



HAL
open science

Investigating the role of hippocampo-thalamic-cortical communication during memory consolidation and fear expression

Charlotte Andrews

► **To cite this version:**

Charlotte Andrews. Investigating the role of hippocampo-thalamic-cortical communication during memory consolidation and fear expression. Neuroscience. Université Paris sciences et lettres, 2023. English. NNT: 2023UPSL045 . tel-04457300

HAL Id: tel-04457300

<https://pastel.hal.science/tel-04457300v1>

Submitted on 14 Feb 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



THÈSE DE DOCTORAT
DE L'UNIVERSITÉ PSL

Préparée à Ecole supérieure de physique et de chimie industrielles de la ville de Paris

Laboratoire Plasticité du Cerveau UMR 8249

Investigating the role of hippocampo-thalamic-cortical communication during memory consolidation and fear expression.

Recherche du rôle de la communication hippocampo-thalamique-corticale pendant la consolidation de la mémoire et l'expression de la peur.

Soutenue par

Charlotte Andrews

Le 14/09/2023

Ecole doctorale n° 158

**Ecole Doctoral Cerveau,
Cognition, Comportement**

Spécialité

Neuroscience

Composition du jury :

Steve Ramirez PR, Boston University	<i>Président du jury</i>
Mathieu Wolff CDR-HDR, Bordeaux Neurocampus	<i>Rapporteur</i>
Andrea Mele PR, La Sapienza Sapienza Università di Roma	<i>Rapporteur</i>
M. Belen, Pardi CDR, Université de Paris	<i>Examinatrice</i>
Sophie, Pezet MCR-HDR, ESPCI-PSL	<i>Examinatrice</i>
Gisella Vetere PR, ESPCI-PSL	<i>Directeur de thèse</i>

Abstract

Memory is a vital component of our daily lives. Some of the experiences that we encounter will be remembered for a lifetime. The process that allows a memory trace to become long-lasting is called memory consolidation.

Classic models of memory consolidation posit that the hippocampus has a crucial role in the first phases of memory formation while the neocortex takes over later in time. Recently, novel discoveries showed that the thalamus may represent a gate hub for hippocampo-cortical communication to form remote memories. The introduction of functional connectomes studies have been of use for discovering new regions involved in memory processes. These studies showed that when mice recall remote fear memories, a strong hippocampo-thalamic-cortical network becomes functionally interconnected and activated. This highlights the need for additional investigation to understand the coordinated role of these 3 subregions in the formation of enduring memories.

Through an examination of the remote memory network of immediate early gene co-active brain regions, dynamic changes in the functional connectivity between the anterodorsal thalamic nucleus (ADn) and the rest of the brain area over time. These findings suggest that ADn inactivation may be necessary for remote memory recall. Indeed, Vetere *et al.* (2021) demonstrated that inhibitory neurons of the CA3 field of the hippocampus projecting to the ADn exhibited increased activity during remote memory recall and that their inactivation impaired memory performances.

In this thesis, I investigated the role of CA3 to ADn projections and how they impact the formation of recent and remote memory traces by influencing the activity of the anterior cingulate cortex (aCC), a cortical region largely studied for its role in recalling remote memory traces.

In particular, I studied spatial fear memories in mice, by training them in a contextual fear conditioning and then testing their recent (1 day after) or remote (28 days after) memory, by analysing freezing behaviour as a proxy for fear memory performances. To investigate the real-time contribution of specific projections during memory recall, I used optogenetics to inactivate different projections of this micro-circuit (CA3-ADn-aCC). I used iC⁺⁺, an inhibitory channelrhodopsin, on a viral promoter that infects excitatory cells, controlled by blue light. At the same time, I analysed c-Fos expression, an immediate early gene and marker of cellular activity, in the 3 regions described above. I also used fiber photometry combined with optogenetics to detect real-time population-level calcium dynamics in freely behaving mice.

I first confirmed that remote memories correlate with aCC activation and ADn inactivation, by analysing their c-Fos levels during memory recall.

I found that: 1. In addition to inhibitory CA3-ADn projections (discovered in Vetere et al, 2021), excitatory CA3-ADn projections also play a significant role in the recall of remote fear memories, but not recent ones: when these excitatory projections are optogenetically inactivated, it results in reduced levels of freezing behaviour, indicating their involvement in the retrieval of remote fear memories; 2. CA3-aCC projections are involved in remote, but not recent, memory recall: similarly to CA3-ADn optogenetic inactivation results, their inactivation leads to lower levels of freezing behaviour; 3. aCC

projections to ADn are necessary for fear memory expression at all times, mediating the recall of both recent and remote fear memories: when these projections were inactivated, it resulted in the suppression of ADn c-Fos values and an increase in freezing behaviour.

Overall, I found a novel cortico-hippocampo-thalamic pathway that regulates fear memory during recent and remote recall. I also identified two different pathways within this circuit: one from the CA3 to both ADn and aCC, important for remote fear memory recall; another one from aCC to ADn, that regulates fear expression (i.e. freezing levels) at both recent and remote time point.

Understanding the mechanisms underlying a fear memory can help further research linked to traumatic memories, such as post traumatic stress disorder and potentially highlight new treatment targets.

Acknowledgements

The simple words of “thank you” do not seem like enough to express how eternally grateful I am to the people in my life (both personal and professional) who have helped me get to this point... but I am a bit sleep deprived from writing this thesis, so they will have to do for now!

Firstly, I would like to acknowledge you, the reader, for taking time to read this thesis and learn about my work. In particular, I would like to thank: Mathieu Wolff, Andrea Mele, M. Belen Pardi, Steve Ramirez and Sophie Pezet for giving their valuable time and expertise to serve as the jury members of my PhD defence. Without whom, this last stage of my journey would not be possible.

I would like to acknowledge the people who have contributed to my academic journey. To Gabrielle Girardeau and Christoph Schmidt-Hieber who served as my committee throughout my PhD and helped guide the direction of my PhD at key stages. To the members of the Brain Plasticity for creating a collaborative work environment, where the goal is to support and help each other. To the secretaries, technicians, IT support, cleaners and all other staff who ensure the smooth daily running of the laboratories to allow us, as researchers, to focus on our work. I would like to thank the past and present members of the C4team. They turned from colleagues to dear friends, who's daily support, advice, collaborations, and companionship truly made the toughest days in the lab just that little bit lighter. I would like to thank my supervisor Gisella Vetere, who saw the potential in me from my master internship in Toronto and has guided, supported

and pushed me to grow throughout my PhD. I have great respect for her as a person and her dedication towards science. Her mentorship and advice has had a positive and everlasting impact on my professional and personal life.

I would like to acknowledge the invaluable role that my friends have had in my journey until this point and will continue to have. I am grateful that my professional journey has given me the opportunity to connect and create friendships in all corners of the world, from Aberdeen, to Toronto, to the “Party Animals” of Paris. As we all grow and move in our different directions, I know that the distance will only strengthen the love and support we have for each other.

I would like to thank my family for their love and support and guidance, especially to my parents who gave me every opportunity in life they could to help chase my dreams and never told me my dreams were too big. To my family-in-law who have accepted me as part of their own family and have encouraged me ever since.

Last but not least, I would like to thank my wife, my better half, Claudia. You followed me to Paris to let me pursue my dream of doing my PhD. We have created our life here together and I am forever grateful for all that you have done for me and our family, including bringing our puppy Pancho into our lives. I love you.

Contents

Abstract	1
Acknowledgements	4
List of Abbreviations	9
Foreword	11
Traduction en Français	13
Résumé	13
Chapitre 1: Mémoire	14
Chapitre 2: Expression de la peur	18
Chapitre 3 : Les techniques	22
Chapitre 4 : Les Resultats	26
Memory and Memory Consolidation	30
1.1 What is Memory?	30
1.2 Memory Consolidation	32
1.2.1 Systems Consolidation	34
1.2.1.1 Standard Theory	35
1.2.1.2 Multiple Trace Theory (MTT)	38
1.2.2 Synaptic Consolidation	41
1.3 Regions involved in memory consolidation	45
1.3.1 Hippocampus	45
1.3.1 The Dentate Gyrus (DG) and Memory Consolidation	45
1.3.2 The CA1 Region and Memory Consolidation	46
1.3.3 The CA3 Region and Memory Consolidation	47
1.3.2 Cortex	49
1.3.3 Thalamus	52
1.3.3.1 Thalamic Regions involved in memory	52
1.3.3.2 Anterior Thalamic Subregion	54
1.3.3.3 Thalamo-cortical and Thalamo-hippocampal communication important for memory consolidation.	57
1.3.3.4 Hippocampal-cortical-thalamic communication important in memory	58
Memory-Related Fear Expression	61
2.1 Why do we need to fear?	61
2.2 Innate vs learnt fear	63
2.3 Regions involved in fear expression	64
2.3.1 Role of cortex in fear expression	64
2.3.2 Role of Hippocampus in fear expression	65
2.3.3 Role of Amygdala in fear expression	66
2.3.4 Role of thalamus in fear expression	67

Techniques	69
3.1 Contextual Fear Conditioning	69
3.2 Optogenetics	71
3.3 Fiber Photometry	74
3.4 Retrobeads	76
Chaptire 4: Les Résultats	77
Hippocampal-cortical-thalamic connections are necessary for memory recall and fear expression	81
4.1 Summary	81
4.2 Participation Statement	82
Supplementary Results	117
5.1 aCC projections to the CA3 are not necessary for memory recall.	117
5.1.1 Introduction	117
5.1.2 Methods	118
5.1.3 Results	121
5.2 CA3 projections to the ADn are necessary for the recall of a generalised memory.	123
5.2.1 Introduction	123
5.2.2 Methods	124
5.2.3 Results	125
Discussion and Conclusion	128
6.1 The CA3	129
6.1.1 The CA3 role in memory.	129
6.1.1.1 Excitatory CA3 cells that project to the ADn are important for remote memory.	131
6.1.1.2 Excitatory CA3 cells that project to the aCC are important for remote memory.	133
6.1.2 The role of the CA3 in generalised memories.	134
6.2 The aCC	135
7.2.1 The aCC is important for memory-related fear expression.	136
6.2.2. The aCC processes and regulates fear expression.	137
6.3 The role of the ADn in memory recall.	139
6.4 Conclusions	141
Spatial Representation Network	145
7.1 Type of Spatial Representation Cells	146
7.1.1 Place Cells	146
7.1.2. Grid Cells	147
7.1.3. Time Cells	147
7.1.4. Head Direction Cells and Angular Head Velocity Cells	147
7.2 Head Direction Cell Regions	149
7.2.1 The Thalamus: A Hub for Head Direction Cells	150
7.2.2 Functional Influences on Head Direction Cells	151

7.2.3 Anticipatory Time Interval and Lesion Studies	152
7.2.3.1 Anticipatory Time Interval (ATI)	152
7.2.3.2 Lesion Studies	152
The Ring Attractor Theory	153
8.1 Properties of the Attractor Network	153
8.2 Integration of Angular Velocity Inputs	154
8.3 Accuracy of Path Integration	154
8.4 Role of Inhibitory and Excitatory Connectivity	155
8.5 Modelling the Ring Attractor Network	155
8.6 Experimental Studies in Flies	155
8.7 Chapter Conclusion	156
The Role of Head Direction Cell Activity on Head Direction Behaviour	158
9.1 Summary	158
9.2 Participation Statement	158
9.3 Abstract	159
9.4 Introduction	160
9.5 Methods	161
9.6 Results	165
9.6.1 Head direction cells in ADn drive the activity of head direction behaviour	165
9.6.2 Is this effect unique to the ADn?	167
9.7 Discussion	170
9.8 Perspective	172
9.8.1 PhD proposal	172
Bibliography	174

List of Abbreviations

aCC	Anterior Cingulate Cortex
ADn	Anterior Dorsal Nucleus of the Thalamus
ATN	Anterior Thalamic Nuclei
ATI	Anticipatory time interval
AP	Anterior-Posterior
CA3	CA3 region of the Hippocampus
ChRs	Channelrhodopsins
CLS	Complementary Learning System
CR	Conditional Response
CS	Conditioned Stimulus
DG	Dentate Gyrus
DV	Dorsal-Ventral
HD	Head Direction
HDCs	Head Direction Cells
ML	Medio-Lateral
MTT	Multiple Trace Theory
NpHRs	Halorhodopsin
NRe	Nucleus Reuniens
NREM	Non-rapid eye movement
PBS	Phosphate-buffered Saline
PFA	Paraformaldehyde
PFC	Prefrontal Cortex

PFD_____Preferred Firing Direction

PVT_____Paraventricular Nucleus of the Thalamus

pPVT_____posterior Paraventricular Nucleus of the Thalamus

RSC_____Retrosplenial Cortex

US_____Unconditional Stimulus

Foreword

This thesis aims to characterise the role of hippocampal-cortical-thalamic projections during memory consolidation and the expression of memory-related fear behaviour. This theme in itself is extremely vast, as these three main brain regions encompass numerous subregions, and their subsequent connections. To this end, this thesis concentrates mainly on three subregions based on previous data showing their involvement in the consolidation of fear spatial memories: for the hippocampus, this thesis will focus on the CA3 field; for the cortex, the anterior Cingulate Cortex (aCC); and for the thalamus, the AnteroDorsal nucleus (ADn).

To help the reader understand the context of the studies presented in the methods and results section, I start by introducing the idea of memory and fear expression in Part I. In Chapter 1 of the introduction, I focus on memory consolidation, with the aim to present the known contributions of the hippocampus, cortex and thalamus, specifically highlighting the respective subregions explored in the thesis. In Chapter 2, I introduce the idea of fear expression and how memories can influence behaviour, highlighting the involvement of the cortex in this process. To help the reader understand the techniques presented in the methods and results section, in Chapter 3 I will introduce the techniques by detailing their background and examples of their application.

In Part II, the main results yielded by this PhD are presented in the form of an article. Following the article, I will present supplementary experiments related to the article that

I conducted during the PhD, and that we decided not to include in the final article. This will then be followed by an extended discussion in Part III.

During my PhD, I conducted a series of experiments investigating the relationship between head direction cells (HDCs) activity and the head direction (HD) behaviour of the animal. The intricacy and scope of these experiments evolved beyond a realistic time scale and secondary project of my PhD; and has now become the foundation for a future PhD thesis.

With the nature of the experiments presented in Part IV differing from that of the main body of work, it is necessary to provide the reader with additional information. To that end there is a secondary introduction. In Chapter 7, I will introduce the broad topic of the spatial representation system, from its definition to the types of cells and the regions involved. Lastly, to hone in on the theory that is the backbone of the research presented, I will explain the attractor ring network in Chapter 8.

I will present the work I conducted in the form of an article, as well as the prospective work in the form of a PhD proposal.

As to comply with regulations for a thesis written in English, the introduction of this thesis will be preceded by a summary of each of the chapters in French.

Traduction en Français

Résumé

La mémoire est une composante essentielle de notre vie quotidienne. Certaines des expériences que nous vivons resteront gravées dans notre mémoire toute notre vie. Le processus qui permet à une trace mnésique de devenir durable s'appelle la consolidation de la mémoire.

Selon les modèles classiques de consolidation de la mémoire, l'hippocampe joue un rôle crucial dans les premières phases de la formation de la mémoire, tandis que le néocortex prend le relais plus tard. Récemment, de nouvelles découvertes ont montré que le thalamus pouvait représenter une plaque tournante pour la communication hippocampo-corticale afin de former des souvenirs à distance. L'introduction d'études sur les connectomes fonctionnels a permis de découvrir de nouvelles régions impliquées dans les processus de mémorisation. Ces études ont montré que lorsque les souris se rappellent des souvenirs de peur à distance, un réseau hippocampo-thalamique-cortical puissant devient fonctionnellement interconnecté et activé. Cela souligne la nécessité de mener des recherches supplémentaires pour comprendre le rôle coordonné de ces trois sous-régions dans la formation de souvenirs durables.

L'examen du réseau de mémoire à distance des régions cérébrales coactives avec les gènes précoces immédiats a révélé des changements dynamiques dans la connectivité fonctionnelle entre le noyau thalamique antérodorsal et le reste de la zone cérébrale au fil du temps. Ces résultats suggèrent que l'inactivation du noyau thalamique

antérodorsal (ADn) pourrait être nécessaire pour le rappel de la mémoire à distance. En effet, Vetere et al. (2021) ont démontré que les neurones inhibiteurs du champ CA3 de l'hippocampe se projetant sur le noyau thalamique antérodorsal présentaient une activité accrue pendant le rappel de souvenirs à distance et que leur inactivation diminuait les performances de la mémoire.

Chapitre 1: Mémoire

Dans ce premier chapitre de ma thèse, je vais introduire les concepts fondamentaux de mon sujet de recherche. La base de mon sujet est la mémoire, et plus précisément le rappel de souvenirs consolidés. Pour commencer, j'éluciderai les distinctions entre les mémoires à court terme et à long terme, et j'approfondirai les mécanismes par lesquels les mémoires sont consolidées. En outre, j'examinerai l'interaction complexe entre les différentes régions du cerveau en fonction de l'âge du souvenir, en mettant en lumière le recrutement de régions spécifiques pendant le rappel du souvenir. En présentant ces concepts fondamentaux et l'implication de l'hippocampe (en particulier le CA3), du cortex (en particulier l'aCC) et du thalamus (en particulier l'ADn) dans la consolidation de la mémoire, ce chapitre vise à préparer le terrain pour les investigations et les résultats ultérieurs de ma recherche.

Les processus moléculaires qui sous-tendent la consolidation synaptique impliquent des modifications complexes des protéines, en particulier celles associées aux récepteurs du N-méthyl-D-aspartate (NMDA-R). La phosphorylation de la sous-unité GluN1, comme l'ont démontré Sans et al. (2003), facilite l'exportation des NMDA-R du

réticulum endoplasmique vers la membrane plasmique, favorisant ainsi une potentialisation synaptique accrue. Ces changements se produisent dans un laps de temps spécifique après l'acquisition de la mémoire, passant d'une forme labile, à court terme, sensible aux inhibiteurs tels que les inhibiteurs de la synthèse des protéines, à un état stable, à long terme. La potentialisation à long terme (PLT), un processus largement étudié pour son rôle dans la consolidation synaptique, présente des similitudes avec les changements synaptiques induits par l'apprentissage. Cependant, malgré les associations entre la PKC, la PKA, l'activité MAP kinase et la potentialisation à long terme tardive, l'élimination de la potentialisation à long terme tardive tout en maintenant la mémoire spatiale à long terme suggère des complexités dans la relation, soulignant la nécessité d'une compréhension globale des mécanismes de consolidation synaptique.

L'hippocampe, mis en lumière par le cas fondateur du patient HM, joue un rôle essentiel dans la consolidation de la mémoire, en transférant les souvenirs à court terme vers le stockage à long terme. Une attention particulière portée aux subdivisions de l'hippocampe révèle des rôles distincts dans ce processus. Le gyrus denté (DG) excelle dans la séparation des formes, les cellules exprimant la somatostatine régulant l'excitabilité. Dans le contexte de la mémoire contextuelle de la peur, l'inhibition de la synthèse protéique du DG conduit à l'amnésie. CA1 contribue aux processus de mémoire spatiale, mettant en évidence la sensibilité temporelle, et joue un rôle central dans la récupération de la mémoire. Des recherches récentes sur les engrammes mettent en évidence l'implication de CA1 dans la séparation des engrammes positifs et

négatifs. La région CA3 est vitale pour la consolidation de la mémoire, la récupération et l'achèvement des schémas, la plasticité médiée par AMPAR étant cruciale pour la consolidation des souvenirs récents. Les capacités d'auto-association de CA3, facilitées par des connexions récurrentes étendues, suggèrent un rôle dans l'encodage et le stockage temporaire des souvenirs avant leur transfert permanent, ce qui correspond au modèle en deux étapes de la formation de la mémoire. Les lésions ou l'inactivation de CA3 affectent l'encodage et la récupération de la mémoire contextuelle, soulignant son rôle distinctif dans les processus de mémorisation.

Le cortex est une région cérébrale centrale dans le processus complexe de consolidation de la mémoire, comme le montrent la théorie standard de la consolidation et la théorie des traces multiples. Les preuves convergentes de ces théories soulignent le rôle central du cortex dans le maintien et le stockage des traces de mémoire à long terme. La consolidation synaptique et la consolidation des systèmes soulignent l'importance du cortex dans les changements structurels au niveau des synapses et la redistribution progressive des représentations de la mémoire depuis l'hippocampe. Cette implication est évidente dans la réorganisation temporelle, le cortex préfrontal (CPF) acquérant et stockant progressivement les informations initialement traitées par l'hippocampe. Les changements structurels dans le CPF et le cortex cingulaire antérieur (CCCA), une sous-région du CPF, correspondent à leur rôle dans la consolidation de la mémoire contextuelle. En particulier, l'activité de l'aCC est cruciale pour les souvenirs lointains, comme le montrent les expériences démontrant une consolidation altérée de la mémoire lors de l'inactivation de l'aCC. En outre, les projections descendantes du

PFC vers la région CA1 jouent un rôle central dans la récupération de la mémoire, soulignant l'interaction dynamique entre les régions corticales dans les processus de mémoire.

Le thalamus, région cérébrale aux multiples facettes, est étroitement impliqué dans la consolidation de la mémoire. Des sous-régions telles que le noyau médiodorsal, le noyau reuniens, le noyau rhomboïde, le noyau supra-géniculé et les noyaux thalamiques antérieurs (NTA) contribuent de manière unique à la formation et au stockage des souvenirs. Le noyau thalamique antérieur, en particulier le noyau dorsal antérieur (NDA), joue un rôle crucial dans le rappel des souvenirs récents et lointains. Les profils d'expression distincts du cFos du NDA et la dynamique de la connectivité fonctionnelle pendant le rappel des souvenirs récents et lointains soulignent son rôle actif dans le processus complexe de la consolidation de la mémoire. En outre, la communication thalamo-corticale et thalamo-hippocampique est vitale, le thalamus servant de plaque tournante pour relayer l'information entre les différentes régions du cerveau. La nécessité de la communication thalamo-hippocampique pour le rappel des souvenirs à distance est mise en évidence par les projections inhibitrices de CA3 vers l'ADn. La cartographie de l'ensemble du cerveau révèle une signature hippocampique, corticale et thalamique pendant le rappel des souvenirs, ce qui suggère qu'une activité coordonnée entre ces régions est nécessaire pour consolider et récupérer les souvenirs à long terme. Les modèles informatiques appuient ces résultats, démontrant que la communication hippocampique-corticale-thalamique est cruciale pour une bonne dynamique de représentation de la mémoire, en accord avec les études biologiques

montrant l'importance de l'activité coordonnée pendant le sommeil pour la formation de la mémoire.

Chapitre 2: Expression de la peur

Les principaux résultats de ma thèse se divisent en deux parties : la consolidation de la mémoire, telle que décrite dans le dernier chapitre, et l'expression d'un comportement de peur lié à la mémoire. Dans ce chapitre, je présenterai les principes de l'expression de la peur et les raisons de son importance. Pour étudier les processus d'expression de la peur, nous avons utilisé le conditionnement contextuel de la peur. La réponse conditionnée associée au conditionnement de peur contextuel est le gel. Ce chapitre étudie la contribution des différentes régions du cerveau et leurs interactions dans la formation des réponses de peur et la modulation des souvenirs de peur, en particulier le comportement de congélation et les souvenirs contextuels.

La peur, en tant qu'émotion, remplit une fonction adaptative cruciale en favorisant l'évitement des menaces et en assurant la survie. La définition de la peur a fait l'objet de débats, notamment sur la question de savoir si elle englobe à la fois des sentiments subjectifs et des processus physiologiques sous-jacents. Dans les études animales, le terme "peur" décrit des réactions comportementales et physiologiques combinées à des signaux liés à la menace. J. LeDoux propose un cadre à deux systèmes, distinguant des circuits sous-corticaux pour les réponses comportementales inconscientes et des circuits corticaux pour les états émotionnels conscients. D'autre part, M. Fanselow définit la peur comme un système fonctionnel neuronal-comportemental qui génère des

comportements défensifs contre les menaces, en mettant l'accent sur un générateur de peur central. Il introduit le concept de continuum menace-imminence, qui organise les réactions défensives en fonction de la proximité d'une menace potentielle.

Les comportements de peur innés sont inhérents et partagés entre les espèces, nécessitant un apprentissage minimal, tandis que les comportements de peur acquis le sont par le biais de l'apprentissage associatif, tel que le conditionnement pavlovien. Le conditionnement pavlovien consiste à associer un stimulus neutre à un stimulus aversif, ce qui entraîne des réponses apprises telles que la congélation en réponse à un contexte autrefois neutre. Si la peur innée implique probablement des éléments appris transmis de génération en génération, elle ne nécessite pas d'associations explicites entre les stimuli et les résultats négatifs.

Le cortex préfrontal (CPF) et ses sous-régions, notamment le cortex cingulaire antérieur (CCA), les régions prélimbiques et infralimbiques, jouent un rôle crucial dans la régulation des réactions de peur. Le CPF est interconnecté avec l'amygdale, ce qui permet un contrôle descendant des réactions de peur. Différentes parties du PFC médian, comme le PFC médian dorsal, sont impliquées dans la modulation de l'expression de la peur, contrôlant les réponses défensives en fonction du contexte. Les oscillations synchronisées des principaux neurones de ce réseau sont essentielles à la transmission d'informations précises et au comportement. Les manipulations optogénétiques, telles que l'inhibition des interneurones GABAergiques, révèlent le rôle du circuit PFC-amygdale dans la régulation de l'expression de la peur. En outre,

l'interaction cortico-thalamique, en particulier les projections vers le nucleus reuniens (NRe), contribue à la modulation de la réponse à la peur, l'inhibition du NRe réduisant les réponses de congélation.

Cette exploration nuancée de la peur porte sur ses diverses définitions, sa signification évolutive et les circuits neuronaux complexes qui orchestrent son expression et sa régulation. L'intégration des connaissances issues du cadre à deux systèmes de LeDoux, du concept de générateur central de peur de Fanselow et du rôle de régions cérébrales spécifiques permet d'approfondir notre compréhension de la peur en tant que phénomène complexe et adaptatif.

L'hippocampe, un composant crucial du système de représentation spatiale, module l'expression de la peur contextuelle. Les études de lésion révèlent que les lésions de l'hippocampe perturbent les réponses de peur conditionnée contextuelle tout en épargnant les réponses aux indices explicites. En particulier, les lésions de l'hippocampe ventral entraînent une réduction de l'expression de la peur, ce qui indique une implication spécifique de cette région. Les lésions de l'hippocampe entier ou de l'hippocampe ventral entraînent une diminution de la peur et du stress par rapport aux lésions de l'hippocampe dorsal. L'ensemble de ces résultats met en évidence le rôle nuancé de l'hippocampe dans l'expression contextuelle de la peur.

L'amygdale fonctionne comme un détecteur rapide de stimuli environnementaux aversifs, coordonnant les réponses aux menaces et générant des comportements

adaptatifs. Chez toutes les espèces, y compris les rongeurs et les humains, les lésions de l'amygdale altèrent la reconnaissance des stimuli effrayants. Des études impliquant des lésions électrolytiques chez des rats et des patients présentant des lésions focales bilatérales de l'amygdale soulignent le rôle de l'amygdale dans l'apprentissage contextuel du conditionnement de la peur et dans la reconnaissance des visages effrayants. L'amygdale est donc une région cérébrale cruciale qui relie les stimuli à une signification émotionnelle et contribue à l'expression de la peur.

Le thalamus est impliqué dans le traitement de la peur, le noyau reuniens (NRe) jouant un rôle crucial dans la régulation de l'expression de la peur. Les projections du PFC vers le NRe contribuent à la modulation de la réponse à la peur, soulignant que le NRe est une structure clé dans l'acheminement des informations liées à la peur du cortex infralimbique vers l'amygdale basolatérale pour l'extinction de la mémoire de la peur à distance. Des études de lésion ont permis d'identifier des régions thalamiques spécifiques impliquées dans l'expression de la peur. La ligne médiane dorsale du thalamus est cruciale pour la régulation de la peur conditionnée, les lésions entraînant des troubles de l'acquisition et du gel de l'expression dans le contexte du conditionnement. Le noyau paraventriculaire du thalamus (PVT), en particulier le PVT postérieur, avec des projections vers l'amygdale, joue un rôle dans l'expression de la peur, les lésions du PVT postérieur entraînant une atténuation de la peur. Dans l'ensemble, les études sur les lésions désignent la ligne médiane dorsale du thalamus et le pPVT comme des régions importantes dans la régulation de l'expression de la peur.

Chapitre 3 : Les techniques

Ce chapitre vise à fournir une exploration complète des techniques employées dans cette thèse de doctorat. En approfondissant leur nature, leurs fonctionnalités et leurs applications pratiques, je vise à doter le lecteur d'une compréhension approfondie des méthodologies qui sous-tendent les recherches menées dans le cadre de cette thèse.

Le conditionnement contextuel de la peur, un paradigme largement étudié en neurosciences comportementales, consiste à associer un stimulus aversif à un contexte neutre, ce qui entraîne des réactions de peur. Dans ce paradigme, le comportement de congélation chez les animaux sert d'indicateur principal de la peur, alors que chez les humains, les comportements de peur sont souvent évalués par des rapports verbaux et des réponses physiologiques. Les circuits neuronaux impliqués dans les souvenirs de peur comprennent le cortex préfrontal prélimbique, l'amygdale basolatérale, l'amygdale centrale et le noyau paraventriculaire du thalamus, montrant des changements dynamiques dans les circuits de rappel sans altération du comportement. L'hippocampe, crucial pour le conditionnement contextuel et le conditionnement de traces, intègre les signaux affectifs de l'amygdale aux informations préexistantes, influençant ainsi la signification des souvenirs de peur. Le choix du conditionnement contextuel de la peur comme paradigme comportemental permet d'explorer la trace hippocampique-thalamique-corticale pendant le rappel de la mémoire, ce qui permet de mieux comprendre les mécanismes neuronaux qui sous-tendent l'expression de la peur et le rappel de la mémoire.

L'optogénétique, une technologie transformatrice dans le domaine des neurosciences, facilite la manipulation précise et rapide d'événements spécifiques au sein de systèmes biologiques complexes, même chez les animaux qui se déplacent librement. Grâce à leur capacité de contrôle optique rapide et de ciblage précis de types de cellules spécifiques, les méthodologies optogénétiques ont révolutionné l'exploration de la biologie, contribuant aux recherches sur la santé et la maladie. Cette technique puissante utilise des protéines sensibles à la lumière, appelées opsines, pour contrôler et manipuler l'activité de neurones spécifiques dans les organismes vivants, offrant un moyen de modulation précise et réversible de l'activité neuronale avec une résolution spatiale et temporelle élevée (Wick et Krook-Magnuson, 2018). Les channelrhodopsines (ChRs) et les halorhodopsines (NpHRs) sont des opsines essentielles utilisées en optogénétique. Les ChRs, en tant que canaux cationiques, induisent une dépolarisation et une activation neuronale, tandis que les NpHRs, agissant comme des pompes à chlorure, provoquent une hyperpolarisation et inhibent l'activité neuronale lorsqu'elles sont activées par la lumière. Cette polyvalence dans la manipulation des processus excitateurs et inhibiteurs fait de l'optogénétique un outil inestimable pour démêler les complexités des circuits neuronaux et comprendre les mécanismes dans diverses conditions physiologiques et pathologiques.

L'avènement d'opsines inhibitrices comme iC++, dérivées d'algues cryptophytes, représente une avancée significative dans le domaine de l'optogénétique. Fonctionnant comme une rhodopsine à canal anionique, iC++ permet une hyperpolarisation efficace de la membrane neuronale grâce à une conduction chlorure gérée par la lumière,

offrant une sensibilité à la lumière et une précision temporelle inégalées pour l'inhibition optogénétique (Berndt et al., 2016 ; Govorunova et al., 2015). Dans mes expériences, iC++ sert d'opsine inhibitrice, complétée par un virus de contrôle n'exprimant qu'un fluorophore eYFP. L'optogénétique, en comparaison avec des alternatives comme la chimiogénétique et la lésion, présente des avantages distincts. La chimiogénétique repose sur des récepteurs de conception activés par des médicaments de conception (DREADD), ce qui nécessite des ligands exogènes pour la modulation neuronale, tandis que l'optogénétique permet un contrôle direct et précis par la lumière, éliminant ainsi la nécessité d'une administration systémique de médicaments. En outre, l'optogénétique permet une haute résolution spatiale dans la manipulation de types de cellules ou de circuits neuronaux spécifiques, ce qui est un défi pour les techniques de lésion. Cependant, l'optogénétique a ses limites, notamment la sélectivité de l'expression et de l'activation de l'opsine dans des types de cellules neuronales spécifiques in vitro et in vivo. En outre, les dommages cellulaires potentiels causés par l'exposition à la lumière laser posent problème, ce qui a un impact sur la fiabilité et la reproductibilité des résultats et nécessite un examen attentif de la conception expérimentale (Wick & Krook-Magnuson, 2018).

La photométrie à fibres s'est imposée comme une technique précieuse pour le suivi en temps réel de l'activité neuronale dans des populations de neurones génétiquement définies, offrant des avantages tels que la compatibilité avec les indicateurs de calcium codés génétiquement et une invasivité minimale par rapport à l'électrophysiologie. Malgré une résolution spatiale inférieure à celle de la microscopie à deux photons, la

photométrie à fibres optiques permet d'obtenir une vue homogène de l'activité des populations, en particulier lorsqu'elle est associée à l'optogénétique pour explorer les relations de cause à effet entre l'activité neuronale et le comportement. Dans ma thèse, j'ai appliqué la photométrie à fibres pour étudier la dynamique du calcium dans l'aCC et l'ADn pendant le comportement de congélation et je l'ai utilisée avec l'optogénétique pour étudier les réponses au niveau de la population dans l'ADn aux projections excitatrices du CA3 pendant le rappel de la mémoire.

Les rétrobilles, microsphères fluorescentes, constituent un outil précieux en neurosciences pour tracer les projections neuronales et explorer la connectivité neuronale. L'injection de rétrobilles dans des régions spécifiques du cerveau permet de marquer et de tracer les projections de neurones, révélant ainsi les connexions avec d'autres régions. Les rétrobilles offrent des avantages par rapport à d'autres méthodes de traçage en raison de la diffusion limitée au site d'injection, ce qui permet de cibler avec précision des populations cellulaires ou des noyaux. En outre, les rétrobilles facilitent l'étude des effets des stimuli sur des populations de neurones spécifiques, mettant en lumière des circuits distincts influencés par différents aspects des stimuli motivants. Dans ma thèse, les rétrobilles ont été utilisées pour colorer de manière rétrograde les projections vers l'ADn, confirmant les connexions des régions aCC et CA3. Cette approche a permis de valider les projections du CA3 vers l'aCC, contribuant ainsi à une compréhension globale des circuits neuronaux impliqués dans l'expression de la peur et le rappel de la mémoire.

Chapitre 4 : Les Résultats

Le recrutement de CA3 pour le rappel de mémoire à distance se fait par un double contrôle de aCC et ADn.

Dans le processus de rappel des souvenirs lointains, l'hippocampe CA3 émerge comme un acteur central, régulé à la fois par aCC et ADn. La consolidation de la mémoire, un processus actif nécessitant des réarrangements synaptiques, est reconnue pour sa nature dépendante du temps. Alors que les points de vue traditionnels suggéraient le désengagement de l'hippocampe au fil du temps, des études récentes proposent un scénario plus complexe où l'activation hippocampique reste nécessaire pour rappeler les souvenirs lointains. La recherche actuelle se concentre sur le champ CA3, révélant l'implication des cellules CA3 à longue portée inhibitrices projetant vers le ADn. De manière surprenante, l'inactivation à la fois des cellules CA3 inhibitrices et excitatrices vers le ADn entraîne une augmentation de l'activité du ADn et des performances mnésiques altérées, remettant en question les hypothèses antérieures. Des investigations supplémentaires sont nécessaires pour comprendre ce résultat inattendu, mais les découvertes soulignent le rôle crucial du CA3 dans la régulation du ADn et du aCC, offrant de nouvelles perspectives sur la dynamique complexe du rappel mnésique.

L'étude met également en lumière la contribution significative du aCC à la consolidation de la mémoire, en particulier lors du rappel des souvenirs lointains. Ce recrutement est indirectement influencé par le recrutement correct de CA3 vers le ADn et directement par les projections de CA3 vers l'aCC. De manière intéressante, le recrutement du aCC se révèle efficace uniquement lors du rappel des souvenirs lointains, soulignant sa

non-nécessité pour le rappel des souvenirs récents. En résumé, la recherche dévoile le microcircuit complexe impliquant CA3, aCC et ADn, révélant leur interaction dans la régulation du rappel des souvenirs lointains. Cette investigation marque une étape cruciale dans la compréhension des dynamiques complexes au sein de l'hippocampe lors du rappel mnésique, fournissant des informations précieuses sur les mécanismes neuronaux régissant les processus mnésiques lointains.

La voie aCC-ADn peut réguler l'occurrence du gel pendant le rappel de la mémoire de la peur.

Notre étude présente des preuves pionnières d'un nouveau rôle des projections de aCC vers le ADn dans l'expression de la peur liée à la mémoire. La confirmation de ces projections par rétro traçage de billes réfléchissantes s'aligne sur des recherches antérieures (Xue et al., 2022). Contrairement aux conclusions antérieures impliquant un contrôle direct de CA3 sur aCC et ADn uniquement à des moments distants, nos résultats révèlent qu'une inhibition optogénétique des connexions de aCC vers l'ADn entraîne une augmentation significative du comportement de gel à la fois aux moments récents et distants. Cela suggère une régulation complexe des comportements liés à la peur dépendant de la mémoire orchestrée par ce microcircuit. Notamment, les projections inactivées de CA3 entraînent systématiquement une altération de la mémoire de la peur à distance, se traduisant par une diminution du comportement de gel, tandis que l'inactivation de aCC vers le ADn produit l'effet inverse, augmentant le comportement de gel aux moments récents et distants.

Pour élucider la corrélation entre le comportement de gel et l'activité de aCC et ADn lors du rappel de la mémoire de la peur à des moments distants, nous avons réalisé des études de photométrie par fibre. Lors de la délivrance du choc, nous avons observé une augmentation significative de l'activité de aCC, en accord avec des recherches antérieures (Pan et al., 2020). Cependant, de manière intrigante, l'activité de ADn semblait diminuer pendant la délivrance du choc, suggérant une réponse coordonnée mais opposée au stimulus non conditionné lors de l'acquisition de la mémoire de la peur dans ces deux régions. Notamment, l'ADn est documenté comme la seule région inactivée lors de la délivrance du choc, fournissant des informations précieuses sur la dynamique complexe entre aCC et ADn lors du traitement de la mémoire de la peur. Des recherches supplémentaires sont nécessaires pour valider et explorer ces résultats préliminaires.

Part I

INTRODUCTION

Chapter 1

In this first chapter of my thesis, I will introduce the fundamental concepts of my research topic. The basis of my topic is memory, specifically the recall of consolidated memories. To begin, I will elucidate the distinctions between short-term and long-term memories, delving into the mechanisms through which memories undergo consolidation. Additionally, I will examine the intricate interplay between different brain regions depending on the age of the memory, shedding light on the recruitment of specific regions during memory recall. By introducing these fundamental concepts and the involvement of the hippocampus (specifically the CA3), the cortex (specifically the aCC), and the thalamus (specifically the ADn) in memory consolidation, this chapter aims to set the stage for the subsequent investigations and findings in my research.

Memory and Memory Consolidation

1.1 What is Memory?

The concept of memory is a fundamental cognitive process that encompasses the encoding, storage, and retrieval of information (Squire *et al.*, 2015). In the field of neuroscience, the study of memory holds great significance as it underlies numerous cognitive functions such as learning, problem-solving, decision-making, and language processing. The intricate nature of memory enables individuals to effectively navigate and interact with their surroundings by retaining and retrieving past experiences, knowledge, and skills.

Memory can be categorised into different types, each serving a specific purpose and exhibiting distinct characteristics. One prevalent classification includes declarative memory and procedural memory. Declarative memory pertains to the recollection of facts, events, and concepts that can be consciously recalled and verbally expressed (Squire *et al.*, 2015). This form of memory can be further subdivided into semantic memory, involving general knowledge and concepts, and episodic memory, encompassing personal experiences and events. Declarative memory plays a critical role in acquiring new information, learning, and maintaining one's sense of personal identity.

In contrast, procedural memory refers to the retention of skills, habits, and procedures that are acquired through practice and repetition. Operating outside of conscious awareness and control, procedural memory is often implicit and automatic. It significantly contributes to the execution of motor skills, such as riding a bike or playing a musical instrument, and plays a vital role in the development and refinement of both motor and cognitive skills (Mochizuki-Kawai, 2008).

Both declarative and procedural memory offer unique advantages and contribute to various aspects of human cognition. Declarative memory enables individuals to acquire and retain knowledge about the world, forming a foundation for problem-solving, decision-making, and language processing. It facilitates the remembrance of facts, concepts, and events, empowering individuals to employ this information in making

informed judgments and choices. Procedural memory, on the other hand, empowers individuals to acquire and automate skills and habits, leading to enhanced efficiency and performance in diverse domains such as sports, music, and professional expertise.

In conclusion, memory represents a fundamental cognitive process involving the encoding, storage, and retrieval of information. This intricate mechanism encompasses various types of memory, including declarative and procedural memory, each serving distinct purposes and offering specific advantages. Declarative memory enables the acquisition and retention of knowledge and events, while procedural memory facilitates the acquisition and automation of skills and habits. Both forms of memory are indispensable to human cognition, playing pivotal roles in diverse cognitive functions.

1.2 Memory Consolidation

Short term memory holds a limited amount of newly learnt information for a brief period of time, where it is susceptible to interference. Short term memory can be consolidated into long term memory, a form of memory that stores information semi-permanently (Baddeley 1966; Waugh and Norman, 1965). In 1900, researchers Müller and Pilzecker, initially proposed the concept of memory consolidation. This theory aimed to explain retroactive interference in humans, which refers to the phenomenon where newly learned information remains susceptible to interference for a certain period after its initial acquisition. Following initial encoding, a series of neurobiological processes occur, leading to the strengthening and stabilisation of memory traces (Squire and Wixted, 2011). The process of consolidation is essential for the transition of memories from a

vulnerable and transient state to a more permanent and resistant form that can be retrieved later (Nader and Einarsson, 2010).

Long-term memories can be categorised into two distinct types: recent and remote memories. The precise point at which one transitions into the other remains uncertain; however, conventionally, recent memories are described as those that form within the initial few hours/days after learning, while remote memories are those retained from the first few days and have the capacity to endure for weeks, months, or even years (Doron and Goshen, 2018; Fig. 1.1).

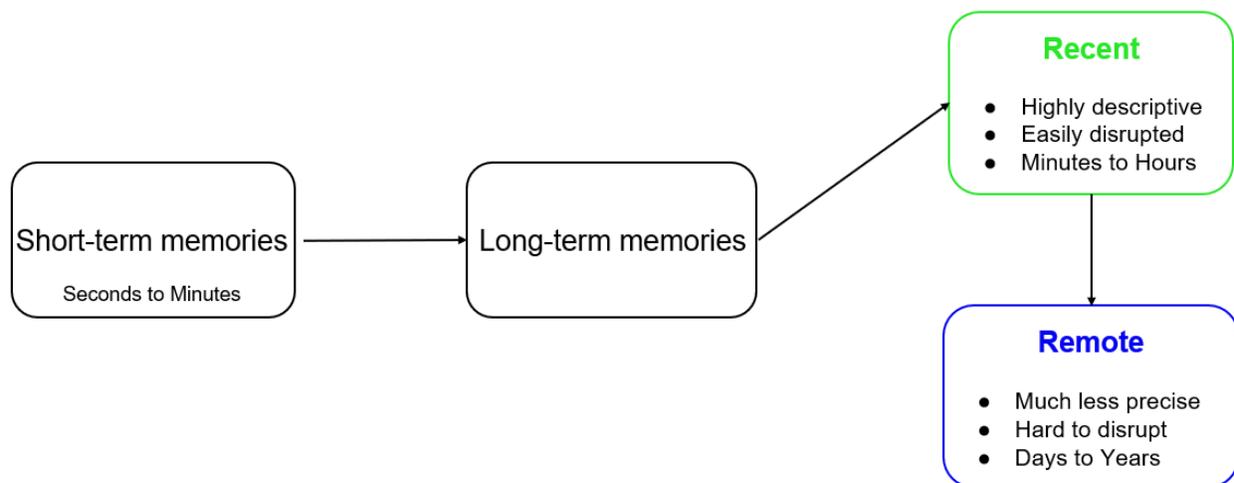


Figure 1.1. Summary of time dependent memories. Highlighting the different categories within long-term memory: recent (green) and remote (blue).

In my thesis, I investigated the difference between the two and I considered recent memories the ones tested 1 day after training and remote memories the ones tested 28 days old.

1.2.1 Systems Consolidation

Systems consolidation is broadly defined as the reorganisation of hippocampal memories over time, with this process thought to unfold over the course of days to years. Typically commencing directly after memory acquisition during periods of quiet wakefulness or sleep (Ben-Yakov et al., 2014; Dudai et al., 2015), the prevailing belief is that during this consolidation process (or processes), the memory trace is redistributed throughout the neocortex (Osada et al., 2008). Consequently, as time progresses, memories become consolidated on a systems level and gradually rely less on the hippocampus (Dudai and Morris, 2000).

Patient HM (Henry Molaison) played a crucial role in inspiring the development of the systems consolidation theory of memory consolidation. HM suffered from severe epilepsy, and in an attempt to alleviate his condition, he underwent brain surgery in 1953 (Squire and Zola-Morgan, 2011). The surgery involved the removal of parts of his medial temporal lobes, including the hippocampus. Following the surgery, HM experienced a significant anterograde amnesia, which impaired his ability to form new long-term memories. Interestingly, his short-term memory and memories formed before the surgery remained intact. This selective memory deficit provided researchers with a unique opportunity to study the role of the hippocampus in memory consolidation. As scientists delved into HM's case, they discovered that the hippocampus was critical for transferring new memories from short-term storage to long-term storage in a process known as memory consolidation. This observation gave rise to the systems

consolidation theory, with the specific achievements and the exact mechanisms underlying these processes subject to debate for many years.

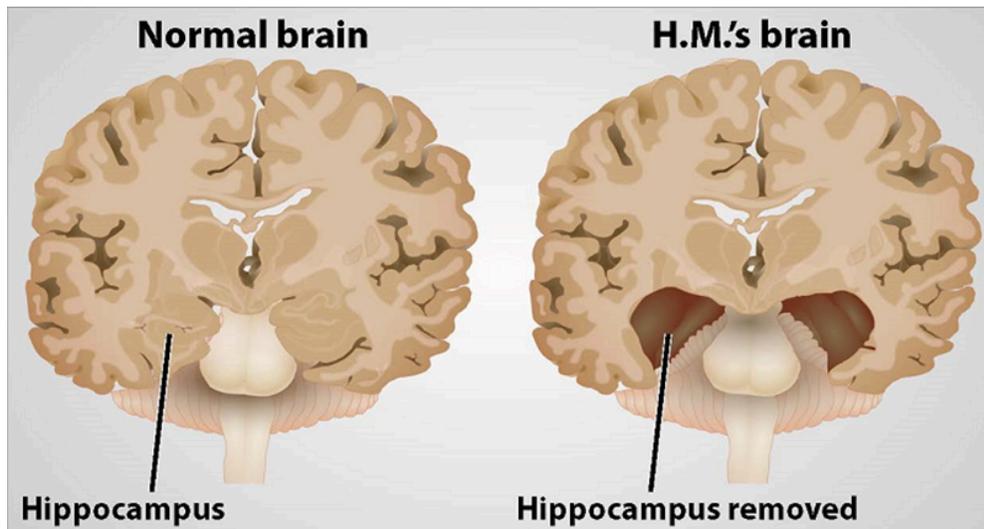


Figure 1.2 Schematic of result of H.M surgery. Highlighting the removal of the hippocampus (right) in comparison to a normal brain (left).

1.2.1.1 Standard Theory

The standard theory of systems-level consolidation (Frankland & Bontempi, 2005; Marr, 1970; McClelland *et al.*, 1995; Squire & Alvarez, 1995), memory processes rely on two separate memory stores to prevent the overwriting of existing knowledge by new information. After encoding new information, the memory traces initially appear in hippocampal patterns of activity, covertly reactivating in hippocampal-neocortical networks. This reactivation robustly establishes cortico-cortical connections, while hippocampal-cortical connections gradually diminish. Consequently, newly formed memory representations integrate into long-term neocortical memory networks,

becoming independent of the hippocampus over time (Frankland & Bontempi, 2005; Marr, 1970; Squire & Alvarez, 1995). This progressive hippocampal independence restores the capacity of the hippocampus to encode new information.

The standard theory leverages the distinction between various aspects of learning and memory formation, presenting memory as a dual system with hippocampal and neocortical components dynamically interacting during memory consolidation (Figure 1.1). The standard theory posits two distinct memory systems. The first system, mediated by the hippocampus and the Medial Temporal Lobe, rapidly encodes sparse representations and experiences, transferring them into a second, long-term system where memory traces integrate with pre-existing knowledge.

The interactions between the two memory systems are considered to be bidirectional; the transfer of new memories into the long-term store doesn't occur solely through hippocampal-neocortical transfer. Instead, an ongoing cross-talk between these systems facilitates a continuous refinement and adjustment of existing knowledge in response to new information (McClelland *et al.*, 1995; Stickgold & Walker, 2013). A fundamental connectionist principle in the standard theory outlines the optimal way the subsystems function to address the potential challenge of introducing new information into the neocortical system, particularly when it conflicts with existing knowledge, potentially disrupting pre-existing patterns. Disruption of these learned patterns can result in catastrophic interference, where the learning of new information erases previously acquired material. The dual nature of the memory systems enables the

preservation of stable memory representations for a more extended period, despite ongoing changes in input form (generalisation) and network structure.

The existence of the hippocampal system provides flexibility and enables the acquisition of new episodes without interference from previously or subsequently learned knowledge. The temporary representations stored in the hippocampus are sparser and independent, allowing for swift learning and supporting the slower and interleaved learning processes within the cortical system. In contrast, the neocortical pathway operates on different principles. Memories, such as procedural skills, gradually form through repeated exposures and can be acquired independently of hippocampal storage.

The standard theory avoids imposing a bottleneck or any 'gating' mechanism that regulates which memories may or may not require hippocampal mediation. Both procedural and declarative memories can be processed by both neocortical and hippocampal routes, provided that the learning aligns with the nature of the respective route (e.g., slow gradual learning for the neocortex; Squire, 1992). Supporting evidence for this perspective comes from studies on amnesic patients. While patients with hippocampal lesions exhibit deficits in forming and retaining new memories, they may still demonstrate unimpaired Hebb repetition effects (Baddeley & Warrington, 1970), reflecting memory of new information acquired through repetitive exposures (Hebb, 1966). Moreover, some knowledge acquired post-lesions, when assessed using familiarity measures, remains intact (Bayley *et al.*, 2008). Investigations into memory

representations in amnesiacs contribute valuable insights into the memory systems involved in memory formation in general.

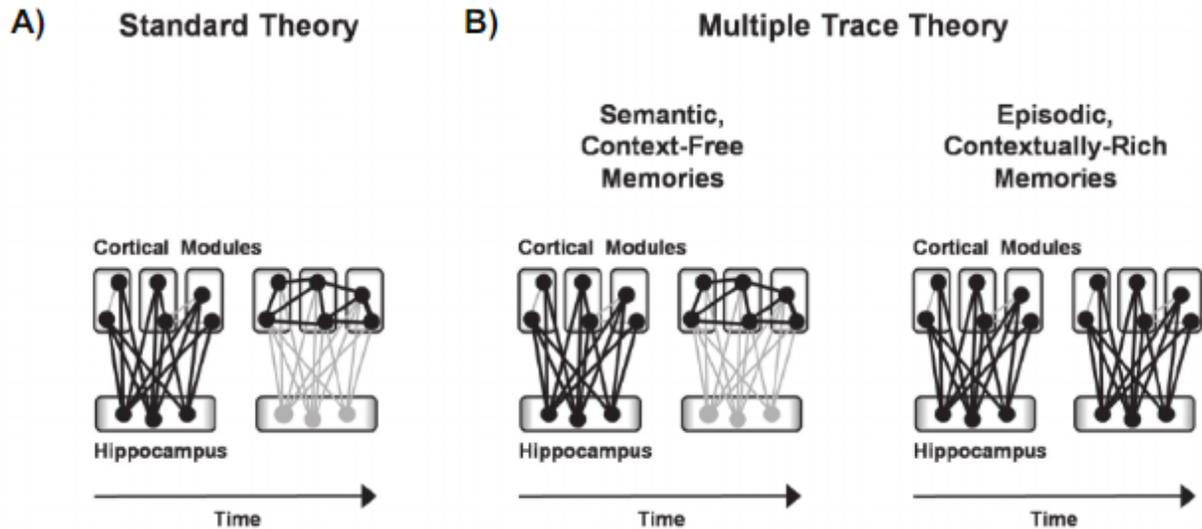


Figure 1.1 This figure, from Akers and Frankland (2009) visually explains the two theories of memory consolidation and the differences between them. A) standard theory and B) Multiple trace theory.

1.2.1.2 Multiple Trace Theory (MTT)

MTT, a memory consolidation model, presents an alternative perspective to the standard theory by suggesting potentially independent processing of semantic and episodic information (Moscovitch & Nadel, 1998; Nadel & Moscovitch, 1997). This model finds support in studies involving amnesic patients, where it is argued that retrograde memory deficits extend over decades and are often contingent on the extent of hippocampal damage and the nature of the declarative memory being tested.

According to MTT, when presented with episodic information, it is encoded in the brain as a unique memory trace comprising a combination of its attributes. The richness of episodic and contextual details relies on multiple memory traces generated in the hippocampus that remain connected to corresponding neocortical networks. As neocortical representations are believed to be context-free or semantic, the retrieval of remote semantic memories is thought to be possible even in the absence of a functioning hippocampus (Figure 1.1). Consequently, the prediction of MTT is that incomplete hippocampal damage should selectively disrupt the retrieval of recent (as opposed to remote episodic or semantic) memories. In contrast, comprehensive hippocampal damage should eliminate all episodic memories, regardless of age, while sparing memories predominantly dependent on the neocortex, such as remote semantic memories (Frankland & Bontempi, 2005).

Supporting this perspective, several reported cases highlight patients with retrograde amnesia after hippocampal damage showing the retrieval of memories spanning up to 35 years back (Maguire *et al.*, 2001). Additionally, research has indicated distinct post-encoding time courses for the consolidation of semantic and episodic memories. For instance, a study on a patient with retrograde amnesia demonstrated a profound impairment of episodic information but a notable retention of words learned during the amnesic period (McCarthy & Warrington, 1988). This study provides evidence supporting the idea of dissociated consolidation processes for episodic and semantic memories.

Neuroimaging research has provided additional support for the MTT. In a study conducted by Bosshardt *et al.* (2005), functional imaging techniques were employed to investigate the involvement of the hippocampus in the retrieval of episodic information. The authors reported that, relative to a one-day interval, episodic memory information exhibited more robust activity in both the hippocampus and neocortex one month after learning. Similarly, in a comparable study, the retrieval of episodic memories showed increased responses in the left hippocampus after a delay of 24 hours compared to a delay of 10 minutes (Bosshardt *et al.*, 2005).

However, it's important to note that this model has been found to have some discrepancies. For instance, Teng and Squire (1999) described a patient with elaborate bilateral lesions of the Medial Temporal Lobe who exhibited exceptional episodic memory from his youth. This suggests that episodic representations have the potential to become entirely independent of the hippocampus. In a more general sense, several studies, including neuroimaging investigations, have reported a reduction in hippocampal activity when testing newly acquired memories at remote time points. For example, Takashima *et al.* (2006) found that activity in the Medial Temporal Lobe observed during the retrieval of previously studied pictures gradually decreased over the course of three months, while activity in the medial prefrontal cortex progressively increased.

In conclusion, despite some differences concerning the time course required for consolidation, both the standard theory and MTT models concur on the existence of systems-level consolidation for semantic memory (Meeter & Murre, 2004), which

unfolds over time. While the systems consolidation theory posits a process extending over months or even years (Dudai, 2004), reports suggest a more gradual scenario. For instance, human neuroimaging research indicates that a substantial amount of memory reorganisation can occur in just one day (Takashima *et al.*, 2009). This contrasts with earlier studies on amnesiacs, which suggested that repetitive learning can lead to long-term memories despite hippocampal damage but over a much longer timeframe.

To reconcile these inconsistent findings, it has been proposed that different neural pathways come into play in the learning processes allowing amnesic patients to acquire new information (Foerde *et al.*, 2013). These pathways would involve interleaved and extensive exposure to new information, enabling some neocortical learning to take place.

1.2.2 Synaptic Consolidation

Synaptic consolidation encompasses both the rapid process of transforming a labile memory into a more stable form through the strengthening of synaptic connections, occurring within the initial hours after learning and reliant on new protein synthesis (Hebb, 1949; Xu *et al.*, 2007; Bailey *et al.*, 2004; Frankland and Bontempi, 2005), as well as the molecular changes at the synapse leading to structural modifications that underlie long-term memory. This phenomenon is highly conserved across species and memory types, as demonstrated in *Aplysia* (Castellucci *et al.*, 1970; Lin and Glanzman, 1994), rodents (Lipp and Wolfer, 1998), and humans (Dudai and Morris, 2000; Goelet *et al.*, 1986). The formation of the memory trace is believed to involve alterations in

synaptic weight or composition (Redondo and Morris, 2011), with enduring traces signifying memories that persist.

Protein synthesis plays a crucial role in synaptic consolidation, as the products of gene expression are essential for synaptic remodelling and growth (Davis and Squire, 1984; Hernandez and Abel, 2008). In the hippocampus, glutamatergic signalling initiates this process, leading to de novo protein synthesis and alterations in synaptic morphology. Activation of α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPA-Rs) on the postsynaptic density (PSD) triggers an influx of sodium ions (Na^+), resulting in membrane depolarization and removal of the N-methyl-D-aspartate-receptor (NMDA-R) magnesium block. Binding of glutamate and D-Serine to the NMDA-R allows an influx of calcium ions (Ca^{2+}), further depolarizing the membrane and, more importantly, activating numerous signalling pathways. These pathways include kinases and GTPases such as protein kinase A (PKA), mitogen-activated protein (MAP) kinase, and p190 Rho GTPase-activating protein (p190 RhoGAP) (Lamprecht and LeDoux, 2004; Lattal and Abel, 2001). These signalling molecules then initiate cytoskeletal alterations and modulate receptor expression and trafficking, ultimately leading to spinogenesis and synaptogenesis (Lamprecht and LeDoux, 2004).

Activity-dependent activation of protein kinase C (PKC) and Ca^{2+} /calmodulin-dependent protein kinase II (CaMKII) results in the phosphorylation of specific GluA1 sequences, driving AMPA-R insertion from extrasynaptic pools. This is followed by small G protein-mediated trafficking of AMPA-Rs and actin polymerization required to support the increased levels of AMPA-Rs (Derkach et al., 2007). Activation of PKC and PKA

also phosphorylates the GluN1 subunit, promoting endoplasmic reticulum export and trafficking of NMDA-Rs to the plasma membrane (Sans *et al.*, 2003). Modifications of both can lead to increased synaptic potentiation, corresponding to the strength of long-term memory expression (Migues *et al.*, 2010).

These outlined molecular changes appear to transpire within a specific time window initiated by memory acquisition, lasting on a scale of minutes to hours (Frey and Morris, 1997). Throughout this period, memories are believed to exist in a short-term form, characterised by a labile nature and sensitivity to factors that may inhibit synaptic processes, such as protein synthesis inhibitors. It is only after the completion of synaptic consolidation processes that the memory transforms into a long-term state, exhibiting stability and resistance to blockade. This holds true for synaptic changes induced by learning, as well as experimentally induced synaptic changes termed long-term potentiation (LTP). The operational definition of the end of synaptic consolidation is the point after learning (or LTP induction) when protein synthesis inhibitors no longer impact memory formation (or LTP). Early-LTP consolidates to late-LTP, and short-term memory consolidates to long-term memory.

Although the relationship between hippocampal LTP and synaptic memory consolidation remains under scrutiny, substantial evidence indicates that LTP is a plausible candidate for synaptic consolidation processes. A significant portion of our understanding of synaptic consolidation is derived from studies investigating LTP mechanisms (Martin *et al.*, 2000). With dendritic spine density, a sign of LTP, dynamic changes in dendritic spine density has been shown in both the hippocampus and the aCC (Restivo *et al.*,

2009; Vetere *et al.*, 2011). These structural changes occur sequentially during memory formation. The time-dependent reorganisation of cortical connections in the aCC contributes to memory expression independently of the hippocampus (Restivo *et al.*, 2009; Vetere *et al.*, 2011).

In experiments where PKC inhibitors were infused into the hippocampus of rats after training on an inhibitory avoidance task, disruptions in long-term memory consolidation occurred on a similar time scale to those observed when using PKC inhibitors to disrupt late-LTP, both *in vitro* and *in vivo* (Colley *et al.*, 1990; Huang *et al.*, 1992; Jerusalinsky *et al.*, 1994).

Similar associations have been observed between late-LTP and long-term memory for PKA and MAP kinase activity during contextual fear conditioning (Schafe *et al.*, 1999) and for cAMP response element binding protein during a Morris Water Maze spatial memory task (Guzowski and McGaugh, 1997). Studies have also reported increases in these proteins in the hours following spatial learning tasks (Porte *et al.*, 2008). It is worth noting that certain studies have successfully eliminated late-LTP *in vivo* while maintaining spatial long-term memory (Meiri *et al.*, 1998), suggesting that the mechanisms underlying late-LTP might not entirely explain the synaptic consolidation of spatial memory. Nevertheless, given the observed similarities between the two processes, LTP remains a likely candidate for the synaptic consolidation of the hippocampal memory trace.

1.3 Regions involved in memory consolidation

1.3.1 Hippocampus

Patient HM (Henry Molaison) was crucial in initiating research into the hippocampus because of the unique memory deficits he experienced after undergoing brain surgery to alleviate severe epilepsy. Following the surgery, HM suffered from profound anterograde amnesia, which impaired his ability to form new long-term memories (Squire and Zola-Morgan, 2011). However, his short-term memory and pre-surgery memories remained intact. Researchers studying HM's case discovered that the hippocampus played a central role in memory consolidation, the process of transferring new memories from short-term to long-term storage. This observation provided strong evidence for the involvement of the hippocampus in memory processes and inspired further research into the brain region's critical role in memory formation and consolidation. To understand how specific cells within the hippocampus take a role in the formation and consolidation of memories, several studies focused on the research of the engram (Josselyn and Tonegawa, 2020), the physical substrate of a memory trace. In this chapter I will explain how each of the main hippocampal subdivisions, the dentate gyrus (DG), the CA1 and the CA3 are involved in memory consolidation.

1.3.1 The Dentate Gyrus (DG) and Memory Consolidation

The DG within the hippocampus is a key player in memory and memory consolidation. Its role extends to pattern separation, a process that transforms similar representations into distinct, non-overlapping memories (O'Reilly & McClelland, 1994). High-resolution

functional magnetic resonance imaging studies have revealed a bias towards pattern separation activity in the DG, emphasising its crucial role in encoding unique memories (Baker *et al.*, 2016). Additionally, somatostatin-expressing cells within the DG regulate its excitability and modulate the efficacy of pattern separation during memory encoding. Inactivation of these somatostatin neurons impairs the discrimination of overlapping episodic-like memories, underscoring the role of the DG in facilitating the encoding of distinct memories (Morales *et al.*, 2019).

It is important to report here that the majority of the studies on the memory engram has been conducted in the DG. When DG neurons are tagged during contextual fear acquisition, their subsequent recall the day after in a new environment, unrelated to the conditioned one, is sufficient to recall the memory trace (Liu *et al.*, 2012; Tonegawa *et al.*, 2015).

The DG also plays a significant role in contextual fear memory consolidation. In fact, when protein synthesis in the DG is inhibited, amnesia for consolidated contextual fear memories can occur (Debiec *et al.*, 2002).

1.3.2 The CA1 Region and Memory Consolidation

Recent studies have delved into unravelling the role of CA1 in the formation of spatial memories, shedding light on its unique contributions. Notably, investigations have shown that CA1 inactivation during the early stages significantly impairs memory performance, whereas activation at later time points is not necessary (Frankland and Bontempi, 2005; Goshen *et al.*, 2011). These findings emphasise the temporal sensitivity of CA1 in spatial memory processes.

Expanding the focus to engram research, CA1 cells have been a subject of interest (Refaeli *et al.*, 2022; Roy *et al.*, 2022). Notably, Shpokayte *et al.* (2022) demonstrated the segregation of positive and negative engrams within the hippocampus, with specific attention to the CA1 region.

In the context of memory retrieval, CA1 plays a pivotal role. Coordinated activity among CA1 neurons supports the replay of previously stored memories, facilitating their consolidation in distributed hippocampal-neocortical circuits (Carr *et al.*, 2012). This underscores the dynamic involvement of CA1 in the retrieval and consolidation of spatial memories.

1.3.3 The CA3 Region and Memory Consolidation

The CA3 region of the hippocampus is a crucial hub for memory processes, encompassing consolidation, retrieval, and pattern completion (Lee *et al.*, 2020). Its unique internal connectivity and interactions with other hippocampal subregions underscore its significance in accurate memory storage and retrieval. AMPAR-mediated synaptic plasticity in CA3 is particularly vital for the consolidation of recently acquired memories (Oussini *et al.*, 2023). When consolidation fails, animals exhibit behaviour as if entirely naive to the task, highlighting the pivotal role of hippocampal synaptic plasticity, especially in CA3, for memory consolidation.

Pattern completion, the ability to retrieve complete memories from partial cues, is a distinctive function of the CA3 region due to its unique internal connectivity, facilitating the formation of autoassociative networks (Cherubini and Miles, 2015). The heterogeneity along the proximodistal axis of CA3 suggests distinct spatial encoding and behavioural roles within CA3 subregions (Sun *et al.*, 2017).

The CA3, with its extensive recurrent connections and plastic CA3-CA3 synapses, serves as the initial storage site for memory traces, functioning as an autoassociative memory network (Paxinos, 1994; Ishizuka *et al.*, 1990; Debanne *et al.*, 1998; Lee *et al.*, 2020). The arborized axon of a CA3 pyramidal cell establishes dendritic contacts with thousands of other CA3 neurons, contributing to its autoassociative capabilities. CA3-CA3 synapses display Hebbian forms of activity-dependent synaptic modification.

The theoretical foundation for CA3 as an autoassociative memory network is supported by experimental studies suggesting that CA3 may encode and temporarily store recent memories in its recurrent circuits before facilitating their permanent transfer to the neocortex (Crick & Mitchison, 1983; Siegel, 2001; Stickgold *et al.*, 2001; Wilson, 2002; Walker & Stickgold, 2004). This aligns with the two-stage model of memory formation, wherein the initial encoding phase occurs during wakefulness, and the transfer phase occurs during sleep. Recent genetic manipulations in rodent models, such as NMDA receptor deletion restricted to CA3, have further emphasised CA3's role in both the encoding and transfer of memories (Nakazawa *et al.*, 2003; Nakashiba *et al.*, 2009).

Lesions or pharmacological inactivation of CA3 can affect the encoding and retrieval of contextual memories, with CA3 neurons specifically involved in retrieving recent and one month old memories, while CA1 is recruited for remembering the gist of events, regardless of memory age (Daumas *et al.*, 2005; Lux *et al.*, 2016).

1.3.2 Cortex

Throughout memory research, the cortex has consistently been implicated as a crucial brain region involved in memory and memory consolidation. Both standard consolidation theory and multiple trace theory share the common belief that long-term memories are ultimately stored in the cortex (Akers and Frankland, 2009; Fig. 1.1). This convergence suggests that the cortex plays a central role in maintaining and storing long-term memory traces. Synaptic consolidation, which involves structural changes at the level of synapses, and systems consolidation, which entails the gradual redistribution of memory representations to the cortex from the hippocampus, collectively underscore the significance of the cortex in the process of long-term memory formation and retention (Frankland *et al.*, 2004; Frankland and Bontempi, 2005; Restivo *et al.*, 2009; Vetere *et al.*, 2011). Together, these theories highlight the integral role played by the cortex in the intricate mechanisms of memory consolidation, solidifying its position as a critical hub for the establishment of enduring memory traces.

Long term memories undergo a time-dependent reorganisation, with PFC gradually learning and storing the information that was initially processed by the hippocampus

(Bontempi et al. 1999; Frankland et al. 2004, 2004; Frankland and Bontempi, 2005; McClelland, McNaughton, and O'Reilly, 1995; O'Reilly and McClelland, 1994; Squire and Alvarez, 1995). This indicates that the PFC is critical for the storage and retrieval of remote spatial memories. Furthermore, structural changes in these regions during the consolidation of spatial memory have been observed, accompanied by a functional disengagement of the hippocampus (Maviel *et al.*, 2004; Restivo *et al.*, 2009; Vetere *et al.*, 2011). This suggests that the cortex undergoes reorganisation and takes over the storage of long-lasting memories.

The anterior cingulate cortex (aCC), a subregion of the PFC, activity is important for remote memories. During memory consolidation, the aCC is recruited, shown by an increase in activity within this region. This is shown through an increase in both (14C)2-deoxyglucose uptake, used to map changes in brain metabolic activity (Bontempi et al. 1999), and c-Fos and Zif268 expression, 2 immediate early genes used to map neuronal activity (Frankland *et al.*, 2004). Furthermore, it has been shown that spine growth in the aCC is necessary for the memory consolidation (Vetere *et al.*, 2011; Restivo *et al.*, 2009). The growth of dendritic spines, which are small protrusions on the surface of neurons, allows for the formation of new synapses and the strengthening of existing ones (Matsuzaki *et al.*, 2004). This process is thought to be important for the storage of information in the brain and the formation of long-term memories (Yuste and Bonhoeffer, 2004). These findings further support that the engagement of the aCC is necessary for remote memories.

aCC inactivation leads to severe impairment of remote memories. This has been shown through the pharmacological (lidocaine) inactivation of the aCC (Teixeira *et al.*, 2006), where the pharmacological inactivation of the aCC leads to a decrease in spatial memory only at remote, but not recent, time point. Furthermore, the same effect has been shown using optogenetics, where the optogenetic inhibition of the aCC resulted in a significant decrease in remote memory recall (Goshen *et al.*, 2011). These results indicate the crucial role of the aCC in remote memories.

PFC top-down projections to the CA1 region play a pivotal role in memory retrieval, as underscored by the findings of Rajasethupathy *et al.* (2015). Their research utilised optogenetic manipulation (explained in detail in section 3.2) of PFC-CA1 projections, revealing a significant impact on contextual memory retrieval. Additionally, the necessity of aCC to CA1 projections for recent memory recall was elucidated through two experiments conducted by Rajasethupathy *et al.* (2015). In the first experiment, retrograded ChR2 was injected into CA1, and cell bodies in aCC were stimulated. The subsequent training in Contextual Fear Conditioning on Day 1 was followed by exposure to Context B on Day 2, with light stimulation resulting in a significant increase in freezing in the experimental group. On Day 3, the return to Context A demonstrated fear memory in both the experimental and control groups, emphasising the robustness of memory recall. In the second experiment, anterograde inhibitory halorhodopsin was injected into aCC, with optrodes strategically positioned above the CA1. Inhibiting aCC-CA1 interactions during recall was found to increase freezing latency, providing

further insights into the intricate dynamics of memory processes at the neural circuit level.

1.3.3 Thalamus

The thalamus plays a multifaceted role in various cognitive processes. It has been linked to learning and memory, inhibitory control, decision-making, and sensory perception responses (Aggleton et al. 2016; Mitchell et al. 2014; Wolff et al. 2021). In particular, alterations in thalamocortical connectivity, notably with the lateral PFC, have been implicated in schizophrenia, impacting working memory and functional activity (Marenco *et al.*, 2012). Moreover, as individuals age, changes in the macrostructure, microstructure, and neural connectivity of the thalamus have been observed, potentially contributing to age-related declines in memory and attention (Fama and Sullivan, 2015).

This section will be on exploring how this critical brain region is intricately involved in the process of memory consolidation. I will explain how subregions of the thalamus are involved, however due to the purpose of my work, I will mainly focus on the ADn.

1.3.3.1 Thalamic Regions involved in memory

Within the thalamus, various subregions have been identified as key players in memory consolidation and recall processes, each contributing uniquely to the formation and storage of memories.

The mediodorsal nucleus has emerged as a critical hub in declarative memory processes. It processes stimulus information for storage and recall, indicating its active

involvement in memory formation (Mitchell and Chakraborty, 2013). By encoding and retrieving memories, the mediodorsal nucleus ensures that essential information is retained and accessible when needed.

The reuniens (NRe) and rhomboid nuclei have been closely associated with learning, contextual memory consolidation (Quet *et al.*, 2020), and behavioural flexibility (Cassel *et al.*, 2013). Their significance becomes evident when considering the impact of lesions or damage, which can lead to memory deficits, underscoring their crucial role in memory processes (Van der Werf *et al.*, 2003). Together, these nuclei play a crucial role in stabilising memories and making them available for future retrieval. Furthermore, The NRe serves as a key regulator for memory extinction, transmitting safety signals to the BLA and contributing to the attenuation of consolidated fear memories (Silva *et al.*, 2021).

The suprageniculate nucleus has been found to encode historical value memory of visual objects, signifying its importance in visual memory processes (Kim *et al.* 2020). As part of memory consolidation, the suprageniculate nucleus ensures that visual information is appropriately stored and accessible for later recollection.

Finally, we turn our attention to the anterior thalamic subregion, which boasts vital connections to the hippocampus and frontal cortex. This region's interconnectivity highlights its role in the modulation and regulation of memory processes (Vetere *et al.*, 2021). Notably, disruptions in this area have been associated with cognitive decline and memory impairments, further emphasising its significance in memory consolidation and recall.

1.3.3.2 Anterior Thalamic Subregion

The anterior thalamic nuclei (ATN), has been shown to be important in spatial memories (Aggleton and Brown, 1999; Jankowski *et al.*, 2013; Saalman and Kastner, 2015). It is important to know at this point that the ATN is part of the spatial representation system, a network that processes the environment around us. However, I will go into more detail of the properties of the ATN relating to the spatial representation network in Chapter 8 of this thesis.

The anterior and intralaminar/lateral thalamic nuclei have distinct functions in memory processes. Through chemogenetic inhibition, it was found that the anterior thalamic nuclei are responsible for recent memory retrieval (Vetere *et al.*, 2021), whereas the lateral mediodorsal nucleus is involved in remote memory retrieval (Lopez *et al.*, 2017; Vetere *et al.*, 2017). On the other hand, the intralaminar/lateral thalamic nuclei play a role in systems consolidation, but they are not involved in memory retrieval or reconsolidation. Furthermore, lesion studies showed that lesions of the ATN can produce anterograde amnesia for spatial memories (Béracochéa and Jaffard, 1994). These results suggest as a whole the ATN are important for spatial memories and different parts of the ATN are recruited at different time points. This section will be mainly dedicated to the ADn, as it is the region of the ATN that was the focus of my experiments.

The ADn, a region within the ATN, is implicated in recent memories and is also part of the spatial representation system, containing head direction cells (this aspect of the

ADn will be covered in a dedicated section in 8.2.1). It has been shown that the activation of the ADn is important for recent memory recall (Vetere *et al.*, 2021; Figure 1.2). Where the ADn is naturally more active at recent time point, as can be seen through cFos expression. The activity of the ADn has been shown to be important in recent memory recall, where the optogenetic inactivation of the ADn at recent, but not remote time point, leads to a decrease in memory recall.

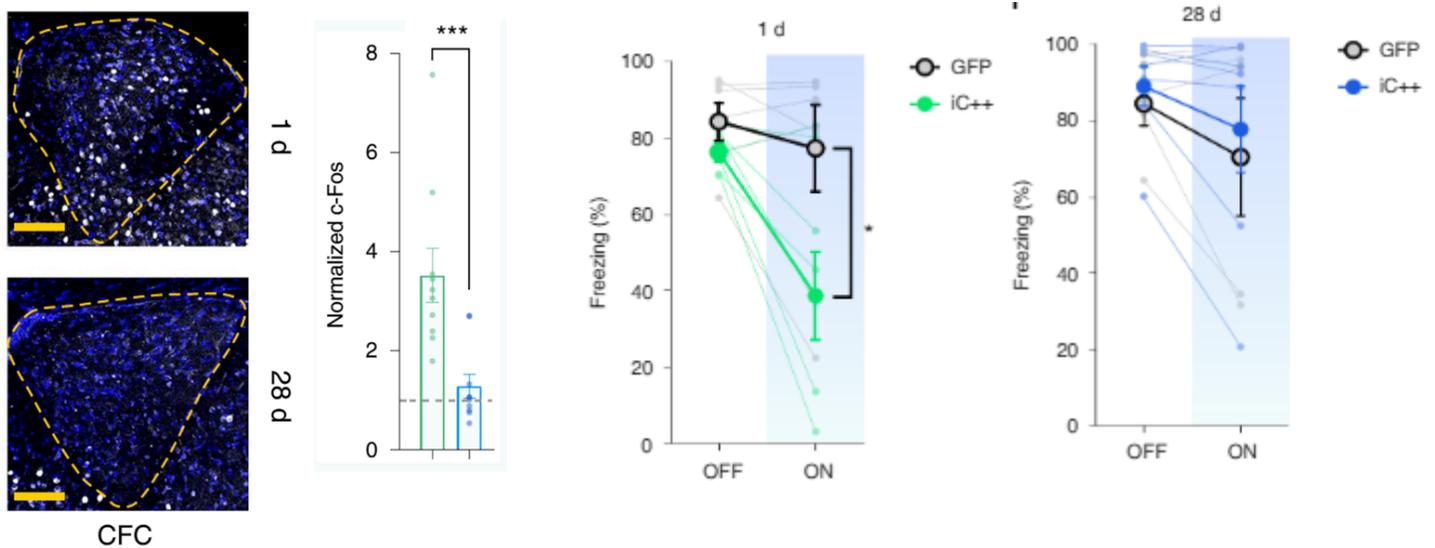


Figure 1.2. Figures edited from Vetere *et al.* (2021). (Left) Histology of cFos expression in the ADn at recent (1 day) and remote (28 days) time point, with statistical analysis (bar graph) showing that there is significantly higher cFos expression, *** = $p < 0.001$, at recent time point compared to remote time point. (Right) optogenetic inhibition of the ADn during recent time point (1 day, green) shows significant decrease in freezing behaviour. There is no significant change in freezing behaviour during optogenetic inhibition of the ADn during remote memory recall (28 days, blue) compared to controls.

Furthermore, using brainwide mapping of immediate early gene expression, the ADn has been implicated in remote memories ((Vetere *et al.*, 2017; Wheeler *et al.*, 2013). The functional connectivity of different brain areas during recent or remote memory recall was analysed and the ADn region exhibited a unique pattern in these networks. In detail, after recent memory recall, ADn c-Fos activity was positively correlated with the activity of the majority of 80+ different regions. However, a striking difference is seen during a remote memory, where the ADn activity is negatively correlated with the activity of the same 80+ regions (Figure 1.3). It is worth stressing that this result was unique to the ADn, no other region behaved this way.

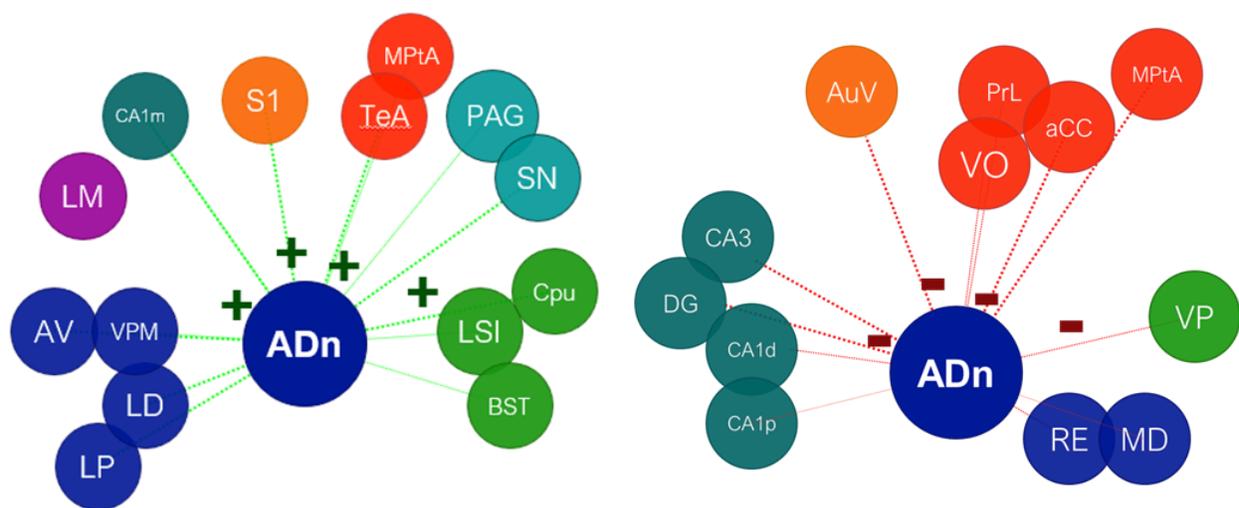


Figure 1.3 Figure representing data from Wheeler *et al.* (2013). The left highlights the positive correlations the ADn has with other regions at recent time point. The right highlights the negative correlations the ADn has with other regions at remote time point.

Putting the cFos expression of the ADn during recent and remote time point, together with functional connectivity analysis, raises the question of does the ADn need to be actively inhibited at remote time point for a memory to be recalled?

1.3.3.3 Thalamo-cortical and Thalamo-hippocampal communication important for memory consolidation.

The thalamus acts as a hub, processing information from various brain regions and relaying it to other areas involved in memory processes, such as the cortex (Kim et al. 2020) and the hippocampus (Aggleton and Brown, 1999). These connections suggest that the thalamus plays a role in memory consolidation.

On that note, thalamo-cortical connectivity during consolidation periods has been shown to influence long-term memory (Brodt et al. 2023). Furthermore, in the process of memory consolidation, there appears to be a mechanism involving coordinated thalamocortical activity, relaying information between the hippocampus and cortex. With this thalamo-cortical coupling during the formation and consolidation of a memory having been linked to a stronger and more durable memory (Wagner *et al.*, 2019).

The thalamus also has strong anatomical projections to the hippocampus, suggesting its involvement in driving hippocampal spindles and contributing to memory consolidation (Aggleton and Brown, 1999; Wagner *et al.*, 2019). The interplay between the thalamus and hippocampus highlights their cooperative role in memory consolidation. It has been shown that hippocampal-thalamic communication is necessary for remote memory recall, with inhibition of inhibitory projections from the CA3 to the ADn results in impaired memory performances (Vetere *et al.*, 2021). This suggests that thalamo-hippocampal communication is important for memory

consolidation. These results start to answer the question I posed at the end of the last section, of, does the ADn need to be actively inhibited at remote time point for a memory to be recalled? Indeed it seems as though the ADn does need to be actively inhibited at remote time point and the inactivation of inhibitory projections results in a decrease in memory recall.

1.3.3.4 Hippocampal-cortical-thalamic communication important in memory

To go further into detail of the brain wide mapping I mentioned earlier, it has been shown that there is a hippocampal-cortical-thalamic signature during recall of memory that appear only at remote time point and not before (Wheeler et al., 2013; Vetere et al., 2017). This suggests that hippocampal-cortical-thalamic signature communication is necessary for consolidating and retrieving long-term memories (see Fig. 1.2).

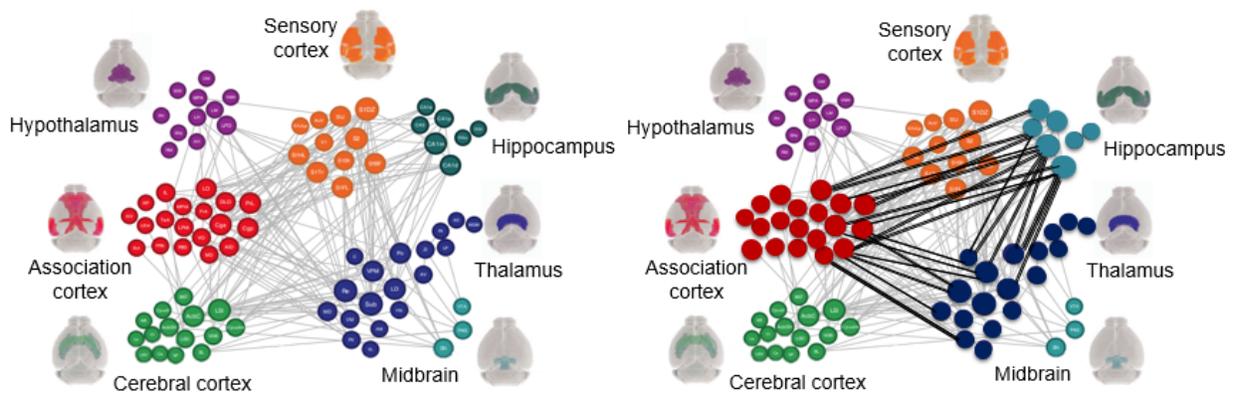


Figure 1.2 Modified from Vetere et al. (2017). Map of the functional connectivity (*c-Fos* expression that co-varies across mice) of 84 different regions during a context based aversive memory. There is a notable increase in connections (*lines*) between the cortex (red areas), the hippocampus (dark green) and the thalamus (dark blue) between a

recent memory (top) and a remote memory (bottom, left). The hippocampal-thalamic-cortical signature is highlighted in bold black lines (left, right).

Indeed, computational models have been used to show that coordinated activity of the thalamus, cortex and hippocampus are necessary for memory consolidation. Tomé *et al.*, (2022) proposed a computational model elucidates the role of the hippocampus, thalamus, and cortex in memory representation dynamics by coordinating synaptic plasticity timescales and incorporating a hippocampus-thalamus-cortex circuit. According to the model, direct, monosynaptic hippocampal to cortical projections cannot fully account for the known interdependencies between engrams in these regions. However, a network with hippocampal-thalamic-cortical communication can overcome this limitation. Specifically, the model demonstrates that hippocampal engram cells, as well as the coupled reactivations of memory representations across brain regions, are crucial for proper memory representation dynamics. This study is not only an example of systems consolidation, but also supports the multiple trace theory, showing that the thalamic communication is necessary for memory consolidation.

This basis of the computational model, that hippocampal-cortical-thalamic communication is necessary for memory, is supported by biological studies. It has been shown that the coordinated activity of the thalamus, cortex and hippocampus during sleep is necessary for memory formation (Contreras and Steriade, 1995; Lara-Vásquez *et al.*, 2016; Latchoumane *et al.*, 2017; Slézia *et al.*, 2011; Varela and Wilson, 2020). The triple phase-locking mechanism that underlies memory formation during sleep

involves the coupling of slow oscillations, spindles, and ripples. Thalamic spindles, when phase-locked to the slow oscillation up-state, boost memory consolidation by coordinating the interplay between these three rhythms. This mechanism is thought to mediate effective hippocampal-to-neocortical information transmission, which serve as long-term storage sites for memories. Such that, thalamic inhibition during slow oscillation up-states can impair memory formation. Optogenetic suppression of thalamic spindles in-phase with the slow oscillation up-state impaired hippocampus-dependent memory. This suggests a causal role for thalamic sleep spindles in hippocampus-dependent memory consolidation.

Chapter 2

The main set of results of my thesis are split into two parts, memory consolidation, as previously described in the last chapter and the expression of memory related fear behaviour. In this chapter I will introduce the principles of fear expression and why it is important. To study the processes of fear expression, we used contextual fear conditioning. The conditioned response associated with contextual fear conditioning is freezing. This chapter investigates the contribution of various brain regions and their interactions in shaping fear responses and modulating fear memories, specifically freezing behaviour and contextual memories.

Memory-Related Fear Expression

2.1 Why do we need to fear?

The term "fear" refers to the emotion that arises when confronted with an imminent threat, giving rise to potent effects that trigger physiological and behavioural changes aimed at promoting threat avoidance and survival. Despite its longstanding interest in the scientific community, the definition of fear has sparked intense debate, particularly regarding whether it encompasses the subjective feeling of fear or its underlying physiological processes (Gross & Canteras, 2012). In animal studies, the term "fear" or "fear response" is used to describe the combined behavioural and physiological reactions observed in the presence of threat-related signals. For example, a threat from predators, this can be represented behaviourally by freezing, or fleeing (De Franceschi

et al., 2016). However, the extent to which this terminology in animal studies accurately corresponds to the human experience of fear is a matter of considerable debate (Gross & Canteras, 2012). Nevertheless, there is an emerging consensus that unravelling the neural circuits responsible for the behavioural and physiological responses to threats holds the key to a better understanding of fear and, by extension, the psychological pathologies rooted in fear.

According to J. LeDoux, fear is a conscious experience of feeling afraid, signifying a subjective state of inner awareness (LeDoux, 2000; 2012; 2014). In support of his concept, there's a suggested distinction between circuits underlying two classes of responses elicited by threats, known as the "two-system framework" (LeDoux & Pine, 2016): one circuitry for behavioural responses and associated physiological changes in the brain and body, and another for conscious feeling states reflecting fear and anxiety. In summary, the first system largely involves subcortical areas and innate circuitry inherited from ancestors, operating unconsciously and leading to behavioural and physiological responses when activated. On the other hand, the second system relies on cortical areas and is primarily involved in generating conscious and subjective feelings. This implies that such a complex and personal experience is specific to humans and challenging to translate to other animals.

However, this definition is challenged by M. Fanselow, who defines fear as a functional neural-behaviour system that generates defensive behaviours evolved to provide protection against environmental threats (Bolles & Fanselow, 1980; Fanselow, 2019). In contrast to LeDoux, he argues that various physiological, behavioural, and

cognitive-emotional responses to danger emerge from a central neuronal circuit, best conceptualised as one single system, namely the "central fear generator" (Fanselow, 2018). He also introduces the concept of a threat-imminence continuum (Fanselow 2018; Fanselow *et al.*, 2019), where defensive reactions are organised along a continuum corresponding to the proximity to fatal contact with the predator. The physical distance to the predator is a prominent factor allowing the prey to assess where it stands on this continuum and adapt its feeding and drinking demands accordingly. In addition to the distance with the predator, its size, identity, effectiveness, probability of occurrence, and timing until the predator gets closer are all elements of predatory imminence.

2.2 Innate vs learnt fear

Innate behaviours, such as reflexes, are shared by all members of a species as part of their heritage and require minimal learning. In contrast, learned behaviours are innate responses under the control of novel stimuli through associative learning, specifically Pavlovian conditioning (Pavlov, 1927; Gross & Canteras, 2012). Pavlovian conditioning, in the form of contextual fear conditioning, is described in detail in section 3.1. To briefly summarise, an animal learns to associate a neutral environment with an aversive stimuli, normally foot shocks. The behavioural result is that the animal freezes in response to once neutral context.

While there is agreement that innate fear has, at some point, been learned, transmitted to offspring, and further developed within organisms through observational learning or

epigenetic factors, it can be deduced that during innate fear, there is no need to establish an association between a stimulus and a negative outcome to later consider the stimulus as a threat.

2.3 Regions involved in fear expression

2.3.1 Role of cortex in fear expression

As previously described, the PFC is important for memory consolidation and remote memory recall. PFC and its subregions (aCC, prelimbic and infralimbic in particular) have also been reported to be important in the regulation of fear responses.

The PFC is interconnected with the amygdala, which provides a top-down pathway for control of fear responses (Courtin *et al.*, 2014; Dejean *et al.*, 2016; Giustino and Maren 2015). To quickly introduce the amygdala, it is a region that is known for emotions, such as acquisition and extinction of conditioned fear response (Pape and Pare, 2010). The prelimbic, infralimbic and dorsal parts of the medial PFC are connected to the amygdala and are involved in the modulation of fear expression (Courtin *et al.*, 2014; Maren, 2011). The dorsal medial PFC controls the passive or active defensive responses depending on the context (Jercog *et al.*, 2021). The synchronised oscillations of principal neurons in the neuronal network essential for the accurate transmission of information that will drive behaviour. The activity of these principal neurons are regulated by GABAergic interneurons. The optogenetic inhibition of these interneurons leads to the disinhibition of the principal neurons that project to the amygdala (Courtin *et al.*, 2014). This disinhibition led to the synchronised firing of the principal neurons and

as a result led to fear expression. Furthermore, it has been found that within synchronised oscillations there is a phase-specific mechanism that can precisely control the timing of fear responses (Dejean *et al.*, 2016). In summary, the PFC-amygdala circuitry is important in the regulation of fear expression.

In the context of cortico-thalamic interaction in regulating fear expression, it has been previously shown that the NRe regulate freezing responses. The NRe is a structure previously associated with fear memory reconsolidation and generalisation (Silva and Gräff, 2023). In detail, the projections to the PFC inhibit the NRe to reduce freezing response in animals (Moscarello, 2020). This can be useful in certain scenarios where there is a chance of escape.

2.3.2 Role of Hippocampus in fear expression

The hippocampus is an important part of the spatial representation system (this aspect of the hippocampus will be covered in section 3.1) and has been implicated in the modulation of contextual fear expression. Lesion studies show that lesions in the hippocampus result in the disrupted contextual conditioned fear responses (freezing and startle response) but not to explicit cue responses (Kim and Fanselow 1992; McNish, Gewirtz, and Davis 1997; Phillips and LeDoux 1992). Moreover, lesion studies within the ventral hippocampus lead to a reduction in fear expression (Kjelstrup *et al.*, 2002). Interestingly, whole hippocampal or ventral hippocampal lesioned rats showed less fear and stress (less defecation and lower corticosterone secretion) compared to rats within the dorsal hippocampus or sham lesions. All together, lesion studies have provided insight into the role of the hippocampus in contextual fear expression.

2.3.3 Role of Amygdala in fear expression

There is a widely accepted understanding that the amygdala functions as a rapid detector of aversive environmental stimuli, coordinating threat responses and subsequently generating adaptive responses adapted to behavioural states (LeDoux, 2007; Maddox, 2019). Studies across human and other species consistently reveal that the structures and functions of the amygdala are highly conserved across species, being activated by both aversive and fear-conditioned stimuli (Mobbs *et al.*, 2010; Janak & Tye, 2015).

Following these observations, lesions in the amygdala in both rodents and humans have been found to result in impaired recognition of fearful stimuli. For example, electrolytic lesions in the amygdala performed in rats impaired contextual fear conditioning learning, leading to decreased conditioned freezing (Kim *et al.*, 1993). Similarly, patients with focal bilateral amygdala lesions showed impairment in their ability to recognize fearful faces and exhibited misjudged social threat signals (Anderson & Adolphs, 2014). All things considered, this underscores the role of the amygdala as a crucial brain region in connecting stimuli with their emotional meaning.

2.3.4 Role of thalamus in fear expression

The thalamus is also implicated in fear processing. The NRe is an important region for fear expression, specifically in reducing the fear expression. It has previously been mentioned that the PFC projections to the NRe regulate fear responses (Moscarello, 2020). The attenuation of fear accompanied by the activation of a circuit centred on the NRe (Silva and Graff, 2023). Furthermore, a thalamo-amygdalar circuit has been identified that underlies the extinction of remote fear memories (Silva *et al.*, 2021). Specifically, it identifies the NRe as a key structure in routing fear-related information from the infralimbic cortex to the basolateral amygdala for remote fear memory extinction. All together, the NRe is an important region for the regulation of fear expression, where its activity is related to fear attenuation.

Lesion studies have been effective in identifying regions in the thalamus that are important for fear expression. For instance, the dorsal midline of the thalamus was shown to be important for the regulation of conditioned fear (Li *et al.*, 2004). With lesions in the dorsal midline of the thalamus causing impairment in the acquisition and freezing expression to the conditioning context. The paraventricular nucleus of the thalamus (PVT) has attracted attention due to its robust connections to brain regions involved in the control of emotional behaviours. More specifically, the posterior PVT (pPVT) has projections to the amygdala (Li and Kirouac, 2008). Lesion in the pPVT led to the attenuation of fear expression (Li *et al.*, 2014). In summary lesion studies have

identified the dorsal midline of the thalamus and the pPVT as important regions in fear expression.

Chapter 3

Techniques

This chapter aims to provide a comprehensive exploration of the techniques employed in this PhD thesis. By delving deeper into their nature, functionalities, and practical applications, I aim to equip the reader with a thorough understanding of the methodologies that underpin the research in this thesis.

3.1 Contextual Fear Conditioning

Pavlovian fear conditioning, a behavioural paradigm, involves organisms learning to anticipate aversive events. This learning process entails associating an aversive stimulus (e.g., an electrical shock) with a neutral context (e.g., a room) or stimulus (e.g., a tone), resulting in fear responses towards the initially neutral stimulus or context. Conditioning is achieved through the pairing of the neutral stimulus with an aversive one, such as an electric shock, loud noise, or unpleasant odour. Subsequently, the neutral stimulus alone can evoke a fear response. In classical conditioning terms, the neutral stimulus or context becomes the "conditioned stimulus" (CS), the aversive stimulus is the "unconditional stimulus" (US), and fear constitutes the "conditional response" (CR).

Extensively studied across various species, from snails (Gainutdinova *et al.*, 2005) to humans (Alvarez *et al.*, 2008), conditioned fear in humans is often assessed through verbal reports and galvanic skin response. In animal models, freezing is one of the most

apparent indicators of fear behaviours. It can be defined as the complete immobilisation of the body, excluding movements related to breathing, and is typically accompanied by a crouching posture (Blanchard & Blanchard, 1969).

Distinct circuits have been shown to be involved in the storage and recall of fear memories. Initially, the prelimbic prefrontal cortex and basolateral amygdala were implicated in memory recall, with subsequent identification of the central amygdala and the paraventricular nucleus of the thalamus in maintaining fear memories (Courtin *et al.*, 2014; Dejean *et al.*, 2016; Giustino and Maren 2015; Pape and Pare, 2010; LeDoux, 2007; Maddox, 2019; Moscarello, 2020; Silva and Graff, 2023; Silva *et al.*, 2021). This research highlights dynamic shifts in circuits governing short-term and long-term recall without altering behaviour or response, solely influencing the memory's recall location.

Certain fear conditioning types, such as contextual and trace conditioning, involve the hippocampus, a region of the brain believed to receive affective signals from the amygdala. The hippocampus integrates these signals with pre-existing information to confer meaningfulness. Theoretical perspectives on traumatic experiences propose that during intense stress, amygdala-driven fear may circumvent the hippocampus, leading to somatic storage or the formation of images.

To explore the hippocampal-thalamic-cortical trace during memory recall, I used contextual fear conditioning (Figure 5.1) as the behavioural paradigm. I chose

contextual fear conditioning as, as previously explained, contextual based fear memories engage the hippocampus.

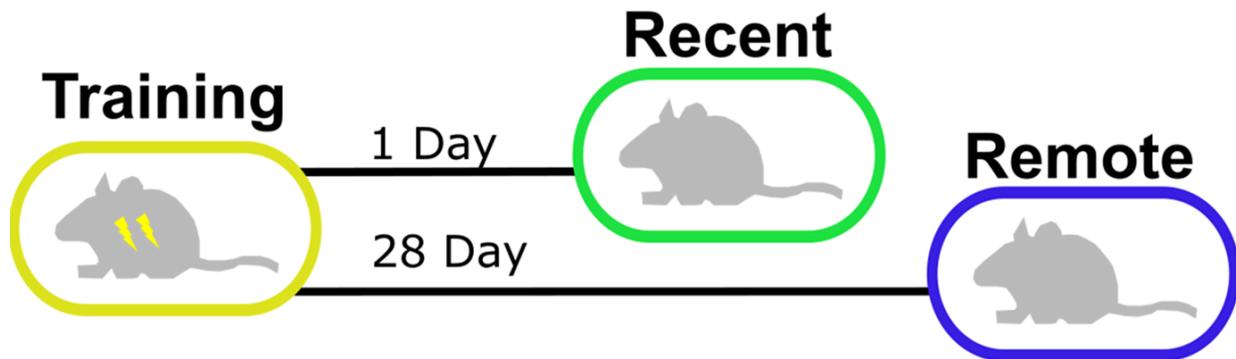


Figure 3.1 Schema explaining the contextual fear conditioning paradigm used in experiments. Mice were trained (yellow) to associate a new and neutral context with an aversive stimuli (2 electrical footshocks, of 0.2mA, lasting for 2 seconds each). Mice were then, either, placed back in the same context 1 day or 28 days later to test their recent (green) or remote (blue) memory, respectively.

3.2 Optogenetics

Optogenetics stands as a technological innovation facilitating precise and swift manipulation of specifically identified occurrences within intricate biological systems, even in freely moving animals. Through the provision of optical control characterised by rapidity in the millisecond range and the requisite precision targeting specific cell types, optogenetic methodologies have ushered in novel frontiers for exploring the realms of biology, encompassing investigations into both health and disease.

Optogenetics is a powerful technique in neuroscience that utilises light-sensitive proteins called opsins to control and manipulate the activity of specific neurons in living organisms. It allows for precise and reversible modulation of neuronal activity with high spatial and temporal resolution (Wick and Krook-Magnuson, 2018).

There are different types of opsins that can be used in optogenetics, including channelrhodopsins (ChRs) and halorhodopsin (NpHRs). Channelrhodopsins are cation channels that, when activated by light, allow the influx of positive ions such as sodium or calcium, leading to neuronal depolarization and activation (Tucker *et al.*, 2022). Halorhodopsins, on the other hand, are chloride pumps that, when activated by light, cause hyperpolarization of the neuronal membrane and inhibition of neuronal activity (see Figure 5.2).

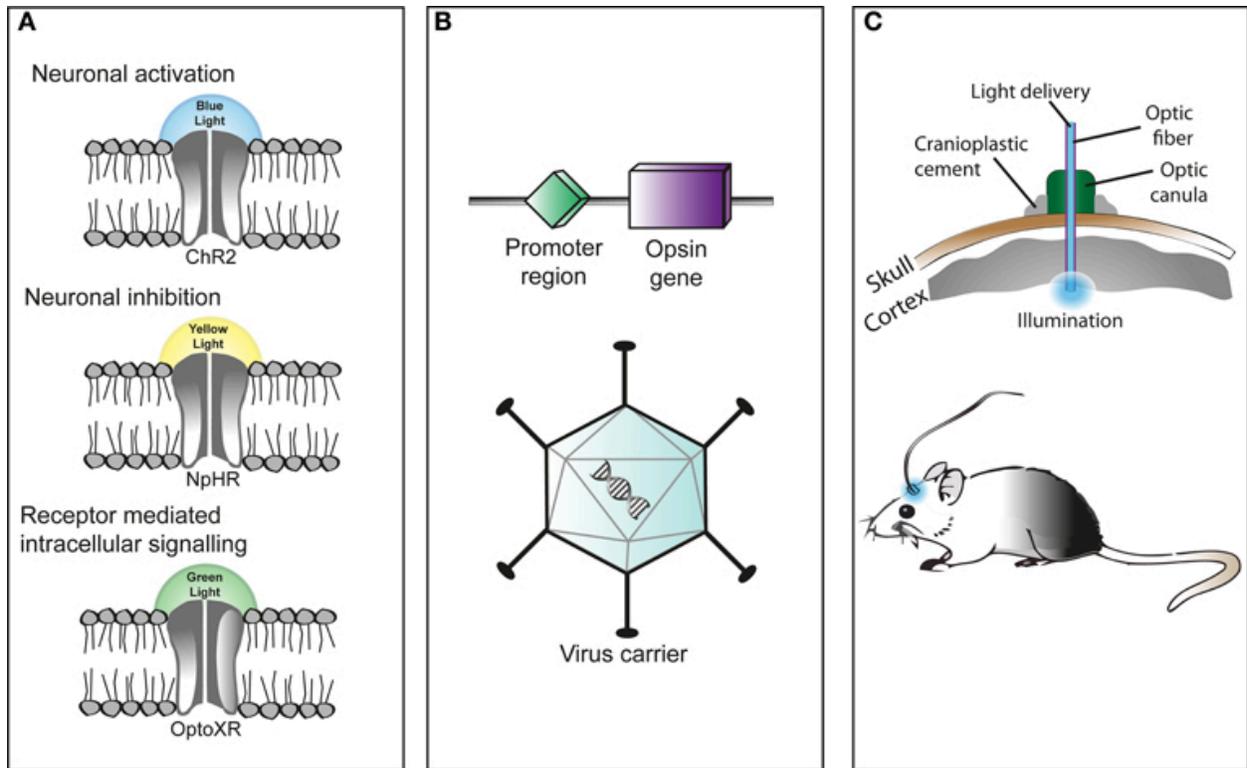


Figure 3.2 Schematic explanation of optogenetics. a) Example of light dependent opsins and their effect on neuronal modulation. (Top) Blue light activates channelrhodopsin which leads to neuronal activation. (Middle) Yellow light activates halorhodopsin which leads to neuronal inhibition. (Bottom) Green light activates which leads to neuronal activation

One of the new inhibitory opsins that has been developed is an inhibitory ChRs, iC⁺⁺. iC⁺⁺ is an anion ChRs derived from cryptophyte algae that allows for efficient and sensitive hyperpolarization of the neuronal membrane through light-gated chloride conduction (Berndt *et al.*, 2016; Govorunova *et al.*, 2015). It provides optogenetic inhibition tools with unprecedented light sensitivity and temporal precision, allowing for precise control of neuronal activity and silencing of specific neural circuits. In my

experiments, I use iC++ as my inhibitory opsin, along with a control virus that only expresses an eYFP fluorophore.

Compared to other techniques such as chemogenetics and lesioning, optogenetics offers several advantages. Chemogenetics relies on the use of designer receptors exclusively activated by designer drugs (DREADDs), which require the administration of exogenous ligands to activate or inhibit neuronal activity (Wick & Krook-Magnuson, 2018). In contrast, optogenetics enables direct and precise control of neuronal activity using light, eliminating the need for systemic drug administration. Additionally, optogenetics allows for the manipulation of specific cell types or neuronal circuits with high spatial resolution, which is not easily achievable with lesioning techniques.

However, there are limitations that need to be taken into consideration when using optogenetics. The power of optogenetics is limited by the degree to which these proteins can be selectively expressed and activated in specific neuronal cell types *in vitro* and *in vivo*. Furthermore, there is a potential that the cells become damaged due to the exposure to laser light, which can hinder the reliability and reproducibility of results.

3.3 Fiber Photometry

Fiber photometry is a technique that allows for the measurement of neural activity in genetically defined populations of neurons by recording calcium dynamics (Formozov *et al.*, 2023). It has gained popularity in recent years due to its ability to provide real-time monitoring of neural activity *in vivo*, particularly in freely behaving animals (Patel *et al.*,

2020). Fiber photometry has evolved as a versatile tool for studying neural circuit dynamics and has several advantages compared to other techniques.

One of the main advantages of fiber photometry is its compatibility with genetically encoded calcium indicators, which allow for the measurement of calcium fluorescence signals that directly reflect changes in neuronal activity (Liang *et al.*, 2017). This technique provides a relatively simple and artefact-free method for simultaneously recording neural signals and can be used to monitor cell-type-specific population activity *in vivo* (Patel *et al.*, 2020). Fiber photometry is also less invasive compared to other techniques such as electrophysiology, as it involves the insertion of an optical fiber into the brain region of interest rather than electrodes (Legaria *et al.*, 2021). This minimises tissue damage and allows for long-term recordings in freely behaving animals.

In comparison to other *in vivo* calcium imaging techniques, such as two-photon microscopy, fiber photometry has lower spatial resolution (Bruno *et al.*, 2021). However, it compensates for this limitation by providing a relatively homogeneous picture of neuronal activity and capturing changes in population activity.

Fiber photometry can be used in conjunction with optogenetics to investigate how the activity of a region as a whole reacts to a stimulus. Optogenetics allows for the precise control of neuronal activity using light-sensitive opsins, while fiber photometry enables the simultaneous measurement of calcium dynamics in response to the optogenetic manipulation (Gunaydin *et al.*, 2014). This combination allows researchers to examine

the causal relationship between neural activity and behaviour by manipulating specific neural circuits and monitoring the resulting changes in calcium fluorescence signals.

In my thesis, I used Fiber photometry to investigate the population level calcium dynamics within the aCC and ADn in response to freezing behaviour. Furthermore, I used fiber photometry, coupled with optogenetics, to study the population level calcium dynamics in the ADn in response to the optogenetic modulation of excitatory projections from the CA3 during memory recall.

3.4 Retrobeads

Retrobeads serve as a valuable tool in neuroscience research, allowing scientists to explore the intricate neural connectivity and functional circuits within the brain. These fluorescent microspheres are employed to label and trace neuron projections in specific brain regions (Aklan *et al.*, 2020; Althammer and Grinevich, 2018; Beier *et al.*, 2015; Ferrara *et al.*, 2022; Hung *et al.*, 2017; Lammel *et al.*, 2011, 2012; Ledderose *et al.*, 2023; Olthof *et al.*, 2019). Retrobeads find application in the exploration of brain region connections and the mapping of neural circuits: Injecting retrobeads into a specific brain region allows researchers to label and trace the neurons projecting to that region, thus shedding light on their connections with other areas. Retrobeads offer advantages over other tracing methods, such as viral vectors, as they have limited diffusion at the injection site due to their larger molecular size. This characteristic enables more precise targeting of specific cell populations or nuclei (Lammel *et al.*, 2012). Moreover,

Retrobeads can be combined with other techniques like in situ hybridization or immunohistochemistry to investigate functional connectivity.

Furthermore, Retrobeads enable investigations into the effects of stimuli on specific neuron populations. For example, Lammel et al. conducted a study where Retrobeads were injected into various target brain regions to examine the modulation of excitatory synapses on dopamine neurons in response to rewarding or aversive stimuli (Lammel *et al.*, 2011). The results indicated that the modulation of synapses on DA neurons depended on the specific brain region to which they projected, suggesting that the mesocorticolimbic DA system consists of distinct circuits influenced by different aspects of motivationally relevant stimuli.

In my thesis, I used retrobeads to retrogradely stain the projections sent to the ADn and confirm that within the regions that send projections are the aCC and the CA3. I used retrobeads in the same vein to confirm projections from the CA3 to the aCC.

Chaptire 4: Les Résultats

Cet article constitue l'aboutissement des principaux résultats obtenus au cours des quatre années de ce doctorat. Il se concentre sur l'étude du rôle d'un nouveau circuit hippocampique-cortical-thalamique pendant le rappel de souvenirs consolidés. Les modèles précédents suggéraient que la consolidation de la mémoire impliquait l'hippocampe dans les premiers stades et le néocortex par la suite. Cependant, des

recherches récentes ont mis en évidence l'importance du thalamus comme plaque tournante de la communication entre l'hippocampe et le cortex pendant la formation des souvenirs à distance. En particulier, on a découvert qu'une nouvelle projection inhibitrice à longue distance de l'hippocampe CA3 vers le thalamus ADn est impliquée dans le rappel des souvenirs de peur à distance. En étendant et en mettant en œuvre ces résultats, ma recherche vise à fournir une compréhension globale de la façon dont l'interaction entre CA3 et ADn a un impact sur l'activité et la fonctionnalité du cortex préfrontal dans le rappel des souvenirs lointains. En outre, j'étudierai la régulation de l'expression de la peur, qui est étroitement liée au rappel de souvenirs lointains.

Dans notre étude sur l'activité neuronale du circuit cortico-hippocampique, nous avons examiné les niveaux d'expression de c-Fos dans le champ CA3 de l'hippocampe et le cortex cingulaire antérieur (aCC) à différents moments liés à la mémoire de peur contextuelle. Les souris ont été entraînées au conditionnement de la peur contextuelle et testées à des moments récents ou éloignés (1 jour ou 28 jours après l'acquisition de la mémoire). La coloration de c-Fos a révélé une augmentation significative de l'activité dans le champ CA3 au moment récent, tandis que le CCA présentait une activité accrue pendant le rappel éloigné. Ces résultats concordent avec des études antérieures et soulignent la nature dynamique de l'implication de CA3 et de l'aCC à des stades distincts de la mémorisation.

De plus, nous avons étudié le rôle régulateur de CA3 dans le noyau dorsal antérieur (ADn) et l'activation neuronale de l'aCC pendant le rappel de la mémoire à distance.

Confirmant les projections inhibitrices de CA3 vers ADn, nous avons inhibé par optogénétique les projections de CA3 vers ADn et observé une diminution significative du rappel de mémoire, spécifiquement à des points temporels éloignés. Cet effet était associé à une augmentation de l'activité de l'ADn, soulignant le rôle des projections du CA3 dans la modulation de l'activité de l'ADn pendant le rappel de mémoire.

De manière intéressante, nous avons constaté que les niveaux de c-Fos de l'aCC étaient significativement plus bas lorsque les projections CA3 vers ADn étaient inhibées par optogénétique pendant le rappel de mémoire, à la fois à des moments récents et à des moments éloignés. Cela suggère que la voie CA3 à ADn peut indirectement influencer le recrutement de l'aCC dans le rappel de mémoire. Ces résultats contribuent à notre compréhension des dynamiques complexes au sein du circuit cortico-hippocampique pendant le traitement et le rappel de la mémoire, en soulignant les contributions spécifiques de CA3 et de ses projections dans l'orchestration de la récupération de la mémoire à distance.

Part II

Results

Chapter 4

Hippocampal-cortical-thalamic connections are necessary for memory recall and fear expression

Charlotte Andrews¹, Maelle Christiaens¹, Camilla Mancini^{1,2}, Fabiola Avila Gamiz^{1,3}, and Gisella Vetere¹

¹ Cerebral Codes and Circuits Connectivity team, Brain Plasticity Unit, CNRS, ESPCI Paris, PSL Research University, 10 Rue Vauquelin, 75005, Paris, France

² University of Camerino, School of Pharmacy, Pharmacology Unit, Camerino, Italy.

³ Psicobiología y Metodología de las Ciencias del Comportamiento, Univ. de Málaga, Málaga, Spain.

4.1 Summary

This article constitutes the output of the main results of 4 years of this PhD. It focuses on the investigation of the role of a novel hippocampal-cortical-thalamic circuitry during the recall of consolidated memories. Previous models suggested that memory consolidation involved the hippocampus in the early stages and the neocortex later on. However, recent research has highlighted the importance of the thalamus as a gate hub for communication between the hippocampus and cortex during the formation of remote memories. In particular, a novel inhibitory long-range projections from the hippocampal CA3 to the thalamic ADn as been discovered to be involved in recall of remote fear memories. By extending and implementing these findings, here my research aims to provide a comprehensive understanding of how the interaction between CA3 and ADn

impacts the activity and functionality of the prefrontal cortex in recalling remote memories. Additionally, I will investigate the regulation of fear expression, which is closely related to remote memory recall.

4.2 Participation Statement

I contributed to the conception of the project and the experimental design. For the optogenetic experiments, I performed all surgeries, behavioural experiments, analysis and generated all the figures. I conducted all of the cFos analysis, from histology, acquisition of images to cell counting. For retrobeads, I acquired the images and analysed the location of injection and projections. For the fiber photometry experiments, I implemented the technique and created the protocol used in the lab, performed the majority of the surgeries and experiments, with significant input into the analysis and the generation of the figures. I, together with my supervisor, wrote the article.

Abstract:

Memory consolidation, the process that stabilises short-term memories into long-lasting ones, is essential in our daily life. This process, initially dependent on hippocampal recruitment, shortly relies on the coordination of activity between hippocampus and neocortex. Recent researches suggested that the thalamus may act as a gateway for memory-dependent hippocampo-cortical communication. Here, we have uncovered a new hippocampo-thalamo-cortical axis that is necessary for both remote fear memory recall and the expression of fear-related behaviour in mice. In our experiments, we trained mice in a contextual fear conditioning paradigm and subsequently re-exposed them to the same conditioned context either 1 day or 28 days later, to assess recent and remote memory, respectively. Freezing behaviour was evaluated as an indicator of fear memory expression.

We confirmed that CA3 hippocampal neurons projecting to the anterodorsal nucleus of the thalamus (ADn) are necessary for the retrieval of consolidated memories since their optogenetic inactivation leads to impairment in memory performance (i.e. decrease of freezing behaviour) during remote, but not recent, fear memory recall. This manipulation indirectly affected anterior cingulate cortex (aCC) activity. Additionally, we found that CA3 also directly affects consolidation-dependent aCC recruitment, since optogenetic inhibition of CA3-to-aCC projections disrupted remote memory recall but had no effect on recent memory. Interestingly, our study provides evidence for a role of aCC-to-ADn circuit in regulating fear-related behaviour associated with memory. Specifically, we found that aCC-to-ADn inactivation results in increased freezing levels at both recent and remote time points. Overall, this study reveals a novel CA3-ADn-aCC pathway that regulates both fear memory consolidation and the expression of fear-related behaviours.

Introduction

Memory consolidation is a fundamental process for the stabilisation and storage of newly acquired information into long-term memory. Traditional models of memory consolidation suggest that the hippocampus plays a crucial role in the initial phases of memory formation and consolidation, with the neocortex assuming control at later stages (Buzsáki 1996; Frankland and Bontempi, 2005; McClelland *et al.*, 1995; Squire and Alvarez, 1995; Winocur *et al.*, 2010). However, functional connectome studies in mice have provided insights into a more complex network of functionally connected brain regions active during remote memory recall, revealing a a robust hippocampo-thalamic-cortical signature that appears at a remote time point (Vetere *et al.*, 2017; Wheeler *et al.*, 2013). Recent studies also highlighted the involvement of the thalamus (Cassel *et al.*, 2013; Jankowski *et al.*, 2013; Schlecht *et al.*, 2022;. Van der Werf *et al.*, 2000) as a relay between the hippocampus and cortex during memory consolidation (Contreras and Steriade, 1995; Lara-Vásquez *et al.*, 2016; Latchoumane *et al.*, 2017; Slézia *et al.*, 2011; Tomé *et al.*, 2022; Varela and Wilson, 2020).

By investigating the differences of the 2 networks of brain regions coactive at recent versus remote time point (Wheeler *et al.*, 2013; Vetere *et al.*, 2017), Vetere *et al.* (2021) identified one specific thalamic region that exhibits dynamic changes in functional connectivity over time, the anterodorsal thalamic nucleus (ADn). These changes showed that ADn, functionally connected with the vast majority of the other brain regions at recent time point, exhibited at remote time point a majority of negative correlations with the rest of them, suggesting that its inactivation during remote memory recall is a requisite for proper memory performances. Following this observation, Vetere *et al.* (2021) indeed demonstrated that inhibitory neurons in the CA3 region of the hippocampus projecting to the ADn are more active during remote memory recall and that their inactivation impairs memory performance. These findings highlight the importance of investigating the functional connectivity and interactions within the CA3-ADn circuitry for a better understanding of memory consolidation processes. Moreover, knowing the role of prefrontal cortex and in particular of the anterior cingulate cortex (aCC) in memory consolidation (Frankland and Bontempi 2005; Maviel *et al.*

2004; Peyrache *et al.*, 2015; Takehara-Nishiuchi and McNaughton, 2008; Vetere et al. 2011), we asked here whether CA3-ADn interaction has a role in aCC engagement during remote memory recall.

Specifically, the aCC has been shown to be involved in the recall of remote memories, particularly in the expression of remote fear spatial memories (Frankland *et al.*, 2004). Pharmacological inactivation of the aCC blocks the expression of remote spatial memory (Teixeira *et al.*, 2006) and disruption of dendritic spine growth in the aCC right after contextual fear conditioning impairs the following consolidation of contextual fear memory and remote fear recall (Vetere et al., 2011).

While the role of CA1 and aCC in memory consolidation has been intensely explored, representing the basis for the classical theories of memory consolidation (Akers and Frankland, 2009), the involvement of CA3 in directly engaging with the aCC and its role in controlling recent and remote memory traces is less known. We seek to address this knowledge gap by investigating the structural connectivity between CA3-aCC-ADn and we employ optogenetic manipulation techniques to gain insights into their respective roles in the formation and consolidation of long-lasting memories.

Results

Neuronal activity of cortico-hippocampal circuitry.

To define the engagement of CA3 and aCC regions during the lifetime of a memory trace, we assessed c-Fos expression levels at different time points. We trained mice in a contextual fear conditioning, and we tested them at recent or a remote time point, 1 day or 28 days after memory acquisition. In 3 different groups of animals, 90 minutes after training (“Training” group), recent recall (“Recent” group) or remote recall (“Remote” group), mice were perfused and we performed c-Fos staining on the CA3 field of the hippocampus and the aCC. A fourth group of mice was perfused without undergoing any behavioural task, to control for c-Fos activity in naive condition (“Control” group) (Fig. 1a). We found a significant increase in c-Fos expression in the CA3 field of the hippocampus (Fig. 1b) at recent time point, compared to all other time points (no memory: 2-tailed unpaired t-test; $t_{18}=3.13$, $p<0.01$; training: 2-tailed unpaired t-test; $t_{19}=3.05$, $p<0.01$; and remote recall: 2-tailed unpaired t-test; $t_{21}=2.17$, $p<0.05$). When analysing aCC neuronal activity in the different groups (Fig. 1c), we found a significant increase in c-Fos expression at remote time point, compared to all other time points (no memory: 2-tailed unpaired t-test; $t_{14}=6.42$, $p<0.001$; training: 2-tailed unpaired t-test; t-test, $t_{10}=4.78$, $p<0.001$; and recent recall: 2-tailed unpaired t-test; t-test, $t_{11}=4.54$, $p<0.001$). These results confirm previous findings (Frankland *et al.*, 2004; Park and Chung, 2019) and provide novel data on CA3 recruitment at selected time points. To note that, while CA3 activity at remote time point is not higher than control levels, Tayler *et al.*, (2013) showed that CA3 neurons active during acquisition are preferentially reactivated at remote, but not recent, time point, and Vetere *et al.* (2021) showed that CA3 subpopulation of cells projecting to ADn is significantly more active at remote time point related to recent. These 2 studies pointed to the presence of fundamental subpopulations within the CA3 playing a disproportionate role in remote memory recall that cannot be identified when analysing CA3 c-Fos overall activity.

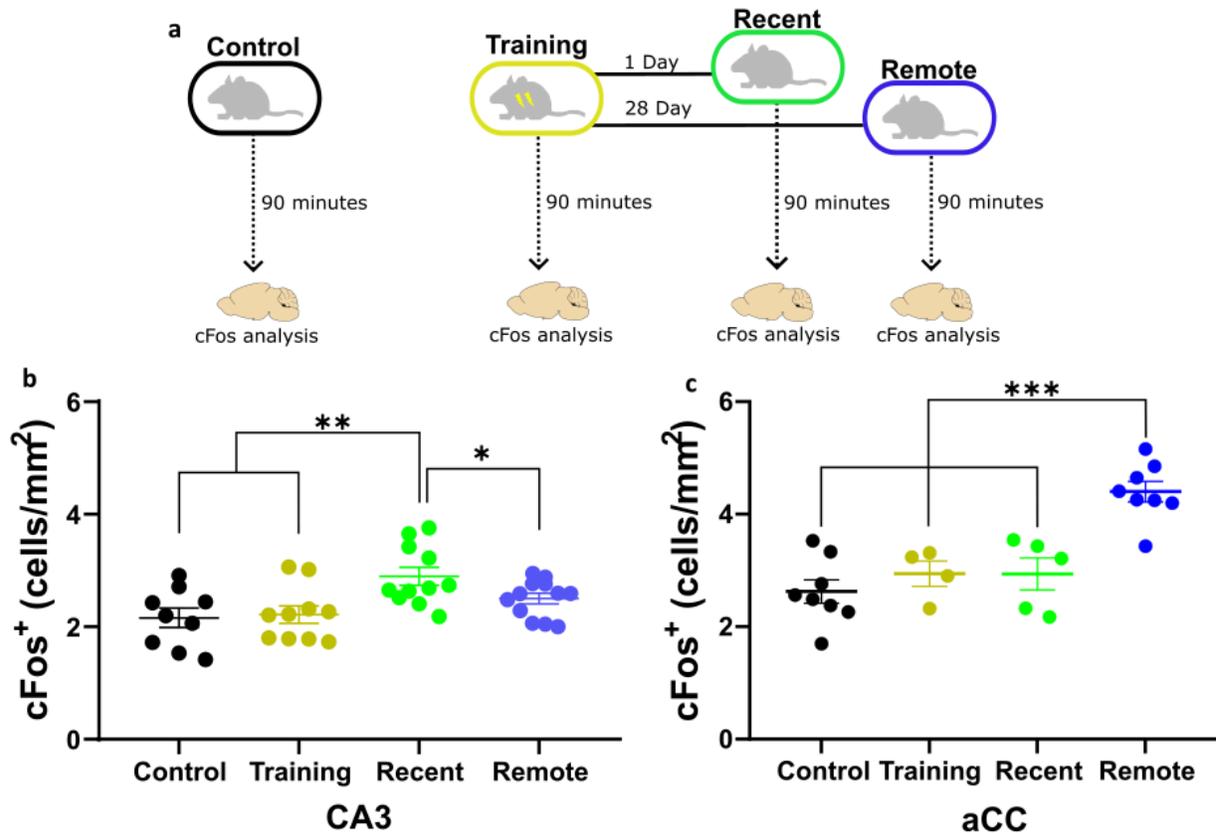


Figure 1. Neuronal activity of CA3 and aCC regions over the lifetime of a memory. **a)** Protocol schema highlighting the 4 experimental groups tested. **b)** CA3 c-Fos levels and **c)** aCC c-Fos levels are compared across groups. ** $p < 0.05$; * $p < 0.01$, *** $p < 0.001$

CA3 regulates both ADn and aCC neuronal activation during remote memory recall.

Vetere *et al.* (2021) showed that CA3 inhibitory projections to ADn, if inactivated, lead to both activation of ADn and impairment in memory performances at remote but not recent time points. Here we asked if excitatory projections from CA3 to ADn also exist and what is their involvement in the recall of fear memories (Fig. 2a). To this aim, we first confirmed the presence of projections from the CA3 to the ADn by unilateral infusion into the ADn of retrobeads and analysis of retrogradely labelled neurons (Fig. 2b). We then injected mice with an inhibitory channelrhodopsin, iC⁺⁺ (AAV-CamkII-iC⁺⁺-eYFP), a light-activated chloride channel with improved chloride

selectivity and conductivity related to other inhibitory opsins (Berndt *et al.*, 2015), or a control virus (AAV-CamkII-iC⁺⁺-eYFP) bilaterally in the CA3 and implanted an optic fiber medial to the ADn (Fig. 2c). Then we fear conditioned mice in a context by exposing them to 2 foot shocks (0.2mA, lasting 2 seconds) at minutes 2 and 3 of the 4 minute training session. Mice were then placed back in the same context, either 1 day or 28 days later to test respectively their recent or remote memory, and subsequently the effect of our optogenetic manipulation on recall. The test lasted 6 minutes, and during minutes 4-6, we optogenetically targeted the projections from the CA3 to the ADn (Fig. 2d).

The optogenetic inhibition of CA3 projections to the ADn leads to a significant decrease in memory recall (i.e., reduction in freezing behaviour compared to control animals) at remote (2-way Repeated Measures ANOVA indicated an effect of laser stimulation, $F_{1,30}=9.33$; $p<0.01$; post-hoc analysis of the ON periods confirmed this: ON eYFP vs ON iC⁺⁺-eYFP, $t_{16}= 2.03$, $p<0.05$) but not recent (2-way Repeated Measures ANOVA, $F_{1,22}=3.07$; *ns*; aCC: $F_{1,22}=3.07$; *ns*) time point (Fig. 2e,f).

During recall of contextual fear memories ADn activity is higher when recent memories are recalled, and equal to control groups when recalling remote memories (Vetere *et al.*, 2021). To test the effect of CA3 to ADn optogenetic silencing, 90 minutes after the tests mice brains were stained for c-Fos. ADn c-Fos expression was significantly higher in iC⁺⁺ mice compared to the eYFP group at both time points (recent: 2-tailed unpaired T-test; $t_6=9.42$, $p<0.001$; remote: 2-tailed unpaired T-test; $t_{30}=2.35$, $p<0.05$; Fig. 2h). No differences were found when we analysed c-Fos activity in the CA3 at either time point (recent: 2-tailed unpaired T-test; $t_6=1.03$, *ns* and remote: 2-tailed unpaired T-test; $t_{34}=0.44$, *ns*; Fig. 2g).

It is well known that aCC is active and necessary for remote memory recall in normal conditions. Therefore, we analysed c-Fos levels in the aCC in the same groups of mice to verify if CA3 to ADn optogenetic inactivation has an effect on aCC recruitment during fear memory recall. Interestingly, we found that aCC c-Fos levels were significantly lower, compared to control, when we optogenetically inactivated CA3 projections to ADn

during memory recall at both time points (recent: 2-tailed unpaired T-test; $t_6=2.53$, $p<0.05$; remote: 2-tailed unpaired T-test; $t_{10}=5.41$, $p<0.05$; Fig. 2i). This suggests that the CA3 to ADn pathway may indirectly control aCC recruitment in memory recall.

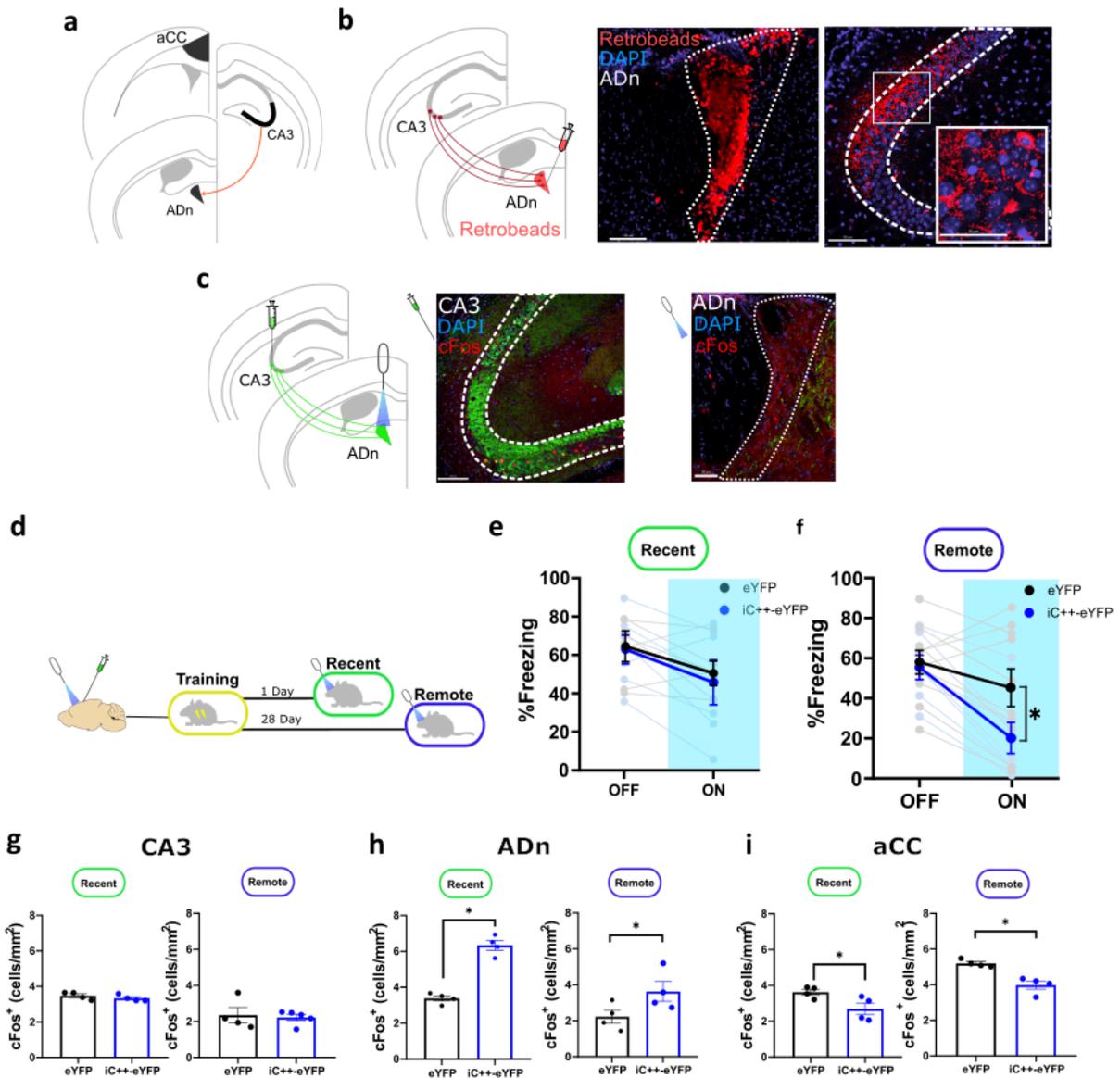


Figure 2. Optogenetic inhibition of CA3 projections to ADn. **a)** Schema of projections targeted in experiment. **b)** Confirmation of projections via retrobead tracing. Injection in the ADn (left) and labelled neurons in the CA3 (right). **c)** Schema of bilateral viral injection in the CA3 and optic fiber implant mediolateral in the ADn. Confirmation of injection in the CA3 and projections in the ADn. Percentage of freezing during

*optogenetic stimulation at e) recent and f) remote time points. The blue shaded area depicts the laser stimulation. Level of c-Fos expression during optogenetic inhibition in the g) CA3 at recent (green, left) and remote (blue, right) time points; in the h) ADn at recent (green, left) and remote (blue, right) time points; in the i) aCC at recent (green, left) and remote (blue, right) time points. * $p < 0.05$. Scale bar = 100 microns*

Building on the data that revealed aCC's indirect inactivation during CA3-ADn opto-manipulation, we were motivated to investigate whether CA3 directly regulated aCC activity during fear memory recall at recent and remote time points (Fig. 3a). To this aim, we first confirmed existing projections from the CA3 to the aCC by unilateral infusion into the aCC of retrobeads and analysis of retrogradely labelled neurons (Fig. 3b). We then injected mice with an inhibitory channelrhodopsin, iC++ (AAV-CamkII-iC++-eYFP), or a control virus (AAV-CamkII-iC++-eYFP) bilaterally in the CA3 and implanted an optic fiber medial to the aCC (Fig. 3c). Then we trained mice in a contextual fear conditioning by exposing them to 2 foot shocks (0.2mA, lasting 2 seconds) at minutes 2 and 3 of the 4 minute training session. Mice were then placed back in the same context, either 1 day or 28 days later to test their recent or remote memory, respectively (Fig. 3d).

The test lasted 6 minutes, and during minutes 4-6, we optogenetically inhibited the targeted projections from the CA3 to the aCC. The optogenetic inhibition of CA3 projections to the aCC lead to a significant decrease in freezing behaviour during memory recall at remote (2-way Repeated Measures ANOVA, indicated an effect of laser stimulation, $F_{1,18}=4.71$; $p < 0.05$; post-hoc analysis of the ON periods confirmed this: ON eYFP vs ON iC++-eYFP, $t_{18}= 3.03$, $p < 0.01$) but not recent (2-way Repeated Measures ANOVA, $F_{1,22}=3.07$; *ns*) time point (Fig. 3e,f).

To test and validate the effect of optogenetic inhibition of CA3 projections to aCC, we analysed c-Fos expression by perfusing mice 90 minutes after the end of the test and staining the brains for c-Fos. aCC c-Fos levels were significantly lower in iC++ mice compared to control at both time points (recent: 2-tailed unpaired T-test; $t_7=2.48$, $p < 0.05$; remote: 2-tailed unpaired T-test; $t_{10}=4.40$, $p < 0.05$, Fig. 3h). Interestingly, we also

analysed c-Fos levels in the ADn after CA3-aCC manipulation and found that c-Fos levels were significantly lower in iC⁺⁺ mice compared to control a remote (2-tailed unpaired T-test; $t_6=3.10$, $p<0.05$) but not recent time point (2-tailed unpaired T-test; $t_7=1.69$, ns ; Fig. 3i). Moreover, when we analysed c-Fos activity in the CA3 we found no differences at either time point (recent: 2-tailed unpaired T-test; $t_7=0.56$, ns and remote: 2-tailed unpaired T-test; $t_6=0.38$, ns ; Fig. 3g).

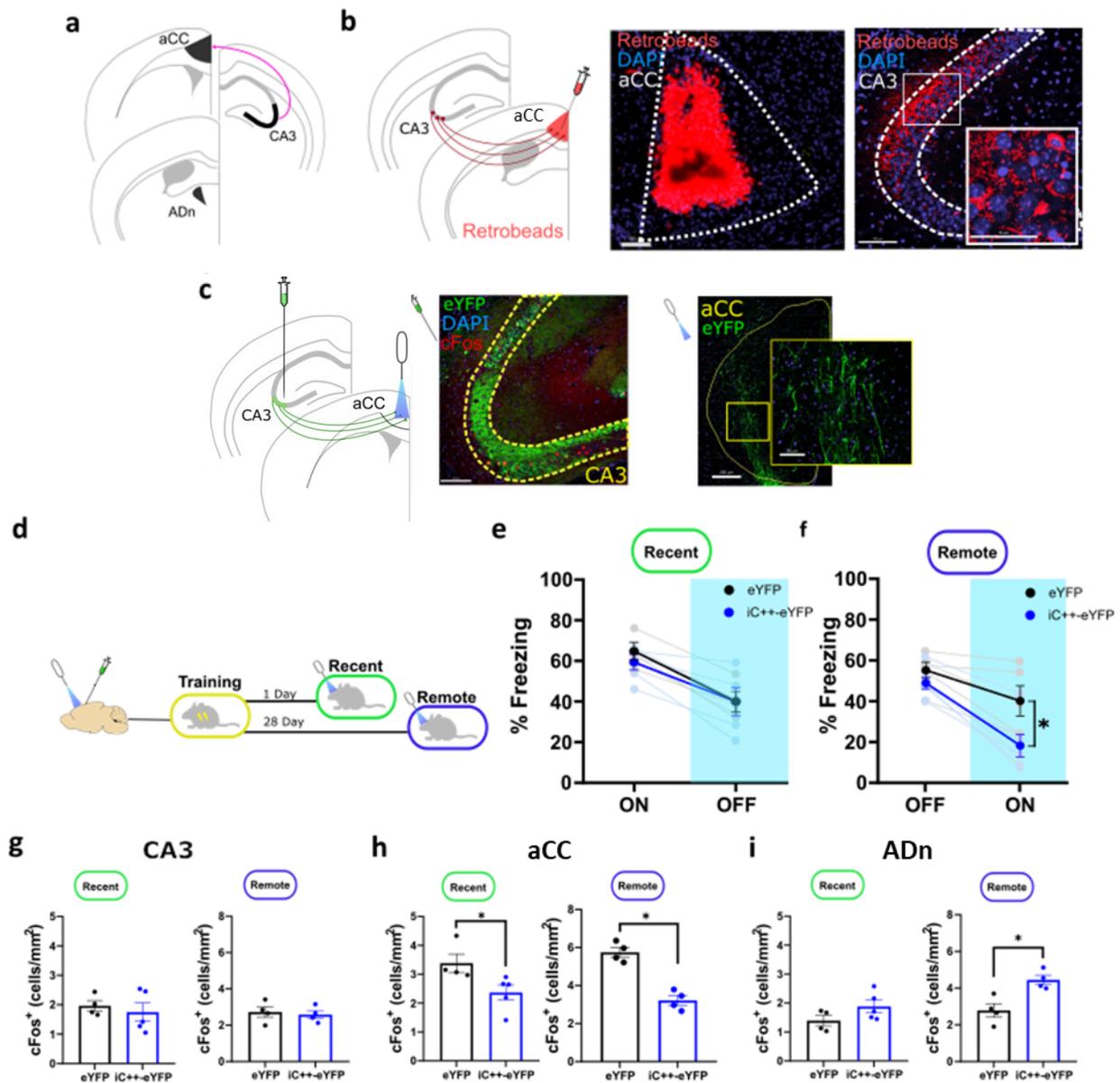


Figure 3. Optogenetic inhibition of CA3 projections to aCC. **a)** Schema of projections targeted in experiment. **b)** Confirmation of projections via retrobead tracing. Injection in the CA3 (left) and labelled neurons in the aCC (right). **c)** Schema of bilateral viral injection in the CA3 and optic fiber implant mediolateral in the aCC. Confirmation of injection in the CA3 and projections in the aCC. Percentage of freezing during optogenetic stimulation at **e)** recent and **f)** remote time points. The blue shaded area depicts the laser stimulation. Level of c-Fos expression during optogenetic inhibition in the **g)** CA3 at recent (green, left) and remote (blue, right) time points; in the **h)** aCC at recent (green, left) and remote (blue, right) time points; in the **i)** ADn at recent (green, left) and remote (blue, right) time points. * $p < 0.05$ scale bar = 100 microns

aCC projections to the ADn are important for fear expression during recall of recent and remote memories.

In the following set of experiments, we asked if aCC activity directly controls ADn and if this pathway has a role in recalling fear memories, with the aim to potentially identify a novel downstream target for aCC activity (Fig. 4a). First, we confirmed existing projections from the aCC to the ADn by injecting unilaterally retrobeads in the ADn and analysing the existence of labelled neurons in the aCC (Fig. 4b). We then injected mice with an inhibitory channelrhodopsin, iC++ (AAV-CamkII-iC++-eYFP), or a control virus (AAV-CamkII-iC++-eYFP) bilaterally in the aCC and implanted an optic fiber medial to the ADn (Fig. 4c). Then we trained mice in a novel context by exposing them to 2 foot shocks (0.2mA, lasting 2 seconds) at minutes 2 and 3 of the 4 minute training session. Mice were then placed back in the same context, either 1 day or 28 days later to test their recent or remote memory, respectively. The test lasted 6 minutes, and during minutes 4-6, we optogenetically targeted the projections from the aCC to the ADn (Fig. 4d).

Surprisingly, we found that during the optogenetic inhibition of the projection from the aCC to the ADn, there was a significant increase in freezing behaviour in the iC++ mice related to eYFP mice at both recent (2-way Repeated Measures ANOVA, indicated an effect of laser stimulation, $F_{1,28}=0.02$; $p < 0.05$; post-hoc analysis of the ON periods

confirmed this: ON eYFP vs ON iC++-eYFP, $t_{28}=2.40$, $p<0.01$) and remote time points (2-way Repeated Measures ANOVA indicated an effect of laser stimulation, $F_{1,16}=5.81$; $p<0.05$; post-hoc analysis of the ON periods confirmed this: ON eYFP vs ON iC++-eYFP, $t_{16}=2.03$, $p=0.05$; Fig. 4e,f), suggesting that aCC-ADn pathway may play a role in regulating fear expression.

We then analysed c-Fos expression in the ADn during the optogenetic inhibition of the projections from the aCC and found significantly lower levels of c-Fos in iC++ mice compared to control at both time points (recent: 2-tailed unpaired T-test; $t_6=8.87$, $p<0.001$; and remote: 2-tailed unpaired T-test; $t_6=3.75$, $p<0.01$; Fig. 4h). No changes in c-Fos expression was found in the CA3 between iC++ and eYFP mice at either time point (recent: 2-tailed unpaired T-test; $t_6=1.03$, *ns*; and remote: 2-tailed unpaired T-test; $t_6=0.44$, *ns*; Fig. 4i) while a decrease in overall activity was detected in the aCC at both time points (recent: 2-tailed unpaired T-test; $t_6=6.92$, $p<0.001$ and remote: 2-tailed unpaired T-test; $t_7=3.61$, $p<0.01$; Fig. 4g). These results suggest that, compared to control animals, the decrease in ADn activity is associated with an increase in freezing. They also suggest that, overall, the CA3 is not affected by the aCC-ADn communication.

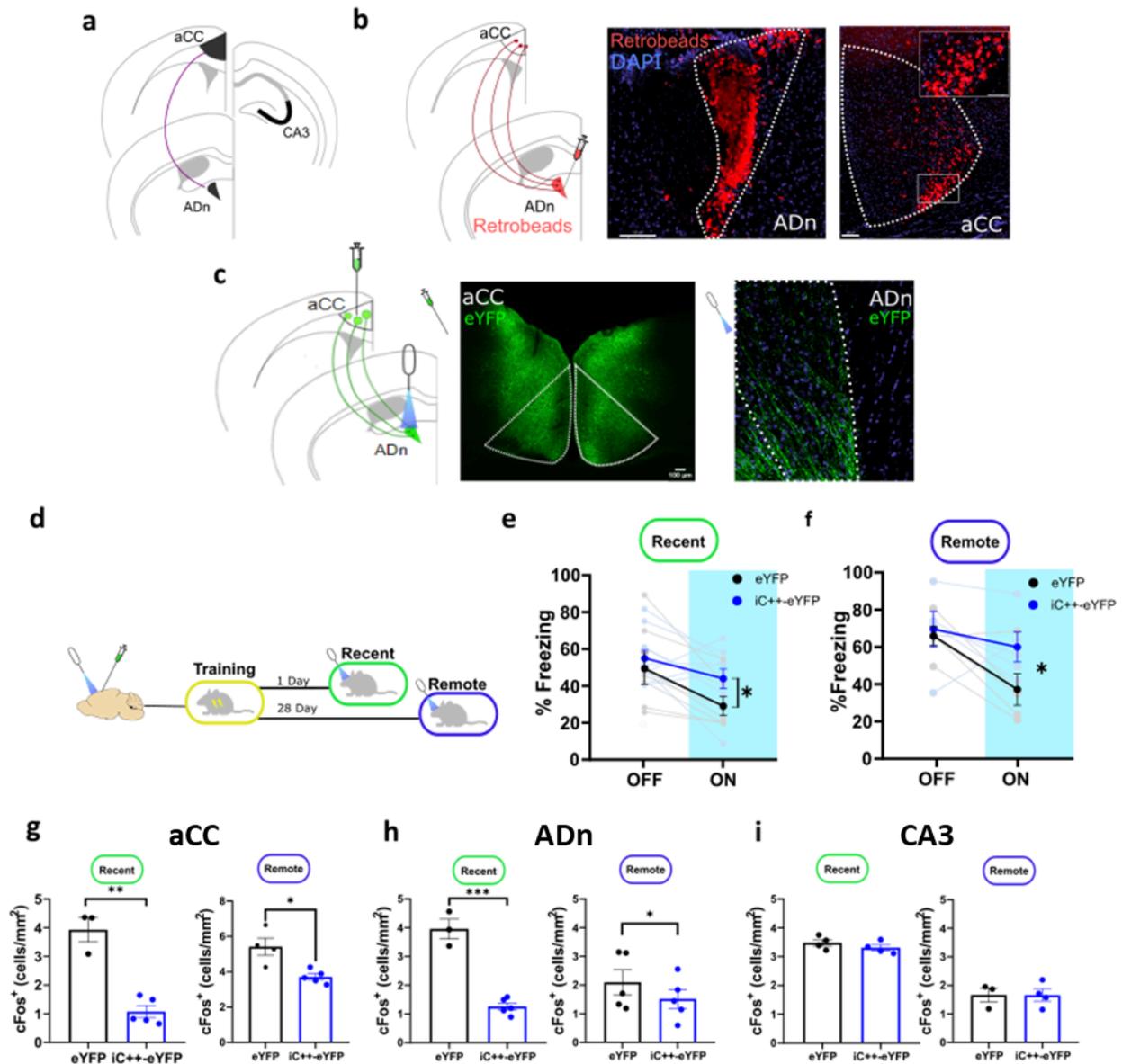


Figure 4. Optogenetic inhibition of aCC projections to ADn. **a)** Schema of projections targeted in experiment. **b)** Confirmation of projections via retrobead tracing. Injection in the aCC (left) and labelled neurons in the ADn (right). **c)** Schema of bilateral viral injection in the aCC and optic fiber implant mediolateral in the ADn. Confirmation of injection in the aCC and projections in the ADn. Percentage of freezing during optogenetic stimulation at **e)** recent and **f)** remote time points. The blue shaded area depicts the laser stimulation. Level of c-Fos expression during optogenetic inhibition in the **g)** aCC at recent (green, left) and remote (blue, right) time points; in the **h)** ADn at recent (green, left) and remote (blue, right) time points; in the **i)** CA3 at recent (green,

left) and remote (blue, right) time points. * $p < 0.05$. Scale bar = 100 microns. * $p < 0.05$
scale bar = 100 microns

The intriguing results of aCC to ADn projection inactivation, which led to an increase in freezing levels, prompted us to explore an intriguing question: could the inactivation of aCC to ADn projections alone drive the occurrence of freezing behaviour? To answer this question, we optogenetically targeted these projections and inhibited them in a new group of naive mice, not trained to fear conditioning, while they were exploring an open field. We found that there was no significant increase in freezing behaviour compared to controls (2-way Repeated Measures Anova; $F_{(1,12)} = 0.16$, *ns*; Fig. 5a,b). These results suggest that aCC projections to ADn influence fear expression only when it is driven by memory recall.

To test the hypothesis that fear expression can be tightly linked with the dynamics of aCC and ADn activity, we analysed the real time changes of neuronal activity of these 2 regions when mice are recalling fear memories and express related freezing behaviour. To this aim, in a different group of mice, we injected AAV-DJ-GcAMP6f-eYFP unilaterally in the aCC ($n = 4$) with a 400 micron optic fiber in the same unilateral aCC. In a different group of mice, we injected AAV-DJ-GcAMP6f-eYFP unilaterally in the ADn ($n = 6$) with a 400 micron optic fiber in the same unilateral ADn. We did this with the aim to analyse calcium changes using fiber photometry (Fig. 5a,c,d). Firstly, we monitored calcium changes during training and we found a significant increase in fluorescence (i.e. increase in calcium influx) in the aCC during shock delivery compared to before (2-tailed paired t-test; t-test $t_3 = 3.89$, $p < 0.05$; Fig. 5e,f). Interestingly, when we analysed ADn calcium changes, we found in some mice, but not all of them, a striking decrease in fluorescence during the shock, however this was not consistent across mice resulting in a not significant trend (2-tailed unpaired t-test $t_{11} = 1.43$, *ns*; Fig. 5g,h).

Secondly, we studied the activity of the aCC and ADn when the mice are in freezing behaviour. We have defined a single period of freezing (i.e. when the mouse is not

moving for a consecutive length of time) as a freezing bout. We found that, when the mouse entered a freezing bout, there was a significant decrease in aCC activity (2-tailed paired t-test; $t_{11}=4.37$, $p<0.01$; Fig. 5i and j), while we found a significant increase in aCC calcium activity right after the end of freezing bout (2-tailed unpaired t-test; $t_{13}=3.71$, $p<0.01$; Fig. 5k and l). In terms of the ADn, we found no significant change in cellular activity during the onset (2-tailed paired t-test; $t_{10} = 0.59$, *ns*; Fig 5g and h) or the offset (2-tailed paired t-test; $t_{11} = 0.16$, *ns*; Fig 5g and h) of freezing bouts. Further analyses need to be conducted to run out the possibility that ADn activity has a role in regulating fear expression in parallel with aCC activity.

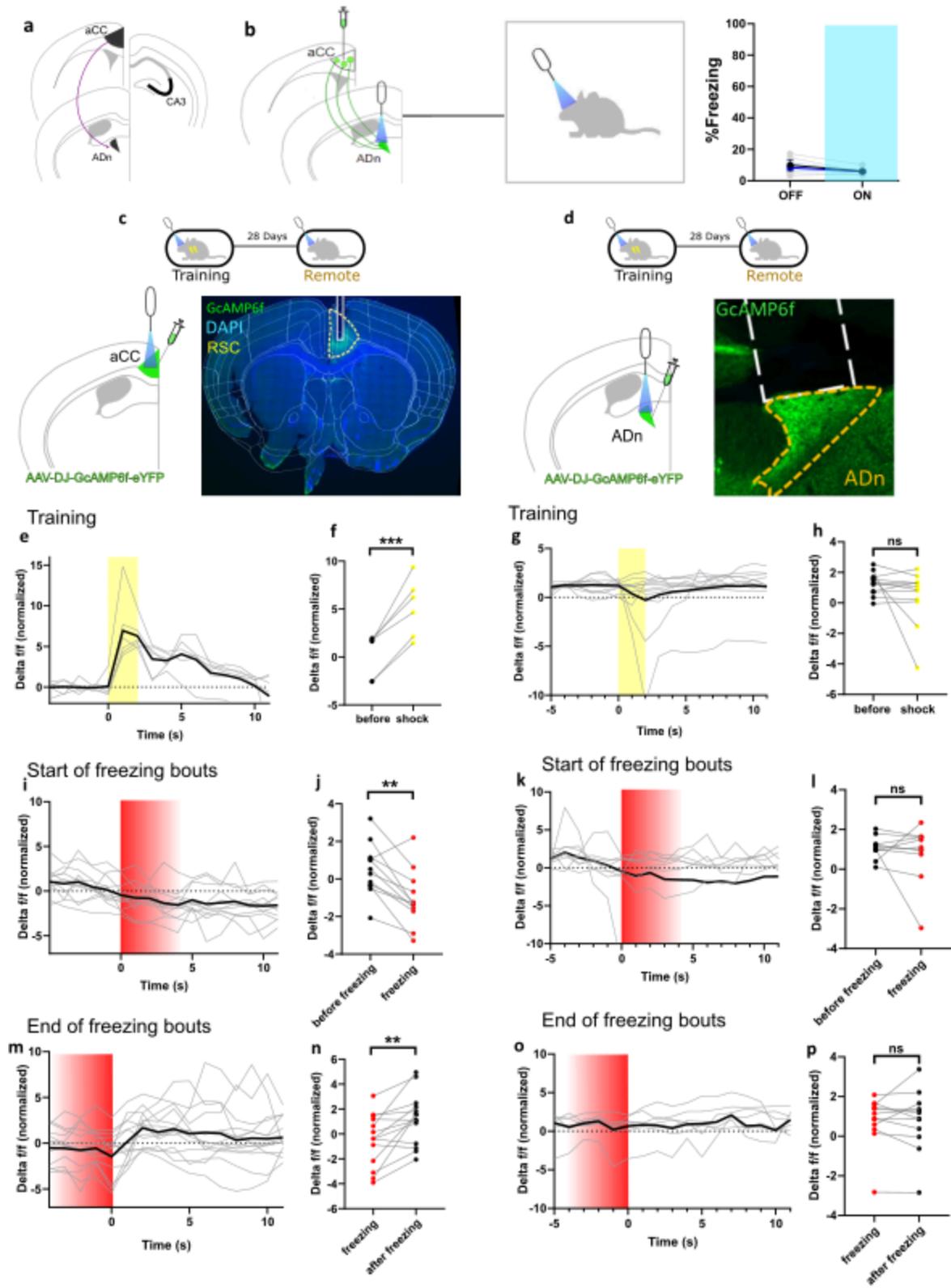


Figure 5. Fiber Photometry recording in the aCC and ADn during training and remote memory recall. **a)** Schema of projections targeted. **b)** Schema of bilateral viral injection in the aCC and optic fiber implant mediolateral in the ADn. Schema of experimental protocol. Percentage of freezing in response to novel environments (right). **c)** Schema of right unilateral viral injection and fiber optic implant in the aCC (left) with histology confirmation (right). **d)** Schema of viral injection and fiber optic implant in the ADn (left) with histology confirmation (right). Traces of normalised delta f/f fluorescence for **e)** aCC and **g)** ADn, during training, with the mean trace represented as a black line. 0 depicts the onset of shock, with the duration of shock (2 seconds) represented in a yellow band on the graph. Average of delta f/f for **f)** aCC and **h)** ADn, before and during the shock. Traces of normalised delta f/f fluorescence for **i)** aCC and **k)** ADn, during onset of freezing bout with the mean trace represented as a black line. Average of delta f/f for **j)** aCC and **l)** ADn, before and during freezing onset. Traces of normalised delta f/f fluorescence for **m)** aCC and **o)** ADn during the offset of a freezing bout, with the mean trace represented as a black line. **g)** Average of delta f/f **n)** aCC and **p)** ADn before and during freezing offset. $p < 0.01$ **** $p < 0.001$

Discussion

CA3 recruitment at remote memory recall occurs through a dual control of aCC and ADn.

Our study presents new evidence to support the hypothesis that the CA3 hippocampus represents a hub region for recalling remote memories.

Memory consolidation occurs as an active process that requires time and synaptic rearrangements to allow memories to become long-lasting (Müller and Pilzecker, 1900; Squire *et al.*, 2015). Memories acquired recently are supported by a network of brain regions that will strongly change if the same memory undergoes consolidation and is recalled at a later time point (Dudai and Morris 2000), supporting the theory of systems consolidation (Dudai and Morris 2013).

While previous hypotheses pointed to a disengagement of the hippocampus at a remote time point (Squire and Alvarez, 1995), later evidence (Goshen *et al.*, 2011; Lux, *et al.*, 2016) and network analysis (Wheeler *et al.*, 2013; Vetere *et al.*, 2017) showed a more complex scenario that may still require hippocampal activation to recall remote memories.

In our study, we focused on the CA3 field of the hippocampus and further explore previous data showing the engagement of inhibitory long-range CA3 projecting cells to the ADn that are active to inhibit ADn activity, a process necessary for a proper recall of remote but not recent memories (Vetere *et al.*, 2021). We added to this work by analysing excitatory CA3 cells projecting to the ADn. We found that also inactivation of excitatory CA3 to ADn at remote, but not recent, time point leads to both ADn inactivation and impairment in memory performances (i.e. decrease of the percentage of freezing). The discovery that inactivating CA3 excitatory neurons to ADn resulted in ADn activation was surprising. Several hypothesis could potentially explain this unexpected outcome. Firstly, it is possible that ADn has an internal regulatory mechanism, leading to an overall increase in its activity when the CA3-ADn circuit is disrupted. Secondly, the observed effect could be attributed to a rebound in activity after optogenetic stimulation, which may manifest as a stronger c-Fos activity when averaged over time. Lastly, downstream regions to ADn may indirectly regulate ADn activity,

causing this counterintuitive response. Further analysis will be conducted to understand this puzzling result. However, it is intriguing to observe that either inhibitory or excitatory projections from CA3 to ADn, if inactivated, lead to the same result: stronger ADn activity and significant decrease in freezing behaviour.

aCC contribution to memory consolidation and recall at a remote time point has been reported extensively (Bontempi *et al.*, 1999; Frankland *et al.*, 2004; Maviel *et al.*, 2004; Takehara-Nishiuchi and McNaughton 2008; Teixeira *et al.*, 2006; Wirt and Hyman 2017). We found here that its recruitment is indirectly controlled by CA3 to ADn correct recruitment (Fig. 2) and directly by CA3 to aCC projections (Fig. 3). This control, while occurring at both recent and remote time point, is effective only when remote memories are recalled confirming previous data showing that the aCC recruitment is not necessary to recall recent memories (Kitamura *et al.*, 2017) while it is crucial to recall remote ones.

Overall, our research has unveiled a critical and indispensable role played by the CA3 field of the hippocampus in regulating the activity of both ADn and aCC, which is pivotal for remote memory recall. Significantly, this is the first investigation of the microcircuit involving CA3-aCC and ADn, shedding new light on the complex interplay between these brain regions during memory retrieval.

The aCC-ADn pathway may regulate freezing occurrence during fear memory recall.

Our study is the first one to bring evidence for a novel role of aCC to ADn projections in the expression of fear related to memory. The confirmation of these projections through retrobead tracing is in line with previous findings (Xue *et al.*, 2022). Our results demonstrate that optogenetic inhibition of aCC to ADn connections led to a significant increase in freezing behaviour at both recent and remote time points. These findings, apparently in contrast with the previous one involving CA3 direct control on both aCC and ADn only at remote time point, underpin a complex regulation of memory-dependent fear-related behaviours that we propose are finely controlled by this microcircuit. To note, CA3 inactivated projections always lead to remote fear memory impairment in the form of lower freezing behaviour while aCC to ADn inactivation leads

to a contrary effect, i.e. increase in freezing behaviour, and at both recent and remote time points.

To unravel the correlation between freezing behaviour and activity of aCC and ADn, we conducted fiber photometry studies during fear memory acquisition and recall at a remote time point. During shock delivery, we observed a significant increase in aCC activity, consistent with findings reported in previous research (Pan *et al.*, 2020). However, intriguingly, we noticed an opposite effect in the ADn, as its activity seemed to decrease during shock delivery (data still preliminary and requiring further confirmation). Intriguingly, this seems to point on a coordinated yet opposite response to the unconditioned stimulus during fear memory acquisition of these 2 regions. Notably, to the best of our knowledge, ADn is the only region documented to be inactivated during shock delivery. These observations offer valuable insights into the intricate dynamics between aCC and ADn during fear memory processing and reveal potential complexities in the neural circuitry underlying fear responses. Further research will be undertaken to validate and explore these preliminary findings.

A substantial body of research focused on the role of the prefrontal cortex in general, and the aCC in particular, in the regulation of fear-related behaviours. aCC can control active defensive behaviour to noxious stimuli (Lee *et al.*, 2022), aCC inputs to the basolateral amygdala selectively control generalised fear (Bian *et al.*, 2019), and its activation is necessary for terminating freezing behaviour (Steenland *et al.*, 2012). These studies clearly reveal that aCC recruitment is not only limited to fear expression when recalling remote memories but that aCC plays a broader role in the regulation of fear responses.

Based on the optogenetic manipulation data, where we observed that inactivation of aCC to ADn projections led to an increase in freezing behaviour during both recent and remote memory recall (but not in an open field context), we sought to investigate correlative patterns between freezing episodes and neuronal activation in the aCC and ADn regions. To this aim, we performed fiber photometry studies in these 2 regions during fear memory recall. Interestingly, during freezing behaviour, aCC and ADn exhibited distinct patterns of activity. The decreased activity in aCC during freezing onset and its subsequent increase upon cessation of freezing may correlate with its

potential role in regulating fear expression. Further analysis is needed to be able to draw conclusions regarding the ADn involvement in the regulation of fear expression during memory recall. Moreover, in the future, it will be necessary to analyse changes in real time occurring when aCC to ADn projections are optogenetically inhibited and correlate the changes with the increased appearance of freezing behaviours.

In summary, our study uncovers a novel cortico-hippocampo-thalamic pathway involving the CA3, ADn, and aCC that regulates ADn activity during recent and remote fear memory recall. The confirmation of this pathway through retrobeads tracing and the functional manipulations using optogenetics solidify the importance of the CA3-ADn-aCC circuitry in memory consolidation and fear expression. These findings enhance our understanding of the intricate hippocampo-thalamic-cortical circuitry underlying the retrieval of contextual fear memories. Future studies can further explore the specific mechanisms by which these regions interact and the precise contributions of each region within this circuitry.

Methods and materials

Mice

7-8 week old male and female hybrid (C57BL/6NRj X 129/SV) mice were used for all experiments. All procedures were carried out during the light phase cycle of the mice. Mice were randomly assigned to different conditions and had within cage viral controls (i.e. the experimental virus and control virus were within different mice of the same cage). Mice were bred in a colony at the ESPCI Paris Institute. At 21 days old, the mice are weaned, separated by sex, and grouped-housed, 2-4 per cage, in a standard home cage.

Surgery

Mice were administered an intraperitoneal injection of ketamine and xylazine (100mg/ml and 2% respectively, 100 mg per kg) and a subcutaneous injection of lurocaïne (20mg/ml). Mice were then placed into stereotaxic frames. As part of post-operative care, mice were injected subcutaneously with an analgesic (metacam, 5mg/ml), and 0.5 ml of 0.9% saline, and 0.3 ml of glucose, to prevent dehydration.

Viral Injection for optogenetic experiment

Mice were injected with 1 µl of AAV-DJ-CamKII-iC++-eYFP or AAV-DJ-CamKII-eYFP per side at a rate of 0.12 µl/min with 10µl hamilton needle, with 5 additional minutes after infusion to ensure that all the virus has infused.

Optrode Implantation for optogenetic experiment

Optrodes were made in-house by inserting 200 µm optical fiber (with a 0.37 numerical aperture) to a 1.25 mm zirconia ferrule, with the optical fiber extending 3mm out of the ferrule. The fibers were secured with epoxy resin. During surgery, the skull was scratched and 2 screws were implanted, to help the grip and adhesion of the black dental cement to the skull. The dental cement is black to minimise light leakage during photostimulation. The location of optrode implantation corresponded to the specific experiment (see table 1).

Experiment	Virus location	Optrode location
Optogenetically inhibit CA3 projections to ADn	Bilateral CA3 (AP: -2.2 mm, ML: +/- 2.7 mm, DV: -2.4 mm)	1 optrode medial to ADn (AP: -0.8 mm, ML: +/- 0.0 mm, DV: -2.8 mm)
Optogenetically inhibit CA3 projections to aCC	Bilateral CA3 (AP: -2.2 mm, ML: +/- 2.7 mm, DV: -2.4 mm)	1 optrode medial to aCC (AP: +0.8 mm, ML: +/- 0.0 mm, DV: -1.7 mm)
Optogenetically inhibit aCC projections to ADn	Bilateral aCC (AP: +0.8 mm, ML: +/- 0.3 mm, DV: -1.8 mm)	1 optrode medial to ADn (AP: -0.8 mm, ML: +/- 0.0 mm, DV: -2.8 mm)

Table 1. Shows the stereotaxic coordinates for viral injections and optrode implantations for the respective experiments.

Optogenetics and fiber photometry targeting CA3 projections in the ADn.

Mice were injected with 500nL AAV5-CamKII-GcAMP6F-eYFP and 500nL AAVrg-CamKII-ArchT-mcherry bi-laterally in ADn (AP: -0.8 mm, ML: +/- 0.75 mm, DV: -2.8 mm) with right-unilateral ADn fiber photometry placement (AP: -0.8 mm, ML: +/- 0.75 mm, DV: -2.8 mm), a mating sleeve was placed on the fiber photometry ferrule before being secured with epoxy resin. The optogenetic optrodes were placed bi-laterally in the CA3 (AP: -2.2 mm, ML: +/- 2.7 mm, DV: -2.3 mm). The fibers were secured using the method described above.

Fiber photometry in the aCC

Mice were injected with 500nL AAV5-CamKII-GcAMP6F-eYFP right-unilaterally in aCC (AP: +0.8 mm, ML: + 0.3 mm, DV: -1.8 mm) with right-unilateral aCC fiber photometry placement (AP: +0.8 mm, ML: + 0.3 mm, DV: -1.7 mm). The fiber was secured using the method described above.

All optrodes were made in-house. Optogenetic optrodes were made using the same method described above. The optrodes for fiber photometry were made by inserting 400 μm optical fiber (with a 0.57 numerical aperture) to a 2.5 mm stainless steel ferrule, with the optical fiber extending 3mm out of the ferrule. The fibers were secured with epoxy resin.

Behavioural Protocol

General Protocol

Training: Mice were placed in a conditioning chamber (31 cm \times 24 cm \times 21 cm) with shock-grid floors inside a sound-insulating box. After a habituation period, mice received two foot shocks (0.2 mA, 2 seconds) at minutes 2 and 3, and remained in the chamber for an additional minute. The training protocol lasted 4 minutes and was controlled by PolyFear software.

Test: Mice were placed back into the conditioning chamber 1 (recent) or 28 (remote) days after training and allowed to explore for 6 minutes without receiving any shocks. Freezing behaviour was recorded and manually scored by experimenters blinded to the group assignment of mice.

During the 6 minute test where mice were allowed to explore the conditioning chamber without receiving any shocks, mice were exposed to laser light (direct current, 10mW) during minute 4, 5 and 6. The laser light stimulates the inhibitory opsin in the projections from the injected region (experiment dependent). These projections are inhibited in the region of interest.

Fiber photometry recording and optogenetic stimulation

Mice were recorded for 2 minutes per day in their homecage, for 3 days, prior to training. Training consisted of 2 minutes of recording in a home cage, being placed in a fearbox and being given 3 shocks of 0.3mA for 2 seconds, at minutes 2, 3 and 4. A TTL

was sent from the POLYFEAR software, to synchronise the fiber photometry signal with the start of training. The test was 7 minutes long, with one minute intervals of stimulation at minutes 1, 3 and 5.

The fiber photometry data was recorded using a Doric fiber photometry recorder, and the raw data was saved in a .csv file format for subsequent analysis. The data was then analysed using PMAT (Bruno et al., 2021), a specialised software tool designed for analysing fiber photometry recordings. Peri-event time histograms (PETH) function of PMAT allowed us to analyse the temporal dynamics of the normalised delta F/F signals. This function allows for the visualisation and quantification of the changes in fluorescence around specific events or stimuli. To visualise and present the results, graphs were created using Prism (Graphpad by Dotmatics). Additionally, statistical analysis, including relevant tests and comparisons, was performed within the Prism software, providing a comprehensive analysis and visualisation package for the fiber photometry data.

Open field

To control for the possibility that optogenetic manipulation of the aCC-ADn pathway alters locomotion in a novel environment, mice were micro-infused with AAV-CamKII-iC⁺⁺-eYFP or control virus (AAV-CamKII-EYFP) in the aCC and implanted with optical fibers in the ADn. 5 weeks after viral infection and optical fiber implant, mice were placed in the centre of an open square arena (45 x 45 x 20 cm[°] (length x width x height)) and allowed to explore for 6 min, when the laser was OFF during the first 3 min, then turned ON during the last 3.

Transcardial Perfusions

At the end of behaviour, mice were perfused transcardially with 1× PBS followed by 4% paraformaldehyde (PFA). We infused 0.9% saline at 13 ml/min until the fluid exiting from the right atrium was clear. We then switched to infusing with the fixative (4% PFA) until the muscles were rigid. Once the mouse is successfully perfused, we decapitate it to

remove the brain. To finish the brains were placed 4% PFA overnight at 4°C before switching them to 30% sucrose until the brain is saturated (2-3 days).

Immunohistochemistry

Once the brains had been saturated in 30% sucrose for 3 days post perfusion, they were sliced coronally at -20°C using a cryostat at 50 micron slices. The 50 micron slices were then incubated with a blocking solution (2.5% BSA, 5% normal goat serum (NGS), 0.3% Triton X-100) for 30 min at room temperature on a shaker. To visualise c-Fos expression, we incubated the slices in a primary antibody for c-Fos (rabbit, 1:500) for 72 hours on a shaker at 4 °C. Next, the sections were washed 3 times for 10 minutes each in 1% Phosphate-buffered saline (PBS). To be able to image c-Fos we incubated with an Alexa 568, an anti-rabbit secondary antibody, raised in goat (1:500) for 24 hours on a shaker at 4 °C. The next day, sections were washed 3 times in PBS for 10 minutes, followed by a 10 minute wash with 4,6-diamidino-2-phenylindole (1:10000, DAPI) to stain the nuclei of the cell. After a final wash in PBS, the slices are mounted on slides and coverslips using PermaFluor mounting medium. The slides were then kept and stored at 4 °C, ready to be imaged on a confocal laser scanning microscope.

c-Fos counting

c-Fos is an immediate early gene (IEG) that is present in a cell 60-90 minutes after it has been active. We stain for c-Fos and quantify its presence in a region as a proxy for its activity during a specific event. To this end, the mice were perfused 90 minutes after the test (recent or remote, depending on their group). To analyse c-Fos-positive nuclei, images of regions of interest (experiment dependent) were taken at ×20 magnification. Some regions, such as the ADn and CA3, were captured using the XY snapshot function, whilst others, the aCC, were captured using the tile scan function. Analysis of c-Fos-positive nuclei was performed using the software Imaris 9.9.1 (Bitplane). Imaris is an automated cell tracking and detection software. We manually defined the borders of the regions using the “Surface” function and used Paxinos and Franklin (2004) mouse

brain atlas for reference. With the “Spots”, we quantified the number of c-Fos positive cells within our defined region.

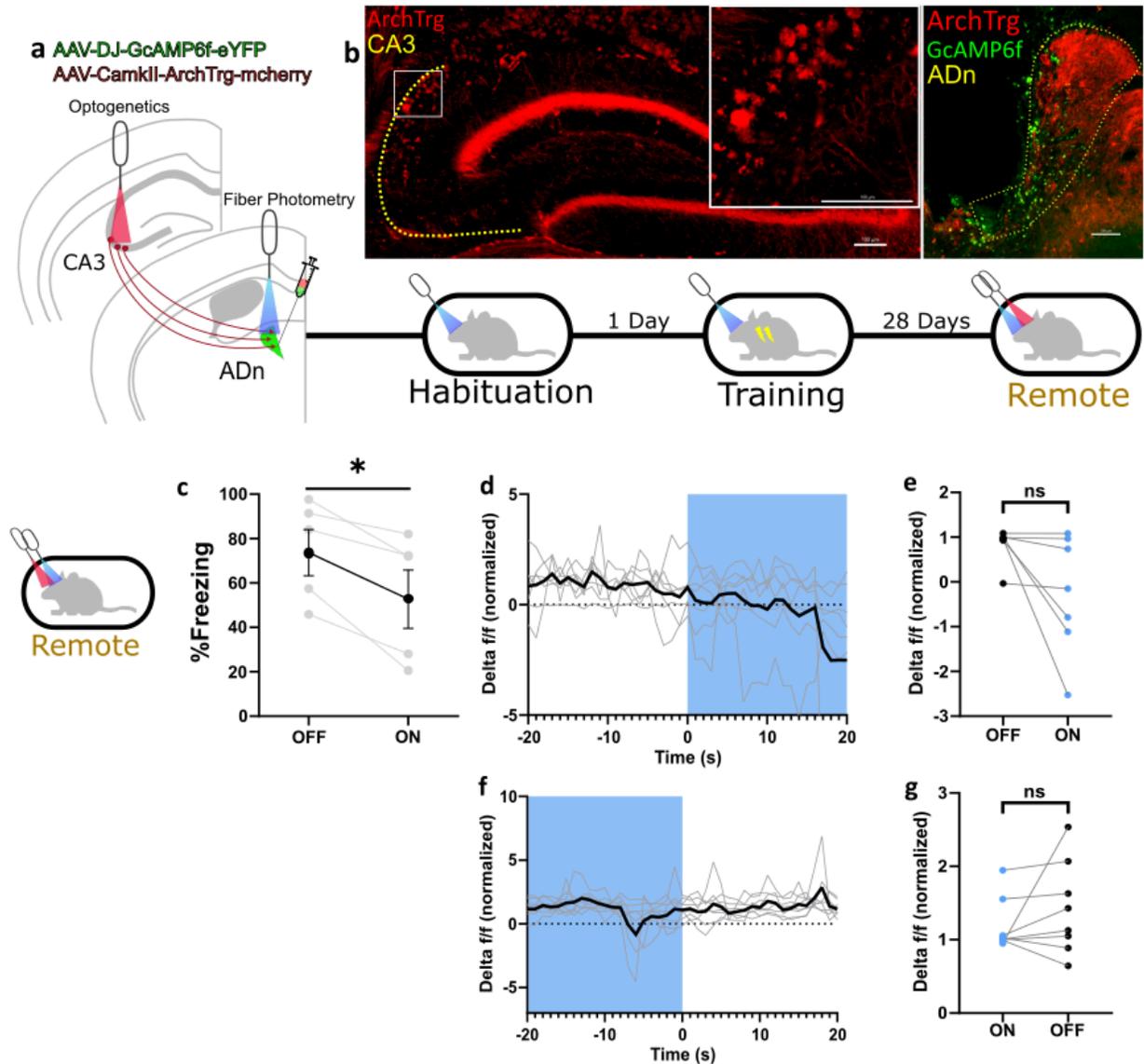
Statistics

The statistical analyses employed in this study included a 2-way repeated measures ANOVA for contextual fear conditioning, with post-hoc Fisher’s LSD tests were performed to compare periods. Mice were randomly assigned to different experimental conditions, ensuring unbiased allocation. Within-cage controls were utilised for comparison purposes. Two-tailed unpaired t-tests were conducted for the c-Fos analyses, and these analyses were performed blind to the experimental condition to minimise potential bias. Inclusion criteria for data analysis encompassed mice that met specific histological criteria, such as correct optrode placement and viral infection limited to the target region. Additionally, mice were required to demonstrate successful task learning, indicated by more than 20% baseline freezing during the first 3 minutes of memory recall. Only data from mice meeting these criteria were included in subsequent analyses.

Supplementary data

The findings from the optogenetic inhibition of direct excitatory CA3 projections to the ADn, a region solely comprising excitatory neurons, were intriguing. The inhibition resulted in an increase in ADn c-Fos expression, indicating heightened neuronal activity, while memory recall exhibited a decrease. To validate and confirm the increased ADn activity during optogenetic inhibition, we employed an in vivo population-level calcium imaging technique, called fiber photometry. By employing fiber photometry, we were able to monitor and measure calcium fluctuations in ADn neurons in real-time during the optogenetic inhibition. To this aim we repeated the optogenetic experiment whilst recording the in vivo in the ADn (Sup. Fig. 1a). To accommodate the fiber photometry recording in the right unilateral ADn and not cause unnecessary noise to the recordings from the additional optic fiber for optogenetics, we injected a viral cocktail of AAV-DJ-GcAMP-eYFP and an inhibitory retrograde halorhodopsin, AAV-CamKII-ArchTrg-mcherry. The optogenetic fibers were placed bilaterally in the CA3, to optogenetically inhibit specifically the somas of the cells that project to ADn (Sup. Fig. 1b). We habituated the mice to the optic fibers for 3 days and recorded for 2 minutes per day in homecage to verify the signal. Before the training and test sessions, we recorded for 2 minutes in homecage. During the test, the projections from the CA3 to the ADn were optogenetically inhibited whilst the activity of the ADn was recorded.

During the test session, we replicated the same behavioural results as our previous experiment, where during optogenetic inhibition of CA3 projections to the ADn resulted in a decrease in memory recall compared to baseline activity (2-tailed paired t-test: t-test, $t_4 = 4.04$, $p < 0.05$; sup. Fig. 1c). Overall, there was no significant change in the activity of the ADn at the onset of stimulation (2-tailed paired t-test: t-test $t_6 = 2.15$, *ns*; sup. Fig. 1e) nor at the offset of stimulation (2-way repeated measures t-test $t_7 = 1.11$, *ns*; sup. Fig. 1g). These results do not bring further insight into the activity of the ADn during optogenetic stimulation. More research is required to draw definitive conclusions.



Supplementary Figure 1. *Fiber Photometry recording in the ADn during optogenetic inhibition of projections from the CA3. a) schema of viral injection and behavioural protocol. b) Histology. Confirmation of GcAMP and ArchTrg infection in the ADn (right) with visualisation of Fiber photometry optic fiber. Confirmation of infected cells in the CA3 (right) with the ArchTrg. c) Behavioural results of optogenetic inhibition. Percentage of freezing during no optogenetic inhibition (OFF) and optogenetic inhibition (ON). d) Traces of normalised delta f/f fluorescence during onset of laser stimulation (optogenetic inhibition of CA3 projections to ADn), with the mean trace represented as a black line. e) Average of delta f/f before and during laser onset. f) Traces of normalised*

delta f/f fluorescence during the offset of laser stimulation, with the mean trace represented as a black line. g) Average of delta f/f before and during laser offset.

Bibliography

- Akers, Katherine, and Paul Frankland. 2009. 'Grading Gradients: Evaluating Evidence for Time-Dependent Memory Reorganization in Experimental Animals'. *Journal of Experimental Neuroscience Neurosciences and Mental Health* 2009.
- Bian, Xin-Lan et al. 2019. 'Anterior Cingulate Cortex to Ventral Hippocampus Circuit Mediates Contextual Fear Generalization'. *Journal of Neuroscience* 39(29): 5728–39.
- Bontempi, B., C. Laurent-Demir, C. Destrade, and R. Jaffard. 1999. 'Time-Dependent Reorganization of Brain Circuitry Underlying Long-Term Memory Storage'. *Nature* 400(6745): 671–75.
- Buzsáki, G. 1996. 'The Hippocampo-Neocortical Dialogue'. *Cerebral Cortex (New York, N.Y.: 1991)* 6(2): 81–92.
- Cassel, Jean-Christophe et al. 2013. 'The Reuniens and Rhomboid Nuclei: Neuroanatomy, Electrophysiological Characteristics and Behavioral Implications'. *Progress in Neurobiology* 111: 34–52.
- Contreras, D., and M. Steriade. 1995. 'Cellular Basis of EEG Slow Rhythms: A Study of Dynamic Corticothalamic Relationships'. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 15(1 Pt 2): 604–22.
- Dudai, Yadin, and Richard G.M. Morris. 2000. 'To Consolidate or Not to Consolidate: What Are the Questions?' In *Brain, Perception, Memory: Advances in Cognitive Neuroscience*, ed. Johan J. Bolhuis. Oxford University Press, 0.
- Frankland, Paul W. et al. 2004. 'The Involvement of the Anterior Cingulate Cortex in Remote Contextual Fear Memory'. *Science (New York, N.Y.)* 304(5672): 881–83.

- Frankland, Paul W., and Bruno Bontempi. 2005. 'The Organization of Recent and Remote Memories'. *Nature Reviews. Neuroscience* 6(2): 119–30.
- Goshen, Inbal et al. 2011. 'Dynamics of Retrieval Strategies for Remote Memories'. *Cell* 147(3): 678–89.
- Kitamura, Takashi et al. 2017. 'Engrams and Circuits Crucial for Systems Consolidation of a Memory'. *Science* 356(6333): 73–78.
- Lara-Vásquez, Ariel et al. 2016. 'Midline Thalamic Neurons Are Differentially Engaged during Hippocampus Network Oscillations'. *Scientific Reports* 6(1): 29807.
- Latchoumane, Charles-Francois V., Hong-Viet V. Ngo, Jan Born, and Hee-Sup Shin. 2017. 'Thalamic Spindles Promote Memory Formation during Sleep through Triple Phase-Locking of Cortical, Thalamic, and Hippocampal Rhythms'. *Neuron* 95(2): 424-435.e6.
- Lee, Jeong-Yun et al. 2022. 'Role of Anterior Cingulate Cortex Inputs to Periaqueductal Gray for Pain Avoidance'. *Current Biology* 32(13): 2834-2847.e5.
- Maviel, Thibault, Thomas P. Durkin, Frédérique Menzaghi, and Bruno Bontempi. 2004. 'Sites of Neocortical Reorganization Critical for Remote Spatial Memory'. *Science (New York, N.Y.)* 305(5680): 96–99.
- McClelland, James L., Bruce L. McNaughton, and Randall C. O'Reilly. 1995. 'Why There Are Complementary Learning Systems in the Hippocampus and Neocortex: Insights from the Successes and Failures of Connectionist Models of Learning and Memory'. *Psychological Review* 102(3): 419–57.
- Müller, GE and Pilzecker. 1900. Experimentelle Beiträge zur Lehre vom Gedächtniss. *Zeitschrift für Psychologie. Ergänzungsband*. 1:1–300

- Pan, Simon et al. 2020. 'Preservation of a Remote Fear Memory Requires New Myelin Formation'. *Nature Neuroscience* 23(4): 487–99.
- Park, Kwanghoon, and ChiHye Chung. 2019. 'Systemic Cellular Activation Mapping of an Extinction-Impaired Animal Model'. *Frontiers in Cellular Neuroscience* 13. <https://www.frontiersin.org/articles/10.3389/fncel.2019.00099>
- Peyrache, Adrien, Marie M. Lacroix, Peter C. Petersen, and György Buzsáki. 2015. 'Internally Organized Mechanisms of the Head Direction Sense'. *Nature Neuroscience* 18(4): 569–75.
- Schlecht, Maximilian, Maanasa Jayachandran, Gabriela E. Rasch, and Timothy A. Allen. 2022. 'Dual Projecting Cells Linking Thalamic and Cortical Communication Routes between the Medial Prefrontal Cortex and Hippocampus'. *Neurobiology of Learning and Memory* 188: 107586.
- Slézia, Andrea, Balázs Hangya, István Ulbert, and László Acsády. 2011. 'Phase Advancement and Nucleus-Specific Timing of Thalamocortical Activity during Slow Cortical Oscillation'. *Journal of Neuroscience* 31(2): 607–17.
- Squire, L. R., and P. Alvarez. 1995. 'Retrograde Amnesia and Memory Consolidation: A Neurobiological Perspective'. *Current Opinion in Neurobiology* 5(2): 169–77.
- Steenland, Hendrik W., Xiang-Yao Li, and Min Zhuo. 2012. 'Predicting Aversive Events and Terminating Fear in the Mouse Anterior Cingulate Cortex during Trace Fear Conditioning'. *Journal of Neuroscience* 32(3): 1082–95.
- Takehara-Nishiuchi, Kaori, and Bruce L. McNaughton. 2008. 'Spontaneous Changes of Neocortical Code for Associative Memory during Consolidation'. *Science (New York, N.Y.)* 322(5903): 960–63.

- Taylor, Kaycie K., Kazumasa Z. Tanaka, Leon G. Reijmers, and Brian J. Wiltgen. 2013. 'Reactivation of Neural Ensembles during the Retrieval of Recent and Remote Memory'. *Current biology: CB* 23(2): 99–106.
- Teixeira, Cátia M. et al. 2006. 'Involvement of the Anterior Cingulate Cortex in the Expression of Remote Spatial Memory'. *Journal of Neuroscience* 26(29): 7555–64.
- Tomé, Douglas Feitosa et al. 2022. 'Dynamic and Selective Engrams Emerge with Memory Consolidation'. : 2022.03.13.484167. <https://www.biorxiv.org/content/10.1101/2022.03.13.484167v1> (June 26, 2023).
- Van der Werf, Y. D., M. P. Witter, H. B. Uylings, and J. Jolles. 2000. 'Neuropsychology of Infarctions in the Thalamus: A Review'. *Neuropsychologia* 38(5): 613–27.
- Varela, Carmen, and Matthew A Wilson. 2020. 'mPFC Spindle Cycles Organize Sparse Thalamic Activation and Recently Active CA1 Cells during Non-REM Sleep' ed. Laura L Colgin. *eLife* 9: e48881.
- Vetere, Gisella et al. 2011. 'Spine Growth in the Anterior Cingulate Cortex Is Necessary for the Consolidation of Contextual Fear Memory'. *Proceedings of the National Academy of Sciences of the United States of America* 108(20): 8456–60.
- Vetere, G., Kenney, J. W., Tran, L. M., Xia, F., Steadman, P. E., Parkinson, J., Josselyn, S. A., & Frankland, P. W. (2017). Chemogenetic interrogation of a brain-wide fear memory network in mice. *Neuron*, 94(2).
- Vetere, G., Xia, F., Ramsaran, A.I. et al. (2021). An inhibitory hippocampal–thalamic pathway modulates remote memory retrieval. *Nature Neuroscience* 24, 685–693
- Wheeler, Anne L. et al. 2013. 'Identification of a Functional Connectome for Long-Term

- Fear Memory in Mice'. *PLoS computational biology* 9(1): e1002853.
- Winocur, Gordon, Morris Moscovitch, and Bruno Bontempi. 2010. 'Memory Formation and Long-Term Retention in Humans and Animals: Convergence towards a Transformation Account of Hippocampal-Neocortical Interactions'. *Neuropsychologia* 48(8): 2339–56.
- Wirt, Ryan A., and James M. Hyman. 2017. 'Integrating Spatial Working Memory and Remote Memory: Interactions between the Medial Prefrontal Cortex and Hippocampus'. *Brain Sciences* 7(4): 43.
- Xue, Man et al. 2022. 'Mapping Thalamic-Anterior Cingulate Monosynaptic Inputs in Adult Mice'. *Molecular Pain* 18: 17448069221087034.

Chapter 5

Supplementary Results

This chapter explores additional research that is closely related to the main article but did not ultimately make it into the final publication. Although these findings were not included, they offer valuable insights and contribute to a deeper understanding into the role of cortical-thalamic communication in memory consolidation, and hippocampal-thalamic communication in memory generalisation.

5.1 aCC projections to the CA3 are not necessary for memory recall.

5.1.1 Introduction

In the same vein as the experiments in the article, I sought to characterise the communication between the cortex and hippocampus during memory recall, focusing on the aCC projections to the CA3. In the end, we decided to leave this out of the article, as the story of the aCC was focused on its role in the expression of fear behaviour during memory recall as opposed to the recall of the memory per se.

5.1.2 Methods

Mice

The study used male and female mice of a hybrid strain (C57BL/6NRj X 129/SV) aged 7-8 weeks. The mice were housed in a temperature-controlled room with a 12-hour light/dark cycle and provided ad libitum access to food and water. They were group-housed in standard home cages, with 2-4 mice per cage.

Surgery

Anaesthesia: Mice were anaesthetised with an intraperitoneal injection of ketamine (100 mg/ml) and xylazine (2%) at a dose of 100 mg/kg. Local analgesic (lurocaïne, 20 mg/ml) was injected subcutaneously. To prevent dehydration, mice received subcutaneous injections of analgesic (metacam, 5 mg/ml) and 0.9% saline solution.

Viral Injection

Mice received bilateral injections of AAV-DJ-CamKII-iC⁺⁺-eYFP or AAV-DJ-CamKII-eYFP (1 µl per side at a rate of 0.12 µl/min) were injected bi-laterally in the aCC (AP: +0.8 mm, ML: +/- 0.3 mm, DV: -1.8 mm) using a Hamilton microsyringe. The infusion was performed over a total duration of 8 minutes, with an additional 5 minute waiting period at the end of infusion, to ensure complete viral distribution.

Optogenetic fiber implant

Optrodes were constructed in-house by attaching a 200 μm optical fiber (0.37 numerical aperture) to a 1.25 mm zirconia ferrule, with the fiber extending 3 mm beyond the ferrule. The optrodes were secured with epoxy resin. During surgery, the skull was prepared by scratching and two screws were implanted to facilitate adhesion of the black dental cement. Optrodes were implanted bi-laterally in the CA3 (AP: -2.2 mm, ML: ± 2.7 mm, DV: -2.3 mm).

Contextual Fear Conditioning

To measure the effect of optogenetic inhibition of aCC projections to CA3, we used the contextual conditioning paradigm. Where, during training mice were placed in a conditioning chamber (31 cm \times 24 cm \times 21 cm) with shock-grid floors inside a sound-insulating box. After a habituation period, mice received two foot shocks (0.2 mA, 2 seconds) separated by a minute, and remained in the chamber for an additional minute. Freezing behaviour was recorded and analysed using automated scoring software. To test the effect of optogenetic inhibition on memory recall, mice were placed back into the conditioning chamber 1 or 28 days after training and allowed to explore for 6 minutes without receiving any shocks. Freezing behaviour was recorded and manually scored by experimenters blinded to the group assignment of mice.

Optogenetic Stimulation during Test

During the test session, optogenetic stimulation was applied to inhibit the projections from the injected region. The stimulation lasted for 3 minutes (minutes 4-6 of the test

session) using a blue laser (direct current, 5V). Behaviour was recorded and analysed to assess the effect of optogenetic inhibition on fear memory.

Transcardial Perfusion

At the end of behaviour, mice were perfused transcardially with 1× PBS followed by 4% paraformaldehyde (PFA). We infused 0.9% saline at 13 ml/min until the fluid exiting from the right atrium was clear. We then switched to infusing with the fixative (4% PFA) until the muscles were rigid. Once the mouse is successfully perfused, we decapitate it to remove the brain. To finish the brains were placed 4% PFA overnight at 4°C before switching them to 30% sucrose until the brain is saturated (2-3 days).

Immunohistochemistry

Once the brains had been saturated in 30% sucrose for 3 days post perfusion, they were sliced coronally at -20°C using a cryostat at 50 micron slices. The 50 micron slices were then incubated with a blocking solution (2.5% BSA, 5% normal goat serum (NGS), 0.3% Triton X-100) for 30 min at room temperature on a shaker. To visualise c-Fos expression, we incubated the slices in a primary antibody for c-Fos (rabbit, 1:500) for 72 hours on a shaker at 4 °C. Next, the sections were washed 3 times for 10 minutes each in 1% Phosphate-buffered saline (PBS). To be able to image c-Fos we incubated with an Alexa 568, an anti-rabbit secondary antibody, raised in goat (1:500) for 24 hours on a shaker at 4 °C. The next day, sections were washed 3 times in PBS for 10 minutes, followed by a 10 minute wash with 4,6-diamidino-2-phenylindole (1:10000, DAPI) to stain the nuclei of the cell. After a final wash in PBS, the slices are mounted on slides

and coverslips using PermaFluor mounting medium. The slides were then kept and stored at 4 °C, ready to be imaged on a confocal laser scanning microscope.

Statistical Analysis

The statistical analyses employed in this study included a 2-way repeated measures ANOVA for contextual fear conditioning. Mice were randomly assigned to different experimental conditions, ensuring unbiased allocation. Within-cage controls were utilised for comparison purposes. Two-tailed unpaired t-tests were conducted for the c-Fos analyses, and these analyses were performed blind to the experimental condition to minimise potential bias. Inclusion criteria for data analysis encompassed mice that met specific histological criteria, such as correct optrode placement and viral infection limited to the target region. Additionally, mice were required to demonstrate successful task learning, indicated by more than 20% baseline freezing during the first 3 minutes of memory recall. Only data from mice meeting these criteria were included in subsequent analyses.

5.1.3 Results

The aCC is known to be an important region in the recall of remote memories (Frankland *et al.*, 2004). Building on our previous results of the CA3 controlling the activity of the aCC at remote time point, we wanted to see if it was reciprocal. To this aim, we injected mice with an inhibitory channelrhodopsin, iC++ (AAV-CamkII-iC++-eYFP), or a control virus (AAV-CamkII-iC++-eYFP) bilaterally in the aCC and implanted an optic fiber bilaterally in the CA3. Then we trained mice in a novel

context by exposing them to 2 foot shocks (0.2mA, lasting 2 seconds) at minutes 2 and 3 of the 4 minute training session. Mice were then placed back in the same context, either 1 day (recent memory) or 28 days (remote memory) later to test their respective memories, and subsequently the effect of our optogenetic manipulation on recall. The test lasted 6 minutes, and during minutes 4-6, we optogenetically targeted the projections from the aCC to the ADn (Fig. 7.1a).

The optogenetic inhibition of aCC projections to the CA3, did not result in a significant change to memory recall at either time point (recent: 2-way Repeated Measures ANOVA, $F_{(1,8)} = 4.55$; *ns*; remote: 2-way Repeated Measures ANOVA, $F_{(1, 18)} = 7.335$; *ns*; Fig. 7.1d,e). These results suggest that the aCC does not have control over the CA3 during memory recall.

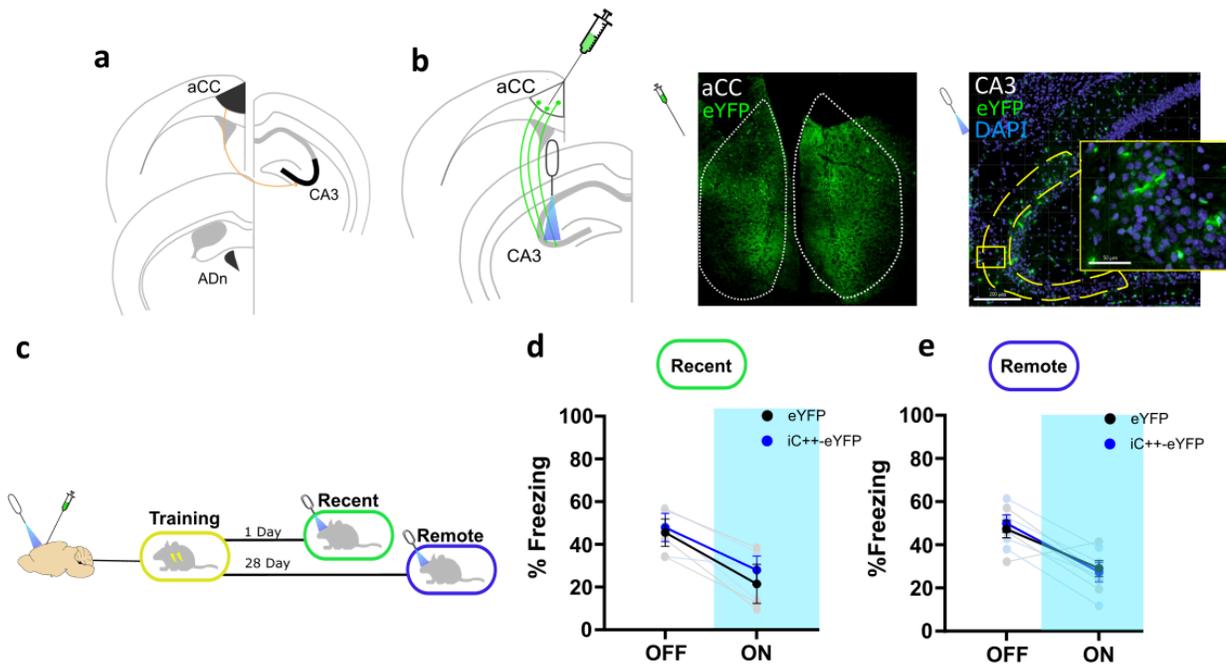


Figure 5.1. Optogenetic inhibition of CA3 projections to ADn during recall of a generalised memory. **a)** Schema of projections targeted in experiment. **b)** Schema of bilateral viral injection in the CA3 and optic fiber implant mediolateral in the ADn. With

*confirmation of injection in the CA3 (left) and projections in the ADn (right). c) Schema of behaviour protocol, mice were trained (yellow) in the conditioned context, with 2 foot shocks at 0.2mA, 2 seconds per shock. Mice were then tested at recent (green) or remote (blue) in a similar, but different context. Percentage of freezing during optogenetic stimulation at d) recent and e) remote time point. The blue shaded area depicts the laser stimulation. * $p < 0.05$*

5.2 CA3 projections to the ADn are necessary for the recall of a generalised memory.

Memory generalisation refers to the process by which information or experiences are generalised or abstracted to form broader representations or concepts that can be applied to similar situations or stimuli (Bowman and Zeithamova, 2018). It involves the ability to extract commonalities or underlying patterns from specific instances and apply them to new situations. Memory generalisation plays a crucial role in cognitive processes such as categorization, concept formation, and the ability to make predictions based on past experiences.

5.2.1 Introduction

As an additional experiment, building upon the discoveries regarding the crucial role of CA3 projections to the anterodorsal nucleus (ADn) in the retrieval of consolidated memories, our curiosity was piqued regarding the significance of these projections in the retrieval of generalised memories. This investigation aimed to provide valuable

insights into the extent of importance of the hippocampal-thalamic projections at a remote temporal point. For the article to maintain its primary focus on memory consolidation and expression of fear memory, it was determined that this preliminary experiment would only encompass a single set of projections.

5.2.2 Methods

See section 7.1.2 for the methods used. Below I have outlined the methods that are different from section 7.1.2.

Contextual Fear Conditioning

To measure the effect of optogenetic inhibition of CA3 projections to ADn during memory generalisation, we used the contextual conditioning paradigm. Where, during training mice were placed in a conditioning chamber (31 cm × 24 cm × 21 cm) with shock-grid floors inside a sound-insulating box. After a habituation period, mice received two foot shocks (0.2 mA, 2 seconds) separated by a minute, and remained in the chamber for an additional minute. To test the effect of optogenetic inhibition on the recall of a generalised memory, mice were placed into a different context 1 or 28 days after training and allowed to explore for 6 minutes without receiving any shocks. Context B is circular with black and white stripes, the floor is textured plastic, the ambient light is blue and the smell of the environment has changed from 70% ethanol to distilled water. Freezing behaviour was recorded and manually scored by experimenters blinded to the group assignment of mice.

5.2.3 Results

The CA3 is known to be an important region in the recall of remote memories, with CA3 inhibition to the ADn necessary for memory recall (Vetere et al., 2021). Building on our previous results of the CA3 controlling the activity of the ADn at remote time point, we wanted to see if these projections were important for the recall of a generalised memory. To this aim, we injected mice with an inhibitory channelrhodopsin, iC++ (AAV-CamkII-iC++-eYFP), or a control virus (AAV-CamkII-iC++-eYFP) bilaterally in the CA3 and implanted an optic fiber mediolaterally in ADn. Then we trained mice in a novel context by exposing them to 2 foot shocks (0.2mA, lasting 2 seconds) at minutes 2 and 3 of the 4 minute training session. Mice were then placed in a novel context, either 1 day (recent memory) or 28 days (remote memory) later to test their respective memories, and subsequently the effect of our optogenetic manipulation on recall. The test lasted 6 minutes, and during minutes 4-6, we optogenetically targeted the projections from the aCC to the ADn (Fig. 7.2a).

The optogenetic inhibition of CA3 projections to the ADn, and to the aCC, leads to a significant decrease in memory recall (i.e., reduction in freezing behaviour compared to control animals) at remote (2-way Repeated Measures ANOVA, indicated an effect of laser stimulation, $F_{(1,14)} = 8.37$; $p < 0.05$; post-hoc analysis of the ON periods confirmed this: ON eYFP vs ON iC++-eYFP, $t_{22} = 2.64$, $p < 0.05$; Fig. 7.2d) but not recent (2-way Repeated Measures ANOVA, ADn: $F_{(1,8)} = 0.07$, *ns*; Fig. 7.2e).

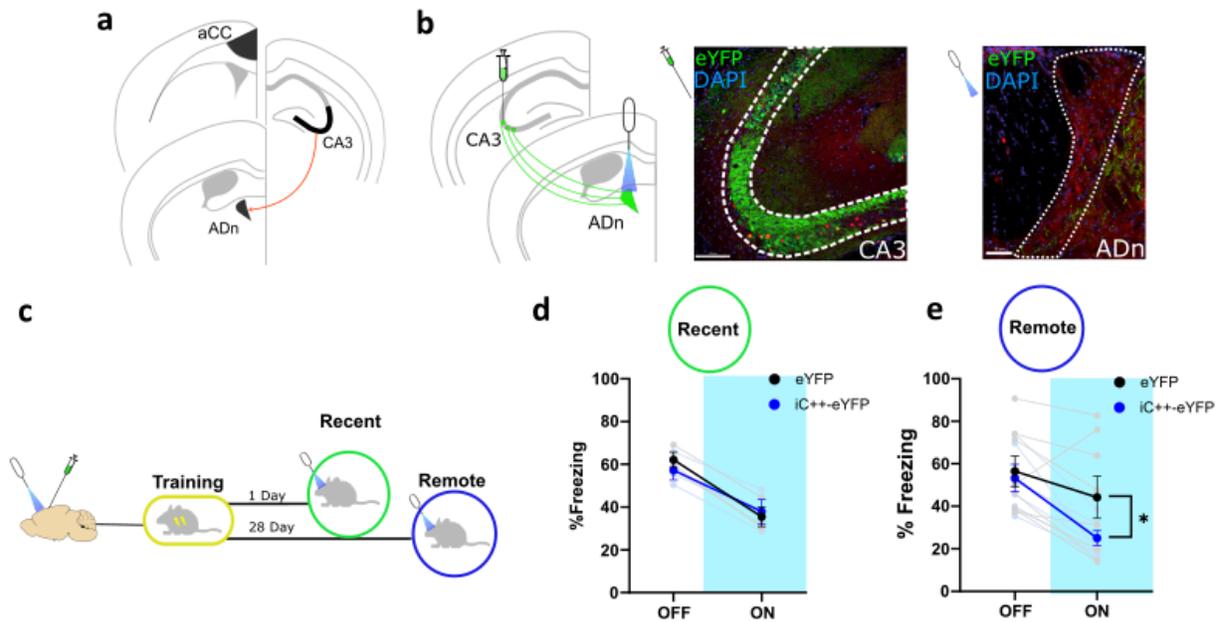


Figure 5.2. Optogenetic inhibition of CA3 projections to ADn during recall of a generalised memory. **a)** Schema of projections targeted in experiment **b)** Schema of bilateral viral injection in the CA3 and optic fiber implant mediolateral in the ADn. With confirmation of injection in the CA3 (left) and projections in the ADn (right). **c)** Schema of behaviour protocol, mice were trained (yellow) in the conditioned context, with 2 foot shocks at 0.2mA, 2 seconds per shock. Mice were then tested at recent (green) or remote (blue) in a similar, but different context. Percentage of freezing during optogenetic stimulation at **d)** recent and **e)** remote time point. The blue shaded area depicts the laser stimulation. * $p < 0.05$

Part III

Discussion

Chapter 6

Discussion and Conclusion

Distributed networks in the brain, involving the hippocampus, thalamus, and cortex, support event memories (Wheeler *et al.*, 2013; Vetere *et al.*, 2017). However, the specific brain regions involved in expressing memories may differ depending on how old the memories are. Previous studies have explored the connection between brain region activation and the age of memories during recall. In this study, we investigated whether the communication between subregions of the hippocampus (CA3), thalamus (ADn) and cortex (aCC) are necessary for memory recall. We conducted a contextual fear conditioning experiment using mice and discovered that the activity of the CA3 during retrieval is diminished at remote time-points (28 days) compared to recent time-points (1 day) after the initial training (Fig. 4.1a (Chapter 4, Fig. 1)) (Lux *et al.*, 2016), with the converse being true for the aCC, where the activity of the aCC increased at remote time points compared to recent time points (Fig. 4.1b) (Frankland *et al.*, 2004). We identified and investigated a long-range pathway from excitatory CA3 neurons to the ADn (Fig. 4.2a). Furthermore, we identified an excitatory pathway from aCC neurons to the ADn (Fig. 4.4a). Lastly, we also identified an excitatory pathway from CA3 neurons to the aCC (Fig. 4.3a).

6.1 The CA3

We found that inhibiting CA3-ADn excitatory pathway impairs memory recall only at remote time point (Fig. 4.2f). We found the same result when we inhibited the CA3-aCC excitatory pathway, that memory is only impaired at remote time point (Fig. 4.3f).

These findings show 2 novel pathways in which the hippocampus controls cortical and thalamic activity during a remote memory. Whilst these projections are present during recent memories, shown by the significant modulation in c-Fos levels in the target regions (for ADn: Fig. 4.2g and aCC: Fig. 4.3g), they do not control the recall of a recent memory. This suggests that these populations of CA3 projecting neurons to the ADn and aCC take control over these regions during memory consolidation.

6.1.1 The CA3 role in memory.

The work presented contributes valuable insights to the existing body of knowledge on memory, particularly within the context of the CA3 region, where we implicate the CA3, or specific projections from the CA3, are important in the regulation of remote contextual memory. Existing research has underscored the significance of CA3 in memory acquisition, with evidence supporting its crucial role in this cognitive process. Studies employing pharmacological inhibition (Stupien *et al.*, 2003; Lee and Kesner, 2006) as well as lesions (Gilbert and Kesner, 2003; Hunsaker *et al.*, 2007) have consistently highlighted the importance of CA3 in facilitating the acquisition of memories.

Furthermore, previous investigations implicate the CA3 in the recall of recent contextual memories (Lux *et al.*, 2016). This supports the notion that CA3 is not only instrumental in memory acquisition but also plays a significant role in the retrieval of information

related to recent experiences. Additionally, the study contributes novel insights by linking the CA3 region to the consolidation of memory (Oussini *et al.*, BioRxiv, 2023).

Lastly, these results support the importance of the CA3 during remote memory recall. They raise an interesting theory that the CA3 is an important hub region within the hippocampal-cortical-thalamic circuitry during remote memory recall. In my experiments, I consider 28 days (1 month) to be a remote memory. Lux *et al.* (2016) and defined 1 month as a “recent remote” memory, so that “very remote” memory is defined as 6 months or 1 year. They found that CA3 neurons are specifically involved in the retrieval of recent (1 day to 1 week) and early remote memories (one month), while CA1 is recruited regardless of the memory's age (6 months, up to 1 year). Our findings are partially inline with this study as we showed that CA3 projections to the ADn and aCC are important for the recall of a 1 month old memory. We can summarise this by saying that whilst as a whole the CA3 is recruited for recent memories (Lux *et al.*, 2016), specific projections in the hippocampal-cortical-thalamic circuitry are important for the recall of remote memories (Wheeler *et al.*, 2013; Vetere *et al.*, 2017).

This notion of “very remote” memories raises an interesting question of until what age of a remote memory is the hippocampal-cortical-thalamic circuitry important for its recall. A potential future experiment would be to repeat my previous experiments with a 6 month or 1 year memory test.

All together, these studies suggest that the CA3 region may play a multifaceted role in various stages of the memory process, from acquisition to recall and consolidation. These findings collectively enrich our understanding of the intricate mechanisms

underlying memory processes, emphasising the multifunctional nature of the CA3 region in the broader memory literature.

6.1.1.1 Excitatory CA3 cells that project to the ADn are important for remote memory.

As previously mentioned, the results have shown that the optogenetic inhibition of the CA3 excitatory cells that project to the ADn leads to a decrease in memory recall. This decrease in memory recall/freezing behaviour, was associated with an increase in cFos expression in the ADn. Whilst the cFos and behavioural results are inline with previous studies (Vetere *et al.*, 2021), that one may have assumed that inhibition of excitatory projections into a region that contains only excitatory cells, there would have been a decrease in cFos expression. This led us to think of possible interpretations for the cFos results.

Firstly, the observed increase in ADn activity during optogenetic inhibition requires us to consider the temporal dynamics captured by the cFos readout. Given that cFos reflects a 90-minute window rather than a precise temporal snapshot, there is potential that the increase in cFos expression is related to a rebound effect after the cessation of the optogenetic stimulation and does not occur during the optogenetic inhibition (Figure 6.1 a). To address this possibility, we can use fiber photometry technique. This will enable a more nuanced and temporally resolved assessment of regional activity, as it measures real-time population level calcium dynamics *in vivo*.

Secondly, it is important to acknowledge the potential non-specificity of the viral vector used for optogenetic manipulation. By this I mean whilst it is commonly accepted that viral promoter CaMKII selectively infects excitatory cells, it may also have off-target effects in inhibitory cells too (Veres *et al.*, 2023; Figure 6.1 b). Either interpretation is interesting: if the virus selectively targets excitatory cells, it implicates both inhibitory and excitatory CA3 in the modulation of ADn activity during recall. Conversely, if inhibitory cells are also affected by the optogenetic manipulation using CaMKII promoter, it underscores the prominence of inhibitory projections. To address this, immunohistochemistry staining targeting both inhibitory and excitatory cells could reveal any co-staining with the viral vector. Lack of co-staining with GABAergic cells would support specificity for excitatory neurons.

Thirdly, the observed changes prompt consideration of an internal or external circuit regulating ADn activity in the absence of CA3 projections during memory recall (Figure 6.1 c). Exploring the existence and nature of such regulatory circuits could provide valuable insights into the intricacies of ADn modulation.

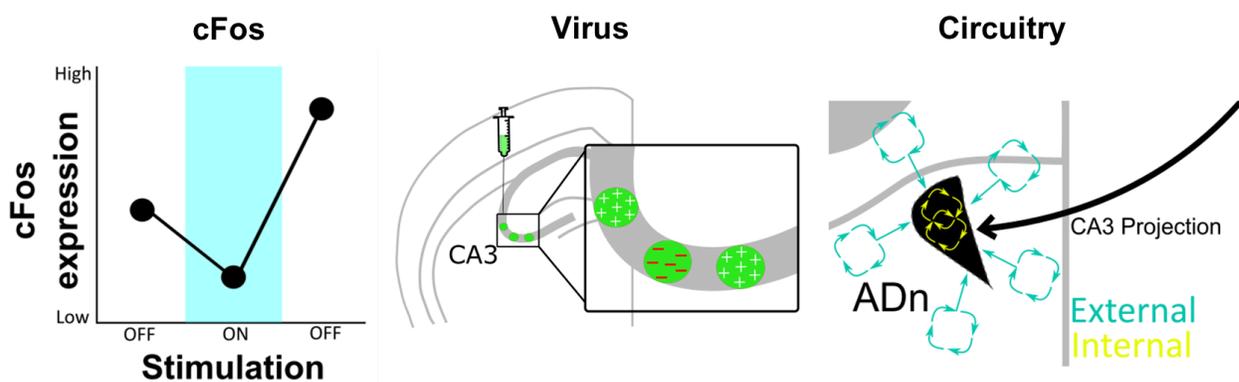


Figure 6.1. *Schema of the possible interpretations of the cFos results. a) Possibility number one, where the cFos expression is linked to a rebound of activity in the ADn after the cessation of the optogenetic inhibition. b) Possibility number two, where the optogenetic virus may have off target expression in inhibitory cells. c) Possibility number three, where there may be an internal or external circuitry regulating the activity of the ADn in the absence of the CA3 projections during remote memory recall.*

6.1.1.2 Excitatory CA3 cells that project to the aCC are important for remote memory.

As previously mentioned, the optogenetic inhibition of CA3 excitatory projections to aCC decreases remote memory recall, and there is an associated decrease in cFos expression in the aCC (Figure 4.3). These results support the theory of the multiple trace theory, where the hippocampus is still engaged at remote time points for contextually rich memories (Moscovitch & Nadel, 1998; Nadel & Moscovitch, 1997; Akers and Frankland, 2009).

A notable observation in studies is discrimination between dorsal and ventral projections of the hippocampus, both presumed to undertake distinct functions in memory processing (Fanslow and Dong, 2010; Bannerman *et al.*, 1999). This divergence in functionality prompts an exploration into potential implications for the spread of infection within the hippocampus, positing that such spread could contribute significantly to the obtained results.

The optogenetic inhibition of CA3 excitatory cells projecting to the aCC demonstrates a consequential decrease during remote memory recall. The results support frameworks

such as the multiple trace theory and the standard model, which implicate the aCC in remote memory recall (Frankland *et al.*, 2004; Frankland and Bontempi, 2005; Vetere *et al.*, 2011). The correlation between decreased memory recall and reduced cFos expression in the aCC further corroborates the findings. This outcome is consistent with prior research demonstrating that optogenetic inhibition of the aCC at remote time points, as opposed to recent ones, is associated with a decline in memory recall (Goshen *et al.*, 2011).

6.1.2 The role of the CA3 in generalised memories.

Taking into consideration our previous findings and the known role of the CA3 in pattern completion, we were interested in whether the projections previously studied were important in generalised memories.

As a memory undergoes consolidation, it becomes generalised, where the specificity of the memory is lost but the gist remains. This means that the event/lesson that happened in a specific environment can now be applied to other similar contexts (Dudai, 2004). Under normal circumstances, this is a useful mechanism as one does not have to learn the same association multiple times for different environments. For example, if you burn your hand on the oven once, you know not to touch any ovens without gloves. The lesson has been generalised to all ovens. However, this mechanism can become pathologic when traumatic memories are generalised and triggered in safe and neutral environments (Asok *et al.*, 2018).

Here, we do not delve into maladapted memory generalisation, but instead, we conducted a preliminary study to investigate whether the excitatory projections from the

CA3 to the ADn are also involved in generalised memories. This was done but optogenetically inhibiting excitatory CA3 projections to the ADn during memory recall in a different but similar context to the context the mice were trained in (Figure 5.2).

We found that there was a significant decrease in memory recall during optogenetic inhibition of a remote but not recent memory. It is not surprising that there was no significant change in memory recall at recent time point, as we did not see a significant change in our previous study (Figure. 4.2e). The result also aligns with literature that shows that mice are able to discriminate between contexts/cues at recent time point and that pattern discrimination is not reliant on the CA3, rather the DG (Wiltgen and Silva, 2007; Leutgeb *et al.*, 2007; Wiltgen *et al.*, 2010).

Furthermore, the finding that there is a significant decrease in memory recall at remote time point compliments our previous finding (Figure 4.2f) and aligns with previous work showing that the hippocampus is important in the representation of generalised memories (Bowman and Zeithamova, 2018).

6.2 The aCC

The aCC is known to be an important region not only in memory consolidation (Frankland *et al.*, 2004; Frankland and Bontempi, 2005; Vetere *et al.*, 2011; Goshen *et al.*, 2011), but also in driving the expression and regulation of fear behaviour and fear responses (Courtin *et al.*, 2014; Herry *et al.*, 2008; Rolls, 2023; Singh and Topolnik, 2023). When conducting the experiment, we originally investigated the aCC projections

to ADn in memory recall at different time points (recent and remote). However, we discovered that they may play a role in the regulation of memory-related fear expression, as there was a significant increase in fear expression during optogenetic inhibition at both recent and remote time points (Fig 6.4e,f). We would expect that, if these projections were involved in the recall of a memory, then we would see a decrease in fear expression (a proxy for memory) at remote, but not recent time point. However we found that when we optogenetically inhibited excitatory aCC projections to the ADn we saw an increase in freezing behaviour at both recent and remote time points. This means that these projections are not involved in memory recall per se, but may be involved in the expression of the fear behaviour itself (i.e. controlling freezing behaviour).

7.2.1 The aCC is important for memory-related fear expression.

The increase in freezing during aCC-ADn projection inhibition prompted an interesting question regarding the role of the aCC-ADn pathway in fear expression. Do these projections play a role in fear expression in all conditions, or, is this pathway only recruited when the fear expression is linked to a memory?

To investigate this, we inhibited the excitatory aCC-ADn projections in a neutral, novel, context (Figure 4.4). If the pathway was involved in the regulation of fear expression in all conditions, we would expect that the optogenetic inhibition in a neutral context would induce freezing. However, this is not the case, there was no significant increase in freezing during optogenetic inhibition.

The results from optogenetic inhibition during contextual memory recall and in a neutral

context, lend to the theory that the aCC-ADn projections are only recruited for fear expression regulation during memory recall.

The aCC works as part of an inhibitory circuitry that regulates fear responses to prevent the overgeneralisation of fear memories (Singh and Topolnik, 2023; Courtin *et al.*, 2014). This is inline with our findings, as the optogenetic inhibition of the aCC projections to the ADn causes a significant decrease in cellular activity (measured by proxy through c-Fos expression) and we see a significant increase in freezing (Fig. 6.4 e,f).

However, the aCC is only one half to this story, as this increase in fear expression occurred during the optogenetic inhibition of aCC projections to the ADn. The ADn needs to be inhibited at a remote time point for a memory to be recalled and an increase in c-Fos expression at remote time point is linked with an increase in movement, a decrease in fear expression (Vetere *et al.*, 2021). With this in mind, the optogenetic inhibition of aCC projections to the ADn resulted in a significant decrease in c-Fos expression in the aCC (Fig 4.4g) and a significant decrease in c-Fos expression in the ADn (Fig 4.4h).

6.2.2. The aCC processes and regulates fear expression.

Following on from our previous discussion, we wanted to understand the real-time relationship between the aCC and ADn during fear expression (i.e. freezing). To this aim, we used the population level *in vivo* calcium imaging technique fiber photometry. Unfortunately, due to variability across mice when recording the ADn we were not able

to yield significant results, despite being able to subjectively see a difference in calcium traces (Fig. 4.5g,h,k,l,o,p). This means that at this point, we cannot draw conclusions regarding the activity of the ADn and expression of freezing. For the aCC, we saw a significant increase in cellular activity during the administration of shocks during the training session (Fig. 4.5e). This fits with work yet to be published in our lab that identifies cells that respond to shock in the aCC and also with previous findings (Etkin *et al.*, 2011). Interestingly, the activity of the aCC was significantly lower at the onset of freezing (Fig. 4.5i,j) and significantly higher when freezing ended (Fig. 4.5m,n), suggesting a possible involvement of this region in regulating fear expression by controlling the initiation and/or termination of each freezing episode.

These findings, together with the c-Fos results, optogenetic results, and previous research, point towards a significant role of aCC projections to the ADn in regulating freezing behaviour. Inhibition of these specific projections appears to have caused the overgeneralization of fear expression. It is important to note that these outcomes are specific to the cell populations within the aCC-ADn pathway, as Goshen *et al.* (2011) demonstrated that if the entire aCC region is optogenetically inhibited during remote memory recall, then it results in an impairment of memory, resulting in a decreased fear expression. To be able to draw definitive conclusions, it is necessary to do further research into the activity of the ADn and its relationship with the aCC during fear expression.

6.3 The role of the ADn in memory recall.

Our findings unveil a dynamic association between ADn activity and fear expression, emphasising its bidirectional modulation (Figure 6.2). During the optogenetic inhibition of excitatory CA3-ADn projections, we observed a significant increase in ADn activity at remote time points, as evidenced by increased c-Fos expression (Fig. 4.2h). Intriguingly, this increase in ADn activity coincided with a decrease in freezing behaviour, indicating that a higher level of ADn activity is associated with decreased memory recall. These findings complement those of Vetere *et al.* (2021). This leads to supporting the hypothesis that the ADn needs to be actively inhibited at a remote time point for a memory to be recalled (Vetere *et al.*, 2017; Vetere *et al.*, 2019).

Conversely, when we inhibited excitatory aCC-ADn projections, we witnessed a significant decrease in ADn activity, as evidenced by reduced c-Fos expression (Fig. 4.4g) accompanied by an increase in freezing behaviour at both recent and remote time points (Fig. 4.4e,f). This unexpected increase in freezing suggests that the aCC-ADn pathway may play a crucial role in modulating fear expression during memory recall, and its inhibition leads to an exaggeration of this response.

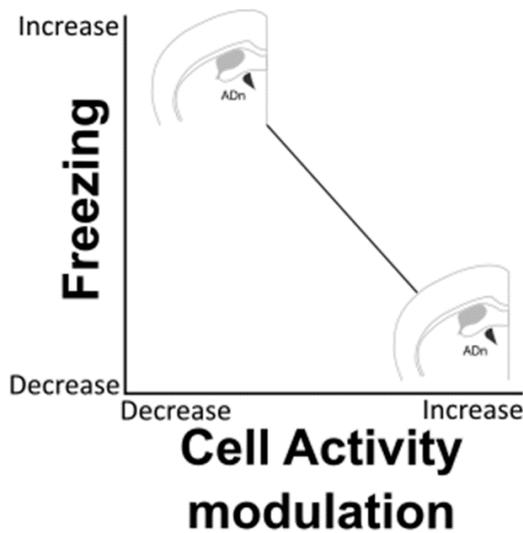


Figure 6.2 *Schema of the potential association of significantly modulated activity in the ADn, compared to control mice, and changed freezing behaviour. Such that, a significant decrease in activity is associated with an increase in freezing and a significant increase in activity is associated with a decrease in freezing behaviour.*

In either instance, these results support that the ADn has to be modulated at remote time point for the proper expression of a memory. These findings support the importance of the thalamus and the hippocampal-thalamic-cortical during remote memory recall (Wheeler *et al.*, 2013; Vetere *et al.*, 2017; Vetere *et al.*, 2021; Tomé *et al.*, 2022).

However, we cannot say for certain if the results seen are controlled by the same pathway, or if it is the same cells within the ADn that receive inputs from both the aCC and the CA3. To answer this, we can do double staining in the aCC and CA3 to see if there is co-localisation of infection in the ADn.

6.4 Conclusions

In summary, our research has revealed a new cortico-hippocampo-thalamic pathway, which involves the CA3, ADn, and aCC regions. This pathway plays a crucial role in regulating ADn activity during the recall of recent and remote fear memories. The use of retrobead tracing, functional manipulations with optogenetics, c-Fos neuronal activity validation and real time calcium imaging study of neuronal activity, has provided strong evidence supporting the significance of the CA3-ADn-aCC circuitry in memory consolidation and fear expression. These findings have advanced our comprehension of the complex hippocampo-thalamic-cortical circuitry that underlies the retrieval of contextual fear memories. Employing advanced techniques such as fiber photometry and immunohistochemistry can enhance the precision and specificity of the findings, which can delve into the specific mechanisms through which these regions interact and determine the precise contributions of each region within this circuitry.

Part IV

Head Direction Cell Project

Overview

Part IV delves into the intricate world of the spatial representation system within the brain, a fundamental network responsible for processing information related to the location and orientation of objects in the environment. The primary focus is on HDCs and their pivotal role in spatial navigation and memory formation. It starts with Chapter 7, a comprehensive introduction to the spatial representation system, elucidating its importance for tasks such as navigation, perception, and motor control.

The spatial representation network is explored through various types of cells, including place cells, grid cells, time cells, and the spotlight on HDCs. Place cells, situated in the hippocampus, and grid cells, found in the medial entorhinal cortex, contribute to the creation of a cognitive map, while time cells integrate temporal aspects into the spatial framework. However, the spotlight shifts to HDCs, particularly those in the ADn, as they are extensively investigated in the subsequent chapters.

There is a detailed exploration of the different types of spatial representation cells, with a particular emphasis on place cells, grid cells, and the crucial HDCs. Place cells exhibit selective firing patterns based on the animal's location, contributing to the formation of spatial memories and supporting goal-directed navigation. Grid cells create a grid-like spatial map, enhancing the animal's ability to track its location. Time cells add a temporal dimension to spatial mapping, firing at successive moments during structured experiences.

Chapter 7 deepens its focus on HDCs, elucidating their role in encoding the allocentric orientation of the animal within its environment. The ADn emerges as a key region housing a significant population of HDCs, emphasising its critical role in spatial orientation. The interconnectedness of HDCs with angular head velocity cells is explored, highlighting their collaboration in providing a stable representation of the animal's head direction and angular movements.

The exploration extends to the thalamus, particularly the ADn, which emerges as a hub for HDCs. Lesion studies underscore the importance of the ADn in maintaining the head direction signal in downstream regions, emphasising its role in integrating head direction information.

Chapter 8 explores the Ring Attractor Theory, a computational model that sheds light on the neural mechanisms underpinning head direction representation. The theory proposes a continuous ring attractor network of HDCs, explaining their stable representation and updating mechanisms. Experimental studies in flies provide additional validation for this theory.

Chapter 9, explains the research done throughout my PhD on the role of HDC activity on head direction behaviour. The project explores whether HDC activity can actively drive head direction orientation, presenting exciting findings that demonstrate a causal relationship between HDC stimulation in the ADn and changes in head direction orientation.

Chapter 7

In this chapter, I will introduce the broad topic of the spatial representation system, from its definition to the types of cells and the regions involved. I will pay specific attention to the HDC system, focusing on the thalamus, particularly to the ADn. This session is an introduction to the topic presented in the Appendix where I investigated the properties of HDCs within the ADn.

Spatial Representation Network

The spatial representation system refers to the neural network in the brain responsible for processing information about the location and orientation of objects in the environment (Moser *et al.*, 2008). It is crucial for tasks such as navigation, perception, and motor control. Spatial navigation is a crucial cognitive function essential for survival in natural surroundings. Animals require an internal understanding of spatial features to accomplish tasks like searching for food or locating suitable shelters. The brain achieves this through the creation of a "cognitive map" of the environment, utilising various populations of neurons that encode environmental features or landmarks. These populations, including place cells, grid cells, and boundary cells, use firing-rate patterns to represent the animal's position in space (Moser *et al.*, 2015). Here I will present only some of the discovered cells that are believed to take part in the formation of an internal spatial map. Major details are dedicated to a specific subpopulation, the head direction cells since they are also the focus of part of my thesis.

7.1 Type of Spatial Representation Cells

7.1.1 Place Cells

Place cells are a type of neuron found in the hippocampus that play a crucial role in spatial navigation and memory formation (Pfeiffer and Foster, 2013). These cells were first discovered by O'Keefe and Dostrovsky (1971) Place cells exhibit increased firing when an animal is in a specific location in its environment, known as the "place field". The firing of place cells is highly selective and specific to particular locations, allowing for the creation of a cognitive map of the environment (Moser *et al.*, 2008).

The firing patterns of place cells are influenced by various factors, including the animal's current location, head direction, speed, and proximity to boundaries. Place cells provide a geometric representation of the current environment and contribute to the formation of spatial memories (Hok *et al.*, 2007). They are involved in encoding the spatial context of retrieved memories, as their activity during memory recall is similar to the activity that represented the locations where the memories were initially encoded (Miller *et al.*, 2013).

Place cells are not only active during actual navigation but also during memory retrieval and planning of future trajectories (Moser *et al.*, 2008). Prior to goal-directed navigation, the hippocampus generates brief sequences of place cell activity that encode spatial trajectories from the animal's current location to a known goal location (Pfeiffer and Foster, 2013). These sequences predict immediate future behaviour and support a goal-directed, trajectory-finding mechanism.

7.1.2. Grid Cells

The formation of place fields in hippocampal pyramidal cells is thought to be influenced by inputs from grid cells in the medial entorhinal cortex (Solstad *et al.*, 2006). Grid cells, discovered in 2005 by Hafting *et al.*, help create a spatial map of the environment. These cells fire in a grid-like pattern as an animal moves around an environment, allowing the animal to keep track of its location relative to the environment.

7.1.3. Time Cells

Time cells were first discovered in 2011 by MacDonald *et al.* These cells fire at successive moments in temporally structured experiences and provide an additional dimension that is integrated with spatial mapping. The firing properties of time cells parallel those of place cells, suggesting a fundamental mechanism for organising the elements of experience into coherent memories (Eichenbaum, 2014).

7.1.4. Head Direction Cells and Angular Head Velocity Cells

The head direction cells encode the allocentric orientation of the animal within its environment. It is composed of cells characterised by a firing rate that is dependent upon the direction of the head relative to an external landmark. Their orientation tuning can be described by a preferred direction (PFD), which is the orientation in which they display their maximum firing rate, and a triangular-shaped distribution of responses to all orientations, which centres around the PFD. HDc are found in many brain regions, including the retrosplenial cortex, subiculum, or the latero- and antero-dorsal thalamic

nuclei (Fig. 7.1). This, along with the fact that they display different tuning properties, suggests that they may have different roles across regions. The ADn is the region in which HDcs are most abundant, as they represent at least 60% of the neuronal population¹, which suggests that it plays a key role in spatial orientation (Wiener and Taube, 2005).

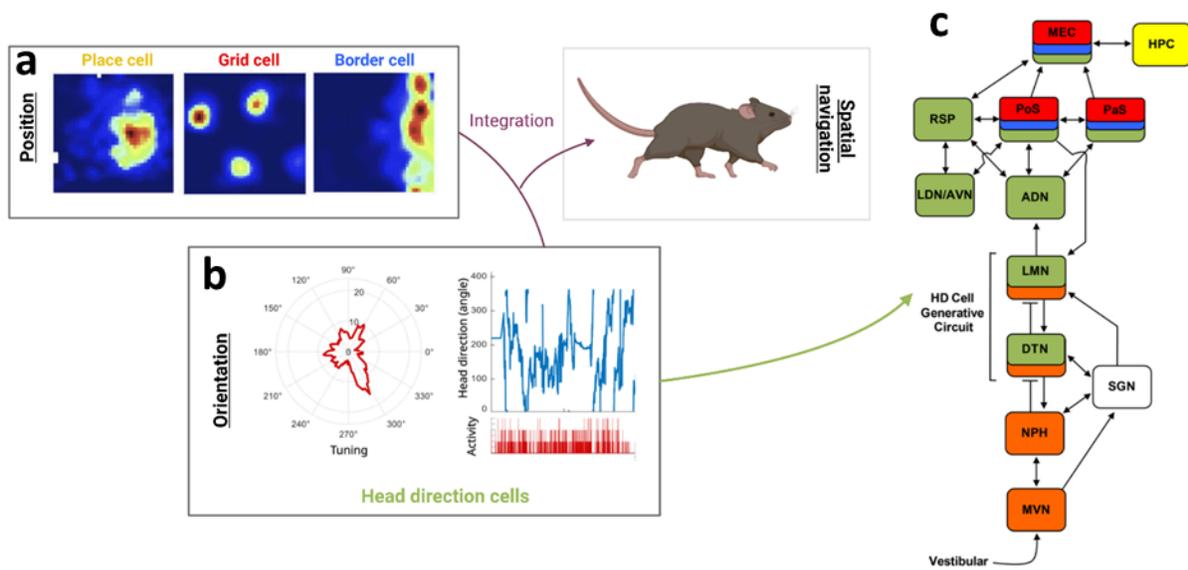


Figure 7.1 Head direction cells and their role in spatial navigation. **a)** Heat map representation of different types of spatial representation cells. **b)** The figure illustrates the electrophysiological data from our laboratory, depicting the firing rate and tuning of head direction cells in the antero-dorsal thalamic nucleus (ADn). **c)** Representation of some of the different regions within the spatial presentation pathway. Head direction cells (green), place cells (yellow), grid cells (red), border cells (blue).

Angular head velocity cells, on the other hand, are neurons that discharge depending on the velocity of head movements (Dillingham *et al.*, 2015). These cells provide information about the speed and direction of head rotations and contribute to the

updating of the head direction representation (Turner-Evans et al. 2017). Angular head velocity cells have been found in regions such as the lateral mammillary nuclei and the dorsal tegmental nucleus (Turner-Evans *et al.*, 2017; Dillingham *et al.*, 2015).

The firing properties of head direction cells and angular head velocity cells reflect the current angular head velocity and the direction of head turning (Stackman and Taube , 1998). Head direction cells maintain their directional firing preference regardless of the angular head velocity, while angular head velocity cells show changes in their directional tuning peak depending on the speed and direction of head turns (Blair and Sharp 1995). These cells work together to provide a stable and continuous representation of the animal's head direction and angular head movements.

The generation of head direction and angular head velocity signals involves the integration of inputs from various sensory systems, including the vestibular system (Angelaki and Cullen, 2008). The vestibular system provides information about head orientation, balance, and navigation, which are important for the functioning of head direction and angular head velocity cells.

7.2 Head Direction Cell Regions

During the 1990s, head direction cells were first identified in the rat brain, marking a significant breakthrough in our understanding of spatial processing (Taube *et al.*, 1990). These neurons respond to an animal's directional heading and contribute to the creation

of a mental map of the environment. The activity of head direction cells remains active even when the animal is stationary, providing a continuous sense of direction.

Head direction cells encode the current direction an animal is facing and play a crucial role in maintaining orientation and spatial awareness. Each cell exhibits a preferred firing direction, forming a directional firing range that varies among cells. The perception of an animal's directional heading is believed to be represented by the collective activity of head direction cells within a specific brain area (Wilson and McNaughton, 1994).

Head direction cells have been observed in several cortical and subcortical regions, including the retrosplenial cortex, posterior parietal cortex, medial entorhinal cortex, precentral cortex, ADn, laterodorsal thalamic nuclei, anteroventral thalamic nuclei, nucleus NRe, dorsal striatum, dorsal tegmental nucleus of Gudden, and lateral mammillary nuclei.

7.2.1 The Thalamus: A Hub for Head Direction Cells

Among the regions that house head direction cells, the thalamus stands out as a key hub for its activity. The anterior dorsal thalamus, including the ADn, has been identified as an essential site for head direction cell representation (Blair and Sharp, 1995). Lesion studies have shown that damage to the ADn disrupts the head direction signal in downstream regions, such as the postsubiculum and medial entorhinal cortex (Goodridge and Taube, 1997; Winter *et al.*, 2015). The ADn has reciprocal connections with the lateral mammillary nuclei (LMN) and receives inputs from various brain regions,

suggesting its pivotal role in integrating head direction information (Blair *et al.*, 1999; Shibata, 1993; van Groen and Wyss, 1995).

The ADn within the anterior thalamic nuclei has been a focus of research regarding head direction cells. Lesions in the retrosplenial cortex have been shown to impair head direction cell representation in the ADn (Clark *et al.*, 2010). On the other hand, intact landmark control and angular path integration by head direction cells in the ADn have been observed after lesions in the medial entorhinal cortex (Clark and Taube, 2011). These findings highlight the specific contributions of the ADn to the spatial representation network and suggest its involvement in cognitive processes beyond spatial learning and navigation (Aggleton and Brown, 1999; Carlesimo *et al.*, 2011).

7.2.2 Functional Influences on Head Direction Cells

Head direction cells can be influenced by both external cues, such as visual landmarks, and internally generated cues, including vestibular, proprioceptive, and motor efference signals. Visual landmarks can shift the preferred firing directions of head direction cells when their orientation changes (Taube, 1995; Taube *et al.*, 1990), while internally generated cues help track changes in directional heading over time (Jeffrey S. Taube 2007).

Motor cues play a significant role in modulating the responses of head direction cells and updating their preferred firing directions. The Interpeduncular nucleus (IPN) is believed to send motor information to the circuitry of head direction cells, as it has reciprocal connections with these cells (Clark *et al.*, 2009). Lesions in the IPN have

been shown to disrupt the maintenance of preferred firing direction during dark sessions and in novel environments (Clark *et al.*, 2009).

7.2.3 Anticipatory Time Interval and Lesion Studies

7.2.3.1 Anticipatory Time Interval (ATI)

The anticipatory time interval (ATI) measures the time between when a head direction cell fires and the predicted head direction change of the animal. Head direction cells in the anterodorsal nucleus (ADn) exhibit ATI values of approximately 25 milliseconds, suggesting their involvement in predicting future head direction changes (Clark *et al.*, 2012).

7.2.3.2 Lesion Studies

Lesion studies have provided insights into the functional significance of head direction cells. Unilateral lesions in the lateral mammillary nuclei have transient effects on head direction firing in the ADn, but recovery of function occurs within a few days (Blair *et al.*, 1999). Lesions in the retrosplenial cortex impair head direction cell representation in the ADn (Clark *et al.*, 2010), while lesions in the medial entorhinal cortex do not affect landmark control and angular path integration by head direction cells (Clark *et al.*, 2010). Furthermore, lesions within the ADn impair place cell representations in the hippocampus (Calton *et al.*, 2003). Together these lesions show that the spatial representation system is highly interconnected, with the proper functioning of different HDCs being crucial for the proper functioning of downstream place cells.

Chapter 8

The Ring Attractor Theory

The ring attractor network theory of head direction is a computational model that aims to explain the neural mechanisms underlying the representation of head direction in the brain. This theory proposes that head direction cells, which are neurons that selectively fire based on the animal's head orientation, are organised in a network with recurrent connections that form a continuous attractor. This network allows for the stable representation and updating of head direction information (Hulse and Jayaraman, 2020).

8.1 Properties of the Attractor Network

Attractor networks are dynamical systems that tend towards one or more equilibrium states over time. In the context of head direction cells, the attractor network is constructed as a continuous ring-like structure, where the connectivity between neurons depends on their preferred head direction. This network can sustain a bump-like activity pattern that represents the animal's current head direction (Esnaola-Acebes *et al.*, 2021; Kim *et al.*, 2017). The ring attractor model is supported by experimental evidence showing that head direction cells rotate together as a tightly coupled network.

8.2 Integration of Angular Velocity Inputs

One key aspect of the ring attractor network theory is the ability to integrate angular velocity inputs to update the head direction representation. Angular velocity signals, which encode the speed and direction of head rotations, are represented as inputs to the ring attractor network. These inputs are received by inhibitory populations within the network, with clockwise and counterclockwise turns represented in separate populations (Seelig and Jayaraman, 2015). The network can accurately update the head direction based on these inputs, allowing for the continuous tracking of head orientation.

8.3 Accuracy of Path Integration

Another important aspect of the ring attractor network theory is its ability to perform accurate path integration. Path integration refers to the ability to estimate one's current position based on self-motion cues, such as velocity and heading direction. The continuous attractor models of head direction cells have been shown to generate regular triangular grid responses, similar to grid cells in the entorhinal cortex, based solely on velocity and heading direction inputs. This suggests that the ring attractor network can support accurate path integration without the need for frequent resets triggered by external sensory cues.

8.4 Role of Inhibitory and Excitatory Connectivity

The connectivity within the ring attractor network plays a crucial role in its functioning. The network consists of excitatory and inhibitory populations, with interconnections between populations but no connections within the neurons of the same population (Seelig and Jayaraman, 2015). The inhibitory connections help maintain the stability of the bump-like activity pattern, while the excitatory connections provide the necessary recurrent feedback to sustain the attractor dynamics. The specific connectivity patterns and strengths determine the emergence of the spatially selective profile and the reliable integration of angular velocity inputs.

8.5 Modelling the Ring Attractor Network

Researchers have developed computational models to simulate and study the dynamics of the ring attractor network theory of head direction. These models incorporate the principles of recurrent connectivity, inhibitory and excitatory interactions, and the integration of angular velocity inputs (Petrucco *et al.*, 2023). By manipulating the parameters and connectivity patterns in these models, researchers can investigate how the network generates and maintains stable representations of head direction.

8.6 Experimental Studies in Flies

Experimental studies in flies have provided insights into the neural mechanisms underlying the ring attractor network theory of head direction. For example, used

two-photon calcium imaging in head-fixed flies walking on a ball in a virtual reality arena to demonstrate that landmark-based orientation and angular path integration are combined in the population responses of neurons in the ellipsoid body, a toroidal structure in the fly brain (Seelig and Jayaraman, 2015). This population of neurons encodes the fly's azimuth relative to its environment, tracking visual landmarks when available and relying on self-motion cues in darkness. Another study combined two-photon calcium imaging with optogenetics to provide physiological evidence of ring attractor dynamics in the fly central brain (Kim *et al.*, 2017). They showed that a network with local excitation and global inhibition enforces a unique and persistent heading representation. These studies have provided additional validation and physiological evidence supporting the ring attractor network theory.

8.7 Chapter Conclusion

The ring attractor network theory of head direction provides a computational framework for understanding how head direction cells in the brain represent and update information about head orientation. The theory proposes that head direction cells are organised in a continuous ring attractor network, where the connectivity between neurons allows for the stable representation and updating of head direction. The network can integrate angular velocity inputs and perform accurate path integration based on self-motion cues. Computational models and experimental studies, particularly in flies, have provided insights into the mechanisms and properties of the ring attractor network

theory. Further research is needed to fully elucidate the detailed mechanisms and functional architecture of the ring attractor network theory of head direction.

Chapter 9

The Role of Head Direction Cell Activity on Head Direction Behaviour

9.1 Summary

One year of my PhD was dedicated to the following project. The project investigates the role played by HDCs activity on HD behaviour. We know that HDCs respond to the orientation of the head but we want to answer if the contrary is true; can the activity of HDCs drive the HD orientation? To this end, I targeted two regions of the spatial representation system (ADn and retrosplenial cortex (RSC)) with an excitatory channelrhodopsin (ChRs) and stimulated bilaterally and observed any changes in HD orientation. I found that when the ADn cells were stimulated, the rate of HD variation increased. Such that the mice passed through 360 degrees consecutively, a behaviour not observed when the the ADn were not stimulated.

9.2 Participation Statement

I contributed to the conception of the project and the experimental design. I helped write the code for the analysis, generate all of the figures, and performed surgeries for the FLiCRE experiments (the next step of the project). I performed all of the optogenetic surgeries and experiments, and established all the behavioural protocols.

9.3 Abstract

The brain's spatial representation system plays a pivotal role in processing information about the location and orientation of objects in the environment, enabling essential functions such as navigation and memory formation. Among the various cell types involved in this system, head direction cells (HDCs) have emerged as key players due to their selective firing patterns in response to an animal's head orientation relative to external landmarks (Moser *et al.*, 2008). While HDCs have been extensively studied for their responsiveness to head movements, a compelling question remains: can the activity of HDCs actively drive the head direction (HD) orientation itself?

In this work, we aim to explore the bidirectional relationship between HDC activity and HD behaviour, investigating whether HDCs have a causal influence on the head direction orientation of animals. To address this question, we targeted specific regions in the spatial representation system, specifically the anterodorsal thalamus (ADn) and retrosplenial cortex (RSC), using optogenetics to selectively stimulate head direction cells.

9.4 Introduction

The brain's ability to represent spatial information is essential for survival, enabling animals to navigate their environment, locate resources, and establish cognitive maps of their surroundings. A critical aspect of this spatial representation system is the selective firing of head direction cells (HDCs), which respond to an animal's head orientation relative to external landmarks. These cells play a significant role in establishing a dynamic cognitive map that guides navigation and spatial memory (Pfeiffer and Foster, 2013; Moser *et al.*, 2008).

The firing patterns of HDCs have been extensively studied in response to head movements, revealing their sensitivity to the animal's directional heading. This sensitivity has led to a profound interest in understanding the role of HDCs in the formation of head direction orientation. While it is clear that HDCs respond to changes in head orientation, an intriguing and less explored question arises: can the activity of HDCs actively drive the head direction orientation itself?

To answer this question, we conducted a comprehensive research project that targeted key regions in the spatial representation system, including the anterodorsal thalamus (ADn) and retrosplenial cortex (RSC). Using optogenetics, we selectively stimulated head direction cells within these regions, allowing us to precisely control HDC activity.

9.5 Methods

Mice

7-8 week old male and female hybrid (C57BL/6NRj X 129/SV) mice were used for all experiments. All procedures were carried out during the light phase cycle of the mice. Mice were randomly assigned to different conditions and had within cage viral controls (i.e. the experimental virus and control virus were within different mice of the same cage). Mice were bred in a colony at the ESPCI Paris Institute. At 21 days old, the mice are weaned, separated by sex, and grouped-housed, 2-4 per cage, in a standard home cage.

Anaesthesia

Mice were administered an intraperitoneal injection of ketamine and xylazine (100mg/ml and 2% respectively, 100 mg per kg) and a subcutaneous injection of lurocaine (20mg/ml). Mice were then placed into stereotaxic frames. As part of post-operative care, mice were injected subcutaneously with an analgesic (metacam, 5mg/ml), and 0.5 ml of 0.9% saline, and 0.3 ml of glucose, to prevent dehydration.

Viral Injection

Mice received bilateral injections of AAV-DJ-CamKII-ChR2-eYFP or AAV-DJ-CamKII-eYFP (1 μ l per side at a rate of 0.12 μ l/min) were injected bi-laterally in the ADn (AP: -0.82 mm, ML: +/- 0.75 mm, DV: -2.8 mm) or RSC (AP: -1mm, ML: +/- 0.4 mm, DV: - 1.9mm) using a Hamilton microsyringe. The infusion was performed over

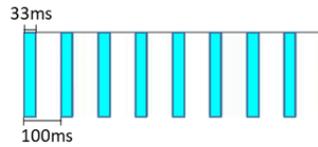
a total duration of 8 minutes, with an additional 5 minute waiting period at the end of infusion, to ensure complete viral distribution.

Optogenetic fiber implant

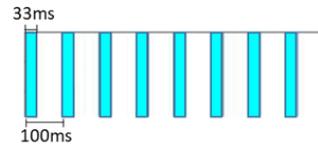
Optrodes were constructed in-house by attaching a 200 μm optical fiber (0.37 numerical aperture) to a 1.25 mm zirconia ferrule, with the fiber extending 3 mm beyond the ferrule. The optrodes were secured with epoxy resin. During surgery, the skull was prepared by scratching and two screws were implanted to facilitate adhesion of the black dental cement. Optrodes were implanted medial lateral to ADn (AP: -0.8 mm, ML: ± 0.0 mm, DV: -2.8 mm) or RSC (AP: -1 mm, ML: ± 0 mm, DV: $- 1.8$ mm).

Optogenetic Stimulation during Test

Mice were placed in the centre of an open square arena (45 x 45 x 20 cm° (length x width x height)) and allowed to explore for 6 min, when the laser was off during the first 3 minutes and then turned on during the last 3 minutes. Mice were repeatedly placed in the open field, with 2 days between sessions. Each session consisted of different stimulation protocols (Table 9.1). The purpose of different stimulation protocols was to try and mimic the natural range of firing rates that are normal for each region.



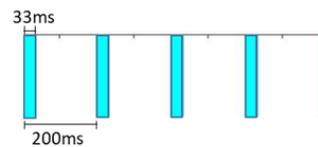
Simulation time: 5 mins
 Frequency: 10Hz
 Power: 10mW



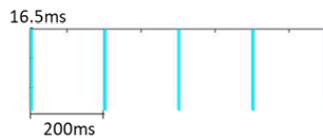
Simulation time: 5 mins
 Frequency: 10Hz
 Power: 5mW



Simulation time: 5 mins
 Frequency: 20Hz
 Power: 5mW



Simulation time: 5 mins
 Frequency: 5Hz
 Power: 5mW



Simulation time: 5 mins
 Frequency: 5Hz
 Power: 5mW

Table 9.1 Describes the different laser stimulations that mice were exposed to per session. The frequency is with the natural firing rate for each region.

Transcardial Perfusion

At the end of behaviour, mice were perfused transcardially with 1× PBS followed by 4% paraformaldehyde (PFA). We infused 0.9% saline at 13 ml/min until the fluid exiting from the right atrium was clear. We then switched to infusing with the fixative (4% PFA) until the muscles were rigid. Once the mouse is successfully perfused, we decapitate it to remove the brain. To finish the brains were placed 4% PFA overnight at 4°C before switching them to 30% sucrose until the brain is saturated (2-3 days).

Immunohistochemistry

Once the brains had been saturated in 30% sucrose for 3 days post perfusion, they were sliced coronally at -20°C using a cryostat at 50 micron slices. The 50 micron slices

were then incubated with a blocking solution (2.5% BSA, 5% normal goat serum (NGS), 0.3% Triton X-100) for 30 min at room temperature on a shaker. To visualise c-Fos expression, we incubated the slices in a primary antibody for c-Fos (rabbit, 1:500) for 72 hours on a shaker at 4 °C. Next, the sections were washed 3 times for 10 minutes each in 1% Phosphate-buffered saline (PBS). Sections were washed for 10 minutes with 4,6-diamidino-2-phenylindole (1:10000, DAPI) to stain the nuclei of the cell. After a final wash in PBS, the slices are mounted on slides and coverslips using PermaFluor mounting medium. The slides were then kept and stored at 4 °C, ready to be imaged on a confocal laser scanning microscope.

Statistics

For the purpose of analysis, the results from each session have been pooled together. Mice were excluded upon histological confirmation of virus and optrode location. Custom script in MATLAB was written to analyse: angular head variation across time; map of animal location during test sessions; cumulative angular variation. Statistical analyses employed in this study included a 2-tailed paired t-test to analyse the mean angular variation and number of spins across mice.

9.6 Results

9.6.1 Head direction cells in ADn drive the activity of head direction behaviour

HDcs play a critical role in spatial navigation as they encode an animal's spatial orientation in relation to external landmarks. The neuronal population of the anterodorsal thalamic nucleus (ADn) is mostly composed of HDc, making this region a crucial hub of the spatial orientation system (Wiener and Taube, 2005). We wanted to investigate the role of the HDcs within the ADn on the HD behaviour of the mouse. To this aim, injected excitatory ChRs, AAV-CamkII-ChR2-eYFP, bilaterally into ADn and placed an optic fiber medial to the ADn. Four weeks after surgery, once the virus had time to express, we placed mice in the centre of an open field and allowed them to explore for 6 minutes, with minutes 4 to 6 being under laser stimulation, where the infected cells were excited (Fig. 9.1a).

During optogenetic excitation of cells within the ADn, we found that the head movement, or head angular variation, significantly increased (2-tailed paired t-test, $t_{30} = 3.099$, $p < 0.05$; Fig. 9.1g) compared to no optogenetic excitation. Most notably during optogenetic excitation of ADn, mice were covering more of the 360 degree head orientation, such that they passed through all 360 degree orientations consecutively. We categorised this behaviour as a "spin" (Fig. 9.c and d). During optogenetic excitation, the mice demonstrated significantly more spins compared to off period (2-tailed paired t-test, $t_{34} = 3.966$, $p < 0.05$; Fig. 9.1e). These results suggest that the activity of HDcs

within the ADn can drive the HD behaviour of the animal. In other words, artificially changing internal signals of the HD system drives external HD orientation.

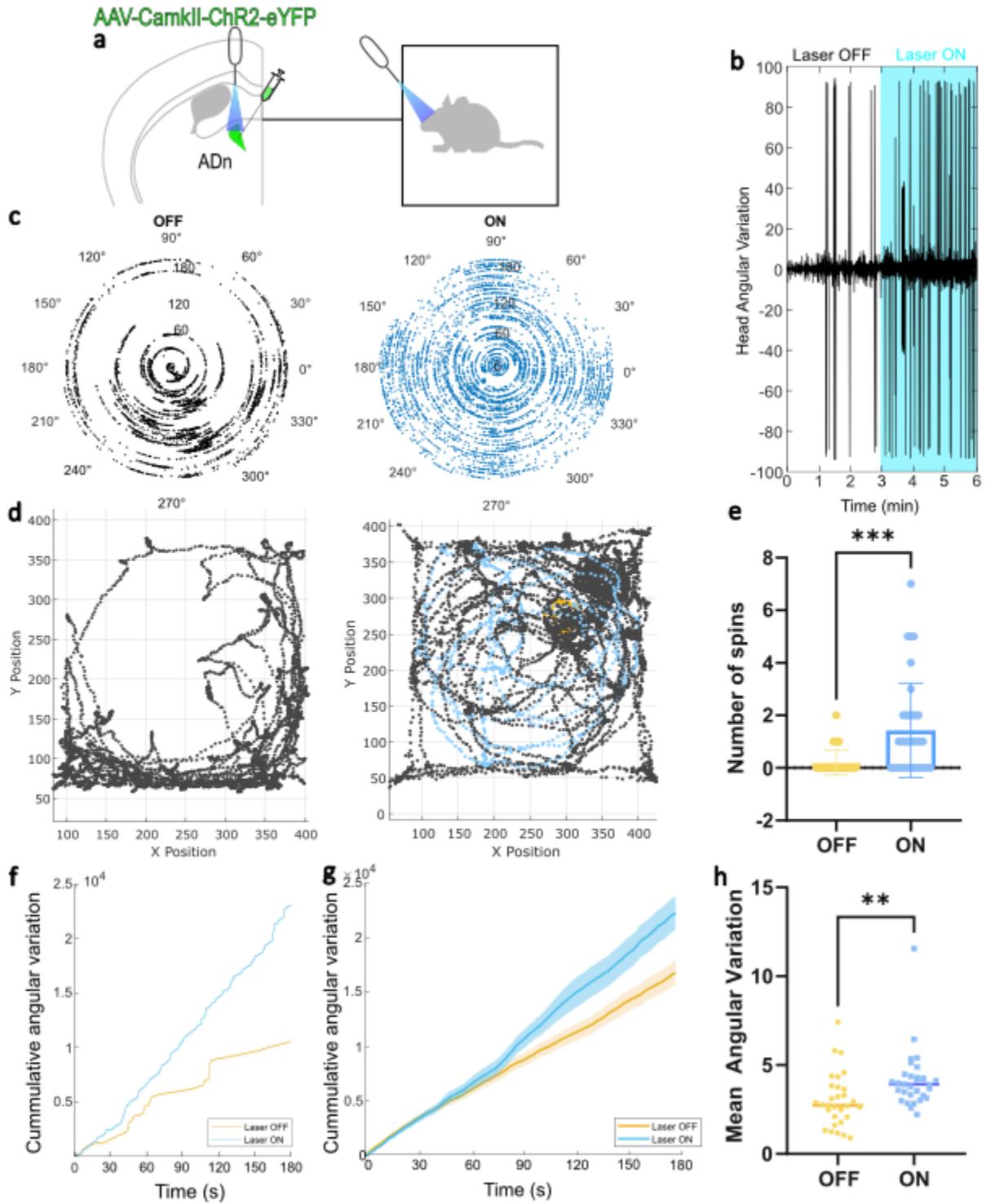


Figure 9.1 *a) Schematic of viral injection and optic fibre implant in the ADn. b) Example of Head Angular Variation trace for one animal. The blue highlighted period indicates laser stimulation. c) Example of polar plot of head direction over time, divided into OFF period (left, black) and ON period (right, blue). d) Example of plot of animal position in open field during OFF period (left) and ON period (right), the coloured lines indicate when the head direction of the mouse passes through 360 degrees between a period of 3 seconds and 20 seconds. e) Summary of number of spins per period across mice. (2-tailed paired t-test; $t_{34} = 3.966$, $p < 0.05$). f) Cumulative angular variation of head direction for the OFF period and ON period for one animal. g) Mean of cumulative angular variation of periods across mice. The shaded area represents the standard error for one animal. h) Mean angular variation per seconds across mice. There is a significant increase in mean angular variation between off and on period (2-tailed paired t-test; $t_{30} = 3.099$, $p < 0.05$).*

9.6.2 Is this effect unique to the ADn?

These striking findings raised a question of whether this effect is unique to the HDCs within ADn or can all HDC containing regions influence HD behaviour to the same extent. To this aim, we chose the RSC. The RSC is a region within the spatial representation network that contains HDCs and place cells. It also has direct downstream connections from the ADn, which allows us to also answer if it is important to activate the ADn HDCs, or if it is sufficient to directly stimulate the downstream cascade. We injected excitatory ChRs, AAV-CamkII-ChR2-eYFP, bilaterally into RSC and placed an optic fiber medial to the ADn. Four weeks after surgery, once the virus

had time to express, we placed mice in the centre of an open field and allowed them to explore for 6 minutes, with minutes 4 to 6 being under laser stimulation, where the infected cells were excited (Fig. 9.2a).

During optogenetic excitation of the RSC, we did not observe the same effect on HD behaviour as we did in the ADn. By this we mean that, there was no significant increase in HD movement, or head angular variation, compared to no stimulation (2-tailed paired t-test; $t_{12} = 0.4672$, *ns*; Fig. 9.2h). The mice pass through 360 degrees consecutively, before or during stimulation. This means that there was no significant difference between the number of spins before and during stimulation (2-tailed paired t-test; $t_9 = 1.000$, *ns*; Fig. 9.2e). These results suggest that the HDCs within the RSC do not drive HD orientation of the animal.

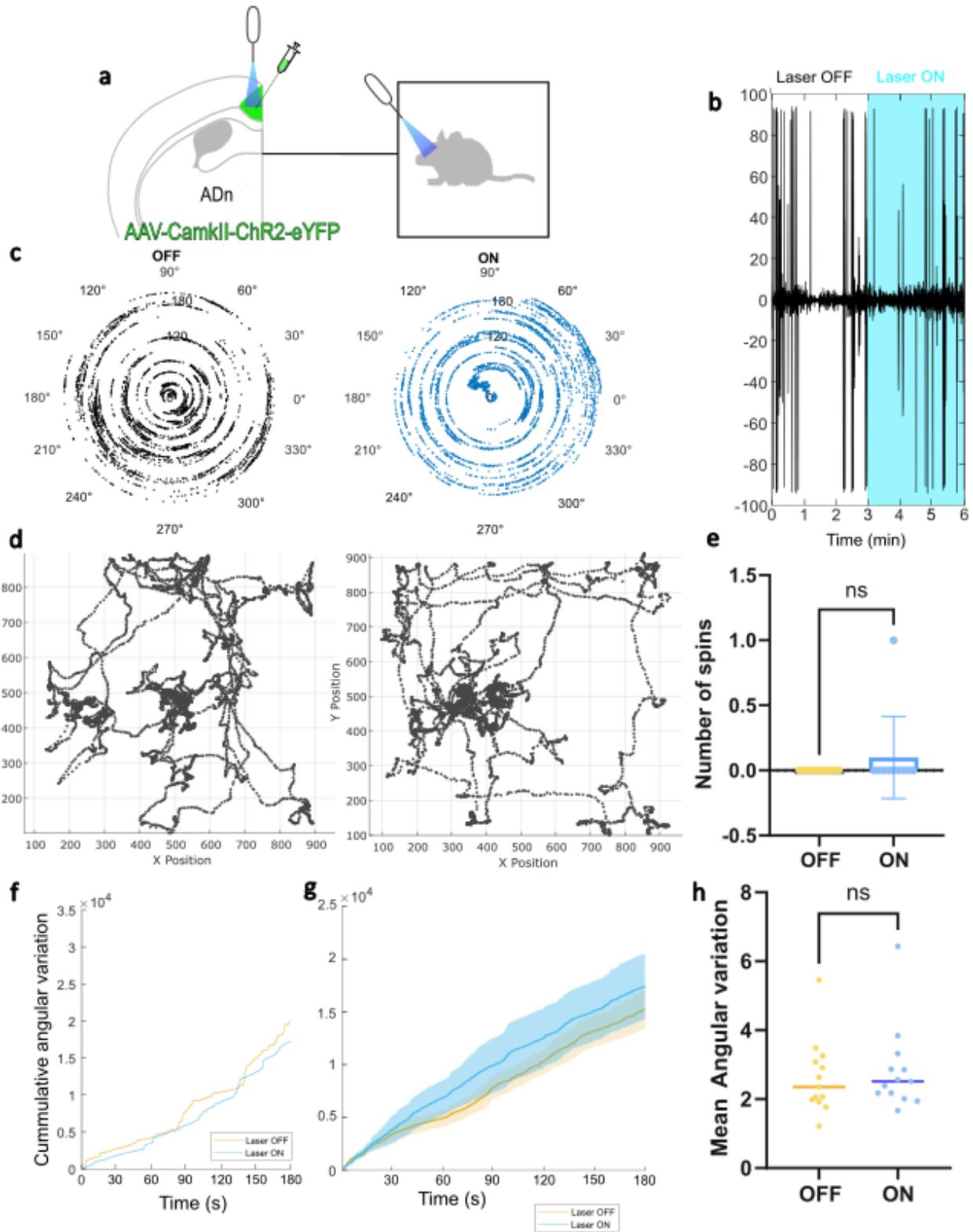


Figure 9.2 **a)** Schema of viral injection and optic fibre implant in the RSC. **b)** Example of Head Angular Variation trace for one animal. The blue highlighted period indicates laser stimulation. **c)** Example of polar plot of head direction over time, divided into OFF period (left, black) and ON period (right, blue). **d)** Plot of animal position in open field during OFF period (left) and ON period (right), the coloured lines indicate when the head direction of the mouse passes through 360 degrees between a period of 3 seconds and 30 seconds. **e)** Summary of number of spins per period for all animals (2-tailed paired t -test, $t_9 = 1.000$, ns) **f)** Cumulative angular variation of head direction for the OFF period and ON period for one animal. **g)** Mean of cumulative angular variation of periods across mice. The shaded area represents the standard error. **h)** Mean angular variation per seconds across mice (2-tailed paired t -test, $t_{12} = 0.4672$, ns) for all mice.

9.7 Discussion

The ring attractor network theory of head direction proposes a computational model to explain the neural mechanisms underlying the representation of head direction in the brain (Boucheny *et al.*, 2005). According to this theory, head direction cells, specialised neurons that fire selectively based on the animal's head orientation (Hulse and Jayaraman, 2020), are organised in a continuous attractor network. This network relies on recurrent connections and inhibitory interactions to sustain a stable bump-like activity pattern, which represents the animal's current head direction (Turner-Evans *et al.*, 2017; Esnaola-Acebes *et al.*, 2021). The connectivity within the network allows for accurate integration of angular velocity inputs, updating the head direction representation, and enabling continuous path integration based solely on self-motion cues (Seelig and

Jayaraman, 2015). Experimental studies, particularly in flies, support the validity of this theory, providing insights into the physiological evidence of ring attractor dynamics in the brain's central regions.

The Ring attractor theory may be an interesting way to explain our results. If we assume that the ring attractor network is within the ADn, then it may explain why the mice produce the spinning behaviour (Fig. 9.1). It would explain the behaviour because the optogenetic stimulation is activating all the cells in the ADn at once within the ring attractor network and therefore to try and compensate with this simultaneous and unnatural activation, the attractor network is cycling through all the HD degree orientations representation. This consecutive cycling through the internal HD degree orientations results in the external HD behaviour mimicking the internal activity.

If this is true, then it raises questions of whether all HD containing regions are organised as attractor networks. The findings from the RSC, where the optogenetic excitation of the RSC did not produce spinning behaviour suggests that not all HD containing regions are organised as attractor networks. However, in the RSC there are other types of cells that may have been activated, as our ChR is not specific for HDCs, it targets all excitatory cells. To be able to answer this question more accurately, we would need to use an excitatory ChR with a HD specific promoter. Nonetheless, our results give a first insight into the different mechanisms that may be in HD regions to process and drive HD behaviour.

9.8 Perspective

The ambitious scope of this project has positioned it as a PhD thesis, with the aim to understand how head direction cells process spatial information to reveal their network organisation. Within the laboratory, an M2 student has taken up the mantle, and started by modelling the attractor network. Additionally, the student used a cutting-edge optogenetic technique, which selectively targets a specific subset of head direction cells, enabling their reactivation at a later point in time. By combining computational modelling with innovative experimental approaches, this project strives to shed new light on the mechanisms behind head direction cell functionality and provide valuable insights into the broader understanding of spatial cognition.

9.8.1 PhD proposal

Understanding how head direction cells process spatial information and revealing their network organisation

This project aims to investigate the function and network organisation of head direction cells (HDc) in the anterodorsal thalamic nucleus (ADn) of mice, which play a crucial role in spatial navigation and encode the animal's spatial orientation. Preliminary results made an intriguing discovery: optogenetic stimulation of the ADn induced a peculiar "spinning" behaviour in mice, suggesting that alterations in HD representation significantly influence behaviour. To understand this phenomenon, the project has two main objectives.

The first objective is to understand how the internal representation of HD in the ADn influences HD behaviour in mice. The researcher plans to use a novel technique called FLiCRE to finely tag specific HDc and then optogenetically reactivate them later to analyse the behavioural response (Kim *et al.*, 2020). Simultaneously, electrophysiological recordings will be performed to study the internal HD representation response to neuronal modulation.

The second objective is to explore whether a ring attractor network, a computational model commonly used to simulate HDc population activity, is implemented in the ADn. The researcher will integrate the optogenetic and electrophysiological data into the established HDc network model and compare its response to the real-time modulation of network activity and animal behaviour.

The project involves various methods, including optogenetic stimulation using FLiCRE, electrophysiological recordings, 3D reconstruction of viral infection sites, and the implementation of a biophysical ring attractor network model. By perturbing the HD representation through optogenetic stimulation and modelling, the researchers aim to gain insights into the structure of the ADn HDc network and its role in behaviour. This research is critical in advancing our understanding of how head direction cells process spatial information and how their network organisation influences spatial navigation in mice.

Bibliography

- Aggleton, J. P., and M. W. Brown. 1999. 'Episodic Memory, Amnesia, and the Hippocampal-Anterior Thalamic Axis'. *The Behavioral and Brain Sciences* 22(3): 425–44; discussion 444-489.
- Aggleton, John P., Agathe Pralus, Andrew J. D. Nelson, and Michael Hornberger. 2016. 'Thalamic Pathology and Memory Loss in Early Alzheimer's Disease: Moving the Focus from the Medial Temporal Lobe to Papez Circuit'. *Brain* 139(7): 1877–90.
- Akers, Katherine, and Paul Frankland. 2009. 'Grading Gradients: Evaluating Evidence for Time-Dependent Memory Reorganization in Experimental Animals'. *Journal of Experimental Neuroscience Neurosciences and Mental Health* 2009.
- Aklan, Iltan et al. 2020. 'NTS Catecholamine Neurons Mediate Hypoglycemic Hunger via Medial Hypothalamic Feeding Pathways'. *Cell metabolism* 31(2): 313-326.e5.
- Althammer, F., and V. Grinevich. 2018. 'Diversity of Oxytocin Neurones: Beyond Magno- and Parvocellular Cell Types?' *Journal of Neuroendocrinology* 30(8): e12549.
- Alvarez FJ, Lafuente H, Rey-Santano MC, Mielgo VE, Gastiasoro E, Rueda M, Pertwee RG, Castillo AI, Romero J, Martínez-Orgado J. 2008. Neuroprotective effects of the nonpsychoactive cannabinoid cannabidiol in hypoxic-ischemic newborn piglets. *Pediatr Res*.
- Anderson DJ, Adolphs R. 2014. A framework for studying emotions across species. *Cell*.
- Angelaki, Dora E., and Kathleen E. Cullen. 2008. 'Vestibular System: The Many Facets of a Multimodal Sense'. *Annual Review of Neuroscience* 31: 125–50.

- Baddeley, A. D. 1966. 'Short-Term Memory for Word Sequences as a Function of Acoustic, Semantic and Formal Similarity'. *The Quarterly Journal of Experimental Psychology* 18(4): 362–65.
- Baddeley, A. D., & Warrington, E. K. (1970). Amnesia and the distinction between long- and short-term memory. *Journal of Verbal Learning & Verbal Behavior*, 9(2), 176–189.
- Baker, Stevenson et al. 2016. 'The Human Dentate Gyrus Plays a Necessary Role in Discriminating New Memories'. *Current biology: CB* 26(19): 2629–34.
- Bayley, P.J., O'Reily, R.C., Curran, T., Squire, L.R. 2008. New semantic learning in patients with large medial temporal lobe lesions. *Hippocampus*. Vol. 18, 6, p. 575-583
- Beier, Kevin T. et al. 2015. 'Circuit Architecture of VTA Dopamine Neurons Revealed by Systematic Input-Output Mapping'. *Cell* 162(3): 622–34.
- Ben-Yakov A, Rubinson M, Dudai Y. 2014. Shifting gears in hippocampus: Temporal dissociation between familiarity and novelty signatures in a single event. *J Neurosci* 34: 12973–12981.
- Berndt, Andre et al. 2016. 'Structural Foundations of Optogenetics: Determinants of Channelrhodopsin Ion Selectivity'. *Proceedings of the National Academy of Sciences* 113(4): 822–29.
- Bian, Xin-Lan et al. 2019. 'Anterior Cingulate Cortex to Ventral Hippocampus Circuit Mediates Contextual Fear Generalization'. *Journal of Neuroscience* 39(29): 5728–39.
- Blair, Hugh T., Jeiwon Cho, and Patricia E. Sharp. 1999. 'The Anterior Thalamic

- Head-Direction Signal Is Abolished by Bilateral But Not Unilateral Lesions of the Lateral Mammillary Nucleus'. *The Journal of Neuroscience* 19(15): 6673–83.
- Blair, Hugh T., and Patricia E. Sharp. 1995. 'Anticipatory Head Direction Signals in Anterior Thalamus: Evidence for a Thalamocortical Circuit That Integrates Angular Head Motion to Compute Head Direction'. *The Journal of Neuroscience* 15(9): 6260–70.
- Blanchard, R. J., & Blanchard, D. C. 1969. Crouching as an index of fear. *Journal of Comparative and Physiological Psychology*, 67(3), 370–375.
- Bontempi, B., C. Laurent-Demir, C. Destrade, and R. Jaffard. 1999. 'Time-Dependent Reorganization of Brain Circuitry Underlying Long-Term Memory Storage'. *Nature* 400(6745): 671–75.
- Bosshardt S, Schmidt CF, Jaermann T, Degonda N, Boesiger P, Nitsch RM, Hock C, Henke K. Effects of memory consolidation on human hippocampal activity during retrieval. *Cortex*. 2005 Aug;41(4):486-98.
- Boucheny, Christian, Nicolas Brunel, and Angelo Arleo. 2005. 'A Continuous Attractor Network Model without Recurrent Excitation: Maintenance and Integration in the Head Direction Cell System'. *Journal of Computational Neuroscience* 18(2): 205–27.
- Bowman, Caitlin R., and Dagmar Zeithamova. 2018. 'Abstract Memory Representations in the Ventromedial Prefrontal Cortex and Hippocampus Support Concept Generalization'. *Journal of Neuroscience* 38(10): 2605–14.
- Brod, Svenja et al. 2023. 'Memory Systems Integration in Sleep Complements Rapid Systems Consolidation in Wakefulness'. : 2023.03.09.531360.

- <https://www.biorxiv.org/content/10.1101/2023.03.09.531360v1> (July 22, 2023).
- Bruno, Carissa A. et al. 2021. 'pMAT: An Open-Source Software Suite for the Analysis of Fiber Photometry Data'. *Pharmacology, Biochemistry, and Behavior* 201: 173093.
- Buzsáki, G. 1996. 'The Hippocampo-Neocortical Dialogue'. *Cerebral Cortex (New York, N.Y.: 1991)* 6(2): 81–92.
- Calton, Jeffrey L. et al. 2003. 'Hippocampal Place Cell Instability after Lesions of the Head Direction Cell Network'. *Journal of Neuroscience* 23(30): 9719–31.
- Carlesimo, Giovanni Augusto, Maria Giovanna Lombardi, and Carlo Caltagirone. 2011. 'Vascular Thalamic Amnesia: A Reappraisal'. *Neuropsychologia* 49(5): 777–89.
- Cassel, Jean-Christophe et al. 2013. 'The Reuniens and Rhomboid Nuclei: Neuroanatomy, Electrophysiological Characteristics and Behavioral Implications'. *Progress in Neurobiology* 111: 34–52.
- Carr MF, Karlsson MP, Frank LM. 2012. Transient slow gamma synchrony underlies hippocampal memory replay. *Neuron*. 75(4):700-13
- Cherubini E, Miles R. 2015. The CA3 region of the hippocampus: how is it? What is it for? How does it do it? *Front Cell Neuroscience*.
- Christenson Wick, Zoé, and Esther Krook-Magnuson. 2018. 'Specificity, Versatility, and Continual Development: The Power of Optogenetics for Epilepsy Research'. *Frontiers in Cellular Neuroscience* 12: 151.
- Clark, Benjamin J., Joshua P. Bassett, Sarah S. Wang, and Jeffrey S. Taube. 2010. 'Impaired Head Direction Cell Representation in the Anterodorsal Thalamus after Lesions of the Retrosplenial Cortex'. *Journal of Neuroscience* 30(15):

5289–5302.

- Clark, Benjamin J., Joel E. Brown, and Jeffrey S. Taube. 2012. 'Head Direction Cell Activity in the Anterodorsal Thalamus Requires Intact Supragenual Nuclei'. *Journal of Neurophysiology* 108(10): 2767–84.
- Clark, Benjamin J., Asha Sarma, and Jeffrey S. Taube. 2009. 'Head Direction Cell Instability in the Anterior Dorsal Thalamus after Lesions of the Interpeduncular Nucleus'. *Journal of Neuroscience* 29(2): 493–507.
- Clark, Benjamin J., and Jeffrey S. Taube. 2011. 'Intact Landmark Control and Angular Path Integration by Head Direction Cells in the Anterodorsal Thalamus after Lesions of the Medial Entorhinal Cortex'. *Hippocampus* 21(7): 767–82.
- Colley PA, Sheu FS, Routtenberg A. 1990. Inhibition of protein kinase C blocks two components of LTP persistence, leaving initial potentiation intact. *J Neurosci.*
- Contreras, D., and M. Steriade. 1995. 'Cellular Basis of EEG Slow Rhythms: A Study of Dynamic Corticothalamic Relationships'. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 15(1 Pt 2): 604–22.
- Courtin, Julien et al. 2014. 'Prefrontal Parvalbumin Interneurons Shape Neuronal Activity to Drive Fear Expression'. *Nature* 505(7481): 92–96.
- Derkach VA, Oh MC, Guire ES, Soderling TR. Regulatory mechanisms of AMPA receptors in synaptic plasticity. *Nat Rev Neurosci.* 2007 Feb;8(2):101-13.
- Debiec, Jacek, Joseph E. LeDoux, and Karim Nader. 2002. 'Cellular and Systems Reconsolidation in the Hippocampus'. *Neuron* 36(3): 527–38.
- De Franceschi, Gioia, Tipok Vivattanasarn, Aman B. Saleem, and Samuel G. Solomon. 2016. 'Vision Guides Selection of Freeze or Flight Defense Strategies in Mice'.

- Current Biology* 26(16): 2150–54.
- Dejean, Cyril et al. 2016. 'Prefrontal Neuronal Assemblies Temporally Control Fear Behaviour'. *Nature* 535(7612): 420–24.
- Dillingham, Christopher M., Aura Frizzati, Andrew J.D. Nelson, and Seralynne D. Vann. 2015. 'How Do Mammillary Body Inputs Contribute to Anterior Thalamic Function?' *Neuroscience and Biobehavioral Reviews* 54: 108–19.
- Doron, Adi, and Inbal Goshen. 2018. 'Investigating the Transition from Recent to Remote Memory Using Advanced Tools'. *Brain Research Bulletin* 141: 35–43.
- Dudai, Yadin, and Richard G. M. Morris. 2013. 'Memorable Trends'. *Neuron* 80(3): 742–50.
- Dudai, Yadin, and Richard G.M. Morris. 2000. 'To Consolidate or Not to Consolidate: What Are the Questions?' In *Brain, Perception, Memory: Advances in Cognitive Neuroscience*, ed. Johan J. Bolhuis. Oxford University Press, 0.
- Eichenbaum, Howard. 2014. 'Time Cells in the Hippocampus: A New Dimension for Mapping Memories'. *Nature Reviews Neuroscience* 15(11): 732–44.
- Esnaola-Acebes, Jose M., Alex Roxin, and Klaus Wimmer. 2021. 'Bump Attractor Dynamics Underlying Stimulus Integration in Perceptual Estimation Tasks'. : 2021.03.15.434192.
- Fama, Rosemary, and Edith V. Sullivan. 2015. 'Thalamic Structures and Associated Cognitive Functions: Relations with Age and Aging'. *Neuroscience and Biobehavioral Reviews* 54: 29–37.
- Fanselow MS. 2018. The Role of Learning in Threat Imminence and Defensive Behaviors. *Curr Opin Behav Sci*.

- Fanselow MS, Hoffman AN, Zhuravka I. 2019. Timing and the transition between modes in the defensive behavior system. *Behav Processes*.
- Ferrara, Nicole C. et al. 2022. 'Developmental Differences in Amygdala Projection Neuron Activation Associated with Isolation-Driven Changes in Social Preference'. *Frontiers in Behavioral Neuroscience* 16.
<https://www.frontiersin.org/articles/10.3389/fnbeh.2022.956102> (July 23, 2023).
- Foerde, K., Race, E., Verfaellie, M., Shohamy, D. 2013. A Role for the Medial Temporal Lobe in Feedback-Driven Learning: Evidence from Amnesia. *Journal of Neuroscience*. 33 (13) 5698-5704.
- Formozov, Andrey, Alexander Dieter, and J. Simon Wiegert. 2023. 'A Flexible and Versatile System for Multi-Color Fiber Photometry and Optogenetic Manipulation'. *Cell Reports Methods* 3(3): 100418.
- Frankland, Paul W. et al. 2004. 'The Involvement of the Anterior Cingulate Cortex in Remote Contextual Fear Memory'. *Science (New York, N.Y.)* 304(5672): 881–83.
- Frankland, Paul W., and Bruno Bontempi. 2005. 'The Organization of Recent and Remote Memories'. *Nature Reviews. Neuroscience* 6(2): 119–30.
- Frey U, Morris RG. 1997. Synaptic tagging and long-term potentiation. *Nature*. 6;385(6616):533-6.
- Giustino, Thomas F., and Stephen Maren. 2015. 'The Role of the Medial Prefrontal Cortex in the Conditioning and Extinction of Fear'. *Frontiers in Behavioral Neuroscience* 9: 298.
- Goodridge, J. P., and J. S. Taube. 1997. 'Interaction between the Postsubiculum and Anterior Thalamus in the Generation of Head Direction Cell Activity'. *The Journal*

- of Neuroscience: The Official Journal of the Society for Neuroscience* 17(23): 9315–30.
- Goshen, Inbal et al. 2011. 'Dynamics of Retrieval Strategies for Remote Memories'. *Cell* 147(3): 678–89.
- Govorunova, Elena G. et al. 2015. 'Natural Light-Gated Anion Channels: A Family of Microbial Rhodopsins for Advanced Optogenetics'. *Science* 349(6248): 647–50.
- Gross CT, Canteras NS. 2012. The many paths to fear. *Nat Rev Neurosci*.
- Gunaydin, Lisa A. et al. 2014. 'Natural Neural Projection Dynamics Underlying Social Behavior'. *Cell* 157(7): 1535–51.
- Guzowski JF, McGaugh JL. 1997. Antisense oligodeoxynucleotide-mediated disruption of hippocampal cAMP response element binding protein levels impairs consolidation of memory for water maze training. *Proc Natl Acad Sci U S A*. 18;94(6):2693-8.
- Hafting, Torkel et al. 2005. 'Microstructure of a Spatial Map in the Entorhinal Cortex'. *Nature* 436(7052): 801–6.
- Herry, Cyril et al. 2008. 'Switching on and off Fear by Distinct Neuronal Circuits'. *Nature* 454(7204): 600–606.
- Hok, Vincent et al. 2007. 'Goal-Related Activity in Hippocampal Place Cells'. *Journal of Neuroscience* 27(3): 472–82.
- Hulse, Brad K., and Vivek Jayaraman. 2020. 'Mechanisms Underlying the Neural Computation of Head Direction'. *Annual Review of Neuroscience* 43(1): 31–54.
- Hung, Lin W. et al. 2017. 'Gating of Social Reward by Oxytocin in the Ventral Tegmental Area'. *Science* 357(6358): 1406–11.

- Janak PH, Tye KM. 2015. From circuits to behaviour in the amygdala. *Nature*.
- Jankowski, Maciej M. et al. 2013. 'The Anterior Thalamus Provides a Subcortical Circuit Supporting Memory and Spatial Navigation'. *Frontiers in Systems Neuroscience* 7: 45.
- Jercog, Daniel et al. 2021. 'Dynamical Prefrontal Population Coding during Defensive Behaviours'. *Nature* 595(7869): 690–94.
- Josselyn, Sheena A., and Susumu Tonegawa. 2020. 'Memory Engrams: Recalling the Past and Imagining the Future'. *Science* 367(6473): eaaw4325.
- Kim, Christina K. et al. 2020. 'A Molecular Calcium Integrator Reveals a Striatal Cell Type Driving Aversion'. *Cell* 183(7): 2003-2019.e16.
- Kim, Jeansok J., and Michael S. Fanselow. 1992. 'Modality-Specific Retrograde Amnesia of Fear'. *Science* 256(5057): 675–77.
- Kim, Sung Soo, Hervé Rouault, Shaul Druckmann, and Vivek Jayaraman. 2017. 'Ring Attractor Dynamics in the Drosophila Central Brain'. *Science* 356(6340): 849–53.
- Kitamura, Takashi et al. 2017. 'Engrams and Circuits Crucial for Systems Consolidation of a Memory'. *Science* 356(6333): 73–78.
- Kjelstrup, Kirsten G. et al. 2002. 'Reduced Fear Expression after Lesions of the Ventral Hippocampus'. *Proceedings of the National Academy of Sciences of the United States of America* 99(16): 10825–30.
- Lamprecht, R., LeDoux, J. Structural plasticity and memory. *Nat Rev Neurosci* 5, 45–54 (2004).
- Lammel, Stephan et al. 2012. 'Input-Specific Control of Reward and Aversion in the Ventral Tegmental Area'. *Nature* 491(7423): 212–17.

- Lammel, Stephan, Daniela I. Ion, Jochen Roeper, and Robert C. Malenka. 2011. 'Projection-Specific Modulation of Dopamine Neuron Synapses by Aversive and Rewarding Stimuli'. *Neuron* 70(5): 855–62.
- Lammel S, Lim BK, Ran C, Huang KW, Betley MJ, Tye KM, Deisseroth K, Malenka RC. 2012. Input-specific control of reward and aversion in the ventral tegmental area. *Nature*.
- Lara-Vásquez, Ariel et al. 2016. 'Midline Thalamic Neurons Are Differentially Engaged during Hippocampus Network Oscillations'. *Scientific Reports* 6(1): 29807.
- Latchoumane, Charles-Francois V., Hong-Viet V. Ngo, Jan Born, and Hee-Sup Shin. 2017. 'Thalamic Spindles Promote Memory Formation during Sleep through Triple Phase-Locking of Cortical, Thalamic, and Hippocampal Rhythms'. *Neuron* 95(2): 424-435.e6.
- Ledderose, Julia MT et al. 2023. 'Layer 1 of Somatosensory Cortex: An Important Site for Input to a Tiny Cortical Compartment'. : 2021.11.26.469979. <https://www.biorxiv.org/content/10.1101/2021.11.26.469979v4> (July 23, 2023).
- Lee H, GoodSmith D, Knierim JJ. 2020. Parallel processing streams in the hippocampus. *Curr Opin Neurobiology*. 64:127–134.
- Lee, Jeong-Yun et al. 2022. 'Role of Anterior Cingulate Cortex Inputs to Periaqueductal Gray for Pain Avoidance'. *Current Biology* 32(13): 2834-2847.e5.
- Legaria, Alex A. et al. 2021. 'Striatal Fiber Photometry Reflects Primarily Non-Somatic Activity'. : 2021.01.20.427525. <https://www.biorxiv.org/content/10.1101/2021.01.20.427525v2> (July 23, 2023).
- Li, Sa, and Gilbert J. Kirouac. 2008. 'Projections from the Paraventricular Nucleus of the

- Thalamus to the Forebrain, with Special Emphasis on the Extended Amygdala'. *The Journal of Comparative Neurology* 506(2): 263–87.
- Li, Xiao Bai, Takeshi Inoue, Shin Nakagawa, and Tsukasa Koyama. 2004. 'Effect of Mediodorsal Thalamic Nucleus Lesion on Contextual Fear Conditioning in Rats'. *Brain Research* 1008(2): 261–72.
- Li, Yonghui, Xinwen Dong, Sa Li, and Gilbert J. Kirouac. 2014. 'Lesions of the Posterior Paraventricular Nucleus of the Thalamus Attenuate Fear Expression'. *Frontiers in Behavioral Neuroscience* 8.
<http://journal.frontiersin.org/article/10.3389/fnbeh.2014.00094/abstract> (July 20, 2023).
- Liang, Zhifeng, Yuncong Ma, Glenn D.R. Watson, and Nanyin Zhang. 2017. 'Simultaneous GCaMP6-Based Fiber Photometry and fMRI in Rats'. *Journal of neuroscience methods* 289: 31–38.
- Liu, Xu et al. 2012. 'Optogenetic Stimulation of a Hippocampal Engram Activates Fear Memory Recall'. *Nature* 484(7394): 381–85.
- Lopez, Joëlle, Karine Gamache, Carmelo Milo, and K. Nader. 2017. 'Differential Role of the Anterior and Intralaminar/Lateral Thalamic Nuclei in Systems Consolidation and Reconsolidation'. *Brain Structure and Function* 223.
<https://consensus.app/details/thalamic-play-roles-memory-processes-nuclei-involved-lopez/c2f3263763df5676afe5de83f98fbe9d/> (July 20, 2023).
- Lux, V., Atucha, E., Kitsukawa, T., Sauvage, M., M. 2016. Imaging a memory trace over half a life-time in the medial temporal lobe reveals a time-limited role of CA3 neurons in retrieval. *eLife* 5:e11862.

- MacDonald, Christopher J., Kyle Q. Lepage, Uri T. Eden, and Howard Eichenbaum. 2011. 'Hippocampal "Time Cells" Bridge the Gap in Memory for Discontiguous Events'. *Neuron* 71(4): 737–49.
- Maguire, Eleanor A., Henson, Richard N., Mummery, Catherine J., Firth, Christopher D. 2001. Activity in prefrontal cortex, not hippocampus, varies parametrically with the increasing remoteness of memories. *Cognitive Neuroscience and Neuropsychology*.
- Mak-McCully, Rachel A. et al. 2017. 'Coordination of Cortical and Thalamic Activity during Non-REM Sleep in Humans'. *Nature Communications* 8(1): 15499.
- Maren, Stephen. 2011. 'Seeking a Spotless Mind: Extinction, Deconsolidation, and Erasure of Fear Memory'. *Neuron* 70(5): 830–45.
- Marenco, Stefano et al. 2012. 'Investigation of Anatomical Thalamo-Cortical Connectivity and fMRI Activation in Schizophrenia'. *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology* 37(2): 499–507.
- Marr, D. 1970. A Theory for Cerebral Neocortex. *Proceedings of the Royal Society of London. Series B, Biological Science*. Vol, 176, No. 1043, pp 161-236
- Martin SJ, Grimwood PD, Morris RG. 2000. Synaptic plasticity and memory: an evaluation of the hypothesis. *Annu Rev Neurosci*.23:649-711
- Matsuzaki, Masanori, Naoki Honkura, Graham C. R. Ellis-Davies, and Haruo Kasai. 2004. 'Structural Basis of Long-Term Potentiation in Single Dendritic Spines'. *Nature* 429(6993): 761–66.
- Maviel, Thibault, Thomas P. Durkin, Frédérique Menzaghi, and Bruno Bontempi. 2004.

- 'Sites of Neocortical Reorganization Critical for Remote Spatial Memory'. *Science (New York, N.Y.)* 305(5680): 96–99.
- McCarthy, R. A., & Warrington, E. K. (1988). Evidence for modality-specific meaning systems in the brain. *Nature*, 334(6181), 428–430.
- McClelland, James L., Bruce L. McNaughton, and Randall C. O'Reilly. 1995. 'Why There Are Complementary Learning Systems in the Hippocampus and Neocortex: Insights from the Successes and Failures of Connectionist Models of Learning and Memory'. *Psychological Review* 102(3): 419–57.
- McNish, K. A., J. C. Gewirtz, and M. Davis. 1997. 'Evidence of Contextual Fear after Lesions of the Hippocampus: A Disruption of Freezing but Not Fear-Potentiated Startle'. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 17(23): 9353–60.
- Meeter, M., & Murre, J. M. J. (2004). Consolidation of Long-Term Memory: Evidence and Alternatives. *Psychological Bulletin*, 130(6), 843–857.
- Migues PV, Hardt O, Wu DC, Gamache K, Sacktor TC, Wang YT, Nader K. 2010. PKMzeta. maintains memories by regulating GluR2-dependent AMPA receptor trafficking. *Nat Neurosci*.
- Miller, Jonathan F. et al. 2013. 'Neural Activity in Human Hippocampal Formation Reveals the Spatial Context of Retrieved Memories'. *Science (New York, N.Y.)* 342(6162): 1111–14.
- Mitchell, Anna S. et al. 2014. 'Advances in Understanding Mechanisms of Thalamic Relays in Cognition and Behavior'. *The Journal of Neuroscience* 34(46): 15340–46.

- Mitchell, Anna S., and Subhojit Chakraborty. 2013. 'What Does the Mediodorsal Thalamus Do?' *Frontiers in Systems Neuroscience* 7: 37.
- Mochizuki-Kawai, Hiroko. 2008. '[Neural basis of procedural memory]'. *Brain and nerve = Shinkei kenkyu no shinpo* 60(7): 825–32.
- Morales, Santiago et al. 2019. 'An fMRI Study of Action Observation and Action Execution in Childhood'. *Developmental Cognitive Neuroscience* 37: 100655.
- Moscarello, Justin M. 2020. 'Prefrontal Cortex Projections to the Nucleus Reunions Suppress Freezing Following Two-Way Signaled Avoidance Training'. *Learning & Memory* 27(3): 119–23.
- Moscovitch, Morris., Nadel, Lynn. 1998. Consolidation and the hippocampal complex revisited: in defense of the multiple-trace model. *Current Opinion in Neurobiology*, Volume 8, Issue 2. P 297-300
- Moser, Edvard I., Emilio Kropff, and May-Britt Moser. 2008. 'Place Cells, Grid Cells, and the Brain's Spatial Representation System'. *Annual Review of Neuroscience* 31(1): 69–89.
- Moser, May-Britt, David C. Rowland, and Edvard I. Moser. 2015. 'Place Cells, Grid Cells, and Memory'. *Cold Spring Harbor Perspectives in Biology* 7(2): a021808.
- Müller, GE and Pilzecker. 1900. Experimentelle Beiträge zur Lehre vom Gedächtniss. *Zeitschrift für Psychologie. Ergänzungsband*. 1:1–300
- Nadel L, Moscovitch M. Memory consolidation, retrograde amnesia and the hippocampal complex. *Current Opinion Neurobiology*. 1997 Apr;7(2):217-27.
- Nader, Karim, and Einar Orn Einarsson. 2010. 'Memory Reconsolidation: An Update'. *Annals of the New York Academy of Sciences* 1191: 27–41.

- O'Keefe, J., and J. Dostrovsky. 1971. 'The Hippocampus as a Spatial Map. Preliminary Evidence from Unit Activity in the Freely-Moving Rat'. *Brain Research* 34(1): 171–75.
- Olthof, Bas M. J., Adrian Rees, and Sarah E. Gartside. 2019. 'Multiple Nonauditory Cortical Regions Innervate the Auditory Midbrain'. *Journal of Neuroscience* 39(45): 8916–28.
- O'Reilly, R. C., and J. L. McClelland. 1994. 'Hippocampal Conjunctive Encoding, Storage, and Recall: Avoiding a Trade-Off'. *Hippocampus* 4(6): 661–82.
- Pan, Simon et al. 2020. 'Preservation of a Remote Fear Memory Requires New Myelin Formation'. *Nature Neuroscience* 23(4): 487–99.
- Pape, Hans-Christian, and Denis Pare. 2010. 'Plastic Synaptic Networks of the Amygdala for the Acquisition, Expression, and Extinction of Conditioned Fear'. *Physiological Reviews* 90(2): 419–63.
- Park, Kwanghoon, and ChiHye Chung. 2019. 'Systemic Cellular Activation Mapping of an Extinction-Impaired Animal Model'. *Frontiers in Cellular Neuroscience* 13. <https://www.frontiersin.org/articles/10.3389/fncel.2019.00099> (July 23, 2023).
- Patel, Amisha A., Niall McAlinden, Keith Mathieson, and Shuzo Sakata. 2020. 'Simultaneous Electrophysiology and Fiber Photometry in Freely Behaving Mice'. *Frontiers in Neuroscience* 14: 148.
- Petrucchio, Luigi et al. 2023. 'Neural Dynamics and Architecture of the Heading Direction Circuit in Zebrafish'. *Nature Neuroscience* 26(5): 765–73.
- Peyrache, Adrien, Marie M. Lacroix, Peter C. Petersen, and György Buzsáki. 2015. 'Internally Organized Mechanisms of the Head Direction Sense'. *Nature*

- Neuroscience* 18(4): 569–75.
- Pfeiffer, Brad E., and David J. Foster. 2013. 'Hippocampal Place-Cell Sequences Depict Future Paths to Remembered Goals'. *Nature* 497(7447): 74–79.
- Phillips, R. G., and J. E. LeDoux. 1992. 'Differential Contribution of Amygdala and Hippocampus to Cued and Contextual Fear Conditioning'. *Behavioral Neuroscience* 106(2): 274–85.
- Quet, Etienne et al. 2020. 'The Reuniens and Rhomboid Nuclei Are Necessary for Contextual Fear Memory Persistence in Rats'. *Brain Structure and Function* 225(3): 955–68.
- Restivo, Leonardo, Gisella Vetere, Bruno Bontempi, and Martine Ammassari-Teule. 2009. 'The Formation of Recent and Remote Memory Is Associated with Time-Dependent Formation of Dendritic Spines in the Hippocampus and Anterior Cingulate Cortex'. *Journal of Neuroscience* 29(25): 8206–14.
- Rolls, Edmund T. 2023. 'Emotion, Motivation, Decision-Making, the Orbitofrontal Cortex, Anterior Cingulate Cortex, and the Amygdala'. *Brain Structure & Function* 228(5): 1201–57.
- Saalmann, Yuri B., and Sabine Kastner. 2015. 'The Cognitive Thalamus'. *Frontiers in Systems Neuroscience* 9.
<https://www.frontiersin.org/articles/10.3389/fnsys.2015.00039> (July 22, 2023).
- Schafe GE, Nadel NV, Sullivan GM, Harris A, LeDoux JE. 1999. Memory consolidation for contextual and auditory fear conditioning is dependent on protein synthesis, PKA, and MAP kinase. *Learning Memory*. 6:97–110.
- Schlecht, Maximilian, Maanasa Jayachandran, Gabriela E. Rasch, and Timothy A.

- Allen. 2022. 'Dual Projecting Cells Linking Thalamic and Cortical Communication Routes between the Medial Prefrontal Cortex and Hippocampus'. *Neurobiology of Learning and Memory* 188: 107586.
- Seelig, Johannes D., and Vivek Jayaraman. 2015. 'Neural Dynamics for Landmark Orientation and Angular Path Integration'. *Nature* 521(7551): 186–91.
- Shibata, Hideshi. 1993. 'Direct Projections from the Anterior Thalamic Nuclei to the Retrohippocampal Region in the Rat'. *Journal of Comparative Neurology* 337(3): 431–45.
- Shpokayte, M., McKissick, O., Guan, X. et al. 2002. Hippocampal cells segregate positive and negative engrams. *Commun Biol* 5, 1009
- Silva, Bianca A. et al. 2021. 'A Thalamo-Amygdalar Circuit Underlying the Extinction of Remote Fear Memories'. *Nature Neuroscience* 24(7): 964–74.
- Silva, Bianca A., and Johannes Gräff. 2023. 'Face Your Fears: Attenuating Remote Fear Memories by Reconsolidation-Updating'. *Trends in Cognitive Sciences* 27(4): 404–16.
- Singh, Sanjay, and Lisa Topolnik. 2023. 'Inhibitory Circuits in Fear Memory and Fear-Related Disorders'. *Frontiers in Neural Circuits* 17.
<https://www.frontiersin.org/articles/10.3389/fncir.2023.1122314> (July 17, 2023).
- Slézia, Andrea, Balázs Hangya, István Ulbert, and László Acsády. 2011. 'Phase Advancement and Nucleus-Specific Timing of Thalamocortical Activity during Slow Cortical Oscillation'. *Journal of Neuroscience* 31(2): 607–17.
- Solstad, Trygve, Edvard I. Moser, and Gaute T. Einevoll. 2006. 'From Grid Cells to Place Cells: A Mathematical Model'. *Hippocampus* 16(12): 1026–31.

- Sun Q, Sotayo A, Cazzulino AS, Snyder AM, Denny CA, Siegelbaum SA. 2017. Proximodistal Heterogeneity of Hippocampal CA3 Pyramidal Neuron Intrinsic Properties, Connectivity, and Reactivation during Memory Recall. *Neuron*.
- Squire, L. R., and P. Alvarez. 1995. 'Retrograde Amnesia and Memory Consolidation: A Neurobiological Perspective'. *Current Opinion in Neurobiology* 5(2): 169–77.
- Squire, Larry R., Lisa Genzel, John T. Wixted, and Richard G. Morris. 2015. 'Memory Consolidation'. *Cold Spring Harbor Perspectives in Biology* 7(8): a021766.
- Squire, Larry R., and John T. Wixted. 2011. 'The Cognitive Neuroscience of Human Memory Since H.M.'. *Annual review of neuroscience* 34: 259–88.
- Stackman, R. W., and J. S. Taube. 1998. 'Firing Properties of Rat Lateral Mammillary Single Units: Head Direction, Head Pitch, and Angular Head Velocity'. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 18(21): 9020–37.
- Steenland, Hendrik W., Xiang-Yao Li, and Min Zhuo. 2012. 'Predicting Aversive Events and Terminating Fear in the Mouse Anterior Cingulate Cortex during Trace Fear Conditioning'. *Journal of Neuroscience* 32(3): 1082–95.
- Takashima A, Petersson KM, Rutters F, Tendolkar I, Jensen O, Zwarts MJ, McNoughton BL, Fernández G. 2006. Declarative memory consolidation in humans: a prospective functional magnetic resonance imaging study. *Proc Natl Acad Sci U S A* 103(3):756-61.
- Takashima A, Nieuwenhuis IL, Jensen O, Talamini LM, Rijpkema M, Fernández G. 2009. Shift from hippocampal to neocortical centered retrieval network with consolidation. *J Neurosci*. 29(32):10087-93.

- Takehara-Nishiuchi, Kaori, and Bruce L. McNaughton. 2008. 'Spontaneous Changes of Neocortical Code for Associative Memory during Consolidation'. *Science (New York, N.Y.)* 322(5903): 960–63.
- Taube, J. S. 1995. 'Head Direction Cells Recorded in the Anterior Thalamic Nuclei of Freely Moving Rats'. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 15(1 Pt 1): 70–86.
- Taube, J. S., R. U. Muller, and J. B. Ranck. 1990. 'Head-Direction Cells Recorded from the Postsubiculum in Freely Moving Rats. II. Effects of Environmental Manipulations'. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 10(2): 436–47.
- Taube, Jeffrey S. 2007. 'The Head Direction Signal: Origins and Sensory-Motor Integration'. *Annual Review of Neuroscience* 30: 181–207.
- Taylor, Kaycie K., Kazumasa Z. Tanaka, Leon G. Reijmers, and Brian J. Wiltgen. 2013. 'Reactivation of Neural Ensembles during the Retrieval of Recent and Remote Memory'. *Current biology: CB* 23(2): 99–106.
- Teixeira, Cátia M. et al. 2006. 'Involvement of the Anterior Cingulate Cortex in the Expression of Remote Spatial Memory'. *Journal of Neuroscience* 26(29): 7555–64.
- Teng E, Squire LR. Memory for places learned long ago is intact after hippocampal damage. *Nature*. 1999 Aug 12;400(6745):675-7.
- Tomé, Douglas Feitosa et al. 2022. 'Dynamic and Selective Engrams Emerge with Memory Consolidation'. : 2022.03.13.484167.
<https://www.biorxiv.org/content/10.1101/2022.03.13.484167v1> (June 26, 2023).

- Tonegawa, Susumu, Xu Liu, Steve Ramirez, and Roger Redondo. 2015. 'Memory Engram Cells Have Come of Age'. *Neuron* 87(5): 918–31.
- Tucker, Kyle, Savitha Sridharan, Hillel Adesnik, and Stephen G. Brohawn. 2022. 'Cryo-EM Structures of the Channelrhodopsin ChRmine in Lipid Nanodiscs'. *Nature Communications* 13(1): 4842.
- Turner-Evans, Daniel et al. 2017. 'Angular Velocity Integration in a Fly Heading Circuit' ed. Alexander Borst. *eLife* 6: e23496.
- Van der Werf, Y. D., M. P. Witter, H. B. Uylings, and J. Jolles. 2000. 'Neuropsychology of Infarctions in the Thalamus: A Review'. *Neuropsychologia* 38(5): 613–27.
- Van der Werf, Ysbrand D. et al. 2003. 'Deficits of Memory, Executive Functioning and Attention Following Infarction in the Thalamus; a Study of 22 Cases with Localised Lesions'. *Neuropsychologia* 41(10): 1330–44.
- Van Groen, Thomas, and J. Michael Wyss. 1995. 'Projections from the Anterodorsal and Anteroveniral Nucleus of the Thalamus to the Limbic Cortex in the Rat'. *Journal of Comparative Neurology* 358(4): 584–604.
- Varela, Carmen, and Matthew A Wilson. 2020. 'mPFC Spindle Cycles Organize Sparse Thalamic Activation and Recently Active CA1 Cells during Non-REM Sleep' ed. Laura L Colgin. *eLife* 9: e48881.
- Vetere, Gisella et al. 2011. 'Spine Growth in the Anterior Cingulate Cortex Is Necessary for the Consolidation of Contextual Fear Memory'. *Proceedings of the National Academy of Sciences of the United States of America* 108(20): 8456–60.
- Vetere, G., Kenney, J. W., Tran, L. M., Xia, F., Steadman, P. E., Parkinson, J., Josselyn, S. A., & Frankland, P. W. (2017). Chemogenetic Interrogation of a Brain-Wide

- Fear Memory Network in Mice. *Neuron* 94(2): 363-374.e4.
- Vetere, G., Xia, F., Ramsaran, A.I. et al. (2021). An inhibitory hippocampal–thalamic pathway modulates remote memory retrieval. *Nature Neuroscience* 24, 685–693
- Wagner, Isabella C., Mariët van Buuren, and Guillén Fernández. 2019. 'Thalamo-Cortical Coupling during Encoding and Consolidation Is Linked to Durable Memory Formation'. *NeuroImage* 197: 80–92.
- Waugh, Nancy C., and Donald A. Norman. 1965. 'Primary Memory'. *Psychological Review* 72(2): 89–104.
- Wheeler, Anne L. et al. 2013. 'Identification of a Functional Connectome for Long-Term Fear Memory in Mice'. *PLoS computational biology* 9(1): e1002853.
- Wiener, Sidney I., and Jeffrey S. Taube, eds. 2005. *Head Direction Cells and the Neural Mechanisms of Spatial Orientation*. The MIT Press.
<https://direct.mit.edu/books/book/2103/Head-Direction-Cells-and-the-Neural-Mechanisms-of> (July 22, 2023).
- Wilson, M. A., and B. L. McNaughton. 1994. 'Reactivation of Hippocampal Ensemble Memories during Sleep'. *Science (New York, N.Y.)* 265(5172): 676–79.
- Winocur, Gordon, Morris Moscovitch, and Bruno Bontempi. 2010. 'Memory Formation and Long-Term Retention in Humans and Animals: Convergence towards a Transformation Account of Hippocampal-Neocortical Interactions'. *Neuropsychologia* 48(8): 2339–56.
- Winter, Shawn S., Benjamin J. Clark, and Jeffrey S. Taube. 2015. 'Spatial Navigation. Disruption of the Head Direction Cell Network Impairs the Parahippocampal Grid Cell Signal'. *Science (New York, N.Y.)* 347(6224): 870–74.

- Wirt, Ryan A., and James M. Hyman. 2017. 'Integrating Spatial Working Memory and Remote Memory: Interactions between the Medial Prefrontal Cortex and Hippocampus'. *Brain Sciences* 7(4): 43.
- Wolff, M. et al. 2021. 'A Thalamic Bridge from Sensory Perception to Cognition'. *Neuroscience & Biobehavioral Reviews* 120: 222–35.
- Xue, Man et al. 2022. 'Mapping Thalamic-Anterior Cingulate Monosynaptic Inputs in Adult Mice'. *Molecular Pain* 18: 17448069221087034.
- Yuste, Rafael, and Tobias Bonhoeffer. 2004. 'Genesis of Dendritic Spines: Insights from Ultrastructural and Imaging Studies'. *Nature Reviews Neuroscience* 5(1): 24–34.

RÉSUMÉ

La mémoire est une composante essentielle de notre vie quotidienne. Certaines des expériences que nous vivons resteront gravées dans notre mémoire toute notre vie. Le processus qui permet à une trace mnésique de devenir durable s'appelle la consolidation de la mémoire. Selon les modèles classiques de consolidation de la mémoire, l'hippocampe joue un rôle crucial dans les premières phases de la formation de la mémoire, tandis que le néocortex prend le relais plus tard. Récemment, de nouvelles découvertes ont montré que le thalamus pouvait représenter une plaque tournante pour la communication hippocampo-corticale afin de former des souvenirs à distance. L'introduction d'études sur les connectomes fonctionnels a permis de découvrir de nouvelles régions impliquées dans les processus de mémorisation. Ces études ont montré que lorsque des souris se rappellent des souvenirs de peur à distance, un réseau hippocampo-thalamique-cortical puissant devient fonctionnellement interconnecté et activé. Cela souligne la nécessité de mener des recherches supplémentaires pour comprendre le rôle coordonné de ces trois sous-régions dans la formation de souvenirs durables. L'examen du réseau de mémoire à distance des régions cérébrales coactives avec les gènes précoces immédiats a révélé des changements dynamiques dans la connectivité fonctionnelle entre le noyau thalamique antérodorsal et le reste de la zone cérébrale au fil du temps. Ces résultats suggèrent que l'inactivation de l'ADn pourrait être nécessaire au rappel de la mémoire à distance. En effet, Vetere et al. (2021) ont démontré que les neurones inhibiteurs du champ CA3 de l'hippocampe se projetant sur l'ADn présentaient une activité accrue pendant le rappel de la mémoire à distance et que leur inactivation diminuait les performances de la mémoire.

MOTS CLÉS

consolidation de la mémoire, optogénétique, hippocampe, cortex, thalamus, fibre photométrique

ABSTRACT

Memory is a vital component of our daily lives. Some of the experiences that we encounter will be remembered for a lifetime. The process that allows a memory trace to become long-lasting is called memory consolidation. Classic models of memory consolidation posit that the hippocampus has a crucial role in the first phases of memory formation while the neocortex takes over later in time. Recently, novel discoveries showed that the thalamus may represent a gate hub for hippocampo-cortical communication to form remote memories. The introduction of functional connectomes studies have been of use for discovering new regions involved in memory processes. These studies showed that when mice recall remote fear memories, a strong hippocampo-thalamic-cortical network becomes functionally interconnected and activated. This highlights the need for additional investigation to understand the coordinated role of these 3 subregions in the formation of enduring memories. Through an examination of the remote memory network of immediate early gene co-active brain regions, dynamic changes in the functional connectivity between the anterodorsal thalamic nucleus and the rest of the brain area over time. These findings suggest that ADn inactivation may be necessary for remote memory recall. Indeed, Vetere *et al.* (2021) demonstrated that inhibitory neurons of the CA3 field of the hippocampus projecting to the ADn exhibited increased activity during remote memory recall and that their inactivation impaired memory performances.

KEYWORDS

memory consolidation, optogenetics, hippocampus, cortex, thalamus, fiber photometry