Inaugural dissertation

for

obtaining the doctoral degree

of the

Combined Faculty of Mathematics, Engineering and Natural Sciences

of the

Ruprecht – Karls – University

Heidelberg

Presented by

Janina Kupke, M.Sc.

Born in Schwelm, Germany

Oral examination 15.09.2023

DNA methylation and transcriptional control in memory formation, persistence and suppression

Referees: Prof. Dr. Hilmar Bading

Dr. Ana Oliveira

Declaration

I hereby declare that I have written the submitted dissertation: "**DNA methylation and transcriptional control in memory formation, persistence and suppression**" myself and in this process, have used no other sources or materials than those explicitly indicated.

This work was performed from January 2019 to September 2023 under the supervision of Dr. Ana Oliveira in the Neurobiology Department at the University of Heidelberg, Germany.

Place, Date

Janina Kupke

We look at the world once, in childhood.
The rest is memory

Louise Glück

Acknowledgements

I like to thank my thesis advisory and my examination committee members; Prof. Dr. Hilmar Bading, Prof. Dr. Valery Grinevich, Prof. Dr. Thomas Kuner, Prof. Dr. Rüdiger Rudolf and Prof. Dr. Christoph Schuster, for their kind interest and guidance in my thesis and work and the fruitful discussions.

I specially want to thank my dearest supervisor – *and lovely Boss ;-)* – **Ana**: I truly appreciate your input and continuous support during my growth as a scientist. Your calm and professionalism are the perfect match for my passionate and heated personality and I truly feel that we are a great team! You were right there to bring me back on track when I got lost in details and all the things I still wanted to do "*on top*". You helped me see the bigger picture and think about "the story" instead of (maybe) meaningless details. I always appreciated your open-door policy and general openness to talk about anything, both scientifically and personally. I know you always appreciated my opinion and valued it. Thank you very much!

AG Oliveira would not be my beloved work group if it were not for **David** & **Kübra**: I am grateful that you became part of my inner circle and that I can call you family. All our scientific and philosophical discussions made each day in the lab perfect and your continuous support is deeply appreciated. I learned so much from you and your passion and enthusiasm for science and life in general. This let us become the best team imaginable! Thank you for just being the best team inside and outside of science. Even being apart, we kept contact and when we see each other, it is as if we were never separated. To all the collaboration of us in the future ;-) Team ***Awemazinating*** rocks!

The addition to AG Oliveira in form of **Stef** was also great. Thank you for always helping me and showing understanding in stressful times, for lending me your ear whenever I felt like complaining and for being the best mouse stitching buddy. I further want to thank all the students who helped me on my journey and with whom I hope I could share my passion for science.

Next, I would like to thank the **Department of Neurobiology**: You guys were always helpful and created a nice environment to work in – especially with all the parties we had. I want to specifically thank **Anna** for becoming a dear friend and **Daniela** for being a great mentor especially in "university politics" and all the unspoken rules in academic research and for becoming a dear friend.

Thank you, **Robert**, for being my first mentor during my scientific journey and growing into a friend.

Beyond all the internal support, I am deeply grateful to have had wonderful friends and times in my 11 years in Heidelberg. Starting at the beginning are my two **Kathas**. Thank you for growing with me during our time in Heidelberg. I will miss our traditional Trash-TV nights but I know I can always count on you. I am very happy to have my dear ***Tiergeräusche*** on board. Thank you, **Katha**, **Franzi** & **David** for being always there. Another heartfelt thank you to **Kai** & **Elisa** for being my ***Gäng***. Just because we geographically move apart, does not mean we will not celebrate Silvester together ;-) And to **Delia** & **Philipp** with their beautiful daughters **Lina** & **Amelie** for having an additional family here.

All of you (and many more) made my time in Heidelberg special. Thank you for all the wonderful dinners, night and parties, for listening to all my complaints and giving me advice and all the beautiful moments, we shared together.

Most importantly I want to thank my family. **Mama**, **Papilie** und **Marvin**: danke, dass ihr immer an mich geglaubt habt und mich in allen meinen Entscheidungen unterstützt. Auch wenn für euch Vieles in der Wissenschaft unverständlich ist und ihr manchmal den Kopf geschüttelt habt, wieso ich das alles mitmache, wart ihr immer für mich da. Ihr seid großartig und danke vielmals. Ich möchte mich auch bei meiner großartigen **Oma** bedanken. Du bist zwar leider für diesen Meilenstein nicht mehr physisch an meiner Seite, aber ich trage dich für immer im Herzen <3

I further *acquired* an additional family. Thank you, **Michael** & **Flavia** for your support to Lukas and me. Also, a warm thank you to *"my"* Italian cousins **Ire** & **Miri**.

Last but with the utmost importance, I reserve the deepest gratitude for my soulmate, cherished confidant and beloved companion, **Lukas**. For your unwavering support, listening to my problems, unyielding belief in me, always giving me advice and for just being there in the bad and the great moments. Thank you for embracing me unconditionally with all my imperfections and for lifting me up, whenever I am down. You are not only my favourite scientist but my anchor. Without you this journey would have been so much harder. I am looking forward to a bright future together – connected through science but so much more.

To the stars who listen and the dreams that are answered.

To whatever end.

Sarah J. Maas

Summary

Memory formation is a complex process regulated by various molecular mechanisms, including unique transcriptional signatures and epigenetic factors. In addition, the brain is equipped with mechanisms that not only promote, but actively constrict memory formation. While the role of epigenetic modifications, such as DNA methylation, in cognition has been established, there are still significant gaps in our understanding of the specific functions of individual DNA methyltransferases (Dnmts) and how their downstream effectors orchestrate memory. Moreover, the molecular mechanisms underlying memory persistence and memory suppression remain largely unexplored.

I investigated the role of specific Dnmts in long-term memory formation, highlighting their unique functions and downstream effects. Additionally, I explored how DNA methylation contributes to the transfer of information from the hippocampus to the cortex for long-term storage and the stabilisation of cortical engrams to drive memory persistence. First, I examined the involvement of Dnmt3a1, the predominant *Dnmt3a* isoform in the adult brain, in hippocampus-dependent long-term memory formation. I identified an activity-regulated Dnmt3a1-dependent gene expression program and found a downstream effector gene (Neuropilin-1) with a previously undescribed function in memory formation. Intriguingly, I found that despite a common requirement for memory formation, Dnmt3a1 and Dnmt3a2 regulate this process via distinct mechanisms - Nrp1 overexpression rescued Dnmt3a1, but not Dnmt3a2, knockdown-driven impairments in memory formation. Next, I investigated the molecular mechanisms underlying memory persistence and systems consolidation, the gradual transfer of information from the hippocampus to the cortex. By modulating DNA methylation processes in the dorsal hippocampus, a short-lasting memory could be converted into a long-lasting one. The applied manipulation resulted in improved reactivation of cortical engrams and increased fear generalisation, mimicking the characteristics of remote memory. These findings provide compelling evidence for the facilitatory role of DNA methylation in memory information transfer to the cortex for long-term storage.

Furthermore, I examined the temporal expression patterns of immediate early genes (IEGs), specifically neuronal PAS domain protein 4 (Npas4), and its potential role in memory suppression. My investigation revealed that highly salient stimuli induced a biphasic expression of *Npas4* in the hippocampus, with the later phase dependent on NMDA receptor activity. Notably, this later phase of *Npas4* expression restricted memory consolidation, suggesting a role in balancing the formation of

highly salient memories and preventing the development of maladaptive behaviours. These findings highlighted the intricate regulatory network by which experience salience modulates IEG expression and thereby fine-tunes memory consolidation.

Overall, this study uncovered the unique functions of distinct Dnmts in memory formation and persistence and shed light on the associated mechanisms that are responsible to facilitate the transfer of information required for long-term storage. This comprehensive understanding of the molecular processes underlying memory formation contributes to our broader knowledge of memory consolidation and may have implications for therapeutic interventions targeting memory-related disorders.

.

Zusammenfassung

Die Gedächtnisbildung ist ein komplexer Prozess, der durch verschiedene molekulare Mechanismen gesteuert wird, darunter einzigartige Transkriptionssignaturen und epigenetische Faktoren. Darüber hinaus ist das Gehirn mit Mechanismen ausgestattet, die die Gedächtnisbildung nicht nur fördern, sondern auch aktiv einschränken. Die Rolle epigenetischer Modifikationen wie der DNA-Methylierung bei der Kognition ist zwar erwiesen, aber unser Verständnis der spezifischen Funktionen einzelner DNA-Methyltransferasen (Dnmts) und der Art und Weise, wie ihre nachgeschalteten Effektoren das Gedächtnis orchestrieren, ist noch sehr lückenhaft. Außerdem sind die molekularen Mechanismen, die der Gedächtnispersistenz und der Gedächtnisunterdrückung zugrunde liegen, noch weitgehend unerforscht.

Ich untersuchte die Rolle spezifischer DNA-Methyltransferasen bei der Bildung des Langzeitgedächtnisses und hob ihre einzigartigen Funktionen und nachgeschalteten Effekte hervor. Darüber hinaus untersuchte ich, wie die DNA-Methylierung zur Übertragung von Informationen aus dem Hippocampus in den Kortex zur Langzeitspeicherung und zur Stabilisierung kortikaler Engramme beiträgt, um die Gedächtnispersistenz zu fördern. Zunächst untersuchte ich die Beteiligung von Dnmt3a1, der vorherrschenden Dnmt3a-Isoform im erwachsenen Gehirn, an der Bildung des Langzeitgedächtnisses. Ich identifizierte ein aktivitätsreguliertes Dnmt3a1-abhängiges Genexpressionsprogramm und fand ein nachgeschaltetes Effektor-Gen (Neuropilin-1) mit einer bisher unbeschriebenen Funktion bei der Gedächtnisbildung. Interessanterweise stellte ich fest, dass Dnmt3a1 und Dnmt3a2 trotz einer gemeinsamen Voraussetzung für die Gedächtnisbildung diesen Prozess über unterschiedliche Mechanismen regulieren - die Überexpression von Nrp1 rettete die durch Reduktion von Dnmt3a1, aber nicht von Dnmt3a2 verursachten Beeinträchtigungen der Gedächtnisbildung. Als Nächstes untersuchte ich die molekularen Mechanismen, die der Gedächtnispersistenz und der allmählichen Übertragung von Informationen aus dem Hippocampus in den Kortex zugrunde liegen. Durch die Modulation von DNA-Methylierungsprozessen im dorsalen Hippocampus konnten kurzlebige Erinnerungen in langanhaltende umgewandelt werden. Die angewandte Manipulation führte zu einer verbesserten Reaktivierung von kortikalen Engrammen und zu einer verstärkten Verallgemeinerung der Furchterinnerung, was die Merkmale der Gedächtnispersistenz nachahmt. Diese Ergebnisse liefern überzeugende Beweise für die unterstützende Rolle der DNA-

Methylierung bei der Übertragung von Gedächtnisinformationen in den Kortex zur Langzeitspeicherung.

Darüber hinaus untersuchte ich die zeitlichen Expressionsmuster sogenannter „*immediate early genes*“ (IEGs), insbesondere des neuronalen PAS-Domänenproteins 4 (Npas4), und seine mögliche Rolle bei der Gedächtnisunterdrückung. Meine Untersuchung ergab, dass starke Salienz eine biphasische Expression von Npas4 im Hippocampus auslösen, wobei die spätere Phase von der NMDA-Rezeptoraktivität abhängig ist. Bemerkenswert ist, dass diese spätere Phase der Npas4-Expression die Gedächtniskonsolidierung einschränkte, was auf eine Rolle beim Ausgleich der Bildung hochsignifikanter Erinnerungen und bei der Verhinderung der Entwicklung maladaptiver Verhaltensweisen hindeutet. Diese Ergebnisse verdeutlichen das komplizierte regulatorische Netzwerk, durch das der Erfahrungswert die IEG-Expression moduliert und dadurch die Gedächtniskonsolidierung fein abstimmt.

Insgesamt deckte diese Studie die einzigartigen Funktionen verschiedener DNA-Methyltransferasen bei der Gedächtnisbildung und -persistenz auf und warf ein Licht auf die damit verbundenen Mechanismen, die für die Erleichterung der für die Langzeitspeicherung erforderlichen Informationsübertragung verantwortlich sind. Dieses umfassende Verständnis der molekularen Prozesse, die der Gedächtnisbildung zugrunde liegen, trägt zu unserem breiteren Wissen über die Gedächtniskonsolidierung bei und könnte Auswirkungen auf therapeutische Maßnahmen zur Behandlung von Gedächtnisstörungen haben.

Abbreviations

4 OHT	4 Hydroxytamoxifen
ACC	anterior cingulate cortex
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
AMPAR	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor
AP-1	activator protein 1
ARC	activity-regulated cytoskeleton-associated protein
ATAC-seq	Assay for Transposase-Accessible Chromatin with high-throughput sequencing
ATF	activating transcription factor
BLA	basolateral amygdala
CA	cornu ammonis
CaMK	Calcium/calmodulin-dependent protein kinases
cAMP	cyclic AMP
catFISH	cellular compartment analysis of temporal activity by fluorescent in situ hybridization
CBP	CREB-binding protein
CCK	Cholecystokinin
CFC	contextual fear conditioning
cFos	c-FBJ osteosarcoma oncogene
ChR2	Channelrhodopsin-2
CNO	Clozapine N-oxide
CREB	cAMP response element-binding protein
CRTC	CREB-regulated transcription coactivator
DEG	differentially expressed gene
DG	dentate gyrus
dHPC	dorsal hippocampus
Dnmt	DNA methyltransferase
DREADD	Designer Receptors Exclusively Activated by Designer Drugs
EC	entorhinal cortex
eGRASP	enhanced GFP Reconstitution Across Synaptic Partners
ERK	extracellular signal-regulated kinase
ESARE	Enhanced synaptic activity-responsive elements
FANS	fluorescent-activated nuclear sorting
Gadd	Growth Arrest and DNA Damage
GO	Gene ontology

HAT	Histone acetyltransferase
HDAC	Histone deacetylase
HPC	Hippocampus
hSyn	human Synapsin
IEG	immediate early gene
KO	Kusabira orange
LRG	late responsive genes
LTD	long-term depression
LTF	long-term facilitation
LTP	long-term potentiation
MAPK	Mitogen-activated protein kinase
NMDA	N-Methyl-D-aspartic acid
NMDAR	N-Methyl-D-aspartic acid receptor
Npas4	Neuronal PAS domain protein 4
Nrp1	Neuropilin 1
PFC	prefrontal cortex
PKA	protein kinase A
PTM	posttranslational modification
PV	Parvalbumin
qRT-PCR	quantitative reverse transcription PCR
rAAV	recombinant adeno-associated virus
RAM	robust activity marking
rtTA	reverse tetracycline-controlled transactivator
SARE	Synaptic activity-responsive elements
Sema3A	Semaphorin 3A
shRNA	short-hairpin RNA
SRF	serum responsive factor
TET	Ten-eleven translocation
TetR	Tetracycline repressor
TORC	transducers of regulated CREB
TRAP	Targeted recombination in active populations
TRE	TPA-responsive element
tTA	Tetracycline-transactivator
WGBS	whole genome bisulfite sequencing

List of Illustrations

Chapter 1: Introduction

Chapter 2: Results

Dnmt3a1 regulates hippocampus-dependent memory via the downstream target Nrp1

DNA methylation promotes memory persistence by facilitating systems consolidation and cortical engram stabilisation.

High salient fear memory induces a biphasic Npas4 expression and results in memory suppression

Chapter 3: Discussion

List of publications

The thesis consists of three planned or submitted publications:

1. **Kupke J**, Klimmt J, Mudlaff F, Schwab M, Sticht C, Oliveira AMM. Dnmt3a1 regulates hippocampus-dependent memory via the downstream target Nrp1.
   Manuscript in preparation.

2. **Kupke J**, Loizou S, Sticht C, Bengtson CP, Oliveira AMM. DNA methylation promotes memory persistence by facilitating systems consolidation and cortical engram stabilisation.
   Manuscript in preparation.

3. Brito DVC*, **Kupke J***, Sokolov R, Cambridge S, Both M, Rozov A, Oliveira AMM. Biphasic Npas4 expression promotes inhibitory plasticity and suppression of fear memory consolidation.
   In revision in Molecular Psychiatry. 10.07.2023

**** Authors contributed equally***

In addition, in the course of this thesis, I contributed to the following publications:

1. Brito DVC, **Kupke J**, Gulmez Karaca K, Oliveira AMM. Regulation of neuronal plasticity by the DNA repair associated GADD45 proteins. Current Research in Neurobiology. 2022. doi.org/10.1016/j.crneur.2022.100031.

2. Gulmez Karaca K, Brito DVC, **Kupke J**, Zeuch B, Oliveira AMM. Engram reactivation during memory retrieval predicts long-term memory performance in aged mice. Neurobiology of Aging. 2021. doi: 10.1016/j.neurobiolaging.2021.01.019.

3. Gulmez Karaca K, **Kupke J**, Oliveira AMM. Molecular and cellular mechanisms of engram allocation and maintenance. Brain Research Bulletin. 2021. doi: 10.1016/j.brainresbull.2021.02.019.

4. Brito DVC, Gulmez Karaca K**, Kupke J**, Frank L, Oliveira AMM. MeCP2 gates spatial learning-induced alternative splicing events in the mouse hippocampus. Molecular Brain. 2020. doi: 10.1186/s13041-020-00695-1.

5. Brito DVC, Gulmez Karaca K, **Kupke J**, Mudlaff F, Zeuch B, Gomes R, Lopes LV, Oliveira AMM. Modeling human age-associated increase in Gadd45γ expression leads to spatial recognition memory impairments in young adult mice. Neurobiology of Aging. 2020. doi: 10.1016/j.neurobiolaging.2020.06.021.

6. Gulmez Karaca K, **Kupke J**, Brito DVC, Zeuch B, Thome C, Weichenhan D, Lutsik P, Plass C, Oliveira AMM. Neuronal ensemble-specific DNA methylation strengthens engram stability. Nature Communications. 2020. doi: 10.1038/s41467-020-14498-4.

7. Brito DVC, **Kupke J**, Gulmez Karaca K, Zeuch B, Oliveira AMM. Mimicking Age-Associated Gadd45γ Dysregulation Results in Memory Impairments in Young Adult Mice. Journal of Neuroscience. 2020. doi: 10.1523/JNEUROSCI.1621-19.2019.

Further, I contributed to the following publications prior to my thesis:

1. Luck R, Karakatsani A, Shah B, Schermann G, Adler H, **Kupke J**, Tisch N, Jeong HW, Müller M, Hetsch F, D'Errico A, De Palma M, Wiedtke E, Grimm D, Acker-Palmer A, von Engelhardt J, Adams RH, Augustin HG, Ruiz de Almodóvar C. The angiopoietin-Tie2 pathway regulates Purkinje cell dendritic morphogenesis in a cell-autonomous manner. Cell Reports. 2021. doi.org/10.1016/j.celrep.2021.109522.

2. Kalamakis G, Brüne D, Ravichandran S, Bolz J, Fan W, Ziebell F, Stiehl T, Catalá-Martinez F, **Kupke J**, Zhao S, Llorens-Bobadilla E, Bauer K, Limpert S, Berger B, Christen U, Schmezer P, Mallm JP, Berninger B, Anders S, Del Sol A, Marciniak-Czochra A, Martin-Villalba A. Quiescence Modulates Stem Cell Maintenance and Regenerative Capacity in the Aging Brain. Cell. 2019. doi: 10.1016/j.cell.2019.01.040.

3. Genovese F, Bauersachs HG, Gräßer I, **Kupke J**, Magin L, Daiber P, Nakajima J, Möhrlen F, Messlinger K, Frings S. Possible role of calcitonin gene-related peptide in trigeminal modulation of glomerular microcircuits of the rodent olfactory bulb. European Journal of Neuroscience. 2017. doi: 10.1111/ejn.13490.

Table of Contents

I. Introduction

We are what our memories make us. They help to build our individual characters and provide us with the power to choose not to engage in unpleasant behaviours by assisting us in making decisions about our future actions based on our prior experiences. This vital role of memory emphasizes the need to explore mechanisms, leading to its continuous and enduring scientific investigation.

1. Categories and definitions in memory research

Memory is commonly referred to as a system that processes information with explicit and implicit meaning. Memory is categorised into declarative (i.e., explicit) and non-declarative (i.e., implicit) forms. Declarative memory is a conscious storage or recollection of knowledge-based facts or events (Sweatt, 2009). It consists of semantic or episodic memory. Episodic memory stores our personal experiences, whereas semantic memory refers more to fact-based knowledge (Squire, 2004). Several brain regions, e.g., the hippocampus (HPC), perirhinal and para-hippocampal cortices are important for declarative memory (Squire & Zola-Morgan, 1991). On the other hand, non-declarative memory is unconsciously retained knowledge. This type of memory is crucial for daily functions and consist of skills and habits, routines, emotional or motor reactions, reflexes and priming. Non-declarative memories typically depend on areas that include the cerebellum, basal ganglia, and motor cortex in addition to being dependent on the cerebral cortex (Squire & Zola-Morgan, 1991).

Memory can further be categorised to its storage capacities. It can last for seconds to couple of hours, which is referred to as short-term memory (Kandel, 2012; Kandel et al., 2014). Whereas long-term memory can be stored for days to weeks or even for a life-time. In addition, long-term memory is subdivided: If the memory is being recalled days after the encoding of memory, this is referred to as the recent memory. Whereas remote memory is another type of long-term memory lasting for weeks to a life-time and it is thought to depend on additional consolidation processes and storage areas in the brain. A more detailed differentiation will be made in the Section 4. *Memory persistence*.

Episodic memory encoding begins as soon as the sensory input from an experience transmitted is received by the brain. During the encoding process, memories are vulnerable and susceptible for disruption via e.g., pharmacological, electrical or behavioural manipulations (Flexner et al., 1962). The memory is then stabilised into

long-term memory via a prolonged process termed consolidation (Izquierdo & Medina, 1997). However, once a memory is being retrieved, it enters again a liable, fragile state that can be altered, erased or updated through a process known as memory reconsolidation (Sweatt, 2009). The primary difference between long-term and short-term memory is the dependency of long-term memory on *de novo* protein synthesis, which causes long-lasting plasticity changes in neurons (Flexner et al., 1962; Kandel, 2001).

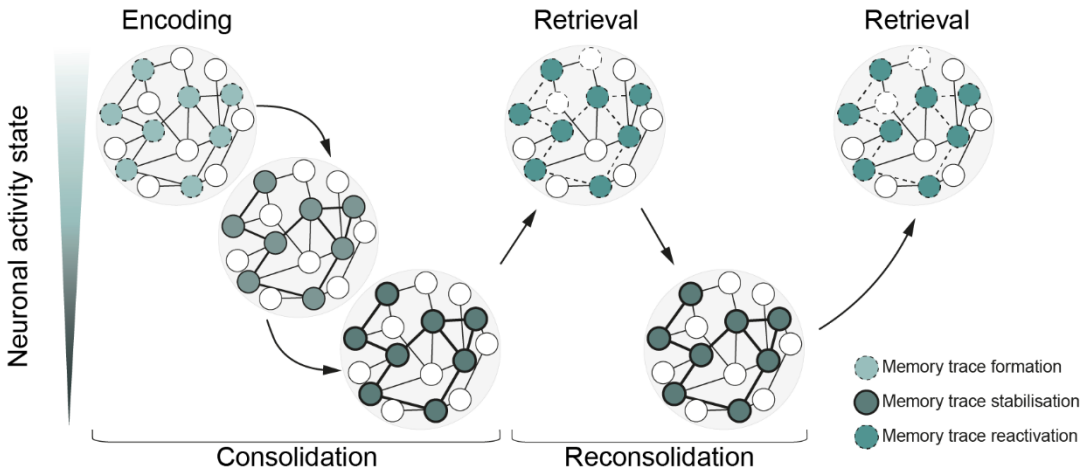


Figure 1. The processes of memory encoding, consolidation and retrieval. Memory encoding involves strengthening of connections between active (green) neurons. These connections become more stable as the consolidation process progresses, increasing the possibility that the same activity pattern may be reproduced at a later time, facilitating effective memory recall. Neuronal activity in neurons of the memory trace decreases during consolidation. Memory retrieval makes the memory trace active once more by increasing neural activity, temporarily destabilising the connectivity network. The memory trace enters reconsolidation and can be updated in this condition.

2. Molecular mechanisms underlying memory processes

Synaptic plasticity is believed to be the underlying mechanism for learning and memory. Early, pioneering studies of Eric Kandel and colleagues on *Aplysia californica* showed that different types of stimulation resulted in short-term and long-term changes in behaviour. These behavioural changes were associated with short-term facilitation and long-term facilitation (LTF) of neurons (in rodents, these terms correlate to short-term or long-term potentiation). Short-term changes involved intracellular signalling molecules and cascades, including cyclic AMP (cAMP) that facilitated neurotransmitter release. While LTF involved the phosphorylation of the transcription factor cAMP response element-binding protein (CREB) downstream of extracellular-signal regulated kinases (ERK) and mitogen-activated protein kinases (MAPK) signalling activation, which then in turn initiated the transcription of genes necessary for long-lasting structural and molecular changes in neurons (Kandel, 2001; Kandel et al., 2014; Sweatt, 2009). This significant research provided valuable

insight into the distinct molecular requirements for short-term and long-term memories and it has suggested that the intensity of molecular and biochemical responses triggered by an experience influences gene expression and synapse function and structure, and consequently affects the resulting behaviour.

2.1 Long-term synaptic plasticity

Synaptic plasticity is a fundamental process in the brain that underlies memory formation and adaptive behaviours. It refers to the ability of synapses to strengthen or weaken in response to activity patterns. Long-term potentiation (LTP) is characterized by a lasting increase in synaptic gain, while long-term depression (LTD) involves a decrease in synaptic strength.

Pioneering work demonstrated several key aspects of LTP. It leads to enhanced synaptic response and neuronal excitability. It requires cooperativity and associativity, enabling nearby synapses to lower the threshold for LTP induction (Bliss et al., 2018). N-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors play a key role in LTP induction. Glutamate binding to both AMPA and NMDA receptors allows passage of Na+ and K+ ions leading to depolarisation. This depolarisation leads to the removal of Mg2+ ion block in the NMDA receptor pore further allowing calcium influx through NMDA receptors. Calcium ions bind to calmodulin, leading to the autophosphorylation of calcium-calmodulin-dependent protein kinase II α (CaMKIIα), a key player in LTP expression. Activated CaMKIIα phosphorylates targets such as NMDA and AMPA receptors, enhancing synaptic transmission. LTP also involves *de novo* gene expression, dendritic spine enlargement and increased spine stability. These changes contribute to the long-lasting increase in synaptic strength. In contrast, LTD is characterized by a long-term decrease in synaptic gain. It occurs by modest NMDA receptor activation and sustained low levels of calcium influx. Phosphatases, such as calcineurin and protein phosphatase 1, play critical roles in LTD. The activation of specific signalling pathways during LTD results in the internalization of AMPA receptors, leading to reduced AMPA receptor-mediated synaptic transmission and shrinkage of dendritic spines. Albeit sounding as a negative mechanism, LTD plays a crucial role in neural circuit refinement, the elimination of unnecessary connections and regulation of synaptic homeostasis. Synaptic plasticity is a cornerstone of neural adaptation that enables the brain to encode, store, and recall information. By adjusting synaptic strength and structure, these processes shape the connectivity and functionality of neural networks.

2.2 Activity-dependent gene transcription

The precise regulation of gene transcription is necessary for cognitive processes such as long-term memory formation. This regulation is orchestrated by mechanisms involving transcription factors (Alberini, 2009; Alberini & Kandel, 2015), epigenetic factors, and 3D-genomic factors such as chromatin structure or nuclear organization (Cholewa-Waclaw et al., 2016; Day & Sweatt, 2011; Medrano-Fernández & Barco, 2016; A. M. M. Oliveira, 2016).

One extensively studied transcription factor involved in learning is CREB. CREB binds to the promoters or enhancers of cAMP-responsive genes, known as cAMP response elements (CRE). Once phosphorylated by protein kinase A (PKA), MAPKs, or Ca2+/calmodulin-dependent protein kinase (CaMK), particularly CaMKII/IV, CREB binds to transcriptional coactivators such as CREB-binding protein (CBP) and initiates gene transcription. Interestingly, CREB-dependent gene expression can also occur independently of CREB phosphorylation through the interaction of CREB-regulated transcriptional coactivators (CRTCs) also known as transducers of regulated CREB (TORC). Numerous studies have revealed that several hundred genes are regulated by CREB activity. These genes include transcription factors, growth factors, signalling molecules and neuronal genes; many of which are crucial for synaptic plasticity processes. Similar to CREB, additional transcription factors that are constitutively expressed have been linked to memory and plasticity pathways. The transcription factor complex activator protein 1 (AP-1) is created when proteins from the Jun (c-Jun, JunB, and JunD) family homodimerize or heterodimerize with proteins from the c-Finkel-Biskis-Jinkis, osteosarcoma oncogene (Fos), activating transcription factors (ATF), families (Alberini, 2009; Hai & Hartman, 2001). Interestingly, many genes associated with neuronal activity responses contain multiple transcription factor binding sites, allowing for redundancy and flexibility in the cellular response. The ERK1/2 and p38 signalling pathways that induce CREB activation also leads to AP-1 activation, suggesting complementary functions of these transcription factors in plasticity mechanisms related to memory formation. Another transcription factor involved in activity-dependent gene expression is the serum response factor (SRF), which binds to serum response elements (SRE). SRF activity plays a critical role in hippocampal functions, dendritic development, and remodelling. Studies have shown that depletion of SRF in mice impairs the induction of immediate early genes (IEGs) in the hippocampus following neuronal activity, leading to deficits in synaptic plasticity and long-term memory.

All these transcriptional events are crucial for the consolidation of long-term memory. Intriguingly, studies have shown that the formation of long-term memory involves at least two rounds of transcriptional activation: the first occurring immediately after the learning experience (i.e., IEGs), and the second taking place 3-6 hours later (i.e., late response genes, LRGs). Blocking transcription at different time points after an experience has revealed that both rounds of transcription are necessary for the long-term storage of memories (Benito & Barco, 2015).

2.2.2 Immediate early genes

Immediate early genes (IEGs) are rapidly and transiently upregulated in response to activity. Their transcription is triggered by calcium influx into neurons through various channels (e.g., NMDA, AMPA, through L-type voltage-sensitive calcium channels) or by Ca2+ release from intracellular stores (West et al., 2001). Transcription factors such as CREB, AP-1 and SRF, which are already present in the cell, undergo post-translational modifications upon calcium influx and initiate the transcription of IEGs because they contain binding motifs for these constitutively expressed transcription factors. Therefore, unlike other genes, the transcription of IEGs does not require *de novo* protein synthesis but relies on the post-translational modifications of existing transcription factors. In absence of activity, IEGs are typically expressed at low levels and increase upon activity. Thus, their expression serves as a proxy for activated neurons (Okuno, 2011). Examples of well-studied IEGs include the activity regulated cytoskeleton (Arc) protein and Fos and the neuronal PAS domain protein 4 (Npas4). Many IEGs are transcription factors that generate a secondary response required for long-term memory formation.

Arc, although not a transcription factor, functions in synaptic processes. Its mRNA is transported to dendrites (Moga et al., 2004) and interacts at the postsynaptic density with protein complexes to regulate AMPA receptor endocytosis (Wall & Corrêa, 2018), influencing plasticity mechanisms like LTP and LTD (DaSilva et al., 2016; Shepherd et al., 2006). Arc has nuclear localization properties and regulates ionotropic receptor AMPA type subunit 1 (GluA1) expression (Korb et al., 2013). An unexpected finding identified virus-like properties of Arc. It was found to form virus-like particles that transport RNA from synapse to synapse (Ashley et al., 2018; Pastuzyn et al., 2018). These findings suggest other mechanisms for Arc-regulated neuroplasticity that still has to be elucidated.

The transcription factor Fos contains both SRE and CRE elements and can be induced by CREB and SRF. It forms the AP-1 complex with members of the Jun

family and acts as an activity-induced transcription factor (E. L. Yap & Greenberg, 2018) that binds to its consensus sequence (TPA-responsive element, (TRE)) and regulates numerous processes, e.g., cell growth, inflammatory responses and repair processes (Alberini, 2009). Transgenic mouse model studies have demonstrated that the expression of Fos is necessary for learning and memory processes (Fleischmann et al., 2003; Paylor et al., 1994). In hippocampal CA1 pyramidal cells, Fos expressing cells help to establish a bidirectional modulation of inhibition by enhancing parvalbumin-expressing interneurons and simultaneously weaken perisomatic inhibition by cholecystokinin-expressing interneurons that in turn creates a network of Fos-activated neurons (E.-L. Yap et al., 2021). In addition, another recent study showed that Fos-induced cells encode precise, persistent, and spatially uniform maps that support hippocampal place codes and that Fos itself plays a causal role in determining these place codes (Pettit et al., 2022).

Another IEG is Npas4. It exhibits unique characteristics distinguishing it from other IEGs since it is exclusively activated by neuronal activity and is thought to control the expression of numerous activity-regulated genes (Sun & Lin, 2016). Also, its role in memory processes is extensively studied. Inhibition of Npas4 expression impaired memory formation (Ploski et al., 2011; Ramamoorthi et al., 2011; Sun et al., 2020; Weng et al., 2018). Further, Npas4 forms heterodimers and action potential bursting or excitatory postsynaptic potentials have distinct effects on this heterodimer formation, which results in unique binding patterns in the genome. This highlights the function of Npas4 as a mediator of synaptic stimuli and in fine-tuning complex cognitive processes (Brigidi et al., 2019). Further, Npas4 was shown to play a role in circadian behaviour and transcriptional responses to light in the suprachiasmatic nucleus (P. Xu et al., 2021) as well as it has a function in activity-dependent DNA repair mechanisms (Pollina et al., 2023).

The importance of stimulus-induced transcription for consolidating significant experiences into long-term memory is widely recognized (Alberini & Kandel, 2015). These transcriptional responses involve the sequential activation of various molecules in memory-related regions, such as the hippocampus and the first transcriptional induction is the expression of IEGs (Alberini & Kandel, 2015; E. L. Yap & Greenberg, 2018). Recent studies have suggested that experiences with different levels of salience and emotional valence trigger distinct patterns of IEG expression (Bekinschtein et al., 2007, 2008; Katche et al., 2010; Mukherjee et al., 2018a; Nakayama et al., 2015; Tyssowski et al., 2018). However, it is still unclear whether the salience of a stimulus triggers transcriptional responses that not only

promote memory consolidation but also act as an activation mechanism to restrict information storage. This could potentially serve as a biological strategy to prevent overly salient stimuli from forming strong memories that may interfere with adaptive behaviour.

2.2.3 Late responsive genes

Late responsive genes (LRGs) are expressed several hours after IEGs because they depend on the transcription factor binding activity of the IEGs and thus this secondary genomic response relies on *de novo* protein synthesis. LRGs encode for effector proteins with functions for dendritic growth, excitatory – inhibitory balance and spine maturation and refinement (E. L. Yap & Greenberg, 2018). Genome-wide studies have identified around 300 to 500 LRGs and moreover, the binding of activity-dependent transcription factors to LRGs appears to be cell-type specific, leading to divergence in the pool of LRGs and adding to the complexity of their regulation (E. L. Yap & Greenberg, 2018). Consequently, research has primarily focused on studying the functions of individual LRGs by classical gain- and loss-of-function approaches. However, since many transcription factors have overlapping functions, understanding the collective actions of LRGs as a whole remains a challenge.

Activity-dependent gene expression involves not only the coordinated activity of transcription factors and effector proteins, but in addition the epigenetic or 3D-genomic landscape of the neurons, which includes the accessibility of gene loci in various cell types (Beagan et al., 2020; Beagan & Phillips-Cremins, 2020; Campbell & Wood, 2019; Gräff & Tsai, 2013a; Zovkic et al., 2013). These alterations can ultimately impact the cellular response to neuronal activity and subsequent synaptic and memory strength. Indeed, epigenetic mechanisms such as DNA methylation have emerged as important regulators for cognitive processes (A. M. M. Oliveira, 2016).

2.3 Epigenetic mechanisms in memory research

Epigenetics is a term defined to influence gene transcription by modifying biochemically DNA or DNA-associated structures, without directly altering the DNA sequence. This allows epigenetic mechanisms to cause enduring or temporary changes in gene expression (Sweatt, 2009). Epigenetic regulation can origin from i) covalent modifications on DNA, such as DNA methylation or DNA hydroxymethylation, ii) post-translational modifications (PTMs) of histone tails that influence the charge of histones, thereby affecting the interaction between DNA and histone proteins as well as histone subunit changes and iii) the 3D organisation of

the chromatin structures as a whole, including interactions between gene promoter-gene enhancer regions, chromatin looping, and nuclear geometry. Not surprisingly, all these mechanisms have been shown to be regulated by neuronal activity and have an impact on long-term memory storage.

Epigenetic regulation happens through molecules responsible for changing the epigenome. They can be classified as *writers* or *erasers*, since they add or remove an epigenetic mark, respectively, or *readers* which are proteins that recognise and interpret the epigenetic changes. Given the beforementioned classification of epigenome changes, epigenetic regulators can further be classified into i) DNA methylation players, ii) histone tail modifiers and iii) chromatin organizers.

Since my thesis primarily emphasises the investigation of DNA methylation players in memory research rather than histone modifications or chromatin organisation, I will provide a concise overview of the latter topics while dedicating detailed attention to DNA methylation.

2.3.1 Histone tail modification

Histone tail modifications include various types such as acetylation, phosphorylation, methylation, sumoylation, ubiquitination and serotonylation (Chatterjee & Abel, 2017; Farrelly et al., 2019), with acetylation being the most studied one in memory research. Histone acetylation consists in the addition of acetyl groups to lysine (K) residues on the N-terminal of histone tail proteins and is carried out by histone acetyltransferases (HATs). This modification recruits remodelling complexes to open the chromatin structure, which in turn facilitates the recruitment of the transcription machinery and is thereby associated with actively transcribed gene loci. Conversely, acetyl groups can be removed by enzymes called histone deacetylases (HDACs). Learning induced neuronal activity have been shown to increase histone acetylation levels and cause the dissociation of HDACs from the chromatin (Chatterjee & Abel, 2017). More specific, histone acetylation occurred on the promoters of genes involved in synaptic plasticity regulation, such as reelin, Bdnf, or Nr4a1 (Chatterjee & Abel, 2017). In addition, HDAC inhibitors promote memory formation, while blocking the activity of HATs impairs memory (Chatterjee & Abel, 2017; Gräff & Tsai, 2013a, 2013b). Nevertheless, histone tail methylation is another mark important for regulation the chromatin state and transcription and important for memory formation (Collins et al., 2019; Gupta et al., 2010). H3K4me together with H3K27ac are the classical defined enhancer marks and trimethylation of H3K4 provides an epigenetic signature for active enhancers and is bound by the general

transcriptional co-activator p300 (Pekowska et al., 2011), thereby regulating activity-dependent transcription important for neuroplasticity processes such as memory. Collectively, these findings suggest that histone modification plays a crucial role in memory research.

2.3.2 3D genomic architecture

Recent research indicates that the 3D-genomic architecture plays a crucial role in synaptic plasticity and memory processes. The dynamic regulation of nuclear and subnuclear geometry, the interaction between structural proteins or transcriptional machinery with chromatin, the positioning of genes in euchromatin or heterochromatin, and chromatin looping that affect gene transcription efficiency are all important mechanisms in this process. Advancements in imaging and sequencing technologies, such the assay for transposase-accessible chromatin with sequencing (ATAC-seq), the chromosome conformation capture (3C) or the high-resolution chromosome-conformation-capture carbon-copy sequencing (5C-seq), have enabled the detailed investigation of these mechanisms at a fine scale and high resolution (Beagan & Phillips-Cremins, 2020; Campbell & Wood, 2019; Fernandez-Albert et al., 2019; Medrano-Fernández & Barco, 2016; Watson & Tsai, 2017).

Studies have shown that chromosomal relocation occurs in response to neuronal activity such as seizures (Borden & Manuelidis, 1988), LTP induction (Billia et al., 1992) or sensory learning (Yamada et al., 2019). Studies using advanced techniques have shown that upon neuronal activity, certain gene loci gain open chromatin accessibility and increase their expression, while others exhibit closed chromatin accessibility and reduced expression (Su et al., 2017). These changes in the chromatin state can persist even after the removal of the neuronal activity-inducing stimulus. Chromatin organization also influences the transcriptional permissiveness of neurons in response to neuronal activity. Chromatin looping, which determine enhancer-promoter interactions, plays a critical role in the transcriptional regulation of IEGs (Joo et al., 2016). A recent study discovered that more than 10% of loops surrounding specific IEGs, LRGs, and synaptic genes were newly formed during cortical neuron activation and that IEGs formed shorter loops, while LRGs formed more complex looping architectures. Additionally, the study identified specific activity-dependent, looped enhancers that were colocalized with genetic variants associated with autism and schizophrenia (Beagan et al., 2020). Chromatin remodelling is not only involved in the activation of genes by neuronal activity but also in the regulation of the transient nature of IEG transcription. Concerning

nucleosome composition, studies have shown that the histone variant H2A.Z negatively regulates long-term memory consolidation in the hippocampus (Zovkic et al., 2014) and its removal after learning allows for the expression of learning-related genes (Stefanelli et al., 2018) These findings highlight the importance of 3D-genomic architecture of chromatin organization in plasticity-related gene expression and cognitive processes.

2.3.3 DNA methylation

DNA methylation primarily occurs at cytosine-guanine dinucleotide sequences (CpG sites) and was historically associated with gene repression. It can further occur at non-CpG sites and recent evidence showed that cytosine methylation can actually promote gene expression (Wu et al., 2010). DNA methylation-dependent mechanisms involve the coordinated activity DNA methylation writers, readers, and erasers.

DNA methylation writers, known as DNA methyltransferases (Dnmts), catalyse the covalent binding of a methyl group to the 5'-position of the cytosine-pyrimidine ring using the methyl donor S-adenosyl-methionine (SAM). Mammals have two different forms of Dnmts, Dnmt1 and Dnmt3. Dnmt1 is primarily thought of as maintenance Dnmt, which is essential for maintaining the DNA's methylation both before and after DNA replication. As they add newly methylation marks to the genome, the Dnmt3 protein family, which consists of Dnmt3a and Dnmt3b, are *de novo* methyltransferases. The *Dnmt3b* gene encodes for different isoforms, but only Dnmt3b1 and Dnmt3b2 have methyltransferase activity (Aoki, 2001) and Dnmt3l is regarded as a regulatory unit assisting other Dnmts in their methyltransferase function. The Dnmt3a genomic locus contains two genes, Dnmt3a1 and Dnmt3a2. Except for the absence of the first 219 amino acids in the protein's N-terminal of Dnmt3a2, it shares the same amino acid sequence as Dnmt3a1. Dnmt3a2 was found to bind to euchromatic areas whereas Dnmt3a1 was found to bind to heterochromatic regions (T. Chen et al., 2002), thus the two isoforms exhibit different expression regulation and genomic binding (Manzo et al., 2017) in response to neuronal activity. Dnmt3a2 is transcribed from an intronic promoter and has IEG properties, whereas the mRNA expression of *Dnmt3a1* is not regulated by activity (Bayraktar et al., 2020; A. M. M. Oliveira et al., 2012, 2016). Despite a lack of its expression regulated by neuronal activity, Dnmt3a1 is expressed at higher levels in the adult brain including in regions required for memory formation (Feng et al., 2005). It is worth noting that the functions of Dnmt3a1 and Dnmt3a2 are not interchangeable, as evidenced by the lack of compensation for memory deficits in Dnmt3a2 knockdown mice despite

normal Dnmt3a1 expression (A. M. M. Oliveira et al., 2012). Thus, the distinct features of Dnmt3a1 and Dnmt3a2 suggest that the two enzymes may be differentially required for long-lasting adaptions in neurons and may target distinct genomic loci during memory formation (Manzo et al., 2017).

DNA methylation erasers are responsible for promoting active DNA demethylation independent of the cell division. The ten-eleven translocation (TET) proteins, including TET1, TET2, and TET3, play a crucial role in this process by converting 5-methylcytosine into its demethylated form through a series of biochemical steps (Alaghband et al., 2016; He et al., 2011; Ito et al., 2011). Tet proteins have been investigated in relation to cognitive functions and have been shown to affect the expression of activity-regulated genes (Alaghband et al., 2016; Rasmussen & Helin, 2016). The growth arrest and DNA damage (Gadd) family has been shown to promote active demethylation. For this, they recruit the demethylation machinery and activate the base excision repair (Gavin et al., 2012; Z. Li et al., 2015) or interacte directly with TET proteins (Kienhöfer et al., 2015). Furthermore, the Gadd family has been shown to be involved in activity-driven synaptic plasticity and learning and memory (Brito et al., 2022; Brito, Gulmez Karaca, et al., 2020; Brito, Kupke, et al., 2020).

MeCP2 was initially thought to repress gene transcription by interacting with other proteins and modifying chromatin structure. However, recent studies have shown that MeCP2 also binds to active gene promoters and has both activating and silencing effects on gene expression (Chahrour et al., 2008). It promotes secondary chromatin structures (Della Ragione et al., 2016), forms chromatin loops and maintains the chromocenter structure (Kernohan et al., 2014). Additionally, MeCP2 plays a role in organizing chromatin structure and is involved in neurological disorders such as Rett syndrome (Sandweiss et al., 2020). These findings highlight the multifaceted functions of MeCP2 in regulating gene expression and chromatin organization (Gulmez Karaca et al., 2019).

2.3.4 DNA methylation in synaptic plasticity and memory processes

DNA methylation has historically been thought of as a static process with significant functions in transcription repression, cell destiny determination and imprinting during early development (Suzuki & Bird, 2008). But it is now well established that DNA methylation is important for controlling the genomic responses in adult neurons (Feng et al., 2010; J. Li et al., 2022; Morris et al., 2014).

Several studies have shown that DNA methylation changes rapidly in response to neural activity, and genetic and pharmacological investigations have linked Dnmt activity to memory formation (Guo et al., 2011; Halder et al., 2015). Particularly, Dnmt3a is known to play a role in cognitive ability (Feng et al., 2010; Miller & Sweatt, 2007; Mitchnick et al., 2015; Morris et al., 2014; Zocher et al., 2021).

Initially, conditional knockout models for Dnmt1 or Dnmt3a did not result in spatial memory impairments (Feng et al., 2010). However, in another study Dnmt3a knock-out mice exhibited spatial memory impairments (Morris et al., 2014). The reasons for the discrepancy in the findings are likely attributed to the different age and genetic background of the mice. Notably, the infusion of a DNA methylation inhibitor into the CA1 region disrupted the firing of place cells and the long-term stability, while leaving the short-term stability of place cell firing intact (Roth et al., 2015). Subsequent studies that specifically reduced one isoform of Dnmt3a – Dnmt3a2 – in the adult mouse hippocampus showed that this reduction caused spatial memory and fear extinction impairments (A. M. M. Oliveira et al., 2012, 2016). Additionally, infusing siRNAs against *Dnmt3a* in the rat hippocampus also resulted in impaired long-term memory (Mitchnick et al., 2015). Further, DNA methylation processes have been proven to be crucial for cognitive mechanisms and that DNA methylation dysregulation occurs in pathological circumstances such as neurodevelopmental (Pohodich & Zoghbi, 2015) and neurodegenerative diseases (De Jager et al., 2014), psychiatric disorders (Gavin & Sharma, 2010), drug addiction (Cannella et al., 2018; LaPlant et al., 2010), and chronic pain (A. M. Oliveira et al., 2019). This emphasizes the significance of further investigating how DNA methylation regulates long-term adaptations.

To understand the molecular mechanisms underlying the impact of DNA methylation on memory and synaptic strength, researchers investigated the link between DNA methylation and transcriptional regulation. Numerous studies have reported the changes in DNA methylation patterns in memory- and plasticity-related gene loci upon neuronal activity. For example, the promoter of the Reelin gene, a positive regulator of memory, was demethylated, leading to an increased expression. In contrast, the promoter of the phosphatase PP1 gene, a negative regulator of memory, was further methylated, resulting in decreased expression (Miller & Sweatt, 2007). Although many studies focused on specific gene promoters, the majority of activity-dependent DNA methylation changes occurred outside of gene promoters (Duke et al., 2017; Guo et al., 2011; Halder et al., 2015). In summary, these findings suggest a strong link between DNA methylation-related mechanisms and activity-

dependent modifications of the transcriptome and underlie that epigenetic mechanisms play a crucial role in neuroplasticity processes necessary for long-term memory storage.

3. Cellular mechanisms underlying memory processes

Memory is the ability to acquire, store, and retrieve information based on experience, and it plays a crucial role in adaptive behaviour. Plato had already speculated that memory leaves imprints of our perceptions and thoughts. In the 20th century, Richard Semon's "engram theory" and Donald Hebb's "synaptic plasticity theory" made significant contributions to understanding the physical basis of memory. According to Semon, an engram is a physical representation of memory in the brain, characterized by enduring changes, the ability to be recalled based on cues, reflecting the content of the memory, and the potential to exist in a dormant state. However, engrams are not fixed, as consolidation and retrieval processes can modify them (Josselyn et al., 2015; Josselyn & Tonegawa, 2020). 30 years before Semon postulated his theory, Ramón y Cajal pioneered and identified areas in the brain where learning-induced changes may take place. According to him, neuronal connections might change over time and experience-related changes would affect dendritic spines, which protrude from neurons (Ramon y Cajal, 1894). In 1949, Hebb's theory proposed that learning leads to the formation of associations among neurons, known as "cell assemblies" or neuronal ensembles, and strengthening synaptic connections facilitates the formation of these ensembles – these cells would “fire together, wire together”. He also suggested that memory retrieval can occur even when only a part of the initial cell assembly is activated (Hebb, 1949). The concept of neuronal ensembles, influenced by Semon's theory, has had a significant impact on modern neuroscience and memory research. Karl Lashley conducted lesion studies in rodents to identify the brain regions involved in memory, but he did not find specific regions responsible for memory impairment, suggesting that ensembles encoding specific memories are distributed throughout the brain (Lashley, 1950). However, subsequent research in humans revealed some specificity in the involvement of certain brain regions in episodic memory. Penfield and Rasmussen's study involved stimulating parts of the lateral temporal cortex in patients, which led to the recall of random episodic memories. Additionally, the case of patient H.M., whose hippocampi was removed, resulting in severe amnesia, provided further evidence for the importance of specific brain regions in memory formation (Scoville & Milner, 2000). The current definition of the engram involves physical and biochemical

changes between active neurons during learning, thus, forming engram cells across different brain regions. In essence, the engram is considered the neuronal substrate of memory.

3.1 Engram technology to find the memory trace

Studies have demonstrated that neuronal activation, either through pharmacological stimulation or behavioural tasks, leads to the upregulation of IEGs such as Arc, cFos, and Egr-1 in different regions of the brain (Guzowski et al., 1999; Ramírez-Amaya et al., 2005; Vann et al., 2000). This indicates a connection between neuronal activity and IEG expression. One particular IEG, Arc, exhibits a specific temporal-spatial distribution of its mRNA upon neuronal activation. It first accumulates in the nucleus within 5 minutes and is then exported to the cytosol within 30 minutes. Using a method called "cellular compartment analysis of temporal activity by fluorescent in situ hybridization" (catFISH), researchers can detect and estimate the approximate time of activation for these neurons.

To address Semon's concept of engram reactivation, experiments were conducted with rats in which they were placed back in a previously explored context or a novel context 20 minutes after the initial exploration. It was found that approximately 90% of the neurons that were activated during the first session were also reactivated during the second session when the rats explored the same environment. However, when the rats explored a novel environment, a different set of neurons were activated, indicating that a specific subpopulation of CA1 neurons was associated with encoding and retrieving information about the explored environment (Guzowski et al., 1999). This finding suggests that activity-dependent IEG expression reflects spatial information and that distinct environments are encoded by distinct subpopulations of neuronal ensembles.

However, the monitoring of neuronal ensembles at extended intervals following the behavioural experience is limited by the short half-life of the IEG transcripts. In order to allow visualisation and manipulation at later time points, the neurons active during encoding need to be marked over an extended period of time. Neuronal capture techniques have been designed employing IEG promoters and taking advantage of activity-regulation to trigger transcription of genetically encoded labels to a temporal interval (Denny et al., 2014; Guenthner et al., 2013; Reijmers et al., 2007). Noteworthy, the tagging window (from hours to days) far outlasts the training period. Therefore, it is likely that these capture techniques overestimate the size of the neuronal ensembles that encode information, a phenomenon known as "over-tagging"

(Josselyn et al., 2015). Tetracycline activator (tTA) expression in the "TetTag" mouse strain is driven by an IEG promoter and regulates the expression of a reporter under the tetracycline responsive element (TRE) promoter. By engineering tTA binding to be regulated by doxycycline, temporal specificity is achieved (Reijmers et al., 2007). Another approach is the “targeted recombination in active populations” (TRAP) system, that takes advantage of a tamoxifen-inducible Cre recombinase ($CreER^{T2}$) system (Denny et al., 2014; Guenthner et al., 2013). When tamoxifen is present, $CreER^{T2}$ translocates to the nucleus and permits the permanent production of EGFP.

Researchers used the TetTag transgenic mouse line to investigate neuronal ensembles involved in fear learning and memory recall. They trained mice without doxycycline to tag activated neurons and found that a significant proportion of these neurons were reactivated during memory recall in the basolateral amygdala (BLA). The reactivation of neurons was positively correlated with long-term fear memory performance, indicating a stable representation of the initial memory trace (Reijmers et al., 2007). Another study using TRAP mouse lines examined hippocampal subregions and found that both the dentate gyrus (DG) and CA3 reactivated a higher percentage of neurons during memory recall in the conditioning context compared to a novel context, but the context-specific engagement of DG was lost 30 days after the fear experience (Denny et al., 2014).

Several research groups have made efforts to enhance neuronal ensemble-reporter systems and develop optimized synthetic activity-dependent promoters for tracking IEG expression. The Synaptic Activity-Responsive Element (SARE) was identified ~7 kb upstream of the Arc gene. It has a distinct genomic structure with binding sites for three major activity-dependent transcription factors (CREB; MEF2; SRF/TCF) and showed robust responsiveness to neuronal stimuli. By combining multiple tandem repeats of this sequence with an appropriate linker, an enhanced SARE promoter was created (ESARE), resulting in significantly higher reporter expression levels compared to the cFos promoter (Kawashima et al., 2013). However, one limitation of this system is the lack of temporal control. Another tool, the Robust Activity Marking (RAM) system, was developed to address this limitation. The RAM system uses a synthetic promoter based on IEGs with an optimized activity-dependent induction profile, allowing improved temporal control of effector gene expression. It incorporates an enhancer module containing AP-1 and Npas4 binding sites upstream of the human FOS minimal promoter, resulting in the synthetic pRAM promoter with the largest fold induction among tested activity-dependent promoters. Additionally, a destabilized version of tTA (d2tTA) was developed to overcome issues with

conventional Tet-Tag systems (Sørensen et al., 2016). The development of these enhanced promoters and tools provides a growing repertoire of resources for memory research and the study of neuronal ensembles.

The correlational investigations were highly informative but they had several limitations in terms of demonstrating the functional causality engram neurons for cognitive processes. To prove that learning-activated neurons are both sufficient and necessary to drive memory recall, as well as that the tagged engram is an accurate representation of memory, causality studies had to be done.

3.2 Engram manipulation using gain- and loss-of-function approaches

By combining tagging techniques with methods to artificially reactivate a specific subpopulation of cells, the content of the particular memory and its behavioural outcome can be predicted. Pioneering work from the Tonegawa laboratory used the TetTag system and combined it with channelrhodopsin 2 (ChR2) mediated optogenetics (Liu et al., 2012) which allowed them to control the activity of the learning-activated neurons at a later time point via light stimulation. Strikingly, when DG ensemble neurons encoding a fear memory of a specific context were stimulated artificially by light in a distinct, neutral context, which has never been paired with foot shock, mice showed freezing behaviour (Liu et al., 2012). Neuronal ensembles can be artificially reactivated not just in the hippocampus but also in other parts of the brain. It was demonstrated that a subset of BLA neurons was more likely to be incorporated into the memory trace when their neuronal activity was artificially increased by the activation of designer receptors exclusively activated by designer drugs (DREADDs), hM3Dq (Yiu et al., 2014). Additionally, synthetically reactivation of hM3Dq-expressing neurons at memory recall was sufficient to induce freezing behaviour. Thus, this demonstrated that the artificially-activated neurons were not simply tentatively a part of the engram, but actually stored the memory information (Yiu et al., 2014). Additionally, neurons in the retrosplenial cortex tagged during CFC, were able to elicit fear memory recall when reactivated (Cowansage et al., 2014).

The advanced technology in engram research allows not only the identification and manipulation of engram neurons, but even a false memory can be created and the valence of a memory can be switched. DG ensembles were marked while context exploring in context A using the TetTag technique and optogenetics. The next day, while employing doxycycline to close the tagging window, mice were shocked in context B while DG ensembles for context A were artificially activated in parallel. Even though they never got a foot shock in context A, animals displayed

freezing behaviour when exposed to this context on day 3 (Ramirez et al., 2013). To rule out the possibility that freezing occurred due to fear generalisation, mice were put in context C and no freezing was observed (Ramirez et al., 2013). Additional studies using this approach showed that optical reactivation of the engram was effective enough to replace negative experiences with positive ones (Ramirez et al., 2013) or vice versa as a rewarding context was switched to a fearful memory (Redondo et al., 2014), as well as to trigger memory recall in a mouse model of Alzheimer's disease (Roy et al., 2016) or during retrograde amnesia (Ryan et al., 2015). Remarkably, Vetere and colleagues were able to elicit a behavioural associative learning response in the absence of an existing sensory cue by optically stimulating the connected brain circuitry (Vetere et al., 2019).

To answer whether engram activity is necessary for a memory recall neuronal ensembles formed upon learning were optogenetically or chemogenetically silenced during memory consolidation or recall. Using the ArcTRAP system, neuronal ensembles active during CFC in the DG or CA3 were tagged and subsequently inactivated using the Cre-dependent light-sensitive neuronal silencer, ArchT which resulted in impaired memory recall (Denny et al., 2014). Similar to this study, inactivation of cFos positive ensembles in the dorsal CA1 expressing Arch-T showed impairment of fear memory recall (Tanaka et al., 2014). Importantly, this research revealed that inhibiting the putative engram impairs memory recall but did not prevent the development of new memories of similar content (Matsuo, 2015; Tanaka et al., 2014). Manipulating the engram is also possible in a contextual memory associated with a positive reinforcer such as cocaine. After learning the context-drug administration, Daun02 injection, that killed previously tagged engram cells, suppressed the context-specific cocaine-induced psychomotor sensitisation in rats (Koya et al., 2009), indicating that inhibiting specific neuronal ensembles impairs a specific memory.

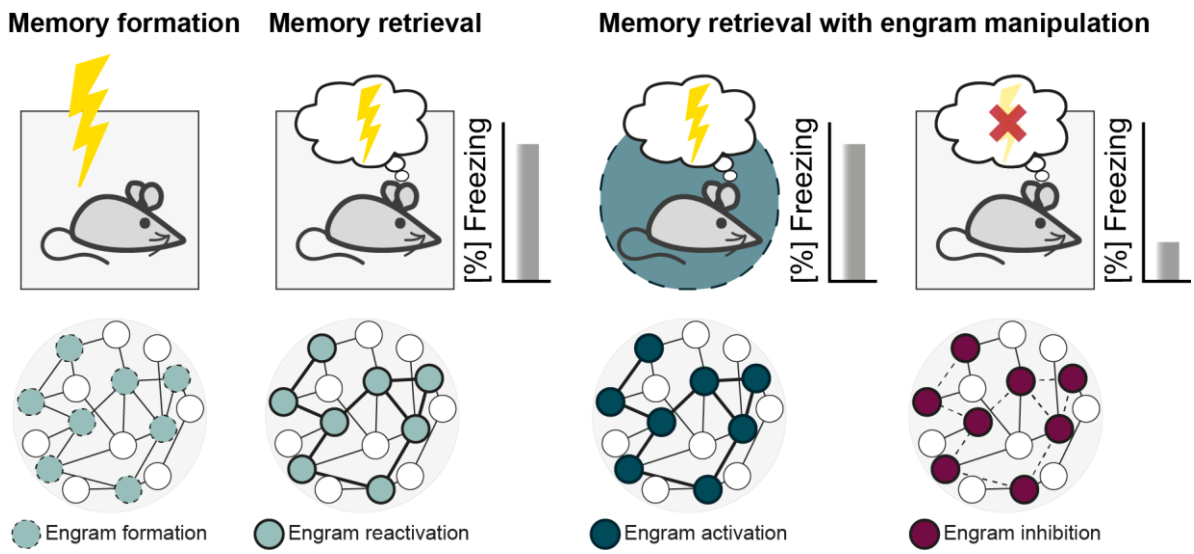


Figure 2. Engram – the cellular substrate of memory. During memory formation, such as in contextual fear conditioning, widely distributed engram neurons are activated. Reactivation of these engrams leads to successful memory retrieval, as indicated by high freezing behaviour. Artificial activation of the engram, through methods like chemo- or optogenetic stimulation, serves as a sufficient cue for memory retrieval and results in freezing behaviour even in a neutral context. On the other hand, if the tagged engram is silenced when mice are returned to the training context, memory retrieval is blocked and the mice exhibit reduced freezing.

Taken together, these studies revealed that neuronal ensembles are active during learning and reactivated during memory recall and their activity is both required for and sufficient for recovering the memory information.

3.3 Structural, functional and transcriptional changes in engram neurons

Engram tagging studies revealed not only that they encode the cellular substrate for a memory, but also that the specific neuronal ensembles involved in memory encoding exhibited notable synaptic changes compared to non-engram cells. These changes included enhanced synaptic strength, potentiated synapses, and increased density of dendritic spines (Ryan et al., 2015). Furthermore, the researchers discovered that inhibiting transcription disrupted the observed synaptic potentiation and impaired memory formation (Ryan et al., 2015). Another study indicated that synaptic potentiation happens selectively in engram neurons of the amygdala upon fear conditioning (Gouty-Colomer et al., 2016). A recent study demonstrated that synaptic strengthening happens selectively between co-active neurons, supporting the Hebbian hypothesis. Using a tool called dual-eGRASP, Choi et al. (2018) labelled individual synapses from non-engram and engram neurons. This allowed them to show that dendritic spine morphology and density were changes in engram-to-engram synapses during consolidation in the CA3-CA1 region. They further

investigated a correlation between engram-to-engram connectivity and memory strength by varying the number and intensity of foot shocks during contextual fear conditioning (Choi et al., 2018). These findings provide valuable insights into the role of engram cells and indicate that during memory consolidation, engram-allocated neurons undergo synapse-specific coordinated structural and functional changes across different brain regions.

Studies have sought to understand the molecular mechanisms involved in maintaining neuronal ensembles during memory consolidation. It is hypothesized that synaptic connections between the active neurons during encoding are strengthened to reconstruct the activity pattern during memory retrieval. Thus, gene transcription and protein synthesis likely regulate this process in engram neurons. Transcriptomic analysis of behaviourally-allocated neuronal ensembles in the DG identified increased expression of IEGs and genes related to the MAPK pathway and post-synaptic density (Lacar et al., 2016). Further, gene expression profiles at different time points after behavioural experiences revealed distinct gene categories: i) early signature genes, that were enriched for IEGs and CREB target genes, ii) sustained signature genes (including transcription factors and epigenetic regulators) and iii) late signature genes (related to dendritic structure and the extracellular matrix) (Jaeger et al., 2018; Rao-Ruiz et al., 2019). Overall, learning triggers long-lasting transcriptional changes in engram neurons.

Nevertheless, transcriptional changes are not the only alterations occurring in engram neurons upon learning. We know that specific epigenetic and 3D-genomic changes play a critical role in the contributions to memory. Therefore, Fernandez-Albert et al. (2019) developed a new approach to investigate the transcriptome and epigenome of engram neurons in the mouse brain using fluorescent-activated nuclear sorting (FANS) and ATAC-seq. They found unique changes in chromatin accessibility and transcription factor binding at genes known to have a function in neuronal plasticity and memory formation processes in engram cells (Fernandez-Albert et al., 2019). Exploration of the epigenomic, genomic architecture and transcriptomic profiles of ensemble neurons revealed that the learning phase led to increased genome accessibility and stable changes in enhancers and whereas, memory encoding triggered an epigenetic priming event without immediate transcriptional changes (Marco et al., 2020). Further during consolidation, large chromatin segments rearranged, bringing promoters and enhancers in proximity and engram reactivation involved specific long-range interactions, where primed enhancers connect with their corresponding promoters to induce transcription (Marco et al., 2020). These findings

highlight the importance to investigate spatial reorganization of chromatin segments and promoter-enhancer interactions in engram neurons to fully grasps the effect on memory.

However, little research was done to investigate whether these transcriptional changes are cause or consequence and thereby lack causality. Rao-Ruiz et al. (2019) confirmed the relevance of CREB-dependent gene expression for memory storage and found overexpressing a dominant negative form of CREB within the fear-encoding DG neuronal ensembles led to impairments in long-term memory (Rao-Ruiz et al., 2019). Further, Matos et al. (2019) demonstrated that activity-dependent expression of this CREB variant disrupted remote memory recall (Matos et al., 2019). And a recent study in our laboratory investigated the impact of epigenomic changes in engram neurons on memory storage (Gulmez Karaca et al., 2020). For this, Dnmt3a2 levels were specifically enhanced in the DG ensemble neurons and examined the effects on memory performance. We showed that reinforcing DNA methylation-related mechanisms in DG engram, but not in non-engram neurons, strengthened long-term memory and improved engram reactivation. Whole-genome bisulfite sequencing (WGBS) revealed that activity-dependent Dnmt3a2 expression modified the methylome of genes involved in synaptic plasticity and memory processes (Gulmez Karaca et al., 2020). These findings demonstrate that epigenetic factors, particularly DNA methylation in engram neurons or CREB-mediated changes, play a crucial role in influencing memory consolidation and the fidelity of engram reactivation during memory recall.

4. Memory persistence

Memory persistence is a key characteristic of some memories and plays a crucial role in shaping our identity and behaviour. The consolidation of these memories occurs over extended periods and involves multiple brain regions (Frankland & Bontempi, 2005). Early studies in psychology demonstrated that recently formed memories were more vulnerable to disruption compared to remote memories (Ribot and Smith, 1882). Memory consolidation refers to the process by which memories stabilise in order to persist for long periods of time (Müller and Pilzecker, 1900). Consolidation is now understood to involve two main stages: cellular or synaptic consolidation and systems consolidation. Cellular consolidation occurs locally in specific brain regions and lasts for minutes to hours, resulting in the restructuring of synaptic connections in activated neurons. Systems consolidation, on the other hand, is a gradual process that takes weeks, months or even years. Despite its

significance, the exact mechanism and brain structures involved in the formation of persistent memories remain largely unknown.

4.1 Systems consolidation theory and engram studies in remote memory

The traditional theory of systems consolidation proposed that the hippocampus is a temporary memory structure necessary for memory formation, while long-lasting memory storage occurs in cortical regions (Wiltgen et al., 2004). According to this theory, during learning, only parahippocampal regions are activated and the hippocampus and cortical regions are weakly connected. Over time, reinforcement processes like replay and sleep, strengthen the hippocampal-cortex connections, thus the memory is no longer only dependent on the hippocampus but further on cortical structures (Alvarez & Squire, 1994; Squire & Wixted, 2011). This theory was supported by studies showing that damage to the hippocampus impairs recently acquired memories but spares distant memories (Bayley et al., 2003; Gonzalez et al., 2013; Scoville & Milner, 2000) and lesioning pre-frontal cortical areas impairs remote but not recent memories (Fitzgerald et al., 2015; Frankland et al., 2004). However, advancements in technology have allowed for more precise investigations of memory consolidation, challenging this traditional view.

A variety of different studies showed that neuronal ensembles are already active in cortical regions during memory encoding (Bero et al., 2014; Cowansage et al., 2014; Kitamura et al., 2017; Lesburguères et al., 2011; Tayler et al., 2013). Precise temporal control of inhibition of the CA1 in the hippocampus showed that blocking hippocampal activity during memory retrieval impaired not only recent but also remote memories (Goshen et al., 2011).

A key study from the Tonegawa group proposed a new concept in 2017. Their study aimed to investigate the neuronal circuit involved in the formation and maturation of cortical memories in interaction with the hippocampus-entorhinal cortex (HPC-EC) network (Kitamura et al., 2017). They examined the nature and dynamics of engram cells in the prefrontal cortex (PFC) and the circuits supporting memory consolidation. The findings revealed that memory engram cells in the PFC were rapidly formed during the initial day of training but were not reactivatable through natural recall cues. However, over the following weeks, these immature PFC engram neurons underwent functional, structural, and physiological maturation, which relied on inputs from the HPC-EC axis. Over time, hippocampal engram cells became silent and were not retrieved by natural recall cues on day 14. The proposed model suggests

that the PFC engram neurons are formed on the first day of training through inputs from the HPC-EC network and the BLA, although they are in an immature state. In contrast to the standard systems consolidation model, which assumes a gradual transfer of memory from the hippocampus to the cortex, this study highlights the role of the hippocampus in the rapid generation of immature engram cells in the PFC during training and their subsequent functional maturation (Kitamura et al., 2017).

4.2 Molecular mechanisms underlying memory persistence

A pioneer study in 1999 demonstrated that long-lasting activity in the hippocampus is necessary for the consolidation of long-term memory (Riedel et al., 1999). Subsequent studies confirmed this and showed that AMPA and NMDA receptors are upregulated upon learning and that this contributes to memory

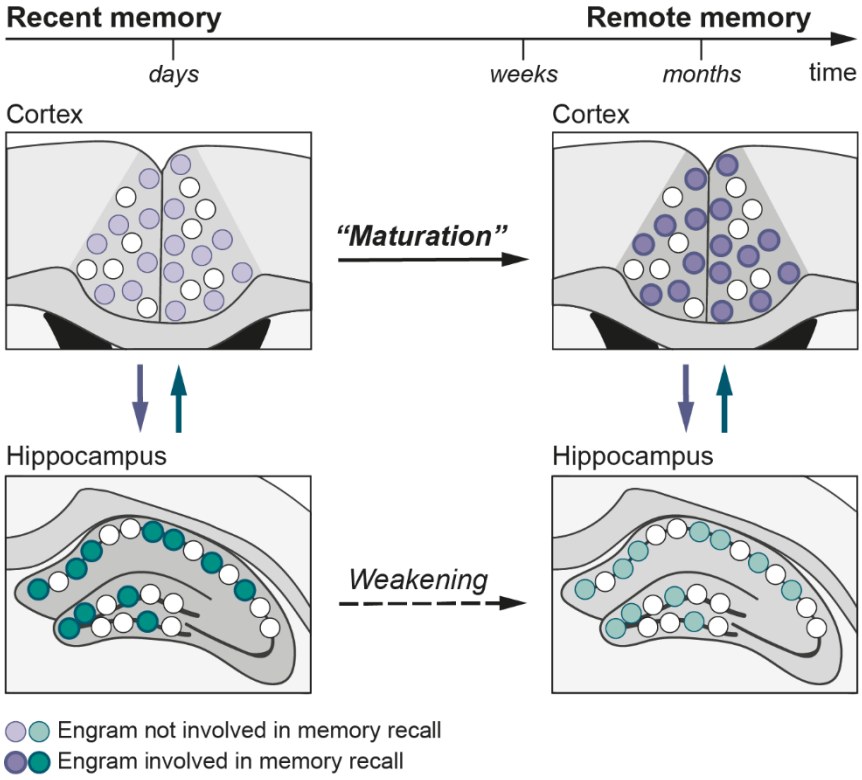


Figure 3. Systems consolidation at the engram level. Memory formation leads to the formation of engrams throughout the entire brain. During memory recall, retrieval cues reactivate the hippocampal engram, facilitating the recall of recent memories. In contrast, the cortical engram remains inactive and cannot be naturally reactivated by cues. However, over time, during the process of system consolidation, the connections of the hippocampal engram weaken while the cortical engram matures. As a result, natural retrieval cues can reactivate the cortical engram during remote memory recall sessions.

maintenance (Mitsushima et al., 2011). The activation of these receptors is dependent on input from other brain regions, with dopaminergic signalling playing a crucial role in the long-term consolidation of memories (Rossato et al., 2009). Recent research has also revealed the involvement of parvalbumin interneurons in the hippocampus

in the consolidation of remote memories, with two distinct phases of activation and signalling contributing to memory persistence (Karunakaran et al., 2016). Neuronal activity triggers molecular changes in plasticity that are crucial for memory formation. These changes involve the activation of signalling cascades and the expression of IEGs. Studies have shown that remote memory consolidation involves similar signalling events and gene expression patterns as memory formation, albeit occurring at different time points. Activation of ERK1/2 occurs in two waves after learning, with the second wave persisting for several hours (Bekinschtein et al., 2008; Trifilieff et al., 2006; TRIFILIEFF et al., 2007). Reactivation of memory through a process called reconsolidation also contributes to memory persistence, with ERK1/2 activation occurring up to 3 hours after memory reactivation (Krawczyk et al., 2016). Delayed gene expression plays a crucial role in remote memory formation. Genes such as Arc and BDNF are upregulated several hours after learning and are necessary for memory persistence. Inhibition of this delayed gene expression impairs memory persistence without affecting memory formation (Bekinschtein et al., 2007, 2008; Ramírez-Amaya et al., 2005).

These findings suggest that the initial process of memory consolidation after a learning event involves signalling cascades that lead to transcriptional changes of memory-related genes that are mostly identical, independently of the longevity of the memory. This supports the hypothesis that additional mechanisms must occur to stabilise the physical substrate of memory. Likely, these mechanisms involve cellular processes within memory-related brain regions that induce communication and maturation process crucial for systems consolidation. Prime candidates are molecular processes that are dynamic but also have the capacity to regulate plasticity-related events over long-time scales. Epigenetic signatures have recently emerged as regulators of synaptic plasticity and memory consolidation (Campbell & Wood, 2019; Sweatt, 2009). In fact, studies of the epigenome, 3D genome architecture and transcriptome of engram neurons showed that learning can trigger chromatin accessibility changes at target genes. These accessibility changes can even persist throughout the late consolidation phase, suggesting a chromatin-based “priming” event that precedes transcriptional changes during memory retrieval (Marco et al., 2020). In addition, different neuronal stimuli seemed to uniquely alter the epigenetic state of genes functionally associated with neuronal plasticity and memory formation (Coda & Gräff, 2020; Fernandez-Albert et al., 2019)

Recent advancements in technology have emphasized the significance of cortical regions during memory formation, while also emphasizing the role of the

hippocampus during memory retrieval, both for recent and remote memories. These findings challenge previous theories that suggested the hippocampus becomes less critical as memories age. It is hypothesized that neocortical regions play a crucial role in this process, while the hippocampus facilitates the transfer of information from short-term to long-term memory storage and epigenetic mechanisms may be involved in regulating the communication between the hippocampus and cortex and the stabilisation of engrams for memory consolidation. Long-lasting forms of memory were previously associated with persistent DNA methylation changes in the PFC and genes that undergo DNA methylation changes in the cortex are functionally associated with structural changes, supporting the idea that DNA methylation plays a role in the rewiring of cortical networks necessary for memory stabilization (Halder et al., 2015; Miller et al., 2010).

However, whether long-lasting memory establishes a unique epigenome code, that drives processes known to be crucial for memory persistence remains to be understood. Furthermore, questions remain regarding the involvement of epigenetic mechanisms, including DNA methylation, in the causal link between memory persistence and engram stabilisation and processes underlying systems consolidation.

5. Memory suppression

Over the past decades, research on learning and memory has mainly focused on understanding the molecules and genes that promote these processes. However, studies have revealed the existence of memory suppressor genes, whose normal function is to limit memory formation. These molecules act as constraints on memory processes (reviewed in (Abel & Kandel, 1998; Cardin & Abel, 1999; Noyes et al., 2021) and understanding memory suppressors is crucial as it provides insights into the neurophysiological mechanisms that regulate memory formation and identifies potential targets for cognitive enhancement or memory-related disorders. This research is particularly relevant for understanding conditions like forms of autism spectrum disorder and post-traumatic stress disorder that involve exceptional or disrupted memory abilities in humans.

How molecules might act as memory suppressors is summarised below: Early research identified ApCREB2 as a memory suppressor molecule in Aplysia, as it represses CREB function. Inhibiting ApCREB2 activity allows for the formation of LTF with single serotonin application (Bartsch et al., 1995). This suggests that ApCREB2 suppresses LTF formation. Studies in mice, such as those involving ATF4,

support the idea that negative regulators of CREB act as memory suppressors (A. Chen et al., 2003; Yin et al., 1994). Further, studies in mice have shown that the loss of ICER (a CREB repressor) enhances weak fear memory (Kojima et al., 2008), indicating its role in preventing weak events from being consolidated into long-term memory. Overexpression of ICER impairs long-term fear memory but not short-term memory (Mioduszewska et al., 2003), further supporting its function as a memory suppressor. Besides ICER, other genes and gene products act as consolidation suppressors by limiting the activation of CREB. The negative regulation of cAMP levels by cyclic phosphodiesterases (PDEs), which prevent excessive cAMP elevation during memory consolidation is such a suppressor. Reduction of PDE activity facilitates long-term memory formation (Hansen et al., 2014; Y.-F. Li et al., 2011; RUTTEN et al., 2006; Scheunemann et al., 2018) and rescues age-dependent cognitive decline in object recognition (Wimmer et al., 2020). Another control point for CREB activity involves negative regulators of PKA. While the role of PKA inhibitors (PKA-I) as memory suppressors is less established, reductions in PKIa mRNA levels suggest that learning may relieve PKIa inhibition, thus promoting PKA-dependent consolidation (de Lecea et al., 1998). Proteins involved in regulating translation serve as control points for consolidation. One such control point is the translation initiation factor eIF2a. Following contextual fear conditioning, eIF2a is rapidly dephosphorylated in the mouse hippocampus, leading to its activation and promoting memory formation (Costa-Mattioli et al., 2007). Phosphorylation of eIF2a suppresses memory formation by either decreasing the production of proteins that promote memory (Boye & Grallert, 2020) or by increasing ATF4 – a CREB inhibitor (Lu et al., 2004; Vattem & Wek, 2004). Memory suppressors control further factors such as PABP (Gray, 2000; Khoutorsky et al., 2013) and mTOR (Hoeffer & Klann, 2010; Jobim et al., 2012) to regulate translation and influence memory formation and synaptic plasticity.

Memory suppressors reduce overload, that allows the brain to focus on essential and important information necessary for optimal evolutionary fitness. However, exceptional cases of individuals with remarkable memories challenge this explanation (Brandt & Bakker, 2018), suggesting that capacity limitations may not be the sole reason for memory suppressors. Memory suppression enables behavioural flexibility in changing environments by allowing the suppression of memories underlying non-adaptive behaviours (Richards & Frankland, 2017). Studies on flies and mice support this idea, showing that memory suppressors play a role in adjusting behaviour to new situations and facilitating behavioural flexibility and inhibiting certain memory suppressor genes enhances both memory and behavioural flexibility

(Berry et al., 2012; X. Chen et al., 2017; Hawasli et al., 2007; Venkitaramani et al., 2011; Zhu et al., 2011). Accurate associations may also be promoted by memory suppression by accepting only authentic associations and reducing the likelihood of forming relationships between unrelated items. Experimental data in mice and flies support this idea, demonstrating the impact of memory suppressors on association strength (Engin et al., 2015), trace conditioning (C. Fan et al., 2020; Konopka et al., 2010) and stimulus generalisation (Yamagata et al., 2021). Furthermore, dysfunction of some memory suppressors can disrupt social behaviour, highlighting their involvement in sociability and social recognition (Blázquez et al., 2019; C. Fan et al., 2020; Ishimoto & Kamikouchi, 2020; Morimura et al., 2017; Venkitaramani et al., 2011).

Overall, research on memory suppressors suggests that they play important roles in various memory operations. While the biological need for limiting and balancing memory formation is evident, further studies are needed to fully understand the mechanisms of action and the reasons behind the existence of memory suppressors, including their role in facilitating behavioural flexibility, promoting accurate memories and modulating social behaviours.

6. Scope of the thesis

The overarching aims of this thesis were to investigate the roles of the different Dnmt3a isoforms in long-term memory formation and to shed light on the molecular mechanisms that control memory persistence and to explore the mechanisms of memory suppression. Specifically, I aimed to determine whether Dnmt3a isoforms have distinct or overlapping functions in memory formation and to identify activity-regulated Dnmt3a-isoform-specific downstream effectors required for memory formation. Additionally, I aimed to elucidate the role of DNA methylation in facilitating the transfer of information from the hippocampus to the cortex for long-term storage (systems consolidation) and its impact on memory duration and engram stabilization. Finally, I set out to explore the mechanisms of memory suppression. Specifically, I investigated the impact of experience salience on the expression of the immediate early gene Npas4 and its role as a memory suppressor. Overall, this study advances our understanding of the intricate mechanisms that regulate memory formation, persistence and suppression and highlights the importance of epigenetic regulation and immediate early genes in these processes.

II. Results

In my thesis I aimed to uncover whether individual DNA methyltransferases (Dnmts) have unique or overlapping roles in long-term memory formation. I found that despite a common requirement for memory formation, Dnmt3a1 and Dnmt3a2 regulate this process via distinct mechanisms. I identified an activity-regulated Dnmt3a1-dependent genomic program and showed that one of these targets, Neuropilin-1 (Nrp1) is required for memory formation. Furthermore, Nrp1 overexpression rescued Dnmt3a1, but not Dnmt3a2, knockdown-driven impairments in memory formation. Thereby revealing a Dnmt3a isoform-specific mechanism in memory formation that underscored the intricate and finely regulated functions of distinct epigenetic regulators in brain function (**Section II 1.** *Dnmt3a1 regulates hippocampus-dependent memory via the downstream target Nrp1*).

Next, I focused on the unknown underlying molecular mechanisms of memory persistence. Since DNA methylation can act as a long-term regulatory signal, it is a prime candidate to regulate memory duration and to stabilise the supporting engram – the physical substrate of a memory. I uncovered that overexpression of Dnmt3a2 in the hippocampus converts short-lasting into long-lasting memory. Moreover, I found an improved reactivation of cortical engrams and increased fear generalisation, mimicking the engram dynamics and behavioural trait of remote memory, respectively. Further, using chemogenetic inhibition of the cortical engram, I proved that the memory trace resides in the cortex. These findings demonstrate that DNA methylation processes facilitate the transfer of information from the hippocampus to the cortex for long-term storage (**Section II 2.** *DNA methylation promotes memory persistence by facilitating systems consolidation and cortical engram stabilisation*).

And lastly, I focused on the topic of memory suppression since the brain is not only equipped with molecular mechanisms that promote, but actively constrain memory formation. This is crucial for behaviour flexibility. I found that high experience salience evokes a biphasic expression of the immediate early gene (IEG) Neuronal PAS Domain Protein 4 (Npas4) several hours after learning. Experiments in which I pharmacologically inhibited or genetically promoted the induction of the biphasic Npas4 expression in a temporally specific manner revealed that Npas4 has a previous unknown function as a memory suppressor gene (**Section II 3.** *High salient fear memory induces a biphasic Npas4 expression and results in memory suppression*).

The **Section II 1.** *Dnmt3a1 regulates hippocampus-dependent memory via the downstream target Nrp1* is published on a preprint server (Kupke et al., 2023). In this, the section about Dnmt3a1 in memory formation is part of two Master thesis. Contribution of co-authors to data acquisition or analysis are stated in the Figure legends.

The **Section II 3.** *High salient fear memory induces a biphasic Npas4 expression and results in memory suppression* is part of a co-first authored manuscript currently (10.07.2023) in revision in Molecular Psychiatry. It contains parts of the doctoral thesis of David V.C. Brito.

Contribution of co-authors to data acquisition or analysis are stated in the Figure legends.

1. Dnmt3a1 regulates hippocampus-dependent memory via the downstream target Nrp1

In this part of my thesis, my aim was to examine the role of Dnmt3a1's neuronal function in memory formation. To accomplish this, I used loss-of-function approaches to selectively reduce the levels of Dnmt3a1 and investigate the impact on cognition as well as RNA sequencing analysis and rescue experiments to identify downstream mechanisms.

1.1 Dnmt3a1 is required for long-term memory formation

First, two shRNAs were designed to knockdown Dnmt3a1 specifically. The efficiency of the shRNAs was confirmed by transfection of primary hippocampal neurons and immunostaining against endogenous Dnmt3a1 (Figure 4A). To further confirm the knockdown efficiency in living organisms, recombinant adeno-associated viruses (rAVV) were generated (Figure 4B) and stereotaxically delivered into the dorsal hippocampus (dHPC) of mice. rAAV1/2 serotype was used due to its established neuronal tropism (R. Xu et al., 2001). In order to track the viral expression, the viral constructs included a GFP marker regulated by the chicken-beta-actin promoter (Figure 4B, C). qRT-PCR and western blot analysis of the dHPC from mice infected with the viral constructs were done and confirmed that both shRNA1 and shRNA2 effectively reduced Dnmt3a1 expression at both the mRNA (Figure 4D, E) and protein (Figure 4F-I) levels, while not affecting other Dnmts (Figure 4D, E).

To assess the importance of Dnmt3a1 in memory performance, memory tests that rely on the hippocampus were performed three weeks after the stereotaxic surgery (Figure 5A). Mice underwent the spatial object recognition test in which they have a habituation session of 6 minutes and can freely explore the training apparatus. Then during training, the mice were exposed to two distinct objects and allowed to explore them for 6 minutes during 3 trials with 3-minute inter-trial intervals. During the testing session, one object was moved to a new location and the preference for the moved object was scored. In the spatial object recognition test, when tested 24 hours after training, mice injected with either Dnmt3a1-shRNA1 or -shRNA2 showed no preference for the displaced object (Figure 5B). However, when evaluated 1 hour after training, mice in the shRNA groups spent a comparable amount of time exploring the displaced object as the control group (Figure 5C). Importantly, the knockdown of Dnmt3a1 did not affect the overall exploration time of the objects during training (Figure 5D, E).

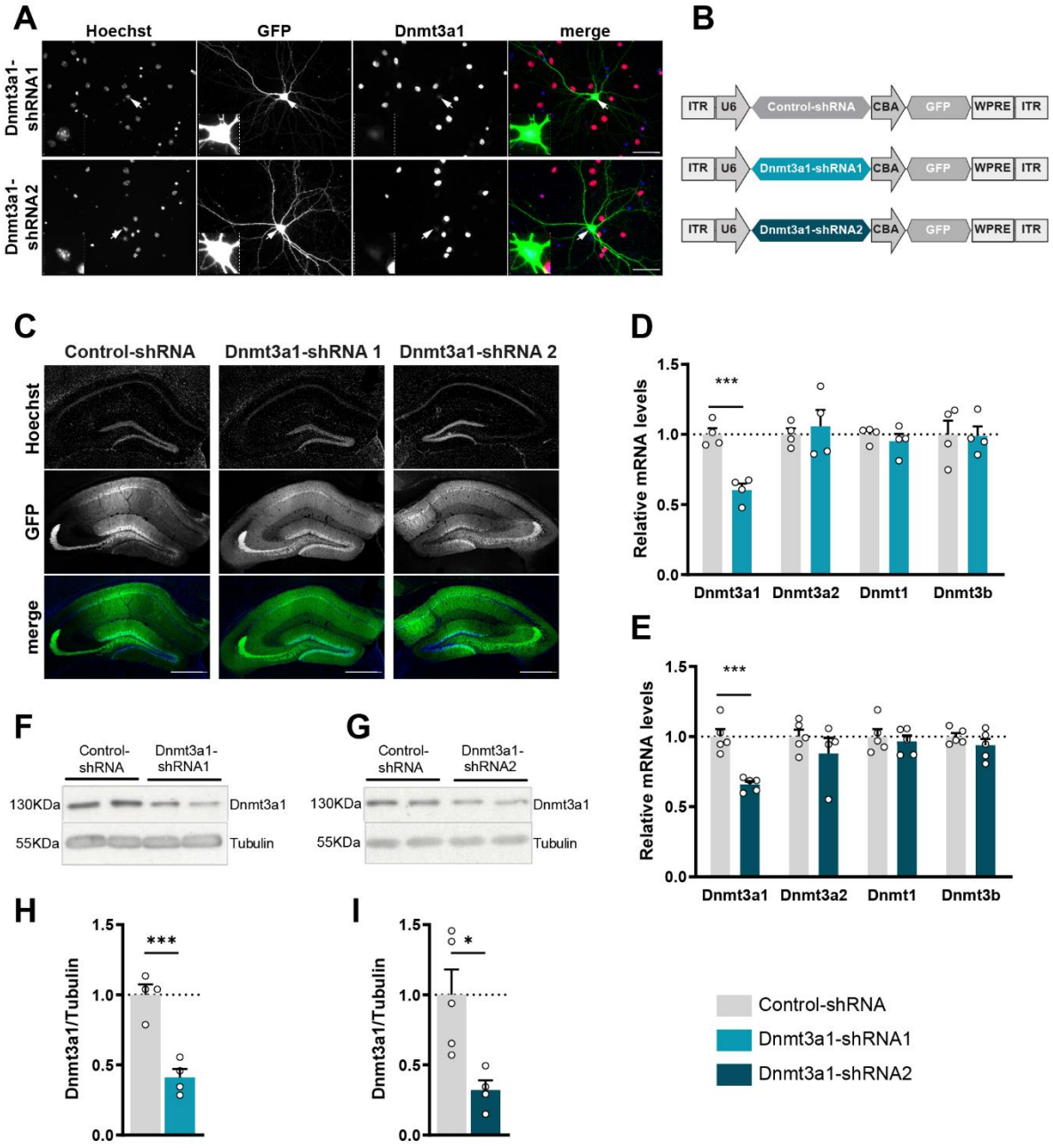


Figure 4. shRNA-based knockdown of Dnmt3a1. (A) Representative image of primary hippocampal cultures transfected with Dnmt3a1-shRNA1 or Dnmt3a1-shRNA2 (green) and immunostained against endogenous Dnmt3a levels (red). Scale bar: 50 µm. **(B)** Schematic representation of viral constructs. **(C)** Representative images of the dorsal hippocampus of mice infected with Control-shRNA, Dnmt3a1-shRNA1 or Dnmt3a1-shRNA2. Scale bar: 500 µm. **(D, E)** Quantitative reverse-transcription PCR (qRT-PCR) analysis of the expression of DNA methyltransferases (Dnmt) in the dHPC of mice infected with rAAVs against Control-shRNA (n=4-5) or either (D) Dnmt3a1-shRNA1 (n=4) or (E) Dnmt3a1-shRNA2 (n=4). Dashed lines represent normalised expression levels to Control-shRNA. ***p≤0.001 by two-tailed, unpaired t-test. **(F, G)** Representative immunoblot scans of the expression of Dnmt3a1 or Tubulin in the dHPC of mice infected with rAAVs against Control-shRNA or either (F) Dnmt3a1-shRNA1 or (G) Dnmt3a1-shRNA2. **(H, I)** Immunoblot quantification of Dnmt3a1 protein levels in the dHPC of mice infected with rAAVs against Control-shRNA (n= 4-5) or either (H) Dnmt3a1-shRNA1 (n=4) or (I) Dnmt3a1-shRNA2 (n=4). Dashed lines represent normalised Dnmt3a1 to Tubulin expression of control group. *p<0.05, ***p≤0.001 by two-tailed, unpaired t-test. *Experiments are part of Julien Klimmt's Master Thesis and were performed and analysed by him.*

To further investigate the role of hippocampal Dnmt3a1 in memory formation, mice were trained in contextual fear conditioning (CFC) 1 week after the spatial object recognition test. In CFC, mice form an association between the context and a foot-shock. Knockdown of Dnmt3a1 using either shRNA1 or shRNA2 impaired the fear response measured in freezing to the context 24 hours after training (Figure 5F), but did not reduce freezing levels compared to the Control-shRNA group when tested 1 hour after training (Figure 5G).

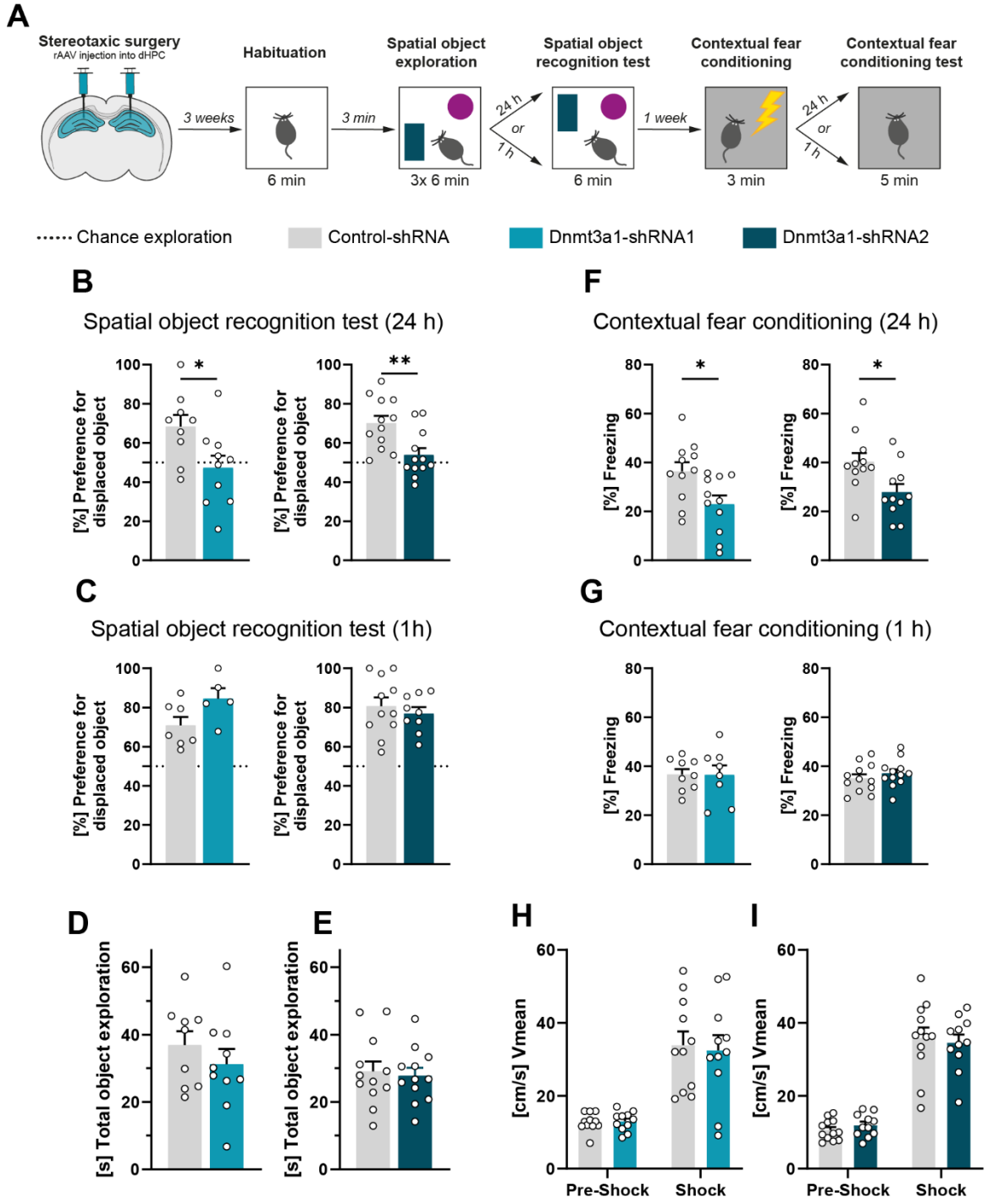


Figure 5. Reduced hippocampal Dnmt3a1 levels lead to memory impairments. (A) Schematic representation of experimental design. *Figure legend continues on next page.*

Figure 5: Reduced hippocampal Dnmt3a1 levels lead to memory impairments. (B, C) (B) Long-term or (C) short-term spatial object recognition memory of mice expressing Control-shRNA (n=7-12), Dnmt3a1-shRNA1 (n=5-10) or Dnmt3a1-shRNA2 (n=9-12). Dashed lines represent equal preference for either object (chance exploration). *p<0.05, **p≤0.01 by two-tailed, unpaired t-test. **(D, E)** Total object exploration time of mice infected with rAAVs against Control-shRNA (n=9-12) or either (D) Dnmt3a1-shRNA1 (n=10) or (E) Dnmt3a1-shRNA2 (n=12) during the three sessions of spatial object recognition training. No significant changes by two-tailed, unpaired t-test. **(F, G)** (F) Long-term or (G) short-term contextual fear memory of mice expressing Control-shRNA (n=9-11), Dnmt3a1-shRNA1 (n=8-11) or Dnmt3a1-shRNA2 (n=11-13). *p<0.05 by two-tailed, unpaired t-test. **(H, I)** Mean velocities during CFC training pre-shock (Pre-Shock) or during shock (Shock) administration of the mice expressing rAAVs against Control-shRNA (n=11-13) or either (H) Dnmt3a1-shRNA1 (n=11) or (I) Dnmt3a1-shRNA2 (n=11). No significant changes by two-tailed, unpaired t-test. *Experiments are part of Julien Klimmt's Master Thesis and were performed and analysed by him.*

This decrease in freezing behaviour was not attributed to the responsiveness to the shock administered during the training session (Figure 5H, I). These findings demonstrate that reducing the expression of Dnmt3a1 disrupts the formation of long-term memory while leaving short-term memory unaffected.

1.2 Temporally-restricted Dnmt3a1 knockdown impairs memory consolidation

The behavioural testing was conducted three weeks following stereotaxic delivery of rAAVs to achieve high viral expression and high knockdown efficiency. However, sustained Dnmt3a1 reduction may lead to network-wide changes and thereby it might impact learning and memory indirectly.

To more directly assign a role for Dnmt3a1 in mature neurons during memory formation, a temporally controlled approach that limits the knockdown of Dnmt3a1 specifically to the behavioural testing phase was applied. One viral construct contained the human synapsin (hSyn) promoter, which controlled the expression of the reverse tetracycline-controlled transactivator (rtTA), the Tet repressor (TetR), and the fluorescent protein Kusabira Orange (KO) as an infection marker. For the second construct, the Dnmt3a1-specific shRNA1 sequence was subcloned into a microRNA-30-based expression cassette (Stegmeier et al., 2005) (Figure 6A).

In the absence of doxycycline, TetR is bound to TRE inhibiting its activity. Upon administration of doxycycline, TetR dissociates from the TRE promoter, which enables the binding of the activator rtTA and thereby the doxycycline-dependent expression of the transgene sequence (Figure 6B) The tight control of transgene expression using the TetON system was verified in hippocampal primary cultures (Figure 6C-F). GFP expression increased over a 72-hour period following doxycycline administration (Figure 6D). Western blot analysis of Dnmt3a1 protein levels at different time points demonstrated a significant reduction after 72 hours (Figure 6E-

F). Consequently, doxycycline was intraperitoneally injected 72 hours prior to the behavioural testing.

To assess whether the importance of Dnmt3a1 in memory formation was due to the sustained reduction of Dnmt3a1 or whether it is crucial during the period of

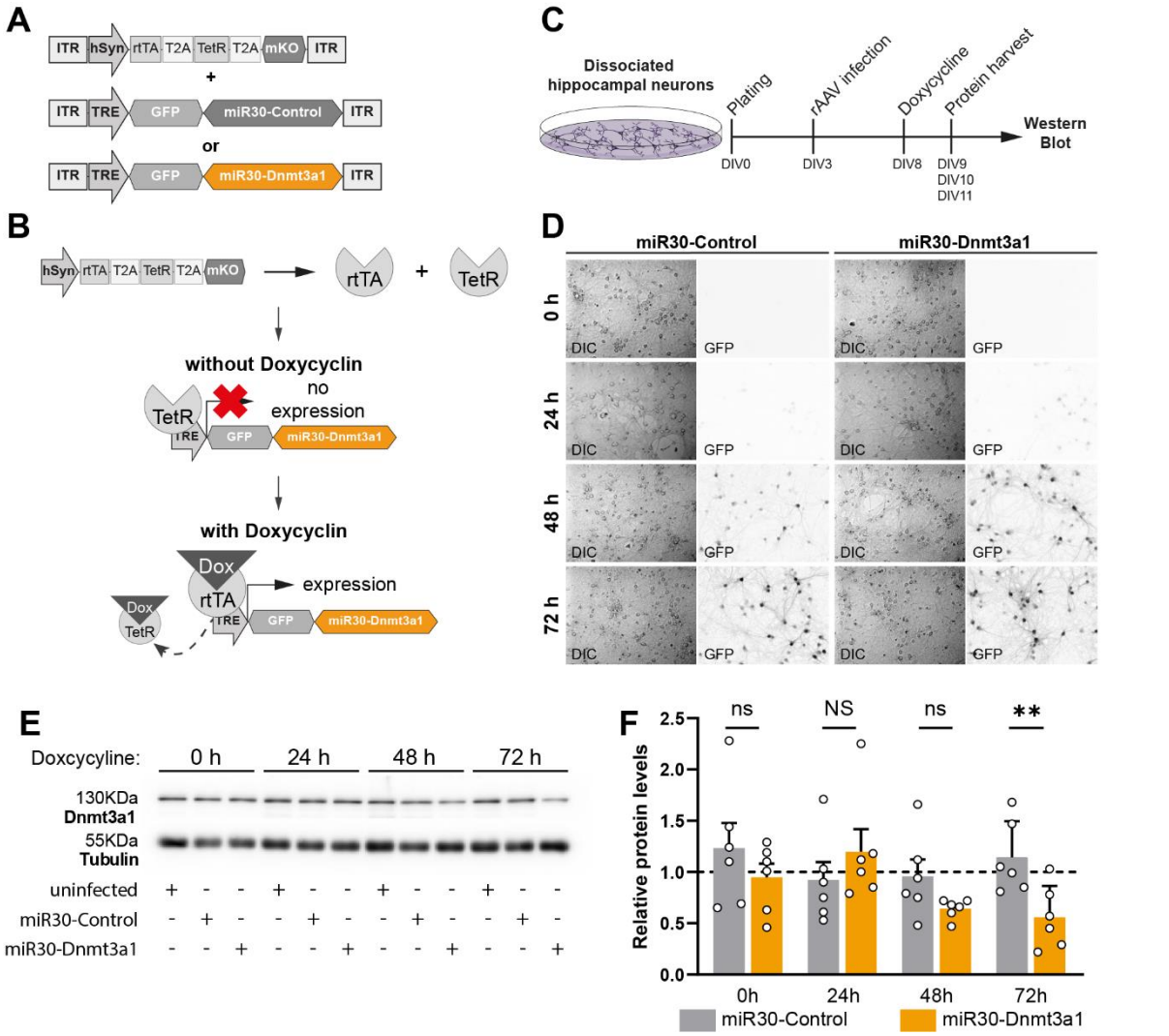


Figure 6. A system to temporally-restrict Dnmt3a1 knockdown. (A) Schematic representation of the viral constructs encoding the TetON-based system. **(B)** The driver construct expresses the Tet repressor (TetR), the reverse tetracycline-controlled transactivator (rtTA) and the infection marker Kusabira Orange (mKO) under the hSyn promoter. The Tet response element (TRE)-dependent construct contains the TRE promoter driving the miR30-based shRNA cassettes. In the absence of doxycycline: TetR is bound to TRE promoting active repression of transgene expression. In the presence of doxycycline: TetR loses its affinity thus allowing rtTA to bind to TRE and activate the expression of the miR30 system. **(C)** Experimental design used to identify the time-point of knock-down using the TetON-based miR30 system. DIV: Day *in-vitro*. **(D)** Representative images of primary hippocampal cells infected with miR30-Control or miR30-Dnmt3a1-shRNA2 sequence. Cultures were treated with doxycycline for 0h, 24h, 48, or 72h. **(E)** Representative immunoblot scans of the expression of Dnmt3a1 and Tubulin of primary hippocampal cultures infected with miR30-Control or miR30- Dnmt3a1-shRNA2 sequence and treated with doxycycline for either 0h, 24h, 48h or 72h. **(F)** Immunoblot quantification of Dnmt3a1 protein levels of primary hippocampal cultures infected with miR30-Control or miR30- Dnmt3a1-shRNA2 sequence and treated with doxycycline for either 0h, 24h, 48h or 72h. Expression levels were normalised to the uninfected controls (dashed line); (n=6 independent neuronal cultures). **p≤0.01, ns: not significant by two-tailed, unpaired t-test. NS: not significant by Mann-Whitney test. *Experiments were performed and analysed by Franziska Mudlaff.*

memory formation, the temporally-controlled TetON system was stereotaxically injected in the dHPC of mice (Figure 7A, B). First, I confirmed the targeting and

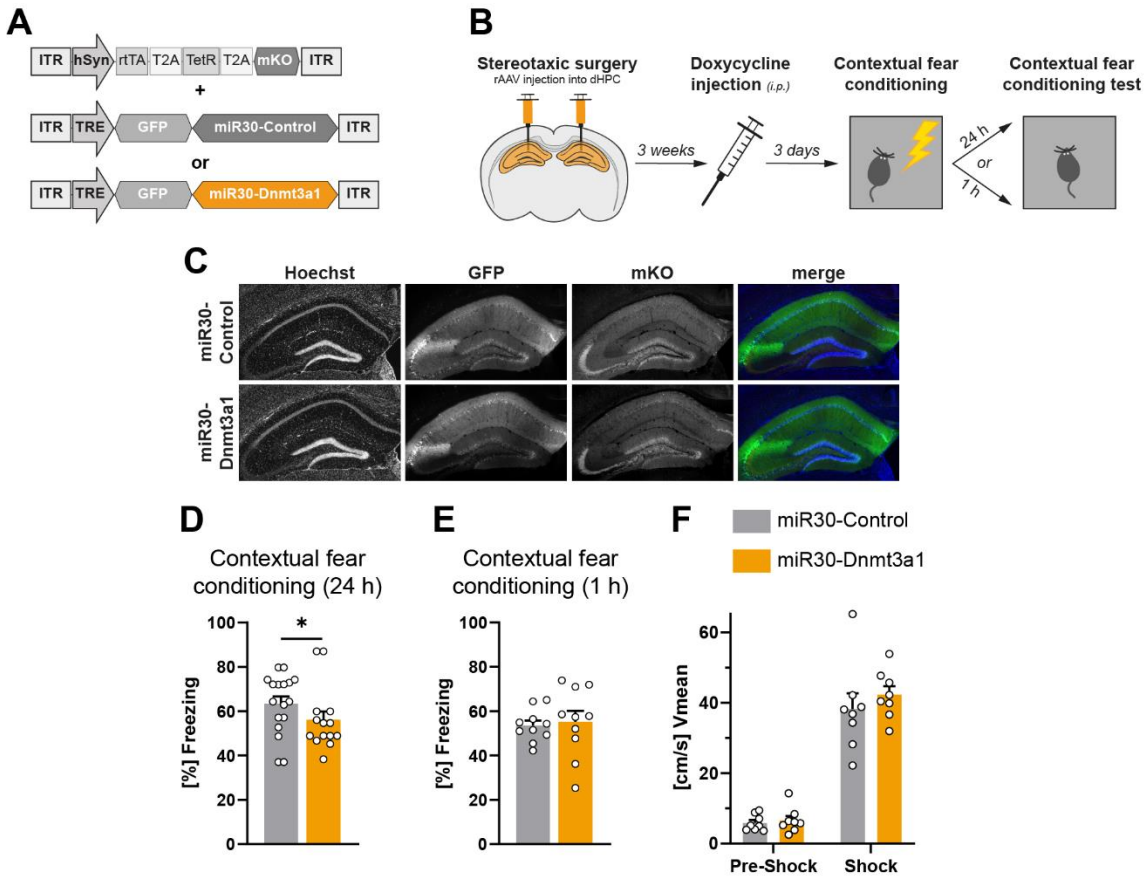


Figure 7. Temporally-restricted Dnmt3a1 knockdown impairs memory consolidation. (A) Schematic representation of the viral constructs. **(B)** Schematic representation of experimental design. (**C**) Representative images of the dHPC of mice infected with rAAVs expressing the miR30-Control shRNA or miR30- Dnmt3a1-shRNA2 sequence. Scale bar: 100 µm. **(D, E)** (D) Long-term or (E) short-term contextual fear memory of mice expressing miR30-Control shRNA (n=12-17) or miR30-Dnmt3a1 shRNA (n=10-14). *p<0.05, by two-tailed, unpaired t-test. **(F)** Mean velocities during CFC training pre-shock (Pre-Shock) or during shock (Shock) administration of the mice expressing miR30-Control shRNA (n=8) or miR30-Dnmt3a1 shRNA (n=8). No significant changes by two-tailed, unpaired t-test. *Experiments were performed and analysed by Franziska Mudlaff and Ana Oliveira.*

expression of the viral constructs in the dHPC via microscopic analysis (Figure 7C). Then, the memory performance of mice was assessed.

Knockdown of hippocampal Dnmt3a1 around the time of fear conditioning reduced freezing levels when mice were tested 24 hours after training (Figure 7D), however, mice froze to a similar extent than the control mice when tested for short-term memory (1h) (Figure 7E) and the mice showed similar responsiveness to the shock administered during the training session (Figure 7F). Overall, these findings further demonstrate a function of Dnmt3a1 during long-term memory formation.

1.3 Dnmt3a1 regulates the expression of activity-dependent genes involved in synaptic plasticity

To elucidate the genomic program through which Dnmt3a1 might regulate memory consolidation processes, RNA sequencing of hippocampal neurons infected with Dnmt3a1-shRNA2 or a Control-shRNA was performed and bicuculline treatment to induce neuronal activity (Figure 8A, B) was done. RNA Sequencing analysis revealed in control conditions, that neuronal activity led to the downregulation of 1981 genes and upregulation of 1935 genes (Figure 8C). I could confirm, that the treatment induced a classical activity-regulated transcriptomic profile, since known IEGs such as Arc, Fos and Npas4 were among the differentially expressed genes (DEGs) (Figure 8C). To identify genes regulated by Dnmt3a1, I focused on genes that showed differential expression between control conditions and reduced Dnmt3a1 levels following neuronal activity by bicuculline treatment. A set of 1241 DEGs, with 436 genes upregulated and 805 genes downregulated was found (Figure 8D). Notably, the expression of classical immediate early genes, including Arc, Fos, and Npas4, was not altered upon Dnmt3a1 knockdown (data not shown). This indicates that the observed changes were not due to a general disruption of neuronal responsiveness to activity, but specifically due to Dnmt3a1 reduction. To identify potential Dnmt3a1 target genes involved in memory formation, I hypothesised that this gene should meet two criteria: a) it should be activity-regulated and b) expressed differently in response to Dnmt3a1 reduction. Therefore, I overlapped the activity-regulated gene set (Figure 8C) with the DEGs upon Dnmt3a1-reduction (Figure 8D). This intersection revealed 327 candidates (Figure 8E). Gene ontology (GO) analysis of these 327 genes revealed a strong enrichment for terms related to structural and functional plasticity, including "*Regulation of neurotransmitter receptor activity*", "*Regulation of signaling receptor activity*" and "*Regulation of cell-substrate adhesion*" (Figure 8F). This suggests that genes downstream of Dnmt3a1 may play a crucial role in synaptic plasticity, learning, and memory. To validate some of the identified Dnmt3a1 effector genes, I performed qRT-PCR on independent biological samples (Figure 8G, H). To further rule out the possibility of off-target effects, I performed the qRT-PCR using the two independent shRNA sequences characterised above. I confirmed that the expression of *Alkal2*, *Cacng5*, and *Nrp1* was regulated by neuronal activity and reduced upon Dnmt3a1 knockdown (Figure 8G). However, other genes such as *Crhbp*, *Npy*, and *Trpc6* did not achieve a significant effect with one of the two shRNA sequences (Figure 8H).

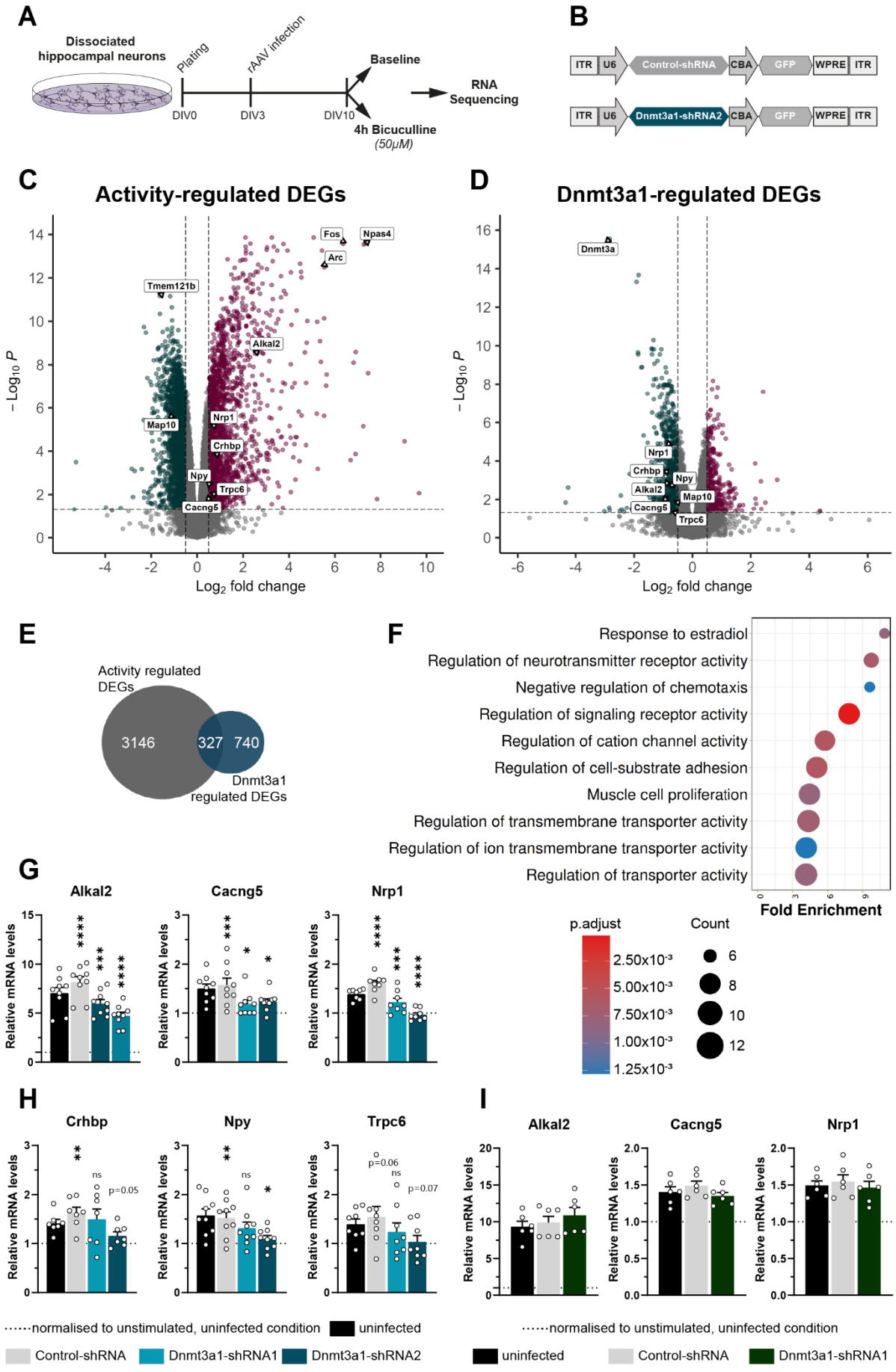


Figure 8. Dnmt3a1 regulates transcription of genes involved in synaptic plasticity processes.

Figure legend continues on next page.

Figure 8. Dnmt3a1 regulates transcription of genes involved in synaptic plasticity processes. (A) Experimental design used to identify activity-regulated genes whose expression is altered upon Dnmt3a1 reduction. DIV: Day *in-vitro*. **(B)** Schematic representation of viral constructs. (**C**) Volcano plot of differentially expressed genes (DEGs) in control condition (infected with rAAVs expressing Control-shRNA) in response to neuronal activity via Bicuculline treatment; n=4 independent neuronal preparation per condition. Log2 fold change cut-off: ± 0.5; adjusted p-value cut-off: 0.05. **(D)** Volcano plot of DEGs upon infection with Control-shRNA or Dnmt3a1-shRNA2 in stimulated condition (4h Bicuculline); n=4 independent neuronal preparation per condition. Log2 fold change cut-off: ± 0.5; adjusted p-value cut-off: 0.05. **(E)** Venn-Diagram of activity-regulated DEGs (C) and Dnmt3a1-regulated DEGs (D). **(F)** GO-Term analysis of overlapping genes between activity-regulated and Dnmt3a1-regulated DEGs. Dot plot illustrates Top 10 GO term enrichment of biological processes. **(G)** qRT-PCR analysis of Alkal2, Cacng5, Nrp1 expression levels in hippocampal cultures infected with Control–shRNA or Dnmt3a1-shRNA1 or -shRNA2 and stimulated 4h with Bicuculline. Expression levels were normalised to the uninfected controls in baseline conditions (dashed line); (n=8-9 independent neuronal cultures). *p<0.05, ***p≤0.001, ****p≤0.0001 by one-way ANOVA test followed by Sidak's multiple comparisons test. **(H)** qRT-PCR analysis of Crhbp, Npy, Trpc6 expression levels in hippocampal-cultured cells infected with Control–shRNA or Dnmt3a1-shRNA1 or -shRNA2 and stimulated 4h with Bicuculline. Expression levels were normalised to the uninfected controls in baseline conditions (dashed line); (n=7-9 independent neuronal cultures). *p<0.05, **p≤0.01, ns: not significant by one-way ANOVA test followed by Sidak's multiple comparisons test. **(I)** qRT-PCR analysis of Alkal2, Cacng5 and Nrp1 expression levels in hippocampal-cultured cells infected with Control–shRNA or Dnmt3a2-shRNA and stimulated 4h with Bicuculline. Expression levels were normalised to the uninfected controls in baseline conditions (dashed line); (n=6 independent neuronal cultures). No significant differences between Control-shRNA and Dnmt3a2-shRNA by two-tailed, unpaired t-test. *RNA Sequencing was analysed by Carsten Sticht. qRT-PCR experiments were partially performed by Celia García-Vilela and Maximilian Schwab.*

Dnmt3a encodes for two distinct isoforms Dnmt3a1 and Dnmt3a2, which are differentially regulated by neuronal activity (Bayraktar et al., 2020; A. M. M. Oliveira et al., 2012). To further investigate whether these isoforms lead to a distinct transcriptomic profile, I reduced Dnmt3a2 levels in primary hippocampal cultures by infecting them with a previously validated shRNA (A. M. M. Oliveira et al., 2012, 2016) and performed qRT-PCR analysis of the expression of the identified Dnmt3a1-regulated genes. I found that the reduction of Dnmt3a2 did not alter the expression of *Alkal2*, *Cacng5*, or *Nrp1* under basal conditions or upon neuronal activity (Figure 8I). Taken together, these findings suggest that the Dnmt3a isoforms regulate distinct genomic program and that Dnmt3a1 is required for the regulation of a synaptic plasticity-related transcriptional profile.

1.4 Neuropilin-1 is a Dnmt3a1 downstream target required for memory formation

My next objective was to determine whether the involvement of Dnmt3a1 in memory consolidation is dependent on the activity of one of its target genes. Specifically, I focused on neuronal genes that have been previously identified as being regulated by exposure to a novel environment in the mouse hippocampus (Jaeger et al., 2018). One such gene of interest was Nrp1. Nrp1 is known to interact with Semaphorin 3a (Sema3A) and Plexin A4, forming a complex involved in signalling

pathways that regulate neuronal morphology (Carulli et al., 2021; Simonetti et al., 2021). Although it has an established role in AMPA receptor trafficking (Jitsuki-Takahashi et al., 2021), whether Nrp1 plays a role in hippocampus-dependent memory formation remains unclear.

In order to elucidate whether Nrp1 is crucial for memory, I performed a shRNA-mediated loss-of-function approach. To this end, I generated two independent shRNA constructs to reduce Nrp1 expression (Figure 9A) and confirmed their knockdown efficiency *in vivo* through stereotaxic delivery of the viral constructs into the dHPC. Both shRNAs significantly reduced the mRNA level of Nrp1 (Figure 9B, C). Next, I assessed the importance of Nrp1 in memory formation. Therefore, three weeks after viral delivery, mice underwent hippocampus-dependent behavioural memory tasks (Figure 9D). Knockdown of Nrp1 using either shRNA resulted in chance-level exploration of the displaced object (Figure 9E) without affecting overall object exploration time (Figure 9F). Mice that have reduced Nrp1 levels showed further

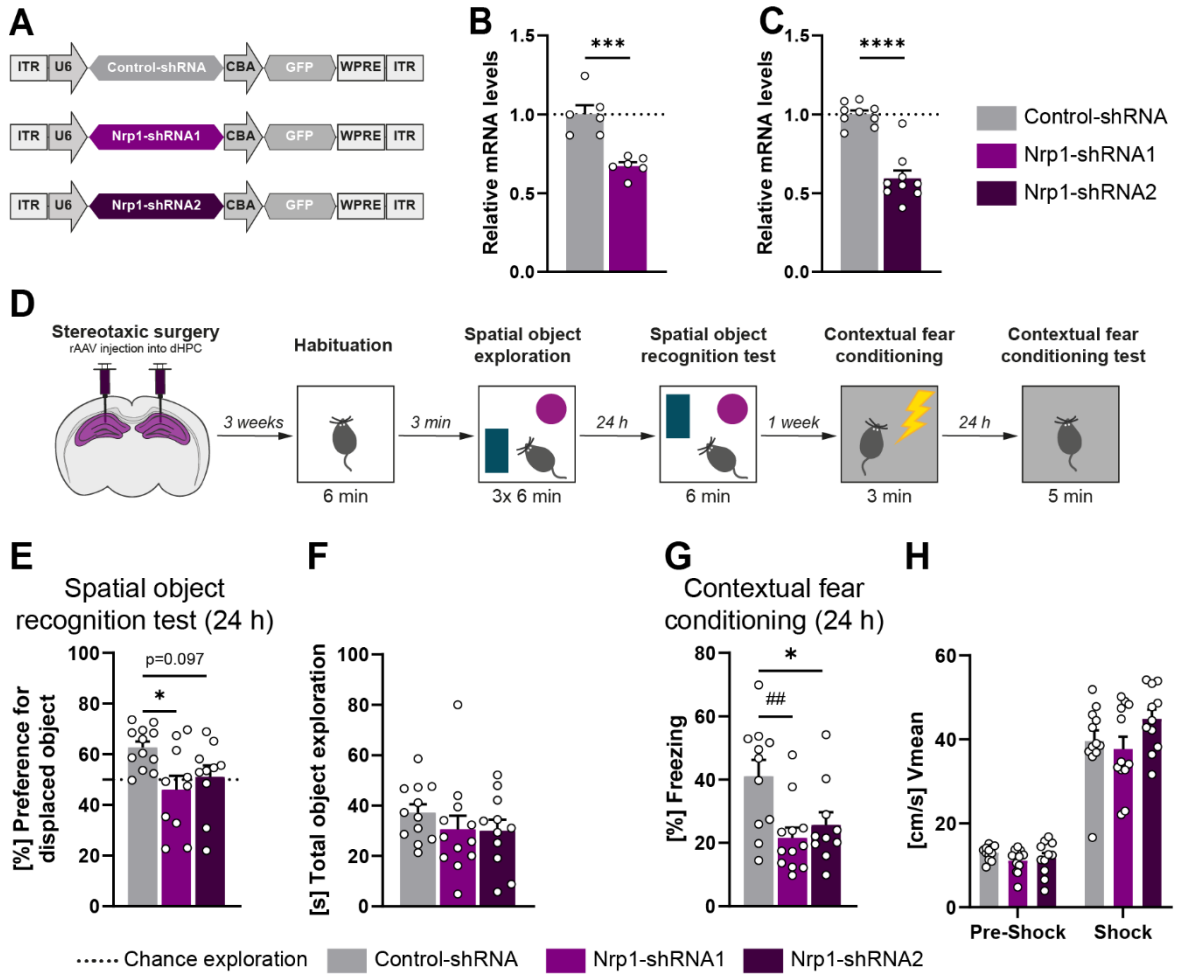


Figure 9. Hippocampal Neuropilin-1 is required for memory formation. (A) Schematic representation of viral constructs. **(B, C)** qRT-PCR analysis of Nrp1 expression in dHPC of mice infected with rAAVs against Control-shRNA (n=6-9), (B) Nrp1-shRNA1 (n=6) or (C) Nrp11-shRNA2 (n=9). Dashed lines represent normalised expression levels to Control-shRNA. ***p≤0.001, ***p≤0.0001, by two-tailed, unpaired t-test. *Figure legend continues on next page.*

Figure 9. Hippocampal Neuropilin-1 is required for memory formation. (D) Schematic representation of experimental design. **(E)** Long-term spatial object recognition memory of mice expressing Control-shRNA (n=12), Nrp1-shRNA1 (n=10) or Nrp1-shRNA2 (n=10). Dashed lines represent equal preference for either object (chance exploration). *p<0.05 by one-way ANOVA test followed by Dunnett's multiple comparisons test. (**F**) Total object exploration time of mice infected with rAAVs against either Control-shRNA (n=12) or Nrp1-shRNA1 (n=12) or Nrp1-shRNA2 (n=11) during the three sessions of spatial object recognition training. No significant changes between Control-shRNA and either Nrp1-shRNA1 or Nrp1-shRNA2 by one-way ANOVA test followed by Dunnett's multiple comparisons test. **(G)** Long-term contextual fear memory of mice infected with rAAVs expressing Control-shRNA (n=11), Nrp1-shRNA1 (n=12) or Nrp11-shRNA2 (n=10). *p<0.05 by one-way ANOVA test followed by Dunnett's multiple comparisons test. ##p≤0.01 by Kruskal-Wallis test followed by Dunn's multiple comparisons test. **(H)** Mean velocities during CFC training pre-shock (Pre-Shock) or during shock (Shock) administration of the mice infected with either Control-shRNA (n=12) or Nrp1-shRNA1 (n=12) or Nrp1-shRNA2 (n=11). No significant changes between Control-shRNA and either Nrp1-shRNA1 or Nrp1-shRNA2 by one-way ANOVA test followed by Dunnett's multiple comparisons test.

decreased freezing rates in the contextual fear conditioning test compared to mice injected with the Control-shRNA (Figure 9G). Importantly, the decrease in freezing was not due to the responsiveness of mice to the shock administration (Figure 9H). In conclusion, this set of experiments revealed a novel role for Nrp1 in long-term memory formation.

1.5 Nrp1 rescues Dnmt3a1-dependent memory impairment

To validate the functional role of Nrp1 as a downstream target of Dnmt3a1 in memory formation, I conducted a rescue experiment. In this experiment, I introduced constructs that overexpressed HA-tagged Nrp1 or a control protein (LacZ) under the control of the hSyn promoter, along with either Dnmt3a1-shRNA2 or the Control-shRNA sequences (Figure 10A, B). First, I certified successful overexpression of Nrp1 in the dHPC using western blot analysis (Figure 10C). In the course of this, I once again confirmed Dnmt3a1 knockdown efficiency (Figure 10C, D). In order to investigate a functional link between Dnmt3a1, Nrp1 and memory formation, I tested if Nrp1 overexpression rescues Dnmt3a1 knockdown-dependent memory impairments. I overexpressed HA-tagged Nrp1 with a Dnmt3a1-specific shRNA sequence in the mouse dorsal hippocampus and performed the spatial object recognition task in mice. Following the stereotaxic delivery of the constructs, mice underwent the spatial object recognition task (Figure 10B). All mice showed similar exploration time during the training sessions (Figure 10E). As previously demonstrated, the reduction of Dnmt3a1 resulted in memory impairments, as indicated by the decreased preference for the displaced object (Figure 10F). Remarkably, this impairment was rescued when Nrp1 was overexpressed (Figure 10F). Mice that overexpressed Nrp1 and expressed a control shRNA sequence showed memory performance similar to the control group (Figure 10F) indicating that Nrp1

overexpression on its own does not enhance memory. These results provide evidence that Nrp1 acts downstream of Dnmt3a1, is essential for memory formation, and can rescue memory impairments caused by Dnmt3a1 depletion, suggesting that Dnmt3a1 regulates memory formation, at least in part, through its downstream target Nrp1.

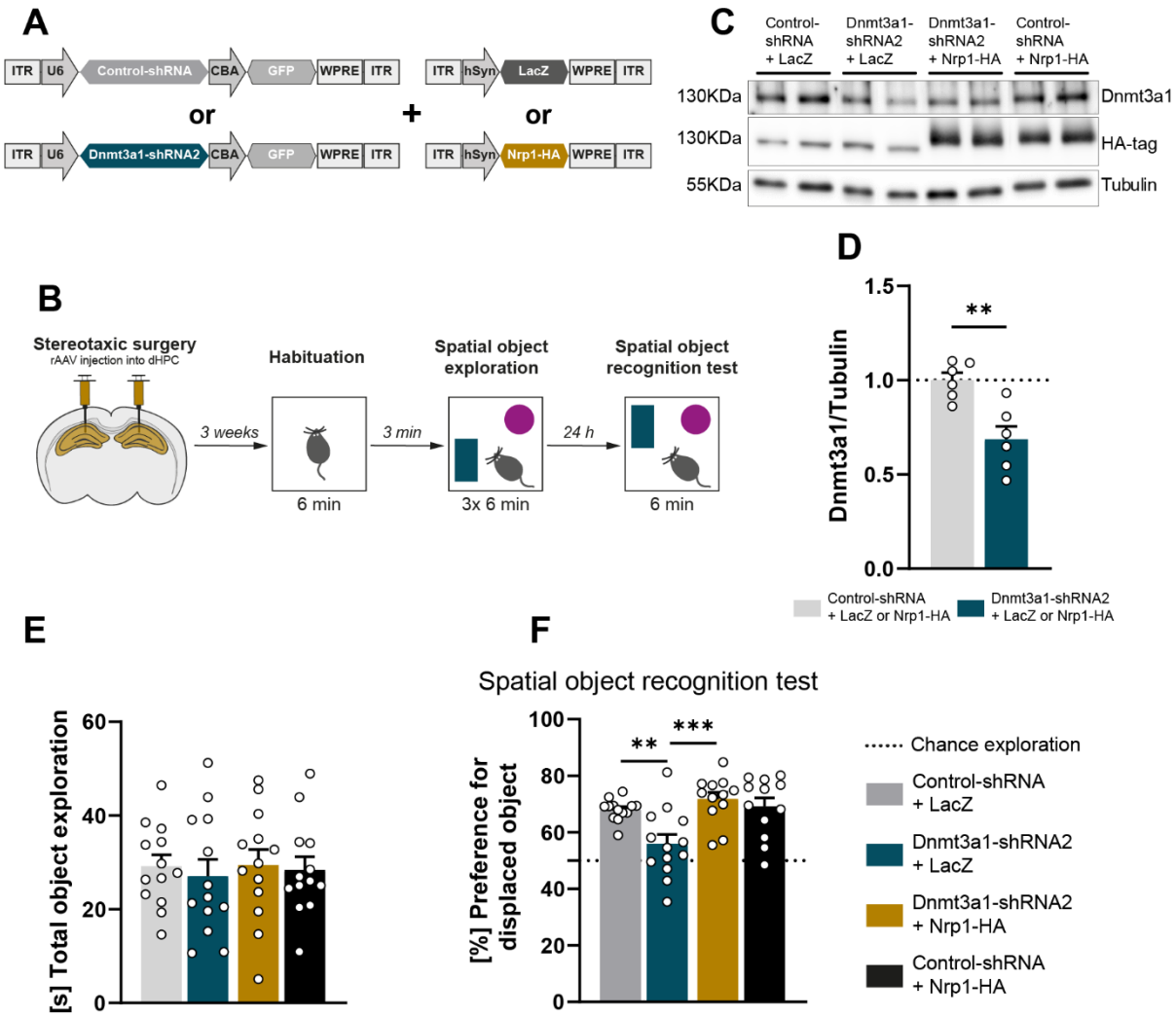


Figure 10. Neuropilin-1 rescues Dnmt3a1-dependent memory impairments. (A) Schematic representation of viral constructs. **(B)** Schematic representation of experimental design. **(C)** Representative immunoblot scans of the expression of Dnmt3a1, HA-tagged Nrp1 or HA-tagged LacZ and Tubulin in the dHPC of mice infected with rAAVs as indicated. **(D)** Immunoblot quantification of Dnmt3a1 protein levels in the dHPC of mice infected with rAAVs against Control-shRNA (n= 6) or Dnmt3a1-shRNA2 (n=6). Dashed lines represent normalised Dnmt3a1 to Tubulin expression of control group. **p≤0.01 by two-tailed, unpaired t-test. **(E)** Total object exploration time of mice infected with Control-shRNA + LacZ (n=13), Dnmt3a1-shRNA2 + LacZ (n=13), Dnmt3a1-shRNA2 + Nrp1-HA (n=13) or Control-shRNA + Nrp1-HA (n=13) during the three sessions of spatial object recognition training. No significant changes by one-way ANOVA test followed by Sidak's multiple comparisons test. **(F)** Long-term spatial object recognition memory of mice expressing Control-shRNA + LacZ (n=13), Dnmt3a1-shRNA2 + LacZ (n=13), Dnmt3a1-shRNA2 + Nrp1-HA (n=12) or Control-shRNA + Nrp1-HA (n=12). Dashed lines represent equal preference for either object (chance exploration). **p≤0.01, ***p≤0.001 by one-way ANOVA test followed by Sidak's multiple comparisons test.

1.6 Dnmt3a2-dependent memory impairments are not rescued by Nrp1

The gene expression analysis hinted that the isoforms Dnmt3a1 and Dnmt3a2 regulate distinct sets of genes. In specific, Nrp1 expression is regulated by Dnmt3a1 but remains unchanged upon knockdown of Dnmt3a2 (see Figure 8G, I). To dissect the involvement of Nrp1 in isoform-specific mechanisms of memory formation, I examined whether Nrp1 could rescue memory impairments caused by Dnmt3a2 knockdown or whether its rescue abilities are Dnmt3a1-dependent. For this, I introduced the Nrp1 overexpression to mice infected with the previously established Dnmt3a2-specific shRNA sequence or the Control-shRNA (Figure 11A) and subjected

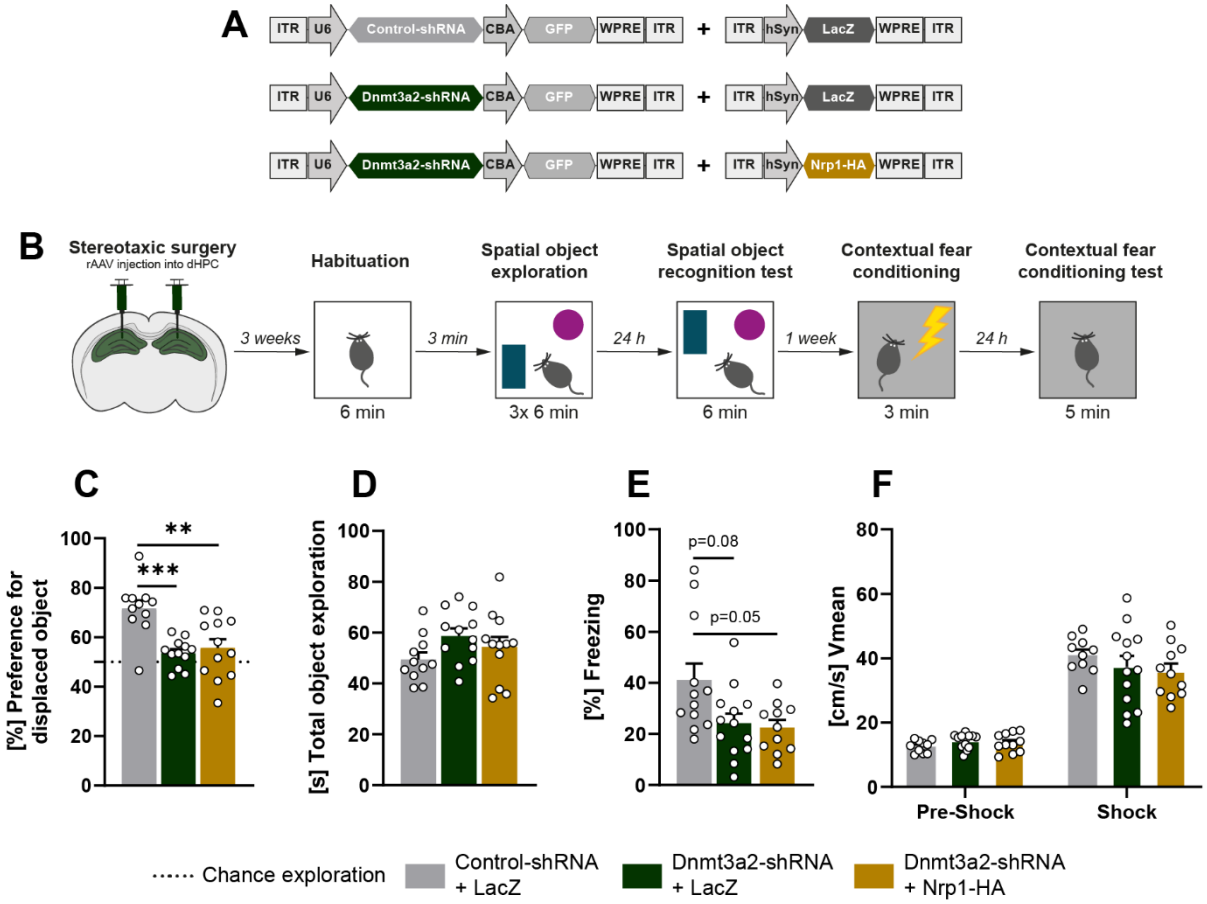


Figure 11. Dnmt3a2-dependent memory impairments cannot be rescued by Neuropilin-1. (A) Schematic representation of viral constructs. **(B)** Schematic representation of experimental design. **(C)** Long-term spatial object recognition memory mice expressing Control-shRNA + LacZ (n=11), Dnmt3a2-shRNA + LacZ (n=12) or Dnmt3a2-shRNA + Nrp1-HA (n=12). Dashed lines represent equal preference for either object (chance exploration). **p≤0.01, ***p≤0.001 by one-way ANOVA test followed by Sidak's multiple comparisons test. **(D)** Total object exploration time of mice infected with Control-shRNA + LacZ (n=11), Dnmt3a2-shRNA + LacZ (n=12), Dnmt3a2-shRNA + Nrp1-HA (n=12) during three sessions of spatial object recognition training. No significant changes by one-way ANOVA test followed by Sidak's multiple comparisons test. **(E)** Long-term contextual fear memory of mice infected with rAAVs expressing Control-shRNA (n=12), Nrp1-shRNA1 (n=13) or Nrp11-shRNA2 (n=11). No significant changes by one-way ANOVA test followed by Sidak's multiple comparisons test. **(F)** Mean velocities during CFC training pre-shock (Pre-Shock) or during shock (Shock) administration of the mice infected with either Control-shRNA + LacZ (n=10), Dnmt3a2-shRNA + LacZ (n=13) or Dnmt3a2-shRNA + Nrp1-HA (n=11). No significant changes by one-way ANOVA test followed by Sidak's multiple comparisons test.

them to memory tests (Figure 11B). I found that in the spatial object recognition test, mice injected with Dnmt3a2-shRNA exhibited memory impairments as previously described (A. M. M. Oliveira et al., 2012) (Figure 11C).

In contrast to the rescue observed with Dnmt3a1 knockdown, Nrp1 overexpression did not rescue these memory impairments caused by Dnmt3a2 knockdown (Figure 11C). There were no significant differences in the overall time spent exploring the objects during training among the different conditions (Figure 11D). To assess further the specificity of Nrp1's rescue abilities, I trained mice in contextual fear conditioning. Again, Dnmt3a2 reduction impaired contextual fear memory response (Figure 11E), which was not due to altered responsiveness to the shock administration during the training session (Figure 11F). Strikingly, this impairment was not rescued upon Nrp1 overexpression (Figure 11E). This finding indicates that the rescue effect obtained through Nrp1 overexpression is not due to a generalised enhancement of memory and further supports the notion that Nrp1 acts downstream of Dnmt3a1 but not Dnmt3a2. Thus, this data uncovers a *Dnmt3a* isoform specific mechanism in memory formation.

In this part, I showed that knockdown of Dnmt3a1 in the adult hippocampus specifically inhibits long-term memory formation and further identified an activity-regulated, Dnmt3a1-dependent genomic program. Several of these discovered genes exclusively rely on Dnmt3a1, but not Dnmt3a2. The role of the effector gene Nrp1 in hippocampus-dependent memory formation was then shown. Further I showed that Nrp1 overexpression rescued Dnmt3a1-driven memory impairments but could not reverse memory deficits that were caused by Dnmt3a2 pointing to an isoform-specific mechanism for memory functions. These discoveries have improved the knowledge of Dnmt3a-regulated effector molecules in memory as well as the necessity for unique Dnmts in mnemonic processes.

2. DNA methylation promotes memory persistence by facilitating systems consolidation and cortical engram stabilisation.

A key aspect of memory is persistence. Memory persistence involves a process called systems consolidation. Traditionally, this process has been described as the gradual transfer of information from the hippocampus to the cortex for long-term memory storage. However, the underlying molecular mechanisms are hardly elucidated. In this part of my thesis, I try to uncover parts of this. I hypothesise that such a mechanism has to be on the one hand dynamic and adaptable for it to be affected by learning and memory. But on the other hand, it has to leave long-term regulatory marks to encode persistency. Epigenetic processes fulfil these criteria. Hence, I probe whether DNA methylation acts as a mechanism for memory duration and affects systems consolidation.

2.1 Establishment of tools to study memory persistence

To probe the molecular mechanism underlying memory persistence, behavioural paradigms had to be established. Since the question is why some memories become long-lasting where others are formed but decay over time, the behavioural tests were designed to induce the formation of memory and to allow either short-lasting (memory decayed by two weeks) or persistent (memory lasted beyond two weeks) memory. To this end, CFC was used due to its properties to change easily the foot shock intensity, that creates memory of different strength. First, mice were trained with a weak (1x 0.2mA) or a strong (3x 0.7mA) protocol or exposed to the chamber in absence of any shock (context-only). Mice had higher freezing levels compared to context only group, indicating that both CFC protocols induced 24h long-term fear memory (data not shown, part of David VC Brito's thesis). Mice were then trained with these protocols and their memory was tested at a recent or remote (14 days after CFC) recall session (Figure 12A). The induction of freezing behaviour at recent recall test was confirmed. Moreover, the strong protocol induced long-lasting fear memory, shown as high freezing behaviour, whereas mice in the weak protocol cohort had decreased freezing at the remote testing session compared to recent memory (Figure 12B). This result demonstrates that both protocols can be used as models to study molecular mechanisms underlying memory strength and duration. After having the behavioural paradigm established, I continued to investigate on the cellular layer – at the level of engram reactivation and thereby at the cellular substrate of memory – how the duration of a memory affects engram stabilisation. To achieve that, I advanced the

doxycycline dependent engram tagging tool, the Robust Activity Marking (RAM) system (Sørensen et al., 2016) to reliably and persistently label neuronal ensembles. I combined the RAM vector with the expression of a H2B-fused GFP to achieve long-lasting tagging and stereotaxically delivered it into the dHPC as well as the anterior cingulate cortex (ACC) (Figure 12A) – brain regions known to be involved in memory encoding and storage. Mice were handled while still on doxycycline diet to ensure that the labelled cells arose exclusively from the CFC training and not during handling. In order to open the window for tagging, doxycycline food was removed for 2 days and CFC was performed. After recall session, mice were sacrificed 90min later and overlap analysis was performed (Figure 12A, C). The percentage of GFP^+ cells correlate to activated neurons during the training. It did not change with strength of the protocol (1x 0.2mA vs. 3x 0.7mA shock) nor over the duration of two weeks (recent vs. remote) in each brain region except in the DG while comparing weak and strong labelling of the cohort tested for remote memory recall (Figure 12D). This might be due to variations in the tagging or viral injection. However, overall, the GFP percentage did not change which points to reliable tagging and successful tagging of neuronal ensembles in different brain regions over time, it also suggests that the size of the engram activated by learning is not dependent on the training strength as shown previously (Choi et al., 2018; Morrison et al., 2016). Further, tagging of ~4% in DG and ~10% in ACC was achieved, which is in line with previous studies (Gulmez Karaca et al., 2020; Karaca et al., 2020; Matos et al., 2019) (Figure 12D). Since consolidation of persistent memories takes place over long periods of time and involves a variety of brain regions (Frankland & Bontempi, 2005), I investigated the recruitment of hippocampal and cortical regions during recent and remote memory recall. For this, I looked at the percentage of $cFos^+$ cells as an indirect marker for activity of that region during memory retrieval. Hippocampal regions were not differently recruited during recent or remote memory recall, whereas the cortex became more engaged during the remote memory retrieval, however only in mice that underwent the CFC protocol, that induces longer-lasting memories (Figure 12E). This result might indicate that indeed long-term storage occurs preferable in cortical regions (Frankland & Bontempi, 2005; Scoville & Milner, 2000; Wiltgen et al., 2004). To investigate the stability of hippocampal and cortical neuronal ensembles during memory persistence, I focused on the reactivation rate of the engram under shorter- and longer-lasting memory conditions and during both recent and remote memory recall to further characterise and understand the role of the different brain regions.

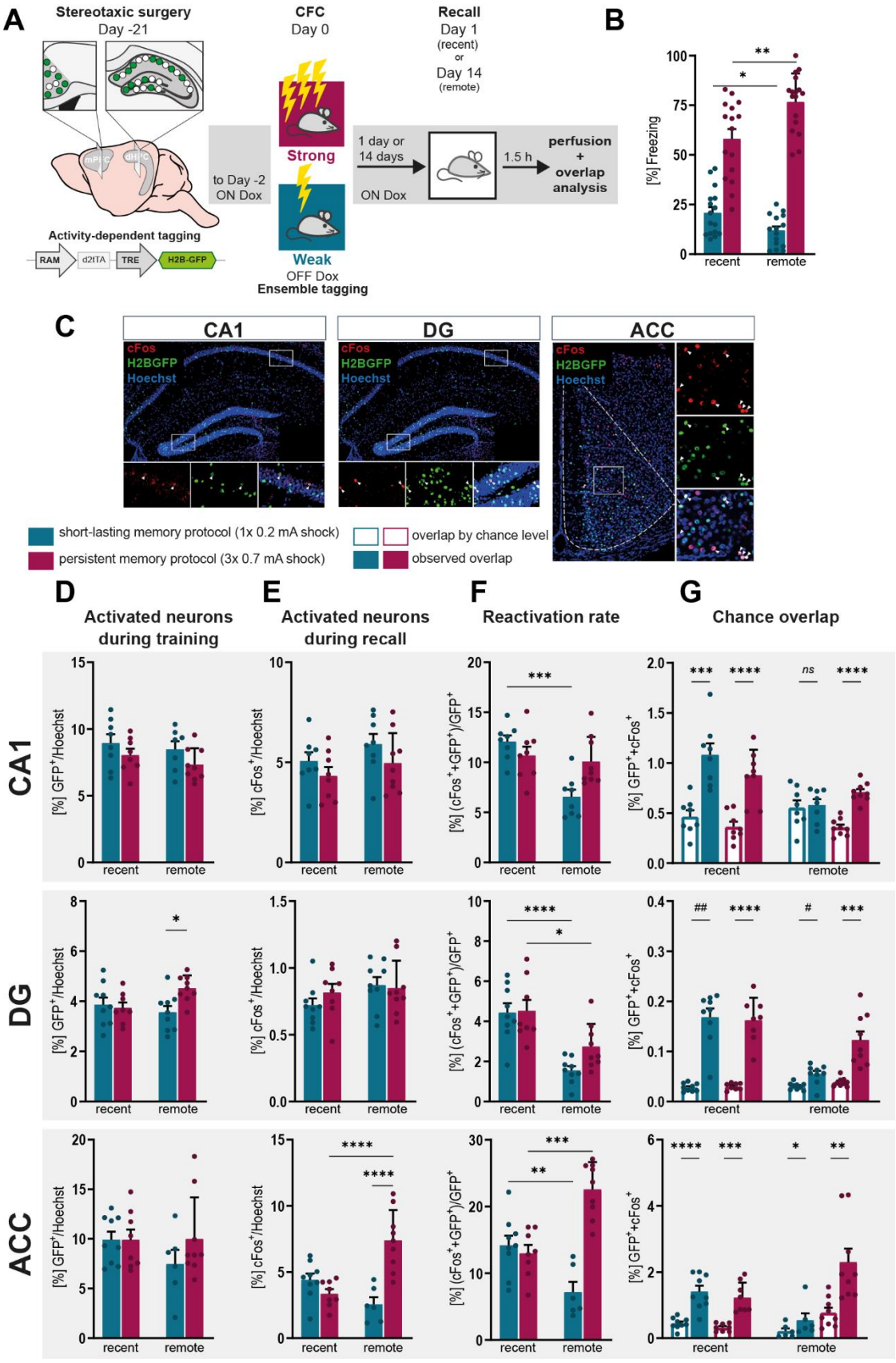


Figure 12. Establishment of tools to study memory persistence. (A) Schematic representation of experimental design. Stereotaxic injection of rAAV to tag neuronal ensembles in dHPC (CA1 and DG) and medial prefrontal cortex (anterior cingulate cortex (ACC)) of mice. Three weeks after surgery, mice were trained in a strong (3x 0.7 mA shock) or weak (1x 0.2 mA shock) CFC paradigm and their memory was tested in a recall session either 1 day or 14 days after. 1.5h post recall, mice were sacrificed for overlap analysis. *Figure legend continues on next page.*

Figure 12. Establishment of tools to study memory persistence. (B) Recent or remote contextual fear memory of mice trained with the weak (n=16; 15) or strong (n=16; 17) CFC protocol. **(C)** Representative images of overlap analysis of CA1, DG and ACC. H2BGFP (green) indicates the activity-regulated neuronal ensemble population from the training session due to the exogenous viral construct. cFos (red) represents the recall activated neurons, immunostained against endogenous cFos. **(D, E, F, G)** Quantitative image analysis in CA1, DG and ACC region of (D) activated neurons during training assessed by GFP signal, (E) activated neurons during recall identified by endogenous cFos labelling, (F) reactivation rate (GFP^++$cFos^+$ neurons in GFP^+ population) and (G) chance overlap (n=6-9). *p<0.05, **p≤0.01, ***p≤0.001, ****p≤0.0001 by one-way ANOVA test followed by Sidak's multiple comparisons test. (G) *p<0.05, **p≤0.01, ***p≤0.001, ****p≤0.0001 ns: not significant by two-tailed, paired t-test. #p<0.05, ##p≤0.01 by Wilcoxon matched-pairs signed rank test.

At recent memory recall, the reactivation rate did not differ in either region upon weak or strong CFC (Figure 12F) and the observed overlap was significantly different from mathematical chance levels (Figure 12G). The reactivation rate significantly dropped over time when mice were trained with the weak protocol (Figure 12F), which is in line with the engram theory that reactivation happens when the memory is being recalled but when there is no memory, the engram reactivation worsen or is at chance levels. Chance reactivation can be found in the CA1 region at remote time when trained with the short-lasting memory protocol (Figure 12G). The reactivation rate of DG engram neurons decreased with time (Figure 12F), which is according to the classical systems consolidation theory stating that memory is encoded in the hippocampus and gradually transferred to the cortex and in agreement with previously published work that showed decreased engram reactivation in DG at remote memory retrieval sessions(Kitamura et al., 2017; Tayler et al., 2013). However, the majority of these studies focused on DG and not the CA1 region leaving an unclear picture of engram dynamics over time in the CA1. I saw that in contrast to the DG, the engram reactivation in CA1 of persistent fear memory does not decrease over time (Figure 12F).

A study from the Tonegawa laboratory proposed that cortical ensemble neurons undergo a maturation process, that means they are unable to be reactivated during recent memory recall and become matured and able to be reactivated at remote memory retrieval (Kitamura et al., 2017). In contrast to their observations, I saw engram reactivation above chance levels in the ACC at recent time points (Figure 12G). Be that as it may, I detected an increase in cortical engram reactivation at remote memory recall mice trained with the strong, persistent fear memory protocol (Figure 12F). These results indicate hippocampal and cortical engram reactivation during recent memory retrieval. However, over time and during system consolidation processes, engram reactivation decreased specifically in DG and increased in cortical regions.

2.2 Hippocampal DNA methylation changes memory duration and stabilises cortical ensemble neurons

Subsequentially, after establishment of engram tagging tools and characterisation of engram dynamics in short-lasting or persistent memory, I now can try to unravel possible molecular mechanisms involved in memory persistence and stabilisation of the supporting neuronal ensembles. It is reasonable that these molecular processes should have the capacity to modulate plasticity-related events for a prolonged period of time to maintain a memory. Epigenetic regulation is a prime candidate due to its capacity for long-lasting changes of gene expression. Indeed, studies have linked DNA methylation to memory persistence (Halder et al., 2015; Miller et al., 2010). Further, previous work of the lab showed that ensembles-specific overexpression of a *de novo* DNA methyltransferases 3a2 (Dnmt3a2) enhanced memory (Gulmez Karaca et al., 2020). With this in mind, I investigated DNA methylation as a potential mechanism to stabilise the memory trace. Therefore, I overexpressed Dnmt3a2 in excitatory neurons in the dHPC and trained mice with the weak CFC protocol (Figure 13A). At recent memory recall, the control cohort as well as the group overexpressing Dnmt3a2 showed similar freezing behaviour and as expected, the memory decayed in the control group over time (Figure 13B). Strikingly, overexpression of Dnmt3a2 in dHPC increased freezing behaviour at remote memory recall (Figure 13B). This indicates that overexpression of an epigenetic regulator is able to convert a short-lasting memory into a persistent memory and affect memory duration.

However so far, I overexpressed an enzyme in the dHPC of mice. To specify and clearly determine whether this effect on memory duration is dependent on DNA methylation and not due to indirect processes, e.g., recruitment of binding partners, I repeated the behavioural experiment on memory duration and included a catalytic-inactive form of Dnmt3a2, that was characterised to contain all qualities as the wild type enzyme except lacking the property to methylate DNA (Dukatz et al., 2019). To this end, mice were trained with the weak CFC protocol after receiving stereotaxic injection of rAAVs expressing a control construct, the overexpression of Dnmt3a2 or the catalytic-inactive form of Dnmt3a2 and their memory was tested 14 days post training (Figure 13C, D). Overexpression of Dnmt3a2 increased freezing behaviour of mice and converted a short-lasting memory into a long-lasting memory and this conversion was completely abolished upon overexpression of the catalytic-inactive mutant (Figure 13D). Thus, this provides a causal link between the conversion of a short-lasting into a persistent memory and Dnmt3a2-driven DNA methylation activity.

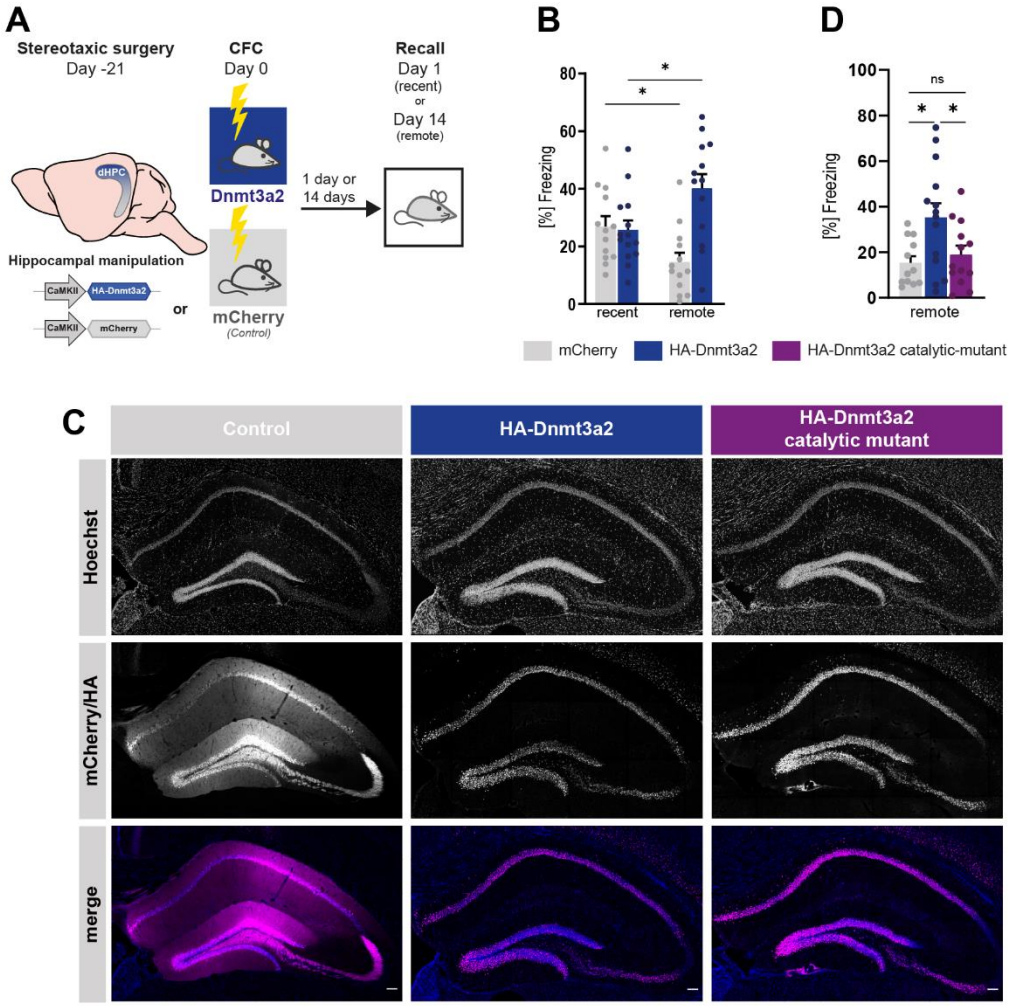


Figure 13. Hippocampal DNA methylation changes memory. (A) Schematic representation of experimental design. Stereotaxic injection of rAAV injection in dHPC to manipulate Dnmt3a2 levels. Three weeks after surgery, mice were trained with the weak (1x 0.2 mA shock) CFC paradigm and their memory was tested in a recall session 14 days after. **(B)** Recent or remote long-term contextual fear memory of mice trained with the weak CFC protocol and infected with rAAVs overexpressing mCherry (n=13; 13) or Dnmt3a2 (n=14; 13). *p<0.05 by by one-way ANOVA test followed by Sidak's multiple comparisons test. **(C)** Representative images of dHPC of mice infected with Control (mCherry), Dnmt3a2 overexpression construct or Dnmt3a2 catalytic mutant variant. Scale bar: 100µm. **(D)** Remote contextual fear memory of mice trained with the weak CFC protocol and infected with rAAVs overexpressing mCherry (n=12), Dnmt3a2 (n=14) or catalytic-inactive form of Dnmt3a2 (n=13). *p<0.05, ns: not significant by *p<0.05 by one-way ANOVA test followed by Sidak's multiple comparisons test.

Naturally, I asked whether this effect on memory duration changes the engram properties. To this end, I additionally tagged hippocampal and cortical neurons with the viral tagging tool and performed overlap analysis after the memory retrieval session (Figure 14A). Overexpression of Dnmt3a2 in dHPC did not change the engram size during encoding (indicated as percentage of GFP$^+$ cells) neither in the manipulated region nor in the cortex (Figure 14B), which was comparable to the previous established engram size (see Figure 12D). The manipulation further did not affect the percentage of cFos$^+$ cells which serves indirect as a marker for activity

during memory retrieval in the hippocampal regions (Figure 14C). These results so far pinpoint that overexpression of Dnmt3a2 did not affect engram recruitment or brain activity patterns. However, I observed an increase in percentage of activated neurons during recall ($cFos^+$ cells) in the ACC (Figure 14C), that is similar to the increase of $cFos^+$ cells mice showed during training with the persistent memory protocol (see Figure 12E) which indicates higher involvement of the ACC during remote memory recall. The engram reactivation over time (recent vs. remote) decreased in all brain regions in the control group (Figure 14D), that is in line with the decay of the memory (see Figure 13B) as well as with the previous engram dynamic (see Figure 12F). The DG engram reactivation rate increased at recent memory recall due to Dnmt3a2 overexpression (Figure 14D). A previous study in the lab observed also a better reactivation rate in DG engram upon overexpression of Dnmt3a2 in said engram neurons, although this was accompanied by better memory performance(Gulmez Karaca et al., 2020). Independently of the increase during recent memory recall, the DG engram reactivation rate decreased significantly over time and in the CA1 region, whilst the reactivation rate did not reduce, the observed overlap was at chance levels (Figure 14E). Remarkably, the cortical engram reactivation increased upon hippocampal Dnmt3a2 overexpression at the remote memory recall (Figure 14D). Taken together, hippocampal Dnmt3a2 overexpression converted a short-lasting memory into a persistent memory and led to improved cortical engram reactivation, suggesting a possible role in cortical engram maturation.

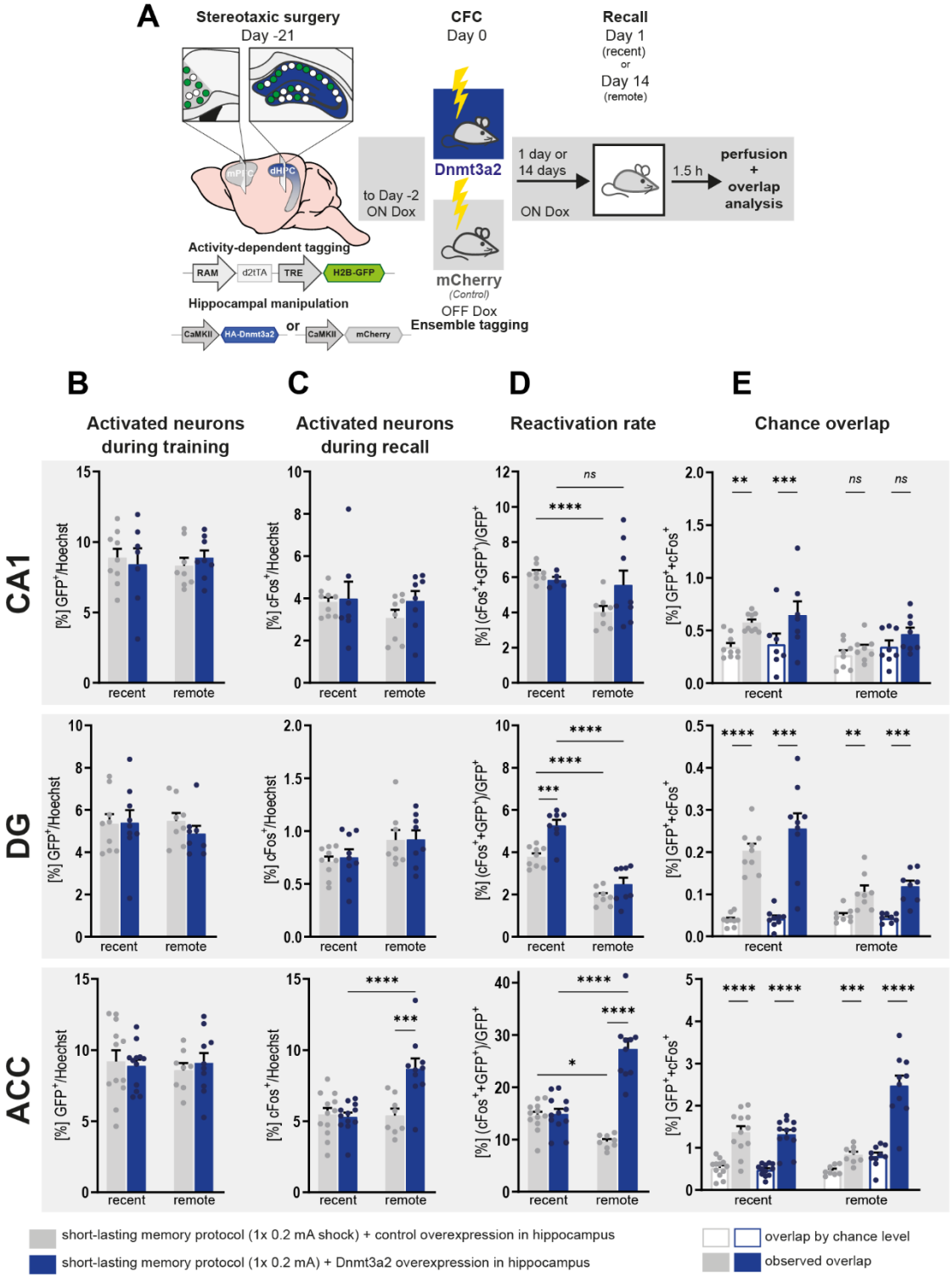


Figure 14. Hippocampal DNA methylation stabilises cortical ensemble neurons. (A) Schematic representation of experimental design. Stereotaxic injection of rAAV to tag neuronal ensembles in CA1, DG and ACC as well as rAAV injection in dHPC to manipulate Dnmt3a2 levels. Three weeks after surgery, mice were trained with the weak (1x 0.2 mA shock) CFC paradigm and their memory was tested in a recall session either 1 day or 14 days after. 1.5h post recall, mice were sacrificed for overlap analysis. **(B, C, D, E)** Quantitative image analysis in CA1, DG and ACC region of (B) activated neurons during training assessed by GFP signal, (C) activated neurons during recall identified by endogenous cFos labelling, (D) reactivation rate (GFP^++$cFos^+$ neurons in GFP^+ population) and (E) chance overlap (n=7-12). *p<0.05, ***p≤0.001, ****p≤0.0001, ns: not significant by one-way ANOVA test followed by Sidak's multiple comparisons test. (G) **p≤0.01, ***p≤0.001, ****p≤0.0001 ns: not significant by two-tailed, paired t-test.

2.3 Dnmt3a2 regulates transcription of genes associated with synaptic transmission

Persistent memories are associated with stable changes in DNA methylation in the cortex (Halder et al., 2015; Miller et al., 2010) and our group showed recently differentially methylated sites on genes associated with functional and structural plasticity upon activity-regulated overexpression of Dnmt3a2 in primary hippocampal cultures (Gulmez Karaca et al., 2020). In order to gain mechanistic insight and to identify the possible downstream effectors through which DNA methylation acts to drive systems consolidation, RNA sequencing analysis of dHPC from mice either overexpressing Dnmt3a2 or a control construct 1 day or 5 days after they underwent the weak CFC protocol was performed (Figure 15A). One day after CFC, 20 DEGs were found (Figure 15B). The number of DEGs increased at day 5 to 249 (Figure 15B). This finding may suggest that transcriptional changes accompany the systems consolidation process. GO term analysis revealed a strong enrichment for terms related to synaptic regulation, e.g., "*Modulation of chemical synaptic transmission*", "*Regulation of trans-synaptic signaling*", "*Synaptic signaling*" and "*Cell-cell signaling*" (Figure 15C).

To validate the RNA sequencing results, I performed qRT-PCR analysis on independent biological samples. To clarify whether the manipulation changes the transcriptome already in baseline condition, home cage controls were included. *Nfix* and *Plxnc1* are genes that were differentially methylated upon Dnmt3a2 overexpression in primary hippocampal cells in our previous study (Gulmez Karaca et al., 2020). I found the expression of *Plxnc1* to be statistically significant upregulated 5 days after CFC and not to be changed at home cage levels or 1 day after CFC (Figure 15D). Whereas *Nfix* expression was downregulated at all time points (Figure 15D) that is in discrepancy to the RNA Sequencing analysis which showed these genes downregulated only 5 days after CFC training. The RNA sequencing revealed further DEGs that have previously been found to be differentially methylated *in vivo* upon CFC (Duke et al., 2017; Halder et al., 2015) (Figure 15 E). In addition, some genes, e-g- *Calb1* and *Fhad1*, are known to increase in DG engram neurons after CFC (Marco et al., 2020; Rao-Ruiz et al., 2019) (Figure 15F). Summarising, these results indicate that Dnmt3a2 overexpression led to transcriptomic changes that might altered synaptic transmission.

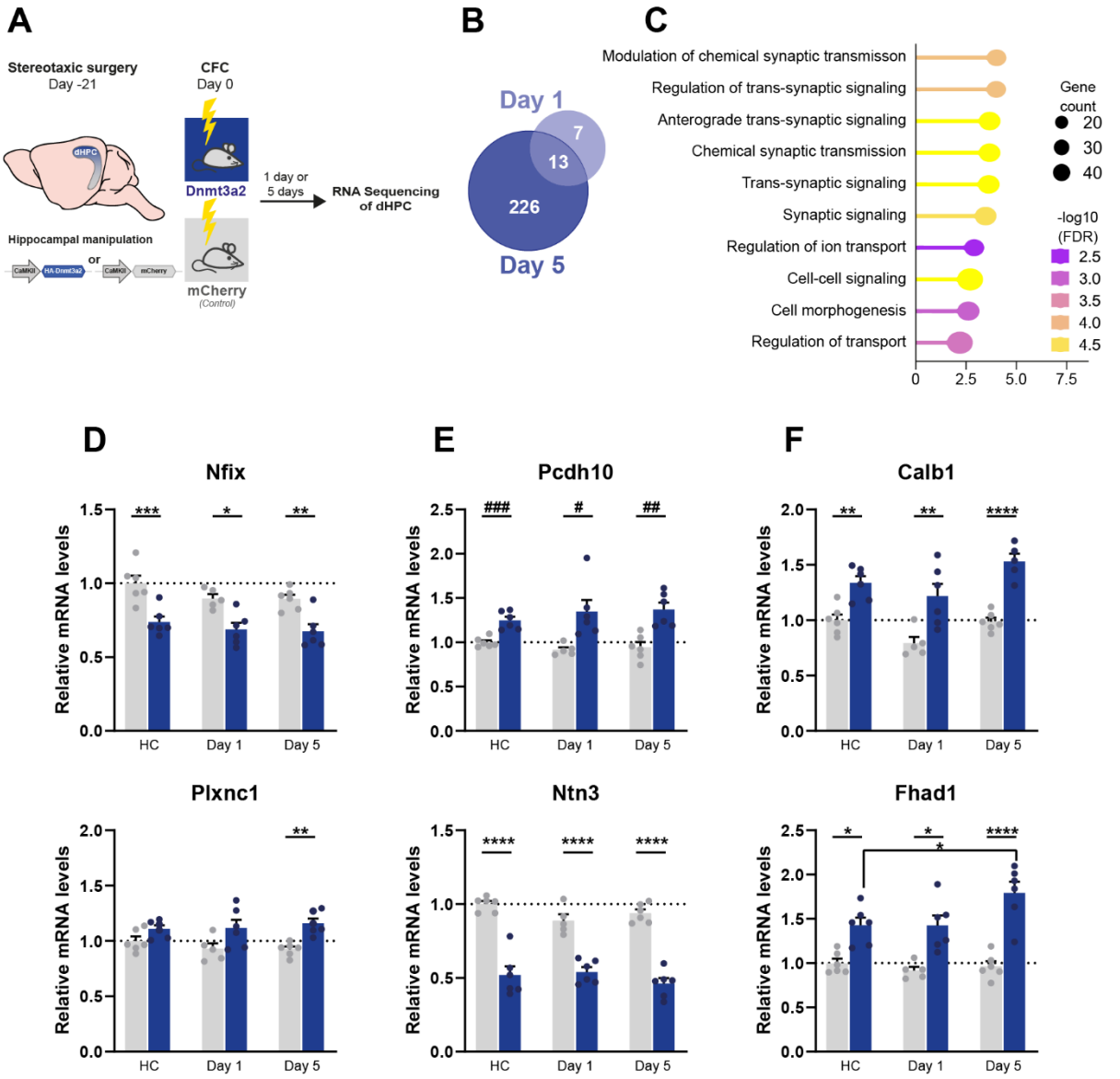


Figure 15. Dnmt3a2 regulates transcription of genes involved in synaptic transmission. (A) Schematic representation of experimental design. Stereotaxic injection of rAAV in dHPC to manipulate Dnmt3a2 levels. Three weeks after surgery, mice were trained with the weak (1x 0.2 mA shock) CFC paradigm and either 1 day or 5 days later dHPC were collected and RNA extracted for RNA Sequencing analysis. **(B)** Venn-Diagram of DEGs 1 day and 5 days after CFC training. **(C)** GO-Term analysis of DEGs. Dot plot illustrates Top 10 GO term enrichment of biological processes. **(D, E, F)** qRT-PCR analysis of genes in dHPC of mice infected with rAAVs to overexpress mCherry (n=5-6) or Dnmt3a2 (n=6) that either stayed in their home cage (HC) or underwent CFC and were sacrificed 1 day or 5 days later. Expression levels were normalised to the mCherry HC group (dashed line). *p<0.05, **p≤0.01, ***p≤0.001, ****p≤0.0001 by one-way ANOVA test followed by Sidak's multiple comparisons test; #p<0.05, ##p≤0.01, ###p≤0.001 by two-tailed, paired t-test. *RNA Sequencing was analysed by Carsten Sticht. RNA extraction was performed by Ana Oliveira and Stefanos Loizou.*

2.4 Hippocampal Dnmt3a2 ensures that the memory trace is transferred to the cortex

A trait for persistent memories is believed to be that the stored information is being gradually transferred from the representation in the hippocampus to the cortex in the process called systems consolidation (Frankland & Bontempi, 2005; Wiltgen et al., 2004). One characteristic of persistent memory stored in the cortex is that the information loses contextual accuracy, likely due to the fact that the hippocampus is not involved in memory retrieval (Wiltgen et al., 2010). As a result, it is known that long-lasting fear memory undergoes contextual generalisation (Asok et al., 2019; Huckleberry et al., 2016). Essentially, mice that underwent CFC in a specific context A show freezing behaviour at remote memory recall in a novel context B. Therefore, to test whether the observed increased reactivation of cortical engram (see Figure 14D) is associated with reduced contextual specificity, which would indicate the transfer of the memory representation towards cortical circuits, I conducted a fear memory generalisation test in mice. To this end, mice were injected with rAAVs expressing either the Dnmt3a2 overexpression or a control construct and trained in the weak CFC and tested for their memory performance at recent or remote memory recall sessions. They were first tested in the specific context to validate that the fear memory was formed (recent) and still retained (remote). The next day, mice were exposed to an altered, novel context that was not associated with the shock. I included a cohort of mice, that was trained with the strong CFC as a positive control for fear generalisation (Figure 16A). In the specific context, control mice that underwent the weak protocol showed the expected memory decay with time (open vs. closed bar); whereas mice overexpressing Dnmt3a2 in dHPC still retained the memory; similar to control mice, that underwent strong memory training, although the percentage of freezing was quite different (Figure 16B). At recent memory recall in the altered context all conditions showed low freezing levels in relation to their freezing in the specific context, indicating that the animals were able to distinguish the novel, altered context (Figure 16C). On the other hand, at day 15 both groups that still exhibit persistent fear memory (Dnmt3a2 overexpression and strong training), froze significantly more compared to the recent memory recall (Figure 16C). This result demonstrates that the persistent memory achieved by hippocampal Dnmt3a2 overexpression undergoes fear generalisation and hints that the memory information is transferred to the cortex.

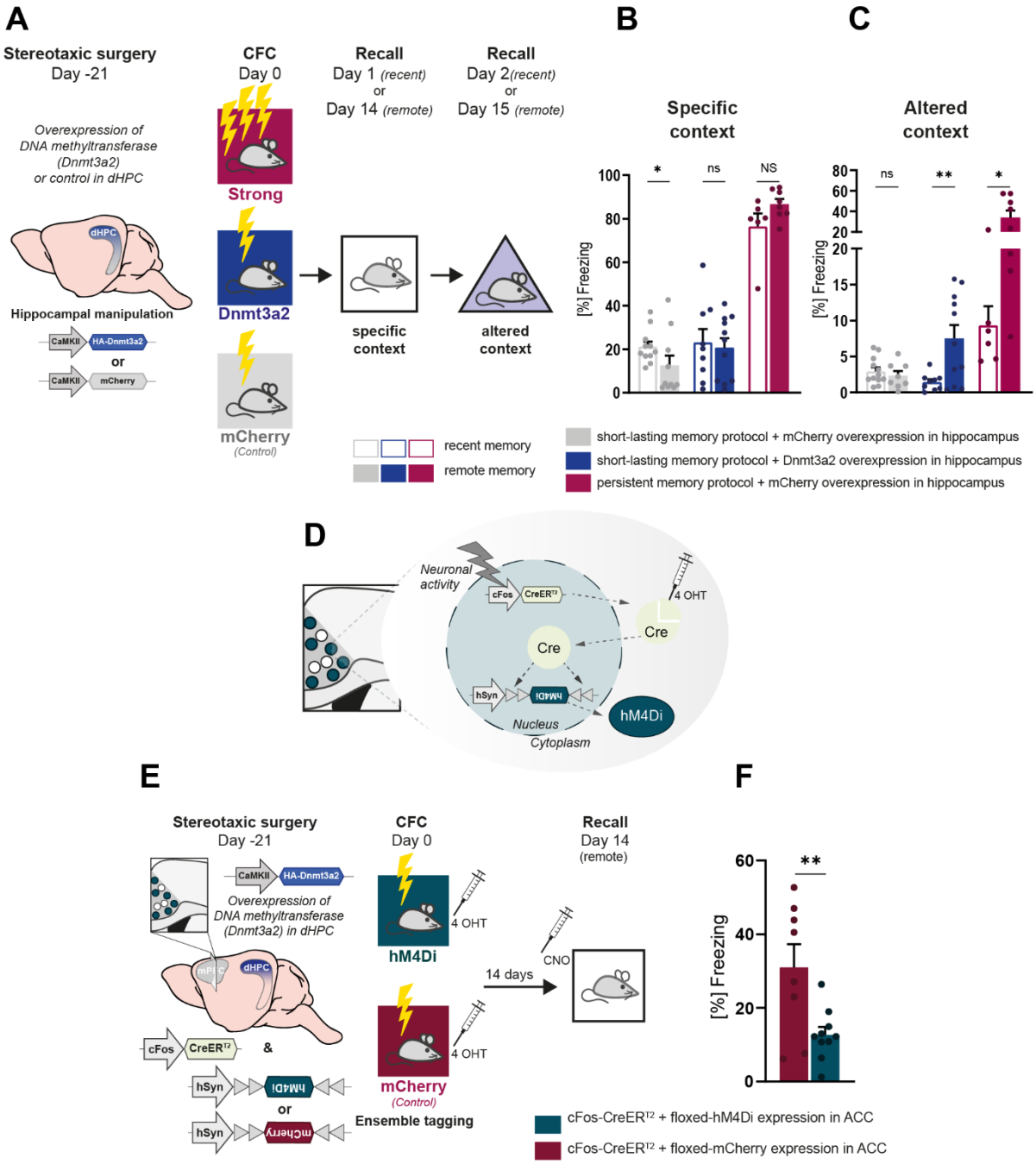


Figure 16. Hippocampal Dnmt3a2 ensures that the memory trace is transferred to the cortex. (A) Schematic representation of experimental design. Stereotaxic injection of rAAV in dHPC to manipulate Dnmt3a2 levels. Three weeks after surgery, mice were trained with the weak or strong CFC paradigm and tested for their memory specificity in the specific context (either day 1 or 14) and an altered context (either day 2 or 15). **(B)** Recent or remote contextual fear memory of the specific context of mice trained with the weak CFC protocol and expressing mCherry (n=10-11) or Dnmt3a2 (n=9-11) or mice trained with the strong CFC protocol and expressing mCherry (n=6-8). *p<0.05, ns: not significant by two-tailed, unpaired t-test. NS: not significant by Mann-Whitney test. **(C)** Freezing behaviour in an altered context of mice trained with the weak CFC protocol and expressing mCherry (n=8-12) or Dnmt3a2 (n=9-11) or mice trained with the strong CFC protocol and expressing mCherry (n=6-8). *p<0.05, **p≤0.01, ns: not significant by two-tailed, unpaired t-test. **(D)** Schematic representation of viral TRAP system. Neuronal activity triggers the activation of the cFos promoter, which causes CreER^{T2} expression. By administering 4-hydroxytamoxifen (4 OHT) systemically, translocation of CreER^{T2} into the nucleus is allowed and irreversible recombination of the Cre-dependent vector is made possible. *Figure legend continues on next page.*

Figure 16. Hippocampal Dnmt3a2 ensures that the memory trace is transferred to the cortex. **(E)** Schematic representation of experimental design. Stereotaxic injection of rAAV to express Dnmt3a2 in dHPC and the viral Targeted Recombination in Active Populations (TRAP) in the ACC. Three weeks after surgery, mice were trained with the weak CFC paradigm. To trap cortical engram neurons, 4-Hydroxytamoxifen (4 OHT) was injected *i.p.* 2h after CFC. 30 min prior memory recall session on day 14, mice were *i.p.* injected with Clozapine N-oxide (CNO) to inhibit the trapped cortical engram neurons and tested for their memory. **(F)** Remote contextual fear memory of mice trained with the weak CFC protocol and overexpressing Dnmt3a2 in dHPC as well as expressing viral trapped control construct (mCherry; n= 8) or the inhibitory DREADD (hM4Di; n=10) in ACC. **p≤0.01 by two-tailed, unpaired t-test.

However, to demonstrate that the memory trace is encoded in the cortical engram, it needs to be shown that remote memory retrieval requires the activation of cortical engram cells. To assess this, I took advantage of the viral Targeted Recombination in Active Populations (TRAP) system (Matos et al., 2019) that expresses the inhibitory Designer Receptors Exclusively Activated by Designer Drugs (DREADD) – hM4Di – only in cortical ensembles activated during CFC training. This technique uses two rAAVs coding for inducible Cre recombinase ($CreER^{T2}$) under the control of the cFos promoter and the inhibitory DREADD hM4Di coding sequence in an inverted open reading frame and flanked by Cre recognition sites. Neuronal activity triggers expression of $CreER^{T2}$ and systemically administration of 4-hydroxytamoxifen (4 OHT) allows coupling of neuronal activity-dependent $CreER^{T2}$-mediated hM4Di expression (Figure 13D). Mice received an rAAV mixture of cFos-driven $CreER^{T2}$ and Cre-dependent hM4Di or a control into the ACC as well as the Dnmt3a2 overexpression construct into the dHPC. To trap cortical engram, CFC was followed by 4 OHT injection intraperitoneal. To find out whether cortical ensemble neurons are involved in remote fear memory expression, remote memory recall session was performed whilst inhibiting CFC-tagged $hM4Di^{+}$ neurons (Figure 13E). Inhibition of the cortical engram by systemic administration of Clozapine-N-oxide (CNO) abolished remote fear memory retrieval compared to control cohort (Figure 13F) and thereby implying a functional link between increased cortical engram reactivation upon hippocampal Dnmt3a2 overexpression and memory performance.

2.5 Endogenous DNA methylation processes regulate memory duration and affect systems consolidation

So far, I was able to show that enhanced hippocampal DNA methylation by Dnmt3a2 is a positive regulator for memory duration and facilitates system consolidation processes. Nevertheless, to determine whether endogenous DNA methylation processes are crucial for memory persistence and systems consolidation, I carried out a loss-of-function approach. Thus, I expressed a former validated and already in **Section II 1.** used shRNA construct (Kupke et al., 2023; A. M. M. Oliveira

et al., 2012) to knockdown Dnmt3a2 in dHPC and examined the effect on memory duration. High salience training may cause overtraining and since it was previously shown that such strong CFC resulted in a prefrontal engram that even via chemogenetic inhibition did not affect memory (Matos et al., 2019), an additional CFC paradigm was used. It consisted of training with 1x 0.7mA foot shock and was prior validated to induce long-lasting fear memory (A. M. M. Oliveira et al., 2012). Stereotaxic surgery to deliver rAAVs expressing either the Control-shRNA or Dnmt3a2-shRNA into the dHPC were performed and in addition, to probe the effect of the loss-of-function on cortical engram stabilisation, the engram tagging tool was injected into the ACC. Two weeks after surgery, mice were trained in CFC, followed by remote memory recall session and engram overlap analysis (Figure 17A, B). First, to exclude the possibility that memory encoding is affected by Dnmt3a2 knockdown, I tested the memory performance during recent (24h) memory retrieval on an independent cohort of mice and found the freezing rates not changed (Figure 17C) implying no effect on memory formation. Remarkably, when tested for their remote memory performance, mice that expressed the viral construct to reduce Dnmt3a2 levels showed memory impairments (Figure 17D) indicating that endogenous DNA methylation is necessary for memory duration. Next, I explored the consequences for cortical engram maturation. Knockdown of hippocampal Dnmt3a2 did not affect the size of the cortical engram during encoding (Figure 17E), nor the endogenous activity during memory retrieval measured indirectly by immunostaining and counting of $cFos^+$ cells (Figure 17F). Intriguingly, however was that the reactivation rate significantly decreased (Figure 17G), in spite of being still above chance levels (Figure 17H). Taken together, the loss-of-function revealed that endogenous DNA methylation processes in the hippocampus are pivotal for memory strength and duration as well as important for cortical engram stabilisation.

In this section of my thesis, I showed that DNA methylation processes in the dorsal hippocampus converted a short-lasting fear memory into a persistent memory. This was accompanied by improved reactivation of the cortex engram over time and a functional chemogenetic experiment proved that the memory trace is transferred to the cortex. These findings verify that DNA methylation processes facilitate systems consolidation mechanisms.

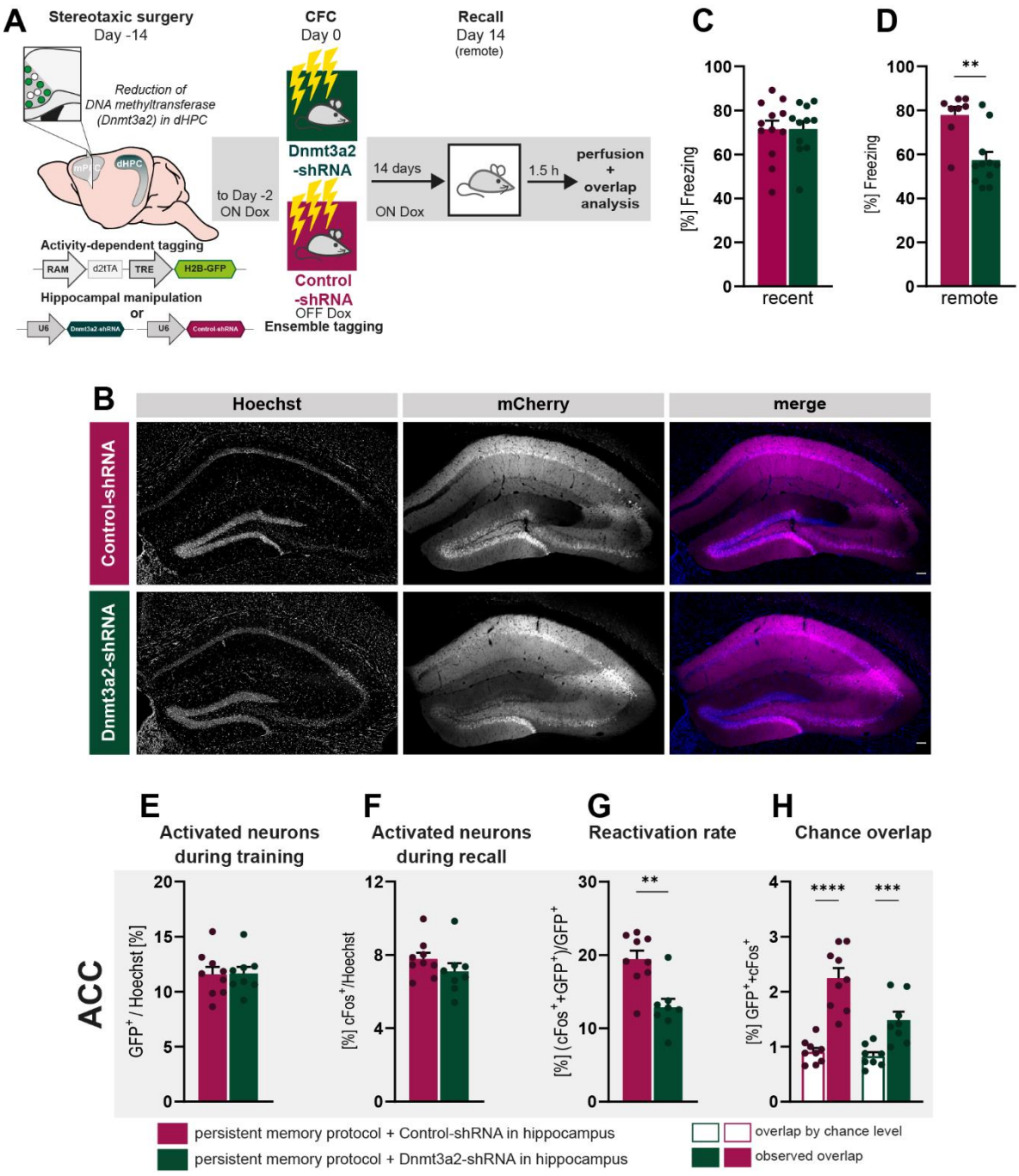


Figure 17. Endogenous DNA methylation processes regulate memory duration and affect systems consolidation. (A) Schematic representation of experimental design. Stereotaxic injection of rAAV to knockdown endogenous levels of Dnmt3a2 in the dHPC. Two weeks after surgery, mice were trained with a strong (1x 0.7 mA shock) CFC paradigm and their memory was tested in a recall session 14 days after. 1.5h post recall, mice were sacrificed for overlap analysis. **(B)** Representative images of dHPC of mice infected rAAVs against Control (shRNA-unc) or shRNA-Dnmt3a2. Scale bar: 100µm. **(C)** Recent contextual fear memory of mice trained with a strong (1x 0.7mA shock) CFC protocol and infected with rAAVs containing shRNA-unc (n=12) or shRNA-Dnmt3a2 (n=11). **(D)** Remote contextual fear memory of mice trained with a strong (1x 0.7mA shock) CFC protocol and infected with rAAVs against shRNA-unc (n=8) or shRNA-Dnmt3a2 (n=11). *p<0.05 by by two-tailed, unpaired t-test. **(E, F, G, H)** Quantitative image analysis in ACC region of (E) activated neurons during training assessed by GFP signal, (F) activated neurons during recall identified by cFos labelling, (G) reactivation rate (GFP^++$cFos^+$ neurons in GFP^+ population) and (H) chance overlap (n=8-9). **p≤0.01 by two-tailed, unpaired t-test. (H) ***p≤0.001, ****p≤0.0001 by two-tailed, paired t-test.

3. High salient fear memory induces a biphasic Npas4 expression and results in memory suppression

This part of my thesis revealed the presence of a molecular mechanism that not only facilitate but actively restrict the formation of memories. It is well-established that IEG expression promotes the consolidation of long-term memory following learning. Additionally, learning induces specific transcriptional signatures that decode the initial salience of the learning event. However, it remains unclear whether stimulus salience triggers gene expression associated with memory suppression.

3.1 High salient fear training is associated with a second Npas4 expression wave

To uncover this, the effect of varying stimulus salience on IEG expression patterns in CA1 of the dHPC was examined. Mice were trained in CFC with the above (Section II 2.) established weak or strong protocol. Subsequently, the expression of IEGs in these mice was analysed using qRT-PCR and Western Blot and compared to their home cage control (Figure 18A). The low salience weak protocol (1x 0.2mA foot shock) induces non-persistent memories, whereas the high salience strong protocol that consists of 3x 0.7mA foot shock for the mice leads to persistent memory (see Figure 12B). For the time course of IEG expression, the focus was on the critical phase of memory consolidation, which is known to be influenced by the salience of the learning stimulus (Igaz et al., 2002; Katche et al., 2010; Mukherjee et al., 2018b) and on the expression of Arc, cFos (data not shown, *part of David VC Brito's thesis*) and Npas4. To account for potential circadian fluctuations in gene expression, home cage controls were sacrificed at the same time of the day. As expected, the IEG expression of trained mice increased compared to their home cage controls within 15 minutes to 1 hour after learning consistent with their fast regulation (Figure 18B-G). Nonetheless, there were visible differences in the induction kinetics between mice trained in the two protocols. In the early phases of gene expression, the weak training led to a more transient Npas4 expression profile (Figure 18B) compared to the strong training (Figure 18C) on mRNA level. Intriguingly, this was the opposite when comparing protein levels (Figure 18D-G). At 1h post training, both protocols induced Npas4 mRNA expression levels similar to baseline (Figure 18B, C). However, mice that underwent strong CFC training displayed a delayed Npas4 expression at 4h on mRNA levels (Figure 18C) and at 6h for Npas4 protein (Figure 18E, G). This delayed Npas4 expression was specific for mice trained with the strong, high salient protocol, as animals that were subjected to the weak, low salient training displayed Npas4

expression comparable to baseline (Figure 18B, D, F). These findings indicate that high salience fear conditioning triggers a biphasic Npas4 expression pattern, whereas such pattern is absent when a low salience stimulus is employed.

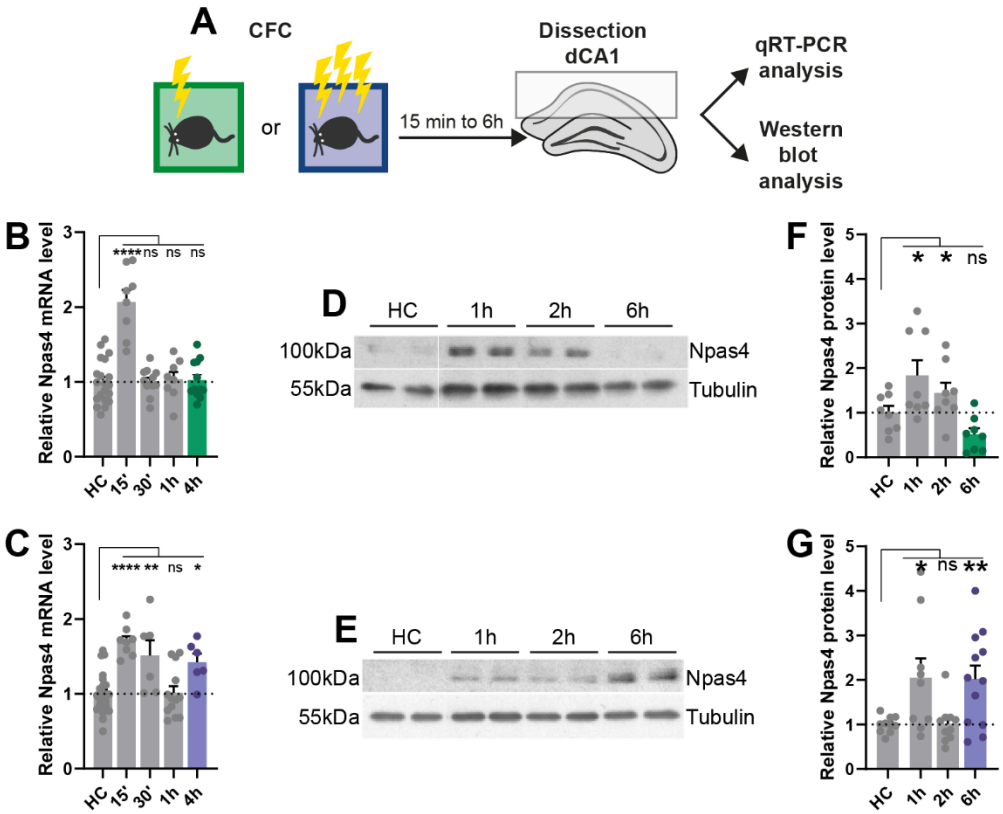


Figure 18. High salience contextual fear learning induces a biphasic Npas4 expression in the dorsal CA1. (A) Schematic representation of experimental design. Mice were trained in low or high salience fear conditioning and were sacrificed after 15min, 30 min, 1h, 2h, 4h or 6h. dCA1 was dissected and mRNA or protein was isolated to perform qRT-PCR or western blot, respectively. Tissue from home cage control mice was collected at same time of day to control for circadian alterations. **(B, C)** qRT-PCR analysis of Npas4 expression of mice that underwent (B) low or (C) high salience fear conditioning (n=8-20). *p<0.05, **p≤0.01, ***p≤0.001, ****p≤0.0001; ns: not significant by one-way ANOVA test followed by Dunnett's multiple comparisons test. **(D, E)** Representative immunoblot scans of the expression of Npas4 or Tubulin in the dCA1 of mice that underwent (D) low or (E) high salience fear conditioning. HC: Home cage. **(F, G)** Immunoblot quantification of Npas4 protein levels in the dCA1 of mice that underwent (D) low (n=8) or (E) high (n=10-12) salience fear conditioning. Dashed lines represent normalised Npas4 to Tubulin expression of HC group. *p<0.05, **p≤0.01, ***p≤0.001, ****p≤0.0001; ns: not significant by one-way ANOVA test followed by Dunnett's multiple comparisons test. *Time course experiment was performed by David Brito and is part of his doctoral thesis.*

3.2 NMDA receptor activity regulates second wave of Npas4 expression.

The delayed Npas4 expression, occurring several hours after the initial learning session, could arise from two potential explanations: Firstly, it is possible that the high salience stimulus triggers a series of molecular events that autonomously lead to the expression of Npas4 within cells, without the need for external stimuli. So, to speak an intrinsic cell autonomous mechanism. Alternatively, it could be hypothesised that neurons receive new inputs hours after learning, which then prompt the late expression of Npas4.

Previous studies have demonstrated that Npas4 expression is regulated by neural activity and relies on N-methyl-D-aspartate receptor (NMDAR) function and calcium influx (E. L. Yap & Greenberg, 2018). Additionally, late expression events of IEGs have been shown to be regulated by neuromodulatory neurotransmitters like dopamine (Karunakaran et al., 2016; Rossato et al., 2009). To address the question whether new activity inputs through NMDAR or dopaminergic D1 receptor is required for second Npas4 transcriptional wave, a pharmacological approach was employed. For this purpose, mice that underwent high salience CFC and were infused with either the NMDA receptor antagonist APV or the dopaminergic antagonist SCH23390 into the CA1 region 3.5 hours after training (Figure 19A). This time point was chosen to assess the impact of the receptor blockage on the late Npas4 expression at 4 hours post training (see Figure 18C). The dorsal CA1 region of these mice was dissected 6

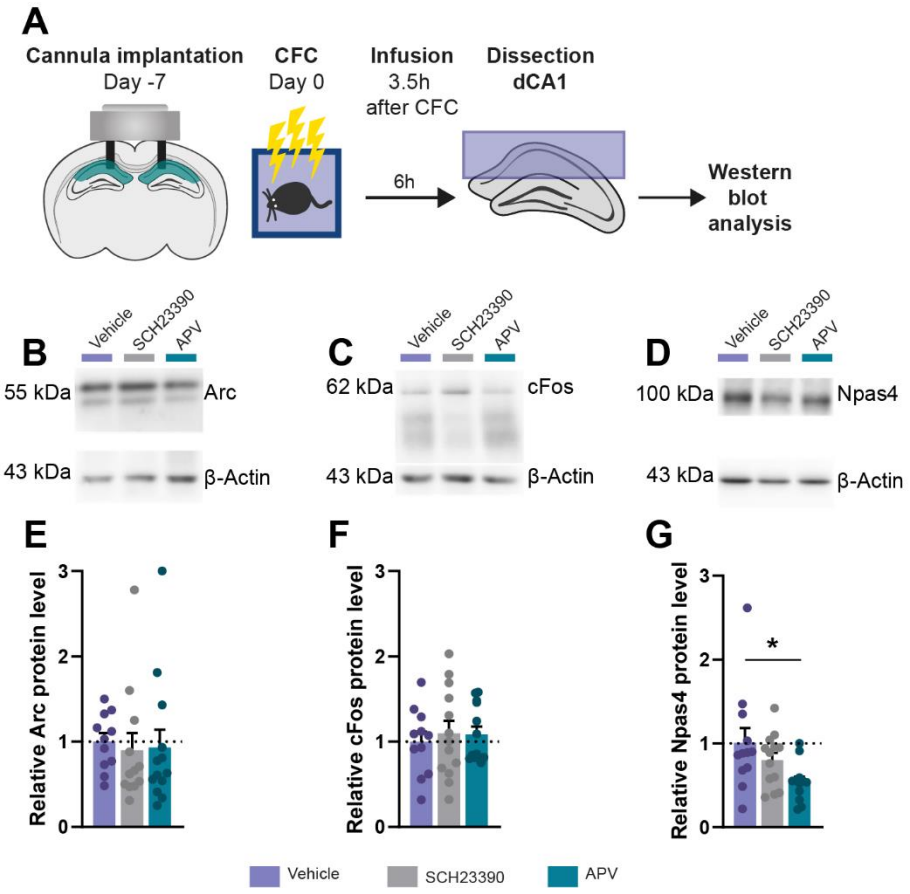
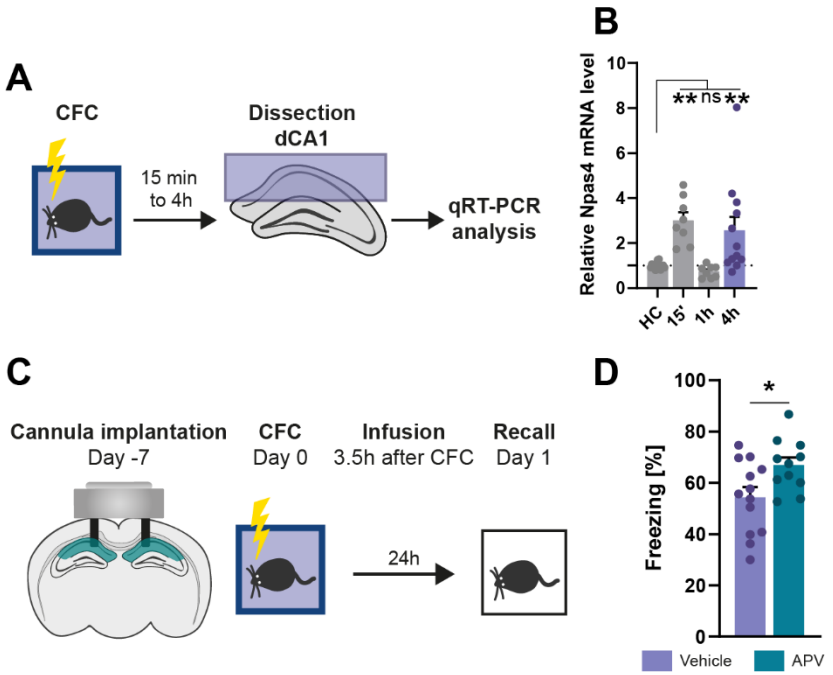


Figure 19. Second wave of Npas4 expression is regulated by NMDA receptor activity. (A) Schematic representation of experimental design. One week after cannula implantation, mice were trained in high salience fear conditioning training and 3.5h after infused with vehicle or pharmacological inhibitors of dopamine D1 receptors (SCH23390) or NMDA receptors (APV). 6h after CFC training, dCA1 were collected and protein isolated for western blot analysis. **(B, C, D)** Representative immunoblot scans of the expression of (B) Arc, (C) cFos and (D) Npas4 and β-Actin of mice trained in high salience CFC and infused with drugs. **(E, F, G)** Immunoblot quantification of (B) Arc, (C) cFos or (D) Npas4 protein levels in the dCA1 (n=11-12). Dashed lines represent normalised protein expression to Actin of Vehicle group. *p<0.05 by one-way ANOVA test followed by Dunnett's multiple comparisons test. *Pharmacological experiments were performed by David Brito and are part of his doctoral thesis.*

hours post training, since it coincided with Npas4 protein expression (see Figure 18E, G) (Figure 19A). The expression of Arc and cFos was also monitored to exclude that the receptor blockage unspecifically affects transcription of IEGs. Neither APV infusion nor SCH23390 affected the expression of Arc (Figure 19B, E) or cFos (Figure 19C, F). Blocking dopaminergic D1 receptor had no effect on the second Npas4 protein expression (Figure 19D, G), whereas blocking the NMDAR with APV infusion significantly reduced Npas4 expression (Figure 19D, G). This finding aligns with recent studies that showed Npas4 expression being highly dependent on neuronal activity and calcium influx rather than induced by signalling pathways downstream of neuromodulators (Lissek et al., 2021; Ramamoorthi et al., 2011) and overall suggests that the biphasic Npas4 expression relies on NMDAR activity.

3.3 Blocking Npas4 second expressional wave enhances memory consolidation

Consequently, I hypothesised whether NMDAR activation driving the second Npas4 expression wave has an impact on memory consolidation. So, to eliminate potential confounding factors associated with overtraining with such a strong protocol, I established another high salience fear conditioning paradigm. Albeit, I had



to confirm the existence of the biphasic Npas4 expression under this second high

Figure 20. Blockade of Npas4 second wave enhances memory consolidation. (A) Schematic representation of experimental design. Mice were trained in a second high salience contextual fear paradigm and their dCA1 was dissected for qRT-PCR analysis. **(B)** qRT-PCR analysis of Npas4 expression (n=8-15). **p≤0.01, ns: not significant by one-way ANOVA test followed by Sidak's multiple comparisons test. **(C)** Schematic representation of experimental design. **(D)** Long-term contextual fear memory of mice trained with high salience CFC and infused with Vehicle or APV (n=11-13). *p<0.05 by two-tailed, unpaired t-test.

salience protocol. To this end, mice were trained with the new CFC protocol that consisted of a 1x 0.7mA foot shock and their CA1 was dissected and analysed for Npas4 expression at different time points compared to home cage controls (Figure 20A). I confirmed the induction of biphasic Npas4 expression (Figure 20B) and was able to now apply this protocol to answer whether the second Npas4 expression wave impacts memory consolidation. Mice trained in this protocol received infusions of either APV or a vehicle into the CA1 region and were tested for memory performance 24 hours later (Figure 20C). Blocking NMDAR activity, and thereby suppressing late Npas4 expression, resulted in enhanced memory (Figure 20D). This suggests that late NMDAR activity hinders the consolidation of highly salient experiences.

3.4 Biphasic Npas4 expression suppresses memory consolidation.

In order to evaluate the functional significance of the late expression of Npas4, I used the previously established TetON system (see Figure 6) to artificially induced the expression of Npas4 (or GFP as control) in the weak protocol, that naturally does not trigger a second Npas4 wave (Figure 21A). First, to assess the expression kinetics of this system *in vivo*, I delivered the viral vectors into the CA1 region and monitored transgene expression by time course analysis of Npas4 protein levels (Figure 21B, C).

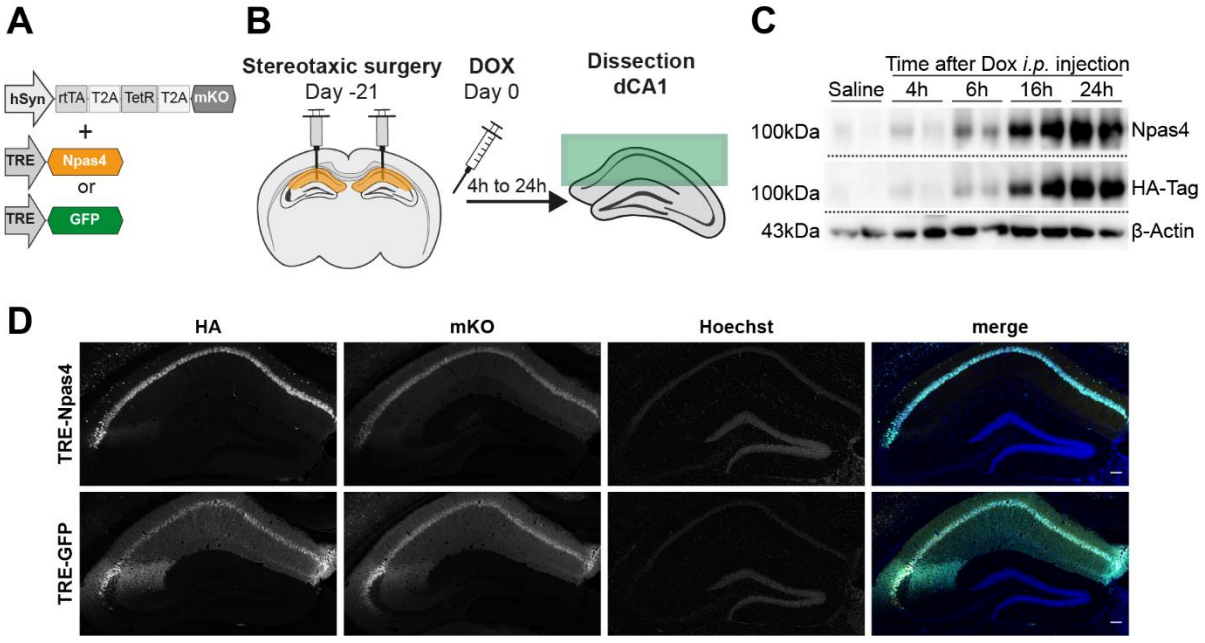


Figure 21. Validation of TetON system to temporally-restricted Npas4 overexpression. (A) Schematic representation of viral constructs. **(B)** Schematic representation of experimental design. rAAVs were delivered into dCA1 of mice. Three weeks later, mice received *i.p.* (intraperitoneal) injections of Saline or Dox and were sacrificed 4h, 6h, 16h or 24h later. **(C)** Representative immunoblot scans of exogenous Npas4 expression. **(D)** Representative images of the dorsal hippocampus of mice infected with TetON system for GFP expression or exogenous Npas4. Scale bar: 100 µm.

Exogenous Npas4 protein was robustly detectable 6 hours after a single intraperitoneal administration of doxycycline and reached its peak between 16 and 24 hours (Figure 21C). Further, I verified successful viral expression and specific targeting to CA1 via immunostaining of HA-tagged exogenous Npas4 (Figure 21D). This established a doxycycline-dependent tool kit to artificially induce the second wave of Npas4. Subsequently, to elucidate whether this artificial induction of late Npas4 expression influences memory consolidation, I delivered the rAAVs expressing GFP or Npas4 into the CA1 region of mice (Figure 22A). Mice were trained with the weak, low salient CFC and in order to express Npas4 at the time point corresponding

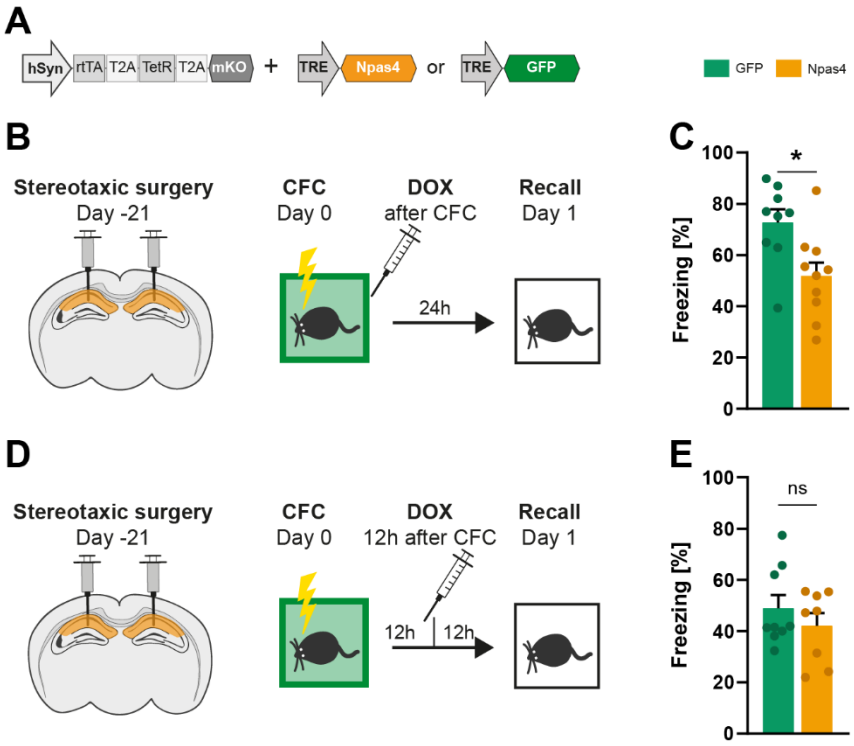


Figure 22. Second wave of Npas4 induces memory suppression. (A) Schematic representation of viral constructs. **(B)** Schematic representation of experimental design. **(C)** Long-term contextual fear memory of mice infected with rAAVs expressing TetON system and GFP (n=9) or Npas4 (n=10) and trained with low salience CFC. *p<0.05 by two-tailed, unpaired t-test. **(D)** Schematic representation of experimental design. **(E)** Long-term contextual fear memory of mice infected with rAAVs expressing TetON system and GFP (n=9) or Npas4 (n=8) and trained with low salience CFC. ns: not significant by two-tailed, unpaired t-test.

to its natural delayed wave due to high salient fear learning - at 6 hours post training (see Figure 18E, G) -, I administered doxycycline immediately after weak CFC (Figure 22B). Mice that expressed late Npas4 within the window of memory consolidation exhibited impairments in long-term memory compared to the control group expressing GFP (Figure 22C). This suggests that biphasic Npas4 expression hampers memory consolidation. To specify whether increased Npas4 expression at 4-6 hours after training is crucial for memory impairment, I temporally shifted the exogenous Npas4 expression using a similar experimental design. In this case, I shifted the

expression of Npas4 to a later stage, which did not overlap with the 4–6-hour time window. To achieve this, mice were injected with rAAVs and trained in the weak CFC and received 12 hours post training a doxycycline injection (Figure 22D). Induction of Npas4 outside of the critical and natural 4-6h window resulted in similar freezing behaviour of mice compared to the control group (Figure 22E), further indicating that the memory suppressor function of Npas4 is associated with the 4–6-hour time point.

In this work, I discovered a unique mechanism that is negatively gated by fear salience and controls memory consolidation. I demonstrated that highly salient experience caused a second Npas4 expression, in contrast to low salience experiences. In addition, I discovered that NMDAR activity is necessary for the late wave of Npas4 expression and pharmacological and genetic experiments pointed out that late Npas4 expression constraints memory consolidation.

III. Discussion

1. Dnmt3a1 regulates hippocampus-dependent memory via the downstream target Nrp1

Rapid and dynamic DNA methylation changes in response to neuronal activity have been shown to be a critical level of regulation of genomic responses during memory formation (Halder et al., 2015; Lubin et al., 2008; Miller & Sweatt, 2007; A. M. M. Oliveira, 2016; A. M. M. Oliveira et al., 2016). However, little is known about how DNA methylation affects synaptic plasticity and memory formation and the specific role of the different Dnmts in this process and how their activity is controlled is still unclear.

In this part of my thesis, I showed that acute knockdown of Dnmt3a1 in the adult hippocampus specifically inhibits long-term memory formation in two hippocampal-dependent tests without impacting short-term memory, revealing a crucial function for this protein in long-term memory formation. I also identified activity-regulated genes which expression is dependent on Dnmt3a1 levels. Moreover, my research indicates that while both Dnmt3a1 and Dnmt3a2 are crucial for memory formation, the two isoforms regulate this process in different manners. Whereas Nrp1 overexpression rescued Dnmt3a1-dependent memory impairments, it did not rescue Dnmt3a2-knockdown driven effects (Figure 23). Together, this research has improved our understanding of the requirement for distinct Dnmts in memory processes as well as uncovered a novel downstream effector molecule involved in memory formation.

The requirement for the activity of Dnmts in memory formation has been demonstrated by studies showing that pharmacological inhibition of Dnmts at the time of learning disrupts memory formation (Lubin et al., 2008; Miller & Sweatt, 2007; Mitchnick et al., 2015), thereby providing the first causal link between the activity of Dnmts and memory formation. To study the specific role of Dnmt3a in the adult brain, genetic studies were conducted. Most used heterozygous constitutive (Christian et al., 2020) or conditional knock-out mice (Feng et al., 2010; J. Li et al., 2022; Morris et al., 2014). This, however deletes Dnmt3a during critical prenatal and postnatal neurodevelopmental phases, which might affect the interpretation of the function of *Dnmt3a*-coded proteins in memory formation in the adult. Nevertheless, these genetic studies indicate a requirement of the *Dnmt3a* gene in memory processes (Feng et al., 2010; J. Li et al., 2022; Morris et al., 2014). In 2015, Mitchnick and colleagues acutely decreased Dnmt3a levels in the adult hippocampus using infusion

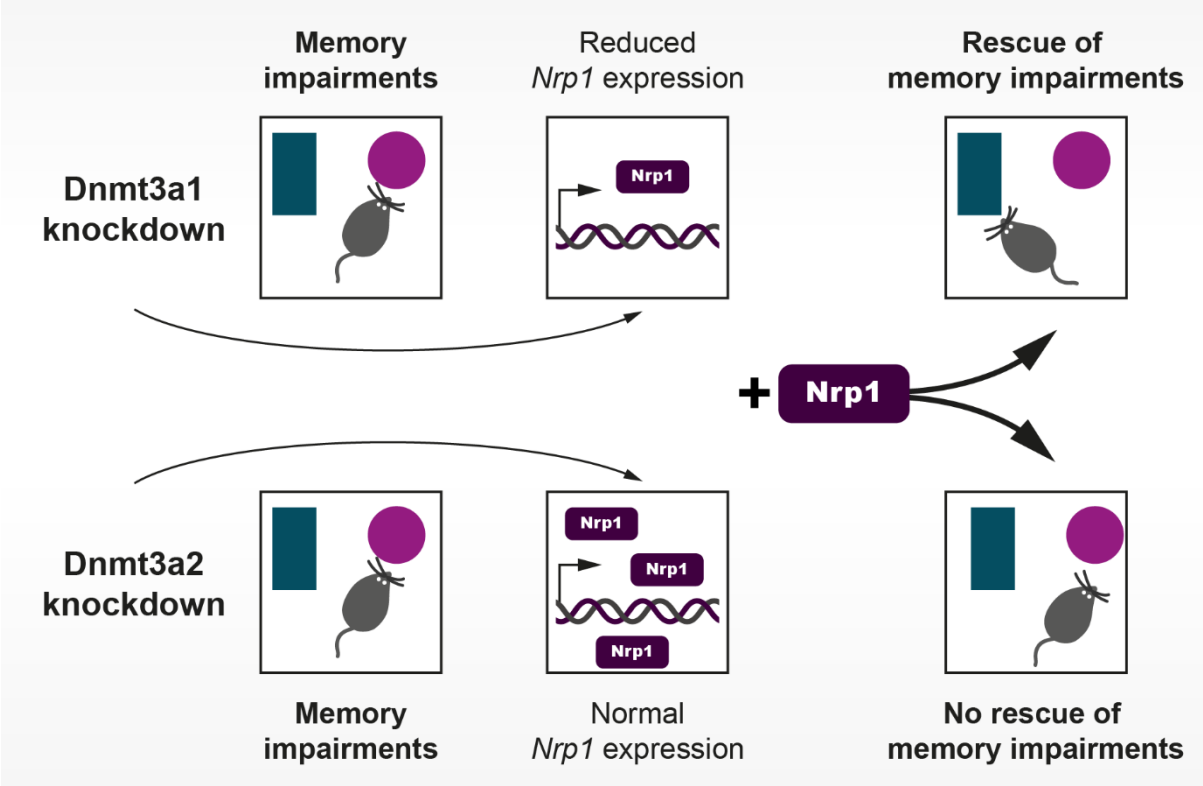


Figure 23. Graphical illustration of main findings of Section 2.1: Dnmt3a1 regulates hippocampus-dependent memory via the downstream target Nrp1. Knockdown of Dnmt3a1 (upper panel) induces memory impairments and reduces Nrp1 expression. Supplementing Nrp1 protein levels by overexpression rescues Dnmt3a1-dependent memory impairments. Knockdown of Dnmt3a2 (lower panel) induces memory impairments and but does not reduce Nrp1 expression. Overexpression of Nrp1 protein levels does not rescue Dnmt3a2-dependent memory impairments.

of small interfering RNAs and found a requirement for Dnmt3a for spatial memory performance (Mitchnick et al., 2015). Yet, these studies did not distinguish between Dnmt3a1 and Dnmt3a2 – the two isoforms of the *Dnmt3a* gene. Studies from our department focused on Dnmt3a2 and indicated its two-sided involvement in memory processes: decreasing its expression induced memory impairments and overexpression rescued age-dependent memory deficits (A. M. M. Oliveira et al., 2012) and enhanced memory performance (Gulmez Karaca et al., 2020; A. M. M. Oliveira et al., 2016). In addition to memory, it also promotes other neuroplasticity-dependent processes such as pain sensitivity (A. M. Oliveira et al., 2019) and drug seeking (Cannella et al., 2018). These studies demonstrated differential regulation of the two isoforms upon neuronal activity and learning. In contrast to Dnmt3a1, Dnmt3a2 mRNA levels are induced in response to action potential bursting and learning (A. M. M. Oliveira et al., 2012), cocaine administration (Cannella et al., 2018) and an inflammatory response (A. M. Oliveira et al., 2019) in different regions of the central nervous system. Despite the lack of activity-dependent transcriptional regulation, Dnmt3a1 is the most abundant form of the Dnmt3 family (Feng et al., 2005) and its protein levels have been shown to be regulated upon neuronal activity (Bayraktar et

al., 2020a). The two isoforms differ in their first 219 amino acid sequence, that is unique to Dnmt3a1, thus enabling specific chromatin recognition (T. Gu et al., 2022; Suetake et al., 2011). Chromatin marks and non-coding RNA species lead to the recruitment of Dnmts and gene-specific methylation (Holz-Schietinger & Reich, 2012; Jurkowska & Jeltsch, 2016; Savell et al., 2016). Further, differences in histone tail marks and the N-terminal binding capacity of Dnmt3a1 may partially be responsible for the isoform-specific differences on the methylome (Manzo et al., 2017; Ooi et al., 2007; Pohodich & Zoghbi, 2015). All this indicates distinct regulatory functions of Dnmt isoforms. Significantly, the absence of compensatory mechanisms by the other isoform, in response to Dnmt3a2-specific knockdown effects on memory, underscores the unique and irreplaceable role of different isoforms. Hence, it was imperative to explore the significance of Dnmt3a1 in memory formation. I uncovered a Dnmt3a1-specific role in hippocampus-dependent memory tasks, thereby confirming the requirement for Dnmt3a in memory formation. Using the doxycycline-dependent miR30 system, I was able to narrow down the time point of its involvement precisely to early memory consolidation processes.

In order to unravel the mechanisms by which activity-induced DNA methylation contribute to memory formation, comprehensive investigations have already examined differential DNA methylation and gene expression following an electroconvulsive shock (Guo et al., 2011) and CFC (Halder et al., 2015). Robust neuronal activity induced significant methylation changes on genes associated with alternative splicing variants, synaptic function, protein phosphorylation and calcium signalling (Guo et al., 2011). One hour after CFC, DNA methylation changes occurred in both the hippocampal CA1 region and the ACC (Halder et al., 2015). Dnmt3a2 is known to be expressed at low baseline levels and at 1 h after novel environment exploration the expression levels were still low and peaked 4 hours after training (A. M. M. Oliveira et al., 2012), whereas Dnmt3a1 is not regulated by learning but is the most abundantly expressed Dnmt3 in the adult mouse brain (Feng et al., 2005). Consequently, the observed methylation changes in the study conducted by Halder et al. (1h after training) are unlikely to be attributed solely to Dnmt3a2-specific methylation, thus suggesting a potential role for Dnmt3a1 in CFC-induced DNA methylation. Yet, it remains unclear how neuronal activity regulates Dnmt3a1 function, since it is not regulated by abundancy. However, it is known that the protein can be posttranslationally modified (Bayraktar et al., 2020; Deplus, Blanchon, et al., 2014; Deplus, Denis, et al., 2014; Ling, 2004). Posttranslational modifications are a known universal regulatory mechanism to regulate enzymatic activities of proteins. This suggests a possibility for activity-dependent posttranslational modification of

Dnmt3a1 to link its methylation function and neuronal activity. Nevertheless, more research to unravel a potential regulatory mechanism of Dnmt3a1 activity in response to neuronal activity has to be conducted.

I uncovered an activity-regulated genomic program modulated by Dnmt3a1 levels in hippocampal neurons. The knockdown of Dnmt3a1 resulted in significant alterations in the expression of multiple genes. It is important to note that these changes in gene expression are likely influenced by both direct effects and indirect effects through the disruption of other transcriptional regulatory processes. I identified that Dnmt3a1 controls the expression of several genes known to be involved in synaptic plasticity and memory consolidation, including *Npy*, *Cort*, and *Trpc6* (Gøtzsche & Woldbye, 2016; Jiang et al., 2017; Xie et al., 2021). Furthermore, GO term analysis revealed a strong enrichment of terms related to structural and functional plasticity, such as "*Regulation of neurotransmitter receptor activity*" "*Regulation of signaling receptor activity*" and "*Regulation of cell-substrate adhesion*". These findings support previous studies that have demonstrated a connection between transcriptional activation and DNA methylation and suggest that downstream genes regulated by Dnmt3a1 may play a critical role in synaptic plasticity, learning, and memory (T. Gu et al., 2022; Suzuki & Bird, 2008; Wu et al., 2010).

I focused on Nrp1 to determine its involvement in memory processes. Nrp1 has been previously identified to be regulated by exposure to a novel environment within DG engram neurons in the mouse hippocampus (Jaeger et al., 2018). This corroborated its activity-regulation that I saw *in vitro* to be true *in vivo*. While the semaphorin family and their receptors (Nrp and plexin) have established roles in neurodevelopment, there is limited evidence regarding their involvement in homeostatic and Hebbian forms of plasticity in the adult hippocampus (Carulli et al., 2021; Jitsuki-Takahashi et al., 2021; Sahay et al., 2005; Wang et al., 2017). It is noteworthy that Nrp1 is localized at synapses in the adult rat hippocampus and secreted semaphorins acting on the sema-Nrp-plexin complex play a modulatory role in synaptic connectivity in granule cells of the DG and pyramidal neurons of CA1 (Sahay et al., 2005), as well as in the trafficking of AMPA receptors in CA3-CA1 synapses (Jitsuki-Takahashi et al., 2021). Additionally, the semaphorin-plexin-Nrp1 complex is engaged in signalling pathways that regulate neuronal morphology (Carulli et al., 2021; Simonetti et al., 2021). This current study now for the first time demonstrated the requirement of Nrp1 for the formation of long-term memory in the hippocampus. Remarkably, I also found that overexpressing Nrp1 in the

hippocampus rescued memory impairments caused by knockdown of Dnmt3a1. Therefore, these findings suggest that the regulation of Nrp1-dependent mechanisms during memory consolidation serves as a mechanism through which Dnmt3a1 contributes to memory formation. Notably, the expression of Nrp1 did not change upon knockdown of Dnmt3a2, thus further indicating a distinct mechanism in the downstream processes regulated by Dnmt3a isoforms. Strikingly highlighted by the fact that Nrp1 overexpression selectively rescued memory impairments promoted by the reduction of Dnmt3a1 levels, but not Dnmt3a2, in the adult hippocampus.

Taken together, in this part of my doctorate thesis I emphasised the diverse and highly regulated role of DNA methylation processes in brain function. The numerous lines of evidence showing that DNA methylation dysregulation underlies a number of pathological conditions, such as neurodevelopmental (Pohodich & Zoghbi, 2015) and neurodegenerative (De Jager et al., 2014) diseases as well as psychiatric conditions (Feng & Nestler, 2013; Nestler et al., 2016) and chronic pain (Denk & McMahon, 2012), further emphasise the relevance to study DNA methylation in the nervous system.

2. DNA methylation promotes memory persistence by facilitating systems consolidation and cortical engram stabilisation

Persistence is a key characteristic of memory that relies on systems consolidation**,** a process classically defined as the gradual transfer of information from the hippocampus to the cortex for long-term memory storage. Although our knowledge of memory formation encompasses a substantial body of literature regarding the initial molecular and cellular processes involved, our understanding of the precise mechanisms responsible for the storage of persistent memories and the factors influencing their longevity or decay over time, remains limited. Since DNA methylation can act as a long-term regulatory signal, it is a prime candidate to regulate memory duration and to stabilise the cellular substrate of a memory.

In this part of my thesis using CFC and engram tagging tools in mice, I uncovered that hippocampal DNA methylation processes regulate memory. I further showed that this regulation is associated with a modulation of cortical engram stability and increased fear generalisation, mimicking the engram dynamics and behavioural trait of remote memory, respectively. To gain further mechanistic insight I identified, using RNA-Sequencing, several genes whose expression is modulated by Dnmt3a2 levels. Using GO analysis, I showed that this genomic program has a strong enrichment of terms related to synaptic signalling, uncovering a possible functional link between DNA methylation regulation and memory persistence. In summary, I found that DNA methylation in dorsal hippocampus facilitates systems consolidation and stabilises of cortical engrams (Figure 24).

Technological advancements in the last decade have identified the cellular substrate of the memory trace and demonstrated that these neuronal ensembles, initially selected during the learning process, retain the memory representation (Josselyn et al., 2015; Josselyn & Tonegawa, 2020). The initial engram studies focused predominantly on memories formed in the recent past, encompassing events occurring within hours to days. The literature that focused on remote memory revealed that engram neurons within brain regions associated with remote memory were already selected during the learning event, reactivated during retrieval of remote memories and had the ability to influence behaviour through optogenetic or chemogenetic stimulation (DeNardo et al., 2019; Denny et al., 2014; Kitamura et al., 2017; Matos et al., 2019; Tayler et al., 2013). Consequently, these studies establish the notion that these engrams remain stable over extended time periods and correlate with memory duration. Here, I showed that the reactivation of engram neurons in HPC and ACC decreases over time when the memory decays, thus following the

proposed idea and literature that engram reactivation correlates with memory. Long-lasting memory induced a different engram reactivation profile. In HPC, DG engram reactivation decreased with time independently of the fact that the memory was still retrievable, whereas in the CA1, the reactivation was unchanged. These findings are in line with the literature (Kitamura et al., 2017; Tayler et al., 2013) and suggest that CA1 engram neurons are still engaged at remote memory recall. Indeed, temporal

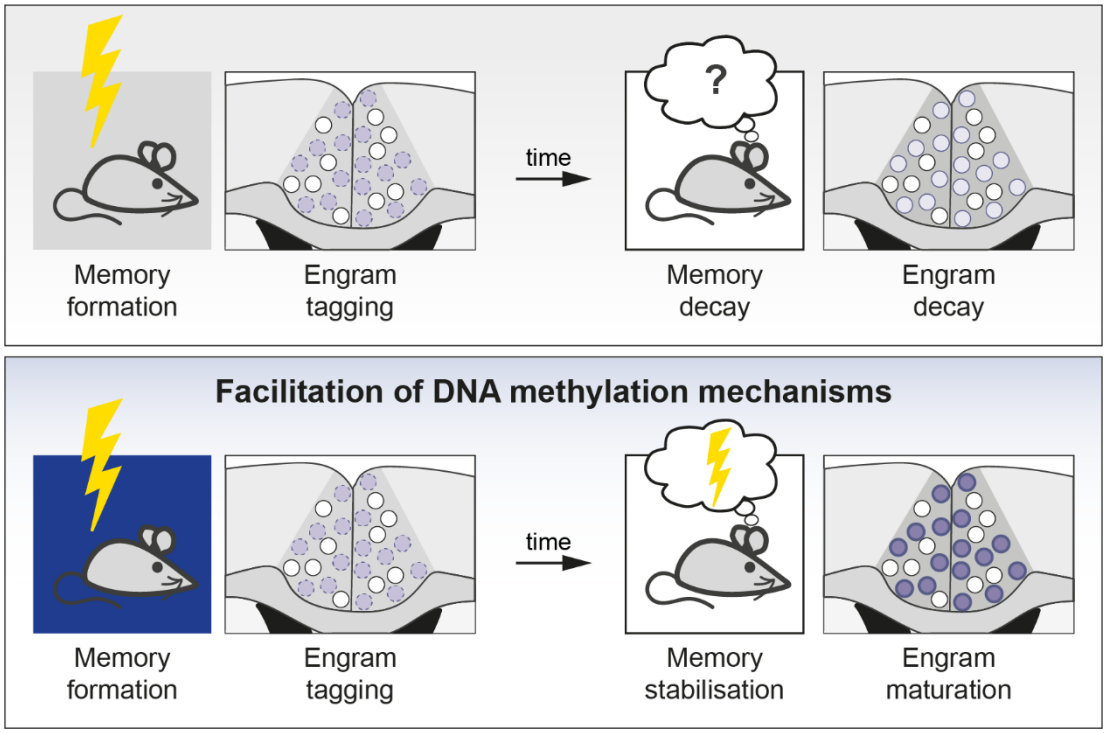


Figure 24. Graphical illustration of main findings of Section 2.2: DNA methylation promotes memory persistence by facilitating systems consolidation and cortical engram stabilisation. Weak contextual fear training (upper panel) induces a fear memory that decays over time. Respectively, cortical engram stability decays. Weak contextual fear training combined with Dnmt3a2 overexpression (lower panel) converts the short-lasting memory into a persistent fear memory and facilitates cortical engram maturation.

inhibition of CA1 activity during memory retrieval impaired not only recent but also remote memories (Goshen et al., 2011). Pioneering work of Kitamura and colleagues uncovered that the prefrontal memory engram generated during learning is in an immature state and through inputs from the HPC-EC axis and the basolateral amygdala (BLA) it undergoes functional and structural maturation and is thereby responsible for the remote memory recall (Kitamura et al., 2017). This led to the proposal that systems consolidation at the engram level is reflected by higher reactivation at remote memory recall compared to recent. In accordance to this study, I observed an increase in ACC engram reactivation at remote time point. Recently three studies replicated the "*cortical maturation*" finding from Kitamura et al. (X. Fan et al., 2022; Lee et al., 2023; Matos et al., 2019). Taken together my findings and the literature depict that engram dynamics correlate with memory duration and reflect

systems consolidation processes. However, the specific molecular and cellular mechanism responsible for the engram stabilisation remains unknown. Promising candidates are molecular processes capable of modulating long-term plasticity-related events, such as the regulation of gene expression.

DNA methylation has emerged as a crucial factor influencing neuroplasticity-related processes and memory formation (Gulmez Karaca et al., 2020; Mitchnick et al., 2015; A. M. M. Oliveira, 2016). Previously published gain-and loss-of-function studies of Dnmt3a2 in the HPC have demonstrated its ability to enhance or impair memory formation, respectively (Gulmez Karaca et al., 2020; A. M. M. Oliveira et al., 2012, 2016). Further, CFC has been shown to induce widespread alterations in both the methylome and transcriptome, particularly affecting genes associated with synaptic transmission (Duke et al., 2017; Halder et al., 2015; Marco et al., 2020; Rao-Ruiz et al., 2019) and we showed previously that overexpression of Dnmt3a2 affects the methylation on genes specifically associated with synaptic plasticity (Gulmez Karaca et al., 2020). Hence, I speculated that Dnmt3a2-driven methylation impacts the duration of memory. I was the first to show that overexpression of Dnmt3a2 in excitatory neurons in the dorsal HPC converted a memory that naturally decayed within 2 weeks to become long-lasting and conversely, reduction of Dnmt3a2 affected the strength of a remote memory. Given that neuronal ensembles serve as the cellular substrates of memory, I investigated their reactivation pattern. Notably, overexpression of Dnmt3a2 in HPC did not lead to an increase in hippocampal engram reactivation during remote memory recall, displaying similar dynamics to the control group suggesting that the trace of the artificially induced long-lasting memory does not reside in HPC. However increased hippocampal Dnmt3a2 led to better DG engram reactivation at recent retrieval session. Despite this fact, there was no effect on recent memory performance which is in contrast to our former study that depicted that increased DG engram reactivation enhances recent contextual fear memory (Gulmez Karaca et al., 2020). As an important remark, that study used a stronger electrical shock, selectively increased the levels of Dnmt3a2 solely in DG engram neurons rather than throughout the entire dorsal HPC, and achieved threefold higher DG engram reactivation compared to control conditions (Gulmez Karaca et al., 2020). Whereas here in my study, DG engram reactivation was only 1.5 times higher. This discrepancy might indicate that different fear intensities engage distinct molecular machinery (Matos et al., 2019) and the mere increase of Dnmt3a2 alone may not be sufficient to drive behavioural effects. Remarkably, I found out that the Dnmt3a2-dependent memory conversion was accompanied by an increase in cortical engram reactivation mimicking cortical engram maturation that occur naturally in a long-

lasting memory (X. Fan et al., 2022; Kitamura et al., 2017; Lee et al., 2023). Importantly, I observed the opposite effect in the loss-of-function experiment. Persistent memory is accompanied by increased spinogenesis and strengthening of connections in cortical engram neurons over time (Kitamura et al., 2017; Lee et al., 2023; Tonegawa et al., 2018) and it remains to be seen whether Dnmt3a2-driven engram maturation changes the morphological and functional properties in the cortex.

Systems consolidation is known to involve the communication between the hippocampus and the cortex and several studies proved the necessity of hippocampal activity to store remote memory in the cortex (Kitamura et al., 2017; Lee et al., 2023; Lesburguères et al., 2011; Matos et al., 2019). Blocking activity of the hippocampus prior to rapid eye movement sleep blocks the upregulation of IEGs in cortical regions upon learning (Ribeiro et al., 2002). It was shown that astrocytic activity in the CA1 region is important for successful remote memory retrieval (Kol et al., 2020) and further a study in 2023 established a correlation between remote memory consolidation and progressive synaptic strengthening between PFC excitatory neurons which was CREB-dependent and required sustained hippocampal activity patterns (Lee et al., 2023). In addition, Matos and colleagues previously identified that PFC engram recruitment involved CREB functioning (Matos et al., 2019). Successful CREB signalling was further crucial within DG engram during memory consolidation and a novel group of CREB target genes was identified (Rao-Ruiz et al., 2019). Here using RNA sequencing, I found some of these CREB target genes e.g., *PENK* to be altered upon enhanced DNA methylation.

Memory consolidation has been linked to rhythmic oscillations, specifically spindle-ripple coupling between the hippocampus and cortex is believed to play a crucial role in systems consolidation and remote memory (Buzsáki, 1996). During rest periods, hippocampal sharp-wave ripples (SWRs) and prefrontal cortical spindles exhibit a temporal correlation, facilitating communication between these regions and this is thought to support the transfer of memories from the hippocampus to the cortex (Buzsáki, 1996; Euston et al., 2007; Schwindel & McNaughton, 2011; Siapas & Wilson, 1998). Parvalbumin (PV)-positive interneuron activity regulates these oscillations and disrupting PV activity in HPC or mPFC eliminated learning-induces ripple-spindle coupling and impaired fear memory consolidation (Xia et al., 2017), thus indicating the contribution of spindle-ripple coupling to systems consolidation and remote memory.

It is tempting to speculate that hippocampal DNA methylation processes facilitate the hippocampal-cortical communication since they induce cortical engram maturation and impact memory duration. Indeed, I found that GO term analysis of DEGs upon Dnmt3a2 overexpression in dHPC revealed a strong enrichment of terms related to synaptic plasticity and morphology, e.g., "*Regulation of trans-synaptic signaling*", "*Synaptic signaling*", "*Regulation of ion transport*", "*Cell-cell signaling*" and "*Cell morphogenesis*". Further, some of the DEGs in my study were found by others to be differentially methylated in the hippocampus upon CFC (Duke et al., 2017; Halder et al., 2015) or in primary hippocampal cultures upon overexpression of Dnmt3a2 itself (Gulmez Karaca et al., 2020). This finding supports the notion that DNA methylation promotes communication between hippocampus and cortex likely through the regulation of expression of genes involved in synaptic transmission. However, it is still unclear whether specific cell-types or a specific gene act as a "master regulator" to functionally drive this communication. Moreover, the temporal requirement of DNA methylation changes for facilitating systems consolidation is still to be determined. In the week immediately following training, inhibiting NMDAR function in the HPC prevented the formation of remote memories. However, suppressing NMDAR function at later time points did not have that effect (Shimizu et al., 2000). Likewise, inhibiting hippocampal activity during the first 12 days post learning in a social transmission of food preference paradigm inhibited remote memory and abolished time-dependent morphological changes at the pre-and post-synapse in the cortex (Lesburguères et al., 2011) and blocking hippocampal PV interneuron activity in the first week, but not the second week post training, interfered with ripple-spindle coupling and remote memory recall (Xia et al., 2017). Besides, five days after CFC, the chromatin architecture of DG engram neurons was associated with the relocation of substantial chromatin segments from inactive to permissive state, alongside the reconfiguration of promoter–enhancer interactions (Marco et al., 2020). In line with this week-long time-window, I found an increase in the number of differentially expressed genes at 5 days post training compared to one day. Overall, these results indicate a crucial time window in which hippocampal activity is important for systems consolidation.

If hippocampal DNA methylation processes facilitate the transfer of information from the hippocampus to the cortex, then the memory trace should reside in cortical engram neurons. Hippocampal activity is needed for a detailed representation of the memory content, whereas with time generalisation to a novel context increases which was associated with the memory trace being transferred to the cortex (Wiltgen & Tanaka, 2013). I saw an increased fear generalisation pointing towards a shift in the

memory storage towards the cortex. Moreover, chemogenetic inhibition of cortical engram cells impaired remote memory, thus providing causal evidence that the memory trace was shifted towards the cortex. Taken together, in this part of my thesis I uncovered a new mechanism in systems consolidation and found out that DNA methylation promotes memory persistence by facilitating systems consolidation and cortical engram stabilisation.

.

3. High salient fear memory induces a biphasic Npas4 expression and results in memory suppression

In this part of my thesis, I uncovered a novel mechanism that negatively gates memory consolidation and is regulated by experience (Figure 25). Using contextual-fear conditioning paradigms, I showed that, unlike low salience experiences, highly salient experiences induced two phases of Npas4 expression. Further, I found that the late wave of Npas4 expression is dependent on NMDAR activation. Moreover, pharmacological and genetic approaches to either artificially induce or block the second Npas4 expressional wave, respectively, indicated that late Npas4 expression constraints fear memory consolidation. Overall, I provided new insight into a molecular mechanism that constrains the formation of otherwise maladaptive fear memories and thus a mechanism that promotes cognitive flexibility.

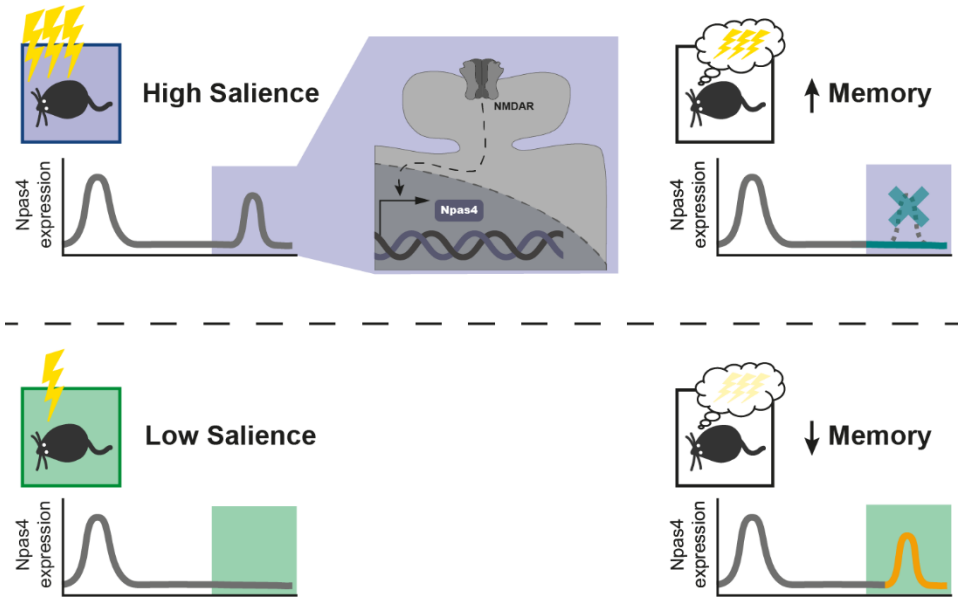


Figure 25. Graphical illustration of main findings of Section 2.3: High salient fear memory induces a biphasic Npas4 expression and results in memory suppression. High salience learning (purple) induces Npas4 biphasic expression in the CA1 region of the hippocampus in a NMDAR-dependent manner. Pharmacologically blocking NMDAR activity and consequently late Npas4 expression, results in enhanced memory performance. Low salience learning (green) leads to one peak of Npas4 expression. Genetically promoting a biphasic expression of Npas4 expression suppresses memory consolidation.

It has been proposed that learning experiences can be stored in long-term memory through genomic responses (Grecksch & Matthies, 1980; Igaz et al., 2002; Tyssowski et al., 2018). Genes that are activated during neuronal activity appear to play a role in defining aspects of the learning experience. A study examined 13 different experiences with distinct properties, such as salience and valence. They identified that each experience is associated with unique gene expression patterns and the expression of five IEGs across five brain areas was sufficient to accurately predict the specific experiences (Mukherjee et al., 2018a). In addition, the duration of neuronal

activity influences expression profiles (Tyssowski et al., 2018). Brief and sustained bursts of activity triggered the "classical" genomic expression of IEGs, while prolonged activity and experiences with high salience led to multiple waves of gene expression hours after the initial experience. These delayed waves of IEG expression have been found to promote memory persistence (Bekinschtein et al., 2007, 2008; Katche et al., 2010, 2013). Here, I found that the expression pattern of the IEG Npas4 is regulated by experience salience. My results depicted that highly salient experiences triggered two phases of Npas4 gene expression, with the late phase being regulated by NMDAR activity. Other studies have also investigated the role of NMDAR activity hours after learning in memory consolidation (Sachser et al., 2016; Shinohara & Hata, 2014; Villarreal et al., 2002). For instance, research conducted by Sachser and colleagues demonstrated that the forgetting of object recognition memory relies on calcium influx, in parts mediated by NMDAR activation after the initial learning and blocking its activity six hours after training led to reduced forgetting in rats (Sachser et al., 2016). Similar results were observed by others (Shinohara & Hata, 2014). My research aligns with these findings. It indicates that blocking NMDAR activity enhances long-term memory, thus suggesting that the delayed wave of Npas4 expression is a mechanism dependent on NMDAR to suppress memory consolidation in highly salient experiences. However, it is possible that other mechanisms associated with NMDAR activation contribute to memory suppression. Despite unsuccessful attempts to selectively abolish the second expression period of Npas4, the gain-of-function approach provides compelling, corroborating evidence that the delayed Npas4 expression between 4 and 6 hours after learning, rather than at later time points, restricts memory consolidation. However, it is not clear what factor drives the NMDA receptor dependent induction of the second Npas4 expression and why this is exclusive for Npas4. Additionally, what transcriptional program does Npas4 expression initiate at this particular time point that leads to the suppressive response on memory consolidation remains to be investigated.

One hypothesis regarding the significance of memory suppressor genes is that they may promote behavioural flexibility (Noyes et al., 2021). Without constraints on memory consolidation, highly salient experiences could generate strong memories that are not adaptive or advantageous in changing environments. As described previously, studies correlated the expression pattern of IEGs with salience of a stimulus and found them to underlie strength of the memory (Mukherjee et al., 2018a; Tyssowski et al., 2018). My current study revealed that highly salient experiences induce two phases of Npas4 gene expression, with potentially opposing roles. In contrast to its known memory promoting role during early wave of Npas4

induction, I found that Npas4 expression four hours after learning actually constrains memory consolidation. Thereby, delayed Npas4 expression acts as an inducible memory suppressor gene. The first wave of Npas4 expression is necessary for memory formation, while the second wave fine-tunes the strength of the memory, preventing the formation of excessively strong maladaptive memories. Recently, Npas4 was proposed to act as a stimulus decoder in the brain (Brigidi et al., 2019). The Bloodgood laboratory discovered that Npas4 builds stimulus-specific heterodimers that bind distinct genomic loci upon either the activity triggered by action potentials or excitatory postsynaptic potentials (Brigidi et al., 2019). This mechanism demonstrates how different neuronal activity can be encoded by the same IEG. It is to be speculated, whether Npas4 expressed immediately or four hours after learning may interact with different partners that are available at those specific time points and thereby trigger a gene response specific to the salience of the experience, thus facilitating or restricting memory consolidation.

Npas4 has unique characteristics. It is only specifically induced by calcium influx, unlike other IEGs (Lin et al., 2008; Ramamoorthi et al., 2011) and in addition, it can be induced in both glutamatergic and GABAergic neurons. It is involved in modulation of activity-dependent synaptic connections at a cellular and circuit level. In excitatory neurons, reducing Npas4 levels decreases the inhibitory synaptic input, while overexpressing Npas4 increases their number (Lin et al., 2008), indicating that Npas4 dynamically regulates inhibitory input. Similarly, in inhibitory neurons, deleting Npas4 reduces the number of excitatory synapses without affecting the total number of inhibitory inputs (Spiegel et al., 2014). These findings suggest that Npas4 expression plays a role in homeostatic plasticity mechanisms, thereby maintaining neural circuit homeostasis (Spiegel et al., 2014). Recent research has shown that Npas4 expression in neurons of CA1 and DG increases inhibitory input specifically from Cholecystokinin (CCK) expressing interneurons onto excitatory neurons (Hartzell et al., 2018; Sun et al., 2020). Optically evoked CCK+ inhibition onto Npas4-expressing neurons 24 hours after learning was found to be stronger in mice that underwent a more intense contextual fear conditioning paradigm (Sun et al., 2020). Although the role of CCK^+ interneurons in memory has not been extensively studied, it suggests a plausible link between highly salient experiences and increased inhibition by CCK+ interneurons during memory consolidation. These studies collectively indicate that Npas4 plays a role in regulating inhibitory synapses to fine-tune neuronal circuits. It opens up a possibility for a Npas4-driven inhibitory mechanism to induce memory suppression and to drive cognitive flexibility.

It is compelling to hypothesise that Npas4's delayed expression contributes to the redistribution of local inhibitory input and the promotion of synaptic homeostasis during memory consolidation, that eventually decreases memory consolidation and weakens the memory trace. It has been hypothesised that the consolidation of salient experiences involves the renormalisation of synaptic strength (Tononi & Cirelli, 2014). This idea is consistent with the described function of Npas4 in excitatory/inhibitory balance. This hypothesis proposes that the absence of cellular homeostasis restoration during memory consolidation might impair learning adaptability and knowledge acquisition. When dealing with emotionally charged situations, this may result in the development of persistent, maladaptive fear memories, which are linked to mental disorders including post-traumatic stress disorder. In summary, this study has uncovered a biological mechanism that modulates the strength of memories related to highly salient experiences. This mechanism may also have implications for regulating resilience and flexibility.

IV. Material and Methods

1. Cloning

1.1. Constructs

For expression of shRNAs, a vector containing the U6 promoter upstream of the shRNA sequence and a chicken β-actin promoter to drive GFP or mCherry expression was used. The constructs to knockdown Dnmt3a1 (Dnmt3a1-shRNA1; Dnmt3a1-shRNA2) were subcloned by Ana Oliveira. The constructs to knockdown Dnmt3a2 (Dnmt3a2-shRNA; shRNA-Dnmt3a2) were subcloned by Dimitri Tkachev and validated (A. M. M. Oliveira et al., 2016). The control constructs (Control-shRNA; shRNA-unc) were subcloned by Dimitri Tkachev and validated (Brito, Kupke, et al., 2020; A. M. M. Oliveira et al., 2016). shRNA sequence can be found in Table 1.

Table 1: shRNA sequences used in the study.

Name	Construct
Control-shRNA	rAAV-U6-**ACTACCGTTGTTATAGGTGCG**-CBA-GFP-WPRE
Control-shRNA	rAAV-U6-**ACTACCGTTGTTATAGGTGCG**-CKII-mCherry-WPRE
Dnmt3a1-shRNA1	rAAV-U6-**GCAGACCAACATCGAATCCAT**-CBA-GFP-WPRE
Dnmt3a1-shRNA2	rAAV-U6-**GGGAGGATGATCGAAAGGAAGGAGA**-CBA-GFP-WPRE
Dnmt3a2-shRNA	rAAV-U6-**ACGGGCAGCTATTTACAGAGC**-CBA-GFP-WPRE
Dnmt3a2shRNA	rAAV-U6-**ACGGGCAGCTATTTACAGAGC**-CKII-mCherry-WPRE
Nrp1-shRNA1	rAAV-U6-**GGAAACCAAGAAGAAATATTA**-CBA-GFP-WPRE
Nrp1-shRNA2	rAAV-U6-**GGGAGAGGAAATCGGAGCTAA**-CBA-GFP-WPRE

For temporally controlled knockdown of Dnmt3a1, a dual-component TetON-based system was used. In detail, this system consisted of a driver plasmid that expresses the transactivator (rtTA), the tetracycline repressor (TetR) and the fluorescent protein Kusabira Orange (KO) that acts as an infection marker under the control of a neuron-specific promoter (hSynapsin). This construct was developed and provided by Dr. Sidney Cambridge, Institute of Anatomy, University of Frankfurt. The second construct contains GFP and a miR30-based shRNA targeting Dnmt3a1 under the control of the tetracycline-responsive promoter (TRE). For the generation of this vector, the plasmids pPRIME-CMV-GFP-FF3(Stegmeier et al., 2005) (a gift from Stephen Elledge (Addgene plasmid # 11663; http://n2t.net/addgene:11663; RRID:Addgene_11663)) and pAAV-PTRE-tight-hM3Dg-mCherry(Zhang et al., 2015) (a gift from William Wisden (Addgene plasmid # 66795; http://n2t.net/addgene:66795; RRID:Addgene_66795)) were used. Specifically, the Dnmt3a1-shRNA1 sequence was

inserted into the pPRIME vector and subsequently, the GFP-miR30-Dnmt3a1-shRNA expression cassette was subcloned into the vector containing the tet-inducible promoter by replacement of the hM3Dq-mCherry insert. This construct was cloned by Özlem Demir.

For the overexpression of Nrp1-HA, I used a vector containing the neuron-specific promoter, hSynapsin, to constitutively express HA-tagged Nrp1. The control construct driving LacZ was kindly provided by Prof. Dr. Daniela Mauceri.

For activity-dependent tagging of neuronal ensembles and reactivation analysis, the H2BGFP sequence (H2B-GFP was a gift from Geoff Wahl (Addgene plasmid # 11680; http://n2t.net/addgene:11680; RRID: Addgene_11680) was subcloned into the pAAV-RAM-d2TTA::TRE-MCS-WPRE-pA construct (kindly deposited to Addgene by Dr. Yingxi Lin) (Addgene plasmid # 63931; http://n2t.net/addgene:63931; RRID: Addgene_63931).

Overexpression of Dnmt3a2 was achieved by using a viral vector that contained the mouse CaMKIIα promoter upstream of the Dnmt3a2 full-length mouse cDNA sequence. As a control vector, a construct containing the CaMKIIα promoter driving the expression of mCherry was used. These constructs were subcloned by Dimitri Tkachev and Anna Hertle and validated (A. M. M. Oliveira et al., 2012).

For the expression of the catalytic-inactive form of Dnmt3a2, Prof. Dr. Albert Jeltsch kindly provided and functionally validated the mutant (Dukatz et al., 2019)and the catalytic domain of the overexpression construct was exchanged using Gibson Cloning.

For the inhibition of cortical ensembles via the viral TRAP system, the construct driving the Cre-recombinase ($CreER^{T2}$) under the cFos promoter was kindly provided and validated by Prof. Dr. Michel van den Oever, Institute of Molecular and Cellular Neuroscience, Vrije University Amsterdam (Matos et al., 2019). The mCherry control construct (pAAV-hSyn-DIO-mCherry) was a gift from Bryan Roth (Addgene plasmid # 50459; http://n2t.net/addgene:50459; RRID: Addgene_50459) and the inhibitory DREADD construct (h4MDi: pAAV-hSyn-DIO-hM4D(Gi)-mCherry) was a gift from Bryan Roth (Addgene plasmid # 44362; http://n2t.net/addgene:44362; RRID: Addgene_44362).

For the temporally-restricted overexpression of Npas4, the dual-component TetON-based system was used. The driver is described above. For the second construct, human influenza hemagglutinin (HA)-tagged GFP or full length Npas4 expression

cassette was subcloned under the control of the tetracycline responsive promoter (TRE) by David Brito and myself.

The infection rate, toxicity, viral titre, and knockdown efficiency for each batch of generated viruses were evaluated. The titre was matched to a final working concentration of around 1-2 x10^{12} viral particles/ml.

1.2. Molecular Cloning and DNA Preparation

Constructs were generated by restriction enzyme based cloning and standard molecular biology procedures. Sticky-end ligation of DNA fragments and plasmids was carried out using T4 DNA ligase (New England Biolabs) at room temperature for 1 hour according to the manufacturer's instructions. Ligation reactions were transformed into NEB 5-alpha Competent E. coli. 100 µl of chemically competent E. coli bacteria were thawed on ice. 5 µl of ligation mix were added to the bacteria which were kept on ice for 20 min. After 45 s of heat shock at 42 °C, 1 ml of LB medium was added and cells were allowed to recover for 1 h at 37 °C; 300 rpm. For selection, transformed bacteria were plated onto LB-medium plates containing 100 µg/ml ampicillin (Thermo Fisher Scientific) and grown over night at 37 °C. Colonies were used to inoculate a 5-10 ml overnight liquid culture. Plasmids were purified from the liquid cultures using the PureLink HiPure according to the manufacturer's instructions. Plasmid DNA concentrations were determined by absorbance measurement using a NanoDrop-11 spectrophotometer (DeNovix).

1.3. PCR and DNA Fragment Purification

PCR products for cloning were amplified using Q5 High Fidelity DNA Polymerase (New England Biolabs) according to the manufacturer's instructions. PCR products were either purified from a 1% agarose gel using the GeneJET gel extraction kit (Thermo Fisher Scientific) or directly from the PCR reaction using the QIAquick PCR Purification Kit (Qiagen).

2. Recombinant adeno-associated virus (rAAVs)

Viral particles were produced and purified as described previously(Gulmez Karaca et al., 2021) and slightly modified. rAAV serotype 2/1 was used to allow preferential infection of mature neurons. Briefly, rAAVs were generated by co-transfecting human embryonic kidney 293 cells (Stratagene, California, USA) with target AAV plasmid and helper plasmids (pFΔ6, pRV1, and pH21) using calcium phosphate precipitation.

60 hours after transfection, the HEK 293 cells were collected and lysed. Remaining viral particles in media were concentrated using 40% Polyethylene glycol (PEG) solution (pH 7,4). For this, medium was added to 40% PEG and stirred for 3h at 4°C. After centrifugation, viral pellet was added to lysed HEK cells and this was purified through heparin affinity columns (HiTrap Heparin HP, GE Healthcare, Uppsala, Sweden) and concentrated using Amicon Ultra-4 centrifugal filters (Millipore, Bedford, MA).

3. Animals

Throughout the study, 3-months-old C57BL/6N male mice (Charles River, Sulzfeld, Germany) were used for this study. Unless there was severe fighting, mice were housed in groups of two or three per cage on a 12h light/dark cycle with *ad libitum* access to water and food, 22 ± 1°C, 55 ± 10% relative humidity, unless severe fighting occurred. With the exception of the recent and remote testing groups, which were kept apart to prevent disturbing the animals on different days of sacrifice, each cage contained mice from different experimental conditions (different rAAV identity). After cannula implantation, cannulated animals were kept singly-housed to avoid cannula removal. From the day of stereotaxic surgery of the recombinant adeno-associated viruses (rAAVs) until two days prior behavioural training, mice that had been injected with rAAVs for expression of doxycycline-dependent transgenes (activity-dependent tagging) were maintained on a doxycycline-containing diet (40mg/kg, BioServ, Flemington, NJ, USA). On experimental training day (day 0), doxycycline food was replaced by regular chow. Mice were once more fed doxycycline-containing food the day following the behavioural training (day 1). All behavioural experiments took place during the light phase. To avoid misinterpretation of the results, sick or injured (from cage-mate fighting) mice were excluded from the analysis of the experiments. Animals were randomly assigned to experimental groups and blinded analysis was performed. All procedures were carried out in accordance with German guidelines for the care and use of laboratory animals and with the European Community Council Directive 86/609/EEC.

4. Surgery

Animals were anesthetized with an intraperitoneal (*i.p.*) injection of sleep mix containing 0.5 mg/kg fentanyl, 0.5 mg/kg medetomidine and 5.0 mg/kg midazolam. Once the animal was immobile, painkiller containing 5mg/kg carprofen was

administered subcutaneously and the animal was placed on a heating pad in the stereotaxic frame. 0.1 % Xylocain was applied underneath the skin at the head for local pain reduction before the cut was made. The surgery started when the animal no longer responded to a foot pinch. Once the stereotaxic injection was done, a wake mix containing 0.75 mg/kg atipamezole, 0.5 mg/kg flumazenil and 1.2 mg/kg naloxone was administered subcutaneously and the animal was returned to its home cage that was placed on a heating plate set to ~39 ^{o}C overnight.

4.1 Stereotaxic delivery of rAAVs in different brain regions

For the experiments investigating the cognitive function of Dnmt3a1 and Nrp1 and targeting the dorsal hippocampus (dHPC) (Figure 5, 7, 9-10), rAAVs were stereotaxically delivered into the dorsal hippocampus at the following coordinates relative to Bregma: AP: −2 mm; ML: ±1.5 mm; DV: −1.7, −1.9 and −2.1 mm. Viral solution was mixed with PBS at 2:1 (virus:PBS) ratio and 1.5 µl of virus mixture was injected per hemisphere (500nl per DV spot) with an injection speed of 200 nl/min through a 33-G Nanofil needle (WPI, Sarasota, FL, USA). Following injections at each hemisphere, the needle was left in place for an additional minute for the diffusion of the fluid before proceeding.

For the experiments involving activity-dependent tagging of neuronal ensembles or hippocampal manipulation of Dnmt3a2 levels (Figure 12-17), rAAVs were injected at the following coordinates relative to Bregma: for the CA1: AP: −2 mm; ML: ±1.4 mm; DV: −1.3 (650 nl); for the DG: AP: −2 mm; ML: ±1.4 mm; DV: –2.1 (300nl); and for the ACC: AP: 0.5 mm; ML: ±0.3 mm; DV: −1.5 (650nl). Viral solution was mixed with PBS at 2:1 (virus:PBS) ratio and injected with a speed of 135 nl/min. For the viral TRAP system, a virus mixture of pAAV-Fos-CreERT2 (titer: 1,4x1012 viral particles/ml) and the Cre-dependent AAV (titer: 5,0-6,0 x1012) were injection in a ratio 1:9. Before and after injections at each individual spot, the needle was left in place for 5min.

For dorsal CA1 overexpression of temporally-restricted Npas4 overexpression (Figure 18-22), rAAVs were injected at the following coordinates relative to Bregma: AP: −2 mm; ML: ±1.5 mm; DV: −1.2. A total volume of 500nl (1:1 ratio of Driver and GFP or Npas4) was injected per hemisphere at 100 nl/min. Before and after injections at each individual site, the needle was left in place for 5min. At the time of behavioural experiments, the experimenter (myself) was blind to the identity of the virus injected into each mouse. After behaviour, histological analysis was performed to confirm

correct targeting and tissue and cellular integrity. Mice that showed absence or missing viral expression were excluded.

4.2 Cannula implantation and infusion

Animals were implanted with a 26-gauge double guide cannula cut 1mm below pedestal (C235G-3.0/Spc, Plastics One, Bilaney) aimed at the CA1 region of the dorsal hippocampus (stereotaxic coordinates: AP: −2 mm; ML: ±1.5 mm; DV: −1.2 mm). Guide cannulas with a dummy cannula without projection (c235g-3 Plastics One, Bilaney) to avoid clogging were placed using 2 screws (00-96x1/16, Plastics One, Bilaney) and HY-bond polycarboxylate cement (9917-1, Shofu). Cement was left to dry for 20min. Animals were allowed to recover from surgery for 7 days before experiments. At the time of drug delivery, internal infusion cannula (C315l/Spc, Plastics One, Bilaney) were tightly fitted into the guides and injections (0.5µl/side) of DL-2-amino-5-phosphonopentanoic acid (DL-APV) (5µg/µl, 22.8mM), SCH23390 (5µg/µl, 15.4mM) or saline were performed at 200nl/min speed with a microinjection pump. The infusion cannulas were left in place for 60 additional seconds to minimize backflow. The placement of the cannulas was verified post-mortem during tissue microdissection. Only data from animals with correct cannula placement were analysed.

5. Behavioural paradigms

Before all behavioural tests, mice were habituated to the experimenter and behavioural room by handling for 3 consecutive days, 2 minute per mouse. Spatial object recognition test and contextual fear conditioning were performed as previously described (A. M. M. Oliveira et al., 2012, 2016). Different mice cohorts were used to test recent (24h) or remote (2 weeks) memory or short-term memory (1h).

5.1 Spatial object recognition

First, mice underwent a habituation session in the training arena (black box; 50 cm × 50 cm × 50 cm) with a visual cue placed on the arena wall in the absence of objects for 6 min and returned to their home cage for 3 min. During training, the animals were exposed to two distinct objects (a glass bottle and a metal tower) and allowed to explore for 6 min during 3 trials with 3 min inter-trial intervals. Animals explored the objects freely during these sessions.

During the testing session, that occurred 24h or 1h later, one object was moved to a new location and exploration of objects was scored for 6min. The experimenter scored manually the amount of time mice spent exploring both of the objects both

during the training and testing phases. As a readout of memory performance, the preference of the mice for the spatial object change was measured using the following formula:

$$\text{Preference for displaced object} = \frac{\text{Time exploring the displaced object x 100}}{(\text{Time exploring the displaced object}) + (\text{Time exploring the displaced object})}$$

If in one experimental batch during the spatial object recognition test control animals did not show a preference, due to day effects, the whole set of animals was excluded from this analysis but was still included in contextual fear conditioning data. Further, if an animal did not explore the objects during the training for at least 2 seconds, that animal was excluded.

5.2 Contextual fear conditioning and drug administration

In CFC, mice were allowed to explore the conditioning chamber (23 × 23 × 35 cm, TSE, Bad Homburg, Germany) followed by a 2s foot shock. They remained for additional 30s in the conditioning chamber before returning to their home cage (HC). The training time and foot shock intensity varied between different CFC protocols and is described in detail below.

For the experiments investigating the cognitive function of Dnmt3a1 and Nrp1 and targeting the dorsal hippocampus (dHPC) (Figure 5, 7, 9-10), mice were allowed to explore the conditioning chamber for 148s until a 0.5mA foot shock was administered for 2s, then animals remained for 30s before returning to their home cage.

For the experiments involving the strong or high salience CFC protocol (Figure 12, 18-20), training consisted on 148s of exploration and 3x 0.7mA shocks for 2s each, spaced by 148s, then animals remained for 60s before returning to their home cage.

For the experiments involving the weak or low salience CFC protocol (Figure 12-22), mice were allowed to explore the conditioning chamber for 148s until a 0.2mA foot shock was administered for 2s, then animals remained for 30s before returning to their home cage.

For the experiments involving activity-dependent tagging of cortical engram neurons and hippocampal reduction of Dnmt3a2 levels (Figure 17), mice were allowed to explore the conditioning chamber for 148s until a 0.7mA foot shock was administered for 2s, then animals remained for 30s before returning to their home cage.

For the experiments involving the APV administration (Figure 20), mice were allowed to explore the conditioning chamber for 448s until a 0.7mA foot shock was administered for 2s, then animals remained for 60s before returning to their home cage.

For the temporally controlled knockdown of Dnmt3a1 (Figure 7) or Npas4 (Figure 22) overexpression, mice received *i.p.* doxycycline hyclate (2.5 mg in 500 µL saline solution, 100mg/kg, Sigma-Aldrich, Munich, Germany) 3 days prior to, immediate after or 12h after CFC.

For viral trapping of cortical engram neurons (Figure 16), mice received *i.p.* 4-Hydroxytamoxifen (4 OHT) (2.5 mg per ml 4 OHT, 5% DMSO, and 1% Tween20 in saline; 25 mg/kg) 2 h after CFC.

When overlap between activity-dependent tagging of neuronal ensembles and endogenous cFos expression was quantified, mice were sacrificed 90min after CFC test session as indicated in the Figure (Figure 12, 14, 17).

For time course expression of Npas4 (Figure 18, 21) and RNA Sequencing experiment (Figure 15), mice were sacrificed at the indicated time point after CFC training without undergoing a memory test.

All testing sessions consisted of exposing the animals to the conditioning chamber for 5 min. As a readout of the memory performance, freezing, defined as the absence of movement except for respiration, was scored manually during the test.

For the altered context experiment (Figure 16), the context was highly dissimilar to the conditioning chamber. It was located in a different experimental room. Moreover, properties of the chamber; floor (white plastic instead of metal grid), scent (lemon detergent instead of ethanol), shape (triangle instead of square) and the light intensity was changed in the altered context.

6. Immunohistochemistry

Animals were anesthetised with Narcoren (2 µl/g body weight; Merial GmbH) and first perfused intracardially with ice-cold PBS and then with ice-cold 4% paraformaldehyde (PFA) (Sigma-Aldrich, Munich, Germany). Brains were collected and post-fixed in 4 % PFA over night at 4 °C, then placed in a 30 % sucrose solution in PBS with 0.01 % Thiomersal (Carl Roth) to achieve cryoprotection. Brains were cut at 30 µm thickness using a Leica CM1950 Cryostat and slices were stored in PBS with 0.01 % Thiomersal at 4 °C. For fluorescent staining, slices were washed in PBS and then blocked in 8 % normal goat serum with 0.3 % Triton X-100 in PBS for 60 min at room temperature and washed once with PBS.

Primary antibody was diluted in 2 % normal goat serum with 0.3 % Triton X-100 in PBS (see Table 2 for dilutions) and incubated with slices over night at 4 °C mildly

shaking. After three washing steps with PBS for 5 min, slices were incubated with secondary antibody (diluted 1:500) in 8 % normal goat serum with 0.3 % Triton X-100 in PBS for 90 min in the dark at room temperature and washed three times with PBS for 5 min. Finally, slices were incubated in Hoechst 33258 (2µg/ml, Serva, Heidelberg, Germany) for 5 min and mounted on glass slides. In order to confirm rAAV-dependent expression, imaging was done using a Leica DM IRBE fluorescence microscope. Whereas for quantification, imaging was using a 20x oil objective on a TCS SP8 confocal microscope (Leica Microsystems, Oberkochen, Germany).

Table 2: Antibodies used for immunostainings.

Antibody	Source	Dilution	Catalog number
Mouse monoclonal anti-HA tag	Covance	*1:1000*	*Cat # MMS-101R*
Rabbit monoclonal anti-c-fos (9F6)	Cell Signaling	*1:2000*	*Cat # 2250*
Goat anti-mouse IgG (H+L), Alexa Fluor 488	Thermo Fisher Scientific	*1:500*	*Cat # A11001*
Goat anti-rabbit IgG (H+L), Alexa Fluor 633	Thermo Fisher Scientific	*1:500*	*Cat # A21071*
Goat anti-chicken IgG (H+L), Alexa Fluor 488	Thermo Fisher Scientific	*1:500*	*Cat # A11039*
Goat anti-rabbit IgG (H+L), Cyanine 3	Thermo Fisher Scientific	*1:500*	*Cat # A10520*

7. Primary hippocampal cultures

Hippocampal cultures from new born C57Bl/6N mice (Charles River, Sulzfeld, Germany) were prepared and maintained by Iris Bünzli-Ehret as previously described(Brito, Kupke, et al., 2020).

Briefly, mice hippocampi were dissociated at P0 by papain digestion and plated onto tissue culture dishes coated with poly-D-lysine and laminin (Sigma-Aldrich, Munich, Germany). After isolation, cells were stored in an incubator at 37 °C and 5 % CO2 in neurobasal A medium (NBA; Invitrogen). This day was counted as day *in vitro* 0 (DIV0). In order to inhibit the proliferation of glial population in the cultures, Cytosine β-D-arabinofuranoside (AraC) (Sigma-Aldrich, Munich, Germany, C1768) was added to the hippocampal cultures at DIV 3. Viral infection occurred on the same

day, at least 6h after AraC treatment. Infection rates, were determined by analysing the respective transgene expression which ranged from 80-90%. Primary cultures were maintained for 8 days in Neurobasal-A medium (GibcoTM) supplemented with 1% rat serum (Biowest), 0.5mM L-glutamine (Sigma-Aldrich, Munich, Germany) and B27 (Gibco™), followed by a medium change containing salt-glucose-glycine solution (10 mM HEPES, pH 7.4, 114 mM NaCl, 26.1 mM NaHCO3, 5.3 mM KCl, 1 mM MgCl2, 2 mM CaCl2, 30 mM glucose, 1 mM glycine, 0.5 mM C3H3NaO3, and 0.001% phenol red) and phosphate-free Eagle's minimum essential medium (9:1 v/v), supplemented with insulin (7.5 µg/ml), transferrin (7.5 µg/ml), and sodium selenite (7.5 ng/ml) (ITS Liquid Media Supplement, Sigma-Aldrich, Munich, Germany) and penicillin-streptomycin. Experiments were performed on DIV 9-11.

Synaptic activity in the cultures was induced by treating the cells with GABA-A inhibitor, Bicuculline, at a concentration of 50 µM (Enzo Life Sciences, Germany).

For transfection, DNA (mg) to Lipofectamine (ml) ratio was 1.6:5 in 1 ml of medium. The Lipofectamine/DNA mixture was left on the cells for 3 h before it was replaced with a transfection medium.

For Doxycycline-dependent expression of constructs, Doxycycline hyclate (25 µM, Sigma-Aldrich, Munich, Germany) was introduced in the medium at DIV 8. To monitor doxycycline dependency, images of infected primary neurons with the TetOn-based miR30 system were acquired (Axio Vert.A1, Zeiss, Oberkochen, Germany).

8. Immunocytochemistry

Primary hippocampal neurons plated on coverslips were rinsed with PBS and fixed with ice-cold solution of 4% paraformaldehyde and 4% sucrose for 15 min at room temperature. After permeabilizing the cells in ice-cold methanol for 6 min, blocking was performed with 10% normal goat serum in PBS for 1 h at room temperature. Next, cells were incubated with primary antibody α-Dnmt3a (1:500, H-295, Santa Cruz, SC-20703 diluted in PBS containing 2% BSA, 0,1% Triton X-100) overnight at 4 °C. The next day, after washing 3x with PBS, the secondary antibody (1:500 goat anti-mouse Alexa488 (Life Technologies, Eugene, OR, USA)) diluted in PBS containing 2% BSA, 0,1% Triton X-100) was incubated for 1 h at room temperature. Finally, coverslips were treated with Hoechst 33258 (2 µg/ml, Serva, Heidelberg, Germany) for 5 min and mounted on glass slides. Images were acquired with a 40x oil objective on a fluorescence microscope (Leica Microsystems, Oberkochen, Germany).

9. Quantitative reverse-transcription PCR

The qRT-PCR primers were designed with Primer3 (https://primer3.ut.ee/) using either the RefSeq curated annotation or the GENCODE VM23 comprehensive transcript annotation, along with the GRCm38/mm10 mouse genome assembly. The specificity and amplicon product size of the primers were verified by BLAST search and *in silico* PCR (UCSC Genome Browser, mm10). Primer pair efficiencies and product melting curves were validated by qRT-PCR on serially diluted cDNA from primary mouse hippocampal cultures. The list of Power SYBR green primers used in the study is provided in Table 3.

For RNA isolation from mouse hippocampal tissue, animals were sacrificed by cervical dislocation and the tissue was rapidly dissected and placed in RNAlater (Sigma, Munich, Germany). The CA1 region, or the infected region was micro dissected prior RNA isolation. RNA was isolated using the RNeasy Plus Mini Kit (Qiagen, Hilden, Germany) with additional on-column DNase I digestion, according to the manufacturer's instructions.

For quantitative reverse-transcription PCR (qRT-PCR) using TaqMan probes, cDNA was synthesised from 1000µg RNA.For qRT-PCR using Power SYBR Green PCR Master Mix (Applied Biosystems), cDNA was synthesized from 400 ng RNA using the Applied Biosystems High-Capacity Complementary DNA Reverse Transcription Kit (ThermoFisher Scientific).

qRT-PCR was performed on a Step One Plus Real Time PCR System (Applied Biosystems, Foster City, CA, USA) using TaqMan gene expression assays (Applied Biosystems, Foster City, CA, USA) for the following mouse genes: *Arc* (Mm00479619_g1), *c-Fos* (Mm00487425_m1), *Npas4* (Mm00463644_m1), Dnmt1 (Mm00599763_m1), Dnmt3a1 (Mm00432870_m1), Dnmt3a2 (Mm00463987_m1) and Dnmt3b (Mm00599800_m1). Expression levels of target genes were normalized to the expression of the housekeeping gene *GusB* (Mm00446953_m1).

Power SYBR Green PCR Master mix was used for the remaining target genes. PCR reactions were run as technical triplicates in 10 µL reactions (96-well format) using 0.5 µM of each primer. 2 µl of diluted cDNA (about 1.25 ng) was added to each reaction. The thermal cycling was conducted with the following settings: a 10-minute incubation at 95 °C, 40 cycles of 10 seconds each at 95 °C, 60 °C, and 72 °C, followed by a 15-second incubation at 95 °C. Melt curves were generated by heating from 60 °C to 90 °C at a ramp rate of 0.6 °C/min. Relative expression levels of each target transcript were determined by the ΔΔCt method using beta-Actin mRNA levels as a reference.

Table 3: qRT-PCR primers used for analysis with Power SYBR Green Mix.

Name		**Sequence**
Actin	5' Primer	TATCCTGACCCTGAAGTACC
	3' Primer	CTCGGTGAGCAGCACAGGG
Alkal 2	5' Primer	TGAAACATCTCACAGGTCCTCT
	3' Primer	GTGCAGTCTCTCGTGTTGTG
Cacng 5	5' Primer	CCCTCGTCAGCCTCTTCTTC
	3' Primer	ACCACAAGAGAGAGGCCAGA
Calb1	5' Primer	GCGAGGAATTCATGAAGACTTGG
	3' Primer	TGTCAGTTCCAGCTTTCCGT
Crhbp	5' Primer	CTGAAGGTATTTGATGGTTGGA
	3' Primer	TCTTCATAGTGGGCAGAGGG
Fhad1	5' Primer	AGACGAAAATGATCCTGACGG
	3' Primer	CTAGGCTCACGATGGTCTGC
Nfix	5' Primer	TGTCCAGCCACATCACATTG
	3' Primer	TGGAAACTTAAGTGCCCGTTG
Ntn3	5' Primer	GCACAATACAGCTGGTCGTC
	3' Primer	TGGCAGTCACAAGCTCTG
Nrp1	5' Primer	GCAAGACTCGAATCCTCCC
	3' Primer	CCAATGTGAGGGCCAACTTC
Npy	5' Primer	CGTGTGTTTGGGCATTCTGG
	3' Primer	TGCCATATCTCTGTCTGGTGA
Pcdh10	5' Primer	TCGCGAGCAAATCTGTAAGC
	3' Primer	AGGAGGGAGGGTTGTCATTG
Plxnc1	5' Primer	GCTGGGAAGGAGGTGAGAAG
	3' Primer	GTGCACCTTTGTAACGGGAG
Trpc6	5' Primer	CCAGCTTCCGGGGTAATGAA
	3' Primer	ACATGTATGCTGGTCCTCGA

10. RNA Sequencing

RNA-seq data processing was performed with R (version 3.6.3) and bioconductor (version 3.9) in Rstudio (version 1.1.463). Quality control of clean sequencing reads was performed using FastQC (Babraham Bioinformatics). Low-quality reads were removed using trim_galore (version 0.6.4). The resulting reads were aligned to the mouse genome version GRCm38.p6 and counted using kallisto version 0.46.1 (Bray et al., 2016). The count data were transformed to log2-counts per million (logCPM) using the voom-function from the limma package(Ritchie et al., 2015). Differential expression analysis was performed using the limma package in R. A false positive rate of α= 0.05 with FDR correction was taken as the level of significance. Volcano plots and heatmaps were created using ggplot2 package (version 2.2.1) and the complexHeatmap package (version 2.0.0)(Z. Gu et al., 2016). For enrichment analysis, we used the fgsea (Korotkevich et al., 2021), the enrichmentbrowser (Geistlinger et al., 2016), and the Enrichr packages (E. Y. Chen et al., 2013).

For GO analysis of DEGs upon Dnmt3a2 overexpression, ShinyGO online platform was used (Ge et al., 2020). Pathway size of 20 was used and GO terms were selected by FDR.

11. Western Blot

Protein extracts were prepared from hippocampal cultures on DIV9-11. After removal of TM, pre-boiled 2x sample buffer (30 % glycerol; 4 % SDS; 160 mM Tris pH 6.8; 0.02 % bromphenol blue) (300 µl per dish or 150 µl per well) supplemented with 10 mM dithiothreitol (DTT) was added and cells were scraped. Samples were boiled for 5 min and stored at -80 °C.

In the case of western blotting of tissue samples, the dorsal hippocampus was quickly dissected in ice-cold PBS and homogenized in RIPA buffer (150 mM NaCl, 1% Triton X-100, 0.5% sodium deoxycholate, 0.1% SDS, 50 mM Tris, pH 8.0) supplemented with 1% protease inhibitor cocktail (Sigma-Aldrich, Munich, Germany). Protein concentration was measured by Bradford assay and 20 µg of denatured protein (in Laemmli buffer at 95°C for 5 min) was loaded and analysed by SDS-PAGE in 10% polyacrylamide gels.

SDS-PAGE was performed at constant voltage. Proteins were then transferred onto nitrocellulose membrane GE Healthcare, Buckinghamshire, UK) by electroblotting for 90 min with 400 mA at 4 °C. Membranes were blocked for 1h at room temperature in 5 % (w/v) low-fat milk in PBS containing 0.1 % Tween20 (Carl Roth) (PBST) and

then incubated with primary antibody (see Table 4 for dilutions) in 5 % milk in PBST overnight at 4 °C on a shaker incubator. Membranes were washed 4x with PBST for 5 min and incubated with secondary antibody (α-mouse or α-rabbit conjugated horseradish peroxidase; 1:5000) in 5 % milk in PBST for 1h room temperature mildly shaking. After washing 4x 5 min with PBST, membranes were incubated with enhanced chemiluminescence substrate (ECL) (mixture at 1:1 ratio Amersham ECL Western Blotting Detection Reagent; GE Healthcare Life Sciences) and either exposed to Hyperfilm (GE Healthcare) using an X-ray processing machine or developed using ChemiDoc™ Imaging System (Bio-Rad, California, USA).

Table 4: Antibodies used for Western Blot analysis.

Antibody	Molecular Weight (kDa)	Dilution	Company
aTubulin	*55*	*1:400000*	*Sigma #t9026*
ß-Actin	*43*	*1:1000*	*Santa Cruz #SC-47778*
Arc	*55*	*1:6000*	*Synaptic Systems #156 003*
cFos	*62*	*1:1000*	*Cell Signaling #2250*
Dnmt3a1	*130*	*1:10000*	*Santa Cruz #SC-20703*
HA	*Not applicable*	*1:1000*	*Biolegend MMS-101R*
Npas4	*100*	*1:500*	*Activity Signaling, AS-AB18A*

12. Image acquisition and analysis

Image acquisition was done using a 20x oil objective on a TCS SP8 confocal microscope (Leica Microsystems, Oberkochen, Germany) and the Leica LAS X LS software. 10% overlap ratio was set to stitch individual tiles of a bigger image. Identical microscope settings (such as gain, offset, stitching overlap ratio) were used between different experimental groups. Image analysis was done using Fiji. At the beginning of each analysis, the background fluorescence of each picture was measured and subtracted.

For overlap analysis, a threshold was defined and particles analysed. Only particles larger than 40-pixel units and above the threshold were included in the analysis. The same threshold of intensity was applied to all images in order to identify cells positive for GFP or cFos. Cell-counter plugin of Fiji was used to count the identified particles in each channel independently. A particle was designated as "overlapping" when it was positively identified in both channels. The total number of

cells (Hoechst$^+$ cells) was calculated using a formula based on the total area of the DG, CA1 or ACC which was developed and generated using cell count and area from 15-20 mice (DG: R2=0.91; CA1 R2=0.96, ACC: R2=0.97).

Throughout the entire study, the image analysis was performed on at least 3 animals per condition and at least 2 to 3 brain slices per animal. The final value for each animal was calculated using the average of its brain slices. The following formulas were used in this study to calculate reactivation rate (Cowansage et al., 2014; Gulmez Karaca et al., 2020):

Observed overlap: $\frac{(GFP+Fos+)}{Hoechst+} \times 100$

Overlap by chance: $\frac{GFP+}{Hoechst+} \times \frac{Fos+}{Hoechst+} \times 100$

Reactivation rate: $\frac{(GFP+Fos+)}{GFP+} \times 100$

13. Statistics

For all of the data analysis throughout the study, at first data was subjected to a statistical test for Gaussian distribution (Shapiro-Wilk normality test, alpha=0.05; $p< 0.05$). For normally distributed data sets, two-tailed unpaired Student's t tests were used to compare two groups. If more than two groups were analysed simultaneously, a one-way ANOVA was used followed by appropriate multiple comparison *post hoc* tests to control for multiple comparisons as specified. Normally distributed significant data was marked with *. In case of a non-Gaussian distribution, two-tailed Mann-Whitney tests were used to compare two distinct groups or a Kruskal-Wallis test followed by Dunn´s *post hoc* test to compare more than 2 groups was used. Non-normal distributed significant data was marked with $^{\#}$. To calculate the statistical difference between the observed overlap (or reactivation rate) and a mathematical chance rate, paired Student's t-test for normally distributed data and Wilcoxon test for non-normally distributed data was used. The sample size was determined based on similar experiments carried-out in the past and in the literature. All plotted data represent mean ± SEM. Statistical analysis was performed using GraphPad Prism for Windows, version 8. For behavioural experiments the investigators were blind to group allocation during data collection and analysis. All behavioural sessions were video recorded and manually scored. The TSE Systems Fear Conditioning program was used to score the mean velocity during the training for CFC. For *in vitro* experiments no blinding was performed since the outcome was dependent on software analysis and not manual scoring. All statistical details of experiments can be found in the respective figure legend.

V. References

Abel, T., & Kandel, E. (1998). Positive and negative regulatory mechanisms that mediate long-term memory storage. *Brain Research Reviews*, *26*(2–3), 360–378. https://doi.org/10.1016/S0165-0173(97)00050-7

Alaghband, Y., Bredy, T. W., & Wood, M. A. (2016). The role of active DNA demethylation and Tet enzyme function in memory formation and cocaine action. *Neuroscience Letters*, *625*, 40–46. https://doi.org/10.1016/j.neulet.2016.01.023

Alberini, C. M. (2009). Transcription Factors in Long-Term Memory and Synaptic Plasticity. *Physiological Reviews*, *89*(1), 121–145. https://doi.org/10.1152/physrev.00017.2008

Alberini, C. M., & Kandel, E. R. (2015). The Regulation of Transcription in Memory Consolidation. *Cold Spring Harbor Perspectives in Biology*, *7*(1), a021741. https://doi.org/10.1101/cshperspect.a021741

Alvarez, P., & Squire, L. R. (1994). Memory consolidation and the medial temporal lobe: a simple network model. *Proceedings of the National Academy of Sciences*, *91*(15), 7041–7045. https://doi.org/10.1073/pnas.91.15.7041

Aoki, A. (2001). Enzymatic properties of de novo-type mouse DNA (cytosine-5) methyltransferases. *Nucleic Acids Research*, *29*(17), 3506–3512. https://doi.org/10.1093/nar/29.17.3506

Ashley, J., Cordy, B., Lucia, D., Fradkin, L. G., Budnik, V., & Thomson, T. (2018). Retrovirus-like Gag Protein Arc1 Binds RNA and Traffics across Synaptic Boutons. *Cell*, *172*(1–2), 262-274.e11. https://doi.org/10.1016/j.cell.2017.12.022

Asok, A., Kandel, E. R., & Rayman, J. B. (2019). The Neurobiology of Fear Generalization. *Frontiers in Behavioral Neuroscience*, *12*. https://doi.org/10.3389/fnbeh.2018.00329

Bartsch, D., Ghirardi, M., Skehel, P. A., Karl, K. A., Herder, S. P., Chen, M., Bailey, C. H., & Kandel, E. R. (1995). Aplysia CREB2 represses long-term facilitation: Relief of repression converts transient facilitation into long-term functional and structural change. *Cell*, *83*(6), 979–992. https://doi.org/10.1016/0092-8674(95)90213-9

Bayley, P. J., Hopkins, R. O., & Squire, L. R. (2003). Successful Recollection of Remote Autobiographical Memories by Amnesic Patients with Medial Temporal Lobe Lesions. *Neuron*, *38*(1), 135–144. https://doi.org/10.1016/S0896-6273(03)00156-9

Bayraktar, G., Yuanxiang, P. A., Confettura, A. D., Gomes, G. M., Raza, S. A., Stork, O., Tajima, S., Suetake, I., Karpova, A., Yildirim, F., & Kreutz, M. R. (2020). Synaptic control of DNA methylation involves activity-dependent degradation of DNMT3A1 in the nucleus. *Neuropsychopharmacology*, *45*(12). https://doi.org/10.1038/s41386-020-0780-2

Beagan, J. A., Pastuzyn, E. D., Fernandez, L. R., Guo, M. H., Feng, K., Titus, K. R., Chandrashekar, H., Shepherd, J. D., & Phillips-Cremins, J. E. (2020). Three-dimensional genome restructuring across timescales of activity-induced neuronal gene expression. *Nature Neuroscience*, *23*(6), 707–717. https://doi.org/10.1038/s41593-020-0634-6

Beagan, J. A., & Phillips-Cremins, J. E. (2020). On the existence and functionality of topologically associating domains. *Nature Genetics*, *52*(1), 8–16. https://doi.org/10.1038/s41588-019-0561-1

Bekinschtein, P., Cammarota, M., Igaz, L. M., Bevilaqua, L. R. M., Izquierdo, I., & Medina, J. H. (2007). Persistence of Long-Term Memory Storage Requires a Late Protein Synthesis- and BDNF- Dependent Phase in the Hippocampus. *Neuron*, *53*(2), 261–277. https://doi.org/10.1016/j.neuron.2006.11.025

Bekinschtein, P., Cammarota, M., Katche, C., Slipczuk, L., Rossato, J. I., Goldin, A., Izquierdo, I., & Medina, J. H. (2008). BDNF is essential to promote persistence of long-term memory storage. *Proceedings of the National Academy of Sciences*, *105*(7), 2711–2716. https://doi.org/10.1073/pnas.0711863105

Benito, E., & Barco, A. (2015). The Neuronal Activity-Driven Transcriptome. *Molecular Neurobiology*, *51*(3), 1071–1088. https://doi.org/10.1007/s12035-014-8772-z

Bero, A. W., Meng, J., Cho, S., Shen, A. H., Canter, R. G., Ericsson, M., & Tsai, L. H. (2014). Early remodeling of the neocortex upon episodic memory encoding. *Proceedings of the National Academy of Sciences of the United States of America*, *111*(32). https://doi.org/10.1073/pnas.1408378111

Berry, J. A., Cervantes-Sandoval, I., Nicholas, E. P., & Davis, R. L. (2012). Dopamine Is Required for Learning and Forgetting in Drosophila. *Neuron*, *74*(3), 530–542. https://doi.org/10.1016/j.neuron.2012.04.007

Billia, F., Baskys, A., Carlen, P. L., & De Boni, U. (1992). Rearrangement of centromeric satellite DNA in hippocampal neurons exhibiting long-term potentiation. *Molecular Brain Research*, *14*(1–2), 101–108. https://doi.org/10.1016/0169-328X(92)90016-5

Blázquez, G., Castañé, A., Saavedra, A., Masana, M., Alberch, J., & Pérez-Navarro, E. (2019). Social Memory and Social Patterns Alterations in the Absence of STriatal-Enriched Protein Tyrosine Phosphatase. *Frontiers in Behavioral Neuroscience*, *12*. https://doi.org/10.3389/fnbeh.2018.00317

Bliss, T. V. P., Collingridge, G. L., Morris, R. G. M., & Reymann, K. G. (2018). Long-term potentiation in the hippocampus: discovery, mechanisms and function. *Neuroforum*, *24*(3), A103–A120. https://doi.org/10.1515/nf-2017-A059

Borden, J., & Manuelidis, L. (1988). Movement of the X Chromosome in Epilepsy. *Science*, *242*(4886), 1687–1691. https://doi.org/10.1126/science.3201257

Boye, E., & Grallert, B. (2020). eIF2α phosphorylation and the regulation of translation. *Current Genetics*, *66*(2), 293–297. https://doi.org/10.1007/s00294-019-01026-1

Brandt, J., & Bakker, A. (2018). Neuropsychological investigation of "the Amazing Memory Man". *Neuropsychology*, *32*(3), 304–316. https://doi.org/10.1037/neu0000410

Bray, N. L., Pimentel, H., Melsted, P., & Pachter, L. (2016). Near-optimal probabilistic RNA-seq quantification. *Nature Biotechnology*, *34*(5). https://doi.org/10.1038/nbt.3519

Brigidi, G. S., Hayes, M. G. B., Delos Santos, N. P., Hartzell, A. L., Texari, L., Lin, P.-A., Bartlett, A., Ecker, J. R., Benner, C., Heinz, S., & Bloodgood, B. L. (2019). Genomic Decoding of Neuronal Depolarization by Stimulus-Specific NPAS4 Heterodimers. *Cell*, *179*(2), 373-391.e27. https://doi.org/10.1016/j.cell.2019.09.004

Brito, D. V. C., Gulmez Karaca, K., Kupke, J., Mudlaff, F., Zeuch, B., Gomes, R., Lopes, L. V., & Oliveira, A. M. M. (2020). Modeling human age-associated increase in Gadd45γ expression leads to spatial recognition memory impairments in young adult mice. *Neurobiology of Aging*, *94*. https://doi.org/10.1016/j.neurobiolaging.2020.06.021

Brito, D. V. C., Kupke, J., Gulmez Karaca, K., & Oliveira, A. M. M. (2022). Regulation of neuronal plasticity by the DNA repair associated Gadd45 proteins. *Current Research in Neurobiology*, *3*, 100031. https://doi.org/10.1016/j.crneur.2022.100031

Brito, D. V. C., Kupke, J., Karaca, K. G., Zeuch, B., & Oliveira, A. M. M. (2020). Mimicking age-associated Gadd45γ dysregulation results in memory impairments in young adult mice. *Journal of Neuroscience*, *40*(6). https://doi.org/10.1523/JNEUROSCI.1621-19.2019

Buzsáki, G. (1996). The Hippocampo-Neocortical Dialogue. *Cerebral Cortex*, *6*(2), 81–92. https://doi.org/10.1093/cercor/6.2.81

Campbell, R. R., & Wood, M. A. (2019). How the epigenome integrates information and reshapes the synapse. In *Nature Reviews Neuroscience* (Vol. 20, Issue 3). https://doi.org/10.1038/s41583-019-0121-9

Cannella, N., Oliveira, A. M. M., Hemstedt, T., Lissek, T., Buechler, E., Bading, H., & Spanagel, R. (2018). Dnmt3a2 in the Nucleus Accumbens Shell Is Required for Reinstatement of Cocaine Seeking. *The Journal of Neuroscience*, *38*(34), 7516–7528. https://doi.org/10.1523/JNEUROSCI.0600-18.2018

Cardin, J. A., & Abel, T. (1999). Memory suppressor genes: enhancing the relationship between synaptic plasticity and memory storage. *Journal of Neuroscience Research*, *58*(1), 10–23.

Carulli, D., de Winter, F., & Verhaagen, J. (2021). Semaphorins in Adult Nervous System Plasticity and Disease. In *Frontiers in Synaptic Neuroscience* (Vol. 13). https://doi.org/10.3389/fnsyn.2021.672891

Chahrour, M., Jung, S. Y., Shaw, C., Zhou, X., Wong, S. T. C., Qin, J., & Zoghbi, H. Y. (2008). MeCP2, a Key Contributor to Neurological Disease, Activates and Represses Transcription. *Science*, *320*(5880), 1224–1229. https://doi.org/10.1126/science.1153252

Chatterjee, S., & Abel, T. (2017). Transcriptional Regulation of Memory Formation. In *Learning and Memory: A Comprehensive Reference* (pp. 329–343). Elsevier. https://doi.org/10.1016/B978-0-12-809324-5.21107-3

Chen, A., Muzzio, I. A., Malleret, G., Bartsch, D., Verbitsky, M., Pavlidis, P., Yonan, A. L., Vronskaya, S., Grody, M. B., Cepeda, I., Gilliam, T. C., & Kandel, E. R. (2003). Inducible Enhancement of Memory Storage and Synaptic Plasticity in Transgenic Mice Expressing an Inhibitor of ATF4 (CREB-2) and C/EBP Proteins. *Neuron*, *39*(4), 655–669. https://doi.org/10.1016/S0896-6273(03)00501-4

Chen, E. Y., Tan, C. M., Kou, Y., Duan, Q., Wang, Z., Meirelles, G. V, Clark, N. R., & Ma'ayan, A. (2013). Enrichr: Interactive and collaborative HTML5 gene list enrichment analysis tool. *BMC Bioinformatics*, *14*. https://doi.org/10.1186/1471-2105-14-128

Chen, T., Ueda, Y., Xie, S., & Li, E. (2002). A Novel Dnmt3a Isoform Produced from an Alternative Promoter Localizes to Euchromatin and Its Expression Correlates with Activede Novo Methylation. *Journal of Biological Chemistry*, *277*(41), 38746–38754. https://doi.org/10.1074/jbc.M205312200

Chen, X., Wang, X., Tang, L., Wang, J., Shen, C., Liu, J., Lu, S., Zhang, H., Kuang, Y., Fei, J., & Wang, Z. (2017). Nhe5 deficiency enhances learning and memory via upregulating Bdnf/TrkB signaling in mice. *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics*, *174*(8), 828–838. https://doi.org/10.1002/ajmg.b.32600

Choi, J.-H., Sim, S.-E., Kim, J., Choi, D. Il, Oh, J., Ye, S., Lee, J., Kim, T., Ko, H.-G., Lim, C.-S., & Kaang, B.-K. (2018). Interregional synaptic maps among engram cells underlie memory formation. *Science*, *360*(6387), 430–435. https://doi.org/10.1126/science.aas9204

Cholewa-Waclaw, J., Bird, A., von Schimmelmann, M., Schaefer, A., Yu, H., Song, H., Madabhushi, R., & Tsai, L.-H. (2016). The Role of Epigenetic Mechanisms in the Regulation of Gene Expression in the Nervous System. *The Journal of Neuroscience*, *36*(45), 11427–11434. https://doi.org/10.1523/JNEUROSCI.2492-16.2016

Christian, D. L., Wu, D. Y., Martin, J. R., Moore, J. R., Liu, Y. R., Clemens, A. W., Nettles, S. A., Kirkland, N. M., Papouin, T., Hill, C. A., Wozniak, D. F., Dougherty, J. D., & Gabel,

H. W. (2020). DNMT3A Haploinsufficiency Results in Behavioral Deficits and Global Epigenomic Dysregulation Shared across Neurodevelopmental Disorders. *Cell Reports*, *33*(8), 108416. https://doi.org/10.1016/j.celrep.2020.108416

Coda, D. M., & Gräff, J. (2020). Neurogenetic and Neuroepigenetic Mechanisms in Cognitive Health and Disease. In *Frontiers in Molecular Neuroscience* (Vol. 13). https://doi.org/10.3389/fnmol.2020.589109

Collins, B. E., Greer, C. B., Coleman, B. C., & Sweatt, J. D. (2019). Histone H3 lysine K4 methylation and its role in learning and memory. *Epigenetics & Chromatin*, *12*(1), 7. https://doi.org/10.1186/s13072-018-0251-8

Costa-Mattioli, M., Gobert, D., Stern, E., Gamache, K., Colina, R., Cuello, C., Sossin, W., Kaufman, R., Pelletier, J., Rosenblum, K., Krnjević, K., Lacaille, J.-C., Nader, K., & Sonenberg, N. (2007). eIF2α Phosphorylation Bidirectionally Regulates the Switch from Short- to Long-Term Synaptic Plasticity and Memory. *Cell*, *129*(1), 195–206. https://doi.org/10.1016/j.cell.2007.01.050

Cowansage, K. K., Shuman, T., Dillingham, B. C., Chang, A., Golshani, P., & Mayford, M. (2014). Direct Reactivation of a Coherent Neocortical Memory of Context. *Neuron*, *84*(2), 432–441. https://doi.org/10.1016/j.neuron.2014.09.022

DaSilva, L. L. P., Wall, M. J., P. de Almeida, L., Wauters, S. C., Januário, Y. C., Müller, J., & Corrêa, S. A. L. (2016). Activity-Regulated Cytoskeleton-Associated Protein Controls AMPAR Endocytosis through a Direct Interaction with Clathrin-Adaptor Protein 2. *Eneuro*, *3*(3), ENEURO.0144-15.2016. https://doi.org/10.1523/ENEURO.0144-15.2016

Day, J. J., & Sweatt, J. D. (2011). Epigenetic Mechanisms in Cognition. *Neuron*, *70*(5), 813–829. https://doi.org/10.1016/j.neuron.2011.05.019

De Jager, P. L., Srivastava, G., Lunnon, K., Burgess, J., Schalkwyk, L. C., Yu, L., Eaton, M. L., Keenan, B. T., Ernst, J., McCabe, C., Tang, A., Raj, T., Replogle, J., Brodeur, W., Gabriel, S., Chai, H. S., Younkin, C., Younkin, S. G., Zou, F., … Bennett, D. A. (2014). Alzheimer's disease: early alterations in brain DNA methylation at ANK1, BIN1, RHBDF2 and other loci. *Nature Neuroscience*, *17*(9), 1156–1163. https://doi.org/10.1038/nn.3786

de Lecea, L., Criado, J. R., Rivera, S., Wen, W., Soriano, E., Henriksen, S. J., Taylor, S. S., Gall, C. M., & Sutcliffe, J. G. (1998). Endogenous protein kinase A inhibitor (PKI?) modulates synaptic activity. *Journal of Neuroscience Research*, *53*(3), 269–278. https://doi.org/10.1002/(SICI)1097-4547(19980801)53:3<269::AID-JNR1>3.0.CO;2-8

Della Ragione, F., Vacca, M., Fioriniello, S., Pepe, G., & D'Esposito, M. (2016). MECP2, a multi-talented modulator of chromatin architecture. *Briefings in Functional Genomics*, elw023. https://doi.org/10.1093/bfgp/elw023

DeNardo, L. A., Liu, C. D., Allen, W. E., Adams, E. L., Friedmann, D., Fu, L., Guenthner, C. J., Tessier-Lavigne, M., & Luo, L. (2019). Temporal evolution of cortical ensembles promoting remote memory retrieval. *Nature Neuroscience*, *22*(3). https://doi.org/10.1038/s41593-018-0318-7

Denk, F., & McMahon, S. B. (2012). Chronic Pain: Emerging Evidence for the Involvement of Epigenetics. *Neuron*, *73*(3), 435–444. https://doi.org/10.1016/j.neuron.2012.01.012

Denny, C. A., Kheirbek, M. A., Alba, E. L., Tanaka, K. F., Brachman, R. A., Laughman, K. B., Tomm, N. K., Turi, G. F., Losonczy, A., & Hen, R. (2014). Hippocampal Memory Traces Are Differentially Modulated by Experience, Time, and Adult Neurogenesis. *Neuron*, *83*(1), 189–201. https://doi.org/10.1016/j.neuron.2014.05.018

Deplus, R., Blanchon, L., Rajavelu, A., Boukaba, A., Defrance, M., Luciani, J., Rothé, F., Dedeurwaerder, S., Denis, H., Brinkman, A. B., Simmer, F., Müller, F., Bertin, B., Berdasco, M., Putmans, P., Calonne, E., Litchfield, D. W., de Launoit, Y., Jurkowski, T. P., … Fuks, F. (2014). Regulation of DNA Methylation Patterns by CK2-Mediated Phosphorylation of Dnmt3a. *Cell Reports*, *8*(3), 743–753. https://doi.org/10.1016/j.celrep.2014.06.048

Deplus, R., Denis, H., Putmans, P., Calonne, E., Fourrez, M., Yamamoto, K., Suzuki, A., & Fuks, F. (2014). Citrullination of DNMT3A by PADI4 regulates its stability and controls DNA methylation. *Nucleic Acids Research*, *42*(13), 8285–8296. https://doi.org/10.1093/nar/gku522

Dukatz, M., Requena, C. E., Emperle, M., Hajkova, P., Sarkies, P., & Jeltsch, A. (2019). Mechanistic Insights into Cytosine-N3 Methylation by DNA Methyltransferase DNMT3A. *Journal of Molecular Biology*, *431*(17), 3139–3145. https://doi.org/10.1016/j.jmb.2019.06.015

Duke, C. G., Kennedy, A. J., Gavin, C. F., Day, J. J., & Sweatt, J. D. (2017). Experience-dependent epigenomic reorganization in the hippocampus. *Learning & Memory*, *24*(7), 278–288. https://doi.org/10.1101/lm.045112.117

Engin, E., Zarnowska, E. D., Benke, D., Tsvetkov, E., Sigal, M., Keist, R., Bolshakov, V. Y., Pearce, R. A., & Rudolph, U. (2015). Tonic Inhibitory Control of Dentate Gyrus Granule Cells by α5-Containing GABA $_A$ Receptors Reduces Memory Interference. *The Journal of Neuroscience*, *35*(40), 13698–13712. https://doi.org/10.1523/JNEUROSCI.1370-15.2015

Euston, D. R., Tatsuno, M., & McNaughton, B. L. (2007). Fast-Forward Playback of Recent Memory Sequences in Prefrontal Cortex During Sleep. *Science*, *318*(5853), 1147–1150. https://doi.org/10.1126/science.1148979

Fan, C., Gao, Y., Liang, G., Huang, L., Wang, J., Yang, X., Shi, Y., Dräger, U. C., Zhong, M., Gao, T.-M., & Yang, X. (2020). Transcriptomics of Gabra4 knockout mice reveals common NMDAR pathways underlying autism, memory, and epilepsy. *Molecular Autism*, *11*(1), 13. https://doi.org/10.1186/s13229-020-0318-9

Fan, X., Song, J., Ma, C., Lv, Y., Wang, F., Ma, L., & Liu, X. (2022). Noradrenergic signaling mediates cortical early tagging and storage of remote memory. *Nature Communications*, *13*(1), 7623. https://doi.org/10.1038/s41467-022-35342-x

Farrelly, L. A., Thompson, R. E., Zhao, S., Lepack, A. E., Lyu, Y., Bhanu, N. V., Zhang, B., Loh, Y.-H. E., Ramakrishnan, A., Vadodaria, K. C., Heard, K. J., Erikson, G., Nakadai, T., Bastle, R. M., Lukasak, B. J., Zebroski, H., Alenina, N., Bader, M., Berton, O., … Maze, I. (2019). Histone serotonylation is a permissive modification that enhances TFIID binding to H3K4me3. *Nature*, *567*(7749), 535–539. https://doi.org/10.1038/s41586-019-1024-7

Feng, J., Chang, H., Li, E., & Fan, G. (2005). Dynamic expression of de novo DNA methyltransferases Dnmt3a and Dnmt3b in the central nervous system. *Journal of Neuroscience Research*, *79*(6), 734–746. https://doi.org/10.1002/jnr.20404

Feng, J., & Nestler, E. J. (2013). Epigenetic mechanisms of drug addiction. *Current Opinion in Neurobiology*, *23*(4), 521–528. https://doi.org/10.1016/j.conb.2013.01.001

Feng, J., Zhou, Y., Campbell, S. L., Le, T., Li, E., Sweatt, J. D., Silva, A. J., & Fan, G. (2010). Dnmt1 and Dnmt3a maintain DNA methylation and regulate synaptic function in adult forebrain neurons. *Nature Neuroscience*, *13*(4), 423–430. https://doi.org/10.1038/nn.2514

Fernandez-Albert, J., Lipinski, M., Lopez-Cascales, M. T., Rowley, M. J., Martin-Gonzalez, A. M., del Blanco, B., Corces, V. G., & Barco, A. (2019). Immediate and deferred epigenomic signatures of in vivo neuronal activation in mouse hippocampus. *Nature Neuroscience*, *22*(10). https://doi.org/10.1038/s41593-019-0476-2

Fitzgerald, P. J., Pinard, C. R., Camp, M. C., Feyder, M., Sah, A., Bergstrom, H. C., Graybeal, C., Liu, Y., Schlüter, O. M., Grant, S. G., Singewald, N., Xu, W., & Holmes, A. (2015). Durable fear memories require PSD-95. *Molecular Psychiatry*, *20*(7), 901–912. https://doi.org/10.1038/mp.2014.161

Fleischmann, A., Hvalby, O., Jensen, V., Strekalova, T., Zacher, C., Layer, L. E., Kvello, A., Reschke, M., Spanagel, R., Sprengel, R., Wagner, E. F., & Gass, P. (2003). Impaired Long-Term Memory and NR2A-Type NMDA Receptor-Dependent Synaptic Plasticity in Mice Lacking c-Fos in the CNS. *The Journal of Neuroscience*, *23*(27), 9116–9122. https://doi.org/10.1523/JNEUROSCI.23-27-09116.2003

Flexner, J. B., Flexner, L. B., Stellar, E., Haba, G. de la, & Roberts†, R. B. (1962). INHIBITION OF PROTEIN SYNTHESIS IN BRAIN AND LEARNING AND MEMORY FOLLOWING PUROMYCIN. *Journal of Neurochemistry*, *9*(6), 595–605. https://doi.org/10.1111/j.1471-4159.1962.tb04216.x

Frankland, P. W., & Bontempi, B. (2005). The organization of recent and remote memories. In *Nature Reviews Neuroscience* (Vol. 6, Issue 2). https://doi.org/10.1038/nrn1607

Frankland, P. W., Bontempi, B., Talton, L. E., Kaczmarek, L., & Silva, A. J. (2004). The Involvement of the Anterior Cingulate Cortex in Remote Contextual Fear Memory. *Science*, *304*(5672), 881–883. https://doi.org/10.1126/science.1094804

Gavin, D. P., & Sharma, R. P. (2010). Histone modifications, DNA methylation, and Schizophrenia. *Neuroscience & Biobehavioral Reviews*, *34*(6), 882–888. https://doi.org/10.1016/j.neubiorev.2009.10.010

Gavin, D. P., Sharma, R. P., Chase, K. A., Matrisciano, F., Dong, E., & Guidotti, A. (2012). Growth Arrest and DNA-Damage-Inducible, Beta (GADD45b)-Mediated DNA Demethylation in Major Psychosis. *Neuropsychopharmacology*, *37*(2), 531–542. https://doi.org/10.1038/npp.2011.221

Ge, S. X., Jung, D., & Yao, R. (2020). ShinyGO: a graphical gene-set enrichment tool for animals and plants. *Bioinformatics*, *36*(8), 2628–2629. https://doi.org/10.1093/bioinformatics/btz931

Geistlinger, L., Csaba, G., & Zimmer, R. (2016). Bioconductor's EnrichmentBrowser: seamless navigation through combined results of set- & network-based enrichment analysis. *BMC Bioinformatics*, *17*(1), 45. https://doi.org/10.1186/s12859-016-0884-1

Gonzalez, C., Kramar, C., Garagoli, F., Rossato, J. I., Weisstaub, N., Cammarota, M., & Medina, J. H. (2013). Medial prefrontal cortex is a crucial node of a rapid learning system that retrieves recent and remote memories. *Neurobiology of Learning and Memory*, *103*, 19–25. https://doi.org/10.1016/j.nlm.2013.04.006

Goshen, I., Brodsky, M., Prakash, R., Wallace, J., Gradinaru, V., Ramakrishnan, C., & Deisseroth, K. (2011). Dynamics of Retrieval Strategies for Remote Memories. *Cell*, *147*(3), 678–689. https://doi.org/10.1016/j.cell.2011.09.033

Gøtzsche, C. R., & Woldbye, D. P. D. (2016). The role of NPY in learning and memory. *Neuropeptides*, *55*, 79–89. https://doi.org/10.1016/j.npep.2015.09.010

Gouty-Colomer, L. A., Hosseini, B., Marcelo, I. M., Schreiber, J., Slump, D. E., Yamaguchi, S., Houweling, A. R., Jaarsma, D., Elgersma, Y., & Kushner, S. A. (2016). Erratum: Arc expression identifies the lateral amygdala fear memory trace. *Molecular Psychiatry*, *21*(8), 1153–1153. https://doi.org/10.1038/mp.2016.91

Gräff, J., & Tsai, L.-H. (2013a). Histone acetylation: molecular mnemonics on the chromatin. *Nature Reviews Neuroscience*, *14*(2), 97–111. https://doi.org/10.1038/nrn3427

Gräff, J., & Tsai, L.-H. (2013b). The Potential of HDAC Inhibitors as Cognitive Enhancers. *Annual Review of Pharmacology and Toxicology*, *53*(1), 311–330. https://doi.org/10.1146/annurev-pharmtox-011112-140216

Gray, N. K. (2000). Multiple portions of poly(A)-binding protein stimulate translation in vivo. *The EMBO Journal*, *19*(17), 4723–4733. https://doi.org/10.1093/emboj/19.17.4723

Grecksch, G., & Matthies, H. (1980). Two sensitive periods for the amnesic effect of anisomycin. *Pharmacology Biochemistry and Behavior*, *12*(5), 663–665. https://doi.org/10.1016/0091-3057(80)90145-8

Gu, T., Hao, D., Woo, J., Huang, T.-W., Guo, L., Lin, X., Guzman, A. G., Tovy, A., Rosas, C., Jeong, M., Zhou, Y., Deneen, B., Huang, Y., Li, W., & Goodell, M. A. (2022). The disordered N-terminal domain of DNMT3A recognizes H2AK119ub and is required for postnatal development. *Nature Genetics*, *54*(5), 625–636. https://doi.org/10.1038/s41588-022-01063-6

Gu, Z., Eils, R., & Schlesner, M. (2016). Complex heatmaps reveal patterns and correlations in multidimensional genomic data. *Bioinformatics*, *32*(18). https://doi.org/10.1093/bioinformatics/btw313

Guenthner, C. J., Miyamichi, K., Yang, H. H., Heller, H. C., & Luo, L. (2013). Permanent Genetic Access to Transiently Active Neurons via TRAP: Targeted Recombination in Active Populations. *Neuron*, *78*(5), 773–784. https://doi.org/10.1016/j.neuron.2013.03.025

Gulmez Karaca, K., Brito, D. V. C., Kupke, J., Zeuch, B., & Oliveira, A. M. M. (2021). Engram reactivation during memory retrieval predicts long-term memory performance in aged mice. *Neurobiology of Aging*, *101*, 256–261. https://doi.org/10.1016/j.neurobiolaging.2021.01.019

Gulmez Karaca, K., Brito, D. V. C., & Oliveira, A. M. M. (2019). MeCP2: A Critical Regulator of Chromatin in Neurodevelopment and Adult Brain Function. *International Journal of Molecular Sciences*, *20*(18), 4577. https://doi.org/10.3390/ijms20184577

Gulmez Karaca, K., Kupke, J., Brito, D. V. C., Zeuch, B., Thome, C., Weichenhan, D., Lutsik, P., Plass, C., & Oliveira, A. M. M. (2020). Neuronal ensemble-specific DNA methylation strengthens engram stability. *Nature Communications*, *11*(1). https://doi.org/10.1038/s41467-020-14498-4

Guo, J. U., Ma, D. K., Mo, H., Ball, M. P., Jang, M.-H., Bonaguidi, M. A., Balazer, J. A., Eaves, H. L., Xie, B., Ford, E., Zhang, K., Ming, G., Gao, Y., & Song, H. (2011). Neuronal activity modifies the DNA methylation landscape in the adult brain. *Nature Neuroscience*, *14*(10), 1345–1351. https://doi.org/10.1038/nn.2900

Gupta, S., Kim, S. Y., Artis, S., Molfese, D. L., Schumacher, A., Sweatt, J. D., Paylor, R. E., & Lubin, F. D. (2010). Histone Methylation Regulates Memory Formation. *The Journal of Neuroscience*, *30*(10), 3589–3599. https://doi.org/10.1523/JNEUROSCI.3732-09.2010

Guzowski, J. F., McNaughton, B. L., Barnes, C. A., & Worley, P. F. (1999). Environment-specific expression of the immediate-early gene Arc in hippocampal neuronal ensembles. *Nature Neuroscience*, *2*(12), 1120–1124. https://doi.org/10.1038/16046

Hai, T., & Hartman, M. G. (2001). The molecular biology and nomenclature of the activating transcription factor/cAMP responsive element binding family of transcription factors: activating transcription factor proteins and homeostasis. *Gene*, *273*(1), 1–11. https://doi.org/10.1016/S0378-1119(01)00551-0

Halder, R., Hennion, M., Vidal, R. O., Shomroni, O., Rahman, R. U., Rajput, A., Centeno, T. P., Bebber, F. Van, Capece, V., Vizcaino, J. C. G., Schuetz, A. L., Burkhardt, S., Benito, E., Sala, M. N., Javan, S. B., Haass, C., Schmid, B., Fischer, A., & Bonn, S. (2015). DNA methylation changes in plasticity genes accompany the formation and maintenance of memory. *Nature Neuroscience*, *19*(1). https://doi.org/10.1038/nn.4194

Hansen, R. T., Conti, M., & Zhang, H.-T. (2014). Mice deficient in phosphodiesterase-4A display anxiogenic-like behavior. *Psychopharmacology*, *231*(15), 2941–2954. https://doi.org/10.1007/s00213-014-3480-y

Hartzell, A. L., Martyniuk, K. M., Brigidi, G. S., Heinz, D. A., Djaja, N. A., Payne, A., & Bloodgood, B. L. (2018). NPAS4 recruits CCK basket cell synapses and enhances cannabinoid-sensitive inhibition in the mouse hippocampus. *ELife*, *7*. https://doi.org/10.7554/eLife.35927

Hawasli, A. H., Benavides, D. R., Nguyen, C., Kansy, J. W., Hayashi, K., Chambon, P., Greengard, P., Powell, C. M., Cooper, D. C., & Bibb, J. A. (2007). Cyclin-dependent kinase 5 governs learning and synaptic plasticity via control of NMDAR degradation. *Nature Neuroscience*, *10*(7), 880–886. https://doi.org/10.1038/nn1914

He, Y.-F., Li, B.-Z., Li, Z., Liu, P., Wang, Y., Tang, Q., Ding, J., Jia, Y., Chen, Z., Li, L., Sun, Y., Li, X., Dai, Q., Song, C.-X., Zhang, K., He, C., & Xu, G.-L. (2011). Tet-Mediated Formation of 5-Carboxylcytosine and Its Excision by TDG in Mammalian DNA. *Science*, *333*(6047), 1303–1307. https://doi.org/10.1126/science.1210944

Hebb, D. (1949). The Organization of Behavior (New York: Wiley & Sons).

Hoeffer, C. A., & Klann, E. (2010). mTOR signaling: At the crossroads of plasticity, memory and disease. *Trends in Neurosciences*, *33*(2), 67–75. https://doi.org/10.1016/j.tins.2009.11.003

Holz-Schietinger, C., & Reich, N. O. (2012). RNA modulation of the human DNA methyltransferase 3A. *Nucleic Acids Research*, *40*(17), 8550–8557. https://doi.org/10.1093/nar/gks537

Huckleberry, K. A., Ferguson, L. B., & Drew, M. R. (2016). Behavioral mechanisms of context fear generalization in mice. *Learning & Memory*, *23*(12), 703–709. https://doi.org/10.1101/lm.042374.116

Igaz, L. M., Vianna, M. R. M., Medina, J. H., & Izquierdo, I. (2002). Two Time Periods of Hippocampal mRNA Synthesis Are Required for Memory Consolidation of Fear-Motivated Learning. *The Journal of Neuroscience*, *22*(15), 6781–6789. https://doi.org/10.1523/JNEUROSCI.22-15-06781.2002

Ishimoto, H., & Kamikouchi, A. (2020). A Feedforward Circuit Regulates Action Selection of Pre-mating Courtship Behavior in Female Drosophila. *Current Biology*, *30*(3), 396-407.e4. https://doi.org/10.1016/j.cub.2019.11.065

Ito, S., Shen, L., Dai, Q., Wu, S. C., Collins, L. B., Swenberg, J. A., He, C., & Zhang, Y. (2011). Tet Proteins Can Convert 5-Methylcytosine to 5-Formylcytosine and 5-Carboxylcytosine. *Science*, *333*(6047), 1300–1303. https://doi.org/10.1126/science.1210597

Izquierdo, I., & Medina, J. H. (1997). Memory Formation: The Sequence of Biochemical Events in the Hippocampus and Its Connection to Activity in Other Brain Structures. *Neurobiology of Learning and Memory*, *68*(3), 285–316. https://doi.org/10.1006/nlme.1997.3799

Jaeger, B. N., Linker, S. B., Parylak, S. L., Barron, J. J., Gallina, I. S., Saavedra, C. D., Fitzpatrick, C., Lim, C. K., Schafer, S. T., Lacar, B., Jessberger, S., & Gage, F. H. (2018). A novel environment-evoked transcriptional signature predicts reactivity in single

dentate granule neurons. *Nature Communications*, *9*(1). https://doi.org/10.1038/s41467-018-05418-8

Jiang, J., Peng, Y., He, Z., Wei, L., Jin, W., Wang, X., & Chang, M. (2017). Intrahippocampal injection of Cortistatin-14 impairs recognition memory consolidation in mice through activation of sst 2 , ghrelin and GABA A/B receptors. *Brain Research*, *1666*, 38–47. https://doi.org/10.1016/j.brainres.2017.04.017

Jitsuki-Takahashi, A., Jitsuki, S., Yamashita, N., Kawamura, M., Abe, M., Sakimura, K., Sano, A., Nakamura, F., Goshima, Y., & Takahashi, T. (2021). Activity-induced secretion of semaphorin 3A mediates learning. *European Journal of Neuroscience*, *53*(10). https://doi.org/10.1111/ejn.15210

Jobim, P. F. C., Pedroso, T. R., Christoff, R. R., Werenicz, A., Maurmann, N., Reolon, G. K., & Roesler, R. (2012). Inhibition of mTOR by rapamycin in the amygdala or hippocampus impairs formation and reconsolidation of inhibitory avoidance memory. *Neurobiology of Learning and Memory*, *97*(1), 105–112. https://doi.org/10.1016/j.nlm.2011.10.002

Joo, J.-Y., Schaukowitch, K., Farbiak, L., Kilaru, G., & Kim, T.-K. (2016). Stimulus-specific combinatorial functionality of neuronal c-fos enhancers. *Nature Neuroscience*, *19*(1), 75–83. https://doi.org/10.1038/nn.4170

Josselyn, S. A., Köhler, S., & Frankland, P. W. (2015). Finding the engram. *Nature Reviews Neuroscience*, *16*(9), 521–534. https://doi.org/10.1038/nrn4000

Josselyn, S. A., & Tonegawa, S. (2020). Memory engrams: Recalling the past and imagining the future. *Science*, *367*(6473). https://doi.org/10.1126/science.aaw4325

Jurkowska, R. Z., & Jeltsch, A. (2016). *Enzymology of Mammalian DNA Methyltransferases* (pp. 87–122). https://doi.org/10.1007/978-3-319-43624-1_5

Kandel, E. R. (2001). The Molecular Biology of Memory Storage: A Dialogue Between Genes and Synapses. *Science*, *294*(5544), 1030–1038. https://doi.org/10.1126/science.1067020

Kandel, E. R. (2012). The molecular biology of memory: cAMP, PKA, CRE, CREB-1, CREB-2, and CPEB. *Molecular Brain*, *5*(1), 14. https://doi.org/10.1186/1756-6606-5-14

Kandel, E. R., Dudai, Y., & Mayford, M. R. (2014). The Molecular and Systems Biology of Memory. *Cell*, *157*(1), 163–186. https://doi.org/10.1016/j.cell.2014.03.001

Karaca, K. G., Brito, D. V. C., Zeuch, B., & Oliveira, A. M. M. (2020). Engram reactivation during memory retrieval predicts long-term memory performance in aged mice. In *bioRxiv*. https://doi.org/10.1101/2020.01.12.903088

Karunakaran, S., Chowdhury, A., Donato, F., Quairiaux, C., Michel, C. M., & Caroni, P. (2016). PV plasticity sustained through D1/5 dopamine signaling required for long-term memory consolidation. *Nature Neuroscience*, *19*(3), 454–464. https://doi.org/10.1038/nn.4231

Katche, C., Bekinschtein, P., Slipczuk, L., Goldin, A., Izquierdo, I. A., Cammarota, M., & Medina, J. H. (2010). Delayed wave of c-Fos expression in the dorsal hippocampus involved specifically in persistence of long-term memory storage. *Proceedings of the National Academy of Sciences*, *107*(1), 349–354. https://doi.org/10.1073/pnas.0912931107

Katche, C., Dorman, G., Gonzalez, C., Kramar, C. P., Slipczuk, L., Rossato, J. I., Cammarota, M., & Medina, J. H. (2013). On the role of retrosplenial cortex in long-lasting memory storage. *Hippocampus*, *23*(4). https://doi.org/10.1002/hipo.22092

Kawashima, T., Kitamura, K., Suzuki, K., Nonaka, M., Kamijo, S., Takemoto-Kimura, S., Kano, M., Okuno, H., Ohki, K., & Bito, H. (2013). Functional labeling of neurons and their projections using the synthetic activity–dependent promoter E-SARE. *Nature Methods*, *10*(9), 889–895. https://doi.org/10.1038/nmeth.2559

Kernohan, K. D., Vernimmen, D., Gloor, G. B., & Bérubé, N. G. (2014). Analysis of neonatal brain lacking ATRX or MeCP2 reveals changes in nucleosome density, CTCF binding and chromatin looping. *Nucleic Acids Research*, *42*(13), 8356–8368. https://doi.org/10.1093/nar/gku564

Khoutorsky, A., Yanagiya, A., Gkogkas, C. G., Fabian, M. R., Prager-Khoutorsky, M., Cao, R., Gamache, K., Bouthiette, F., Parsyan, A., Sorge, R. E., Mogil, J. S., Nader, K., Lacaille, J.-C., & Sonenberg, N. (2013). Control of Synaptic Plasticity and Memory via Suppression of Poly(A)-Binding Protein. *Neuron*, *78*(2), 298–311. https://doi.org/10.1016/j.neuron.2013.02.025

Kienhöfer, S., Musheev, M. U., Stapf, U., Helm, M., Schomacher, L., Niehrs, C., & Schäfer, A. (2015). GADD45a physically and functionally interacts with TET1. *Differentiation*, *90*(1–3), 59–68. https://doi.org/10.1016/j.diff.2015.10.003

Kitamura, T., Ogawa, S. K., Roy, D. S., Okuyama, T., Morrissey, M. D., Smith, L. M., Redondo, R. L., & Tonegawa, S. (2017). Engrams and circuits crucial for systems consolidation of a memory. *Science*, *356*(6333). https://doi.org/10.1126/science.aam6808

Kojima, N., Borlikova, G., Sakamoto, T., Yamada, K., Ikeda, T., Itohara, S., Niki, H., & Endo, S. (2008). Inducible cAMP Early Repressor Acts as a Negative Regulator for Kindling Epileptogenesis and Long-Term Fear Memory. *Journal of Neuroscience*, *28*(25), 6459–6472. https://doi.org/10.1523/JNEUROSCI.0412-08.2008

Kol, A., Adamsky, A., Groysman, M., Kreisel, T., London, M., & Goshen, I. (2020). Astrocytes contribute to remote memory formation by modulating hippocampal–cortical communication during learning. *Nature Neuroscience*, *23*(10), 1229–1239. https://doi.org/10.1038/s41593-020-0679-6

Konopka, W., Kiryk, A., Novak, M., Herwerth, M., Parkitna, J. R., Wawrzyniak, M., Kowarsch, A., Michaluk, P., Dzwonek, J., Arnsperger, T., Wilczynski, G., Merkenschlager, M., Theis, F. J., Köhr, G., Kaczmarek, L., & Schütz, G. (2010). MicroRNA Loss Enhances Learning and Memory in Mice. *The Journal of Neuroscience*, *30*(44), 14835–14842. https://doi.org/10.1523/JNEUROSCI.3030-10.2010

Korb, E., Wilkinson, C. L., Delgado, R. N., Lovero, K. L., & Finkbeiner, S. (2013). Arc in the nucleus regulates PML-dependent GluA1 transcription and homeostatic plasticity. *Nature Neuroscience*, *16*(7), 874–883. https://doi.org/10.1038/nn.3429

Korotkevich, G., Sukhov, V., Budin, N., Shpak, B., Artyomov, M. N., & Sergushichev, A. (2021). Fast gene set enrichment analysis. *BioRxiv*, 060012. https://doi.org/10.1101/060012

Koya, E., Uejima, J. L., Wihbey, K. A., Bossert, J. M., Hope, B. T., & Shaham, Y. (2009). Role of ventral medial prefrontal cortex in incubation of cocaine craving. *Neuropharmacology*, *56*, 177–185. https://doi.org/10.1016/j.neuropharm.2008.04.022

Krawczyk, M. C., Navarro, N., Blake, M. G., Romano, A., Feld, M., & Boccia, M. M. (2016). Reconsolidation-induced memory persistence: Participation of late phase hippocampal ERK activation. *Neurobiology of Learning and Memory*, *133*, 79–88. https://doi.org/10.1016/j.nlm.2016.06.013

Kupke, J., Klimmt, J., Mudlaff, F., Schwab, M., Sticht, C., & Oliveira, A. M. M. (2023). Dnmt3a1 regulates hippocampus-dependent memory via the downstream target Nrp1. *BioRxiv*, 2023.05.22.541739. https://doi.org/10.1101/2023.05.22.541739

Lacar, B., Linker, S. B., Jaeger, B. N., Krishnaswami, S. R., Barron, J. J., Kelder, M. J. E., Parylak, S. L., Paquola, A. C. M., Venepally, P., Novotny, M., O'Connor, C., Fitzpatrick, C., Erwin, J. A., Hsu, J. Y., Husband, D., McConnell, M. J., Lasken, R., & Gage, F. H. (2016). Nuclear RNA-seq of single neurons reveals molecular signatures of activation. *Nature Communications*, *7*(1), 11022. https://doi.org/10.1038/ncomms11022

LaPlant, Q., Vialou, V., Covington, H. E., Dumitriu, D., Feng, J., Warren, B. L., Maze, I., Dietz, D. M., Watts, E. L., Iñiguez, S. D., Koo, J. W., Mouzon, E., Renthal, W., Hollis, F., Wang, H., Noonan, M. A., Ren, Y., Eisch, A. J., Bolaños, C. A., … Nestler, E. J. (2010). Dnmt3a regulates emotional behavior and spine plasticity in the nucleus accumbens. *Nature Neuroscience*, *13*(9), 1137–1143. https://doi.org/10.1038/nn.2619

Lashley, K. S. in Society of Experimental Biology Symposium, No. 4: Psychological Mechanisms in Animal Behavior (eds Danielli, J. F. & Brown, R.) 454–482 (Academic Press, 1950).

Lee, J.-H., Kim, W. Bin, Park, E. H., & Cho, J.-H. (2023). Neocortical synaptic engrams for remote contextual memories. *Nature Neuroscience*, *26*(2), 259–273. https://doi.org/10.1038/s41593-022-01223-1

Lesburguères, E., Gobbo, O. L., Alaux-Cantin, S., Hambucken, A., Trifilieff, P., & Bontempi, B. (2011). Early tagging of cortical networks is required for the formation of enduring associative memory. *Science*, *331*(6019). https://doi.org/10.1126/science.1196164

Li, J., Pinto-Duarte, A., Zander, M., Cuoco, M. S., Lai, C.-Y., Osteen, J., Fang, L., Luo, C., Lucero, J. D., Gomez-Castanon, R., Nery, J. R., Silva-Garcia, I., Pang, Y., Sejnowski, T. J., Powell, S. B., Ecker, J. R., Mukamel, E. A., & Behrens, M. M. (2022). Dnmt3a knockout in excitatory neurons impairs postnatal synapse maturation and increases the repressive histone modification H3K27me3. *ELife*, *11*. https://doi.org/10.7554/eLife.66909

Li, Y.-F., Cheng, Y.-F., Huang, Y., Conti, M., Wilson, S. P., O'Donnell, J. M., & Zhang, H.-T. (2011). Phosphodiesterase-4D Knock-Out and RNA Interference-Mediated Knock-Down Enhance Memory and Increase Hippocampal Neurogenesis via Increased cAMP Signaling. *The Journal of Neuroscience*, *31*(1), 172–183. https://doi.org/10.1523/JNEUROSCI.5236-10.2011

Li, Z., Gu, T.-P., Weber, A. R., Shen, J.-Z., Li, B.-Z., Xie, Z.-G., Yin, R., Guo, F., Liu, X., Tang, F., Wang, H., Schär, P., & Xu, G.-L. (2015). Gadd45a promotes DNA demethylation through TDG. *Nucleic Acids Research*, *43*(8), 3986–3997. https://doi.org/10.1093/nar/gkv283

Lin, Y., Bloodgood, B. L., Hauser, J. L., Lapan, A. D., Koon, A. C., Kim, T.-K., Hu, L. S., Malik, A. N., & Greenberg, M. E. (2008). Activity-dependent regulation of inhibitory synapse development by Npas4. *Nature*, *455*(7217), 1198–1204. https://doi.org/10.1038/nature07319

Ling, Y. (2004). Modification of de novo DNA methyltransferase 3a (Dnmt3a) by SUMO-1 modulates its interaction with histone deacetylases (HDACs) and its capacity to repress transcription. *Nucleic Acids Research*, *32*(2), 598–610. https://doi.org/10.1093/nar/gkh195

Lissek, T., Andrianarivelo, A., Saint-Jour, E., Allichon, M., Bauersachs, H. G., Nassar, M., Piette, C., Pruunsild, P., Tan, Y., Forget, B., Heck, N., Caboche, J., Venance, L., Vanhoutte, P., & Bading, H. (2021). Npas4 regulates medium spiny neuron physiology and gates cocaine-induced hyperlocomotion. *EMBO Reports*, *22*(12). https://doi.org/10.15252/embr.202051882

Liu, X., Ramirez, S., Pang, P. T., Puryear, C. B., Govindarajan, A., Deisseroth, K., & Tonegawa, S. (2012). Optogenetic stimulation of a hippocampal engram activates fear memory recall. *Nature*, *484*(7394), 381–385. https://doi.org/10.1038/nature11028

Lu, P. D., Harding, H. P., & Ron, D. (2004). Translation reinitiation at alternative open reading frames regulates gene expression in an integrated stress response. *Journal of Cell Biology*, *167*(1), 27–33. https://doi.org/10.1083/jcb.200408003

Lubin, F. D., Roth, T. L., & Sweatt, J. D. (2008). Epigenetic Regulation of *bdnf* Gene Transcription in the Consolidation of Fear Memory. *The Journal of Neuroscience*, *28*(42), 10576–10586. https://doi.org/10.1523/JNEUROSCI.1786-08.2008

Manzo, M., Wirz, J., Ambrosi, C., Villaseñor, R., Roschitzki, B., & Baubec, T. (2017). Isoform-specific localization of DNMT3A regulates DNA methylation fidelity at bivalent CpG islands. *The EMBO Journal*, *36*(23), 3421–3434. https://doi.org/10.15252/embj.201797038

Marco, A., Meharena, H. S., Dileep, V., Raju, R. M., Davila-Velderrain, J., Zhang, A. L., Adaikkan, C., Young, J. Z., Gao, F., Kellis, M., & Tsai, L. H. (2020). Mapping the epigenomic and transcriptomic interplay during memory formation and recall in the hippocampal engram ensemble. *Nature Neuroscience*, *23*(12). https://doi.org/10.1038/s41593-020-00717-0

Matos, M. R., Visser, E., Kramvis, I., van der Loo, R. J., Gebuis, T., Zalm, R., Rao-Ruiz, P., Mansvelder, H. D., Smit, A. B., & van den Oever, M. C. (2019). Memory strength gates the involvement of a CREB-dependent cortical fear engram in remote memory. *Nature Communications*, *10*(1). https://doi.org/10.1038/s41467-019-10266-1

Matsuo, N. (2015). Irreplaceability of Neuronal Ensembles after Memory Allocation. *Cell Reports*, *11*(3), 351–357. https://doi.org/10.1016/j.celrep.2015.03.042

Medrano-Fernández, A., & Barco, A. (2016). Nuclear organization and 3D chromatin architecture in cognition and neuropsychiatric disorders. *Molecular Brain*, *9*(1), 83. https://doi.org/10.1186/s13041-016-0263-x

Miller, C. A., Gavin, C. F., White, J. A., Parrish, R. R., Honasoge, A., Yancey, C. R., Rivera, I. M., Rubio, M. D., Rumbaugh, G., & Sweatt, J. D. (2010). Cortical DNA methylation maintains remote memory. *Nature Neuroscience*, *13*(6). https://doi.org/10.1038/nn.2560

Miller, C. A., & Sweatt, J. D. (2007). Covalent Modification of DNA Regulates Memory Formation. *Neuron*, *53*(6), 857–869. https://doi.org/10.1016/j.neuron.2007.02.022

Mioduszewska, B., Jaworski, J., & Kaczmarek, L. (2003). Inducible cAMP early repressor (ICER) in the nervous system - a transcriptional regulator of neuronal plasticity and programmed cell death. *Journal of Neurochemistry*, *87*(6), 1313–1320. https://doi.org/10.1046/j.1471-4159.2003.02116.x

Mitchnick, K. A., Creighton, S., O'Hara, M., Kalisch, B. E., & Winters, B. D. (2015). Differential contributions of *de novo* and maintenance DNA methyltransferases to object memory processing in the rat hippocampus and perirhinal cortex - a double dissociation. *European Journal of Neuroscience*, *41*(6), 773–786. https://doi.org/10.1111/ejn.12819

Mitsushima, D., Ishihara, K., Sano, A., Kessels, H. W., & Takahashi, T. (2011). Contextual learning requires synaptic AMPA receptor delivery in the hippocampus. *Proceedings of the National Academy of Sciences*, *108*(30), 12503–12508. https://doi.org/10.1073/pnas.1104558108

Moga, D. E., Calhoun, M. E., Chowdhury, A., Worley, P., Morrison, J. H., & Shapiro, M. L. (2004). Activity-regulated cytoskeletal-associated protein is localized to recently activated

excitatory synapses. *Neuroscience*, *125*(1), 7–11. https://doi.org/10.1016/j.neuroscience.2004.02.004

Morimura, N., Yasuda, H., Yamaguchi, K., Katayama, K., Hatayama, M., Tomioka, N. H., Odagawa, M., Kamiya, A., Iwayama, Y., Maekawa, M., Nakamura, K., Matsuzaki, H., Tsujii, M., Yamada, K., Yoshikawa, T., & Aruga, J. (2017). Autism-like behaviours and enhanced memory formation and synaptic plasticity in Lrfn2/SALM1-deficient mice. *Nature Communications*, *8*(1), 15800. https://doi.org/10.1038/ncomms15800

Morris, M. J., Adachi, M., Na, E. S., & Monteggia, L. M. (2014). Selective role for DNMT3a in learning and memory. *Neurobiology of Learning and Memory*, *115*, 30–37. https://doi.org/10.1016/j.nlm.2014.06.005

Morrison, D. J., Rashid, A. J., Yiu, A. P., Yan, C., Frankland, P. W., & Josselyn, S. A. (2016). Parvalbumin interneurons constrain the size of the lateral amygdala engram. *Neurobiology of Learning and Memory*, *135*, 91–99. https://doi.org/10.1016/j.nlm.2016.07.007

Mukherjee, D., Ignatowska-Jankowska, B. M., Itskovits, E., Gonzales, B. J., Turm, H., Izakson, L., Haritan, D., Bleistein, N., Cohen, C., Amit, I., Shay, T., Grueter, B., Zaslaver, A., & Citri, A. (2018a). Salient experiences are represented by unique transcriptional signatures in the mouse brain. *ELife*, *7*. https://doi.org/10.7554/eLife.31220

Mukherjee, D., Ignatowska-Jankowska, B. M., Itskovits, E., Gonzales, B. J., Turm, H., Izakson, L., Haritan, D., Bleistein, N., Cohen, C., Amit, I., Shay, T., Grueter, B., Zaslaver, A., & Citri, A. (2018b). Salient experiences are represented by unique transcriptional signatures in the mouse brain. *ELife*, *7*. https://doi.org/10.7554/eLife.31220

Müller, G.E. and Pilzecker, A. (1900) Experimentelle Beiträge zur Lehre vom Gedächnis. Zeitschrift für Psychologie. Ergänzungsband. 1900, 1:1-300.

Nakayama, D., Iwata, H., Teshirogi, C., Ikegaya, Y., Matsuki, N., & Nomura, H. (2015). Long-Delayed Expression of the Immediate Early Gene Arc/Arg3.1 Refines Neuronal Circuits to Perpetuate Fear Memory. *The Journal of Neuroscience*, *35*(2), 819–830. https://doi.org/10.1523/JNEUROSCI.2525-14.2015

Nestler, E. J., Peña, C. J., Kundakovic, M., Mitchell, A., & Akbarian, S. (2016). Epigenetic Basis of Mental Illness. *The Neuroscientist*, *22*(5), 447–463. https://doi.org/10.1177/1073858415608147

Noyes, N. C., Phan, A., & Davis, R. L. (2021). Memory suppressor genes: Modulating acquisition, consolidation, and forgetting. *Neuron*, *109*(20), 3211–3227. https://doi.org/10.1016/j.neuron.2021.08.001

Okuno, H. (2011). Regulation and function of immediate-early genes in the brain: Beyond neuronal activity markers. *Neuroscience Research*, *69*(3), 175–186. https://doi.org/10.1016/j.neures.2010.12.007

Oliveira, A. M., Litke, C., Paldy, E., Hagenston, A. M., Lu, J., Kuner, R., Bading, H., & Mauceri, D. (2019). Epigenetic control of hypersensitivity in chronic inflammatory pain by the de novo DNA methyltransferase Dnmt3a2. *Molecular Pain*, *15*, 174480691982746. https://doi.org/10.1177/1744806919827469

Oliveira, A. M. M. (2016). DNA methylation: a permissive mark in memory formation and maintenance. *Learning & Memory*, *23*(10), 587–593. https://doi.org/10.1101/lm.042739.116

Oliveira, A. M. M., Hemstedt, T. J., & Bading, H. (2012). Rescue of aging-associated decline in Dnmt3a2 expression restores cognitive abilities. *Nature Neuroscience*, *15*(8). https://doi.org/10.1038/nn.3151

Oliveira, A. M. M., Hemstedt, T. J., Freitag, H. E., & Bading, H. (2016). Dnmt3a2: A hub for enhancing cognitive functions. *Molecular Psychiatry*, *21*(8). https://doi.org/10.1038/mp.2015.175

Ooi, S. K. T., Qiu, C., Bernstein, E., Li, K., Jia, D., Yang, Z., Erdjument-Bromage, H., Tempst, P., Lin, S.-P., Allis, C. D., Cheng, X., & Bestor, T. H. (2007). DNMT3L connects unmethylated lysine 4 of histone H3 to de novo methylation of DNA. *Nature*, *448*(7154), 714–717. https://doi.org/10.1038/nature05987

Pastuzyn, E. D., Day, C. E., Kearns, R. B., Kyrke-Smith, M., Taibi, A. V., McCormick, J., Yoder, N., Belnap, D. M., Erlendsson, S., Morado, D. R., Briggs, J. A. G., Feschotte, C., & Shepherd, J. D. (2018). The Neuronal Gene Arc Encodes a Repurposed Retrotransposon Gag Protein that Mediates Intercellular RNA Transfer. *Cell*, *172*(1–2), 275-288.e18. https://doi.org/10.1016/j.cell.2017.12.024

Paylor, R., Johnson, R. S., Papaioannou, V., Spiegelman, B. M., & Wehner, J. M. (1994). Behavioral assessment of c-fos mutant mice. *Brain Research*, *651*(1–2), 275–282. https://doi.org/10.1016/0006-8993(94)90707-2

Penfield, W., and Rasmussen, T. (1950). The cerebral cortex of man: a clinical study of localization of function (New York: Macmillan).

Pekowska, A., Benoukraf, T., Zacarias-Cabeza, J., Belhocine, M., Koch, F., Holota, H., Imbert, J., Andrau, J.-C., Ferrier, P., & Spicuglia, S. (2011). H3K4 tri-methylation provides an epigenetic signature of active enhancers. *The EMBO Journal*, *30*(20), 4198–4210. https://doi.org/10.1038/emboj.2011.295

Pettit, N. L., Yap, E.-L., Greenberg, M. E., & Harvey, C. D. (2022). Fos ensembles encode and shape stable spatial maps in the hippocampus. *Nature*, *609*(7926), 327–334. https://doi.org/10.1038/s41586-022-05113-1

Ploski, J. E., Monsey, M. S., Nguyen, T., DiLeone, R. J., & Schafe, G. E. (2011). The Neuronal PAS Domain Protein 4 (Npas4) Is Required for New and Reactivated Fear Memories. *PLoS ONE*, *6*(8), e23760. https://doi.org/10.1371/journal.pone.0023760

Pohodich, A. E., & Zoghbi, H. Y. (2015). Rett syndrome: disruption of epigenetic control of postnatal neurological functions. *Human Molecular Genetics*, *24*(R1), R10–R16. https://doi.org/10.1093/hmg/ddv217

Pollina, E. A., Gilliam, D. T., Landau, A. T., Lin, C., Pajarillo, N., Davis, C. P., Harmin, D. A., Yap, E.-L., Vogel, I. R., Griffith, E. C., Nagy, M. A., Ling, E., Duffy, E. E., Sabatini, B. L., Weitz, C. J., & Greenberg, M. E. (2023). A NPAS4–NuA4 complex couples synaptic activity to DNA repair. *Nature*, *614*(7949), 732–741. https://doi.org/10.1038/s41586-023-05711-7

Ramamoorthi, K., Fropf, R., Belfort, G. M., Fitzmaurice, H. L., McKinney, R. M., Neve, R. L., Otto, T., & Lin, Y. (2011). Npas4 Regulates a Transcriptional Program in CA3 Required for Contextual Memory Formation. *Science*, *334*(6063), 1669–1675. https://doi.org/10.1126/science.1208049

Ramirez, S., Liu, X., Lin, P.-A., Suh, J., Pignatelli, M., Redondo, R. L., Ryan, T. J., & Tonegawa, S. (2013). Creating a False Memory in the Hippocampus. *Science*, *341*(6144), 387–391. https://doi.org/10.1126/science.1239073

Ramírez-Amaya, V., Vazdarjanova, A., Mikhael, D., Rosi, S., Worley, P. F., & Barnes, C. A. (2005). Spatial Exploration-Induced *Arc* mRNA and Protein Expression: Evidence for Selective, Network-Specific Reactivation. *The Journal of Neuroscience*, *25*(7), 1761–1768. https://doi.org/10.1523/JNEUROSCI.4342-04.2005

Ramon y Cajal, S. The Croonian lecture: la fine structure des centre nerveux. Proc. R. Soc. Lond. 55, 444–468 (in French) (1894).

Rao-Ruiz, P., Couey, J. J., Marcelo, I. M., Bouwkamp, C. G., Slump, D. E., Matos, M. R., van der Loo, R. J., Martins, G. J., van den Hout, M., van IJcken, W. F., Costa, R. M., van den Oever, M. C., & Kushner, S. A. (2019). Engram-specific transcriptome profiling of contextual memory consolidation. *Nature Communications*, *10*(1). https://doi.org/10.1038/s41467-019-09960-x

Rasmussen, K. D., & Helin, K. (2016). Role of TET enzymes in DNA methylation, development, and cancer. *Genes & Development*, *30*(7), 733–750. https://doi.org/10.1101/gad.276568.115

Redondo, R. L., Kim, J., Arons, A. L., Ramirez, S., Liu, X., & Tonegawa, S. (2014). Bidirectional switch of the valence associated with a hippocampal contextual memory engram. *Nature*, *513*(7518), 426–430. https://doi.org/10.1038/nature13725

Reijmers, L. G., Perkins, B. L., Matsuo, N., & Mayford, M. (2007). Localization of a stable neural correlate of associative memory. *Science*, *317*(5842). https://doi.org/10.1126/science.1143839

Ribeiro, S., Mello, C. V., Velho, T., Gardner, T. J., Jarvis, E. D., & Pavlides, C. (2002). Induction of Hippocampal Long-Term Potentiation during Waking Leads to Increased Extrahippocampal *zif-268* Expression during Ensuing Rapid-Eye-Movement Sleep. *The Journal of Neuroscience*, *22*(24), 10914–10923. https://doi.org/10.1523/JNEUROSCI.22-24-10914.2002

Richards, B. A., & Frankland, P. W. (2017). The Persistence and Transience of Memory. *Neuron*, *94*(6), 1071–1084. https://doi.org/10.1016/j.neuron.2017.04.037

Riedel, G., Micheau, J., Lam, A. G. M., Roloff, E. v. L., Martin, S. J., Bridge, H., Hoz, L. de, Poeschel, B., McCulloch, J., & Morris, R. G. M. (1999). Reversible neural inactivation reveals hippocampal participation in several memory processes. *Nature Neuroscience*, *2*(10), 898–905. https://doi.org/10.1038/13202

Ritchie, M. E., Phipson, B., Wu, D., Hu, Y., Law, C. W., Shi, W., & Smyth, G. K. (2015). Limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Research*, *43*(7). https://doi.org/10.1093/nar/gkv007

Rossato, J. I., Bevilaqua, L. R. M., Izquierdo, I., Medina, J. H., & Cammarota, M. (2009). Dopamine Controls Persistence of Long-Term Memory Storage. *Science*, *325*(5943), 1017–1020. https://doi.org/10.1126/science.1172545

Roth, E. D., Roth, T. L., Money, K. M., SenGupta, S., Eason, D. E., & Sweatt, J. D. (2015). DNA methylation regulates neurophysiological spatial representation in memory formation. *Neuroepigenetics*, *2*, 1–8. https://doi.org/10.1016/j.nepig.2015.03.001

Roy, D. S., Arons, A., Mitchell, T. I., Pignatelli, M., Ryan, T. J., & Tonegawa, S. (2016). Memory retrieval by activating engram cells in mouse models of early Alzheimer's disease. *Nature*, *531*(7595). https://doi.org/10.1038/nature17172

RUTTEN, K., PRICKAERTS, J., & BLOKLAND, A. (2006). Rolipram reverses scopolamine-induced and time-dependent memory deficits in object recognition by different mechanisms of action. *Neurobiology of Learning and Memory*, *85*(2), 132–138. https://doi.org/10.1016/j.nlm.2005.09.002

Ryan, T. J., Roy, D. S., Pignatelli, M., Arons, A., & Tonegawa, S. (2015). Engram cells retain memory under retrograde amnesia. *Science*, *348*(6238). https://doi.org/10.1126/science.aaa5542

Sachser, R. M., Santana, F., Crestani, A. P., Lunardi, P., Pedraza, L. K., Quillfeldt, J. A., Hardt, O., & de Oliveira Alvares, L. (2016). Forgetting of long-term memory requires activation of NMDA receptors, L-type voltage-dependent Ca2+ channels, and calcineurin. *Scientific Reports*, *6*(1), 22771. https://doi.org/10.1038/srep22771

Sahay, A., Kim, C.-H., Sepkuty, J. P., Cho, E., Huganir, R. L., Ginty, D. D., & Kolodkin, A. L. (2005). Secreted Semaphorins Modulate Synaptic Transmission in the Adult Hippocampus. *The Journal of Neuroscience*, *25*(14), 3613–3620. https://doi.org/10.1523/JNEUROSCI.5255-04.2005

Sandweiss, A. J., Brandt, V. L., & Zoghbi, H. Y. (2020). Advances in understanding of Rett syndrome and MECP2 duplication syndrome: prospects for future therapies. *The Lancet Neurology*, *19*(8), 689–698. https://doi.org/10.1016/S1474-4422(20)30217-9

Savell, K. E., Gallus, N. V. N., Simon, R. C., Brown, J. A., Revanna, J. S., Osborn, M. K., Song, E. Y., O'Malley, J. J., Stackhouse, C. T., Norvil, A., Gowher, H., Sweatt, J. D., & Day, J. J. (2016). Extra-coding RNAs regulate neuronal DNA methylation dynamics. *Nature Communications*, *7*(1), 12091. https://doi.org/10.1038/ncomms12091

Scheunemann, L., Plaçais, P.-Y., Dromard, Y., Schwärzel, M., & Preat, T. (2018). Dunce Phosphodiesterase Acts as a Checkpoint for Drosophila Long-Term Memory in a Pair of Serotonergic Neurons. *Neuron*, *98*(2), 350-365.e5. https://doi.org/10.1016/j.neuron.2018.03.032

Schwindel, C. D., & McNaughton, B. L. (2011). *Hippocampal–cortical interactions and the dynamics of memory trace reactivation* (pp. 163–177). https://doi.org/10.1016/B978-0-444-53839-0.00011-9

Scoville, W. B., & Milner, B. (2000). Loss of recent memory after bilateral hippocampal lesions. 1957. *The Journal of Neuropsychiatry and Clinical Neurosciences*, *12*(1). https://doi.org/10.1176/jnp.12.1.103-a

Semon, R. (1923). Mnemic Philosophy (Allen & Unwin).

Shepherd, J. D., Rumbaugh, G., Wu, J., Chowdhury, S., Plath, N., Kuhl, D., Huganir, R. L., & Worley, P. F. (2006). Arc/Arg3.1 Mediates Homeostatic Synaptic Scaling of AMPA Receptors. *Neuron*, *52*(3), 475–484. https://doi.org/10.1016/j.neuron.2006.08.034

Shimizu, E., Tang, Y.-P., Rampon, C., & Tsien, J. Z. (2000). NMDA Receptor-Dependent Synaptic Reinforcement as a Crucial Process for Memory Consolidation. *Science*, *290*(5494), 1170–1174. https://doi.org/10.1126/science.290.5494.1170

Shinohara, K., & Hata, T. (2014). Post-acquisition hippocampal NMDA receptor blockade sustains retention of spatial reference memory in Morris water maze. *Behavioural Brain Research*, *259*, 261–267. https://doi.org/10.1016/j.bbr.2013.11.016

Siapas, A. G., & Wilson, M. A. (1998). Coordinated Interactions between Hippocampal Ripples and Cortical Spindles during Slow-Wave Sleep. *Neuron*, *21*(5), 1123–1128. https://doi.org/10.1016/S0896-6273(00)80629-7

Simonetti, M., Paldy, E., Njoo, C., Bali, K. K., Worzfeld, T., Pitzer, C., Kuner, T., Offermanns, S., Mauceri, D., & Kuner, R. (2021). The impact of Semaphorin 4C/Plexin-B2 signaling on fear memory via remodeling of neuronal and synaptic morphology. *Molecular Psychiatry*, *26*(4). https://doi.org/10.1038/s41380-019-0491-4

Sørensen, A. T., Cooper, Y. A., Baratta, M. V, Weng, F. J., Zhang, Y., Ramamoorthi, K., Fropf, R., Laverriere, E., Xue, J., Young, A., Schneider, C., Gøtzsche, C. R., Hemberg, M., Yin, J. C. P., Maier, S. F., & Lin, Y. (2016). A robust activity marking system for exploring active neuronal ensembles. *ELife*, *5*(September). https://doi.org/10.7554/eLife.13918

Spiegel, I., Mardinly, A. R., Gabel, H. W., Bazinet, J. E., Couch, C. H., Tzeng, C. P., Harmin, D. A., & Greenberg, M. E. (2014). Npas4 Regulates Excitatory-Inhibitory Balance within Neural Circuits through Cell-Type-Specific Gene Programs. *Cell*, *157*(5), 1216–1229. https://doi.org/10.1016/j.cell.2014.03.058

Squire, L. R. (2004). Memory systems of the brain: A brief history and current perspective. *Neurobiology of Learning and Memory*, *82*(3), 171–177. https://doi.org/10.1016/j.nlm.2004.06.005

Squire, L. R., & Wixted, J. T. (2011). The Cognitive Neuroscience of Human Memory Since H.M. *Annual Review of Neuroscience*, *34*(1), 259–288. https://doi.org/10.1146/annurev-neuro-061010-113720

Squire, L. R., & Zola-Morgan, S. (1991). The Medial Temporal Lobe Memory System. *Science*, *253*(5026), 1380–1386. https://doi.org/10.1126/science.1896849

Stefanelli, G., Azam, A. B., Walters, B. J., Brimble, M. A., Gettens, C. P., Bouchard-Cannon, P., Cheng, H.-Y. M., Davidoff, A. M., Narkaj, K., Day, J. J., Kennedy, A. J., & Zovkic, I. B. (2018). Learning and Age-Related Changes in Genome-wide H2A.Z Binding in the Mouse Hippocampus. *Cell Reports*, *22*(5), 1124–1131. https://doi.org/10.1016/j.celrep.2018.01.020

Stegmeier, F., Hu, G., Rickles, R. J., Hannon, G. J., & Elledge, S. J. (2005). A lentiviral microRNA-based system for single-copy polymerase II-regulated RNA interference in mammalian cells. *Proceedings of the National Academy of Sciences of the United States of America*, *102*(37). https://doi.org/10.1073/pnas.0506306102

Su, Y., Shin, J., Zhong, C., Wang, S., Roychowdhury, P., Lim, J., Kim, D., Ming, G., & Song, H. (2017). Neuronal activity modifies the chromatin accessibility landscape in the adult brain. *Nature Neuroscience*, *20*(3), 476–483. https://doi.org/10.1038/nn.4494

Suetake, I., Mishima, Y., Kimura, H., Lee, Y.-H., Goto, Y., Takeshima, H., Ikegami, T., & Tajima, S. (2011). Characterization of DNA-binding activity in the N-terminal domain of the DNA methyltransferase Dnmt3a. *Biochemical Journal*, *437*(1), 141–148. https://doi.org/10.1042/BJ20110241

Sun, X., Bernstein, M. J., Meng, M., Rao, S., Sørensen, A. T., Yao, L., Zhang, X., Anikeeva, P. O., & Lin, Y. (2020). Functionally Distinct Neuronal Ensembles within the Memory Engram. *Cell*, *181*(2), 410-423.e17. https://doi.org/10.1016/j.cell.2020.02.055

Sun, X., & Lin, Y. (2016). Npas4: Linking Neuronal Activity to Memory. *Trends in Neurosciences*, *39*(4), 264–275. https://doi.org/10.1016/j.tins.2016.02.003

Suzuki, M. M., & Bird, A. (2008). DNA methylation landscapes: provocative insights from epigenomics. *Nature Reviews Genetics*, *9*(6), 465–476. https://doi.org/10.1038/nrg2341

Sweatt, J. D. (2009). Experience-Dependent Epigenetic Modifications in the Central Nervous System. In *Biological Psychiatry* (Vol. 65, Issue 3). https://doi.org/10.1016/j.biopsych.2008.09.002

Tanaka, K. Z., Pevzner, A., Hamidi, A. B., Nakazawa, Y., Graham, J., & Wiltgen, B. J. (2014). Cortical Representations Are Reinstated by the Hippocampus during Memory Retrieval. *Neuron*, *84*(2), 347–354. https://doi.org/10.1016/j.neuron.2014.09.037

Tayler, K. K., Tanaka, K. Z., Reijmers, L. G., & Wiltgen, B. J. (2013). Reactivation of neural ensembles during the retrieval of recent and remote memory. *Current Biology*, *23*(2). https://doi.org/10.1016/j.cub.2012.11.019

Tonegawa, S., Morrissey, M. D., & Kitamura, T. (2018). The role of engram cells in the systems consolidation of memory. *Nature Reviews Neuroscience*, *19*(8), 485–498. https://doi.org/10.1038/s41583-018-0031-2

Tononi, G., & Cirelli, C. (2014). Sleep and the Price of Plasticity: From Synaptic and Cellular Homeostasis to Memory Consolidation and Integration. *Neuron*, *81*(1), 12–34. https://doi.org/10.1016/j.neuron.2013.12.025

TRIFILIEFF, P., CALANDREAU, L., HERRY, C., MONS, N., & MICHEAU, J. (2007). Biphasic ERK1/2 activation in both the hippocampus and amygdala may reveal a system consolidation of contextual fear memory. *Neurobiology of Learning and Memory*, *88*(4), 424–434. https://doi.org/10.1016/j.nlm.2007.05.004

Trifilieff, P., Herry, C., Vanhoutte, P., Caboche, J., Desmedt, A., Riedel, G., Mons, N., & Micheau, J. (2006). Foreground contextual fear memory consolidation requires two independent phases of hippocampal ERK/CREB activation. *Learning & Memory*, *13*(3), 349–358. https://doi.org/10.1101/lm.80206

Tyssowski, K. M., DeStefino, N. R., Cho, J.-H., Dunn, C. J., Poston, R. G., Carty, C. E., Jones, R. D., Chang, S. M., Romeo, P., Wurzelmann, M. K., Ward, J. M., Andermann, M. L., Saha, R. N., Dudek, S. M., & Gray, J. M. (2018). Different Neuronal Activity Patterns Induce Different Gene Expression Programs. *Neuron*, *98*(3), 530-546.e11. https://doi.org/10.1016/j.neuron.2018.04.001

Vann, S. D., Brown, M. W., Erichsen, J. T., & Aggleton, J. P. (2000). Fos Imaging Reveals Differential Patterns of Hippocampal and Parahippocampal Subfield Activation in Rats in Response to Different Spatial Memory Tests. *The Journal of Neuroscience*, *20*(7), 2711–2718. https://doi.org/10.1523/JNEUROSCI.20-07-02711.2000

Vattem, K. M., & Wek, R. C. (2004). Reinitiation involving upstream ORFs regulates *ATF4* mRNA translation in mammalian cells. *Proceedings of the National Academy of Sciences*, *101*(31), 11269–11274. https://doi.org/10.1073/pnas.0400541101

Venkitaramani, D. V., Moura, P. J., Picciotto, M. R., & Lombroso, P. J. (2011). Striatal-enriched protein tyrosine phosphatase (STEP) knockout mice have enhanced hippocampal memory. *European Journal of Neuroscience*, *33*(12), 2288–2298. https://doi.org/10.1111/j.1460-9568.2011.07687.x

Villarreal, D. M., Do, V., Haddad, E., & Derrick, B. E. (2002). NMDA receptor antagonists sustain LTP and spatial memory: active processes mediate LTP decay. *Nature Neuroscience*, *5*(1), 48–52. https://doi.org/10.1038/nn776

Wall, M. J., & Corrêa, S. A. L. (2018). The mechanistic link between Arc/Arg3.1 expression and AMPA receptor endocytosis. *Seminars in Cell & Developmental Biology*, *77*, 17–24. https://doi.org/10.1016/j.semcdb.2017.09.005

Wang, Q., Chiu, S.-L., Koropouli, E., Hong, I., Mitchell, S., Easwaran, T. P., Hamilton, N. R., Gustina, A. S., Zhu, Q., Ginty, D. D., Huganir, R. L., & Kolodkin, A. L. (2017). Neuropilin-2/PlexinA3 Receptors Associate with GluA1 and Mediate Sema3F-Dependent Homeostatic Scaling in Cortical Neurons. *Neuron*, *96*(5), 1084-1098.e7. https://doi.org/10.1016/j.neuron.2017.10.029

Watson, L. A., & Tsai, L.-H. (2017). In the loop: how chromatin topology links genome structure to function in mechanisms underlying learning and memory. *Current Opinion in Neurobiology*, *43*, 48–55. https://doi.org/10.1016/j.conb.2016.12.002

Weng, F.-J., Garcia, R. I., Lutzu, S., Alviña, K., Zhang, Y., Dushko, M., Ku, T., Zemoura, K., Rich, D., Garcia-Dominguez, D., Hung, M., Yelhekar, T. D., Sørensen, A. T., Xu, W., Chung, K., Castillo, P. E., & Lin, Y. (2018). Npas4 Is a Critical Regulator of Learning-Induced Plasticity at Mossy Fiber-CA3 Synapses during Contextual Memory Formation. *Neuron*, *97*(5), 1137-1152.e5. https://doi.org/10.1016/j.neuron.2018.01.026

West, A. E., Chen, W. G., Dalva, M. B., Dolmetsch, R. E., Kornhauser, J. M., Shaywitz, A. J., Takasu, M. A., Tao, X., & Greenberg, M. E. (2001). Calcium regulation of neuronal gene expression. *Proceedings of the National Academy of Sciences*, *98*(20), 11024–11031. https://doi.org/10.1073/pnas.191352298

Wiltgen, B. J., Brown, R. A. M., Talton, L. E., & Silva, A. J. (2004). New circuits for old memories: The role of the neocortex in consolidation. In *Neuron* (Vol. 44, Issue 1). https://doi.org/10.1016/j.neuron.2004.09.015

Wiltgen, B. J., & Tanaka, K. Z. (2013). Systems consolidation and the content of memory. *Neurobiology of Learning and Memory*, *106*, 365–371. https://doi.org/10.1016/j.nlm.2013.06.001

Wiltgen, B. J., Zhou, M., Cai, Y., Balaji, J., Karlsson, M. G., Parivash, S. N., Li, W., & Silva, A. J. (2010). The Hippocampus Plays a Selective Role in the Retrieval of Detailed Contextual Memories. *Current Biology*, *20*(15), 1336–1344. https://doi.org/10.1016/j.cub.2010.06.068

Wimmer, M. E., Blackwell, J. M., & Abel, T. (2020). Rolipram treatment during consolidation ameliorates long-term object location memory in aged male mice. *Neurobiology of Learning and Memory*, *169*, 107168. https://doi.org/10.1016/j.nlm.2020.107168

Wu, H., Coskun, V., Tao, J., Xie, W., Ge, W., Yoshikawa, K., Li, E., Zhang, Y., & Sun, Y. E. (2010). Dnmt3a-Dependent Nonpromoter DNA Methylation Facilitates Transcription of Neurogenic Genes. *Science*, *329*(5990), 444–448. https://doi.org/10.1126/science.1190485

Xia, F., Richards, B. A., Tran, M. M., Josselyn, S. A., Takehara-Nishiuchi, K., & Frankland, P. W. (2017). Parvalbumin-positive interneurons mediate neocortical-hippocampal interactions that are necessary for memory consolidation. *ELife*, *6*. https://doi.org/10.7554/eLife.27868

Xie, R., Wang, Z., Liu, T., Xiao, R., Lv, K., Wu, C., Luo, Y., Cai, Y., & Fan, X. (2021). AAV Delivery of shRNA Against TRPC6 in Mouse Hippocampus Impairs Cognitive Function. *Frontiers in Cell and Developmental Biology*, *9*. https://doi.org/10.3389/fcell.2021.688655

Xu, P., Berto, S., Kulkarni, A., Jeong, B., Joseph, C., Cox, K. H., Greenberg, M. E., Kim, T.-K., Konopka, G., & Takahashi, J. S. (2021). NPAS4 regulates the transcriptional response of the suprachiasmatic nucleus to light and circadian behavior. *Neuron*, *109*(20), 3268-3282.e6. https://doi.org/10.1016/j.neuron.2021.07.026

Xu, R., Janson, C. G., Mastakov, M., Lawlor, P., Young, D., Mouravlev, A., Fitzsimons, H., Choi, K. L., Ma, H., Dragunow, M., Leone, P., Chen, Q., Dicker, B., & During, M. J. (2001). Quantitative comparison of expression with adeno-associated virus (AAV-2) brain-specific gene cassettes. *Gene Therapy*, *8*(17). https://doi.org/10.1038/sj.gt.3301529

Yamada, T., Yang, Y., Valnegri, P., Juric, I., Abnousi, A., Markwalter, K. H., Guthrie, A. N., Godec, A., Oldenborg, A., Hu, M., Holy, T. E., & Bonni, A. (2019). Sensory experience remodels genome architecture in neural circuit to drive motor learning. *Nature*, *569*(7758), 708–713. https://doi.org/10.1038/s41586-019-1190-7

Yamagata, N., Ezaki, T., Takahashi, T., Wu, H., & Tanimoto, H. (2021). Presynaptic inhibition of dopamine neurons controls optimistic bias. *ELife*, *10*. https://doi.org/10.7554/eLife.64907

Yap, E. L., & Greenberg, M. E. (2018). Activity-Regulated Transcription: Bridging the Gap between Neural Activity and Behavior. In *Neuron* (Vol. 100, Issue 2). https://doi.org/10.1016/j.neuron.2018.10.013

Yap, E.-L., Pettit, N. L., Davis, C. P., Nagy, M. A., Harmin, D. A., Golden, E., Dagliyan, O., Lin, C., Rudolph, S., Sharma, N., Griffith, E. C., Harvey, C. D., & Greenberg, M. E. (2021). Bidirectional perisomatic inhibitory plasticity of a Fos neuronal network. *Nature*, *590*(7844), 115–121. https://doi.org/10.1038/s41586-020-3031-0

Yin, J. C. P., Wallach, J. S., Del Vecchio, M., Wilder, E. L., Zhou, H., Quinn, W. G., & Tully, T. (1994). Induction of a dominant negative CREB transgene specifically blocks long-term memory in Drosophila. *Cell*, *79*(1), 49–58. https://doi.org/10.1016/0092-8674(94)90399-9

Yiu, A. P., Mercaldo, V., Yan, C., Richards, B., Rashid, A. J., Hsiang, H.-L. L., Pressey, J., Mahadevan, V., Tran, M. M., Kushner, S. A., Woodin, M. A., Frankland, P. W., & Josselyn, S. A. (2014). Neurons Are Recruited to a Memory Trace Based on Relative Neuronal Excitability Immediately before Training. *Neuron*, *83*(3), 722–735. https://doi.org/10.1016/j.neuron.2014.07.017

Zhang, Z., Ferretti, V., Güntan, I., Moro, A., Steinberg, E. A., Ye, Z., Zecharia, A. Y., Yu, X., Vyssotski, A. L., Brickley, S. G., Yustos, R., Pillidge, Z. E., Harding, E. C., Wisden, W., & Franks, N. P. (2015). Neuronal ensembles sufficient for recovery sleep and the sedative actions of α 2 adrenergic agonists. *Nature Neuroscience*, *18*(4). https://doi.org/10.1038/nn.3957

Zhu, P. J., Huang, W., Kalikulov, D., Yoo, J. W., Placzek, A. N., Stoica, L., Zhou, H., Bell, J. C., Friedlander, M. J., Krnjević, K., Noebels, J. L., & Costa-Mattioli, M. (2011). Suppression of PKR Promotes Network Excitability and Enhanced Cognition by Interferon-γ-Mediated Disinhibition. *Cell*, *147*(6), 1384–1396. https://doi.org/10.1016/j.cell.2011.11.029

Zocher, S., Overall, R. W., Berdugo-Vega, G., Rund, N., Karasinsky, A., Adusumilli, V. S., Steinhauer, C., Scheibenstock, S., Händler, K., Schultze, J. L., Calegari, F., & Kempermann, G. (2021). *De novo* DNA methylation controls neuronal maturation during adult hippocampal neurogenesis. *The EMBO Journal*, *40*(18). https://doi.org/10.15252/embj.2020107100

Zovkic, I. B., Guzman-Karlsson, M. C., & Sweatt, J. D. (2013). Epigenetic regulation of memory formation and maintenance. *Learning & Memory*, *20*(2), 61–74. https://doi.org/10.1101/lm.026575.112

Zovkic, I. B., Paulukaitis, B. S., Day, J. J., Etikala, D. M., & Sweatt, J. D. (2014). Histone H2A.Z subunit exchange controls consolidation of recent and remote memory. *Nature*, *515*(7528), 582–586. https://doi.org/10.1038/nature13707

Urlaub fürs Gehirn

K.I.Z.