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Sarah Rose Thomas March 28, 2022

Sex Differences in the Ventral Hippocampus to Lateral Septum Pathway in Modulating Social Recognition Memory

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An abstract of
a thesis submitted to the Faculty of Emory College of Arts and Sciences
of Emory University in partial fulfillment
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Abstract

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The ability to discriminate between novel and familiar individuals is central to human social life. Deficits in social recognition memory are core symptoms in several neurological diseases such as Alzheimer's disease and Autism Spectrum Disorder. Despite the debilitating nature of these diseases, the neural circuits underlying social recognition memory remain poorly understood. The ventral hippocampus has been implicated in social recognition memory. The ventral hippocampus, however, projects to several downstream regions including the lateral septum, a region involved in regulating a wide number of behaviors such as aggression, feeding, and kinship behavior. Previous studies performed by our lab have established that the ventral hippocampus to lateral septum pathway modulates social recognition memory in male mice. Yet, it remained unknown whether the pathway plays a similar role in female mice. In this study, we combined intersectional viral strategies, chemogenetics, and histology in rodent models to probe the role of the ventral hippocampus to lateral septum pathway in mediating social recognition memory in female mice. Similar to results seen in male mice, we found that inhibition of the pathway disrupted social recognition memory in female mice, suggesting that the ventral hippocampus to lateral septum circuit regulates social recognition memory in both male and female mice. Future studies that further examine this pathway will shed light on the neural circuits underlying social recognition memory and answer the fundamental question, "How do we recognize each other?"

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Acknowledgements

I would like to thank Dr. Malavika Murugan for accepting me into her lab and mentoring me on cutting-edge research techniques. My time in her lab has been an exciting and memorable part of my undergraduate experience.

I would like to thank Maha Rashid for being both my mentor and friend for the past two years. Without her constant encouragement and baked pastries, I would not have been able to complete my project.

I would like to thank my fellow lab members, Sonia Karkare for her puns that always made me laugh, and Jenni Isaac for her invaluable conversations and insight. I would also like to thank all the members of the Murugan lab for supporting me through my project and making my time in lab enjoyable and fun.

I would like to thank my grandfather, Daniel George, and my grandmother, Saramma George, for their constant prayers and unwavering belief in my abilities.

I would like to thank my father, Thomas George, my mother, Minimol Thomas, and my sister, Mariya Thomas for being my anchor in difficult times and for always believing in me despite my own doubts.

Finally, I would like to thank my friends, Lingyuan Wu, Kris Stallings, and Gracelyn Chen, for helping me finish my project and giving me the best senior year.

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Introduction

Social recognition memory, the ability to discriminate between a novel and familiar conspecific, is fundamental to mammalian social life. It is necessary for the survival of many species as a wide range of social behaviors such as finding mates, establishing hierarchies, and forming communities depend on the ability to recognize a conspecific. In humans, social recognition deficits often accompany neurological diseases such as Alzheimer's disease, a form of dementia characterized by progressive memory loss, Autism Spectrum disorder, a disorder marked by impaired social abilities, and prosopagnosia, an inability to recognize familiar faces (Endo et al., 2007; Mu & Gage, 2011). Although the symptoms of these conditions are well-known, the neural circuits underlying social recognition memory remain poorly understood. Previous studies in our lab have investigated the role of the ventral hippocampus (vHPC) and its projection to the lateral septum (LS) in mediating social recognition memory. However, there has been a notable lack of study of social recognition memory in female mice, and it remains unclear whether the vHPC-LS pathway in female mice is involved in discriminating familiar from novel animals. In this study, we used inhibitory chemogenetic methods to investigate if the vHPC-LS pathway plays a necessary role in mediating social recognition memory in both male and female mice.

Hippocampus Anatomy

A key brain region implicated in social recognition memory is the hippocampus (HPC) (Meira et al., 2018; Okuyama et al., 2016). Named after the Greek word for horse and sea-monster due to the structure's resemblance to a seahorse, the HPC is located deep in the medial temporal lobe and consists of the dentate gyrus, the hippocampus proper (cornu ammonis fields),

and the subiculum (Engelhardt, 2016). The HPC receives information from other parts of the medial temporal lobe such as the perirhinal cortex, parahippocampal cortex, and entorhinal cortex (Broadbent et al., 2004). The entorhinal cortex receives sensory inputs and projects to excitatory granule cells of the dentate gyrus via the perforant pathway, marking the beginning of the notable "trisynaptic circuit" within the HPC (Witter et al., 2017). In turn, the dentate gyrus sends signals to excitatory pyramidal cells of the CA3 region via the mossy fiber pathway. The CA3 region synapses onto the CA1 region via Schaffer collaterals, and CA1 projects back to the entorhinal cortex, creating a loop (Fig 1) (Knierim, 2015). While information through the trisynaptic circuit was originally thought to flow in a unidirectional manner, anatomical studies have revealed that information flow through the hippocampus is more complex and interconnected than previously thought (Deguchi et al., 2011). The extensive connectivity found within the HPC points to its role in establishing memories.

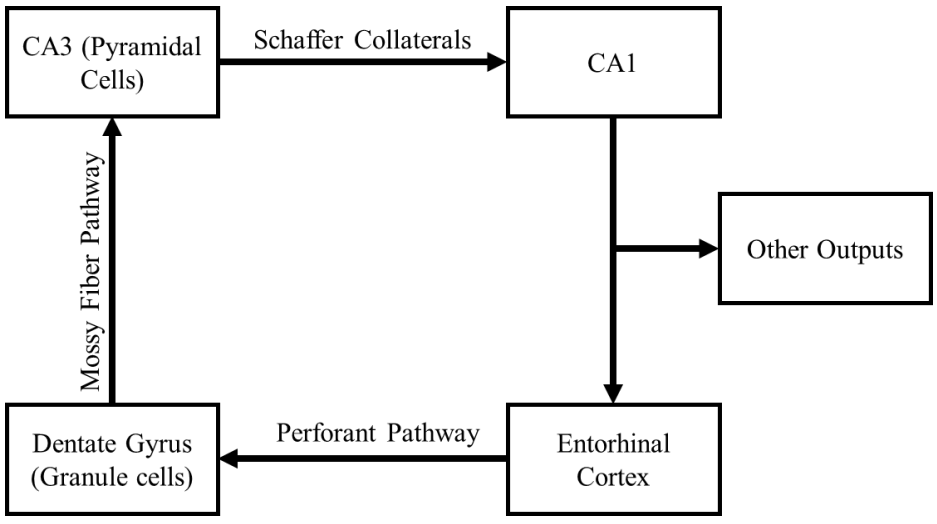

Fig 1: *A simplified example of the trisynaptic circuit found within the HPC.* The entorhinal cortex, via the perforant pathway, projects to the dentate gyrus, which in turn projects to CA3 through the mossy fiber pathway. Schaffer collaterals of the CA3 project to CA1, and CA1 finally projects back to the entorhinal cortex.

Hippocampus Function

Historically, there have been two views regarding the function of the HPC. On the one hand, it has been thought to be involved in declarative and spatial memory. Declarative memory consists of both episodic memory, the ability to remember experiences, and semantic memory, the ability to recall facts and general information. The most famous case underscoring the role of the HPC in declarative memory comes from the study of patient H.M., who was plagued by severe seizures (Scoville & Milner, 1957). To stop his seizures, he underwent surgery to remove his medial temporal lobe, which includes the HPC. After his surgery, patient H.M. no longer suffered from serious seizures, but rather developed profound anterograde amnesia. Although he retained memories from before his surgery, he was incapable of forming new memories, including an inability to remember novel faces. The case of patient H.M., as well as numerous subsequent lesion studies, have highlighted the essential role that the medial temporal lobe, including the HPC, plays in forming and consolidating declarative memories. Furthermore, while the HPC is necessary for the formation of memories, over time, memories become independent of the hippocampus and become distributed to other brain regions (Lehmann et al., 2009). As such, damage to the HPC can prevent the formation of certain types of memories.

In addition to declarative memory, the HPC has been known to be involved in spatial memory. Lesion studies have revealed that damage to the HPC often leads to poor performance in spatial tasks like the Morris water maze in both rodents and humans (Astur et al., 2002; Broadbent et al., 2004; E. Moser et al., 1993). The discovery of place cells and grid cells in the HPC further reinforce the role that the HPC plays in spatial memory (E. Moser et al., 2008). Place cells, which were first recorded in rats, are neurons in the HPC that only fire when the

animal is in a specific location. Neighboring place cells fire at different locations in an environment, providing the animal with an adaptable map of its environment (O'Keefe & Dostrovsky, 1971). Similar to place cells, grid cells, which are found in the entorhinal cortex, also provide spatial information. Unlike place cells, however, these cells fire at multiple locations, forming hexagonal grids that span the entire environment (Fyhn et al., 2004). Together, place cells and grid cells are able to create a dynamic system to confer spatiotemporal information. Thus, the HPC appears to play a significant role in spatial memory.

The HPC has also been closely linked to emotion and stress regulation. Early interest in the role of the HPC in emotion comes from its inclusion in Papez's limbic circuit, and subsequent studies have shown that the HPC plays a major role in emotion (Fanselow & Dong, 2010; Papez, 1937). The HPC is highly susceptible to stress as evidenced by individuals with psychiatric disorders, such as post-traumatic stress disorder (PTSD), often possessing hippocampal alterations (Sala et al., 2004; Shin et al., 2004). Primates with lesions to the region exhibit abnormal emotional responses such as reduced defensive behaviors in response to threatening stimuli (Chudasama et al., 2008). Additionally, the HPC projects to the amygdala, suggesting that the HPC is involved in forming and interpreting emotional memories as well as consolidating memories in a context-dependent manner (Phelps, 2004). The HPC also modulates stress by providing negative feedback to the hypothalamic-pituitary-adrenal (HPA) axis (Jacobson & Sapolsky, 1991). The HPA axis is a homeostatic mechanism in the body that responds to stressors through the release of cortisol that can induce physiological changes such as increasing blood pressure, suppressing the immune system, and raising blood sugar. Glucocorticoid receptors in the HPC respond to increase in HPA axis activity and inhibit its

activity (Zhu et al., 2014). Overall, the HPC has been shown to regulate both emotion and stress.

Thus, there is a rich body of evidence suggesting that the HPC is involved in memory as well as emotion and stress regulation. This functional duality likely arises as a result of differences between the dorsal and ventral regions of the HPC.

Dorsal Hippocampus

The HPC was initially thought to be a single large structure (Moser & Moser, 1998). However, more recent work suggests that the dorsal and ventral regions of the HPC have distinct inputs, outputs, and functions (Fanselow & Dong, 2010). The dorsal hippocampus (dHPC), which corresponds to the posterior hippocampus in primates, receives inputs from regions like the striatum, thalamus, hypothalamus (HPT), and supramammillary nucleus (SUM) and sends excitatory projections to regions like the LS, retrosplenial cortex (RSC), subiculum, and anterior cingulate cortex (ACC) (Fig 2) (Fanselow & Dong, 2010). Studies have implicated the dHPC in spatial memory. The dHPC in rodents was shown to have a greater density of place fields than the ventral HPC (Jung et al., 1994). Consistent with these findings from rodents, taxi drivers had larger posterior hippocampi than control subjects and showed differential activation of the left posterior hippocampus compared to the anterior hippocampus when recalling complex routes (Maguire et al., 2000). Primates showed more activation of the posterior hippocampus during spatial tasks (Colombo et al., 1998). Additionally, lesions to the dHPC, but not ventral HPC, in rodents impaired performance in the Morris water maze (E. Moser et al., 1993). Thus, previous research suggests that the dHPC is involved in spatial memory.

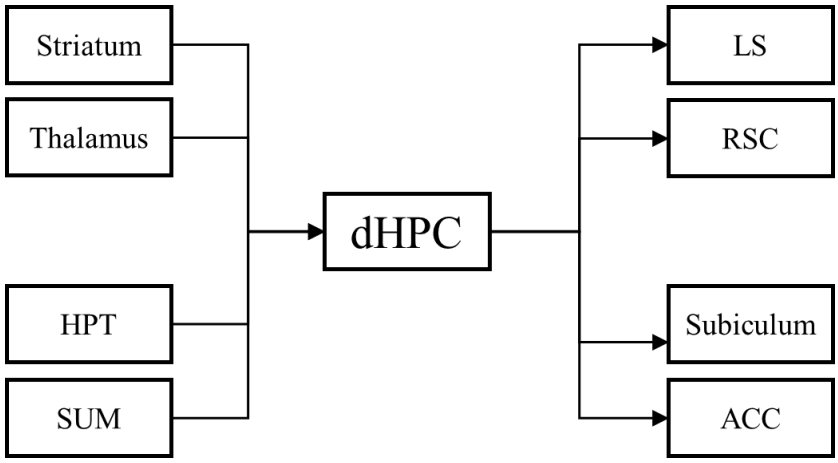

Fig 2: *Notable inputs and outputs of the dHPC.* The dHPC receives inputs from the striatum, thalamus, hypothalamus (HPT), and supramammillary nucleus (SUM) and projects to the lateral septum (LS), retrosplenial cortex (RSC), subiculum, and anterior cingulate cortex (ACC).

Ventral Hippocampus

The ventral hippocampus (vHPC), which corresponds to the anterior hippocampus in primates, is associated with stress and emotional regulation. It receives inputs from regions such as the amygdala, infralimbic cortex (IL), thalamus, and insular cortex and outputs to the olfactory bulb, medial prefrontal cortex (mPFC), nucleus accumbens (NAc), lateral septum (LS), and bed nucleus of the stria terminalis (BNST) (Fig 3) (Fanselow & Dong, 2010). Primate studies revealed that early-life adversity decreased expression of protein kinase C zeta, a kinase thought to be important for long term memory, in the vHPC and led to more timid responses to intruders (Fulton et al., 2021). Following damage to the vHPC, rats spend more time in the open arms of the elevated plus-maze, which is an indicator of low anxiety levels, and have lower corticosterone, indicating that lesions to the vHPC have an anxiolytic effect (Henke, 1990; Kjelstrup et al., 2002). Furthermore, lesions to the vHPC in rodents led to a decrease in fear responses such as freezing and crouching while lesions to the dHPC did not alter fear responses

(Pentkowski et al., 2006). Thus, the vHPC plays a role in modulating stress and emotion.

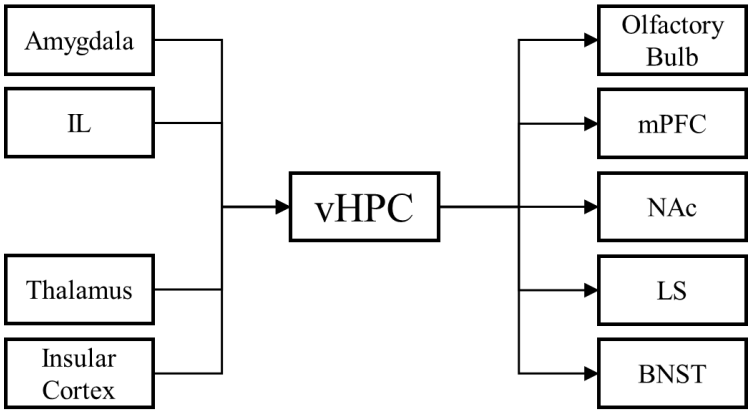

Fig 3: *Notable inputs and outputs of the vHPC.* The vHPC receives inputs from the amygdala, infralimbic cortex (IL), thalamus, and insular cortex and projects to the olfactory bulb, medial prefrontal cortex (mPFC), nucleus accumbens (NAc), lateral septum (LS), and bed nucleus of the striata terminalis (BNST).

More recent evidence has implicated the vHPC in social memory. A projection from the dCA2 to the vHPC was shown to be necessary for social memory. Inhibition of the dCA2-vHPC pathway disrupted social recognition memory in mice. Mice naturally spend more time with a novel mouse than a familiar mouse, and dCA2-vHPC inhibition caused mice to spend an equal amount of time with a novel and familiar conspecific (Meira et al., 2018). In another study, inhibition of the vHPC, but not dHPC, disrupted social recognition memory (Okuyama et al., 2016). Notably, the vHPC has outputs to regions, such as the mPFC, NAc, and LS, traditionally associated with social behaviors. The mPFC is involved in higher order processing of memory in humans and has been implicated in sexual recognition of a conspecific and social investigation in rodents (Euston et al., 2012; Kingsbury et al., 2020; Murugan et al., 2017). The NAc is most known for its role in reward and motivation, but a recent study has indicated that disruption of the vCA1-NAc pathway leads to deficits in social memory in mice (Okuyama et al., 2016). In

this study, we were most interested in the projection of the vHPC to the LS, a brain region that receives dense inputs from the vHPC (Risold et al., 1997).

Lateral Septum

The LS is an under-researched brain region that has been implicated in driving motor and social behavior. It receives inputs from the amygdala, HPT, thalamus, midbrain, hindbrain, and HPC and projects to many brain regions such as the HPT, VTA, NAc, medial septum, and the periaqueductal gray (PAG) (Fig 4) (Deng et al., 2019). Based on its projections from the HPC, the LS can be divided into four subregions: dorsomedial (dm), dorsolateral (dl), ventrolateral (vl), and ventromedial (vm). All subregions predominantly contain inhibitory GABAergic projection neurons and interneurons, which indicates that the LS plays an important modulatory role within itself and throughout the brain (Rizzi-Wise & Wang, 2021).

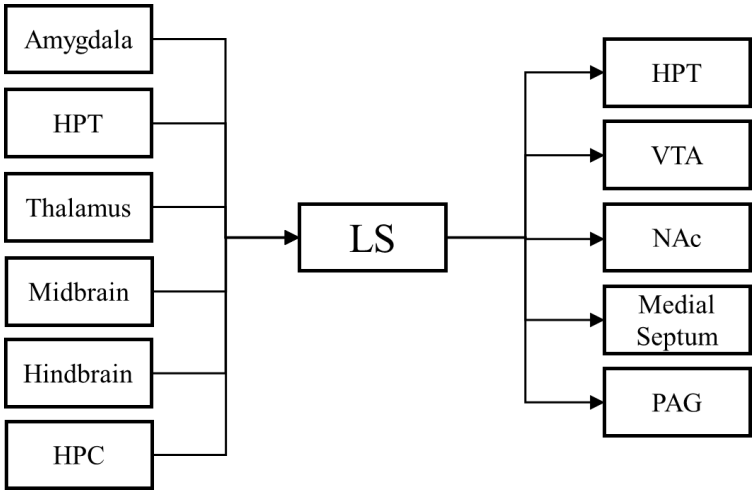

Fig 4: *Notable inputs and outputs of the LS.* The LS receives inputs from the amygdala, hypothalamus (HPT), thalamus, midbrain, hindbrain, and hippocampus (HPC) and projects to the HPT, ventral tegmental area (VTA), nucleus accumbens (NAc), medial septum, and the periaqueductal gray (PAG).

The LS is involved in a wide range of behaviors such as reward, aggression, feeding and

anxiety. Rats self-stimulated the LS, and inhibition of the brain region led to a decrease in drug-seeking behavior, pointing to the role of the LS in reward (Olds & Milner, 1954; Pantazis & Aston-Jones, 2019). Early studies revealed that lesions to the lateral septum led to septal rage where animals exhibit highly aggressive behaviors (Sheehan et al., 2004). More recent evidence has shown that vasopressin increased social aggression by binding to CA2 terminals in the LS (Leroy et al., 2018). Additionally, the LS contains receptors for ghrelin and glucagon-like peptide 1 (GLP-1), neuropeptides that mediate feeding behavior (Terrill et al., 2016, 2018). Furthermore, activation of the glutamatergic vHPC-LS pathway reduced food intake in rodents (Sweeney & Yang, 2015). Activation of the paraventricular hypothalamus (PVN) to ventral LS pathway decreased feeding in mice while inhibition of the pathway led to an increase in feeding (Xu et al., 2019). Studies have revealed that chemogenetic activation of the vHPC-LS pathway decreased anxiety while inhibition of the pathway promoted anxiety (Parfitt et al., 2017). Notably, optogenetic activation of certain LS neurons has been shown to increase anxiety while inhibition had the opposite effect (Anthony et al., 2014). The conflicting role of the LS in modulating anxiety may point to the competing activity of different subregions.

In this study, we were interested in the role of the LS in mediating memory, a function that remains poorly understood. The LS is enriched in neuropeptide receptors for oxytocin and vasopressin (Bredewold et al., 2014). Studies have shown that vasopressin receptors in the LS were necessary and sufficient for social recognition (Bielsky et al., 2005). Furthermore, mice lacking the oxytocin gene exhibited social amnesia, suggesting that the neuropeptide is involved in social memory (Ferguson et al., 2000). More recently, a study revealed that lesions to the LS disrupted sibling preference in young rats, highlighting the role of the LS in kinship behavior and

social memory (Clemens et al., 2020). Although both the vHPC and LS have been separately involved in memory, no study has elucidated the role of the vHPC to LS pathway in regulating social recognition memory.

Previous experiments in the lab have established that the vHPC-LS pathway mediates social recognition memory in male mice. Yet, it remains unclear whether the pathway plays a similar role in female mice. Previous research indicates the vasopressin system is sexually dimorphic with males having more vasopressin fibers in the lateral septum than females, which could influence social behavior (De Vries & Panzica, 2006). The estrous cycle and stress have been shown to be involved in spatial memory with acute stress impairing performance in spatial tasks in male mice but not female mice (Conrad et al., 2004). Furthermore, ovariectomized rats performed worse on spatial and non-spatial memory tasks, suggesting that ovarian hormones play a role in maintaining memory in female mice (Wallace et al., 2006). While earlier studies have investigated whether hormones modulate behavior in female mice, such studies often involved removal of key regions or administration of hormones, which can have widespread and non-specific effects. Additionally, female subjects have been historically excluded from research over concerns regarding changing hormones and estrous cycles. While the U.S. National Institutes of Health now requires the inclusion of females in clinical studies, a recent study has revealed that sex differences are often misreported leading to exaggerated or overlooked differences between male and female subjects (Garcia-Sifuentes & Maney, 2021). For these reasons, there is a need to study this historically excluded and misrepresented population.

In this study, we explored if there is a sex difference in the vHPC-LS pathway in mediating social recognition memory through viral targeting strategies, chemogenetics,

behavioral assays, and histology in rodent models. We found that inhibition of the vHPC-LS pathway disrupted the recall of social recognition memory in both male and female mice. Furthermore, inhibition did not influence general investigative behavior, object preference, or anxiety levels. Ultimately, the study aimed to examine the neural circuits underlying social recognition memory and investigate the fundamental question, “How do we recognize each other?”

Materials and Methods

All protocols conformed to the Guidelines for the Care and Use of Laboratory Animals of the National Institutes of Health and were approved by the Emory University Institutional Animal Care and Use Committee.

Subjects

Subjects used in the study were C57BL/6J wild-type male and female mice obtained from the Jackson Laboratory. All mice were group housed in a 12 hour (light: 7am – 7pm) reverse light/dark cycle with food and water. All experiments were performed during the dark cycle of the animals.

Stereotaxic Surgery

Mice were anaesthetized using 5% isoflurane. Viruses were injected using a glass micropipette attached to a Nanoject III to microinject. The Nanoject was used to control the speed and volume of virus injections. Mice were given meloxicam (5 mg/kg) as an analgesic and allowed to recover for three weeks before all subsequent experiments. For behavioral experiments, bilateral viral delivery was aimed at coordinates relative to Bregma. vHPC

injections were targeted to AP: ± 3 mm, ML: -3.250 mm, DV: - 4.2 mm while LS injections were targeted to AP: ± 0.4 mm, ML: 0.00 mm, DV: - 2.8 mm.

Designer Receptors Exclusively Activated by Designer Drugs (DREADDs)

The vHPC-LS pathway was inhibited in both male mice and female mice using Designer Receptors Exclusively Activated by Designer Drugs (DREADDs). DREADDs is a technique that can selectively manipulate brain regions through engineered G protein-coupled receptors (GPCRs) that are activated by inert molecules such as clozapine-N-oxide (CNO). In this study, Retro AAV-hSyn Cre (500 nL) was injected in the LS while Retro AAV5-hSyn-DIO-hM4D(Gi)-mCherry (500 nL) was injected in the vHPC. Control mice were injected with Retro AAV-hSyn-Cre (500 nL) in the LS and AAV5-hSyn-DIO-mCherry (250 nL) in the vHPC. Retro AAV-Cre is a retrogradely transporting adeno-associated virus (AAV) expressing Cre recombinase. When injected in the LS, it enables the expression of inhibitory Cre-dependent hM4D(Gi) proteins in the vHPC-LS pathway. For this study, the virus was allowed to express in target neuronal populations for three weeks before behavioral assays were performed. The mice were given intraperitoneal 0.1 mL injections of saline (control) or CNO (1 mg/kg) at least 30 minutes before beginning behavioral assays. CNO is a biologically inert molecule that is metabolized by mice and removed from the body in approximately 24 hours, causing its effects to cease.

Social Discrimination Task (SDT)

Social recognition behavior was measured using the well-established social discrimination task (SDT) in four conditions: novel-familiar conspecific, novel-novel conspecific, novel object, and novel-familiar food (Fig 5). In the novel-familiar conspecific SDT, a test mouse freely roamed a chamber containing a novel mouse and a familiar mouse for five

minutes to measure social recognition memory (Fig 5A). The amount of time the test mouse spent with each social target was determined using Bonsai and MATLAB. The target mice were placed in two pencil holders (diameter = 7.62 cm) at opposite ends of a chamber (58.42 cm x 25.4 cm x 22.86 cm). Test mice were pair-housed with social targets for 72 hours to familiarize. In the novel-novel SDT, test mice roamed a chamber containing two novel mice for five minutes to examine general investigative behavior (Fig 5B). In the novel object task, test mice roamed a chamber with a novel toy and familiar toy to measure preference for objects (Fig C). Mice were housed with familiar toys for 72 hours to familiarize. In the novel-familiar food SDT, test mice freely roamed a chamber containing novel food (fruit loops) and familiar food (standard rodent diet) for five minutes to measure food preference (Fig 5D).

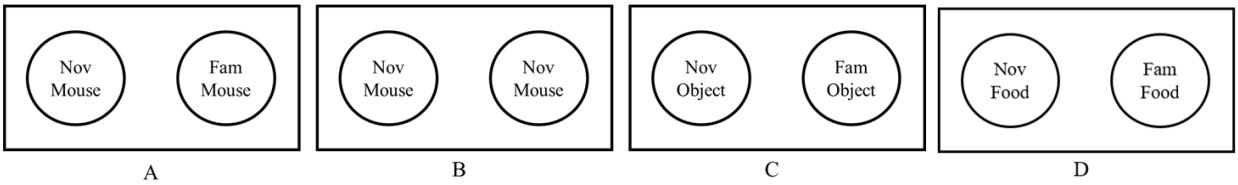

Fig 5: *The SDT was performed in four different conditions*: A) novel-familiar conspecific, B) novel-novel conspecific, C) novel object, D) and novel-familiar food.

Open Field Assay

Anxiety levels were measured using the open field assay. Mice were allowed to freely roam in a square chamber (40.64 cm) for ten minutes. The amount of time a mouse spends in the middle of the box as opposed to the edges of the box were determined through Bonsai and MATLAB. Greater time spent at the edges of the box served as an indicator of higher anxiety levels.

Histology and Imaging

After all behavioral assays were completed, the mice were perfused with 4%

paraformaldehyde (PFA), a fixative agent that preserved the brain, in phosphate buffered saline (PBS). After extraction, each brain was stored in PFA for 24-48 hours and then transferred to sucrose. Histology was performed on a microtome at 50 µm, and coronal brain slices are mounted on glass slides. Slides were cover slipped with DAPI to seal. Brain slices were imaged using a Keyence (BZ-X810) to confirm viral targeting and expression of virus. The brains were imaged in DAPI and mCherry.

Statistical Analysis

Behavioral data was analyzed using MATLAB and GraphPad Prism. The social discrimination tasks were analyzed using a two-way repeated measures analysis of variance (ANOVA). We were interested in determining whether there was a significant difference in time spent near a social target across virus type (mCherry/hM4Di) and conspecific identity (novel/familiar) within male and female mice. A post-hoc paired t-test was used to analyze which comparisons resulted in the largest change in time spent. Results from the open field assay were analyzed using an ordinary one-way ANOVA and Tukey's test to determine if there was a significant difference across virus type.

Results

Inhibition of the vHPC-LS pathway disrupted social recognition in both male and female mice

Previous experiments performed in the lab have revealed that the vHPC-LS pathway modulates recall of social recognition memory in male mice. To determine, whether the circuit plays a similar role in female mice, we inhibited the vHPC-LS pathway in both male and female mice using Designer Receptors Exclusively Activated by Designer Drugs (DREADDs). Both male and female mice were bilaterally injected with the inhibitory Cre-dependent protein,

hM4Di, in the vHPC and a retrogradely transporting Cre (retro AAV-Cre) in the LS, allowing us to specifically inhibit neurons in the vHPC-LS pathway. Control mice were injected with Cre-dependent mCherry in the vHPC and retro Cre in the LS. Viral injections were confirmed through histology (Fig 6AD).

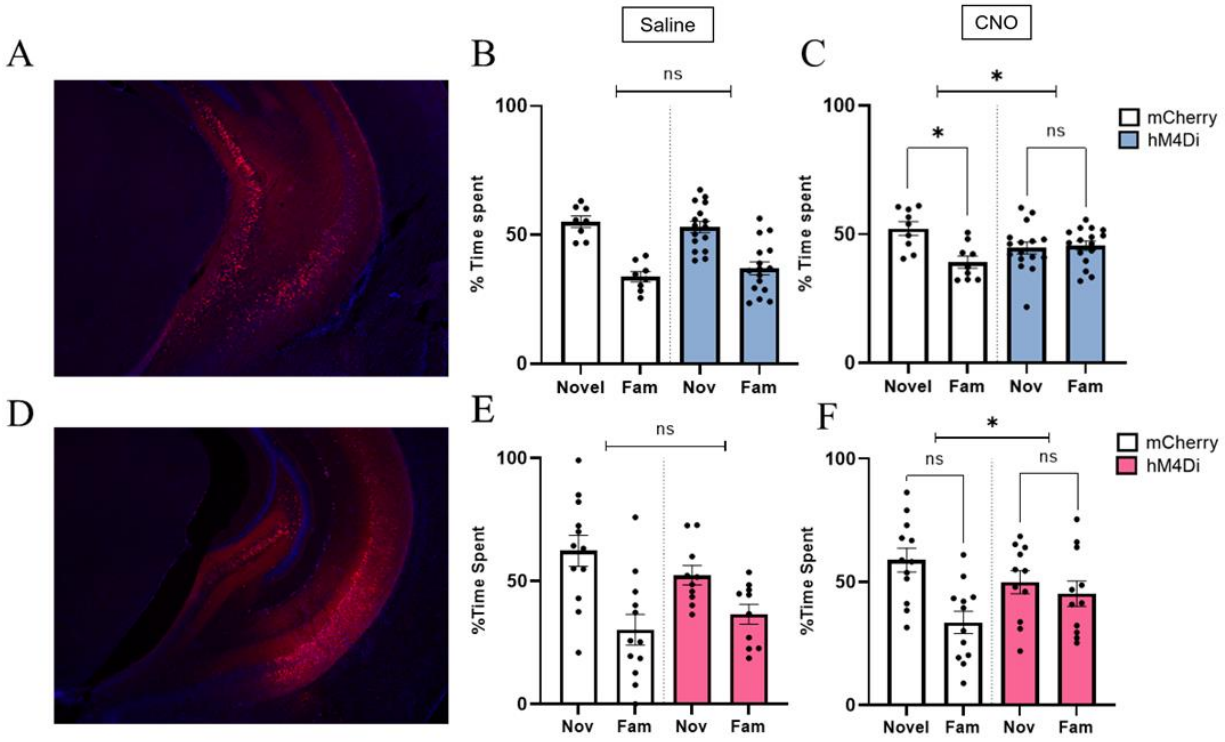

Fig 6: *Inhibition of the vHPC-LS pathway disrupted social recognition memory.* A) vHPC section showing hM4Di-mCherry expression in an example male mouse. B) Percent time male control (n=9) and DREADDs (n = 16) mice spent interacting with a novel or familiar conspecific in the saline condition of the novel-familiar SDT. C) Percent time male control and DREADDs mice spent interacting with a novel or familiar conspecific in the CNO condition of the novel-familiar SDT. D) vHPC section showing hM4Di-mCherry expression in a female mouse. E) Percent time female control (n=12) and DREADDs (n = 11) mice spent interacting with a novel or familiar conspecific in the saline condition of the novel-familiar SDT. F) Percent time female control and DREADDs mice spent interacting with a novel or familiar conspecific in the saline condition of the novel-familiar SDT.

Social recognition behavior was measured using the novel-familiar conspecific social discrimination task (SDT). Mice freely explored a chamber containing an encaged novel mouse on one end and an encaged familiar mouse at the other end, and the amount of time test mice spent in the vicinity of each social target was determined (Fig 5A). When saline was administered, both male control and DREADDs mice spent more time with a novel mouse than a familiar mouse (2-way ANOVA, p=0.3179), which was expected as mice naturally spend more time with a novel rather than a familiar conspecific (Fig 6B). Inhibition through CNO administration caused male DREADDs mice (paired t-test, p=0.8276), but not control mice (paired t-test, p=0.0311), to spend an equal amount of time with both novel and familiar conspecifics, suggesting deficits in recall of social recognition memory (Fig 6C). Similarly, in the saline condition, female mice interacted more with a novel conspecific (2-way ANOVA, p=0.1478) as expected (Fig 6E). When the vHPC-LS pathway was inhibited (2-way ANOVA, p=0.0374), female DREADDs mice showed no preference for a novel social target (paired t-test, p=0.6420), indicating that social recognition memory was impeded (Fig 6F). Notably, control female mice in the CNO condition spent more time with novel mice as expected when viewing the data visually. However, the trend was not statistically significant which may be due to the small sample size (paired t-test, p=0.0716). Overall, the results suggest that inhibition of the vHPC-LS pathway disrupted recall of social recognition memory in both male and female mice, which supported our hypothesis. Yet, it was unclear if inhibition could be altering investigation rather than memory.

Inhibition did not influence investigation

To explore whether inhibition of the vHPC-LS pathway could influence general investigation, we performed the novel-novel SDT. Mice explored a chamber containing two novel mice on each end (Fig 5B). We expected test mice to display no preference for either social target. In the saline condition, male (2-way ANOVA, *p=0.6585)* and female (2-way ANOVA, *p*=0.5556) mice spent the same amount of time with both novel social targets (Fig 7AC). When CNO was administered, male (2-way ANOVA, *p*=0.5212) and female (2-way ANOVA, *p*=0.5529) mice continued to spend the same amount of time with each social target, suggesting that inhibition did not greatly alter general investigative behavior in mice (Fig 7BD). Next, we examined whether the vHPC-LS pathway would alter object preference.

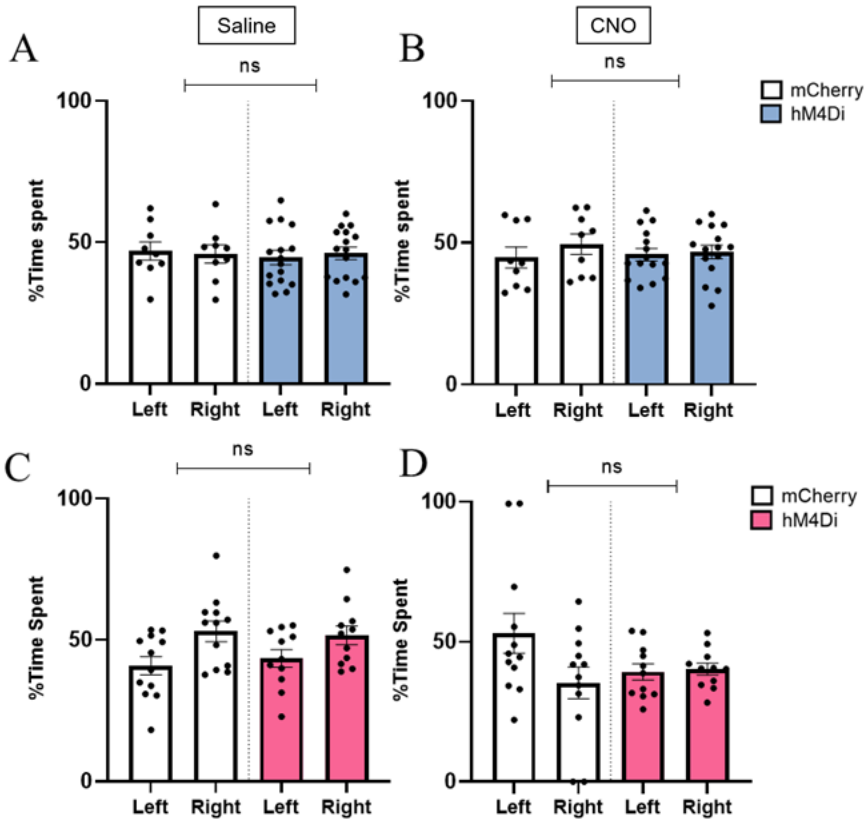

Fig 7: *Inhibition of the vHPC-LS pathway did not influence investigation.* A) Percent time male control (n = 9) and

DREADDs (n = 16) mice spent investigating two novel mice in the saline condition of the novel-novel SDT. B) Percent time male control and DREADDs mice spent investigating two novel mice in the CNO condition of the novel-novel SDT. C) Percent time female control (n = 12) and DREADDs (n = 11) mice spent investigating two novel mice in the saline condition of the novel-novel SDT. D) Percent time female control and DREADDs mice spent investigating two novel mice in the CNO condition of the novel-novel SDT.

Inhibition did not impact preference for objects

We determined whether inhibition could modify investigation of objects by allowing mice to explore a chamber containing a novel and familiar toy (Fig 5C). We expected mice to spend more time with a novel object rather than a familiar object. Surprisingly, male control and DREADDs mice in the saline condition spent the same amount of time with each object (2-way ANOVA, p=0.7664) (Fig 9A). Inhibition with CNO did not modify preference across groups (2-way ANOVA, p=0.4833) (Fig 9B). Similarly, female control and DREADDs mice had no preference for either object in the saline condition (2-way ANOVA, p=0.0701), and inhibition did not alter this preference (2-way ANOVA, p=0.9641) (Fig 9CD). The results suggest that inhibition does not alter preference for objects.

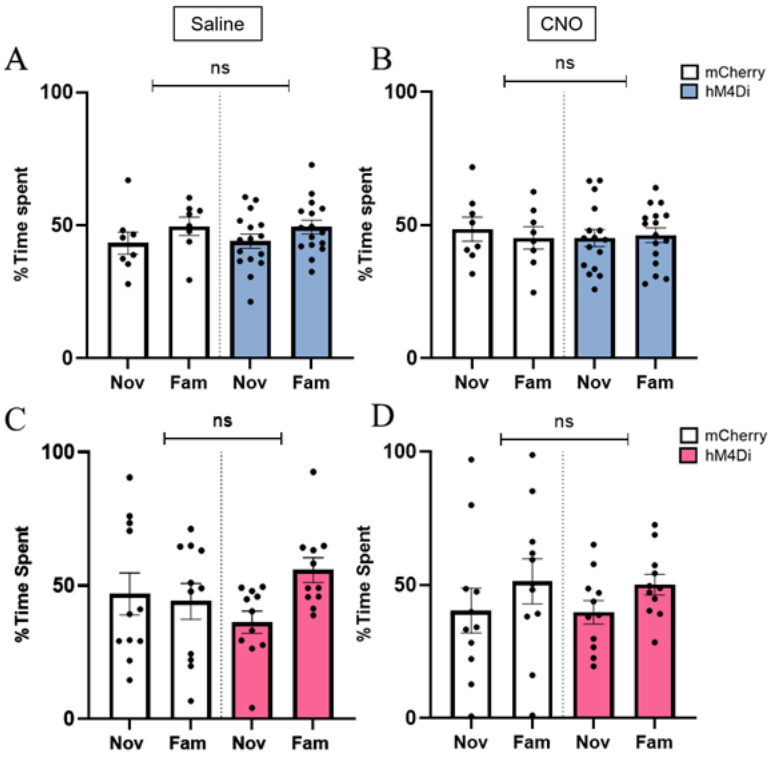

Fig 8: *Inhibition did not influence preference for objects.* A) Percent time male control (n = 8) and DREADDs (n = 16) mice spent interacting with a novel and familiar toy in the saline condition of the novel-familiar object SDT. B) Percent time male control and DREADDs mice spent interacting with a novel and familiar toy in the CNO condition of the novel-familiar object SDT. C) Percent time female control (n = 11) and DREADDs (n =11) mice spent interacting with a novel and familiar toy in the saline condition of the novel-familiar object SDT. D) Percent time female control and DREADDs mice spent interacting with a novel and familiar toy in the CNO condition of the novel-familiar object SDT.

Inhibition and food preference

Since the LS has been implicated in feeding behaviors, we performed the novel-familiar food SDT to explore if inhibition would modify food preference. Mice were placed in an arena containing encaged fruit loops (novel food) and standard rodent diet (familiar food), and the amount of time test mice spent with each food type was determined (Fig 5D). When saline was

administered, male control and DREADDs mice (2-way ANOVA, p=0.2021) spent the same amount of time with both food types (Fig 8A). When CNO was administered, both control and DREADDs male mice spent slightly more time with the familiar food than the novel food (Fig 8B). Yet, there was no statistical difference across the two groups (2-way ANOVA, p=0.3767). On the other hand, when saline was administered to female mice, there was significant difference between the control and DREADDs group (2-way ANOVA, p=0.0157) (Fig 8C). Control mice exhibited no preference for either food type (paired t-test, p=0.6856) while DREADDs mice spent significantly more time with familiar food than novel food (paired t-test, p=0.0228). In the CNO condition, there was no difference between the control and DREADDs group (2-way ANOVA, p=0.3717) (Fig 8D). Similar to the male group, both groups of mice spent more time with the familiar food than the novel food. The discrepancies in the results suggest that the food assay is too variable to determine the effects of inhibition of food preference. Additional experiments will be necessary to fully parse out the role of the vHPC-LS pathway in mediating social recognition memory.

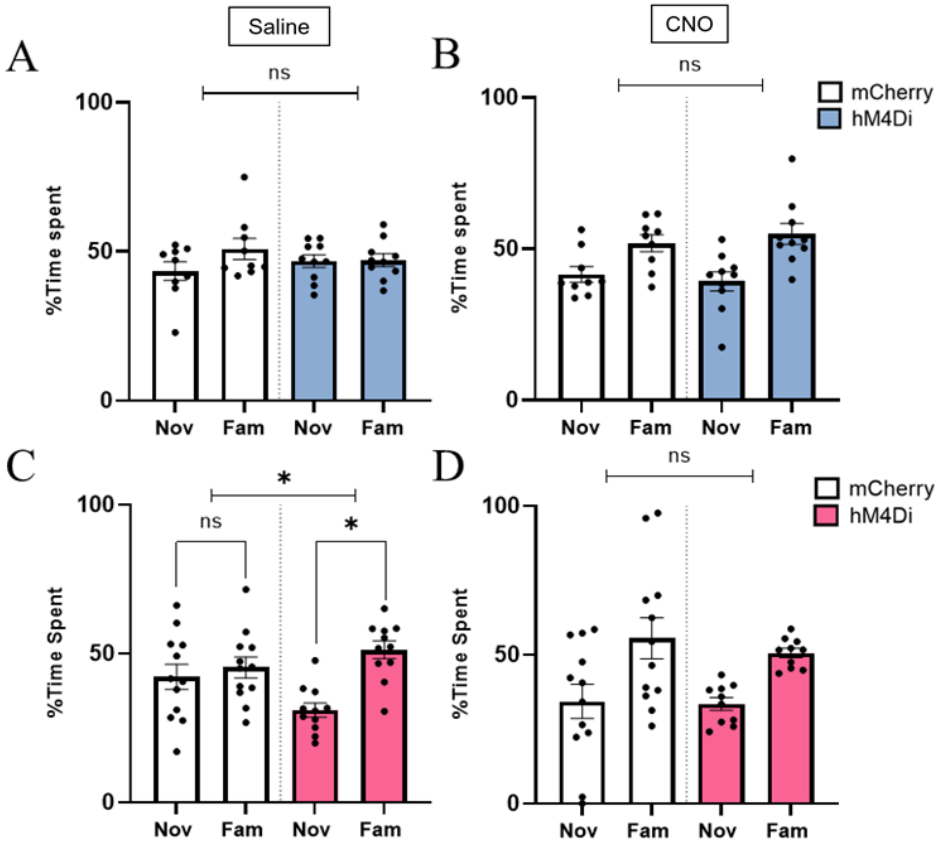

Fig 9: *Inhibition and food preference*. A) Percent time male control (n = 9) and DREADDs (n = 10) mice spent interacting with a novel (fruit loops) and familiar (standard rodent diet) food in the saline condition of the novel-familiar food SDT. B) Percent time male control and DREADDs mice spent interacting with novel and familiar food in the CNO condition of the novel-familiar food SDT C) Percent time female control (n = 12) and DREADDs (11) mice spent interacting with novel and familiar food in the saline condition of the novel-familiar food SDT. D) Percent time female control (n = 12) and DREADDs (11) mice spent interacting with novel and familiar food in the saline condition of the novel-familiar food SDT.

Inhibition of the vHPC-LS pathway is not anxiogenic

A previous study found that chemogenetic inhibition of the vHPC-LS pathway caused an increase in anxiety-related behaviors (Parfitt et al., 2017). As such, we performed the open field assay to investigate whether inhibition could influence anxiety and locomotion. Mice were allowed to freely explored a large chamber, and the amount of time spent in the center of the

chamber was determined as a measure of anxiety. We also measured the total distance traveled by the mice to investigate locomotive activity. Male control and DREADDs mice spent the same amount of time in the center zone in the saline (1-way ANOVA, p=0.9871) and CNO (1-way ANOVA, p=0.7814) condition, suggesting that inhibition did not influence anxiety in male mice (Fig 10A). DREADDs male mice travelled significantly more distance in the saline condition than control male mice (1-way ANOVA, p=0.0110) (Fig 10B). When CNO was administered, however, there was no difference between the control and DREADDs group, suggesting that locomotion was not influenced (1-way ANOVA, p=0.7814). Both control and DREADDs mice spent the same amount of time in the center zone regardless of condition (1-way ANOVA, saline: p=0.6234, CNO: p=0.9998) (Fig 10C). Female mice also travelled the same distance after saline (1-way ANOVA, p=0.1304) and CNO (1-way ANOVA, p=0.1082) administration (Fig 10D). Overall, the results suggest inhibition did not greatly influence anxiety or locomotion in both male and female mice.

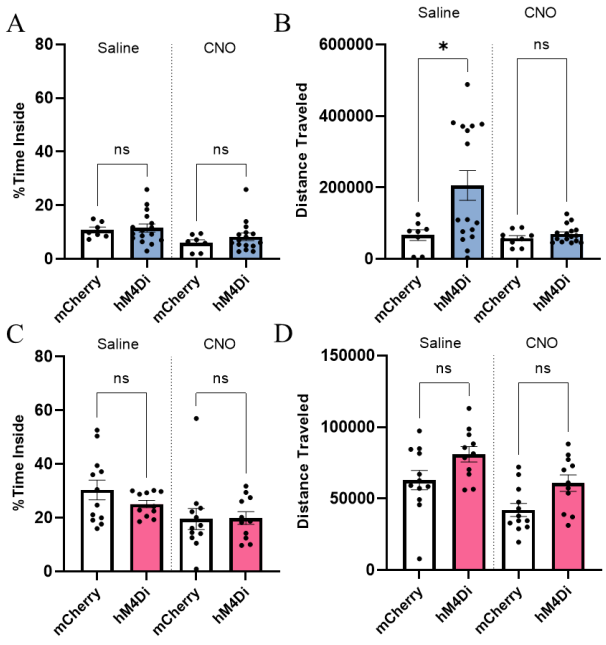

Fig 10: *Inhibition of the vHPC-LS pathway did not alter anxiety*. A) Percent time male control (n = 7) and DREADDs (n = 16) mice spent in the center zone of the open field assay. B) Distance travelled by male mice in the open field assay. C) Percent time female control (n = 12) and DREADDs (n = 11) mice spent in the center zone of the open field assay. D) Distance travelled by female mice in the open field assay.

Discussion

In this study, we found that inhibition of the vHPC-LS pathway led to deficits in recall of social recognition memory in both male and female mice. Inhibition did not alter general investigative behavior, object preference, anxiety levels, or locomotion. or alter preference for objects, suggesting that the pathway is socially specific. Overall, the results indicate that the vHPC-LS pathway is necessary for the recall of social recognition memory in both male and female mice.

Social Recognition Memory

The ability to discriminate between novel and familiar conspecifics is fundamental for the formation and maintenance of social bonds. Yet, the neural circuits that underlie social recognition memory are poorly understood. Numerous studies have shown that the HPC is essential for declarative memory (Fanselow & Dong, 2010; Knierim, 2015). The LS, on the other hand, is implicated in a wide range of distinct behaviors such as anxiety, feeding, and kinship memory (Clemens et al., 2020; Parfitt et al., 2017; Sweeney & Yang, 2015). The LS not only receives large projections from the HPC but also outputs to downstream regions involved in social behaviors such as the NAc, VTA, and mPFC (Kingsbury et al., 2020; Okuyama et al., 2016). The widespread connectivity of the LS enables it link the HPC to key brain regions and modulate social behaviors like social recognition memory. While previous studies performed by

our lab have suggested that the vHPC-LS pathway is necessary for recall of social recognition memory in male mice, this study revealed that the pathway is also necessary in female mice.

Yet, many questions remain about the nature of the social information found in the vHPC-LS pathway. Inhibition of the pathway caused mice to spend an equal amount of time with both a novel mouse and a familiar mouse, suggesting that inhibition may make a novel mouse appear familiar or a familiar mouse appear novel. Inhibition may prevent mice from accessing the memory of a familiar conspecific, which would disrupt recall, or alter the saliency of a novel or familiar conspecific, which may alter social motivation. The circuit might also be involved in encoding representations or engrams of social memory (Tonegawa et al., 2015). Our results suggest that the vHPC-LS pathway is a promising circuit for future studies into memory.

Since the ability to discriminate between novel and familiar is a complex process, the vHPC-LS pathway may be involved in a larger network of brain regions that modulate social recognition memory. A recent study found that neurons in the dorsal CA2 (dCA2) of the HPC encode both social familiarity and identity of a conspecific (Boyle et al., 2022). The dCA2-vHPC pathway was also shown to be necessary for social recognition memory (Meira et al., 2018). Additionally, our lab has found through monosynaptic rabies tracing experiments that the vHPC projects to the LS, which in turn sends distinct projections to both the NAc and VTA, brain regions implicated in social investigation (Isaac, unpublished; Okuyama et al., 2016). An interesting point of study would be to investigate the role of regions downstream of the LS.

Social Investigation and Object Preference

Could inhibition modify factors besides social recognition memory? We found that manipulation of the vHPC-LS pathway did not influence general investigative behavior in the novel-novel

SDT nor alter preference for objects in the novel object task. Although we expected mice to spend more time with a novel object rather than a familiar object in the novel object SDT, mice surprisingly spent an equal amount of time with both objects. Social investigation is a dynamic process because it contains elements of smell, noise, and interaction. Having the objects encaged and inaccessible to the mice may make it difficult for the mice to discriminate between the objects, leading to a lack of preference. Future experiments where the mice have access to the objects may resolve the discrepancies seen in the control conditions.

Food Preference

A previous study found that chemogenetic inhibition of the vHPC-LS pathway increased food intake in rodents (Sweeney & Yang, 2015). We performed the novel-familiar food SDT to determine whether inhibition could influence response to a salient feature such as food. Since mice are neophobic, we expected them to spend more time near the vicinity of familiar food than novel food. However, the assay did not yield meaningful results due to high variability. The discrepancies in the data may be due to the small sample size or the results may be dependent on the hunger state of the mice. While we cannot make any conclusions about the impact of inhibition on food preference, future experiments with larger sample sizes and that control for hunger states may provide more insight into the role of this pathway in food behaviors.

Anxiety and Locomotion

Previous experiments have implicated the vHPC and the LS in regulating anxiety levels. A recent study found that chemogenetic inhibition of the vHPC-LS pathway increased anxiety-related behaviors in the open field assay and elevated plus maze (Parfitt et al., 2017). Thus, we wanted to determine whether the changes in preference were due to alterations in memory or due

to alterations in anxiety and velocity through the open field assay. Contrary to the previous study, inhibition did not increase anxiety-related behaviors in either sex. Notably, the previous study used a different adeno-associated virus (AAV) serotype than the one used in this study. While the previous study used an AAV8 strain, we utilized an AAV5 strain. The deviation between the two studies may be due to the differing AAV strains as different serotypes can have varying infectivity and levels of expression (Hammond et al., 2017).

Limitations and Future Directions

DREADDs technology alone cannot sufficiently parse out the function of the vHPC-LS circuit. Interestingly, a study found that CNO may not be pharmacologically inert in mice, indicating that administration of the drug may have unintended behavioral effects (Manvich et al., 2018). Off-target effects of CNO may explain some of the variation seen during behavioral assays. However, control mCherry mice that were administered CNO did not exhibit large changes in behavior, suggesting that CNO did not greatly influence behavior in our experiments. Additionally, DREADDs technology is not temporally specific and cannot reveal the type of social information encoded by the vHPC-LS pathway. Utilizing a technique with temporal specificity, such as optogenetics, and recording neural activity through calcium imaging will further shed light on the role of this pathway in modulating social recognition memory.

Social recognition memory is most likely an emergent property that arises from the activity of many different brain regions working together. Thus, it is necessary to study other brain regions and their connections to fully understand the neural circuits underlying social recognition memory. Our results express that the vHPC-LS pathway is a promising pathway for the study of social recognition memory, and future studies should not only elucidate the function

of the vHPC-LS pathway but also investigate the necessity and sufficiency of further downstream circuits.

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