the sensory preconditioning paradigm have shown that the perirhinal and postrhinal cortices are necessary for associating cues during preconditioning and retrieving the associations during conditioning. However, the question of whether these regions are also required for retrieval when subjects are re-exposed to the preconditioning cue had not been addressed. Using a chemogenetic approach, we temporarily suppressed the perirhinal or postrhinal cortex in rats during the test phases. Both groups showed significantly reduced preferential responding compared to sham surgery controls, suggesting impaired sensory preconditioning. This finding expands our knowledge of the roles these parahippocampal cortices play in integrating latently acquired associations into goal-directed higher-order conditioning.

Introduction

The medial temporal lobe plays pivotal roles in a range of memory functions underpinning human and animal behavior. In the rodent medial temporal lobe, the perirhinal cortex (PER) and postrhinal cortex (POR, homologous to the parahippocampal cortex in primates) are critically involved in various forms of learning and memory (Burwell et al., 1995; Estela-Pro & Burwell, 2021; Peng & Burwell, 2021). Both regions receive polymodal and unimodal associational input from other cortical and subcortical structures, with the PER receiving information from all sensory modalities and the POR receiving mostly visuospatial information (Burwell & Amaral, 1998a; Furtak et al., 2007; Tomas Pereira et al., 2016). The PER and POR project to different divisions of the entorhinal cortex, through which information eventually reaches the hippocampus. The PER and POR also project directly to hippocampal CA1 and the subiculum and receive direct feedback from CA1 (Agster & Burwell, 2013). In recognition memory, the PER is considered part of the pathway that specializes in processing information about "what", whereas the POR belongs to the pathway that processes information about "where". Information from these two segregated streams is thought to be bound together by the hippocampus (Diana et al., 2007; Eichenbaum et al., 2012; Eichenbaum et al., 2007; Yonelinas et al., 2022). It should be noted, though, anatomical, behavioral, and electrophysiological evidence indicates significant cross-talk between the "what" and "where" streams. Importantly, there are robust, reciprocal connections between the PER and POR, upstream from the hippocampus (Burwell & Amaral, 1998b; Estela, 2020; Furtak et al., 2012; Heimer-McGinn et al., 2017). In addition to involvement in

recognition memory, both areas also play key roles in context-dependent behavior in rodents (Peng & Burwell, 2021).

Sensory preconditioning is a form of second-order conditioning that occurs when multiple associations formed through separate experiences are integrated to support behavior (Brogden, 1939). A typical sensory preconditioning procedure includes three phases. In the preconditioning phase, subjects receive paired presentation of two neutral stimuli. Next, in the conditioning phase, subjects undergo first-order conditioning between one of the neutral stimuli and an unconditioned stimulus (US); this previously neutral stimulus hence becomes a conditioned stimulus (CS). Finally, in the test phase, the other neutral stimulus (the preconditioning stimulus, PCS) is presented to the subject. Successful sensory preconditioning occurs when the PCS elicits a response from the subject, despite having never been directly paired with the US. The ability of the PCS to generate responses develops via its association with the CS during preconditioning, in the absence of an explicit US.

Two types of cognitive mechanisms are thought to support sensory preconditioning. The PCS may become directly associated with the US via "representation-mediated learning" (Holland, 1981), when the CS evokes a representation of the PCS during conditioning and this representation is associated with the US. Alternatively, the PCS may elicit responding indirectly via an "associative chain" composed of the PCS-CS association formed during preconditioning and the CS-US association formed during conditioning

(Rizley & Rescorla, 1972). Multiple factors seem to influence the mechanism on which a sensory preconditioning procedure primarily relies (Holmes et al., 2022), though we are not aware of any study that has systematically manipulated these factors and evaluated their influence. Moreover, it is unclear whether different neural substrates are engaged by these two mechanisms. Both the PER (Holmes et al., 2013; Nicholson & Freeman Jr, 2000; Wong et al., 2019) and the POR (Taylor-Yeremeeva et al., 2021) are shown to be critically involved in sensory preconditioning. In these studies, however, the experimental procedures likely favored representation-mediated learning over associative chaining as the primary underlying mechanism. Therefore, whether the contributions of the PER and POR to sensory preconditioning extend to associative chaining is an open question.

In the current study, we utilized a sensory preconditioning procedure previously shown to rely on associative chaining at the time of PCS testing (Jones et al., 2012) and investigated the involvement of the PER and POR. Since we reasoned that PER and POR contribution to sensory preconditioning underpins their broader roles in latent learning (i.e. learning in the absence of explicit reward or punishment), we hypothesized that they should be required for retrieving neutral associations for chaining as well. Therefore, reduced responding to the PCS was predicted when either the PER or POR was temporarily unavailable during the PCS test. We employed the Designer-Receptor-Exclusively-Activated-by-Designer-Drugs (DREADDs) technique to create temporary suppression by systematic injection of clozapine-*N*-oxide prior to behavioral sessions (Smith et al., 2016; Urban & Roth, 2015).

Methods

Subjects

The subjects were 34 adult male Long-Evans rats (*Rattus norvegicus*) acquired from Charles River Laboratories (Wilmington, MA). Upon arrival at our facility, they were pair-housed in ventilated cages, given *ad libitum* access to food and water, and placed on a reverse 12-hour light/dark cycle. Starting one week before surgeries, they were food restricted to 85-90% free-feed body weight until the end of the experiments. All subjects were around 3-4 months old at the time of surgeries, weighing between 300g to 400g. Subjects were handled by multiple lab personnel before surgery and during post-surgical recovery. All procedures were conducted according to NIH guidelines and protocols approved by the Brown University Institutional Animal Care and Use Committee.

Surgery

The timeline for the procedures is shown in Figure 2.1 (Panel A). Subjects were randomly assigned to the PER group, POR group, or Sham group before surgery. Pre-operative preparation included injections of glycopyrrolate, diazepam, and buprenorphine, and the subjects' heads were shaved to expose the surgery site. Subjects were anesthetized with isoflurane and secured with ear bars in a stereotaxis apparatus. The bregma and lambda were arranged in the same horizontal plane (+/- 0.1 mm) by adjusting the incisor bar. Craniotomies over the injection coordinates were made using a

dental drill, and the dura was pierced prior to pipette insertion. For rats in the PER and POR group, bilateral injections of the DREADD virus AAV8-CaMKIIa-hM4d(Gi)-mCherry were made using a Hamilton syringe pump at 0.1 µl/min for 5 minutes per site (Table 2). The pipette tip remained at the injection coordinate for an additional 10 minutes before slowly being retracted. Rats in the Sham group received craniotomies only. Afterwards, gel foam soaked in sterile saline was placed over the cortex, and the incision was sutured. All subjects were given at least 2 weeks to recover from the surgery before behavioral procedures began.

Site	AP (mm)	ML (mm)	DV (mm)	Angle
PER1	3.2	+/- 5.0	6.3	13°
PER2	4.4	+/- 5.0	6.2	13°
PER3	5.6	+/- 5.0	5.9	13°
PER4	6.8	+/- 5.0	5.5	13°
POR1	-0.5*	+/- 5.0	4.5	16°
POR2	0.2*	+/- 5.0	2.75	16°

Table 2 Stereotaxic coordinates for bilateral DREADD virus injections. In the anterior-posterior axis (AP), positive values indicate locations posterior to the reference point, and negative values indicate locations anterior to the reference. The bregma was used as reference for PER sites, whereas the lambda was the reference for POR sites (marked by *). Dorsal-ventral (DV) values indicate distances from the tip of the pipette to the top surface of the skull.

Apparatus

Behavioral procedures were performed in conditioning chambers (Med Associates) placed inside sound attenuating cabinets. The chambers were made with two aluminum side panels, three Plexiglas panels as front, back, and the ceiling. A food port located on

one of the side panels was used to deliver pellets and detect the frequency and duration of nose pokes via infrared sensors. A speaker and a mechanical relay were located on the same side panel near the roof to produce the auditory stimuli used in the behavioral experiments. Four auditory stimuli (around 75 dB) were used for sensory preconditioning. The white noise and click (0.5 Hz) were used as PCS+ and PCS-, whereas a tone (2.5 kHz) and a siren (alternating between 1 kHz and 4 kHz) were used as CS+ and CS-. The stimulus arrangement was counter-balanced across subjects. The floor was made of stainless steel rods attached to a shock generator and scrambler that were used to deliver foot shocks for fear conditioning. Video cameras mounted behind the back Plexiglas panel captured the fear conditioning sessions and saved the videos to a hard drive. Additional cues added to the chamber for the fear conditioning experiment to differentiate the new contexts from the base context included laminated sheets with distinctive visual patterns and artificial odors such as vanilla and almond.

Behavioral procedures

Sensory preconditioning. The general procedure was based upon prior studies from Schoenbaum and colleagues (Hart et al., 2020; Jones et al., 2012; Sadacca et al., 2018). Subjects first underwent a magazine training session, in which a total of 20 pellets were delivered via a variable-interval schedule. For this and all following sessions, the inter-trial intervals were chosen pseudo-randomly from a list of values ranging from 3 to 6 minutes. The sensory preconditioning procedure contains 4 phases (Figure 2.2, panel A). First, subjects were placed individually into conditioning chambers for two daily sessions of 12 preconditioning trials each. Each trial contained a serial presentation of one of two

pairs of auditory stimuli. No reward was delivered. Next in the conditioning phase, subjects received 6 sessions of Pavlovian conditioning, each with 12 trials. During the CS+ trials, 3 food pellets total were delivered into the food port at 5, 7, 9 seconds after CS+ onset. No pellet was delivered during CS-. The third phase was the PCS testing phase. Around 30 minutes prior to testing, all subjects received an intraperitoneal injection of clozapine n-oxide (CNO, 5mg per 1kg of body weight) and were returned to the home cage. The first 6 trials were CS+ and CS- reminder trials identical to trials in the conditioning phase. Then came 12 PCS probe trials arranged in four 3-trial blocks, in which PCS+ and PCS- were presented without rewards. Finally, 48 hours after PCS

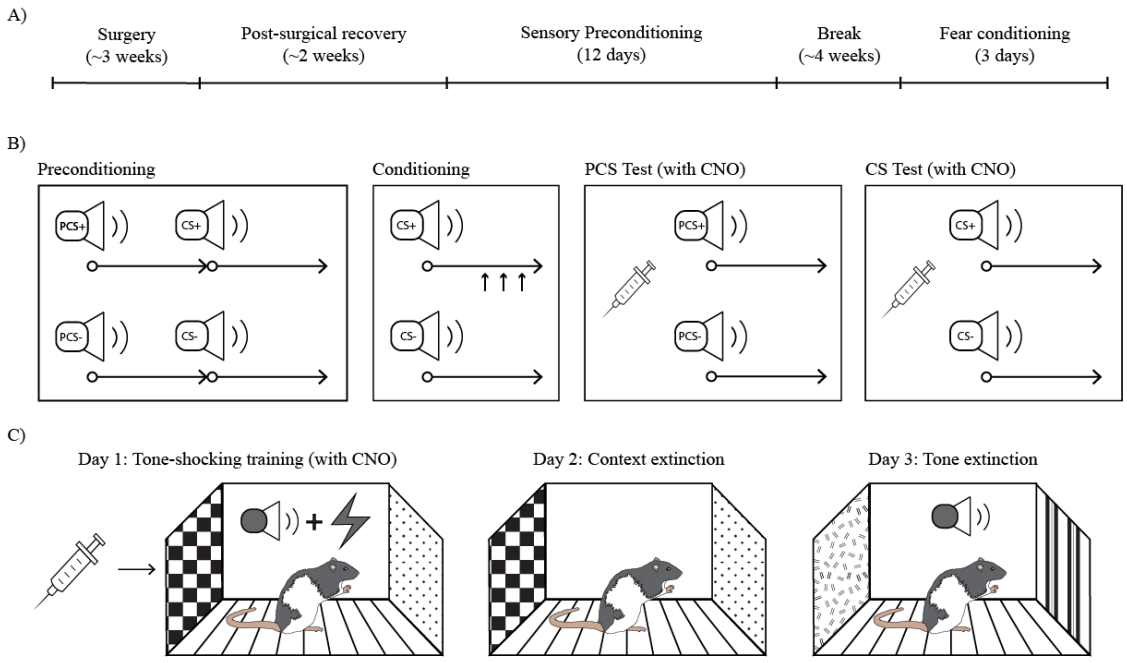

Fig. 2.1. Schematics for experimental timeline and behavioral procedures. A) Overall timeline for the experiment, from the start of the surgeries until the end of behavioral testing. B) The Pavlovian sensory preconditioning procedure based on studies such as Jones et al. (2012) combined with CNO injections, indicated by the syringe symbol. Pellets were delivered only during the last 5 s of CS+ trials, indicated by upward arrows. C) A standard one-session fear conditioning procedure, followed with context extinction and tone extinction. CNO injections were only given on the first day.

testing, a 12-trial CS probe session was conducted, also around 30 minutes after intraperitoneal CNO injections. In each trial, subjects were presented with CS+ or CS without reward delivery. All trial types within each session were counter-balanced.

Fear conditioning. At least 30 days after the CS test session, a fear conditioning procedure was conducted to behaviorally verify the efficacy of DREADDs inhibition (Figure 2.2, panel B). Though the same apparatus was used, each subject was assigned a conditioning chamber different from the one where they underwent sensory preconditioning. Multiple visual, tactile, and olfactory cues were also introduced into the chambers to distinguish them from previous sensory preconditioning contexts. On day 1 (shock training with CNO), subjects received signaled fear conditioning 30 minutes after CNO injections. Subjects were placed into context A. After 3 minutes, 3 tones (10s, 2 kHz, 75dB) were delivered, each of which co-terminated with a foot shock (1s, 1.0 mA). The inter-stimulus-interval was 1 minute. Subjects remained in the chamber for an additional minute after the last tone-shock pairing before being returned to their home cages. On day 2 (context extinction without CNO), subjects were again placed into context A for 9 minutes without tone or shock delivery. On day 3 (tone extinction without CNO), subjects were put into context B. After 2 minutes, they received a continuous tone presentation lasting for 7 minutes. The specific configurations of context A and B were counter-balanced among subjects. All sessions were recorded with Ethovision 15 and saved to a computer hard drive for analysis.

Behavioral and statistical analysis

Sensory preconditioning. Custom MED-PC scripts were written to control the stimuli and generate output containing the timestamps of all nose pokes into the food port. The output file was then analyzed by a custom-written Python script to compute the latency and total duration of nose pokes during periods of interest. For preconditioning, the nose poke time percentage differences were calculated between PCS+ and PCS-, and between CS+ and CS-. A positive difference score indicated a higher nose poke time percentage during PCS+ versus PCS-, or CS+ versus CS-. Separate one-way ANOVAs were conducted to detect whether any initial preference existed before conditioning started. For conditioning trials in the conditioning phase and CS reminder trials in PCS test, pellets were delivered during the second half (5 s) of CS+, therefore conditioned responding was assessed by comparing the nose poke time percentages during the first half of CS+ and CS-. For PCS and CS test trials, nose poke time percentages during the second half of the stimuli were compared. The difference scores were computed. First, a mixed-design ANOVA was conducted on difference scores from the conditioning sessions, with GROUP (Sham, PER, POR) as the between-subject factor and SESSION (1 to 6) as the within-subject factor. This test examined whether any group difference occurred during first-order Pavlovian conditioning, even though no CNO injection was made prior to the conditioning sessions. Second, a one-way ANOVA with GROUP being the between-subject factor was conducted on difference scores from PCS trials in the PCS test session under inactivation. Further, one-sample t tests were conducted to compare each group's PCS difference score to 0. The Bonferroni-Holm procedure was applied to control for familywise type I error rate (Abdi, 2010). These tests provided additional evidence on

whether sensory preconditioning was successful in the Sham group, and whether suppression of the PER or POR led to deficits. The same analysis was conducted for CS test trials, also under DREADD suppression. These tests evaluated whether first-order conditioning was successful in the Sham group, and whether suppression of the PER or POR led to deficits. Finally, response latencies during PCS+ and CS+ trials were tested for group differences with one-way ANOVA to determine whether attentional deficits contributed to group differences in nose poke time percentage.

Fear conditioning. The sessions were broken into 1-minute blocks, then a motion analysis software custom-written in Python was used to measure the total duration of freezing within each minute. Briefly, adjacent video frames were contrasted and processed to detect changes in pixel intensity that indicated movement from the subject. The subject was determined to be freezing when the period without movement exceeded a pre-set threshold. This software was validated with human observation in prior fear conditioning experiments (data not included). Expression of contextual fear was evaluated by analyzing freezing behavior on day 2 (context extinction). A mixed-design ANOVA was conducted with BLOCK as the within-subject factor and GROUP as the between-subject factor. Post hoc two-sided Dunnett's t tests were used to compare each of the PER and POR group to the Sham group. Tone-elicited fear behavior on day 3 (tone extinction) was examined using the same tests.

Histology

After the last fear conditioning session, the subjects were deeply anesthetized with isoflurane and received an overdose of Beuthanasia-D (100mg/kg, i.p.). They were then perfused with phosphate-buffered saline, followed by a 10% formalin solution. The brains were post-fixed for at least 48 hours in formalin before being transferred to a 30% sucrose solution for at least another 48 hours. The brains were sectioned on a microtome into three series of 40μm sections. For each brain, one series was later stained with thionine, and another was stained with DAPI for fluorescence microscopy. Fluorescent images were taken in blue and red channels showing DAPI-stained neurons and neurons transfected by the DREADD virus, respectively. For histological analysis, each brain was scored based on expressions in the rostral and caudal halves of the target area and the degree of extra-target spread.

Results

Histology

For the eight of eleven rats in the PER group, viral transduction was observed bilaterally, and along the rostrocaudal extent of the target region (Figure 2.2, temporary). For two cases, there was moderate involvement in the ventral temporal association cortex and caudal hippocampus in both hemispheres. For three cases, there was not sufficient transduction. These five cases were removed from the study. For the nine of ten rats in the POR group, viral transduction was observed bilaterally, and along the rostrocaudal

extent of the region (Figure histology). For three cases, there was minimal involvement in caudal PER and caudal entorhinal cortex. In two cases, there was no observed transduction in the POR, and these two cases were removed from the study. In some cases there was involvement of the temporal association cortex (TEv) dorsal to the POR, but this involvement was unilateral and these cases were retained in the study.

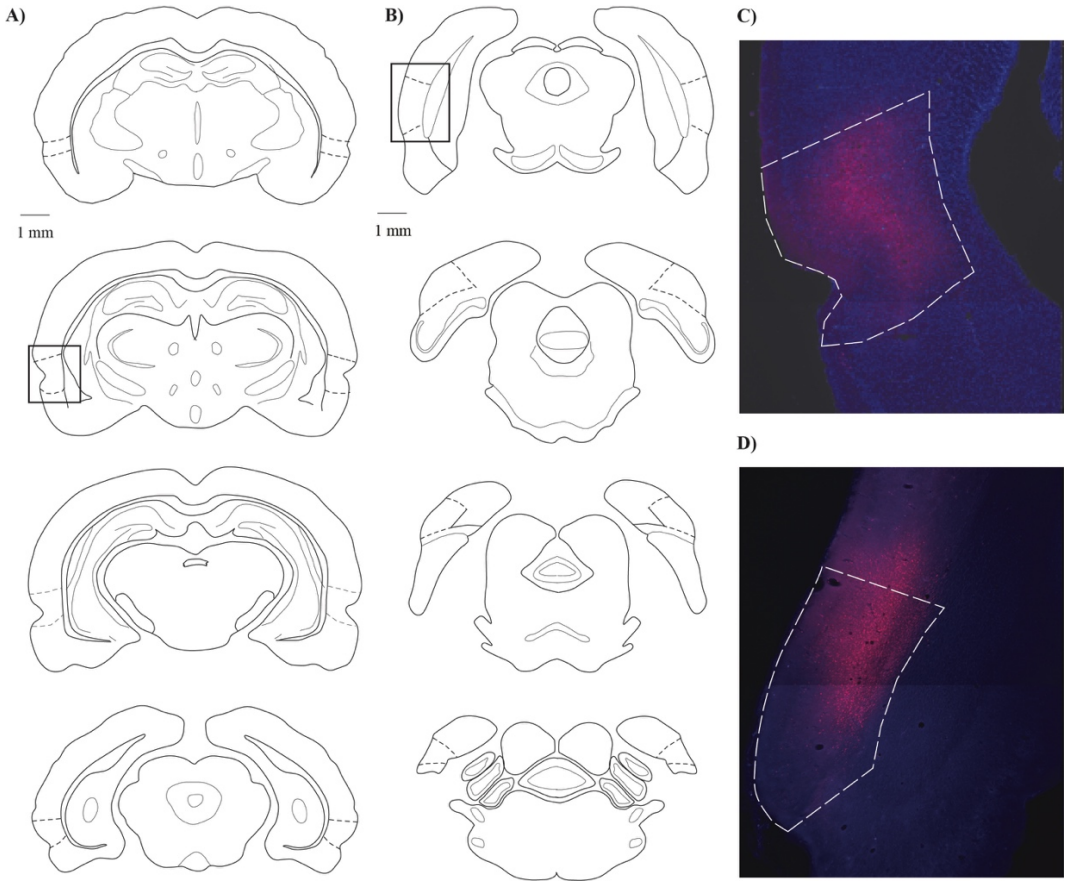

Figure 2.2. Schematics of PER and POR showing target locations along with representative labeling of viral transduction. A) Four levels of the PER with the target regions illustrated by dashed lines. Labeling was distributed across the rostrocaudal extent (see text for details). B) Four levels of the POR with the target regions illustrated by dashed line. Again, labeling was distributed across the rostrocaudal extent (see text for details). C) Example of labeling in PER. See inset in A for location. D). Example of labeling in POR. See inset in B for location. DAPI staining is captured in the blue channel, and the DREADD virus is captured in the red channel.

Preconditioning and conditioning

Behavioral data from four subjects were excluded from this and all following analysis: data for three were removed due to equipment malfunction, and one was removed due to adverse reactions to the CNO injection prior to PCS test. A mixed-design ANOVA on nose poke time percentage differences during preconditioning sessions confirmed that the groups did not differ in their initial preference for PCS+ versus PCS-, or CS+ versus CS-. Results did not reveal significant DIFFERENCE*GROUP interaction ($p = 0.792$), or main effects for DIFFERENCE ($p = 0.625$) or GROUP ($p = 0.99$). Then, a mixed-design ANOVA on nose poke time percentage differences for the 6 conditioning sessions (Fig. 2.3) showed a significant main effect for SESSION, $F_{(5, 100)} = 4.61$, $p = 0.001$, but not for SESSION*GROUP interaction ($p = 0.247$) or GROUP ($p = 0.849$). This suggested that all groups significantly increased nose poke time during the first 5s of CS+, compared to CS-, and the changes were not significantly different among groups. Therefore, as expected, no impairment was found in any group for first-order conditioning.

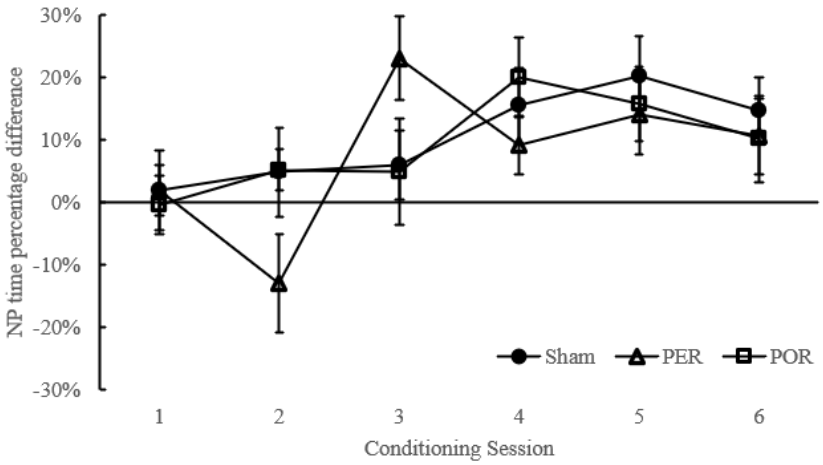

Figure 2.3. Nose poke time percentage difference during the conditioning phase. Data was only analyzed for the first half (5 s) of CS+ and CS- because pellets were delivered during the second half of CS+. Error bars indicate standard error.

PCS test

The PCS test was conducted 30 minutes after the subjects received a CNO injection. The test started with 6 reminder trials that were identical to conditioning trials in that pellets were delivered during the last 5s of the CS+. The main measure for the sensory preconditioning effect was nose poke time the percentages during PCS+ and PCS- (Fig. 2.4A). A one-way ANOVA was conducted to compare the nose poke time percentage difference between the last 5s of PCS+ versus PCS- (Fig. 2.4B). The result indicated significant differences existed among groups, $F_{(2,20)} = 3.82$, $p = 0.039$. Planned comparisons revealed a marginally significant difference when the Sham group is compared to the PER group, $t_{(20)} = 2.02$, $p = 0.057$, and a significant difference between the Sham and the POR group, $t_{(20)} = 2.53$, $p = 0.020$. Further, one-sample t tests showed that only the sham group had a significantly positive nose poke time percentage

difference, $t_{(9)} = 3.71$, $p = 0.005$. Neither the PER, $t_{(5)} = 0.97$, $p = 0.378$, nor POR group $t_{(6)} = 0.001$, $p = 0.999$, was significantly different from 0. Thus, after CNO injections, the sham group had significantly higher responding to PCS+ than PCS- during the last 5 s, whereas the PER and POR groups did not. This result provided evidence for an impairment in retrieving the PCS-CS association when either the PER or POR is under temporary DREADDs suppression.

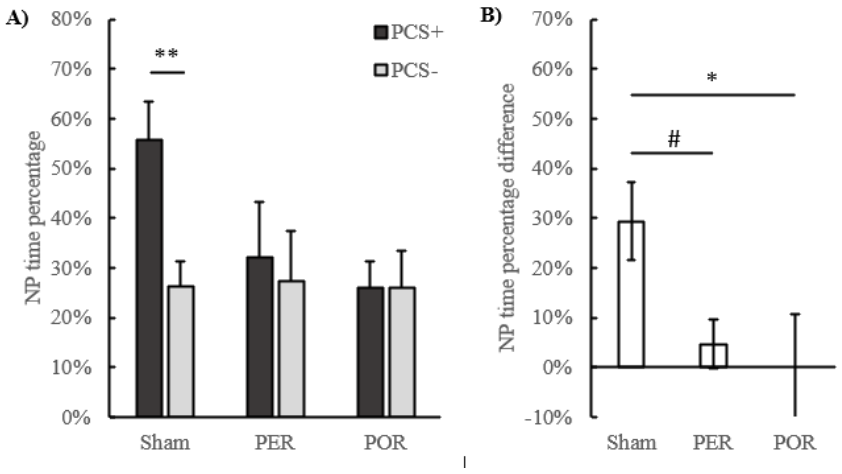

Figure 2.4. Nose poke time percentage during the last 5s of PCS trials in the PCS test phase with DREADD suppression. A) nose poke time percentages during PCS+ and PCS-. P-value symbols indicate significant differences between stimuli. B) nose poke time percentage difference between responding during PCS+ and PCS-. Positive values indicate higher responding during PCS+. P-value symbols indicate significant differences between groups. #: $p < 0.10$, *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$. Error bars indicate standard error.

CS test

A CS test session was conducted 48 hours after the PCS test, also following CNO injections (Fig. 2.5). Nose poke time during the last 5s of the CS+ and CS- were analyzed (Fig. 2.5A). The differences between CS+ and CS- were calculated and analyzed with

one-way ANOVA (Fig. 2.5B). The omnibus ANOVA was not significant, $F_{(2,20)} = 2.39$, $p = 0.117$. Planned comparisons revealed no significant difference between the Sham and PER group, $t_{(20)} = 1.66$, $p = 0.112$), and but a marginally significant difference between the Sham and POR group, $t_{(20)} = 2.01$, $p = 0.064$. One-sample t tests showed that all groups had significantly positive difference scores during the CS test: sham, $t_{(9)} = 7.96$, $p < 0.001$; PER, $t_{(5)} = 3.40$, $p = 0.019$; POR, $t_{(6)} = 6.79$, $p < 0.001$. Overall, all groups demonstrated significantly longer nose poke time percentage during the CS+, compared with the CS-, indicating successful first-order conditioning.

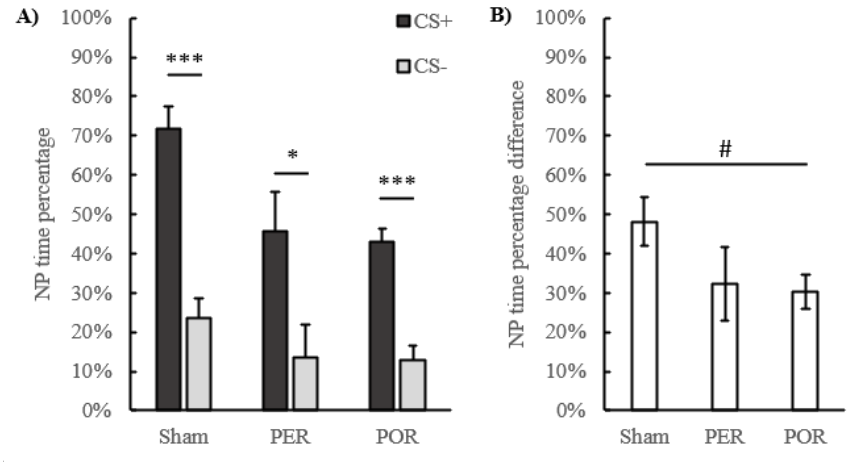

Figure 2.5. Nose poke time percentages during the last 5s of CS trials in the CS test with DREADD suppression. A) nose poke time percentages during CS+ and CS-. Asterisks indicate significant one-sample t-tests on the difference between CS+ and CS-. B) nose poke time percentage differences between CS+ and CS-. Error bars indicate standard error.

PCS and CS response latency

To confirm that DREADD suppression did not lead to attentional deficits in the PER or the POR group, nose poke latencies after stimulus onset during the PCS+ and CS+ test trials were analyzed separately with one-way ANOVA (Figure 3.6). The omnibus

ANOVA was not significant for CS+ trials (p = 0.308) or PCS+ trials (p = 0. 379), and none of the planned comparisons between the Sham and virus groups was significant. Therefore, deficits observed in nose poke time percentages can't be explained by delayed reaction in response to stimulus onset.

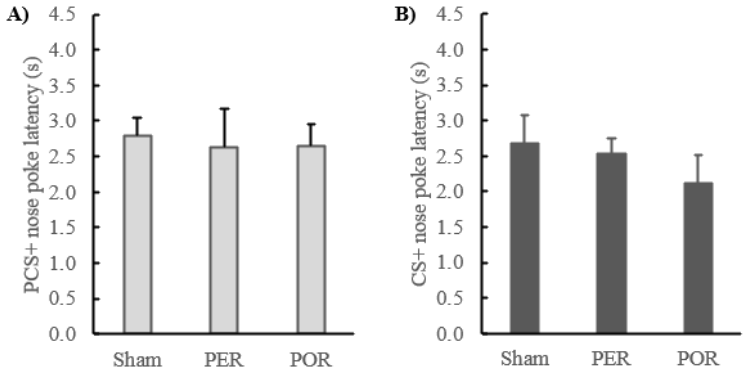

Figure 2.6. Latencies of nose poking in the test sessions under suppression. A) nose poke latencies since the onset of PCS+ during test trials. B) nose poke latencies since the onset of CS+ during test trials. Error bars indicate standard error.

Fear conditioning

In a fear conditioning paradigm, subjects received an injection of CNO (i.p.) on day 1, followed by tone-shock conditioning in context A. On day 2, they received extinction in context A without the tone. And on day 3, they underwent tone extinction in context B. No CNO injections were made on day 2 or 3. For freezing time on day 2 (Fig. 2.6A), a mixed-design ANOVA did not reveal a significant MINUTE*GROUP interaction, $F_{(16, 160)} = 0.968$, $p = 0.494$. However, there was a significant main effect of GROUP, $F_{(2, 20)} = 6.87$, $p = 0.005$, as well as a main effect of MINUTE, $F_{(4.90, 160)} = 2.47$, $p = 0.039$. Further simple contrast tests revealed a significant difference between the Sham and the PER

group, $p = 0.002$, but not between the Sham and the POR group, $p = 0.79$. Therefore, when re-exposed to the shock context, the PER group displayed a significantly lower amount of freezing when compared to the sham group, indicative of impaired contextual fear memory. No reduction in freezing was observed in the POR group, indicating normal contextual fear memory. Since DREADDs suppression was induced only during tone-shock training (day 1) but not on day 2, this suggests that the acquisition of contextual fear was impaired in the PER group, but not in the POR group, when compared to the Sham group.

Freezing to the tone on day 3 was also analyzed by a mixed-design ANOVA to detect the presence of tone-elicited fear (Fig. 2.6B). A significant main effect of MINUTE was revealed, $F_{(3.74, 74.81)} = 11.76$, $p < 0.001$, but no significant MINUTE *GROUP interaction, $F_{(7.48, 74.81)} = 1.34$, $p = 0.203$, or main effect of GROUP, $F_{(2, 20)} = 1.77$, $p = 0.196$. Simple contrast tests revealed a marginally significant difference between the Sham and the PER group ($p = 0.82$). Further tests revealed a significant linear trend for MINUTE, $F_{(1,20)} = 36.60$, $p < 0.001$. The results indicated that all three groups gradually displayed less freezing to the tone, with no significant group difference. Therefore, fear conditioning to the tone was not impaired by PER or POR suppression during conditioning.

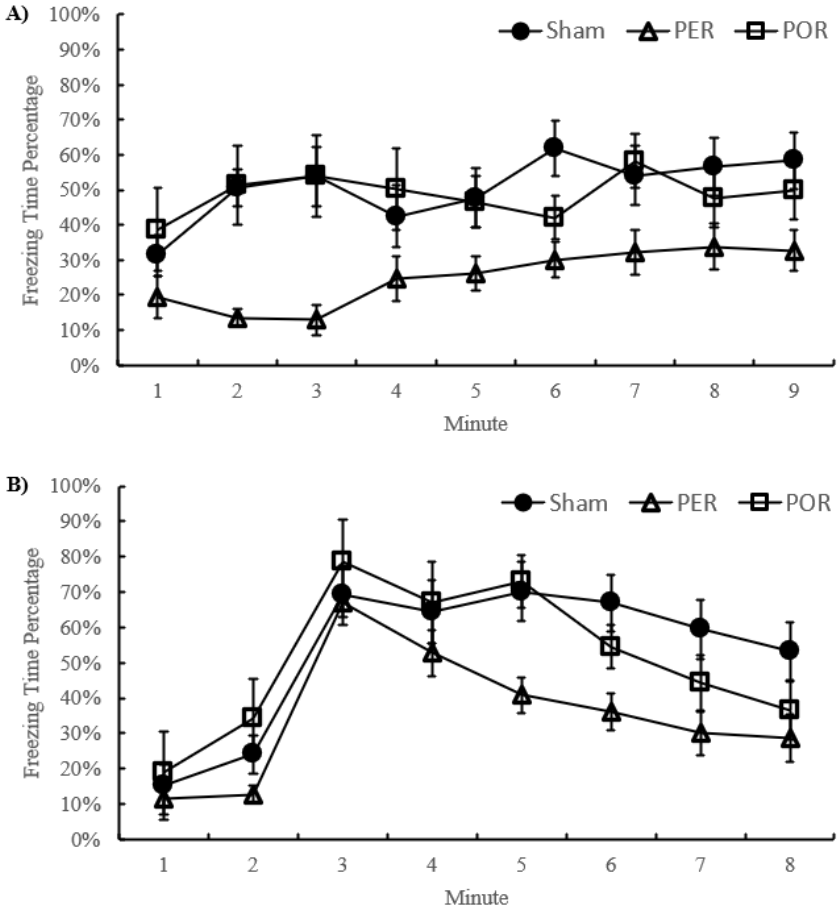

Figure 2.7. Freezing time percentage in each minute during context extinction (day 2, panel A) and tone extinction (day 3, panel B). The tone was presented from the start of the third minute during tone extinction. Error bars indicate standard error.

Discussion

In the current study, chemogenetically suppressing the bilateral PER or POR during the PCS test disrupted the retrieval of PCS-US associations formed when both stimuli were neutral and led to impaired sensory preconditioning. Rats in the Sham group had

significantly higher nose poke time percentage during PCS+ than during PCS-. In contrast, no preference existed in the PER or POR group after CNO administration. First-order conditioning was not affected in any group under the influence of CNO, as indicated by higher responding to CS+ than to CS- during the CS test. The overall results suggested that although neither the PER nor POR is necessary for conditioned responding to a directed conditioned stimulus, both are necessary for retrieving neutral S-S associations when re-exposed to the PCS. These findings appear partially inconsistent with existing studies with temporary PER or POR suppression. However, we argue that this is because the sensory preconditioning effect in our study relied on associative chaining rather than representation-mediated learning, as did procedures in previous studies. Implications of our findings are discussed in relation to the significance of the PER and POR in context processing and episodic-like memory in the animal models.

Whereas existing studies collectively showed that the PER and POR are necessary for sensory preconditioning if it is (at least partially) supported by representation-mediated learning, our finding suggests they are crucial for associative chaining as well. Our sensory preconditioning procedure was slightly modified from a previous study in which sensory preconditioning deficit occurred when temporarily suppressing the orbitofrontal cortex during the PCS test (Jones et al., 2012). This pattern of deficit typically indicates it is likely associative chaining that supports responding to the preconditioning cue (Holmes et al., 2022; Jones et al., 2012). In contrast, previous studies on the PER or POR employed procedures that likely relied on another mechanism, representation-mediated learning (Holland, 1981). A recent study investigated the role of the POR in multiple

forms of sensory preconditioning and found reduced responding to the PCS if chemogenetic suppression took place during preconditioning, or conditioning, but not if occurred during testing (Taylor-Yeremeeva et al., 2021). In Wong et al. (2019), reduced sensory preconditioning was observed when a sodium channel blocker was injected into the PER cortex before the conditioning phase or before the test phase (albeit of a smaller degree). The authors concluded that memory integration in their procedure occurred primarily via mediated learning during conditioning, requiring active participation of the PER; however, it wasn't ruled out that some associative chaining also took place at the time of testing. Collectively, these studies and ours suggest the PER and POR are necessary for retrieving neutral S-S associations in sensory preconditioning, regardless of whether these associations are used for mediated learning or associative chaining. Moreover, both of them were shown to be necessary for encoding association between neutral stimuli during preconditioning (Holmes et al., 2013; Taylor-Yeremeeva et al., 2021). Therefore, the overall evidence points to the central role of the PER and POR in sensory preconditioning in both encoding and retrieving the associations formed when the stimuli involved are neutral.

The ability to associate neutral stimuli in the absence of explicit reward or punishment fits well with the well-known roles of the PER and POR in automatically representing the spatial context and using contextual cues to guide spontaneous or learned behavior (Peng & Burwell, 2021). At the most basic level, the context exists as a loose collection of environmental elements that contain multi-sensory information without meaningful connections. For example, the aluminum, plastic side panels and the background noise

generated by the fan, though all being part of a conditioning chamber, are not inherently associated with each other. However, with the contribution from the medial temporal lobe structures such as the PER and POR, associations are formed between the elements to generate a cohesive representation of the context. Importantly, the context representation is automatically established even when the elements do not have inherent biological significance or learning history. For example, neurons in the POR show increased spiking when the rat encounters a particular object at a particular location, but not when encountering the same object at other locations or other objects at the same location (Furtak et al., 2007). These "object-location" cells likely emerge as object information from the PER is combined with spatial information from POR via direct interconnectivity (Agster & Burwell, 2013; Heimer-McGinn et al., 2017). Indeed, the PER and POR are necessary in a variation of sensory preconditioning in which the preconditioning phase requires establishing an association between a neutral cue and the context (Iordanova et al., 2009; Taylor-Yeremeeva et al., 2021).

Context learning and S-S learning in sensory preconditioning both involve forming associations. However, one important difference does exist between the two. In typical context learning paradigms, the context is usually composed of static stimuli that remain constant throughout a session. In contrast, associations formed in preconditioning and later retrieved are among transient stimuli with clear onsets and offsets. Therefore, the critical involvement of the PER and POR in sensory preconditioning suggests their contributions extend beyond the well-known ability to automatically learn about the local context; they may also be capable of representing transient cues inside the context. In

other words, they are not only able to represent stimuli existing in the three-dimensional *space*, but also stimuli in the dimension of *time*. This is consistent with significant advances regarding how events and sequences are represented in the medial temporal lobe, such as the entorhinal cortex (Robinson et al., 2017) and the hippocampus (Allen et al., 2016; MacDonald et al., 2013; Pastalkova et al., 2008). The PER is necessary for bridging the temporal gap between segments of a discontinuous auditory stimulus (Bang & Brown, 2009; Kholodar-Smith, Allen, et al., 2008) and between a CS and US in trace fear conditioning (Kholodar-Smith, Boguszewski, et al., 2008). It is likely supported by persistent firing PER neurons that can maintain activation for seconds to several minutes (Navaroli et al., 2012). The PER is also important for incidental learning of odor sequences (Allen et al., 2020; Jayachandran et al., 2019). It may be speculated that the POR cortex also plays a role in processing temporal events if there is a significant spatial component associated with them, in which case it may cooperate with the PER cortex via their functional connectivity (Heimer-McGinn et al., 2017).

Combining the evidence that both areas are necessary for incidental learning of stimulus associations together with their well-established roles in representing context, we propose that the rodent PER and POR may collectively construct and maintain neural representations of cue sequences and contexts that serve as rudimentary forms of event representation. These representations contain salient elements of an event, such as the occurrence of transient cues, their associations and temporal relationships, and the context. In other words, these "event elements" in the PER and POR cortex constitutes the "gist", but not the perceptual aspects of an event (Moscovitch et al., 2016; Robin &

Moscovitch, 2017). These representations may be transmitted to the hippocampus as the "backbone" for episodic memory (Agster & Burwell, 2013; Furtak et al., 2007). Alternatively, they may be made available to other areas for behaviors not depending on the hippocampus, such as expressing remote fear memory (Burwell et al., 2004), or when hippocampal functioning is disrupted (Fanselow, 2010; Wiltgen et al., 2006). This proposal does not detract from the key role of the hippocampus in episodic memory (Eichenbaum, 2017; Eichenbaum et al., 2012; Moscovitch et al., 2016; Robin & Moscovitch, 2017). Instead, it fits into the idea that two types of episode memory representations dynamically co-exist in the brain, with one being "schematic" or "gist-like" that mostly resides in the neocortex, and the other one being detail-rich and hippocampal-dependent (Moscovitch et al., 2016; Robin & Moscovitch, 2017). The PER and POR have connections with the hippocampus and various prefrontal areas (Agster & Burwell, 2013; Furtak et al., 2007; Hwang et al., 2018), therefore they potentially serve as one of the regions where the two types of episodic representation overlap or interact. Other cortical areas such as the orbitofrontal cortex and the retrosplenial cortex may be involved as well (Hart et al., 2020; Jones et al., 2012; Robinson et al., 2014; Todd et al., 2019).

Results from the contextual fear conditioning with DREADD inactivation during training were partially inconsistent with existing literature. Prior studies found that normal contextual fear learning required both the PER and POR (Bucci et al., 2000; Bucci et al., 2002). However, in the current study the PER group indeed displayed less freezing during the context extinction test, whereas the POR group had similar levels of context-

elicited freezing compared to the Sham group. We argue this discrepancy regarding effects of POR inactivation was because the fear conditioning contexts were not made sufficiently distinctive from the sensory preconditioning context. Even though we did introduce various visual and olfactory cues to the conditioning chambers, certain environmental features remained constant for sensory preconditioning and fear conditioning, such as the grid floor, the geometry of the chamber, the clear plexiglass panels etc. Consequently, unlike in typical fear conditioning procedures, where subjects experience electric shocks in a novel context, our subjects were already partially familiar with the fear conditioning context. Therefore, the ability to associate a familiar context with shocks may be spared even without contribution from the POR, while still requiring participation from the PER. Alternatively, it was possible that the shocks were associated with only the novel elements of the context, but not the familiar ones, due to latent inhibition (Lubow & Moore, 1959; Lubow & Weiner, 2010). In this case, POR contribution might not be critical to support freezing, due to over-generalization of the fear memory from newly introduced environmental cues.

In conclusion, we utilized the DREADD technique to show that both the PER and POR are necessary for retrieving neutral S-S associations in a sensory preconditioning procedure that likely depends on the chaining of associations at PCS testing. Combined with their well-established roles in context processing, these findings raise the possibility that the PER and POR cortex collectively support a rudimentary, gist-like representation of the elements needed for episodic-like memory in rodents. Although latent learning paradigms such as sensory preconditioning do not capture the full spectrum of episodic-

like memory in animals (Clayton et al., 2003), they nevertheless provide us with a powerful tool to investigate the neural mechanisms underlying different aspects of episodic-like memory.

References

Agster, K. L., & Burwell, R. D. (2013). Hippocampal and subicular efferents and afferents of the perirhinal, postrhinal, and entorhinal cortices of the rat. *Behavioural brain research*, *254*, 50-64.

Allen, L. M., Lesyshyn, R. A., O'Dell, S. J., Allen, T. A., & Fortin, N. J. (2020). The hippocampus, prefrontal cortex, and perirhinal cortex are critical to incidental order memory. *Behav Brain Res*, *379*, 112215. https://doi.org/10.1016/j.bbr.2019.112215

Allen, T. A., Salz, D. M., McKenzie, S., & Fortin, N. J. (2016). Nonspatial sequence coding in CA1 neurons. *Journal of Neuroscience*, *36*(5), 1547-1563.

Bang, S. J., & Brown, T. H. (2009). Perirhinal cortex supports acquired fear of auditory objects. *Neurobiol Learn Mem*, *92*(1), 53-62. https://doi.org/10.1016/j.nlm.2009.01.002

Brogden, W. J. (1939). Sensory pre-conditioning. *Journal of Experimental Psychology*, *25*(4), 323-332. https://doi.org/10.1037/h0058944

Bucci, D. J., Phillips, R. G., & Burwell, R. D. (2000). Contributions of postrhinal and perirhinal cortex to contextual information processing. *Behav Neurosci*, *114*(5), 882-894. https://doi.org/10.1037//0735-7044.114.5.882

Bucci, D. J., Saddoris, M. P., & Burwell, R. D. (2002). Contextual fear discrimination is impaired by damage to the postrhinal or perirhinal cortex. *Behavioral Neuroscience*, *116*(3), 479-488. https://doi.org/10.1037/0735-7044.116.3.479

Burwell, R. D., & Amaral, D. G. (1998a). Cortical afferents of the perirhinal, postrhinal, and entorhinal cortices of the rat. *Journal of comparative neurology*, *398*(2), 179-205.

Burwell, R. D., & Amaral, D. G. (1998b). Perirhinal and postrhinal cortices of the rat: interconnectivity and connections with the entorhinal cortex. *Journal of comparative neurology*, *391*(3), 293-321.

Burwell, R. D., Bucci, D. J., Sanborn, M. R., & Jutras, M. J. (2004). Perirhinal and postrhinal contributions to remote memory for context. In (Vol. 24, pp. 11023-11028).

Burwell, R. D., Witter, M. P., & Amaral, D. G. (1995). Perirhinal and postrhinal cortices of the rat: a review of the neuroanatomical literature and comparison with findings from the monkey brain. *Hippocampus*, *5*(5), 390-408.

Clayton, N. S., Bussey, T. J., & Dickinson, A. (2003). Can animals recall the past and plan for the future? *Nature Reviews Neuroscience*, *4*(8), 685-691.

Diana, R. A., Yonelinas, A. P., & Ranganath, C. (2007). Imaging recollection and familiarity in the medial temporal lobe: a three-component model. *Trends in cognitive sciences*, *11*(9), 379-386.

Eichenbaum, H. (2017). Prefrontal-hippocampal interactions in episodic memory. *Nat Rev Neurosci*, *18*(9), 547-558. https://doi.org/10.1038/nrn.2017.74

Eichenbaum, H., Sauvage, M., Fortin, N., Komorowski, R., & Lipton, P. (2012). Towards a functional organization of episodic memory in the medial temporal lobe. *Neurosci Biobehav Rev*, *36*(7), 1597-1608. https://doi.org/10.1016/j.neubiorev.2011.07.006

Eichenbaum, H., Yonelinas, A. P., & Ranganath, C. (2007). The medial temporal lobe and recognition memory. *Annu Rev Neurosci*, *30*, 123-152. https://doi.org/10.1146/annurev.neuro.30.051606.094328

Estela-Pro, V. J., & Burwell, R. D. (2021). The anatomy and function of the postrhinal cortex. *Behavioral Neuroscience*.

Estela, V. J. (2020). *Neural correlates of early- and late-stage learning in the hippocampus and postrhinal cortex* [Neural correlates of early- and late-stage learning in the hippocampus and postrhinal cortex, Brown University]. Providence, RI.

Fanselow, M. S. (2010). From contextual fear to a dynamic view of memory systems. *Trends in cognitive sciences*, *14*(1), 7-15.

Furtak, S. C., Ahmed, O. J., & Burwell, R. D. (2012). Single neuron activity and theta modulation in postrhinal cortex during visual object discrimination. *Neuron*, *76*(5), 976-988. https://doi.org/10.1016/j.neuron.2012.10.039

Furtak, S. C., Wei, S. M., Agster, K. L., & Burwell, R. D. (2007). Functional neuroanatomy of the parahippocampal region in the rat: the perirhinal and postrhinal cortices. *Hippocampus*, *17*(9), 709-722. https://doi.org/10.1002/hipo.20314

Hart, E. E., Sharpe, M. J., Gardner, M. P., & Schoenbaum, G. (2020). Responding to preconditioned cues is devaluation sensitive and requires orbitofrontal cortex during cue-cue learning. *Elife*, *9*. https://doi.org/10.7554/eLife.59998

Heimer-McGinn, V. R., Poeta, D. L., Aghi, K., Udawatta, M., & Burwell, R. D. (2017). Disconnection of the perirhinal and postrhinal cortices impairs recognition of objects in context but not contextual fear conditioning. *Journal of Neuroscience*, *37*(18), 4819-4829.

Holland, P. C. (1981). Acquisition of representation-mediated conditioned food aversions. *Learning and Motivation*, *12*(1), 1-18.

Holmes, N. M., Parkes, S. L., Killcross, A. S., & Westbrook, R. F. (2013). The basolateral amygdala is critical for learning about neutral stimuli in the presence of danger, and the perirhinal cortex is critical in the absence of danger. *J Neurosci*, *33*(32), 13112-13125. https://doi.org/10.1523/JNEUROSCI.1998-13.2013

Holmes, N. M., Wong, F. S., Bouchekioua, Y., & Westbrook, R. F. (2022). Not "either-or" but "which-when": A review of the evidence for integration in sensory preconditioning. *Neuroscience & Biobehavioral Reviews*.

Hwang, E., Willis, B. S., & Burwell, R. D. (2018). Prefrontal connections of the perirhinal and postrhinal cortices in the rat. *Behav Brain Res*, *354*, 8-21. https://doi.org/10.1016/j.bbr.2017.07.032

Iordanova, M. D., Burnett, D. J., Aggleton, J. P., Good, M., & Honey, R. C. (2009). The role of the hippocampus in mnemonic integration and retrieval: complementary evidence from lesion and inactivation studies. *Eur J Neurosci*, *30*(11), 2177-2189. https://doi.org/10.1111/j.1460-9568.2009.07010.x

Jayachandran, M., Linley, S. B., Schlecht, M., Mahler, S. V., Vertes, R. P., & Allen, T. A. (2019). Prefrontal Pathways Provide Top-Down Control of Memory for Sequences of Events. *Cell Rep*, *28*(3), 640-654 e646. https://doi.org/10.1016/j.celrep.2019.06.053

Jones, J. L., Esber, G. R., McDannald, M. A., Gruber, A. J., Hernandez, A., Mirenzi, A., & Schoenbaum, G. (2012). Orbitofrontal cortex supports behavior and learning using inferred but not cached values. *Science*, *338*(6109), 953-956. https://doi.org/10.1126/science.1227489

Kholodar-Smith, D. B., Allen, T. A., & Brown, T. H. (2008). Fear conditioning to discontinuous auditory cues requires perirhinal cortical function. *Behav Neurosci*, *122*(5), 1178-1185. https://doi.org/10.1037/a0012902

Kholodar-Smith, D. B., Boguszewski, P., & Brown, T. H. (2008). Auditory trace fear conditioning requires perirhinal cortex. *Neurobiol Learn Mem*, *90*(3), 537-543. https://doi.org/10.1016/j.nlm.2008.06.006

Lubow, R., & Moore, A. (1959). Latent inhibition: the effect of nonreinforced pre-exposure to the conditional stimulus. *Journal of comparative and physiological psychology*, *52*(4), 415.

Lubow, R., & Weiner, I. (2010). *Latent inhibition: Cognition, neuroscience and applications to schizophrenia*. Cambridge University Press.

MacDonald, C. J., Carrow, S., Place, R., & Eichenbaum, H. (2013). Distinct hippocampal time cell sequences represent odor memories in immobilized rats. *J Neurosci*, *33*(36), 14607-14616. https://doi.org/10.1523/JNEUROSCI.1537-13.2013

Moscovitch, M., Cabeza, R., Winocur, G., & Nadel, L. (2016). Episodic Memory and Beyond: The Hippocampus and Neocortex in Transformation. *Annual Review of Psychology*, *67*(1), 105-134. https://doi.org/10.1146/annurev-psych-113011-143733

Navaroli, V. L., Zhao, Y., Boguszewski, P., & Brown, T. H. (2012). Muscarinic receptor activation enables persistent firing in pyramidal neurons from superficial layers of dorsal perirhinal cortex. *Hippocampus*, *22*(6), 1392-1404.

Nicholson, D. A., & Freeman Jr, J. H. (2000). Lesions of the perirhinal cortex impair sensory preconditioning in rats. *Behavioural brain research*, *112*(1-2), 69-75.

Pastalkova, E., Itskov, V., Amarasingham, A., & Buzsaki, G. (2008). Internally generated cell assembly sequences in the rat hippocampus. *Science*, *321*(5894), 1322-1327.

Peng, X., & Burwell, R. D. (2021). Beyond the hippocampus: the role of parahippocampal-prefrontal communication in context-modulated behavior. *Neurobiology of Learning and Memory*, *185*, 107520.

Rizley, R. C., & Rescorla, R. A. (1972). Associations in second-order conditioning and sensory preconditioning. *Journal of comparative and physiological psychology*, *81*(1), 1.

Robin, J., & Moscovitch, M. (2017). Details, gist and schema: hippocampal–neocortical interactions underlying recent and remote episodic and spatial memory. *Current opinion in behavioral sciences*, *17*, 114-123.

Robinson, N. T. M., Priestley, J. B., Rueckemann, J. W., Garcia, A. D., Smeglin, V. A., Marino, F. A., & Eichenbaum, H. (2017). Medial Entorhinal Cortex Selectively Supports Temporal Coding by Hippocampal Neurons. *Neuron (Cambridge, Mass.)*, *94*(3), 677-688.e676. https://doi.org/10.1016/j.neuron.2017.04.003

Robinson, S., Todd, T. P., Pasternak, A. R., Luikart, B. W., Skelton, P. D., Urban, D. J., & Bucci, D. J. (2014). Chemogenetic silencing of neurons in retrosplenial cortex disrupts sensory preconditioning. *J Neurosci*, *34*(33), 10982-10988. https://doi.org/10.1523/JNEUROSCI.1349-14.2014

Smith, K. S., Bucci, D. J., Luikart, B. W., & Mahler, S. V. (2016). DREADDS: Use and application in behavioral neuroscience. *Behav Neurosci*, *130*(2), 137-155. https://doi.org/10.1037/bne0000135

Taylor-Yeremeeva, E. M., Wisser, S. C., Chakoma, T. L., Aldrich, S. J., Denney, A. E., Donahue, E. K., Adelman, J. S., Ihle, P. C., & Robinson, S. (2021). Appetitive and aversive sensory preconditioning in rats is impaired by disruption of the postrhinal cortex. *Neurobiology of Learning and Memory*, *183*, 107461.

Todd, T. P., Fournier, D. I., & Bucci, D. J. (2019). Retrosplenial cortex and its role in cue-specific learning and memory. *Neuroscience and Biobehavioral Reviews*, *107*, 713-728. https://doi.org/10.1016/j.neubiorev.2019.04.016

Tomas Pereira, I., Agster, K. L., & Burwell, R. D. (2016). Subcortical connections of the perirhinal, postrhinal, and entorhinal cortices of the rat. I. afferents. *Hippocampus*, *26*(9), 1189-1212. https://doi.org/10.1002/hipo.22603

Urban, D. J., & Roth, B. L. (2015). DREADDs (designer receptors exclusively activated by designer drugs): chemogenetic tools with therapeutic utility. *Annu Rev Pharmacol Toxicol*, *55*, 399-417. https://doi.org/10.1146/annurev-pharmtox-010814-124803

Wiltgen, B. J., Sanders, M. J., Anagnostaras, S. G., Sage, J. R., & Fanselow, M. S. (2006). Context fear learning in the absence of the hippocampus. *Journal of Neuroscience*, *26*(20), 5484-5491.

Wong, F. S., Westbrook, R. F., & Holmes, N. M. (2019). 'Online' integration of sensory and fear memories in the rat medial temporal lobe. *Elife*, *8*. https://doi.org/10.7554/eLife.47085

Yonelinas, A. P., Ramey, M. M., Riddell, C., Kahana, M., & Wagner, A. (2022). Recognition memory: The role of recollection and familiarity. In: Oxford University Press.

CHAPTER 3

THE CONTRIBUTIONS OF PERIRHINAL AND POSTRHINAL CONNECTIONS WITH ORBITOFRONTAL CORTEX IN SUPPORT OF GOAL-DIRECTED BEHAVIOR

Abstract

In the previous experiment, sensory preconditioning was impaired in rats with bilateral perirhinal (PER) or postrhinal (POR) suppression at the time of the PCS test, indicating that both areas are necessary for retrieving associations established between neutral stimuli during preconditioning. The orbitofrontal cortex (OFC) plays important roles in goal-directed behavior, for instance, by performing value inference based on associative models. OFC activity is also necessary during PCS testing. Therefore, a disconnection experiment was conducted to investigate whether the OFC interacts with the PER or POR during the test phase of the same sensory preconditioning procedure. Adult rats received unilateral injections of a DREADD virus into the lateral OFC and the ipsilateral PER or POR, or contralateral PER or POR; a group of rats received sham-surgeries to act as controls. When temporary suppression was induced by CNO injections before testing, impaired sensory preconditioning was observed in rats with contralateral or ipsilateral OFC-PER inactivation and rats with ipsilateral OFC-POR inaction. This pattern of results is partially consistent with those observed in other asymmetrical lesion or inactivation experiments; however, it does reveal the functional significance for inter-hemispheric pathways between the OFC and both the PER and POR. Overall, it was concluded that the PER and POR indeed interacts with the OFC, likely to support value inferencing based on associative models.

Introduction

The orbitofrontal cortex (OFC) plays important roles in goal-directed behavior, including sensory preconditioning (Hart et al., 2020; Jones et al., 2012), reinforcer devaluation (Pickens et al., 2003), and reversal learning (Schoenbaum et al., 2002). In recent years, Schoenbaum and colleagues proposed that, rather than representing outcome values, the core functions of the OFC may be constructing and updating a structured representation of associative relationships between cues and using it to guide reward-driven decision-making (Costa et al., 2023; Gardner & Schoenbaum, 2021; Wilson et al., 2014; Zhou et al., 2021). Moreover, these structured OFC representations share a number of features with so-called "cognitive map" in the hippocampus (Wikenheiser et al., 2021; Wikenheiser & Schoenbaum, 2016). The PER and POR are directly and reciprocally connected with the OFC and CA1 of the hippocampus (Agster & Burwell, 2013; Burwell & Amaral, 1998a; Hwang et al., 2018). Therefore, they are prime candidates for not only supporting cognitive maps in the OFC and the hippocampus but also for being part of a network in which the prefrontal region and medial temporal lobe cooperate in the service of flexible goal-directed behavior.

One of the paradigms for assessing goal-directed behavior is sensory preconditioning (Brogden, 1939). Briefly, sensory preconditioning is a form of higher-order conditioning that starts with un-reinforced pairing of two neutral stimuli, followed by first-order conditioning with one of the stimuli (CS). Successful sensory precondition occurs when the other neutral stimulus (PCS) elicits responding from the subject, even without direct

pairing with a US. Previous studies showed that the OFC is necessary for the initial encoding of PCS-CS associations (Hart et al., 2020; Sadacca et al., 2018) as well as for testing during PCS testing (Jones et al., 2012). Also, preferential responding to the PCS was reduced after aversion-induced devaluation, suggesting that the PCS acquired value based the underlying associative model involving the PCS-CS-US chain (Hart et al., 2020). In the previous chapter, we examined the effects of PER and POR suppression using a highly similar sensory preconditioning procedure, and results suggested that the PER and POR functions are also critical during the PCS test phase. These findings raised the possibility of the OFC interacting with the PER or POR during the PCS test phase to support value inference in sensory preconditioning.

We used the asymmetrical inactivation approach to test this possibility by creating cross-hemispheric disconnections between the OFC and either PER or POR with the DREADD virus (Figure 3.1). Previous studies have used this approach to demonstrate the functional significance of multiple pathways in cognition in rats (Barker et al., 2007; Heimer-McGinn et al., 2017; Lasseter et al., 2011) and monkeys (Eldridge et al., 2016; Oyama et al., 2022). It should be noted that this approach does not provide information regarding the directionality of the communication. Prior to the PCS test and CS test, all rats received intraperitoneal CNO injections. It was predicted that deficits in PCS responding would occur in rats with contralateral OFC-PER or contralateral OFC-POR inactivation. In contrast, sham-surgery rats and rats with ipsilateral OFC-PER or ipsilateral OFC-POR inhibition would display normal sensory preconditioning. No impairment was predicted in first-order conditioning in any group.

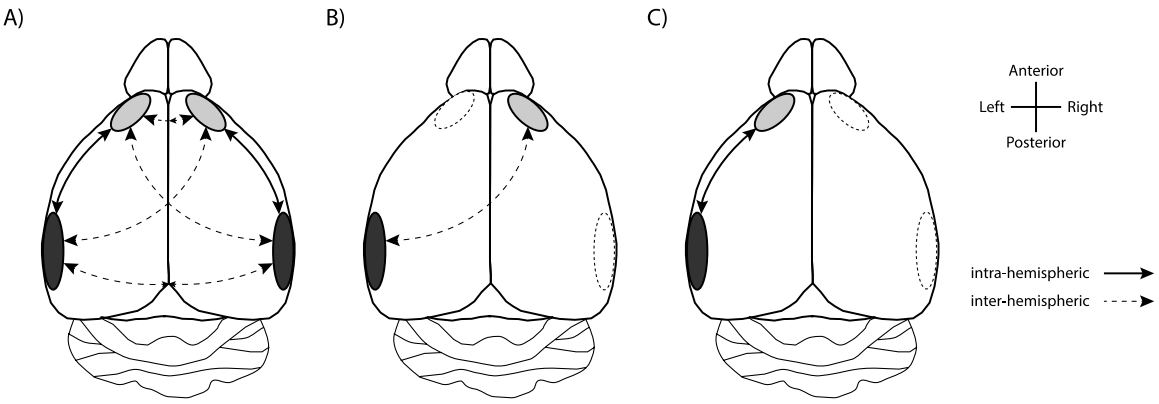

Figure 3.1. Top view schematic of a rat brain showing the orbitofrontal-parahippocampal connections. A. Ipsilateral (intra-hemispheric) corticocortical connections are reciprocal and robust. In comparison, contralateral connects are substantially weaker. B. Contralateral inactivation will effectively disconnect orbitofrontal and parahippocampal regions by disrupting the primary intra-hemispheric corticocortical connections. C. Ipsilateral inactivation leaves the most robust intra-hemispheric connections intact in one hemisphere and theoretically should spare functions not relying on bilateral intra-hemispheric interactions.

Methods

Thirty-nine adult male Long-Evans rats (*Rattus norvegicus*) were acquired from Charles River Laboratories (Wilmington, MA). The housing conditions were identical to the previous experiment. The timeline and surgical procedures were mostly identical to the previous experiment. Rats were randomly assigned to one of five groups. Except for the sham surgery group, four groups of rats received unilateral injections of an inhibitory DREADD virus (AAV8-CaMKIIa-hM4D-mCherry) into the lateral OFC as well as unilateral injections into the ipsilateral PER or POR, or contralateral PER or POR (Table 3). Two injection sites were used to target the whole rostral-caudal extent of the lateral OFC because both rostral and caudal portions have connections with the PER or POR (Hwang et al., 2018). The coordinates for lateral OFC were chosen to cover the target areas from other OFC studies (Costa et al., 2021; Hart et al., 2020; Jones et al., 2012).

The same set of conditioning chambers were used for sensory preconditioning. No additional cues were introduced to the basic context of the chambers. The sensory preconditioning procedure and statistical analysis were identical to the previous experiment. These rats did not undergo fear conditioning at the end of the experiment.

Site	AP	ML	DV	Angle
OFC 1	-4.0	3.0	3.6	0°
OFC 2	-2.7	3.5	5.0	0°
PER 1	3.4	5.0	6.4	13°
PER 2	5.0	5.0	6.2	13°
PER 3	6.6	5.0	5.8	13°
POR 1	-0.5*	4.6	4.5	16°
POR 2	0.2*	4.3	2.75	16°

Table 3 Stereotaxic coordinates for unilateral DREADD virus injections. In the anterior-posterior axis (AP), positive values indicate locations posterior to the reference point, and negative values indicate locations anterior to the reference. The bregma was used as reference for OFC and PER sites; the lambda was the reference for POR sites (marked by *). Dorsal-ventral (DV) values indicate distances from the pipette tip to the top surface of the skull.

Results

Histology

Out of thirty-nine rats, seven did not show sufficient expression in all target areas, therefore they were excluded from behavioral analysis. All remaining rats showed adequate viral expression in the target areas (Fig. 3.2). Viral expression in the lateral

OFC was overall similar to those in prior studies (Costa et al., 2023; Costa et al., 2021; Hart et al., 2020; Jones et al., 2012). We did not detect bilateral viral expression in any case.

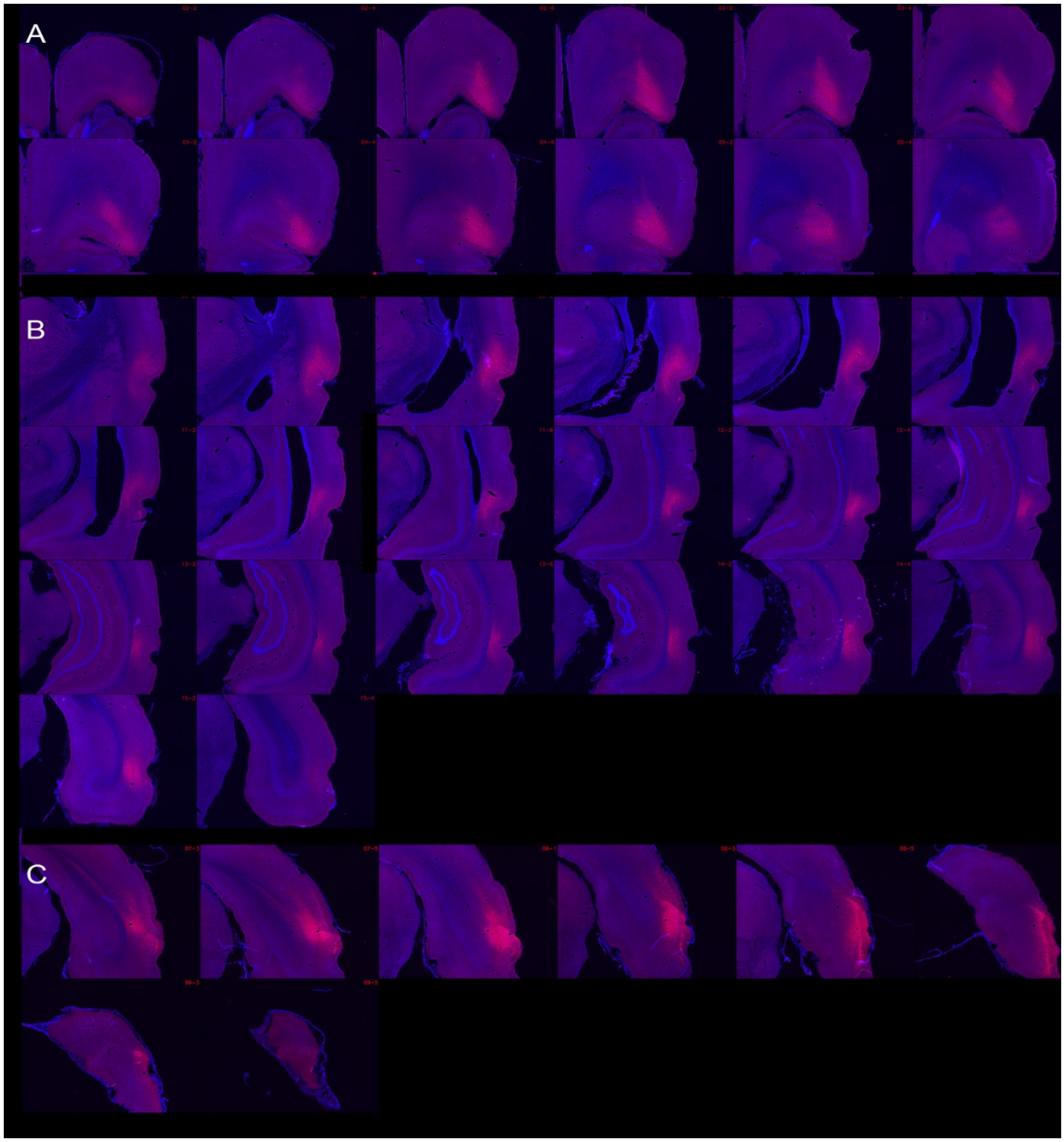

Figure 3.2. Representative placements of viral transduction in the lateral OFC (A), PER (B), and POR (C). DAPI staining is captured in the blue channel, and the DREADD virus is captured in the red channel.

Preconditioning and conditioning

Data from one rat was excluded from all analyses for being a statistical outlier in PCS responding. One-way ANOVA on nose poke time percentage differences during preconditioning sessions confirmed that the groups did not differ in initial preferences for PCS+ versus PCS- ($p = 0.187$), or CS+ versus CS- ($p = 0.188$). Next, a mixed-design ANOVA on nose poke time percentage differences for the 6 conditioning sessions (Fig. 3.3) revealed a significant main effect for SESSION, $F_{(5, 130)} = 5.96$, $p < 0.001$, but not for SESSION*GROUP interaction ($p = 0.832$) or GROUP ($p = 0.659$). This suggested that all groups performed similarly in the acquisition of first-order conditioning.

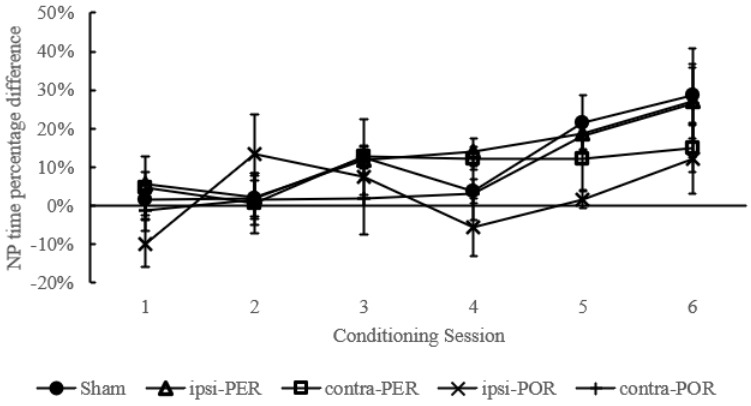

Figure 3.3. Nose poke time percentage difference during the conditioning phase. Data was only analyzed for the first half (5 s) of CS+ and CS- because pellets were delivered during the second half of CS+. Error bars indicate standard error.

PCS test

All subjects received an intraperitoneal CNO injection 30 minutes prior to the PCS test. For PCS trials, one-way ANOVA with planned comparisons was conducted to compare

the nose poke time percentage difference between the last 5s of PCS+ versus PCS- (Fig. 3.4B). Planned comparisons revealed a significant difference only between the Sham and ipsilateral-POR group, $t_{(26)} = -2.47$, $p = 0.020$. One-sample t tests showed significantly positive nose poke time percentage differences for the Sham group, $t_{(9)} = 3.83$, $p = 0.004$, and the contralateral-POR group, $t_{(9)} = 7.11$, $p = 0.001$. Therefore, whereas the Sham and contralateral-POR group demonstrated significantly higher responding to PCS+ than PCS-, neither the ipsilateral-POR group nor either of the PER groups showed successful sensory preconditioning.

CS test

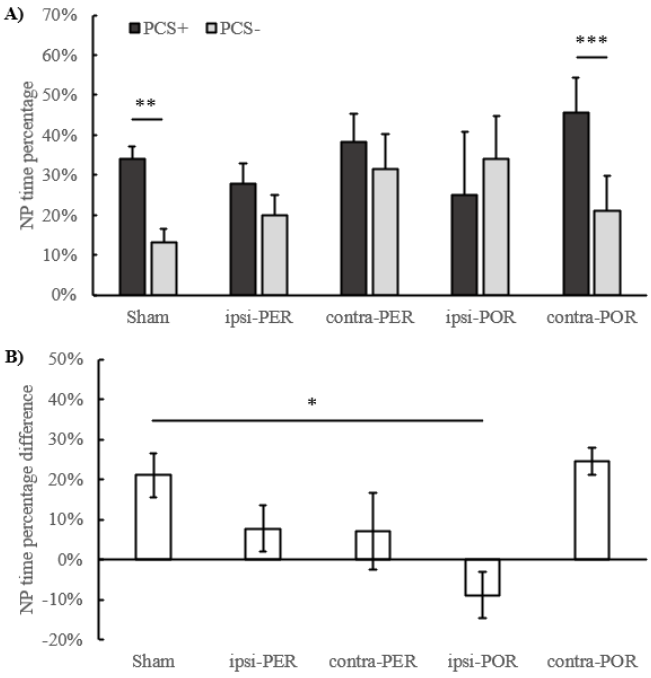

Figure 3.4. Nose poke time percentage during the last 5s of PCS trials in the PCS test phase under suppression. A) nose poke time percentages during PCS+ and PCS-. P-value symbols indicate significant differences between stimuli. B) nose poke time percentage difference between PCS+ and PCS-. Error bars indicate standard error. P-value symbols indicate significant differences between groups. #: $p < 0.10$, *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$.

Forty-eight hours after the PCS test, the CS test session was conducted following CNO injections (Fig. 3.5). The nose poke time difference between the last 5s of the CS+ and CS- were analyzed. The one-way ANOVA was not significant, $p = 0.380$, nor were any

of the planned comparisons between the Sham and virus groups. One-sample t tests showed that all groups except the ipsilateral-POR group ($p = 0.161$) had significantly positive difference scores for the last 5s of the CS test trials: Sham, $t_{(9)} = 7.14$, $p < 0.001$; ipsilateral-PER, $t_{(4)} = 7.41$, $p = 0.005$; contralateral-PER, $t_{(7)} = 3.40$, $p = 0.011$; contralateral-POR, $t_{(5)} = 5.36$, $p = 0.003$. Collectively, these results indicated no robust differences between the Sham and any of the virus groups regarding nose poke times during the last 5s of CS+ compared with the CS-.

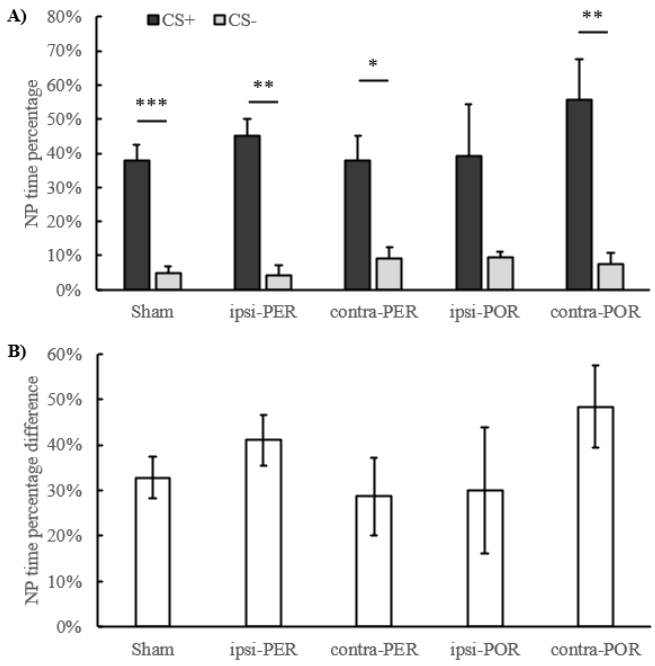

Figure 3.5. Nose poke time percentage during the last 5s of CS trials in the CS test phase under suppression. A) nose poke time percentages during PCS+ and PCS-. P-value symbols indicate significant differences between stimuli. B) nose poke time percentage difference between CS+ and CS-. Error bars indicate standard error. P-value symbols indicate significant differences between groups. #: $p < 0.10$, *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$.

PCS and CS response latency

Nose poke latencies after stimulus onset during the CS+ and PCS+ trials during tests were analyzed to assess possible attentional deficits caused by temporary suppression

(Figure 3.6). ANOVAs for CS+ trials ($p = 0.308$) and for PCS+ trials ($p = 0.379$) were not significant. None of the planned comparisons between the Sham and virus groups was significant.

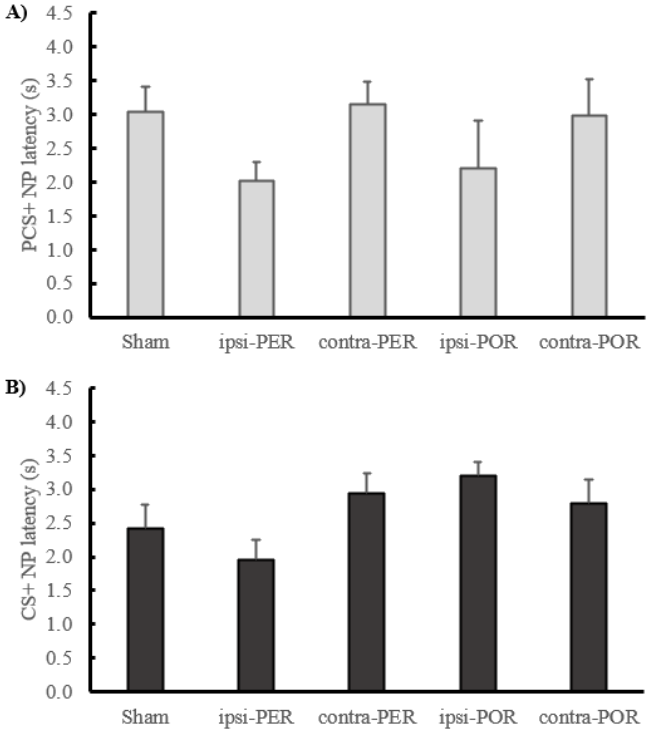

Figure 3.6. Latencies of nose poking in the test sessions under suppression. A) nose poke latencies since onset of PCS+ during test trials. B) nose poke latencies since onset of CS+ during test trials. Error bars indicate standard error.

Discussion

The current study used a DREADD-based asymmetrical inactivation approach to temporarily disrupt OFC-PER and OFC-POR communication during the test phases of sensory preconditioning. Impaired sensory preconditioning was observed in rats with

ipsilateral OFC-PER, contralateral OFC-PER, and ipsilateral OFC-POR inactivation, as indicated by non-significant one-sample t-tests on nose poke time percentage differences between PCS+ and PCS-. Although planned comparisons only revealed a significant difference when the sham-surgery control group was compared to the ipsilateral OFC-POR group, but not the other two groups, it was partly due to the Sham group showing a slightly weaker sensory preconditioning effect relative to sham rats in the previous experiment. No deficit was found in rats with contralateral OFC-POR inactivation. All groups displayed normal response latencies and first-order conditioning.

Although we had expected impaired sensory preconditioning under temporary OFC disconnection with both the PER and POR, the pattern of impairments shown by the virus groups was only partially consistent with our predictions. Specifically, whereas the contralateral OFC-PER group displayed a deficit as we predicted, unexpected deficits were found in both the ipsilateral OFC-PER and OFC-POR groups. In typical rodent disconnection experiments with cross-lateral lesions or inactivation, the ipsilateral group is usually intended as a control to which the contralateral group is compared. Strong conclusions regarding the functional interdependency between two structures may be drawn when deficits are observed in the contralateral group, but not in the ipsilateral or sham-surgery group. This reasoning is predicated on the assumption that monosynaptic communication between two rodent brain structures is mostly accomplished via intra-hemispheric pathways, and the inter-hemispheric pathways either do not exist or are functionally insignificant. Therefore, an impairment may emerge when the intra-hemispheric projections are disturbed in the contralateral group, but not in the ipsilateral

group. However, inter-hemispheric projections have indeed been reported between heterotopic regions in the rodent brain, though usually at only a fraction of the intensity of their intra-hemispheric connections (Hoover & Vertes, 2007; Markowitsch & Guldin, 1983; Vertes et al., 2023).

Studies with ipsilateral lesions or inactivation do support the functional significance of inter-hemispheric heterotopic connectivity in rodents, even though reports of behavioral impairment have been inconsistent across different areas and behaviors under investigation. Our lab previously examined the effects of PER-POR disconnections using crossed unilateral NMDA injections into the PER and POR. Although the overall results pointed to impaired context processing for the contralateral group compared to both the sham and ipsilateral lesion group, a mild impairment was observed in the ipsilateral group in the 3-dimentional contextual spontaneous object recognition (SOR) task (Heimer-McGinn et al., 2017). In another study, ipsilateral OFC-BLA inactivation reduced context renewal of cocaine-seeking behavior in rats to the same extent as contralateral OFC-BLA inactivation (Lasseter et al., 2011). These findings are particularly informative not only because they involve the same brain regions as the current experiment, but also because these are tasks in which behaviors are strongly modulated by the context. Context processing is one of the core functions of the PER and POR (Estela-Pro & Burwell, 2021; Heimer-McGinn et al., 2017; Peng & Burwell, 2021) and spatial mapping has also been reported in the OFC (Wikenheiser et al., 2021). Even though no study has demonstrated context-specificity in the current sensory preconditioning procedure, other latent learning paradigms have been shown to be

context-specific, such as latent inhibition (Lubow & Weiner, 2010). Therefore, it may be speculated that, although often being overlooked, inter-hemispheric connectivity for areas like the OFC, PER, and POR is crucial for latent learning, especially when the context-modulation of behavior is required.

Other than the proposed functional significance of the inter-hemispheric OFC-PER and OFC-POR connections, an alternative explanation is that unilaterally suppressing any of the OFC, PER, or POR is sufficient to create deficits in the current sensory preconditioning procedure. In other words, these areas may operate independently but this task demands all of them to be active in both hemispheres. However, this is unlikely because no deficit was observed in the contralateral OFC-POR group compared to the Sham group. Therefore, the loss of unilateral OFC or POR contribution cannot account for the deficit shown by the ipsilateral OFC-POR group. Based on current results, however, we cannot strictly rule out the possibility that the loss of PER activity in one hemisphere was the sole cause of the impairment displayed by the ipsilateral and contralateral OFC-PER groups. If this was the case, then any possible effect of OFC-PER disconnection would have been masked by the detriments of unilateral PER suppression. Direct evidence for this possibility is lacking. Unilateral lidocaine injections into the rostral PER were found to mildly impair recency discrimination (Hannesson et al., 2004). The impairment, however, could be due to the lidocaine blocking fibers of passage in the area, instead of suppressing the PER per se. It is notable that impairments have been reported in rats with unilateral lesions in the lateral entorhinal cortex (LEC), which is another MTL area that receives strong input from the PER and the POR (caudal LEA),

and then projects to the hippocampus. Both the PER and LEC are part of a pathway that specializes in processing item information for recognition and episodic memory (Eichenbaum et al., 2012; Eichenbaum et al., 2007). Unilaterally inactivating the LEC led to impaired trace eyeblink conditioning in rats (Tanninen et al., 2013). In another study, deficits in the contextual SOR task created by unilateral LEC lesions were as severe as those after bilateral damages (Wilson et al., 2013). Moreover, deficits in trace and contextual fear conditioning have been reported after unilateral ventral hippocampal inactivation (Gilmartin et al., 2012). Although these studies focused on MTL structures with strong connectivity with the PER, their findings do not provide direct insight into the functional consequence of unilateral PER inactivation. Future studies are required to fully address this possibility.

Although our results point to the functional significance of monosynaptic OFC-PER and OFC-POR pathways, due to the inherent limitation of the asymmetrical disconnection method, it remains an open question exactly how the OFC interacts with the PER and POR during the retrieval of PCS-CS associations in sensory preconditioning. It may be speculated, however, that the PER and POR collectively support OFC neurons to reduce the complexity and to increase the efficiency when encoding associative relationships among events. Particularly, the PER "unitizes" complex or discontinuous sensory information from external stimuli into efficient neural representations for associative learning and other memory functions (Bang & Brown, 2009; Kholodar-Smith, Allen, et al., 2008; Lindquist et al., 2004). Taking advantage of these stimulus representations from the PER, the OFC may organize task-relevant cues and associational structures into

a more concise cognitive map by trimming down the excess sensory details. As a real-world example, even a map the size of A4 paper can contain the spatial relationships among dozens of cities because each city can be efficiently represented by a simple dot, rather than by images of its every constituent. The process of searching through the map space will also be more efficient. While the PER specializes in representing and processing items, the POR might contribute in similar ways in the spatial domain (Furtak et al., 2012). Even though not tested in the current experiment, OFC-PER/POR cooperation might first occur during the preconditioning phase when associations between neutral cues are encoded by the OFC (Hart et al., 2020; Sadacca et al., 2018). Prior studies indicate that PER and POR involvement is also crucial during preconditioning (Holmes et al., 2013; Taylor-Yeremeeva et al., 2021).

In conclusion, building upon our findings from the previous experiment, we examined the effects of temporary OFC-PER and OFC-POR disconnections during the PCS test phase of sensory preconditioning. Under the influence of CNO, deficits in PCS responding were observed in rats with virus injections in ipsilateral OFC-PER, contralateral OFC-PER, and ipsilateral OFC-POR. Unexpectedly, no impairment was found in the contralateral OFC-POR group. Overall, the results suggest that both intra- and inter-hemispheric connections between the OFC and PER are necessary for retrieving PCS-CS associations and support sensory preconditioning, whereas for the POR only its inter-hemispheric OFC pathways are critical. Future studies may employ electrophysiological recording techniques to further understand the functional significance of the PER, POR, and their interaction with the OFC.

References

Agster, K. L., & Burwell, R. D. (2013). Hippocampal and subicular efferents and afferents of the perirhinal, postrhinal, and entorhinal cortices of the rat. *Behavioural brain research*, *254*, 50-64.

Bang, S. J., & Brown, T. H. (2009). Perirhinal cortex supports acquired fear of auditory objects. *Neurobiol Learn Mem*, *92*(1), 53-62. https://doi.org/10.1016/j.nlm.2009.01.002

Brogden, W. J. (1939). Sensory pre-conditioning. *Journal of Experimental Psychology*, *25*(4), 323-332. https://doi.org/10.1037/h0058944

Burwell, R. D., & Amaral, D. G. (1998). Cortical afferents of the perirhinal, postrhinal, and entorhinal cortices of the rat. *Journal of comparative neurology*, *398*(2), 179-205.

Costa, K. M., Scholz, R., Lloyd, K., Moreno-Castilla, P., Gardner, M. P., Dayan, P., & Schoenbaum, G. (2022). The role of the lateral orbitofrontal cortex in creating cognitive maps. *Nature Neuroscience*, 1-9.

Eichenbaum, H., Sauvage, M., Fortin, N., Komorowski, R., & Lipton, P. (2012). Towards a functional organization of episodic memory in the medial temporal lobe. *Neurosci Biobehav Rev*, *36*(7), 1597-1608. https://doi.org/10.1016/j.neubiorev.2011.07.006

Eichenbaum, H., Yonelinas, A. P., & Ranganath, C. (2007). The medial temporal lobe and recognition memory. *Annu Rev Neurosci*, *30*, 123-152. https://doi.org/10.1146/annurev.neuro.30.051606.094328

Estela-Pro, V. J., & Burwell, R. D. (2021). The anatomy and function of the postrhinal cortex. *Behavioral Neuroscience*.

Gilmartin, M. R., Kwapis, J. L., & Helmstetter, F. J. (2012). Trace and contextual fear conditioning are impaired following unilateral microinjection of muscimol in the ventral hippocampus or amygdala, but not the medial prefrontal cortex. *Neurobiology of Learning and Memory*, *97*(4), 452-464.

Hannesson, D., Howland, J. G., & Phillips, A. G. (2004). Interaction between perirhinal and medial prefrontal cortex is required for temporal order but not recognition memory for objects in rats. *The Journal of Neuroscience*, *24*(19), 4596-4604. https://doi.org/10.1523/jneurosci.5517-03.2004

Hart, E. E., Sharpe, M. J., Gardner, M. P., & Schoenbaum, G. (2020). Responding to preconditioned cues is devaluation sensitive and requires orbitofrontal cortex during cue-cue learning. *Elife*, *9*. https://doi.org/10.7554/eLife.59998

Heimer-McGinn, V. R., Poeta, D. L., Aghi, K., Udawatta, M., & Burwell, R. D. (2017). Disconnection of the perirhinal and postrhinal cortices impairs recognition of objects in context but not contextual fear conditioning. *Journal of Neuroscience*, *37*(18), 4819-

4829.

Holmes, N. M., Parkes, S. L., Killcross, A. S., & Westbrook, R. F. (2013). The basolateral amygdala is critical for learning about neutral stimuli in the presence of danger, and the perirhinal cortex is critical in the absence of danger. *J Neurosci*, *33*(32), 13112-13125. https://doi.org/10.1523/JNEUROSCI.1998-13.2013

Hoover, W. B., & Vertes, R. P. (2007). Anatomical analysis of afferent projections to the medial prefrontal cortex in the rat. *Brain Struct Funct*, *212*(2), 149-179. https://doi.org/10.1007/s00429-007-0150-4

Hwang, E., Willis, B. S., & Burwell, R. D. (2018). Prefrontal connections of the perirhinal and postrhinal cortices in the rat. *Behav Brain Res*, *354*, 8-21. https://doi.org/10.1016/j.bbr.2017.07.032

Jones, J. L., Esber, G. R., McDannald, M. A., Gruber, A. J., Hernandez, A., Mirenzi, A., & Schoenbaum, G. (2012). Orbitofrontal cortex supports behavior and learning using inferred but not cached values. *Science*, *338*(6109), 953-956. https://doi.org/10.1126/science.1227489

Kholodar-Smith, D. B., Allen, T. A., & Brown, T. H. (2008). Fear conditioning to discontinuous auditory cues requires perirhinal cortical function. *Behav Neurosci*, *122*(5), 1178-1185. https://doi.org/10.1037/a0012902

Lasseter, H. C., Wells, A. M., Xie, X., & Fuchs, R. A. (2011). Interaction of the basolateral amygdala and orbitofrontal cortex is critical for drug context-induced reinstatement of cocaine-seeking behavior in rats. *Neuropsychopharmacology*, *36*(3), 711-720. https://doi.org/10.1038/npp.2010.209

Lindquist, D. H., Jarrard, L. E., & Brown, T. H. (2004). Perirhinal cortex supports delay fear conditioning to rat ultrasonic social signals. *J Neurosci*, *24*(14), 3610-3617. https://doi.org/10.1523/JNEUROSCI.4839-03.2004

Lubow, R., & Weiner, I. (2010). *Latent inhibition: Cognition, neuroscience and applications to schizophrenia*. Cambridge University Press.

Markowitsch, H. J., & Guldin, W. O. (1983). Heterotopic interhemispheric cortical connections in the rat. *Brain research bulletin*, *10*(6), 805-810. https://doi.org/10.1016/0361-9230(83)90212-5

Peng, X., & Burwell, R. D. (2021). Beyond the hippocampus: the role of parahippocampal-prefrontal communication in context-modulated behavior. *Neurobiology of Learning and Memory*, *185*, 107520.

Pickens, C. L., Saddoris, M. P., Setlow, B., Gallagher, M., Holland, P. C., & Schoenbaum, G. (2003). Different roles for orbitofrontal cortex and basolateral amygdala in a reinforcer devaluation task. *Journal of Neuroscience*, *23*(35), 11078-11084.

Sadacca, B. F., Wied, H. M., Lopatina, N., Saini, G. K., Nemirovsky, D., & Schoenbaum, G. (2018). Orbitofrontal neurons signal sensory associations underlying model-based inference in a sensory preconditioning task. *Elife*, *7*. https://doi.org/10.7554/eLife.30373

Schoenbaum, G., Nugent, S. L., Saddoris, M. P., & Setlow, B. (2002). Orbitofrontal lesions in rats impair reversal but not acquisition of go, no-go odor discriminations. *Neuroreport*, *13*(6), 885-890.

Tanninen, S. E., Morrissey, M. D., & Takehara-Nishiuchi, K. (2013). Unilateral lateral entorhinal inactivation impairs memory expression in trace eyeblink conditioning. *PLoS One*, *8*(12), e84543.

Taylor-Yeremeeva, E. M., Wisser, S. C., Chakoma, T. L., Aldrich, S. J., Denney, A. E., Donahue, E. K., Adelman, J. S., Ihle, P. C., & Robinson, S. (2021). Appetitive and aversive sensory preconditioning in rats is impaired by disruption of the postrhinal cortex. *Neurobiology of Learning and Memory*, *183*, 107461.

Vertes, R. P., Hoover, W. B., Witter, M. P., Yanik, M. F., Rojas, A. K., & Linley, S. B. (2023). Projections from the five divisions of the orbital cortex to the thalamus in the rat. *Journal of comparative neurology*, *531*(2), 217-237.

Wikenheiser, A. M., Gardner, M. P., Mueller, L. E., & Schoenbaum, G. (2021). Spatial representations in rat orbitofrontal cortex. *Journal of Neuroscience*, *41*(32), 6933-6945.

Wikenheiser, A. M., & Schoenbaum, G. (2016). Over the river, through the woods: cognitive maps in the hippocampus and orbitofrontal cortex. *Nature Reviews Neuroscience*, *17*(8), 513-523.

Wilson, D. I., Langston, R. F., Schlesiger, M. I., Wagner, M., Watanabe, S., & Ainge, J. A. (2013). Lateral entorhinal cortex is critical for novel object-context recognition. *Hippocampus*, *23*(5), 352-366.

Wilson, R. C., Takahashi, Y. K., Schoenbaum, G., & Niv, Y. (2014). Orbitofrontal cortex as a cognitive map of task space. *Neuron*, *81*(2), 267-279. https://doi.org/10.1016/j.neuron.2013.11.005

Zhou, J., Gardner, M. P., & Schoenbaum, G. (2021). Is the core function of orbitofrontal cortex to signal values or make predictions? *Current opinion in behavioral sciences*, *41*, 1-9.

CHAPTER 4

CONCLUSION AND FUTURE DIRECTIONS

In the previous chapters, I described two experiments that examined the functional significance of the rodent PER and POR in sensory preconditioning and whether their contributions were dependent upon direct interaction with the OFC. In the first experiment, adult male rats received sham surgeries or bilateral injections of a DREADD virus into the PER or POR. Afterwards, they underwent a sensory preconditioning procedure with auditory stimuli that was previously shown to be critically reliant upon the OFC (Hart et al., 2020; Jones et al., 2012). No inhibition was induced during the preconditioning and conditioning phases. Inhibition was induced only before the test phases when the rats were injected with an exogenous ligand to bind with the DREADD ion channels to create temporary PER or POR suppression. Whereas the sham-surgery rats demonstrated normal sensory preconditioning, both the PER and POR groups displayed an impairment when responding to the PCS. This suggested that both areas are required for retrieving the neutral stimulus-stimulus associations acquired during preconditioning. Earlier studies have demonstrated their necessary involvement during the initial encoding of associations between naïve, neutral stimuli (Holmes et al., 2013; Taylor-Yeremeeva et al., 2021). These results provide evidence that, in addition to being crucial for associating transient external events during latent learning, the PER and POR are also necessary for retrieving the association for subsequent learning.

In the second experiment, I adopted the asymmetrical inactivation approach to examine the effects of temporarily disrupting OFC-PER or OFC-POR communication in the same sensory preconditioning task. With contralateral inactivation, the intra-hemispheric pathways remain functional while the inter-hemispheric pathways are blocked. Rats with ipsilateral viral injections were intended as a second control group, in addition to rats that

received sham surgeries. Unexpectedly, impaired sensory preconditioning was observed in the ipsilateral OFC-POR group and both the ipsilateral and contralateral OFC-PER group. In contrast, the contralateral OFC-POR and the Sham group retained normal behavior during the PCS test. Although the functional significance of inter-hemispheric pathways between heterotopic areas has not been extensively investigated in rodents, similar reports do exist in which behavioral deficits were observed in rats with ipsilateral lesions or inactivation (Heimer-McGinn et al., 2017; Lasseter et al., 2011). Therefore, although the pattern of deficits was not as predicted, the results point to functional interdependencies in sensory preconditioning between the OFC and both the PER and POR during the PCS test.

Further studies are needed to reveal specifically how the PER and POR contribute to latent learning and goal-directed behavior. Electrophysiological recordings of PER or POR neurons will provide particularly useful information. A large portion of existing electrophysiological studies on the PER revolve around its prominent role in object recognition memory. In comparison, although lesion and inactivation studies have provided ample evidence on the importance of the PER beyond object recognition (Kent & Brown, 2012; Peng & Burwell, 2021), the question of specifically how the PER contributes to other cognitive processes remains largely unanswered. For example, although results from the second experiment indicated functional cooperation between the OFC and PER in sensory preconditioning, it is unknown what type of information is transmitted between these two areas. Future experiments may involve bilateral PER inhibition combined with electrophysiological recording in the OFC, or vice versa. Studies like these will not only add to our existing knowledge on the functions of the

PER and POR, but they will also advance our understanding of how the medial temporal lobe memory system interact with other brain systems for flexible, adaptive behavior.

Even though throughout most parts of this thesis latent learning and goal-directed decision-making are treated as two separate cognitive processes that occasionally interact, the "cognitive map" theory in fact has put forward the co-implementation of these two processes inside the OFC (Gardner & Schoenbaum, 2021; Wilson et al., 2014; Zhou et al., 2021). A notable distinction between the "cognitive map" theory and previous theories of OFC functions is that although cognitive maps can be used for reward-driven behavior, the construction and updating of the maps can occur in the absence of actual rewards or reward expectations, i.e., during latent learning. This notion significantly expands the role of the OFC from largely being limited to value-related processing to automatically representing a structured model of the world, regardless of value. Besides sensory preconditioning, latent inhibition is another associative learning paradigm in which latent learning plays a crucial role. Latent learning occurs during the pre-exposure phase for the not-yet-conditioned stimulus in the latent inhibition procedure (Lubow & Moore, 1959; Lubow & Weiner, 2010). This phase is similar to the preconditioning phase of sensory preconditioning because they involve presentations of single stimulus or stimulus pairs without explicit reinforcement. Interestingly, just as with the preconditioning phase of sensory preconditioning (Hart et al., 2020), OFC involvement is also critical during the CS pre-exposure phase for successful latent inhibition to occur, consistent with the idea that the OFC is required for hidden state inference (Costa et al., 2021).

Though largely focused on functions of the OFC, recent versions of the "cognitive map" theory propose that the OFC (or at least, lateral OFC) alone is not responsible for all processes surrounding the utilization of associative models for decision-making (Costa et al., 2023; Gardner & Schoenbaum, 2021; Zhou et al., 2021). Therefore, building upon the findings from the sensory preconditioning experiments in this thesis, I argue that it may be beneficial to consider the "cognitive map" theory not as a functional theory for the OFC per se, but instead, for a network of structures that collectively sustain the operations related to cognitive mapping and re-mapping. As part of the network, the PER and POR (possibly with areas of the entorhinal cortex) may provide non-spatial and spatial information, respectively, to the OFC for initially generating the cognitive map. Once the map has been established, the OFC may disengage from maintaining the map and transfer it into other cortical areas for storage. The anterior cingulate cortex and the retrosplenial cortex are among the likely candidates (Fournier et al., 2020; Hart et al., 2022; Robinson et al., 2011; Robinson et al., 2014). These areas are also connected with the PER and POR (Agster & Burwell, 2009; Burwell & Amaral, 1998a; Hwang et al., 2018). OFC involvement may be required to update the map should new information appear and to utilize the map for value inferences. Notably, since the PER and POR (and the entorhinal cortex) also provide information to the hippocampus, it is possible that their output can be used for cognitive mapping in two parallel systems. The OFC and hippocampal system possibly compete for input from the PER and POR to construct cognitive maps (Zong et al., 2023).

To conclude, although the PER and POR have long been known for their involvement in context processing and context-modulated behavior, particularly as sources of indirect

and direct input to the hippocampus, evidence suggests that their contributions extend beyond the medial temporal lobe. Chemogenetic suppression of the PER and POR showed that both areas are crucial for retrieving associations formed between neutral stimuli in sensory preconditioning. Moreover, evidence from the temporary disconnection experiment indicated that the PER and POR may collectively act as a support circuitry that functions in cooperation with the OFC in reward learning. Further studies will be beneficial to the advancement of our understanding of the parahippocampal region and the medial temporal lobe.

References

Abdi, H. (2010). Holm's sequential Bonferroni procedure. *Encyclopedia of research design*, *1*(8), 1-8.

Agster, K. L., & Burwell, R. D. (2009). Cortical efferents of the perirhinal, postrhinal, and entorhinal cortices of the rat. *Hippocampus*, *19*(12), 1159-1186. https://doi.org/10.1002/hipo.20578

Agster, K. L., & Burwell, R. D. (2013). Hippocampal and subicular efferents and afferents of the perirhinal, postrhinal, and entorhinal cortices of the rat. *Behavioural brain research*, *254*, 50-64.

Allen, L. M., Lesyshyn, R. A., O'Dell, S. J., Allen, T. A., & Fortin, N. J. (2020). The hippocampus, prefrontal cortex, and perirhinal cortex are critical to incidental order memory. *Behav Brain Res*, *379*, 112215. https://doi.org/10.1016/j.bbr.2019.112215

Allen, T. A., Salz, D. M., McKenzie, S., & Fortin, N. J. (2016). Nonspatial sequence coding in CA1 neurons. *Journal of Neuroscience*, *36*(5), 1547-1563.

Bang, S. J., & Brown, T. H. (2009). Perirhinal cortex supports acquired fear of auditory objects. *Neurobiol Learn Mem*, *92*(1), 53-62. https://doi.org/10.1016/j.nlm.2009.01.002

Barker, G. R., Bird, F., Alexander, V., & Warburton, E. C. (2007). Recognition memory for objects, place, and temporal order: a disconnection analysis of the role of the medial prefrontal cortex and perirhinal cortex. *J Neurosci*, *27*(11), 2948-2957. https://doi.org/10.1523/JNEUROSCI.5289-06.2007

Brogden, W. J. (1939). Sensory pre-conditioning. *Journal of Experimental Psychology*, *25*(4), 323-332. https://doi.org/10.1037/h0058944

Bucci, D. J., Phillips, R. G., & Burwell, R. D. (2000). Contributions of postrhinal and perirhinal cortex to contextual information processing. *Behav Neurosci*, *114*(5), 882-894. https://doi.org/10.1037//0735-7044.114.5.882

Bucci, D. J., Saddoris, M. P., & Burwell, R. D. (2002). Contextual fear discrimination is impaired by damage to the postrhinal or perirhinal cortex. *Behavioral Neuroscience*, *116*(3), 479-488. https://doi.org/10.1037/0735-7044.116.3.479

Burwell, R. D., & Amaral, D. G. (1998a). Cortical afferents of the perirhinal, postrhinal, and entorhinal cortices of the rat. *Journal of comparative neurology*, *398*(2), 179-205.

Burwell, R. D., & Amaral, D. G. (1998b). Perirhinal and postrhinal cortices of the rat: interconnectivity and connections with the entorhinal cortex. *Journal of comparative neurology*, *391*(3), 293-321.

Burwell, R. D., Bucci, D. J., Sanborn, M. R., & Jutras, M. J. (2004). Perirhinal and postrhinal contributions to remote memory for context. In (Vol. 24, pp. 11023-11028).

Burwell, R. D., Witter, M. P., & Amaral, D. G. (1995). Perirhinal and postrhinal cortices of the rat: a review of the neuroanatomical literature and comparison with findings from the monkey brain. *Hippocampus*, *5*(5), 390-408.

Clayton, N. S., Bussey, T. J., & Dickinson, A. (2003). Can animals recall the past and plan for the future? *Nature Reviews Neuroscience*, *4*(8), 685-691.

Costa, K. M., Scholz, R., Lloyd, K., Moreno-Castilla, P., Gardner, M. P., Dayan, P., & Schoenbaum, G. (2023). The role of the lateral orbitofrontal cortex in creating cognitive maps. *Nature Neuroscience*, *26*(1), 107-115.

Costa, K. M., Sengupta, A., & Schoenbaum, G. (2021). The orbitofrontal cortex is necessary for learning to ignore. *Current Biology*, *31*(12), 2652-2657. e2653.

Diana, R. A., Yonelinas, A. P., & Ranganath, C. (2007). Imaging recollection and familiarity in the medial temporal lobe: a three-component model. *Trends in cognitive sciences*, *11*(9), 379-386.

Eichenbaum, H. (2017). Prefrontal-hippocampal interactions in episodic memory. *Nat Rev Neurosci*, *18*(9), 547-558. https://doi.org/10.1038/nrn.2017.74

Eichenbaum, H., Sauvage, M., Fortin, N., Komorowski, R., & Lipton, P. (2012). Towards

a functional organization of episodic memory in the medial temporal lobe. *Neurosci Biobehav Rev*, *36*(7), 1597-1608. https://doi.org/10.1016/j.neubiorev.2011.07.006

Eichenbaum, H., Yonelinas, A. P., & Ranganath, C. (2007). The medial temporal lobe and recognition memory. *Annu Rev Neurosci*, *30*, 123-152. https://doi.org/10.1146/annurev.neuro.30.051606.094328

Eldridge, M. A., Lerchner, W., Saunders, R. C., Kaneko, H., Krausz, K. W., Gonzalez, F. J., Ji, B., Higuchi, M., Minamimoto, T., & Richmond, B. J. (2016). Chemogenetic disconnection of monkey orbitofrontal and rhinal cortex reversibly disrupts reward value. *Nature Neuroscience*, *19*(1), 37-39.

Estela-Pro, V. J., & Burwell, R. D. (2021). The anatomy and function of the postrhinal cortex. *Behavioral Neuroscience*.

Estela, V. J. (2020). *Neural correlates of early- and late-stage learning in the hippocampus and postrhinal cortex* [Neural correlates of early- and late-stage learning in the hippocampus and postrhinal cortex, Brown University]. Providence, RI.

Fanselow, M. S. (2010). From contextual fear to a dynamic view of memory systems. *Trends in cognitive sciences*, *14*(1), 7-15.

Fournier, D. I., Monasch, R. R., Bucci, D. J., & Todd, T. P. (2020). Retrosplenial Cortex Damage Impairs Unimodal Sensory Preconditioning. In (Vol. 134, pp. 198-207).

Furtak, S. C., Ahmed, O. J., & Burwell, R. D. (2012). Single neuron activity and theta modulation in postrhinal cortex during visual object discrimination. *Neuron*, *76*(5), 976-988. https://doi.org/10.1016/j.neuron.2012.10.039

Furtak, S. C., Wei, S. M., Agster, K. L., & Burwell, R. D. (2007). Functional neuroanatomy of the parahippocampal region in the rat: the perirhinal and postrhinal cortices. *Hippocampus*, *17*(9), 709-722. https://doi.org/10.1002/hipo.20314

Gardner, M. P., & Schoenbaum, G. (2021). The orbitofrontal cartographer. *Behavioral Neuroscience*, *135*(2), 267.

Gilmartin, M. R., Kwapis, J. L., & Helmstetter, F. J. (2012). Trace and contextual fear conditioning are impaired following unilateral microinjection of muscimol in the ventral hippocampus or amygdala, but not the medial prefrontal cortex. *Neurobiology of Learning and Memory*, *97*(4), 452-464.

Hannesson, D., Howland, J. G., & Phillips, A. G. (2004). Interaction between perirhinal and medial prefrontal cortex is required for temporal order but not recognition memory for objects in rats. *The Journal of Neuroscience*, *24*(19), 4596-4604. https://doi.org/10.1523/jneurosci.5517-03.2004

Hart, E. E., Gardner, M. P., & Schoenbaum, G. (2022). Anterior cingulate neurons signal neutral cue pairings during sensory preconditioning. *Current Biology*, *32*(3), 725-732.

e723.

Hart, E. E., Sharpe, M. J., Gardner, M. P., & Schoenbaum, G. (2020). Responding to preconditioned cues is devaluation sensitive and requires orbitofrontal cortex during cue-cue learning. *Elife*, *9*. https://doi.org/10.7554/eLife.59998

Heimer-McGinn, V. R., Poeta, D. L., Aghi, K., Udawatta, M., & Burwell, R. D. (2017). Disconnection of the perirhinal and postrhinal cortices impairs recognition of objects in context but not contextual fear conditioning. *Journal of Neuroscience*, *37*(18), 4819-4829.

Holland, P. C. (1981). Acquisition of representation-mediated conditioned food aversions. *Learning and Motivation*, *12*(1), 1-18.

Holmes, N. M., Parkes, S. L., Killcross, A. S., & Westbrook, R. F. (2013). The basolateral amygdala is critical for learning about neutral stimuli in the presence of danger, and the perirhinal cortex is critical in the absence of danger. *J Neurosci*, *33*(32), 13112-13125. https://doi.org/10.1523/JNEUROSCI.1998-13.2013

Holmes, N. M., Wong, F. S., Bouchekioua, Y., & Westbrook, R. F. (2022). Not "either-or" but "which-when": A review of the evidence for integration in sensory preconditioning. *Neuroscience & Biobehavioral Reviews*.

Hoover, W. B., & Vertes, R. P. (2007). Anatomical analysis of afferent projections to the medial prefrontal cortex in the rat. *Brain Struct Funct*, *212*(2), 149-179. https://doi.org/10.1007/s00429-007-0150-4

Hwang, E., Willis, B. S., & Burwell, R. D. (2018). Prefrontal connections of the perirhinal and postrhinal cortices in the rat. *Behav Brain Res*, *354*, 8-21. https://doi.org/10.1016/j.bbr.2017.07.032

Iordanova, M. D., Burnett, D. J., Aggleton, J. P., Good, M., & Honey, R. C. (2009). The role of the hippocampus in mnemonic integration and retrieval: complementary evidence from lesion and inactivation studies. *Eur J Neurosci*, *30*(11), 2177-2189. https://doi.org/10.1111/j.1460-9568.2009.07010.x

Jayachandran, M., Linley, S. B., Schlecht, M., Mahler, S. V., Vertes, R. P., & Allen, T. A. (2019). Prefrontal Pathways Provide Top-Down Control of Memory for Sequences of Events. *Cell Rep*, *28*(3), 640-654 e646. https://doi.org/10.1016/j.celrep.2019.06.053

Jones, J. L., Esber, G. R., McDannald, M. A., Gruber, A. J., Hernandez, A., Mirenzi, A., & Schoenbaum, G. (2012). Orbitofrontal cortex supports behavior and learning using inferred but not cached values. *Science*, *338*(6109), 953-956. https://doi.org/10.1126/science.1227489

Kent, B. A., & Brown, T. H. (2012). Dual functions of perirhinal cortex in fear conditioning. *Hippocampus*, *22*(10), 2068-2079. https://doi.org/10.1002/hipo.22058

Kholodar-Smith, D. B., Allen, T. A., & Brown, T. H. (2008). Fear conditioning to discontinuous auditory cues requires perirhinal cortical function. *Behav Neurosci*, *122*(5), 1178-1185. https://doi.org/10.1037/a0012902

Kholodar-Smith, D. B., Boguszewski, P., & Brown, T. H. (2008). Auditory trace fear conditioning requires perirhinal cortex. *Neurobiol Learn Mem*, *90*(3), 537-543. https://doi.org/10.1016/j.nlm.2008.06.006

Lasseter, H. C., Wells, A. M., Xie, X., & Fuchs, R. A. (2011). Interaction of the basolateral amygdala and orbitofrontal cortex is critical for drug context-induced reinstatement of cocaine-seeking behavior in rats. *Neuropsychopharmacology*, *36*(3), 711-720. https://doi.org/10.1038/npp.2010.209

Lindquist, D. H., Jarrard, L. E., & Brown, T. H. (2004). Perirhinal cortex supports delay fear conditioning to rat ultrasonic social signals. *J Neurosci*, *24*(14), 3610-3617. https://doi.org/10.1523/JNEUROSCI.4839-03.2004

Lubow, R., & Moore, A. (1959). Latent inhibition: the effect of nonreinforced pre-exposure to the conditional stimulus. *Journal of comparative and physiological psychology*, *52*(4), 415.

Lubow, R., & Weiner, I. (2010). *Latent inhibition: Cognition, neuroscience and applications to schizophrenia*. Cambridge University Press.

MacDonald, C. J., Carrow, S., Place, R., & Eichenbaum, H. (2013). Distinct hippocampal time cell sequences represent odor memories in immobilized rats. *J Neurosci*, *33*(36), 14607-14616. https://doi.org/10.1523/JNEUROSCI.1537-13.2013

Markowitsch, H. J., & Guldin, W. O. (1983). Heterotopic interhemispheric cortical connections in the rat. *Brain research bulletin*, *10*(6), 805-810. https://doi.org/10.1016/0361-9230(83)90212-5

Moscovitch, M., Cabeza, R., Winocur, G., & Nadel, L. (2016). Episodic Memory and Beyond: The Hippocampus and Neocortex in Transformation. *Annual Review of Psychology*, *67*(1), 105-134. https://doi.org/10.1146/annurev-psych-113011-143733

Navaroli, V. L., Zhao, Y., Boguszewski, P., & Brown, T. H. (2012). Muscarinic receptor activation enables persistent firing in pyramidal neurons from superficial layers of dorsal perirhinal cortex. *Hippocampus*, *22*(6), 1392-1404.

Nicholson, D. A., & Freeman Jr, J. H. (2000). Lesions of the perirhinal cortex impair sensory preconditioning in rats. *Behavioural brain research*, *112*(1-2), 69-75.

Oyama, K., Hori, Y., Mimura, K., Nagai, Y., Eldridge, M. A., Saunders, R. C., Miyakawa, N., Hirabayashi, T., Hori, Y., & Inoue, K.-i. (2022). Chemogenetic disconnection between the orbitofrontal cortex and the rostromedial caudate nucleus disrupts motivational control of goal-directed action. *Journal of Neuroscience*, *42*(32), 6267-6275.

Pastalkova, E., Itskov, V., Amarasingham, A., & Buzsaki, G. (2008). Internally generated cell assembly sequences in the rat hippocampus. *Science*, *321*(5894), 1322-1327.

Peng, X., & Burwell, R. D. (2021). Beyond the hippocampus: the role of parahippocampal-prefrontal communication in context-modulated behavior. *Neurobiology of Learning and Memory*, *185*, 107520.

Pickens, C. L., Saddoris, M. P., Setlow, B., Gallagher, M., Holland, P. C., & Schoenbaum, G. (2003). Different roles for orbitofrontal cortex and basolateral amygdala in a reinforcer devaluation task. *Journal of Neuroscience*, *23*(35), 11078-11084.

Rizley, R. C., & Rescorla, R. A. (1972). Associations in second-order conditioning and sensory preconditioning. *Journal of comparative and physiological psychology*, *81*(1), 1.

Robin, J., & Moscovitch, M. (2017). Details, gist and schema: hippocampal–neocortical interactions underlying recent and remote episodic and spatial memory. *Current opinion in behavioral sciences*, *17*, 114-123.

Robinson, N. T. M., Priestley, J. B., Rueckemann, J. W., Garcia, A. D., Smeglin, V. A., Marino, F. A., & Eichenbaum, H. (2017). Medial Entorhinal Cortex Selectively Supports Temporal Coding by Hippocampal Neurons. *Neuron (Cambridge, Mass.)*, *94*(3), 677-688.e676. https://doi.org/10.1016/j.neuron.2017.04.003

Robinson, S., Keene, C. S., Iaccarino, H. F., Duan, D., & Bucci, D. J. (2011). Involvement of retrosplenial cortex in forming associations between multiple sensory stimuli. *Behav Neurosci*, *125*(4), 578-587. https://doi.org/10.1037/a0024262

Robinson, S., Todd, T. P., Pasternak, A. R., Luikart, B. W., Skelton, P. D., Urban, D. J., & Bucci, D. J. (2014). Chemogenetic silencing of neurons in retrosplenial cortex disrupts sensory preconditioning. *J Neurosci*, *34*(33), 10982-10988. https://doi.org/10.1523/JNEUROSCI.1349-14.2014

Sadacca, B. F., Wied, H. M., Lopatina, N., Saini, G. K., Nemirovsky, D., & Schoenbaum, G. (2018). Orbitofrontal neurons signal sensory associations underlying model-based inference in a sensory preconditioning task. *Elife*, *7*. https://doi.org/10.7554/eLife.30373

Schoenbaum, G., Nugent, S. L., Saddoris, M. P., & Setlow, B. (2002). Orbitofrontal lesions in rats impair reversal but not acquisition of go, no-go odor discriminations. *Neuroreport*, *13*(6), 885-890.

Smith, K. S., Bucci, D. J., Luikart, B. W., & Mahler, S. V. (2016). DREADDS: Use and application in behavioral neuroscience. *Behav Neurosci*, *130*(2), 137-155. https://doi.org/10.1037/bne0000135

Tanninen, S. E., Morrissey, M. D., & Takehara-Nishiuchi, K. (2013). Unilateral lateral entorhinal inactivation impairs memory expression in trace eyeblink conditioning. *PLoS One*, *8*(12), e84543.

Taylor-Yeremeeva, E. M., Wisser, S. C., Chakoma, T. L., Aldrich, S. J., Denney, A. E., Donahue, E. K., Adelman, J. S., Ihle, P. C., & Robinson, S. (2021). Appetitive and aversive sensory preconditioning in rats is impaired by disruption of the postrhinal cortex. *Neurobiology of Learning and Memory*, *183*, 107461.

Todd, T. P., Fournier, D. I., & Bucci, D. J. (2019). Retrosplenial cortex and its role in cue-specific learning and memory. *Neuroscience and Biobehavioral Reviews*, *107*, 713-728. https://doi.org/10.1016/j.neubiorev.2019.04.016

Tomas Pereira, I., Agster, K. L., & Burwell, R. D. (2016). Subcortical connections of the perirhinal, postrhinal, and entorhinal cortices of the rat. I. afferents. *Hippocampus*, *26*(9), 1189-1212. https://doi.org/10.1002/hipo.22603

Urban, D. J., & Roth, B. L. (2015). DREADDs (designer receptors exclusively activated by designer drugs): chemogenetic tools with therapeutic utility. *Annu Rev Pharmacol Toxicol*, *55*, 399-417. https://doi.org/10.1146/annurev-pharmtox-010814-124803

Vertes, R. P., Hoover, W. B., Witter, M. P., Yanik, M. F., Rojas, A. K., & Linley, S. B. (2023). Projections from the five divisions of the orbital cortex to the thalamus in the rat. *Journal of comparative neurology*, *531*(2), 217-237.

Wikenheiser, A. M., Gardner, M. P., Mueller, L. E., & Schoenbaum, G. (2021). Spatial representations in rat orbitofrontal cortex. *Journal of Neuroscience*, *41*(32), 6933-6945.

Wikenheiser, A. M., & Schoenbaum, G. (2016). Over the river, through the woods: cognitive maps in the hippocampus and orbitofrontal cortex. *Nature Reviews Neuroscience*, *17*(8), 513-523.

Wilson, D. I., Langston, R. F., Schlesiger, M. I., Wagner, M., Watanabe, S., & Ainge, J. A. (2013). Lateral entorhinal cortex is critical for novel object-context recognition. *Hippocampus*, *23*(5), 352-366.

Wilson, R. C., Takahashi, Y. K., Schoenbaum, G., & Niv, Y. (2014). Orbitofrontal cortex as a cognitive map of task space. *Neuron*, *81*(2), 267-279. https://doi.org/10.1016/j.neuron.2013.11.005

Wiltgen, B. J., Sanders, M. J., Anagnostaras, S. G., Sage, J. R., & Fanselow, M. S. (2006). Context fear learning in the absence of the hippocampus. *Journal of Neuroscience*, *26*(20), 5484-5491.

Wong, F. S., Westbrook, R. F., & Holmes, N. M. (2019). 'Online' integration of sensory and fear memories in the rat medial temporal lobe. *Elife*, *8*. https://doi.org/10.7554/eLife.47085

Yonelinas, A. P., Ramey, M. M., Riddell, C., Kahana, M., & Wagner, A. (2022). Recognition memory: The role of recollection and familiarity. In: Oxford University Press.

Zhou, J., Gardner, M. P., & Schoenbaum, G. (2021). Is the core function of orbitofrontal cortex to signal values or make predictions? *Current opinion in behavioral sciences*, *41*, 1-9.

Zong, W., Zhou, J., Gardner, M. P., Costa, K. M., Zhang, Z., & Schoenbaum, G. (2023). Schema cell formation in orbitofrontal cortex is suppressed by hippocampal output. *bioRxiv*, 2023.2005. 2003.539307.