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Epigenetic memory aids: Synaptic and molecular effects of HDAC inhibition that support memory formation

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Abstract

Learning and memory rely on synaptic communication in which intracellular signals are transported to the nucleus to stimulate transcriptional activation. Memory induced transcriptional increases are accompanied by alterations to the epigenetic landscape and can be pharmacologically mimicked to ameliorate memory in both healthy and cognitively impaired animals. Of particular interest in this regard is histone acetylation, as this epigenetic modification is enriched during memory formation and is readily amenable to pharmacological manipulation by means of histone deacetylase inhibitors (HDACis). Although multiple studies have shown that systemic HDACi administration can improve memory formation, their mode of action is not yet fully understood.

In this study, we tested whether HDACis – given systemically – augment epigenetic, transcriptional and electrophysiological responses that are induced by learning, thus amplifying naturally occurring responses via cognitive epigenetic priming. To do this, we combined a system-wide HDACi treatment with a subthreshold contextual fear conditioning (CFC) paradigm, a weakened Pavlovian modal that, alone, does not lead to long term memory formation.

We found that, in combination, HDACi and CFC improve recent contextual memory that is accompanied by enhanced long-term potentiation in the hippocampus. Conversely, combined HDACi-CFC treatment induced no such synaptic strengthening in the striatum, a brain region that is not directly activated by fear conditioning. We then used bulk and single nuclear RNA-sequencing to show that HDACi activates unique transcriptional pathways, both between the two brain regions and between cell types within the hippocampus, indicating that HDACi does not act indiscriminatingly, but instead supports cellular processes that are already occurring in response to the conditioning. Finally, we show that HDACi treatment paired with contextual fear conditioning enriches H3K27ac at enhancers of known memory-related genes within the hippocampus but that not all these genes are necessarily more transcriptionally active at the same time point.

These results indicate that systemic HDACi administration acts as a cognitive enhancer by amplifying brain-region specific processes that are naturally induced during memory formation but that these results may not be a direct effect of enhancer histone acetylation. These findings

shed light on the mechanisms of HDACi-mediated cognitive epigenetic priming and help to pave the way for potential new therapies for memory-related disorders.

Keywords: Memory, contextual fear conditioning, hippocampus, epigenetics, HDACi, long-term potentiation, next-generation sequencing

Résumé

L'apprentissage et la mémoire reposent sur la communication synaptique, au cours de laquelle des signaux intracellulaires sont transportés dans le noyau cellulaire pour stimuler l'activation de la transcription. L'augmentation de la transcription induite par un apprentissage est accompagnée par des altérations du paysage épigénétique qui peuvent être imitées par une intervention pharmacologique, afin d'améliorer la mémoire chez des individus sains ou cognitivement diminués. A cet égard, l'acétylation des histones présente un intérêt particulier car cette modification épigénétique est naturellement augmentée lors d'un apprentissage, et qu'elle peut être facilement manipulée pharmacologiquement grâce aux inhibiteurs d'histones déacétylases (HDACis). Bien que de nombreuses études ont démontré que la formation de la mémoire peut-être améliorée par l'administration systémique d'HDACis, leur mode d'action n'a pas encore été décrit.

Au cours de cette étude, nous avons testé si les HDACis, administrés de façon systémique, peuvent augmenter les réponses épigénétiques, transcriptionnelles et électrophysiologiques qui sont induites par l'apprentissage, permettant ainsi d'amplifier les réponses naturellement existantes au moyen d'un priming cognitif épigénétique. Pour y parvenir, nous avons combiné un traitement systémique d'HDACi avec un protocole faible de conditionnement à la peur par contexte (CPC), basé sur un conditionnement pavlovien, mais qui, seul, ne conduit pas à la formation d'un souvenir.

Nous avons observé qu'en combinant le CPC avec un HDACi, cela améliorait la mémoire récente pour le contexte associé, et augmentait la potentialisation à long terme dans l'hippocampe. A l'inverse, l'association CPC-HDACi n'induisait aucun renforcement de l'activité synaptique dans le striatum, une région du cerveau qui n'est pas directement activée par le CPC. Ensuite, nous avons séquencé l'ARN soit en gros, soit dans des noyaux cellulaires uniques, pour montrer que le traitement à l'HDACi active des voies de transcriptions uniques, à la fois entre ces deux régions du cerveaux et entre les différentes types cellulaires de l'hippocampe. Cela indique que l'HDACi n'agit pas de façon générale, mais plutôt qu'il permet de soutenir les processus cellulaires spécifiques qui ont déjà lieu en réponse au protocole comportemental. Finalement, nous avons montré que l'association CPC-HDACi induit dans l'hippocampe l'augmentation d'H3K27ac au niveau des enhancers de gènes connus pour être

liés aux processus d'apprentissage. Cependant, tous ces gènes ne sont pas nécessairement plus transcrits à ce moment là.

Dans leur ensemble, ces résultats indiquent que d'administration systémique d'HDACi agit comme un exhausteur cognitif en amplifiant des processus spécifiques et locaux qui ont lieu naturellement lors de l'apprentissage. Cependant, ces effets ne sont pas nécessairement dus directement à l'acétylation des histones au niveau des enhancers. Ces découvertes mettent en lumière les mécanismes impliqués dans le priming cognitif épigénétique via un traitement à l'HDACi, et permettent d'ouvrir la voie à de nouvelles thérapies pour les troubles liés à la mémoire.

Mots-clés: Mémoire, conditionnement à la peur par contexte (CPC), hippocampus, épigénétique, HDAC, potentialisation à long terme (PLT), séquençage de prochaine génération

Contents

List of Abbreviations

AD	Alzheimer's disease
CA1	CA1 field (Cornu Ammonis)
CA3	CA3 field (Cornu Ammonis)
CEP	Cognitive epigenetic priming
CFC	Contextual fear conditioning
CI-994	Benzamide-type class I HDAC inhibitor
ChIP-seq	Chromatin immuno-precipitation followed by sequencing
CPM	Counts per million
CREB	cAMP response element binding
DG	Dentate gyrus
E-LTP	Early LTP
ERK	Extracellular signal related kinase
FANS	Fluorescence activated neuronal sorting
fEPSP	Field excitatory postsynaptic potential
FSC	Forward scatter (aka Forward scattered light)
HAT	Histone acetyltransferase
H3K27ac	Acetylated lysine 27 histone3
HDAC	Histone deacetylase
HDACi	Histone deacetylase inhibitor
HFS	High frequency stimulation
HIP	Hippocampus
IEG	Immediate early gene
IHC	Immunohistochemistry
I/O	Synaptic transmission input/output
I.P.	Intraperitoneal injection
LTP	Long-term potentiation
L-LTP	Late LTP
MAPK	Mitogen-activated protein kinase
MWM	Morris water maze
NOR	Novel object recognition
PCA	Principal component analysis
PolII	RNA polymerase II

PolII CTD	C-terminal domain of RNA polymerase II
PPF	Paired pulse facilitation
PTM	Post-translational modification
RNA	Ribonucleic acid
RNA-seq	RNA-sequencing of pooled cell populations (bulk)
snRNA-seq	Single nuclear RNA-sequencing
STR	Striatum
TPM	Transcripts per kilobase million
TSS	Transcription start site
VEH	Vehicle

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Chapter 1 Introduction

In 1984, Francis Crick hypothesized that the time span of human memory, which can last decades, could not be stored directly in neuronal proteins due to their relatively transient nature (Crick 1984). While he proposed a few solutions to this problem, he felt that the most likely was that molecules can "interact in such a way that they can be replaced with new material, one at a time, without altering the overall state of the structure". This theory shares the defining idea of self-perpetuation with the epigenetic property of DNA methylation and broadly foreshadowed the budding new field of neuroepigenetics. Since then, we have advanced our understanding of the relationship between memory and epigenetics, showing that massive changes both in DNA methylation and histone acetylation occur in response to learning induced neuronal activation. In this thesis, I will discuss the concepts of memory and epigenetics and how drugs that alter the epigenetic state, particularly that of histone acetylation, can improve long term memory formation.

1.1 Memory

Memory refers to the brain's ability to encode, store and retrieve data (Squire 2009), however, our understanding of the mechanisms that regulate memory remain incomplete. Neuroscientists often approach the study of memory from two lines of inquiry: first, by directly examining animal behavior after memory formation (Pavlov 2006) and after manipulation of either the memory tests or the underlying brain anatomy (Landeira-Fernandez et al. 2006; J. Kim and Fanselow 1992); and second, by measuring the cellular and molecular processes that accompany those behaviors and manipulations (Carew, Castellucci, and Kandel 1971). Based on these studies, we can conclude that memory is not a unitary contribution of the full brain but is composed of many levels of systems and interactions which contribute to varying aspects of learning.

The study of the systems and interactions involved in learning can be simplified by classifying memory based on its underlying properties. For example, memory can be broken into two stages: short-term, which only lasts a few moments without continued active maintenance; and long-term, that can last a life-time (Squire 1984). Both forms of memory rely on the strength of pre-existing synaptic connections, however, long-term memory uniquely relies on

downstream transcriptional activation and protein synthesis (Mayford, Siegelbaum, and Kandel 2012). Beyond the temporal aspects of a memory, it can be classified as either an implicit or explicit memory. Implicit memory refers to unconscious recollections that are often the result of repeated exposure or movement. They include perceptual and motor skills and can be expressed through performance – for example, by speaking to another person or riding a bike. On the other hand, explicit memories define the learning of semantic information, such as facts learned in school, or episodic information, in which memories of events are associated with information about their spatio-temporal context (Kandel 2001). We focus on the hippocampus and the essential role that it plays in the acquisition and retrieval of long-term episodic memory (Bird and Burgess 2008). This type of memory is often measured using a Pavlovian contextual fear conditioning (CFC) paradigm in which a neutral stimulus, a novel context, is paired with an aversive stimulus, a mild foot shock, thus implicitly linking the two. To measure the strength of the memory, we re-expose the animal to the context at a later time point and measure the fear response, which manifests as freezing. During this process, the hippocampus plays a crucial role in converting a transient short term memory to a more robust long-term memory (Kandel 2001), a process known as consolidation.

Consolidation is the gradual stabilization of memories that begins immediately after memory acquisition and involves many processes at various levels of organization (Lee and Silva 2009), from synaptic communication to intra-cellular and nuclear signaling. At the level of the synapse, a persistent strengthening of synaptic communication in response to an experience is known as long-term potentiation (LTP) and is one of the mechanisms underlying learning and memory. During this process, neurotransmitter release from the pre-synaptic cell signals a post-synaptic cell to increase glutamate membrane receptors, thus sensitizing it to future pre-synaptic signals. This process strengthens future connectivity between the two cells, thus increasing synaptic plasticity (Lee and Silva 2009). In addition to synaptic plasticity, memory may also be accompanied by changes in structural plasticity. Dendritic spines are the small protrusions emerging from principal neurons and are the main sites of excitatory synaptic contacts innervating those neurons (Alvarez and Sabatini 2007). In particular, CFC and long term memory formation lead to extensive synaptic remodeling and an increase in spine density in the hippocampus (Abate et al. 2018; Restivo et al. 2009). On the other hand, many neurological disorders are accompanied by changes in dendritic spine distribution and neuronal loss (Fiala, Spacek, and Harris 2002). This suggests that both changes in structural plasticity and synaptic plasticity are important players in memory formation.

At the cellular level, the memory engram also contributes as a potential mechanism for memory formation (Josselyn, Köhler, and Frankland 2015; Miry, Li, and Chen 2021). The engram refers to a sparse ensemble of neurons that are activated at the time of memory formation and, as a result, undergo structural and functional modifications. This allows them to enduringly maintain the activation memory so that they may then reactivate upon a recall exposure to the initial experience (Tonegawa et al. 2015). Learning during CFC relies on the hippocampal engram, as optogenetically silencing those neurons that were recruited during encoding reduced freezing during the recall session (Denny et al. 2014). Conversely, artificially activating hippocampal engram cells elicited the fear response without any external cues (X. Liu et al. 2012). There is some interplay between synaptic activity and engram allocation, as cells that are highly excitable before a learning event are more likely to be recruited into the engram (J. Li et al. 2020). Additionally, cells with high activity of the cAMP response element-binding protein (CREB), a transcription factor that leads to enhance memory when overexpressed (Josselyn et al. 2001), are also more likely to be recruited into the engram (J. Han et al. 2007). This suggests that the processes underlying synaptic plasticity and engram allocation may be intertwined and likely rely on the molecular mechanisms occurring within the neurons.

The synaptic signaling induced by CFC also initiates an intra-cellular signaling cascade in the post-synaptic cell. In particular, LTP induces the activation of the mitogen-activated protein kinase (MAPK) pathway (Atkins et al. 1998) which carries these synaptic signals to the nucleus where they activate the transcription factor, CREB (Lee and Silva 2009; Kandel 2001). This leads to the activation of memory specific transcriptional pathways, including immediate early genes (IEGs) that are known markers of ensemble neurons (Josselyn, Köhler, and Frankland 2015). So, just as repeated inter-cellular signaling strengthens synaptic connections, it is believed that it also enhances the intra-cellular and transcriptional sensitivity of the post-synaptic cell. Although the mechanisms that underlie the regulation of memory-activated transcription are not fully characterized, researchers have turned to studying the epigenome to describe the methods by which neurons encode, store and retrieve information about the long-lasting alterations in gene regulation (Lee and Silva 2009).

1.2 Epigenetics in learning and memory

The term “epigenetics” was originally coined by Conrad Waddington in which he described changes in phenotype that were unaccompanied by changes in genotype (Waddington 1957). While Waddington was referring to the epigenetic changes that drive their differentiation trajectories through development, it has since been accepted that epigenetic changes are also a method by which we can integrate environmental signals to prompt a transcriptional response in fully differentiated cells (Goldberg, Allis, and Bernstein 2007).

The epigenetic control of chromatin landscape is under the control of cumulative modifications, many of which are known to contribute to learning and memory. For example, DNA methylation is increased during learning, correlates with transcriptional changes and is necessary for down-stream protein synthesis (Duke et al. 2017; Halder et al. 2016; Pearce et al. 2017). DNA methylation seems to mediate stable changes after memory formation as inhibition of DNA methyltransferases (DNMTs) 1 month after a learning event disrupted remote memory (C. A. Miller et al. 2010). Further, upregulation of Dnmt3a2 in the hippocampal dentate gyrus (DG) was shown to enhance fear memory and alter the methylation profile at memory-specific genes. When Dnmt3a2 overexpression was done in an engram-specific manner it stabilized the recall-dependent reactivation, further showing the importance of DNA methylation in learning and memory (Gulmez Karaca et al. 2020). More transient epigenetic modifications can also occur on histone tails and can regulate the underlying transcriptional state by altering chromatin conformation. Methylation of lysine or arginine residues of histone tails can activate or repress transcription, depending on which residue is methylated (Campbell and Wood 2019). Although the role of histone methylation in learning and memory is not as well studied as other epigenetic modifications, it’s dysregulation has been implicated in impaired cognition and neurodevelopmental disorders (Schaefer et al. 2009; Collins et al. 2019).

Alternatively, histone acetylation, an epigenetic mark that occurs primarily on lysine residues and is associated with transcriptional activation, has been strongly implicated in learning and memory. It is increased in the hippocampus during memory forming tasks, such as CFC or novel object recognition (NOR) (Levenson et al. 2004; Villain, Florian, and Roullet 2016). Whereas, impaired cognition, such as that seen in Alzheimer’s disease and age related cognitive decline, is coupled with a reduction in hippocampal acetylation (Eva Benito et al. 2015; Gräff, Rei, et al. 2012; Peleg et al. 2010). Many of these changes occur at memory

related genes (Gräff et al. 2014). Previous work has shown that manipulating the enzyme ratios that regulate histone acetylation can alter memory formation. For example, inactivating histone acetyltransferases (HATs), enzymes that add acetylation to histone tails, decreases acetylation and hinders the hippocampal transition from short-term to long-term memory (Alarcón et al. 2004). On the other hand, blocking histone deacetylases (HDACs), enzymes that remove acetylation from histone tails, ameliorates memory, both in healthy and cognitively impaired individuals. (Villain, Florian, and Roullet 2016). Due to this phenomenon, administration of HDAC inhibitors (HDACis) has been adopted as a means of enhancing memory formation. However, the mechanisms by which inhibiting HATs and HDACs improves memory is still unknown. One previously proposed theory is called, “cognitive epigenetic priming”.

1.3 Cognitive epigenetic priming

This section represents the following published review article which has been formatted to fit with the style of the thesis:

Burns AM, and Gräff J. (2021) Cognitive epigenetic priming: Leveraging histone acetylation for memory amelioration. *Current Opinion in Neurobiology*, 67:75-84 https://doi.org/10.1016/j.conb.2020.08.011

Contribution: I researched and wrote this review with discussion and editing provided by Professor Johannes Gräff.

1.3.1 Abstract

Multiple studies have found that increasing histone acetylation by means of histone deacetylase inhibitor (HDACi) treatment can ameliorate memory and rescue cognitive impairments, but their mode of action is not fully understood. In particular, it is unclear how HDACis, applied systemically and devoid of genomic target selectivity, would specifically improve memory-related molecular processes. One theory for such specificity is called cognitive epigenetic priming (CEP), according to which HDACis promote memory by facilitating the expression of neuroplasticity-related genes that have been stimulated by learning itself. In this review, we summarize the experimental evidence in support of CEP, describe newly discovered off-target effects of HDACis and highlight similarities between drug-induced and naturally occurring CEP. Understanding the underlying mechanisms of CEP is important in light of the preclinical premise of HDACis as cognitive enhancers.

1.3.2 Introduction

Memory formation relies both on synapse-to-synapse communication and synapse-to-nucleus signaling. While short-term memory is primarily the result of the former and independent of the latter, long-term memory depends on synaptic messages being integrated via downstream signaling cascades to induce transcription in the nucleus (Kandel 2001). Transcriptional induction requires the access of the transcriptional machinery and regulatory transcription factors to the chromatin. For this, chromatin conformation must be in an “open” state, which is controlled by stimulus and time-specific epigenetic mechanisms. Some epigenetic markers, such as DNA methylation, are primarily associated with closed and un-transcribed regions of the chromatin (Klose and Bird 2006). Others, like histone acetylation – the primary focus of

this review – are associated with open regions and an increase in gene transcription at the affected locations.

Histone acetylation is maintained by "writing" enzymes, known as histone acetyltransferases (HATs), "erasing" enzymes, called histone deacetylases (HDACs), and "reading" enzymes that contain bromodomains that recognize and localize to acetylated lysine residues. The ratios of "writer" and "eraser" activity can be artificially altered by treating animals with HDAC inhibitors (HDACis), leading to global changes in histone acetylation. These histone acetylation changes not only open the chromatin to be more accessible but also re-allocate the targeting of "readers" such as the transcriptional co-activators, bromodomain-containing protein 4 (Brd4) and cAMP response element-binding protein (CBP) (Peixoto and Abel 2013), thereby leading to increased transcription.

In vivo, histone acetylation in the hippocampus, an active brain region in spatial and contextual learning, has been shown to positively correlate with contextual fear conditioning, novel object recognition and spatial memory formation (Levenson et al. 2004; Villain, Florian, and Roullet 2016; Takuma et al. 2014; Bousiges et al. 2010). Conversely, disorders defined by reduced cognitive abilities, such as Alzheimer's Disease and age-related cognitive decline are associated with reduced histone acetylation in the hippocampus in both pre-clinical models and in human *post-mortem* brain samples (Janczura et al. 2018; Peleg et al. 2010; Panikker et al. 2018).

Correspondingly, HDACis were found to improve performance in contextual fear conditioning and extinction learning, as well as to rescue memory impairments in neurodegenerative disorders (Levenson et al. 2004; Fischer et al. 2007; Kwapis et al. 2017; Yuan et al. 2015; Valiati et al. 2017; Gräff et al. 2014; Whittle et al. 2016; Wilson et al. 2014; Bowers et al. 2015; Agís-Balboa et al. 2017). Based on these positive effects, several HDACis are currently being tested in clinical trials to treat memory disorders in arachnophobia (NCT02789813), schizophrenia (NCT03263533), and Alzheimer's disease (NCT03056495). Despite promising results for HDACis in the treatment of preclinical models of neurodegeneration and memory loss, the mechanisms by which HDAC inhibition improves memory formation are still not fully elucidated. This is important to understand as in some cases HDACis were found to exacerbate negative symptoms of neurodegenerative disorders such as aggression and agitation (Herrmann et al. 2007).

1.3.3 Cognitive epigenetic priming

"Cognitive epigenetic priming" (CEP) is a recently proposed theory that aims to explain how, when paired with a stimulus such as learning, HDAC inhibition ameliorates memory formation (Gräff and Tsai 2013). This model is derived from the experimentally well-established concept of "epigenetic priming" in development and cellular differentiation, which describes three conformationally defined chromatin states: closed states, that are tightly wound and allow limited access to underlying genes; permissive or primed states, in which pioneer transcription factors and histone modifications allow for initial relaxing of chromatin; and open states, that are easily accessible to the transcription machinery (Klemm, Shipony, and Greenleaf 2019). In development, this theory characterizes the coordinated epigenetic and transcriptional signals needed to transition through different states during cell lineage diversification (A. Wang et al. 2015) (**Box 1**).

Box 1: Conventional definition of epigenetic priming

Traditionally, epigenetic priming is a phenomenon that explains how cells maintain the ability to appropriately respond to inductive cues during cell type differentiation (A. Wang et al. 2015). The theory states that priming occurs before gene activation, allows for a more fine-tuned temporal regulation of downstream gene expression in response to a stimulus and maintains the memory of the previous gene activity (Bonifer and Cockerill 2017). Bivalent, or poised, promoters and enhancers, in which genetic loci maintain both active and repressive epigenetic marks are a likely mechanism of epigenetic priming. In development, H3K4me1 marks poised enhancers and recruits pioneer transcription factors which in turn allow for H3K27ac activation and the further rapid recruitment of transcription factors that induce gene activation (A. Wang et al. 2015). This sequence of events has not directly been studied in the brain *in vivo*. However, there is some evidence that – similar to developmental processes(J. Liu et al. 2017) – RNA polymerase II is paused at IEGs in order to be more quickly induced after activation in primary neuronal cultures (Saha et al. 2011).

Similarly, CEP defines cell states during memory formation, albeit with more flexible epigenetic modifications owing to the faster timescale required for learning. In particular, this theory focuses on histone acetylation induced by HDAC inhibition during memory formation and states that the HDACi-induced epigenetic changes sensitize memory-related genes so that they are more likely to be transcribed in response to a targeting stimulus, such as neuronal

activation (Gräff and Tsai 2013). Indeed, most HDACi treatments are administered orally during clinical trials or interperitoneally (i.p.) in animal experiments, from which follows that they affect, in principle, histone acetylation indiscriminatingly between brain areas, cell types and gene loci. Accordingly, HDACis would relax the overall chromatin conformation, thus priming genes for potential activation. However, genes that are already characterized by histone acetylation, namely by learning-induced neuronal activity, would be more likely to reach the threshold necessary for transcription. In turn, this would lead to a targeted activation of plasticity-related pathways and thereby to enhanced cognition (**Figure 1.1**).

CEP was first described in extinction learning (Gräff et al. 2014), where pairing CI-994, a benzamide-type class I HDACi (**Box 2**), with an otherwise inefficient extinction protocol, helped to reduce long-lasting traumatic memories in mice. Importantly, the effects of HDAC inhibition were stimulus dependent, as HDACi treatment without memory recall did not ameliorate the traumatic memory. Since then, other studies have revealed similar memory-promoting effects using different HDACis in conjunction with learning, both in healthy conditions and those characterized by cognitive impairment, and have thereby helped to not only elucidate the mechanisms of action of HDACis as cognitive enhancers but also to assess the experimental evidence for CEP.

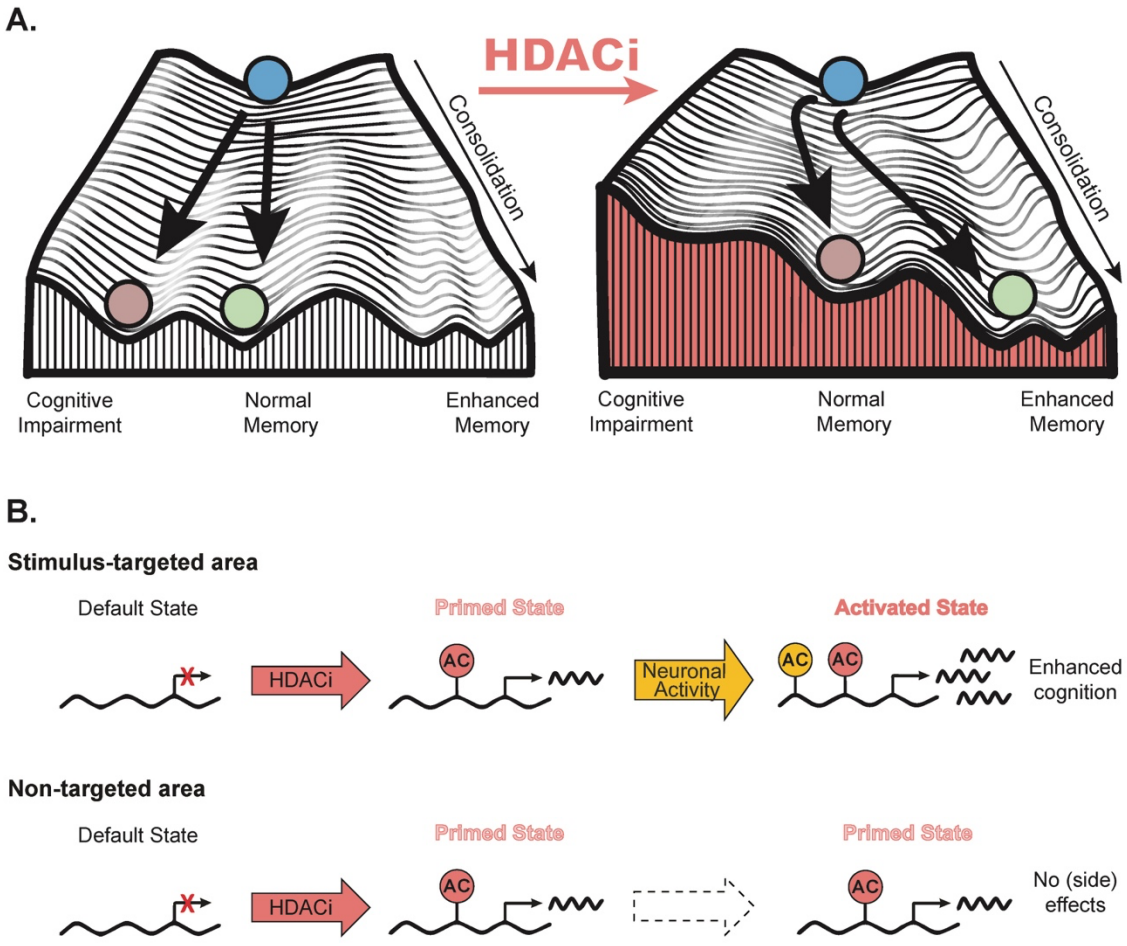


Figure 1.1 Putative model of epigenetic influence on memory performance.

A) (Left) Akin to Waddington's traditional epigenetic landscape during development (Waddington 1957), which illustrates how undifferentiated stem cells develop into physiologically mature cells by acquiring epigenetically defined cell fates, memory performance can take different trajectories depending on the epigenetic makeup of its neuronal substrates. The valleys represent different cognitive outcomes, while the hills represent epigenetic boundaries consisting of, among others, histone acetylation. (Right) There are numerous examples in the literature summarized in this review showing that HDAC inhibitors (HDACi) can not only rescue memory deficits, but also enhance memory when applied to cognitively healthy animals. Many of these studies indicate that such HDACi-mediated cognitive enhancement occurs through alterations in the epigenetic histone acetylation landscape. **B)** CEP and the effects of HDACi are stimulus and time-specific. System-wide treatment with an HDACi likely increases histone acetylation indiscriminately across the genome. In doing so, the HDACi treatment induces a "primed state". Genes that are then targeted by a stimulus such as neuronal activity – known to also induce histone acetylation - will be more likely to reach the threshold necessary to induce transcription, in effect, leading to an "activated state" and enhanced cognition.

Box 2: Class I vs Pan-HDACis

There are currently 11 known HDAC proteins that are divided into 4 different subgroups based on their DNA sequence. Historically, the most commonly used HDACis inhibit several subgroups, which could lead to an increase in off-target effects and may account for some of the changes seen in tau aggregation in response to HDAC inhibition (Jeong et al. 2019).

Many of the cognitive enhancements that are caused by HDAC inhibition can also be seen when individual Class I HDACs, such as HDAC2 (Guan et al. 2009) and HDAC3 (Bowers et al. 2015; Janczura et al. 2018; Krishna, Behnisch, and Sajikumar 2016; McQuown et al. 2011), are inhibited, suggesting that Class I specific inhibitors are sufficient for memory enhancing effects. Future clinical trials should use more targeted HDACis in order to reduce unwanted effects.

HDAC inhibitor	Class of inhibition	Phase of memory	Ref.
TSA	Pan	Formation and rescue	(Jeong et al. 2019; Levenson et al. 2004; Vaid, Wen, and Mannervik 2020; Valiati et al. 2017; Vecsey et al. 2007)
Vorinostat (SAHA)	Pan	Formation and rescue	(Agís-Balboa et al. 2017; Eva Benito et al. 2015; Guan et al. 2009; Huang et al. 2014; Jeong et al. 2019; Peleg et al. 2010)
Valproic acid	Pan	Extinction and rescue	(Jeong et al. 2019; Wilson et al. 2014)
M344	Pan	Rescue	(Volmar et al. 2017)
CI-994	Class I	Formation, extinction and rescue	(Gräff et al. 2014; Jeong et al. 2019; Zhao et al. 2018)
Sodium Butyrate	Class I	Formation and reconsolidation	(Cao et al. 2018; Fischer et al. 2007; Levenson et al. 2004; Villain, Florian, and Roullet 2016)
RGFP963	Class I	Extinction	(Bowers et al. 2015)
MS-275	Class I	Formation and extinction	(Yuan et al. 2015; Whittle et al. 2016)
RGFP966	HDAC3	Extinction and rescue	(Bowers et al. 2015; Janczura et al. 2018)

1.3.4 Potential mechanisms of HDAC inhibition

The molecular and physiological mechanisms underlying CEP have predominantly been studied at three levels. First, as the epigenetic changes induced by HDACis and their priming effects on the chromatin landscape; second, as the ensuing changes in gene transcription and activity; and third, as the corresponding alterations in synaptic plasticity, in particular long-term potentiation (LTP) (**Figure 1.2**).

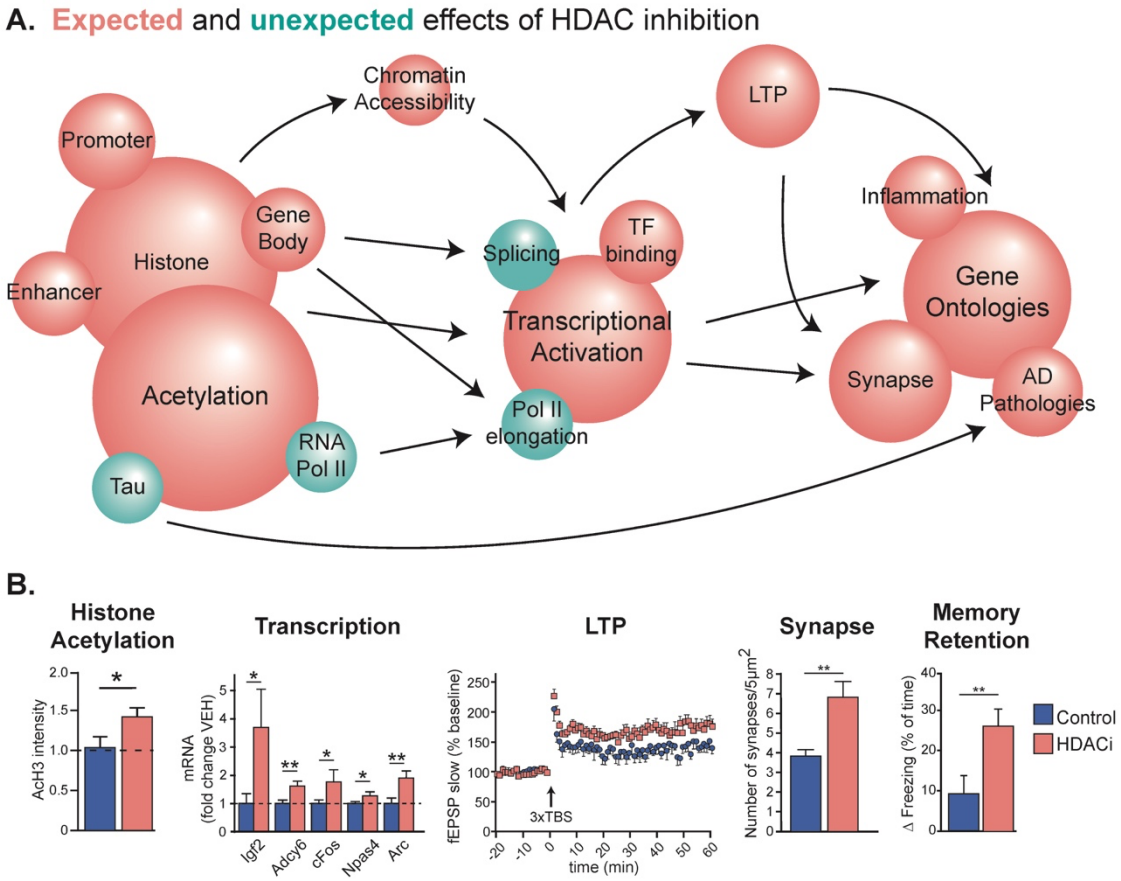


Figure 1.2 Mechanisms underlying HDACi-mediated cognitive epigenetic priming (CEP).
A) To enhance memory, HDAC inhibitors (HDACis) are not only found to alter histone acetylation (pink), but also that of other unexpected substrates (teal), which in turn alters chromatin accessibility as well as transcriptional activation and ultimately results in different gene ontologies being affected. Node size represents the number of publications since 2013 (summarized in this review) that describe the node's role in learning and memory following HDACi-mediated CEP. Arrows represent interactions between connecting nodes. **B**) Experimental evidence of the role of HDAC inhibition at different target levels (Gräff et al. 2014). All readouts are measured 2 hours after HDACi and 1 hour after extinction training between HDACi and vehicle-treated animals. From left to right: Histone acetylation in the hippocampal CA1 measured by IHC. RT-PCR confirmation of the transcriptional expression of several neuroplasticity related genes in the hippocampus. LTP measured at Schaffer collaterals. Quantification of synapse formation taken from hippocampal brain sections by transmission electron microscopy. Differences in freezing behavior between remote fear memory recall and 1 hour after extinction training, indicating improved memory retention.

HDAC inhibition increases histone acetylation

Combining HDAC inhibition with learning paradigms was repeatedly found to increase histone acetylation in multiple conditions. When paired with extinction training, CI-994 (i.p.) not only improved the consolidation of extinction memories, but also increased total hippocampal H3 acetylation as revealed by immunohistochemistry (IHC) (Gräff et al. 2014). Importantly, no histone acetylation changes were observed when the HDACi was applied without extinction training, which provides evidence for the stimulus-dependency of CEP (**Figure 1.1B**). Further studies have found that the class I HDACi, MS-275 (i.p.), also enhances the consolidation of recent fear memory extinction, and concomitantly increases total H4 acetylation in the cortex and amygdala as revealed by IHC (Whittle et al. 2016). Beyond extinction training, global histone acetylation increases were also observed, via western blot analysis, in the hippocampus of healthy animals following learning, where chronic sodium butyrate (NaB) (i.p.) administration enhanced and maintained memory for up to one month after contextual fear conditioning compared to vehicle treated animals (Cao et al. 2018).

Another intriguing application of HDAC inhibition lies in the amelioration of cognitive disorders in preclinical models of Alzheimer's disease (AD) and age-related cognitive decline, both of which are characterized by an impairment in spatial memory and a depreciation in these task's ability to induced histone acetylation (Eva Benito et al. 2015; Peleg et al. 2010). In 3xTg AD and APP/PS1 mice, two models of AD, chronic SAHA treatment (oral) rescued their memory impairments (Eva Benito et al. 2015), an effect that was recapitulated by the more specific HDACis RGFP-966 (i.p.) (Janczura et al. 2018) and M344 (i.p.) (Volmar et al. 2017). Furthermore, SAHA administration was found to reverse the dominant histone hypo-acetylation (revealed by chromatin immunoprecipitation followed by sequencing, ChIP-seq) in hippocampal area CA1 of these mouse models (Eva Benito et al. 2015), which was also observed in western blots for RGFP-966 in HEK/APPsw cell lines (Janczura et al. 2018) and for M344 in wild-type mice (Volmar et al. 2017). Finally, SAHA (intrahippocampal and oral administration) also improved the cognitive defects and the H4K12ac reductions in the hippocampi of aged mice (Peleg et al. 2010; Eva Benito et al. 2015).

Notwithstanding, in order to better understand whether and to which extent genomic regions transition from a primed state to an activated state during CEP, it is important to determine which specific loci are affected. Using ChIP-qPCR approaches, H3K9/14ac was found to be increased at the promoter regions of immediate early genes (IEGs) such as cFos and Npas4 after combined HDAC inhibition and extinction training (Gräff et al. 2014). Furthermore, although H4K12ac was reduced *en masse* at neuronal transcriptional start sites (TSSs) in

cognitively impaired animals, this reduction could be rescued by long-term oral administration of SAHA as revealed by ChIP-seq (Eva Benito et al. 2015; Peleg et al. 2010).

In addition, several studies indicate that acetylation at enhancers are also relevant in CEP. Both H3K27ac and H3K9ac are associated with active enhancers, which play a critical role in transcriptional activation (Lopez-Atalaya et al. 2013; Greer et al. 2015). Recent work using a CRISPR-based approach, in which the dead mutant of Cas9 was fused to the HAT p300, showed that increasing acetylation in the enhancer region of cFos alone was sufficient to upregulate this IEG and its downstream targets (Chen et al. 2019). This suggests that CEP at the level of enhancer histone acetylation could be sufficient to improve memory. Future studies of CEP will need to corroborate this and also measure changes at intergenic regions in order to determine which genes are truly in the primed state.

HDAC inhibition enhances transcription

According to the CEP hypothesis, the genes epigenetically primed by HDACi treatment will subsequently be more readily activated by the targeting stimulus, i.e., learning. In the following studies, this was indeed the case. After remote fear extinction training, the IEGs showing increased promoter histone acetylation were also transcriptionally upregulated. What is more, genome-wide, learning and synaptic-related pathways were among the most upregulated ontologies, suggesting that such specific activation may have been facilitated by the HDACi treatment (Gräff et al. 2014). Further evidence for such a targeting effect in CEP comes from a study comparing divergent transcriptional profiles in two conditions of cognitive impairment, AD and aging. While AD was found to inhibit genes involved in synaptic plasticity (Eva Benito et al. 2015; Volmar et al. 2017; Janczura et al. 2018), downregulated genes in aging were mainly involved in RNA transcription, protein modification and cellular metabolism (Eva Benito et al. 2015; Peleg et al. 2010). Intriguingly, in both ailments, SAHA was found to selectively rescue the mis-regulated genes (Eva Benito et al. 2015), likely as a consequence of HDACis having a stronger effect on genes that are already defined by hypoacetylation. The HDACi-induced global reallocation of histone acetylation likely alters transcription by redistributing the binding of acetylation “readers”, such as Brd4, a protein that binds regions of acetylated histones and recruits elongation factors involved in transcriptional induction (Greer et al. 2015). In line, inhibiting Brd4 with the small-molecule inhibitor, JQ1, also ameliorates memory formation in AD-like animals (E. Benito et al. 2017), suggesting that memory enhancement might also be driven by the recruitment and localization of the transcriptional machinery in addition to acetylation-induced chromatin conformation changes.

Nevertheless, multiple studies have reported a surprisingly high number of down-regulated genes as shown in RNA-sequencing experiments following HDACi-supported contextual fear conditioning and extinction training (Lopez-Atalaya et al. 2013; Gräff et al. 2014). The mechanisms and roles played by HDAC inhibition in this unexpected decrease in transcription are not clear, but could be the result of compensatory mechanisms regulating transcriptional homeostasis (Eva Benito et al. 2015), in particular since little specificity was found among the gene ontologies of the downregulated genes (Lopez-Atalaya et al. 2013). In line, one study showed that in human cell lines, HDACi treatment prevents histone deacetylation at gene bodies and intergenic regions, and that this indiscriminating increase in acetylation may mask other genetic elements such as promoters and enhancers for stimulus-specific histone acetylation increments (Greer et al. 2015).

In addition to reallocating the transcriptional and epigenetic machinery, HDACi treatment may also alter transcriptional profiles by ameliorating splicing defects and isoform switching induced by AD. For example, HDACi treatment was found to reinstate H4K12ac at mis-regulated exon-intron junctions in a mouse model of neurodegeneration (Eva Benito et al. 2015), which coincided with the reestablishment of the proper isoform usage. The role of HDAC inhibition on isoform switching, in particular promoter usage, has not been described in healthy individuals, however it could be relevant for genes like BDNF, for which different isoforms play distinct roles in synaptic plasticity in the hippocampus (An et al. 2008). Investigating these mechanisms further could shed light on the role of HDAC inhibition in transcriptional dynamics and the downstream products that play a role in memory formation.

HDAC inhibition facilitates synaptic plasticity

The HDACi-mediated upregulation of transcriptional cascades that involve IEGs and synaptic transmission suggests that this treatment can also enhance synaptic plasticity. Indeed, HDACi application during extinction training increased LTP at Schaffer Collaterals, which was accompanied by an increase in structural plasticity such as enhanced dendritic branching and synaptic density (Gräff et al. 2014). Furthermore, SAHA treatment rescued the LTP reduction observed in age-related cognitive decline (Eva Benito et al. 2015) and RGFP-966 enhanced LTP to wild-type levels after Aβ oligomer-induced reduction (Zhiyun Wei et al. 2020; Krishna, Behnisch, and Sajikumar 2016).

Importantly, the positive effects of HDAC inhibition on synaptic potentiation both in AD models and healthy animals were only observed after LTP induction and not without, speaking to the stimulus-dependency of CEP (Levenson et al. 2004; Krishna, Behnisch, and Sajikumar 2016).

Furthermore, they relied on transcriptional activation and downstream protein synthesis (Krishna, Behnisch, and Sajikumar 2016): Co-treating HDACis with the transcriptional inhibitor, actinomycin D (Levenson et al. 2004; Vecsey et al. 2007), or a Brd4 inhibitor, JQ1 (Sartor et al. 2019), blocked the HDACi-mediated enhancement of LTP. This could be a consequence of blocked IEG induction and subsequently, reduced activation of genes involved in synaptic plasticity (Gräff et al. 2014; Eva Benito et al. 2015), as for example ion channel promoters are bound and activated by cFos and other members of the AP-1 complex (Su et al. 2017).

Based on the above, HDACis influence histone acetylation and regulate downstream changes in transcription and LTP. Even so, due to the broad functions of HDACs (Haberland, Montgomery, and Olson 2009), it cannot be discounted that some of the consequences of CEP are due to non-histone acetylation.

Non-histone targets of HDACis

Although the range of action of HATs and HDACs are mostly associated with histone acetylation, these enzymes can also substantially modify non-histone proteins (Falkenberg and Johnstone 2014). To illustrate this, pan-HDACis, such as TSA and SAHA, were found to increase cytoplasmic tau acetylation and aggregation, hallmarks of many neurodegenerative diseases (Jeong et al. 2019). This is surprising considering that HDACi treatment ameliorates the behavioral deficits in AD-like pathologies (Volmar et al. 2017; Janczura et al. 2018; Eva Benito et al. 2015). These discrepancies may be explained by the evidence that more specific HDACis, such as RGFP-966 and M344 (see box 2), decrease levels of phosphorylated tau and the toxic form of amyloid beta in the cytoplasm, this latter likely via altering the expression of amyloid precursor protein (APP)-processing genes (Volmar et al. 2017; Janczura et al. 2018). Nevertheless, as even the pan-HDACi VPA was found to ameliorate levels of reactive oxygen species (ROS) and pro-inflammatory cytokines in PTSD and AD-like animal models (Wilson et al. 2014), it remains unclear whether using more specific HDACis represents a viable strategy to eliminate off-target, i.e., non-histone acetylation-mediated effects.

What is more, even within the nucleus, HDACis can target non-histone proteins. HATs and HDACs have been found to modify acetylation on the C-terminal domain (CTD) of RNA polymerase II (RNA Pol II) in mouse embryonic stem cells (ESCs), inducing the transition from polymerase pausing to elongation, particularly at IEGs (Schröder et al. 2013). TSA impedes the deacetylation of the RNA Pol II CTD, and thus likely maintains a higher ratio of activated polymerases, which in turn reduces pausing and increases levels of transcription. This

particular example illustrates that in addition to, or even bypassing the epigenome, HDAC inhibition can affect transcriptional activation. Furthermore, there is a plethora of evidence outside of the brain suggesting that HDACs can target pathways that are involved in DNA damage repair, cell division, protein folding and metabolism, processes that also play a role in learning and memory (Narita, Weinert, and Choudhary 2019). Future studies must explore whether these findings are also relevant to HDACis in the brain.

1.3.5 CEP beyond HDAC inhibition

Based on the experimental evidence gathered thus far, HDACi-supported CEP appears to promote memory formation by imitating epigenetic processes that occur naturally following neuronal activation. For example, histone acetylation, chromatin opening and transcription are increased in the hippocampus within an hour after contextual learning or following electroconvulsive seizures (Levenson et al. 2004; Halder et al. 2016; Su et al. 2017), and these epigenetic changes are further enhanced by HDACi treatments (Cao et al. 2018; Whittle et al. 2016). Likewise, extinction learning *per se* induces hippocampal histone acetylation, an effect that is reinforced by HDACi application (Gräff et al. 2014; Bredy and Barad 2008). Therefore, it is not surprising that other behavioral paradigms may be able to improve subsequent learning when inducing similar epigenetic changes to those elicited by HDACis. Indeed, both environmental enrichment and nicotine exposure enhance hippocampal histone acetylation, LTP, and long-term memory in response to either cocaine or contextual learning alone (Huang et al. 2014; Mews, Walker, and Nestler 2018; Robison and Nestler 2011), and thus act as "natural" CEP stimuli. In both circumstances, "natural" CEP was found to lead to memory improvement. While in the case of environmental enrichment, the CEP effect was beneficial to fight cognitive decline occurring in neurodegeneration (Fischer et al. 2007), priming via drugs of abuse was found to lead to greater drug dependency (Robison and Nestler 2011).

Beyond HDAC inhibition, histone acetylation can also be altered by increasing or decreasing levels of HAT activity. For example, activating the HAT CBP/p300 using the HAT activator CSP-TTK21, reinstated levels of H2B acetylation, rescued transcription of genes involved in ion transport and learning and restored spatial memory in a mouse model for tauopathy, THY-Tau22 (Chatterjee et al. 2018). Interestingly, enhancing HAT activity may have an even broader priming effect than HDAC inhibition, as HDAC activity was found to be limited only to locations that had previously been acetylated by HATs in human $CD4^+$ T cells (Z. Wang et al. 2009), however this has not yet been assessed in the brain.

What is more, CEP effects may not be unique to histone acetylation alone. For example, mice that lack certain histone methyltransferases exhibit impairments in contextual and motor learning (Day and Sweatt 2012), suggesting an important role for histone methylation in learning and memory. Likewise, memory formation also induces changes in DNA methylation at genes involved in synaptic transmission (Halder et al. 2016; Duke et al. 2017), while inhibition of DNA methylation via DNMT inhibitors can impede memory enhancements induced by a sub-threshold pre-training in *Aplysia* (Pearce et al. 2017). Lastly, the control of activity-dependent gene transcription and it's role on downstream learning and memory may also rely on protein turnover and the incorporation of histone variants that alter chromatin conformation (Maze et al. 2015; Zovkic et al. 2014). Although, these epigenetic modifications beyond histone acetylation are out of the scope of this review, these examples of non-acetylation-based CEP may provide further insight into the mechanisms of drug-induced and natural epigenetic priming and could help improve future therapies both for healthy and cognitively impaired individuals.

1.3.6 Discussion

There is now considerable evidence supporting the theory of CEP as a mechanism by which HDAC inhibition improves learning in a stimulus-dependent manner. However, there are still a number of details that must be elucidated. At the cellular level, some studies suggest that, in the brain, neurodegeneration and HDAC inhibition specifically alter acetylation in neurons while having no effect on non-neuronal cell types (Eva Benito et al. 2015; Whittle et al. 2016). Future research will also need to decipher these effects in other neuronal cell types such as astrocytes, which are crucial for proper memory formation (Adamsky and Goshen 2018), and *bona fide* memory-related cell populations, so-called engrams (Josselyn, Köhler, and Frankland 2015; Josselyn and Tonegawa 2020). At the molecular level, it will be important to investigate which acetylation residues and genomic locations are most affected by each HDACi. For example, one study has used IHC to show that one hour after i.p. administration and prior to any conditioning, the HDACis RGFP-966 and RGFP-963 reduce total H3ac and H4ac levels in the hippocampus, whereas they increase H3ac and have no effect on H4ac in the amygdala (Bowers et al. 2015). Future experiments will need to more quantitively determine whether HDACis can indeed penetrate brain regions differentially and whether certain HDACis have differential effects on histone marks within and between brain regions.

In addition, the sequence of molecular events taking place in response to combined HDAC inhibition and learning must be better determined. It is clear that both HDACis and learning first alter histone acetylation. However, it is less evident whether this in turn alters chromatin

conformation to permit transcription factor localization and activation or whether acetylation-induced transcription precedes changes in chromatin conformation. Although this has not yet been tested in the brain, it has been shown that HDAC inhibition alone can induce changes in histone acetylation and transcription that precede moderate changes in chromatin accessibility in Drosophila S2 cell lines (Vaid, Wen, and Mannervik 2020). Nevertheless, given the well-established interplay between acetylation and chromatin conformation the more likely scenario is that changes in chromatin conformation precede transcriptional changes (Gräff and Tsai 2013; Hong et al. 1993). This also fits with the recently proposed theory of "genomic metaplasticity", in which activity-induced changes in epigenetic marks prime genetic locations for the transcriptional activity induced by later neuronal activation (Baker-Andresen, Ratnu, and Bredy 2013). After contextual fear conditioning, many of the newly accessible regions overlap with enhancers and promoters of IEGs and channel proteins involved in synaptic signaling. While transcription of these genes usually endures for less than 24 hours (Su et al. 2017; Fernandez-Albert et al. 2019), a subset of neuronal activity-induced opened chromatin sites, so-called gained open sites, was found to be maintained for at least 48 hours. These gained-open regions overlapped with the AP-1 binding motif, and were enriched for binding of members of the AP-1 binding transcription factors, such as cFos, FosB and Jun (Su et al. 2017; Fernandez-Albert et al. 2019). Consequently, cFos overexpression was partially able to mimic the neuronal activity-induced natural chromatin opening, and genes upregulated by cFos-induced chromatin opening were similar to those induced by neuronal activation (Su et al. 2017). In turn, such long-term maintenance of opened regions and chromatin binding may act to prime their underlying architecture for facilitated transcriptional reactivation in response to a recall event, thus facilitating memory retention, but this remains to be experimentally determined. In future studies, it is therefore important to disentangle these processes in learning and memory in order to clearly separate "primed" and "active" locations and their role in consolidation.

Finally, despite isolated reports of HDACis' inability to penetrate the blood brain barrier *in vivo* (Hanson et al. 2013) and some reported toxicity in clinical trials (Gardner et al. 2009), preclinical research agrees that HDAC inhibition alters neuronal acetylation *in vivo,* which leads to memory enhancements in both healthy and cognitively impaired conditions. These enhancements are likely due to the joint interactions of acetylation of histones and other proteins that enhance transcription. Future studies that further elucidate our understanding of these mechanisms will help us understand how we learn and how we can harness those mechanisms to improve our own memory.

1.4 Aim of the thesis

In this body of work we aim to demonstrate the differential priming properties of systemic HDACi treatment in connection with CFC and to characterize the mechanism by which this priming improves subthreshold contextual fear memory in mice. We approach this question at three different levels.

First, we aim to explore the role of HDACi-mediated priming in neuronal communication by comparing differences in synaptic communication between the hippocampus, the main brain area involved in contextual learning, and the striatum, a brain region that regulates motor activity (Groenewegen 2003; Durieux, Schiffmann, and De Kerchove D'Exaerde 2012), after CFC and rotarod training. This will help us to establish a priming effect of systemic HDACi treatment at the level of neuronal synaptic communication.

Second, we intend to use bulk RNA-sequencing and single nuclear RNA-sequencing (snRNA-seq) to compare differences in transcriptional activation after HDACi paired with CFC in the hippocampus and striatum, as well as between cell types within the hippocampus. This will help us to show that HDACi treatment primes the synaptic response by strengthening the underlying processes occurring after CFC.

Finally, we aim to characterize histone acetylation changes, particularly H3K27ac, in neurons of the hippocampal DG to see whether or not CFC and HDACi mediated epigenetic changes are driving the transcriptional activation that leads to priming in the hippocampus.

We believe that these experiments will help to improve our understanding of HDACi specificity and can contribute to ongoing work and efforts to study the efficacy and safety of HDACi treatment as an epigenomic memory aid.

Chapter 2 Methods and Results

2.1 The HDAC inhibitor, CI-994, acts as a molecular memory aid by facilitating synaptic and intra-cellular communication after learning

This section represents the following submitted article which has been formatted to fit with the style of the thesis:

Burns AM, Farinelli-Scharly M, Hugues-Ascery S, Sanchez-Mut JV, Santoni G and Gräff J. (2021) The HDAC inhibitor CI-994 acts as a molecular memory aid by facilitating synaptic and intra-cellular communication after learning. *Submitted to BioRxiv*.

Contribution: I conceptualized the project and experiments with Professor Johannes Gräff and Jose Sanchez-Mut. With the exception of the electrophysiological experiments performed by E-PHY-SCIENCE, I performed all animal experimentation, all molecular techniques (except bulk RNA-seq and snRNA-seq library preparations) and analyzed all data. I also wrote this paper with the discussion and editing from Professor Gräff.

2.1.1 Abstract

Long-term memory formation relies on synaptic plasticity, activity-dependent transcription and epigenetic modifications. Multiple studies have shown that HDAC inhibitor (HDACi) treatments can enhance individual aspects of these processes, and thereby act as putative cognitive enhancers. However, their mode of action is not fully understood. In particular, it is unclear how systemic application of HDACis, which are devoid of substrate specificity, can target pathways that promote memory formation. In this study, we explore the electrophysiological, transcriptional and epigenetic responses that are induced by CI-994, a class I HDAC inhibitor, combined with contextual fear conditioning (CFC) in mice. We show that CI-994-mediated improvement of memory formation is accompanied by enhanced long-term potentiation in the hippocampus, a brain region recruited by CFC, but not in the striatum, a brain region not

primarily implicated in contextual memory formation. Furthermore, using a combination of bulk and single cell RNA sequencing, we find that synaptic plasticity-promoting gene expression cascades are more strongly engaged in the hippocampus than in the striatum, but only when HDACi treatment co-occurred with CFC, and not by either treatment alone. Lastly, using ChIP-sequencing, we show that the combined action of HDACi application and conditioning is required to elicit enhancer histone acetylation in pathways that may underlie improved memory performance. Together, our results indicate that systemic HDACi administration amplifies brain-region specific processes that are naturally induced by learning. These findings shed light onto the mode of action of HDACis as cognitive enhancers.

2.1.2 Significance Statement

Memory formation relies on a plethora of functions, including epigenetic modifications. Over the past years, multiple studies have indicated the potential of HDAC inhibitors (HDACi) to act as cognitive enhancers, but their mode of action is not fully understood. Here, we tested whether HDACi treatment improves memory formation via “cognitive epigenetic priming”, stipulating that HDACis – without inherent target specificity – specifically enhance learning-induced plasticity-related processes. We found that combining HDACi with fear learning, but not either treatment alone, enhances synaptic plasticity as well as memory-promoting transcriptional signaling in the hippocampus, a brain area known to be recruited by fear learning, but not elsewhere. These results lend experimental support to the theory of “cognitive epigenetic priming”.

2.1.3 Introduction

Long-term memory is a product of synaptic communication as well as activity-dependent transcription that is regulated by epigenetic signaling (Kandel 2001; Lee and Silva 2009; Gräff and Tsai 2013; Campbell and Wood 2019; Levenson and Sweatt 2005). For example, memory forming tasks, such as contextual fear conditioning (CFC), are paralleled by gene expression and histone acetylation changes in the hippocampus (Levenson et al. 2004; Villain, Florian, and Roullet 2016; Bousiges et al. 2010), while impaired cognition, as seen in Alzheimer’s Disease and age-related cognitive decline, is coupled with a reduction in hippocampal histone acetylation and plasticity-related gene expression (Janczura et al. 2018; Eva Benito et al. 2015; Volmar et al. 2017; Peleg et al. 2010; Gräff, Rei, et al. 2012). Some of these epigenetic and transcriptional changes can be augmented by systemic HDAC inhibitor (HDACi) treatment, which improves memory in both healthy and cognitively impaired mice (Vecsey et al. 2007; Gräff et al. 2014; Peleg et al. 2010; Eva Benito et al. 2015; Volmar et al. 2017). Although the use of HDACis in these studies testifies to their suitability as pharmacological

memory aids, the mechanisms by which HDACi enhances memory are not fully understood. In particular, it is unclear how systemic application of HDACis, most of which are devoid of substrate specificity *per se*, can target pathways that promote memory formation.

One proposed theoretical mode of action for HDACis as cognitive enhancers is called "cognitive epigenetic priming" (Gräff and Tsai 2013; Burns and Gräff 2021). This model is inspired by evidence from cancer research, where several HDACis have been shown to improve target efficacy of anti-cancer treatments (Scandura et al. 2011; Terranova-Barberio et al. 2017), and from addiction research, where chronic drug abuse was found to durably enrich histone acetylation, which relaxes the chromatin structure into a primed state and thereby lowers the activation threshold for gene expression changes during subsequent drug exposures (Mews, Walker, and Nestler 2018; Robison and Nestler 2011). Analogously, for cognition, this theory stipulates that by broadly increasing histone acetylation, HDACi treatment leads to an overall primed state. Memory-induced neuronal activity, which is inherently characterized by a high degree of target specificity (Lee and Silva 2009), would then further enrich HDACi-induced histone acetylation and recruit the transcriptional machinery specifically to synaptic plasticity-related genes.

In this study, we tested the concept of "cognitive epigenetic priming" in mice on three different levels. First, we investigated whether systemic HDACi treatment elicits brain-region specific electrophysiological and transcriptional responses after contextual fear conditioning, a hippocampus-dependent memory task. Second, we assessed whether and to which extent specific cell types are affected by the HDACi treatment in combination with learning using single nuclear RNA-sequencing (snRNA-seq) of the hippocampus; and third, we determined which gene loci are epigenetically regulated by HDACi treatment using chromatin immunoprecipitation (ChIP) followed by sequencing. These experiments were designed to better understand the underlying mechanisms of HDACis as potential cognitive enhancers.

2.1.4 Materials and Methods

Animals. All procedures, including animal experiments, were handled according to protocols approved by Swiss animal licenses VD2808/2808.1, VD2875/2875.1, VD3169 and VD3413 and according to the standard operating procedures of E-PHY-SCIENCE SAS (ENV/JM/MNO (2077)). Ten-week-old C57BL/6J male mice were purchased from Janvier Labs and allowed an acclimatization and handling period in the EPFL animal house for two weeks before experimentation. All animals were housed in groups of 4-5 animals at 22-25° C on a 12-hour light-dark cycle with food and water ad libitum. Mice were randomly assigned a drug treatment,

and experimental conditions were randomly split by cage so that all mice in one cage underwent the same fear conditioning protocol.

Drug administration. The class I HDAC inhibitor, CI-994 (synthesized at the Broad Institute with a purity of >95% by HPLC analysis)(Gräff et al. 2014), was dissolved in 10% dimethyl sulfoxide (Sigma-Aldrich, D2438), 30% Kolliphor (Sigma-Aldrich, C5135), and 60% 0.9% saline (Braun, 395158) Its vehicle (VEH) solution consisted of all of the above, without CI-994. One hour before contextual fear conditioning, each animal was interperitoneally (i.p.) injected with either 30mg/kg of CI-994 or a corresponding volume of VEH pre-heated to 37˚C on a thermomixer. Solutions were made fresh before each experiment and stored at -20˚C until use.

Contextual fear conditioning (CFC). All behavioral testing was performed between 9AM and 1PM. CFC for behavior, electrophysiology and sequencing experiments consisted of a 3 min habituation to the conditioning chamber (TSE Systems GmbH at EPFL for all molecular experiments; Imetronic (Pessac, France) for electrophysiology experiments) followed by two 1 s foot shocks (0.2mA) with an interval of 29 s and a final 15 s in the chamber. The context groups in all experiments were exposed to the conditioning chamber for the same amount of time with no shocks. The chamber was cleaned with 5% ethanol between each animal.
To measure the effect of CI-994 on fear learning, 9-12 animals per group were re-exposed to the chamber for 3 min, 24 h after the initial exposure. Percentage of time spent freezing over the total habituation period was automatically calculated with an infrared beam detection system (MultiConditioning System, TSE Systems GmbH). Freezing was quantified when absence of movement was detected for longer than 1 sec. Animal velocity (average cm/s) and distance travelled (total cm) during the habituation phases were calculated TSE system. Changes in anxiety were determined by dividing the conditioning chamber int 36 sections and calculating the percent of total time each animal spent in the inner 16 section (no bordering wall) of the fear conditioning chamber during the initial habituation phase.
For all other molecular experiments, animals were left in their home cage for 1 hour after CFC. Then animals were sacrificed and respective brain regions were manually dissected and immediately frozen on dry ice. Brain regions were stored at -80˚C until further processing.

Rotarod. Motor performance was measured using a rotarod apparatus (Bioseb, model LE8200). Mice were placed on the rotating rod, and the latency to fall was measured while the speed was accelerating from 4 to 40 rpm. Trials began when mice were placed on the rod and rotation began. Each trial ended, and latency was recorded, when the mouse fell off the rod. Mice were tested for 4 trials with a 1 minute inter-trial interval (Kheirbek et al. 2009).

Electrophysiology. For electrophysiological experiments, 8 animals were used per group. One hour after CFC or Rotarod experiments mice were anesthetized with isoflurane and decapitated. Heads were immediately immersed in ice-cold freshly prepared artificial cerebrospinal fluid (aCSF; 124 mM NaCl, 1.3 mM MgSO4, 4 mM KCl, 1.0 mM Na2HPO4, 2.0 mM CaCl2, 26 mM NaHCO3, and 10 mM D-glucose) for at least 2 mins before brain extraction. Acute slices (350 µm thick) were prepared with a vibratome (VT 1000S; Leica Microsystems, Bannockburn, IL) in ice-cold gassed aCSF. Sections were kept at room temperature (RT) for at least 1 h before recording.

For electrophysiological recordings, a single slice was placed in the recording chamber, submerged and continuously super-fused with gassed (95% O2, 5% CO2) aCSF at a constant rate (2 ml/min). fEPSPs were evoked by an electrical stimulation at 0.25 Hz (100μsec duration) in the perforant path, Schaffer collaterals or the cortico-striatal pathway. Downstream extracellular fEPSPs were recorded in the Dentate Gyrus (DG), CA1 and striatum, respectively, using a glass micropipette filled with aCSF. Synaptic transmission input/output (I/O) curves were constructed at the beginning of each experiment to asses basal synaptic transmission. For the I/O, a stimulus ranging from 0 to 100 µA by 10 µA steps was applied and measured every 5 secs. Paired Pulse Facilitation (PPF) was performed to assess short-term plasticity. For PPF, two stimulations were applied and measured at 50, 100, 150, 200, 300 and 400 ms intervals. Stable baseline fEPSPs were recorded by stimulating at 30% maximal field amplitude (single stimulation (0.25 Hz) every 30 secs). The same intensity of stimulation was kept for the reminder of the experiment. After a 10-15 min stabilization, high-frequency stimulation (HFS: 3 trains of 100-Hz stimulation, each train having a 1 sec duration and 2 trains separated by 20 sec) was delivered. Following these conditioning stimuli, a 90 min test period was recorded where responses were again elicited by a single stimulation every 30 sec at the same stimulus intensity. Signals were amplified with an Axopatch 200B amplifier (Molecular Devices, Union City, CA) digitized by a Digidata 1322A interface (Axon Instruments, Molecular Devices, US) and sampled at 10 kHz. Recordings were acquired using Clampex (Molecular Devices) and analyzed with Clampfit (Molecular Devices). Experimenters were blinded to treatment groups for all the experiments.

Data Processing. Off-line data analysis of hippocampal and striatal basal synaptic transmission and synaptic plasticity was processed using Clampfit (Molecular Devices). For I/O data, fEPSPs slopes were measured at each intensity of stimulation (from 0 to 100 µA). Normalized fEPSP slopes were plotted against different intensities of stimulation. PPF measurements were normalized by setting the first fEPSP slope to 1 and comparing it with the

second fEPSP slope. LTP was measured as percent of baseline fEPSP slope recorded over a 10-min period before HFS delivery. This value was taken as 100% of the excitatory post-synaptic potential slope and all recorded values were normalized to this baseline.

HDACi assay. Hippocampal and striatal hemispheres, collected 1 hour after CFC consisting of three 2 sec foot shocks (0.8mA), were thawed and homogenized in RIPA buffer (150mM NaCl, 50mM Tris-HCl ph8, 0.1% SDS, 0.5% deoxycolate, 1% NP-40) on ice for 30 min. Proteins were then extracted from the nuclei by adding HDAC buffer (50mM Tris-HCl pH8, 137nM NaCl; 2.7mM KCl; 1mM MgCl2; 1mg/mL BSA) and sonicating at full strength for 15 min (Diagenode, Bioruptor Plus). Protein concentration was measured using a Bradford Assay and normalized so that all assay inputs contained the same amount of protein. Pan-HDAC enzyme activity was determined using the Fluor de Lys HDAC fluorometric activity assay kit (Enzo Life Science, BML-AK500) according to the manufacturer's protocol. Protein extracts were incubated with the Fluor De Lys Substrate for 30 min and then with the Fluor De Lys Developer for 15 min. Fluorescence intensity (380nm excitation; 510 nm emission) was measured on a the Infinite M200 Pro fluorometric reader (Tecan). Mice treated with VEH and not undergoing fear conditioning were considered as representing baseline HDAC activity (normalized to one-fold). Assays were run in triplicate from 3 independent experiments.

Western Blots. Animals underwent drug administration and subthreshold CFC as described above. Full hippocampi were dissected and flash frozen 1 h after CFC. Frozen hippocampal hemispheres were cut in half and homogenized and incubated for 30 min on ice in 500μl RIPA buffer (150mM NaCl, 50mM Tris-HCl ph8, 0.1% SDS, 0.5% deoxycolate, 1% NP-40) with 20μl 20x protease inhibitor (Complete mini, EDTA-free, Sigma Aldrich Cat#11836170001). Nuclei were collected by centrifugation (max speed, 20min, 4˚C) and cytoplasm (supernatant) was transferred to a new tube. The nuclear pellet was mixed with 50μl 1x Laemmli buffer, sonicated for 10 min at full power and boiled for 10min at 90˚C or until samples were no longer viscous. Protein quantifications were performed using a DC assay. For each sample, 10μg protein was added to SDS-PAGE gel (12.5% acrylamide in Resolving Gel and 4.5% in stacking gel) and run at 25A for ~1.5 h. Proteins were then transferred to nitrocellulose membrane for 2 h at 4˚C and blocked for 1 h in 5% milk in PBS-Tween20. Primary antibodies (1:2500 H4K12ac (ab46983), 1:500 H3K9ac (ab10812), and 1:5000 H3K27ac (ab4729) in 2% milk + PBS-Tween20) were incubated with the membrane overnight at 4˚C (except 1:5000 total H3 (ab1791), incubated for only 30 min at RT). Then membranes were washed 3x in TBS-Tween20 and secondary antibodies (1:10,000 Goat anti-rabbit in 2% milk) for 1 h at RT. Membranes were washed and incubated with chemiluminescent ECL Plus (GE Healthcare,

Cat# RPN2232SK) for 5 mins before visualization on the Fusion FX Vilber Laurmat imaging system. Due to similar sizes of histone markers, blotting was done separately and stripped between each antibody.
To quantify chemiluminescence, images were analyzed using “Set Measurements” in ImageJ. For each blot, percent of total luminescence was calculated for each band and normalized to the respective H3 total luminescence. Technical replicates (same samples, 2 western blots) were averaged together for each antibody and per biological replicate (6 replicates per treatment).

RNA-seq. *RNA extraction and library preparation.* Single frozen hippocampal and striatal hemispheres from four biological replicates (one mouse each) were isolated after CFC. Samples were homogenized and total RNA was isolated using Trizol Reagent (Life Technologies) according to the manufacturer’s protocol. RNA was further purified by an on-column DNAse digestion using the RNase-Free DNase Set (Qiagen, Cat# 79254) and two rounds of washes using the RNAeasy Mini Kit (Qiagen, Cat# 74106). Total RNA concentration was determined with the Nanodrop 1000 (v3.8.1, Thermo Fisher).
Libraries were prepared using the TruSeq Stranded mRNA Preparation Kit (Illumina) starting from 900ng of RNA. Libraries were quantified with the dsDNA HS Assay kit (Qubit, Cat# Q32851) and profile analysis was performed using the TapeStation (Agilent, TS4200) D500 Screen Tape System (Agilent, Cat#5067- 5588 and 5067-5589). Finally, libraries were multiplexed and sequenced across five lanes on the Illumina HiSeq 4000 (Illumina), yielding 100-bp, paired-end reads, at EPFL’s gene expression core facility.

RNA-seq analysis. Truseq adapter sequences were trimmed from raw FASTQ files using bcl2fastq (v2.20.0, Illumina). STAR (v2.6)(Dobin et al. 2013) was used to align FASTQ reads to the mouse mm10 reference genome with annotations downloaded from Ensembl release 93 (Zerbino et al. 2018). A custom R script was used to count reads mapping to the exonic regions of genes and to define transcript abundance. Reads were only considered if they overlap a single gene region. Differential expression and downstream analysis were performed using DEseq2 (Love, Huber, and Anders 2014) and custom R scripts. Genes were considered differentially expressed if they had an adjusted p-value ≤ 0.05 and a $|\log_2 FC| \geq 0.04$. For the trajectory analysis, all experimental groups were compared to samples coming from animals that were treated with the VEH and context paradigm (baseline). Genes were grouped into trajectory pathways using custom-written decision tree clustering in R.

Nuclear Extraction. Nuclear extraction was performed for both ChIP and single nuclear sequencing experiments. All steps of nuclear extraction were done on ice. First, frozen brain

tissue was homogenized in a douncer filled with 6ml Solution D (0.25M Sucrose, 25mM KCl, 5mM $MgCl_2$, 20mM Tris-HCl, pH 7.5). For snRNA-sequencing, 5μg/mL actinomycin D (Sigma, Cat# A4262) was added to Solution D to block transcription induced by disassociation. Samples were centrifuged for 1 min at maximum speed and pellets were resuspended in 4ml Solution D and 2ml Optiprep (Serumwerk Bernburg). Samples were then pelleted by centrifugation for 10 min at 3,200g, and the supernatant was discarded. Optiprep purification was performed a second time. After the final centrifugation, pellets were resuspended and filtered into 5ml polysterene tubes with filter (75mm) snap-caps (Corning, Cat# 352235). For the ChIP-seq experiments the final resuspension occurred in in PBS-T (0.1% Tween 20) and for snRNA-seq the final resuspension occurred in N-PBS (PBS, 5% BSA, 5μg/mL actinomycinD and 0.2U/μl RNAse inhibitor (Thermo Fisher Scientific, Cat#N8080119)).

ChIP-seq. *Nuclear sorting.* ChIP-seq was performed on 3 replicates per treatment and each replicate consisted of individually pooled dentate gyri from 5 mice. After nuclear extraction (see above), filtered nuclei were cross-linked by incubating with 1% formaldehyde (AppliChem, A08770) for 5 min at RT. Cross-linking was quenched with 125mM glycine (VWR, 101196X) and nuclear structural quality was assessed using an EVOS FL cell imaging system (Life Technologies).

For each sample, approximately 750,000 nuclei were resuspended in 500μl PBS-T (PBS, 0.1% Tween 20). Nuclei were stained with 1:50 Alexa Fluor488 conjugated anti-NeuN antibody (Millipore, MAB377X) for 30 min. Then nuclei were spun down (1250rcf, 4˚C, 5 min) and washed in PBS-T (0.1% Tween 20) twice. Finally, nuclei were resuspended and stored in 200μl PBS-T until sorting.

Flow cytometry was performed on the FACSAriaIII (BD Bioscience) by the EPFL Flow Cytometry Core Facility (FCCF). Before sorting, samples were passed through a 26G needle 5 times. Hoechst (1:1000) was mixed into each sample and incubated on ice for 10 mins. Debris was first excluded by gating using forward and side scatter pulse area parameters (FSC-A and SSC-A). Multiplets were then excluded by gating FSC-H vs. FSC-W and SSC-H vs. SSC-W. Single nuclei were sorted by Hoechst intensity, elicited by 405 nm wavelength excitation and measured at 425-475nm (450/50-A). Finally, NeuN+ nuclei were sorted into ice-cold Eppendorf tubes containing 100μl PBS-T.

Chromatin immunoprecipitation (ChIP). After sorting, nuclei were pelleted by centrifugation (4˚C, 1250g, 5 min) and lysed by incubating in 750μl RIPA buffer on ice for 10 min. Samples were sonicated on an E220 Focused-ultra-sonicator (Covaris) for 20 min (Peak power = 140W, Duty = 5, Cycle/Burst = 200). Sonication efficiency was measured by decrosslinking 125μl of

chromatin in 500μl of TL-Brain Buffer(10mM Tris-HCl pH7.5, 10mM EDTA 200mM NaCl), 50μl of 10% SDS and 1μl of RNAseA (Thermo Fisher, Cat#EN0531) and incubating at 65˚C and 650rpm overnight. 10μl recombinant, PCR-grade Proteinase K (Roche, Cat#03115828001) was added and incubated at 45˚C and 650 rpm for another hour. DNA was extracted with AcNH4 (100μl of 10M), 20μl glycogen (10μg/μl) and 1ml cold isopropanol and then pelleted by centrifuging at 14000rfc, 4˚C for 20min. DNA was further purified in 1ml 70% EtOH and centrifuged (14000rfc, 4˚C, 10 min). Sonicated DNA size was assessed on a 1.5% agarose gel.

The rest of the ChIP experiment (beginning from "Protein G Agarose Bead Preparation") was carried out using the reagents and protocols from the Low Cell ChIP-Seq Kit (Active Motif, 53084). In brief, 400μl of sonicated chromatin was first cleared by incubating with pre-cleared Protein G agarose beads for 2 h on a rotator at 4˚C. Half was kept as input for each sample. The other half was immunoprecipitated overnight at 4˚C with 3μl of H3K27ac (Abcam, ab4729). After precipitation, pre-cleared Protein G agarose were added for 3 h, and both input and IP samples were washed following the kit specifications. Cross-linking was reversed by incubating samples with 5μl 5M NaCl and 2μl proteinase K at 65˚C, 300rpm overnight. DNA was purified using phenol-chloroform.

Library preparation. To prepare libraries for both input and IP samples, the Next Gen DNA Library Kit (Active Motif, Cat# 53216) and Next Gen Indexing Kit (Active Motif, Cat# 53264) were used according to the manufacturer's instructions. After adaptor ligation, fragments were amplified (1 cycle, 30s at 98˚C; 14 cycles, 10s at 98˚C, 30s at 60˚C and 60s at 68˚C) and DNA was cleaned and purified using magnetic SPRI beads (Beckman Coulter, Ca# B23317). Libraries were resuspended in 25μl Low EDTA TE buffer and concentration was measured using a Qubit dsDNA HS Assay Kit. DNA fragments size was determined using a Fragment analyzer (NGS High Sensitivity kit (DNF-474), Agilent). Libraries were sequenced, paired-end, on the Illumina NextSeq 500 at EPFL's gene expression core facility**.**

ChIP-seq analysis. The Next Gen DNA Library Kit (Active Motif) includes molecular identifiers (MIDs), a 9-base random N sequence that is added with the P5 adaptor, to allow for removal of PCR duplicates from sequencing data. While R1 (75bp) contains the sequence information, R2 (9 bp) contains the MID information. To conserve MID information during mapping, the MID sequence from R2 was appended to the FASTQ header in R1 using a custom R-script. Adapter sequences and low quality regions from R1 were removed using Trimmomatic (v0.38)(Bolger, Lohse, and Usadel 2014) in single end mode with the following parameters: ILLUMINACLIP:Y2_adapter_seq.fa:0:6:6 SLIDINGWINDOW:10:20 MINLEN:36.

The processed FASTQ file (R1) was then aligned to the mm10 genome using Bowtie2 (v2.3.5)(Langmead and Salzberg 2012) in single-end mode and using default parameters. SAMtools (v1.9) (H. Li et al. 2009) was used to convert SAM files to BAM format and then to sort BAM files. PCR duplicated alignments were removed from the BAM files using a script provided by Active Motif. Finally, multi-mapping and low-quality reads ($\geq$ 40) were removed and BAM files were re-indexed using SAMtools.

Open chromatin peaks were defined using MACS2(Yong Zhang et al. 2008) in broad peak mode. Differentially acetylated regions (DARs) were identified using Diffbind (v2.16.2) (Stark and Brown 2011) and DEseq2 (v 1.28.1) (Love, Huber, and Anders 2014) with default parameters. Peaks were considered differentially enriched if they had a false discover rate (FDR) $\leq$ 0.05 and $|\log_2\text{FoldChange}| \geq 1$.

Since H3K27ac is a marker for both promoters and enhancers, ChromHMM (v1.22) (Ernst and Kellis 2012) was used to establish a chromatin state model that identified enhancers and promoters. The program was run, allowing for 8 states, on independently published ChIP-sequencing data (Halder et al. 2016), taken from bulk hippocampal tissue 1 h after CFC. We combined groups to get 5 chromatin states (control regions, repressed regions, promoter regions, poised enhancers and active enhancers) based on the combination of histone marks. This information was aligned with our own peak information to define differentially expressed enhancers and promoters. We assigned enhancers to genes using HOMER (v4.11) annotatePeaks.pl (Heinz et al. 2010). Downstream trajectory analysis was performed (as described in the *RNA-sequencing Analysis* section) separately for peaks in different chromatins states.

Single-nuclear RNA-seq. *Library Preparation.* For single-nuclear RNA-sequencing (snRNA-seq) animals were treated with either VEH or HDACi and exposed to CFC. For each sample, both hippocampal hemispheres from 5 mice were pooled into two replicates each of VEH and HDACi treated groups. Nuclear extractions were performed as described. Nuclear structural quality was checked using an EVOS cell imaging system and nuclei were counted and diluted to 1,000 nuclei/μl.

Library Sequencing. Library constructions were performed using Chromium SingleCell 3'Reagent Kit v3 chemistry (10x Genomics) according to the manufacturers protocol (CG000183 - Rev A). All 4 libraries were multiplexed to reduce batch effects and sequenced across 2 NextSeq 500 (v2.5) chips for 75 cycles. FASTQ files were generated using cellranger mkfastq (CellRanger v3.0.1), yielding an R1 length of 28nt and an R2 length of 56 nucleotides.

snRNA-seq analysis. To generate single cell feature counts cellranger count (CellRanger v3.0.1) was run to align FASTQ files to the mm10 pre-mRNA genome (created using cellranger mkref (CellRanger v3.0.1)) using the following settings: expect-cells=4000, chemistry = SC3Pv3, r2-length = 56. Downstream analysis was performed using custom R-scripts. Seurat (v4.0.3) (Hao et al. 2021) was used to calculate quality control metrics. DoubletFinder(McGinnis, Murrow, and Gartner 2019) was used to find and remove doublets and normalization and variance stabilization was done using SCTransform(Hafemeister and Satija 2019). Seurat was then used to perform UMAP and TSNE clustering, to define clusters using molecular identifiers. Replicate comparisons for both HDACi and Vehicle treated cells were performed by comparing cluster profiles and transcriptional differences between replicates (data not shown). Differential expression analysis between VEH and HDACi treated groups was performed for each cell type using the logistic regression framework, to remove batch effects between replicates, in Seurat's FindMarkers() command. Augur (Squair et al. 2021; Skinnider et al. 2021) was used with default commands to calculate perturbation prioritization for each cell type and scProportionsTest (S. A. Miller and Policastro 2020) to compute cell type composition changes between HDACi and VEH treated samples.

All in-house analysis code can be found at https://github.com/allie-burns/2021_Burns_etal.

KEGG pathway visualization. The *Mus musculus* MAPK KEGG pathway (mmu04010) was downloaded from the KEGG PATHWAY Database and drawn using the Bioconductor package, Pathview (Luo and Brouwer 2013). Differential expression of genes (or enhancers associated with genes) within this pathway are indicated by colors within each box representing a gene: the leftmost color is the log_2FC value for the active enhancers from the ChIP analysis; the middle color is the log_2FC for the bulk RNA-seq; and the right most color is the snRNA-seq. The pathway was manually redrawn for visualization purposes and simplified by only plotting MAPK subpathways containing at least one differentially acetylated or transcribed gene.

Statistics. Statistical details are included in the main text and figure legends, including *P*-values, statistical tests used, 'n's for each experiment, and a description of what 'n' refers to. Biological replicates refer to biological material from different mice or pools of mice and technical replicates refer to technical repetition using the same material from biological replicates.

2.1.5 Results

Systemic HDACi treatment enhances memory consolidation after subthreshold contextual fear conditioning

To investigate the mechanisms by which systemic HDACi treatment enhances fear memory, we treated mice with the HDACi CI-994, before subjecting them to a subthreshold contextual fear conditioning (CFC) task, a modified Pavlovian conditioning paradigm that, alone, does not induce memory formation (Parsons and Davis 2012). CI-994 is a class I HDACi that selectively impedes HDACs 1-3 (Bradner et al. 2010), promotes functional recovery after stroke (S. Li et al. 2019), and that has shown promise against cognitive dysfunctions in preclinical animal models (Gräff et al. 2014; Sada et al. 2020; Cooper et al. 2020). When i.p. injected it crosses the blood-brain-barrier and remains in the brain at concentrations greater than 1000nm for up to 5 hours (Gräff et al. 2014). One hour prior to CFC or Context only exposure (Context), mice were interperitoneally (i.p.) injected with 30mg/kg of CI-994 or its vehicle (VEH) (**Fig. 2.1A**). One day later, freezing was measured during a 3 min context exposure. We found that pairing the subthreshold CFC paradigm with the HDACi significantly improved memory retention ($P = 0.0002$) compared to the CFC paradigm alone ($P = 0.172$), and compared to HDACi treatment alone ($P = 0.997$, Tukey's HSD test following one-way ANOVA, $F_{(3,39)} = 10.16$, $P = 4.44e\text{-}05$) (**Fig. 2.1B**). There were no freezing differences between context and CFC exposure for VEH-treated animals ($P = 0.172$). Furthermore, HDACi treatment had no effect on speed ($F_{(3,39)} = 1.71$, $P = 0.18$) or distance travelled ($F_{(3,39)} = 1.69$, $P = 0.184$) and did not affect anxiety levels as measured by an open field test at the time of initial encoding ($F_{(3,39)} = 0.536$, $P = 0.66$) (**Supplemental Fig. 2.1**). These results indicate that the HDACi treatment can elevate an otherwise inefficient learning paradigm above threshold and lead to long-term memory retention.

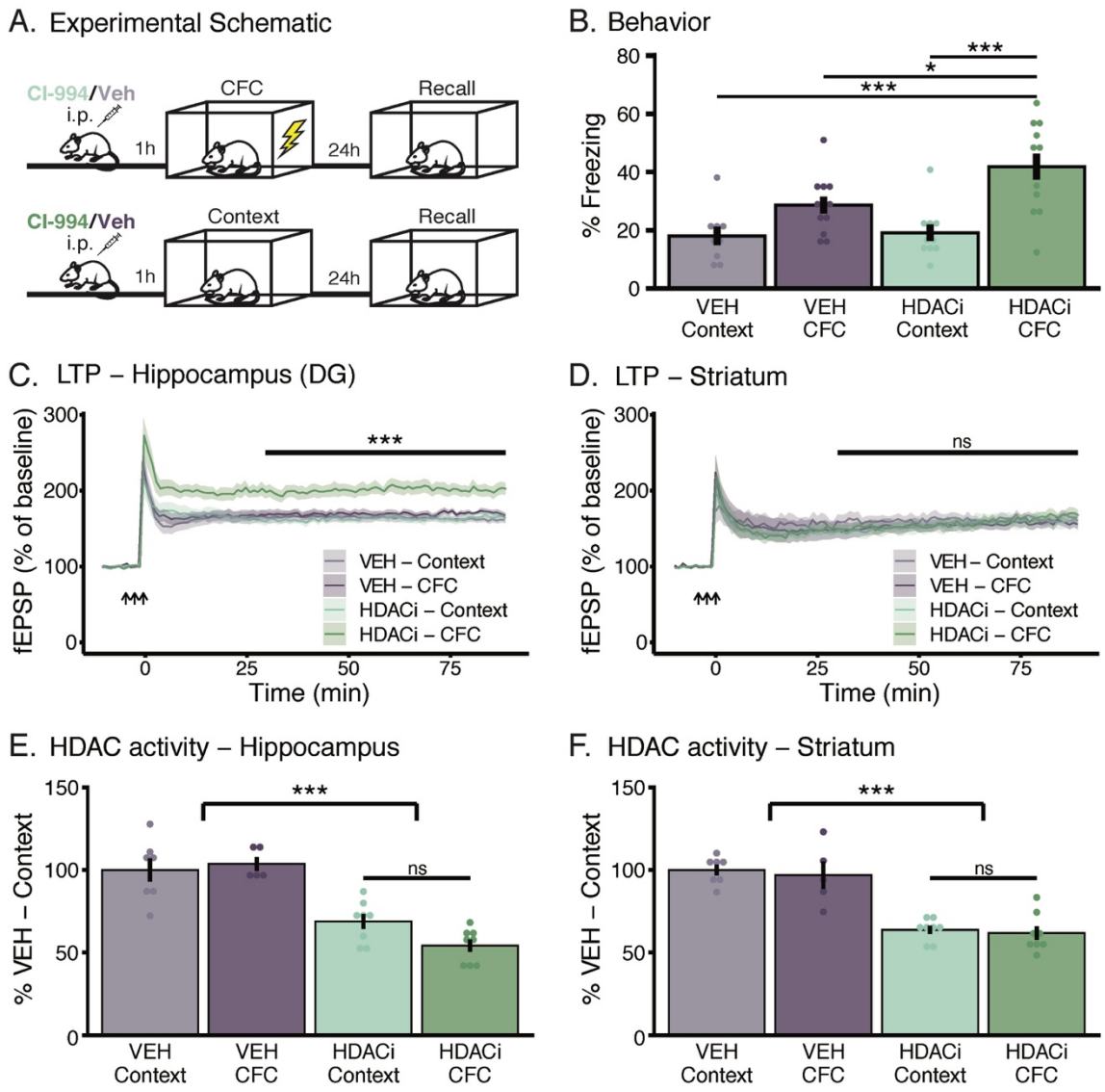


Figure 2.1 HDACi treatment enhances long term potentiation in the hippocampus, but not the striatum, despite reducing HDAC activity in both brain regions.
(A) Schematic representation of the behavioral paradigm for subthreshold contextual fear conditioning (CFC) (top) and Context only exposure (bottom). All animals received an i.p. injection of either vehicle or of the HDACi, CI-994 (30mg/kg). One hour later, animals underwent sub-threshold CFC (2x 0.2mA – 1s shocks) and fear memory was measured one day later by re-exposing animals to the conditioning context in the absence of the foot shock. **(B)** HDACi combined with CFC increases the percent of time spent freezing (> 1s) during 3-minute re-exposure to the conditioning chamber. n= 9-12 animals/group. **(C and D)** HDACi combined with CFC enhances LTP in response to 3 trains of high frequency stimulation (HFS – arrows) in the perforent pathway of the dentate gyrus **(C)** but not in the cortical-striatal pathway **(D)** one hour after conditioning. Statistical differences were calculated for the 30 minutes (end of short-term-potentiation) to 90 minutes (end of recording) for each mouse. n = 8 animals/group. **(E and F)** HDAC activity was reduced after HDACi in both the hippocampus **(E)** and striatum **(F)** with no further reduction in HDAC activity in response to CFC. One or two-way ANOVA with Tukey's HSD multiple-comparisons test was used for analysis. Graphs represent mean ± SEM. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Systemic HDACi treatment regulates long term potentiation in an activity-specific manner

To explore whether HDACi treatment improves memory via cognitive epigenetic priming, we first assessed its mode of action on synaptic plasticity. To this end, we measured the effects of HDACi on long term potentiation (LTP) in the hippocampus, a brain region activated by CFC (Phillips and LeDoux 1992), and the striatum, a brain region that is not directly involved in contextual memory formation (Ferreira et al. 2003) one hour after CFC. We found a significant increase in LTP at perforant path synapses of the dentate gyrus (DG) of the hippocampus when CFC was paired with the HDACi treatment (**Fig. 2.1C**; one-way ANOVA, $F_{(3,28)} = 10.57$, $P = 8.09e\text{-}05$). Without CFC, the HDACi had no effect on LTP; similarly, CFC alone did not facilitate LTP. Conversely, at cortico-striatal fibers, the HDACi treatment had no effect regardless of the behavioral paradigm ($F_{(3,28)} = 0.234$, $P = 0.872$) (**Fig. 2.1D**). Neither paired pulse facilitation (PPF) nor input/output (I/O) relationships were changed in either brain region (**Supplemental Fig. 2.2A-D**). Importantly, combining CFC with HDACi also enhanced LTP at Schaffer collaterals of the CA1, another hippocampal subregion (one-way ANOVA, $F_{(3,28)} = 5.213$, $P = 0.005$) both after sub-threshold CFC (**Supplemental Fig. 2.3**), and when using a stronger CFC paradigm (one-way ANOVA, $F_{(3,33)} = 3.663$, $P = 0.0221$) (**Supplemental Fig. 2.4**).

These findings indicate a brain area-specific effect of the HDACi treatment, with only brain areas engaged by CFC displaying enhanced synaptic plasticity. Interestingly, this brain region-specific effect on synaptic plasticity occurred in spite of the same degree of HDAC activity inhibition in both brain regions. HDAC activity was reduced by about 50% in both the hippocampus ($F_{(1,24)} = 60.15$, $P = 5.44e\text{-}08$) (**Fig. 2.1E**) and the striatum ($F_{(1,24)} = 68.96$, $P = 1.62e\text{-}08$) (**Fig. 2.1F**) in response to HDACi, with no difference in HDAC activity induced by learning itself. Thus, despite the same extent of HDAC inhibition induced by the HDACi, synaptic plasticity was only altered in the brain area directly engaged by CFC.

To confirm these findings in a different task, and to show that the HDACi does not only improve plasticity and performance in a hippocampus-specific manner, we also tested HDACi treatment during rotarod training, a motor skill learning task known to depend on the cortico-striatal pathway (Costa, Cohen, and Nicolelis 2004). Animals were i.p. injected with HDACi or VEH one hour before training (**Supplemental Fig. 2.5A**). We found that HDACi-treated animals were able to stay on the apparatus for longer than their VEH-treated counterparts (one-way ANOVA, $F_{(1,120)} = 12.155$, $P = 0.0007$) (**Supplemental Fig. 2.5B**), indicating improved motor learning. While neither training nor HDACi had any effect on hippocampal or

striatal LTP (**Supplemental Fig. 2.5C-D**), we found that HDACi paired with rotarod training selectively increased striatal PPF (two-way ANOVA, $F_{(3,192)}$ = 12.217, P = 2.37e-07) (**Supplemental Fig. 2.5E-F**), which is known to underlie motor learning in the striatum (Assous et al. 2019). In addition, there were no major differences in I/O in the striatum or the hippocampus (**Supplemental Fig. 2.5G-H**). These electrophysiological data are thus in support of the cognitive epigenetic priming hypothesis at the level of these two brain areas, insofar as the HDACi application *per se* did not yield any measurable difference, but necessitated task-specific neuronal activity to reveal its potentiating effect.

HDACi activates different transcriptional cascades in response to CFC in the hippocampus and striatum

To further understand the molecular mechanisms by which epigenetic priming leads to improved memory performance, we used bulk RNA-sequencing in the hippocampus and striatum to determine which genes are activated when CFC is combined with HDACi treatment. We chose to perform RNA-seq on the full brain regions both in order to compare to previously published characterizations of CFC (Halder et al. 2016) and because changes in synaptic plasticity occurred in both the DG (**Fig. 2.1C**) and the CA1 (**Supplemental Fig. 2.3)** of the hippocampus, For this, we extracted and sequenced total mRNA from whole-tissue homogenates one hour after CFC or context only exposure, using the same experimental setup as for the electrophysiological recordings **(Fig. 2.1A)**. The Illumina HiSeq4000 was used to generate four replicate libraries for each group with a minimum of 28M uniquely mapping paired reads per sample (**Supplemental Fig. 2.6A**). In total, 26,020 genes were expressed by greater than 1 count per million (CPM) in at least 4 of the libraries. Principal component analysis for the top 1000 most variable genes across all libraries revealed that 93% of the variance results from inter-brain region differences (**Supplemental Fig. 2.6B, C**).

In the hippocampus, consistent with previous data (Halder et al. 2016), we found no differentially expressed genes (DEGs) ($P \leq 0.05$; $|\log_2 FC| \geq 0.4$) between CFC and context only exposure in VEH-treated animals (**Fig. 2.2A, left panel**). Likewise, when comparing CFC with the context-only group in HDACi-treated animals, no DEGs were detected, indicating that subthreshold CFC alone is not sufficient to induce detectable transcriptional changes (**Fig. 2.2A, middle left panel**). Conversely, when context exposure was paired with CI-994, we found 1002 and 1679 genes significantly up- and downregulated, respectively, indicating that the addition of the HDACi alone alters the transcriptional landscape (**Fig. 2.2A, middle right panel**). When HDACi-CFC was compared to VEH-CFC, we detected 1336 up-regulated

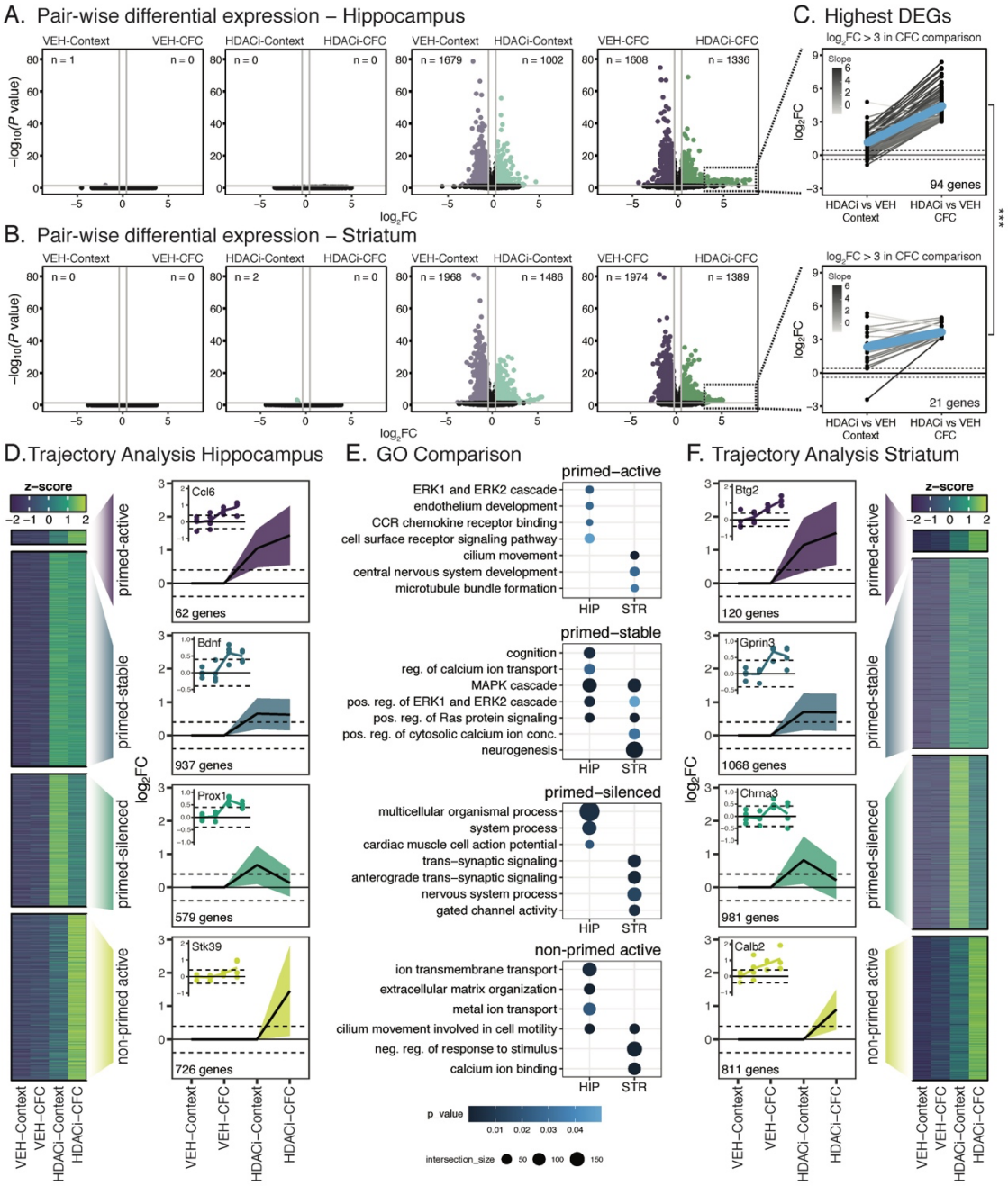


Figure 2.2 HDACi activates brain region-specific transcription in response to CFC.
(A and B) Volcano plots of magnitude of differential expression (log_2FC) versus statistical significance ($-log_{10}$ *P*-value) of pairwise comparisons labelled above each plot in the hippocampus **(A)** and striatum **(B)**. n-values in corners represent number of DEGs ($log_2FC \geq 0.4$; adjusted *P*-value ≤ 0.05) in the corresponding corner label. **(C)** Comparisons of DEGs that have ≥ 3 log_2FC in the HDACi-CFC compared to VEH-CFC (right column) in the hippocampus (top) and striatum (bottom). Log_2FC values are plotted for those same genes in the HDACi-Context compared to VEH-Context in the left column. Lines connect the same gene in each comparison and are colored by log_2FC difference between the two comparisons (slope). The blue line represents the average slope for each brain region. Student's t-test comparing slopes between hippocampus and striatum (right of plots). **(D)** Heat map of z-scores of average gene counts in the hippocampus (left). Line graphs in trajectory plots represent significant log_2FC values for each group (right). Count in lower left corner indicates number of genes in each cluster. Line plots shown as mean ±SEM. Insets represent DEGs from each cluster. Normalized counts for each replicate were compared to average normalized count for VEH-Context. **(E)** Gene ontology (GO) analysis of hippocampal (left) and striatal (right). **(F)** Gene cluster analysis for striatum RNA-seq data as in D. n = 4 biologically independent samples. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

genes, a 25% increase when compared to the context only contrast, but a similar number (1608) of downregulated genes (**Fig. 2.2A, right panel**).

In the striatum, there were no DEGs between the CFC and context only exposure in either the VEH or HDACi treated animals (**Fig. 2.2B, left panels**). Similar to the hippocampus, when HDACi-Context was compared to VEH-Context, 1486 and 1968 genes were significantly up- and downregulated (**Fig. 2.2B, middle right panel**). In contrast to the hippocampus, however, no further increase in the number of DEGs was detected when HDACi was paired with CFC (1389 genes were upregulated, and 1974 genes were downregulated) (**Fig. 2.2B, right panel**). Lists of pair-wise differential expression for both the hippocampus and striatum can be found in **Supplemental Table 1**.

When focusing on strongly up-regulated genes ($P \leq 0.05$, $\log_2FC \geq 3$), we detected 4.5x more genes in the hippocampus than in the striatum (**Fig. 2.2C**). Furthermore, these genes were more strongly activated in the hippocampus than in the striatum (Student's t-test of slope values, P = 2.304e-05) (**Fig. 2.2C**). The strongly upregulated hippocampal genes (**Supplemental Table 2**) were enriched in ion-transport ontologies and included transthyretin, *Ttr*, which has been shown to provide neuroprotection in aged mice and to be associated with enhanced memory (Liz et al. 2020; Brouillette and Quirion 2008), and synaptotagmin 13 (*Syt13*), a gene previously shown to be up-regulated after CFC (S. Han et al. 2012). In contrast, the striatal genes were primarily predicted genes (**Supplemental Table 2**) and GO analysis did not yield any enriched pathways. This indicates that in the hippocampus, the expression of these genes is further enhanced when the HDACi is paired with CFC, while pairing HDACi with CFC had no such effect in the striatum.

Next, we set out to identify transcriptional patterns by selecting genes that were differentially expressed ($P <= 0.05$, $\log_2FC >= 0.4$) when compared to the baseline group (VEH-Context). For this, all DEGs underwent decision tree clustering as described in the materials and methods. Considering that we aim to specifically understand the targets of epigenetic priming, we focused on genes that are up-regulated by HDACi treatment in our analyses, however the other clusters, including down-regulated ones, as well as their associated genes, can be found in **Supplemental Fig. 2.7** and **Supplemental Table 3**. Four major clusters were identified as trajectories of interest (**Fig. 2.2D**). In the hippocampus, we found 1) 62 genes that were up-regulated by the HDACi treatment alone (i.e., in the HDACi-VEH group) and further increased when HDACi was paired with CFC (i.e, the HDACi-CFC group), which we termed *primed-active*; 2) 937 genes that were increased by HDACi treatment but showed no further CFC-driven increase, which we termed *primed-stable*; 3) 579 genes that were enriched by HDACi

treatment, but were reduced when the HDACi was paired with CFC, which we termed *primed-silenced*; 4) and 726 genes that were only activated when combining HDACi with CFC, but not by either condition alone, which we termed *non-primed active* (**Fig. 2.2D**). In the striatum, the order of magnitude of DEGs was similar (**Fig. 2.2F**). There were 120 genes in the *primed-active* cluster, 1068 genes in the *primed-stable* cluster, 981 genes in the *primed-silenced* cluster and 811 genes in the *non-primed active cluster* (**Fig. 2.2F**).

We next performed a Gene Ontology (GO) analysis (**Fig. 2.2E**) to identify enriched pathways in each cluster in both the hippocampus and the striatum. In the hippocampus, the *primed-active* cluster was enriched for the Erk1 and Erk2 cascade, which has been implicated in synaptic plasticity as well as learning and memory (Peng et al. 2010; Davis et al. 2000). This cluster included cytokine genes, such as *Ccl6* (**Fig. 2.2D**), which is involved in the p38-MAPK pathway (Hsu et al. 2013; Dolgachev et al. 2007) and which plays a role in cell survival (Koul, Pal, and Koul 2013). Conversely, in the striatum, the *primed-active* cluster was not enriched for any ontologies involved in MAPK/ERK signaling or learning and memory.

Furthermore, the *primed-stable* cluster was characterized by learning and memory-related pathways such as cognition and regulation of calcium ion transport in the hippocampus, but not the striatum (**Fig. 2.2E**). Hippocampal DEGs in this cluster included brain derived neurotrophic factor (*Bdnf*) and the proto-oncogene Jun (*Jun*), both immediate early genes (IEGs) induced by neuronal activity and implicated in synaptic plasticity as well as learning and memory. *Bdnf* plays a critical role in hippocampal CFC (J. Liu et al. 2017) and enhances synaptic strength at the Schaffer collateral-CA1 synapses (Kang and Schuman 1995), while *Jun* is a member of the AP-1 transcriptional activator complex, which binds enhancers and regulates chromatin opening during CFC (Su et al. 2017; Fernandez-Albert et al. 2019). In the striatum, this cluster did not include memory-related IEGs. It did, however, contain pathways involved in intracellular signal transduction that also regulate learning and memory (Levenson et al. 2004; Adams and Sweatt 2002) such as the “MAPK cascade”, “Ras protein signal transduction” and “Erk1 and Erk2 cascade”. This comparison stipulates that HDACi similarly primes the MAPK pathway in both the hippocampus and the striatum but further potentiates only the *primed-active* genes in the hippocampus.

In the *primed-silenced* or *non-primed active* states, no ontologies associated with synaptic signal transduction were found (**Fig. 2.2E**). Finally, the hippocampal *non-primed active* cluster, represented by genes that are only transcribed after combined HDACi-CFC, is enriched for “metal ion transport” and “ion transmembrane transport” pathways, while in the striatum, it is enriched for genes involved in a “negative response to stimulus”. This could indicate that the

combination of HDACi treatment and CFC increases inhibitory signaling in the striatum, possibly related to decreased motor response conditioning**.** Of note, none of the clusters in which HDACi reduced transcription included pathways that are involved in learning and memory or synaptic plasticity in either the hippocampus or the striatum. Taken together, these results illustrate that HDACi treatment, and not CFC, is the major driver of differential transcription between the hippocampus and the striatum. It enhances the Mapk/Erk signaling pathway in both the hippocampus and the striatum as seen in the comparisons of the *primed-stable* groups, but is able to further induce learning-specific genes in the *primed-active* state in the hippocampus when paired with CFC, but not the striatum.

HDACi activates different transcriptional cascades across cell types within the hippocampus.

Next, we aimed to understand which cell types within the hippocampus are most responsive to HDACi treatment. To do so, we used snRNA-seq on isolated hippocampi from animals that were treated with either HDACi or VEH one hour before undergoing CFC. Since transcriptional differences were most prominent in the HDACi-CFC versus the VEH-CFC groups (**Fig. 2.2C**), we focused on only this comparison. We performed dimensionality reduction using uniform manifold approximation and projection (UMAP) and clustered nuclei by the k-nearest neighbors. We removed clusters containing fewer than 50 nuclei, revealing 30 distinct clusters consisting of 15,339 total nuclei and expressing a total of 24,271 genes (**Supplemental Fig. 2.8A**). These clusters were then assigned to known cell types by comparing expression of cell-type specific genes taken from previously published snRNA-seq datasets (Zalocusky et al. 2021; Jaeger et al. 2018; Hrvatin et al. 2017; Ye Zhang et al. 2014) and the Allen Brain Atlas (Lein et al. 2007) (**Supplemental Fig. 2.8B**). This analysis identified 10 distinct cell types: 4 clusters of excitatory neurons that split based on location within the hippocampus (5175 DG nuclei, 2871 CA1 nuclei, 1657 CA3 nuclei, and 507 nuclei with no location marker); 1 cluster of 794 inhibitory neurons; 4 glial clusters (1960 Oligodendrocytes, 254 oligo-precursors, 763 astrocytes and 880 microglia); and a final cluster of 478 nuclei (NA) which could not be assigned to a single cell type based on its expression profile (**Fig. 2.3A and Supplemental Fig. 2.8C**). In line with previous work (Zeisel et al. 2015), neuronal clusters had more expressed genes than glial clusters (**Supplemental Fig. 2.8D**) and the proportions of cell types were similar to those reported for the hippocampal region in the Blue Brain Atlas (Erö et al. 2018) (**Supplemental Fig. 2.8E**).

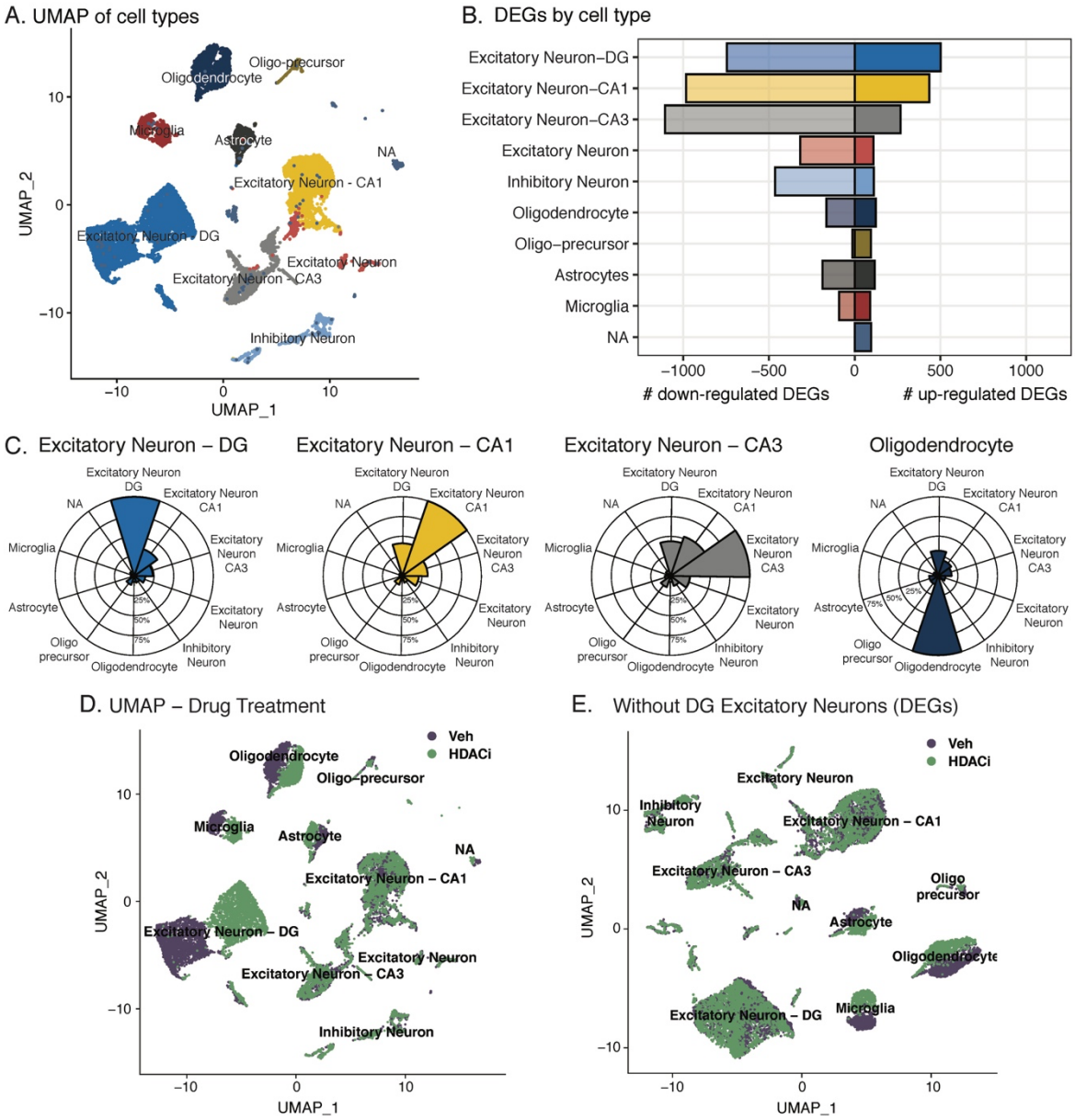


Figure 2.3 HDACi activates different transcriptional cascades within hippocampal cell types. **(A)** Uniform manifold approximation and projection (UMAP) visualization of 15,339 nuclei from the full hippocampus colored by 10 identified cell-types. NA refers to nuclei that could not be assigned a cell type based on expression of marker genes. **(B)** Number of up-regulated (right; $log_2FC \geq 1$; FDR ≤ 0.05) and down-regulated (left; $log_2FC \leq -1$; FDR ≤ 0.05) genes in each cell type when comparing HDACi-CFC to VEH-CFC. **(C)** Radar plots showing overlap of up-regulated genes across cell types. (Left) Percent overlap of Excitatory Neurons - DG with others. (Middle left) Percent overlap of Excitatory Neurons – CA1 with other clusters. (Middle right) Percent overlap of Excitatory Neurons – CA3 with other clusters. (Right) Percent overlap of Oligodendrocytes with other clusters. **(D)** UMAP visualization of nuclei from the full hippocampus colored by sample drug treatment. **(E)** UMAP visualization, colored by drug treatment, after removing the 501 up-regulated genes in the DG excitatory neurons and re-clustering. n = 2 biological replicates per group (HDACi-CFC and VEH-CFC).

We then explored whether pairing CFC with HDACi induces distinct responses across cell types. Augur, a tool that prioritizes a population's responsiveness to an experimental perturbation (Squair et al. 2021), reported a similar global responsiveness for all clusters (**Supplemental Fig. 2.9A**), and with the exception of oligo-precursors, HDACi treatment did not significantly change cell type composition within clusters (**Supplemental Fig. 2.9B**). However, HDACi treatment differentially regulated a distinct set of genes in each cell type, with excitatory neurons having the largest HDACi response (**Fig. 2.3B, Supplemental Fig. 2.9C** and **Supplemental Table 4**). These DEGs were highly cluster specific: We found that excitatory neurons of the DG share 36% and 24% of their up-regulated DEGs with excitatory neurons of the CA1 and CA3, respectively, and fewer than 15% with each of the other cell types (**Fig. 2.3C, left panel**). This low overlap of HDACi induced up-regulation was also seen in other cell types (**Fig. 2.3C** and **Supplemental Fig. 2.9D**). Among excitatory neurons, 45%, 38% and 27% of genes were uniquely up-regulated in the DG, CA1 and CA3, respectively, while among glial cells, 68%, 53% and 47% were uniquely upregulated among microglia, astrocytes and oligodendrocytes, respectively (**Supplemental Fig. 2.9E)**. Down-regulated genes also appeared to be cluster specific, although to a lower magnitude than the up-regulated genes (**Supplemental Fig. 2.9F**).

Interestingly, we found an HDACi-specific separation for excitatory neurons in the DG and for glia, but not for any other cluster (**Fig. 2.3D**). This split was mainly mediated by the upregulated genes within the DG, as removing those genes and re-running the dimension reduction re-merged the split DG cluster (**Fig. 2.3E**). Conversely, there was no cluster re-merging when removing up-regulated DEGs from CA1, glia or from any other cell type (**Fig. 2.3F and Supplemental Fig. 2.10A**). Furthermore, DG cluster re-merging was specific to the up-regulated genes, as removing only downregulated genes had no effect (**Supplemental Fig. 2.10B**). Together, these results provide supporting evidence that pairing CFC with HDACi treatment transcriptionally activates different gene sets across cell types, with a particularly strong response among upregulated genes in the DG. For this reason, we continued our analysis of epigenetic priming by focusing on excitatory neurons of the DG.

HDACi combined with CFC enriches H3K27ac at genes involved in synaptic communication.

Given the strong up-regulation of genes involved in excitatory neurons of the DG, we characterized histone acetylation in this region by chromatin immunoprecipitation followed by sequencing (ChIP-Seq). We focused on H3K27ac, a known marker of active enhancers that

is enriched at activity-dependent regulatory elements after neuronal activation (Tyssowski et al. 2018; Chen et al. 2019; Malik et al. 2014; Halder et al. 2016; Fernandez-Albert et al. 2019), correlates with gene transcription (Creyghton et al. 2010; Halder et al. 2016) and often co-occurs with H3K9ac, a marker of active promoters (Coda et al. 2017). Furthermore, in line with previous studies (Gräff et al. 2014; Cooper et al. 2020; Zhou et al. 2018; Jin et al. 2018), we found that HDACi treatment increased global H3K27ac, alongside H3K9ac and H4K12ac as revealed by western blotting (Two-way ANOVA, $F_{(3,60)} = 22.47$, $P = 1.11e\text{-}13$) (**Supplemental Fig. 2.11**). For ChIP-seq, we had 3 replicates, each from the pooled DG from 5 mice and sorted postmitotic neuronal nuclei (NeuN+) by fluorescence activated nuclear sorting (FANS) (**Fig. 2.4A** and **Supplemental Fig. 2.12**). Libraries for the H3K27ac- immunoprecipitated samples were prepared and processed as described in the materials and methods.

Differential enrichment analysis (Diffbind, DEseq2, data in **Supplemental Table 5**) revealed that CFC in VEH-treated animals led to only marginal changes in H3K27ac enrichment (**Fig. 2.4B**), in line with the transcriptional data (**Fig. 2.2A**). Conversely, when CFC occurred in the presence of the HDACi, more then 10,000 and 15,000 regions were significantly down and up-regulated, respectively, indicating that in the presence of HDACi, the behavioral paradigm *per se* can trigger substantial epigenetic changes. Furthermore, the HDACi treatment itself also enriched a significant number of regions – approximately 10,500 regions in both context and CFC treated groups (**Fig. 2.4B**, **right plots**) – suggesting that both CFC and HDACi treatment alter H3K27ac enrichment. This data is in contrast with the transcriptional results, in which only HDACi treatment, and not the behavioral condition alone, induced transcriptional changes. In addition, while there was equal down and up-regulation of transcription after HDACi treatment (**Fig. 2.2A, right plots**), we see a higher amount of H3K27ac accumulation after HDACi (**Fig. 2.4, right plots**).

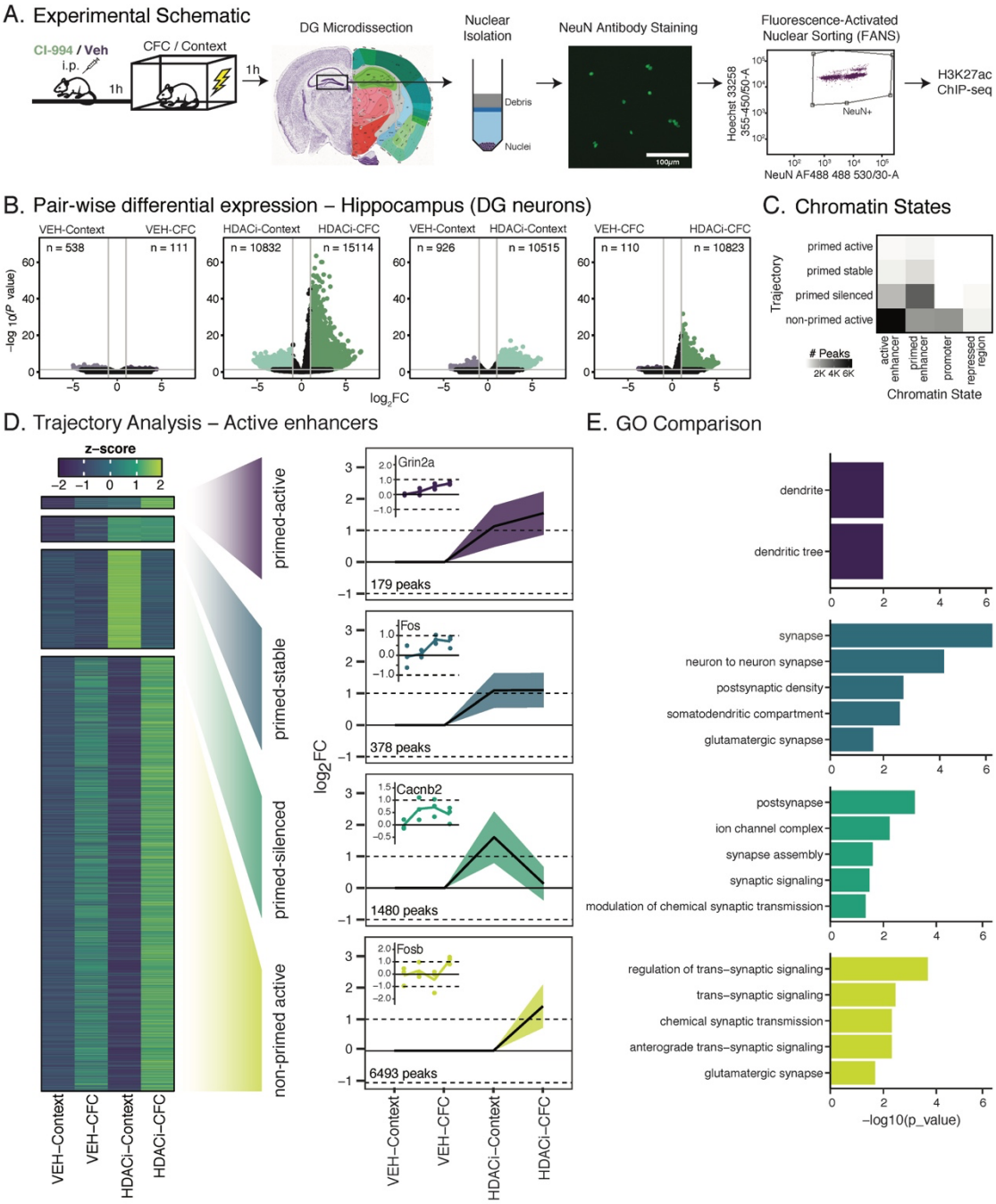


Figure 2.4 HDACi enriches H3K27ac at genes involved in neuronal synaptic communication. **(A)** Schematic of experimental outline. **(B)** Volcano plots showing the magnitude of differential H3K27ac enrichment (log_2FC) versus statistical significance ($-log_{10}$ *P*-value) for pairwise comparisons (labelled above each plot) for each peak. n-values in corners represent the number of peaks that are enriched ($log_2FC \geq 1$; adjusted *P*-value ≤ 0.05). *P*-values were calculated by the Wald test and corrected for multiple comparisons using FDR. **(C)** Heat map representing number of peaks that are in the trajectories of interest (y-axis) and in each chromatin state (x-axis). **(D)** Heat map of z-scores of the average normalized H3K27ac peak counts for all 4 clusters of interest. Peak sets underwent decision tree clustering based on significant log_2FC values for associated peaks in each group when compared to VEH-Context. Line graphs in trajectory plots represent significant log_2FC values for each group in clusters of interest. Count in lower left corner indicates number of peaks. Line plots shown as mean ± SEM. Insets represent differentially enriched active enhancer peaks from each cluster. Normalized counts for each replicate were compared to average normalized count for VEH-Context. (E) Gene ontology (GO) analysis of genes associated with H3K27ac peaks. n = 3 biologically independent samples. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

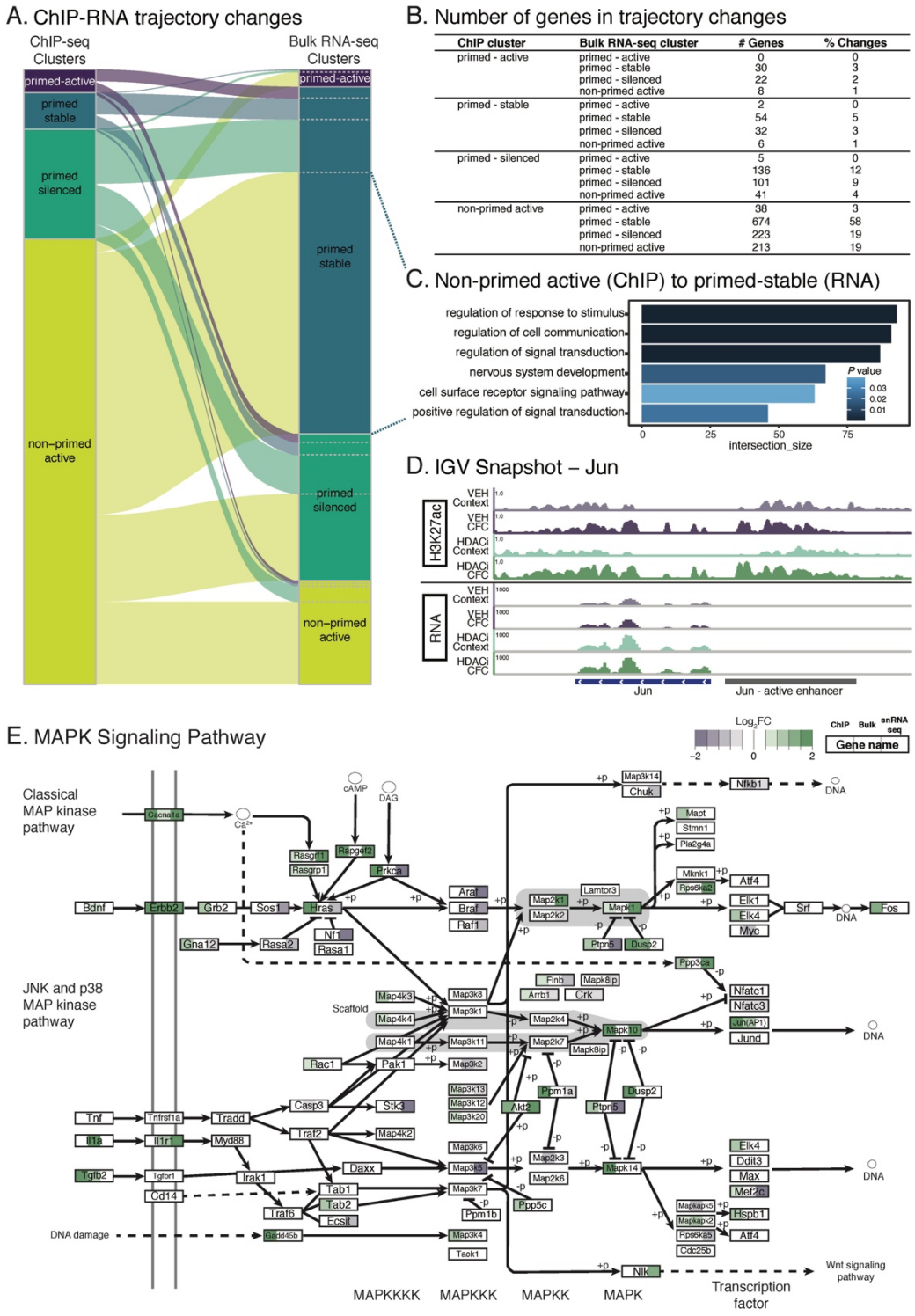
In order to determine the chromatin state and the corresponding gene for each H3K27ac peak, we used ChromHMM (Ernst and Kellis 2012) on previously published histone post-translational modifications (PTMs) from bulk hippocampal tissue collected after CFC (Halder et al. 2016). The entire mouse genome was assigned to one of five chromatin states: Control regions; repressed regions; promoter regions; poised enhancers; and active enhancers (**Supplemental Fig. 2.13A**). We calculated the state overlap for each peak and assigned the peak to the state that covered the highest proportion (**Supplemental Fig. 2.13B**). Doing so, 70.5% of bases assigned as active enhancers in ChromHMM were enriched for H3K27ac in our dataset; 44% and 34.9% of bases assigned as poised enhancers promoters, respectively, were also enriched for H3K27ac, while only 2.8% and 2.9% of control regions and repressed regions had H3K27ac peaks (**Supplemental Fig. 2.13C**).

Next, we performed a decision tree analysis for each chromatin state, focusing on the same four trajectories as before: *primed-active*, *primed-stable*, *primed-silenced* and *non-primed active* (**Fig. 2.2D-F**). Since active enhancers contained the largest number of peaks (**Fig. 2.4C**), we chose to analyze this subset of peaks in depth (**Fig. 2.4D**), but other chromatin states are included in **Supplemental Figure 2.14**. The *primed-active* cluster for active enhancers was the smallest, containing 179 peaks (**Fig. 2.4D**). This cluster represented ontologies associated with dendritic locations in the cell (**Fig. 2.4E**) and included peaks associated with NMDA receptor 2A (*Grin2a*) and Calcium Voltage-Gated Channel Subunit (*Cacna1e*). In the *primed-stable* cluster, there were 378 active enhancers for which H3K27ac was increased after HDACi treatment but not further enriched after CFC (**Fig. 2.4D**). This cluster included ontologies specific to synaptic locations, and a previously described enhancer of *cFos*, whose regulation by histone acetylation was recently validated by targeted dCas9-p300 manipulations (Chen et al. 2019). The 1480 enhancers of the *primed-silenced* cluster were also associated with genes that are involved in synaptic assembly and signaling, although noticeably fewer enhancers were associated with IEGs (**Supplemental Table 6**). Finally, the *non-primed active* cluster was the largest and contained 6493 active enhancer peaks, the ontologies for which were also associated with regulation of synaptic signaling. This cluster included enhancers for many genes that are specific for memory and synaptic plasticity: for example, *Fosb*, *Jun*, *Junb* and *JunD*, which are members of the AP-1 complex and known to be involved in neuronal plasticity processes during CFC (Su et al. 2017; Fernandez-Albert et al. 2019); calcium dependent protein kinases, which are crucial for signaling at glutamatergic synapses (Hinds et al. 2003); and genes in the MAPK/ERK signaling cascade, which regulates H3 acetylation during CFC and helps to establish the stabilization of long-term memory (Levenson et al. 2004; Adams and Sweatt 2002; Davis et al. 2000).

Taken together, these data show that HDACi-induced H3K27ac enrichment after context or CFC is highly specific to neuronal signaling processes. However, in contrast to the RNA-seq data, the H3K27ac enrichments appear to be most relevant in the *non-primed active* cluster, indicating that it is most responsive to combined HDACi and CFC treatment, which closely resembles the behavioral and electrophysiological results. This is interesting insofar as we would expect changes in acetylation, or our priming step, to be relevant in all HDACi treated groups, but transcriptional activation to be more specific to the paired HDACi and CFC experiments. Thus, to better understand this disconnect between transcriptional activation and acetylation enrichment, we directly compared which genes are both enriched and activated and which genes are only enriched for H3K27ac at enhancer regions.

Transcriptional activation and H3K27ac accumulation occur at genes involved in synaptic communication.

To better understand the relationship between HDACi-induced epigenetic changes and transcriptional activation after CFC, we related H3K27ac accumulation at active enhancers to the expression changes of their associated genes. Doing so, we found that multiple genes underwent a change in their trajectory (**Fig. 2.5A, B**). The most pronounced trajectory change occurred for genes associated with active enhancers that were in the *non-primed active* cluster in the ChIP dataset, of which 58% changed to being transcriptionally activated by HDACi regardless of whether CFC had occurred or not (*primed-stable*). This indicates that, while CFC was needed to drive their acetylation changes, CFC was no longer required for their transcription changes. These genes were enriched for ontologies including “positive regulation of signal transduction” and “nervous system development” (**Fig. 2.5C**), whereas ontology analysis for genes that switched between other clusters did not yield any significant hits. Genes in this group included several voltage-gated potassium channels such as *Kcna1*, the transcription factor *Neurod2*, which is crucial for fear learning (Lin et al. 2005), as well as the IEG and AP-1 complex member, *Jun* (**Fig. 2.5D**). In addition, the *non-primed active* enhancer to *primed-stable* transcriptional cluster switch was enriched for various genes belonging to the MAPK signaling pathway such as Mapk4, Jun and Rapgef2 (**Fig. 2.5E and Supplemental Tables 3 and 6**).



ChIP cluster	Bulk RNA-seq cluster	# Genes	% Changes
primed - active	primed - active	0	0
	primed - stable	30	3
	primed - silenced	22	2
	non-primed active	8	1
primed - stable	primed - active	2	0
	primed - stable	54	5
	primed - silenced	32	3
	non-primed active	6	1
primed - silenced	primed - active	5	0
	primed - stable	136	12
	primed - silenced	101	9
	non-primed active	41	4
non-primed active	primed - active	38	3
	primed - stable	674	58
	primed - silenced	223	19
	non-primed active	213	19

Figure 2.5 Overlap between HDACi-induced acetylation and transcriptional changes.
(A) Sankey plot showing the change in trajectory association for the 1585 active-enhancer associated genes present in both the ChIP (left) and bulk RNA-seq (right) and in the trajectory clusters of interest. **(B)** Number of genes changing between the ChIP-seq clusters and the bulk RNA-seq clusters. **(C)** Gene ontologies for the 674 genes in the ChIP non-primed active cluster shifted to the RNA-seq primed-stable cluster. **(D)** Example genome track of the H3K27ac (top 4 tracks) and mRNA expression (bottom 4 tracks) for Jun. Jun's active enhancer is labelled in grey and the Jun gene is labelled in blue. **(E)** MAPK signaling KEGG pathway visualization. Colors in each box represent significant log_2FC values for the HDAC-CFC compared to the VEH-CFC comparison in the ChIP (left color), bulk RNA (middle color) and snRNA-sequencing (right color).

When comparing H3K27ac enrichment to the transcriptional activation in single nuclei of the DG after combined HDACi-CFC (**Supplemental Fig. 2.15A**), we found that only 199 of the 4594 genes were up-regulated in both analyses after combined HDACi-CFC treatment **(Supplemental Fig. 2.15B**). Despite this being a small subset of the total number of genes, which is likely due to technical differences between the bulk and single nuclei preparations, these genes were relevant to learning and memory in that they included NMDA receptors (*Grin2a* and *Grin2b*), a calcium voltage gated ion channel (*Cacna1e*) and, again, members of the MAPK pathway including *Mapk10* and Ras-guanine-nucleotide releasing factor 1(*Rasgrf-1*) (**Fig. 2.5E**), all of which contribute to glutamatergic synapse communication.

Lastly, when comparing all three datasets together, namely enhancer acetylation, bulk and snRNA-seq transcriptional changes, the MAPK pathway emerged as being predominantly activated (**Fig. 5E**). ERK-mediated MAPK pathway is necessary for memory consolidation (Atkins et al. 1998; Blum et al. 1999) and, once activated, ERK phosphorylates protein targets that are implicated in gene transcription, protein synthesis and synaptic plasticity (Davis et al. 2000), as well as histone acetylation (Levenson et al. 2004). In addition to the MAPK-pathway, 18 genes were increased after combined HDACi-CFC in both the snRNA-seq and bulk-seq and had increased enhancer H3K27ac (**Supplemental Table 7**). Interestingly, two of these genes, autism susceptibility candidate 2 (*Auts2*) and cortactin binding protein 2 (*Cttnbp2*) protect against autism like behavior and impaired object recognition memory (Hori et al. 2015; Shih et al. 2020), while the rest did not seem related to synaptic signaling. Taken together these data suggest that genes involved in synaptic communication and MAPK pathway signaling are epigenetically and transcriptionally activated by HDACi, which suggests that these pathways underlie HDACi-mediated memory enhancement.

2.1.6 Discussion

In this study, we aimed to determine the mechanisms by which HDACi application facilitates memory formation, and thereby to assess the concept of “cognitive epigenetic priming”. We found that the HDACi, CI-994, improves behavioral responses to a subthreshold CFC paradigm (**Fig. 1B**) and following rotarod training (**Supplemental Fig. 5B**), regulated by the hippocampus and striatum respectively. In both behavioral paradigms, CI-994 selectively enhanced unique aspects of synaptic communication within each brain region (**Fig. 1C** and **Supplemental Fig. 5F**) despite these brain areas showing comparably reduced HDAC activity (**Fig. 1E-F**). At the molecular level, HDACi treatment transcriptionally activated distinct gene subsets in each brain region (**Fig. 2**) and between different cell types within the hippocampus (**Fig. 3**). Finally, in DG neurons, HDACi treatment enriched H3K27ac at the enhancers of

genes associated with synaptic function (**Fig. 4**), particularly at those involved in MAPK signaling (**Fig. 5**). Together, these findings indicate that CI-994 – although applied systemically – results in brain region, cell type and pathway-specific effects.

As these effects were predominantly observed when HDACi treatment was combined with CFC, but not by either paradigm alone, they support the notion that CI-994 at least partly acts via "cognitive epigenetic priming" (Gräff and Tsai 2013; Burns and Gräff 2021). This model has been inspired by evidence from cancer research, where HDACi application – inherently devoid of target specificity – improves the efficacy of ongoing cancer treatments, while *per se* having no beneficial effects (Scandura et al. 2011; Terranova-Barberio et al. 2017). Analogously, here, we found the HDACi application itself to have minimal effects; but when applied jointly with subthreshold CFC, the HDACi treatment elicited electrophysiological, transcriptional and epigenetic changes that paralleled the improved memory performance.

The brain region-specific electrophysiological effects likely occur because the HDACi treatment reinforces behaviorally relevant cellular pathways per brain area. When paired with CFC, HDACi specifically enhances hippocampal LTP, which is known to underlie contextual fear learning (Bliss and Collingridge 1993; Sacchetti et al. 2002; Whitlock et al. 2006; Nabavi et al. 2014); whereas when paired with rotarod training, HDACi enhances cortico-striatal PPF, which is known to underlie motor learning (Assous et al. 2019; Haber 2016; Cataldi et al. 2021). This specialization is further supported by the differential transcriptional programs activated in the hippocampus and striatum. While HDACi addition enriched the MAPK pathway in both brain regions irrespective of whether the animals were fear conditioned or only context exposed, the learning and memory-related ERK1 and ERK2 cascade as well as *Bdnf* and *Jun*, which are both involved in MAPK/ERK signaling pathway (Peng et al. 2010; Sütterlin et al. 2013; Revest et al. 2014; Chen et al. 2019), were only enriched in the hippocampus in combination with contextual learning. This suggests that HDACi generally targets the MAPK pathway but that, when paired with CFC, it leads to a further transcriptional enhancement thereof.

At the epigenetic level, we found a matching correlation between improved contextual memory formation, hippocampal LTP and enhancer H3K27ac enrichment when HDACi treatment was paired with CFC (**Fig. 4**). But even after HDACi treatment alone, we observed H3K27ac-enriched pathways to be mainly associated with synaptic functions. Interestingly, past results have indicated that either HDACi (Lopez-Atalaya et al. 2013) or CFC alone (Halder et al. 2016)

enrich histone acetylation at regions that were already acetylated in baseline conditions. This suggests that the HDACi – although broadly inhibiting HDAC activity – acts by reinforcing acetylated regions, which is likely, given that HDACs are known to be predominantly recruited to and act upon previously activated chromatin regions (Z. Wang et al. 2009). Furthermore, H3K27ac enrichment also occurred at enhancers of the MAPK pathway (**Fig. 5**), which expands on previous findings linking HDACi treatment to this pathway (Levenson et al. 2004; Chwang et al. 2007), and testifies to the importance of H3K27ac-induced epigenetic priming for improved memory performance.

At the same time, we observed that H3K27ac changes were not always translated into transcriptional changes (**Fig. 5**), which indicates such gene activation to be independent of H3K27ac priming at this time post-learning. This observation bears striking resemblance to a recent study which described an initial increase in engram enhancer accessibility following CFC, which was not yet paralleled by transcriptional changes, but only after several days post-conditioning (Marco et al. 2020). In turn, this stipulates that HDACi-induced epigenetic priming might become more important at later stages of memory consolidation. Alternatively, the apparent de-coupling between H3K27ac and the transcriptional changes implies that these changes also rely on other epigenetic modifications. Indeed, several studies have shown that general chromatin rearrangements, a product of combined histone post translational modifications and DNA methylation changes, are necessary for memory formation and occur soon after CFC (C. A. Miller, Campbell, and Sweatt 2008; Gupta et al. 2010; Halder et al. 2016; Duke et al. 2017; Pearce et al. 2017; Su et al. 2017; Fernandez-Albert et al. 2019; Marco et al. 2020).

Given the multifactorial physiological and molecular underpinnings of learning and memory there are several open questions emerging from this study. For example, while we only assessed histone acetylation changes in the DG, we cannot exclude the role of other hippocampal subregions, in particular CA1 (**Supplemental Fig. 4**), to be epigenetically altered by HDACi in response to CFC (Sacchetti et al. 2002). Another limitation is the possibility that measuring mRNA and histone acetylation changes 1 hour after CFC might be more representative of secondary-wave effects of HDACi application and CFC training, considering that many IEGs, acting as transcription factors themselves, are already up-regulated 30 minutes after CFC (Tyssowski et al. 2018; Saha et al. 2011). Additionally, HDACi effects may reach beyond histone acetylation. For example, HDACi treatment is known to stimulate RNA polymerase II (Pol II) elongation at transcriptionally poised genes by altering PolII acetylation

in vitro (Schröder et al. 2013; Ali et al. 2019). Since many IEGs associated with learning and memory have been found to be in a poised PolII state and are subsequently released in response to neuronal activation in cultures (Saha et al. 2011), this scenario warrants further investigation *in vivo* as well.

Another interesting observation is the substantial transcriptional down-regulation in response to HDACi (**Fig. 2A)**, which is surprising given that HDACs are members of protein complexes involved in transcriptional silencing (Nakayama and Hayakawa 2011; Hoffmann and Spengler 2019). Although this phenomenon has been observed in previous studies investigating HDACi treatment alone (Lopez-Atalaya et al. 2013) or when combined with memory extinction (Gräff et al. 2014), it remains to date without definite explanation. Likewise, it remains to be determined whether similar molecular and physiological cascades are triggered by other HDACis or in conditions characterized by impaired cognition.

These open questions notwithstanding, the findings presented here shed light on the mechanisms by which systemic HDACi treatment can lead to specific memory-promoting effects. By enhancing neuronal activity-induced epigenetic and transcriptional cascades, HDACi treatment reaches a high level of target specificity despite being devoid of such specificity *per se.*

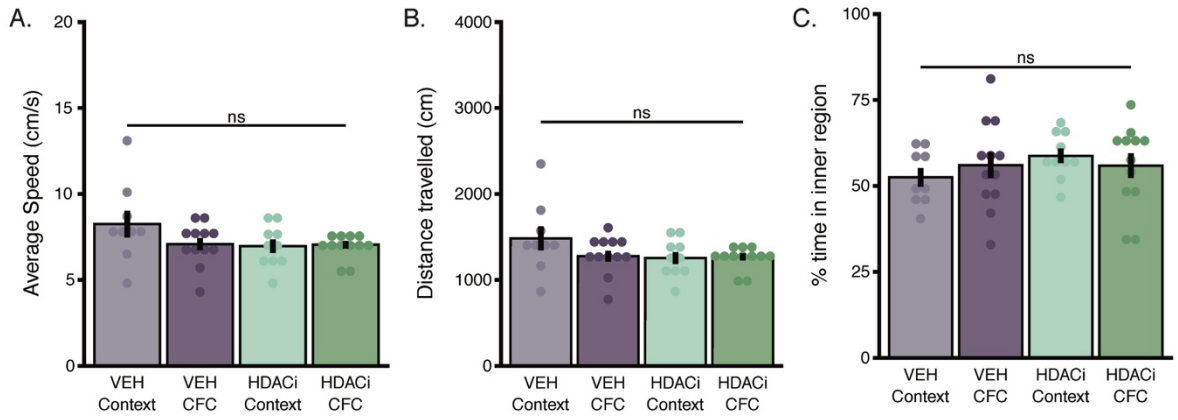
2.1.7 Acknowledgements

We would like to thank all past and current members of the Laboratory of Neuroepigenetics at EPFL for their support and discussion throughout this project, in particular, Paola Arguello and Diego Camacho for their contribution to molecular protocols and analysis. We would also like to thank the EPFL Gene Expression Core Facility (GECF) for their technical assistance with experiment planning and sequencing, the EPFL Flow Cytometry Core Facility (FCCF) for providing the nuclear sorting and the EPFL Center of Phenogenomics (CPG) for ensuring the welfare of the laboratory animals. **Funding**: This work in the laboratory of JG is supported by the European Research Council (ERC-2015-StG 678832), the Swiss National Science Foundation (SNSF, 31003A_155898), the National Competence Center for Research SYNAPSY (51NF40-185897) and the Floshield and Dragon Blue Foundations.

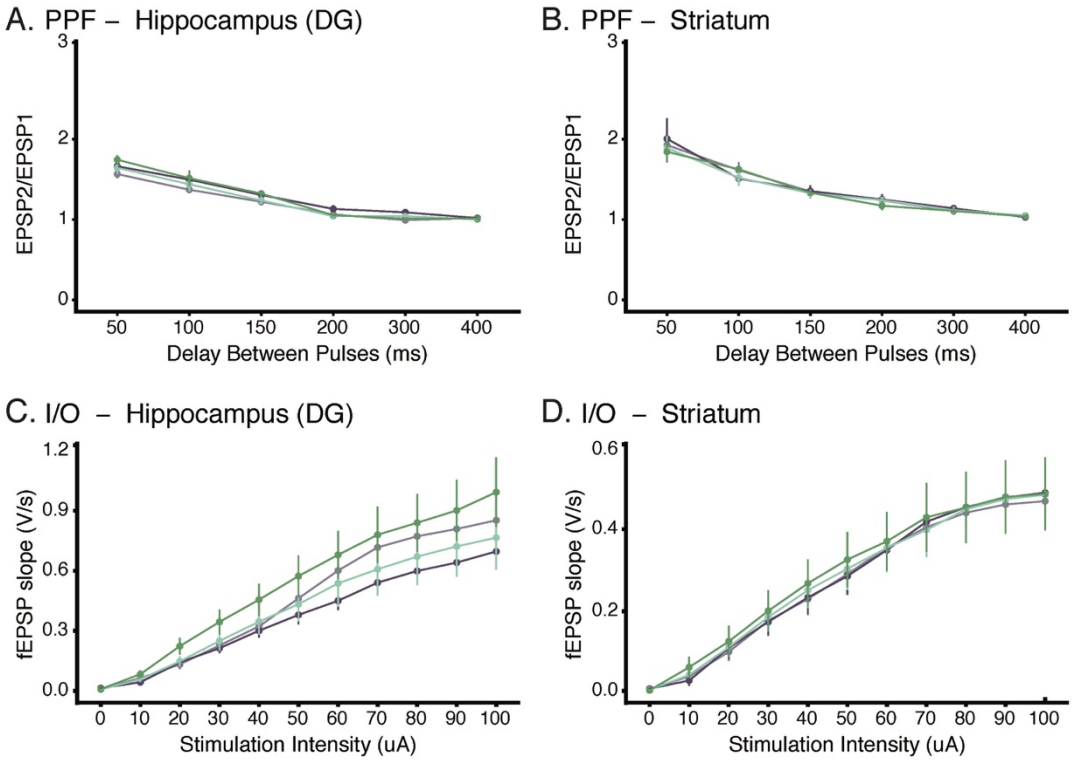
2.1.8 Supplementary Information

All supplementary tables can be downloaded from the BioRxiv repository:
https://www.biorxiv.org/content/10.1101/2021.09.21.460970v1.supplementary-material

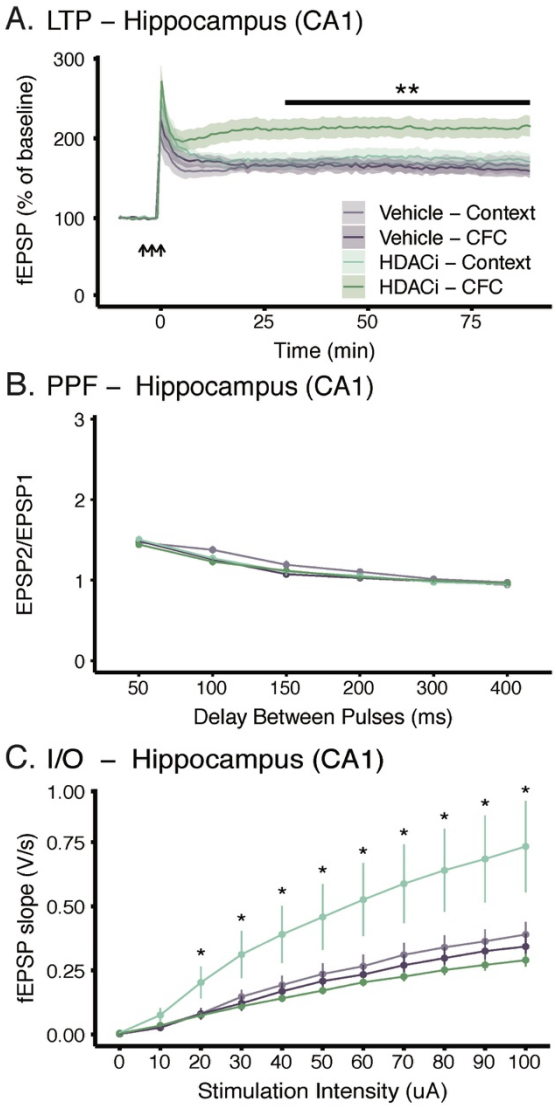
Supplemental Figures:



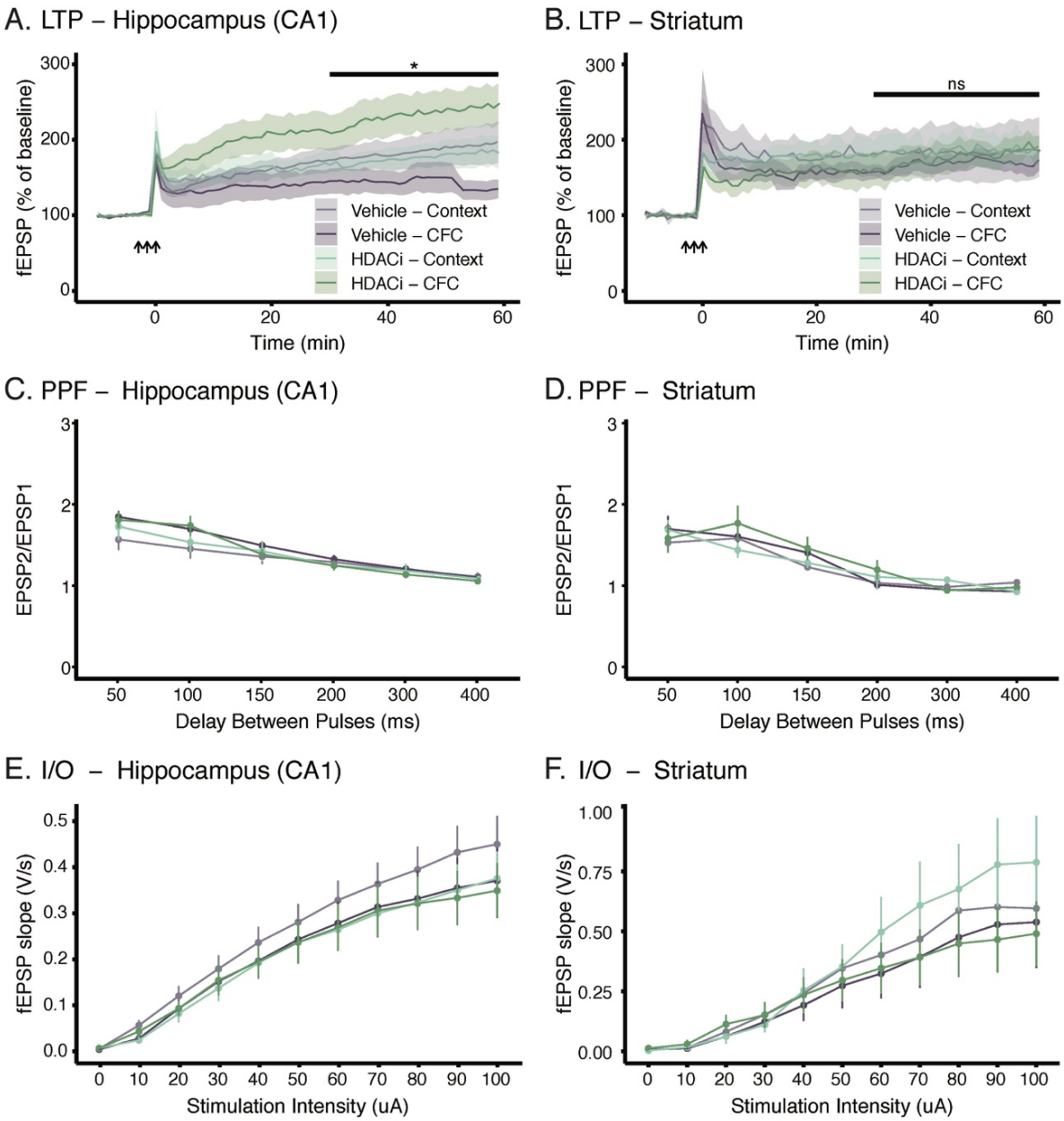
Supplementary Figure 2.1 HDACi treatment does not affect speed, distance travelled or anxiety levels.
(A) Average animal speed (cm/s) during the 3-minute habituation of initial behavioral conditioning was not affected 1 hour after i.p. injection of Vehicle or CI-994. **(B)** Average distance travelled (cm) during the 3-minute habituation of the initial behavioral conditioning was not different 1 hour after i.p. injection of Vehicle or CI-994. **(C)** Time spent in inner regions of the conditioning chamber during the 3-minute habituation of initial conditioning did not change 1 hour after i.p. injection of Vehicle or CI-994. One or two-way ANOVA with Tukey's HSD multiple comparisons test was used for analysis. Graphs represent mean ± SEM. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns = not significant. n = 9-12 animals/group.



Supplementary Figure 2.2 HDACi does not alter PPF or I/O in the hippocampus or striatum after sub-threshold CFC.
(A and B) Paired pulse facilitation (PPF) in the DG **(A)** and striatum **(B)** 1 hour after CFC. **(C and D)** Input/output (I/O) relationship in the DG **(C)** and striatum **(D)** 1 hour after CFC. Graphs represent mean ± SEM. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns = not significant. n = 8 animals/group.

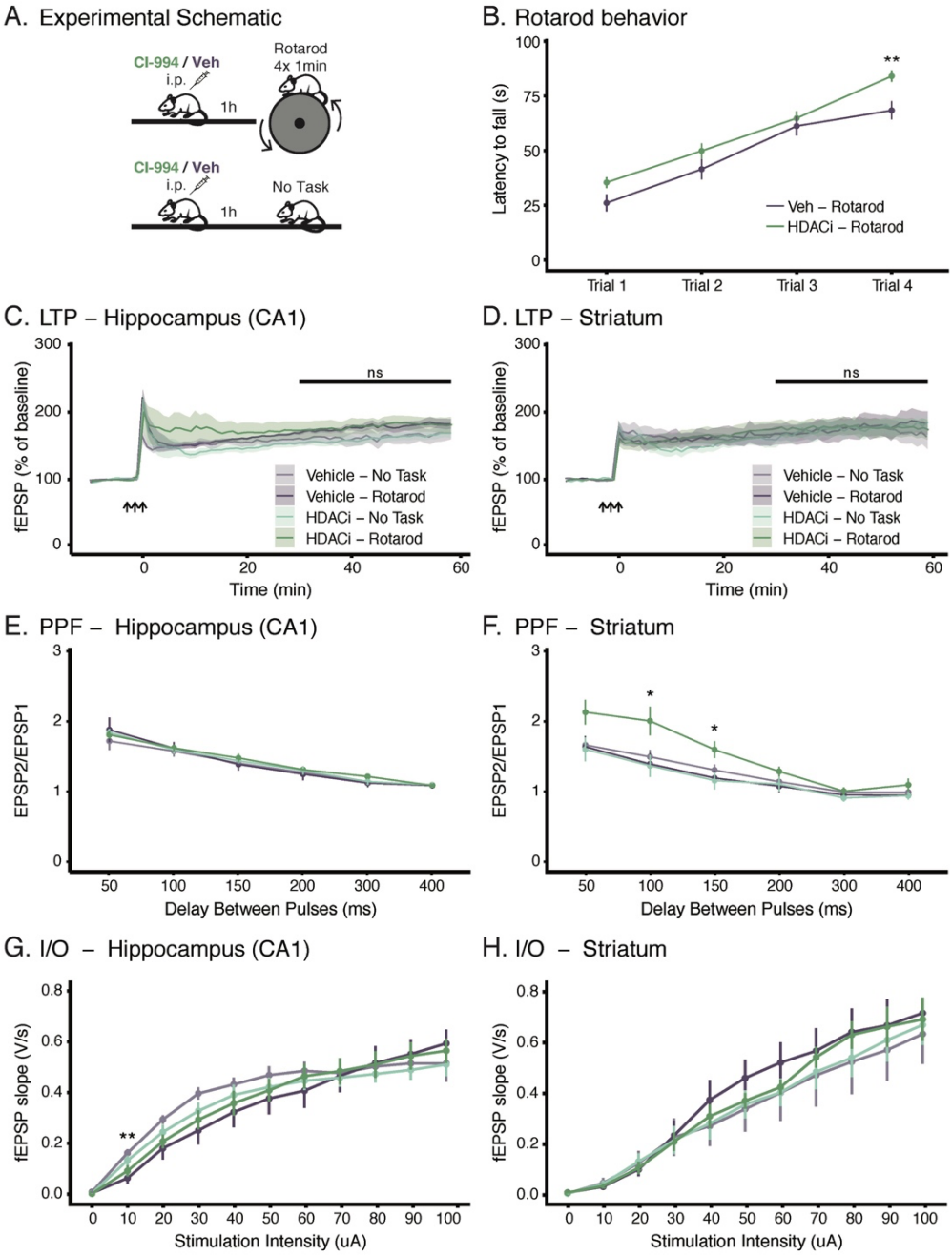


Supplementary Figure 2.3 HDACi combined with sub-threshold CFC enhances LTP in hippocampal area CA1.
(A) HDACi combined with CFC enhanced LTP in response to 3 trains of high frequency stimulation (HFS – arrows) at Schaffer Collaterals of the hippocampal CA1 one hour after initial behavioral conditioning. Statistical differences were calculated for the 30 minutes (end of short-term-potentiation) to 90 minutes (end of recording) for each mouse. **(B)** There were no treatment-induced differences in PPF in the CA1. **(C)** HDACi-Context did have a larger I/O relationship than other groups in the CA1. Graphs represent mean ± SEM. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. n = 8 animals/group.



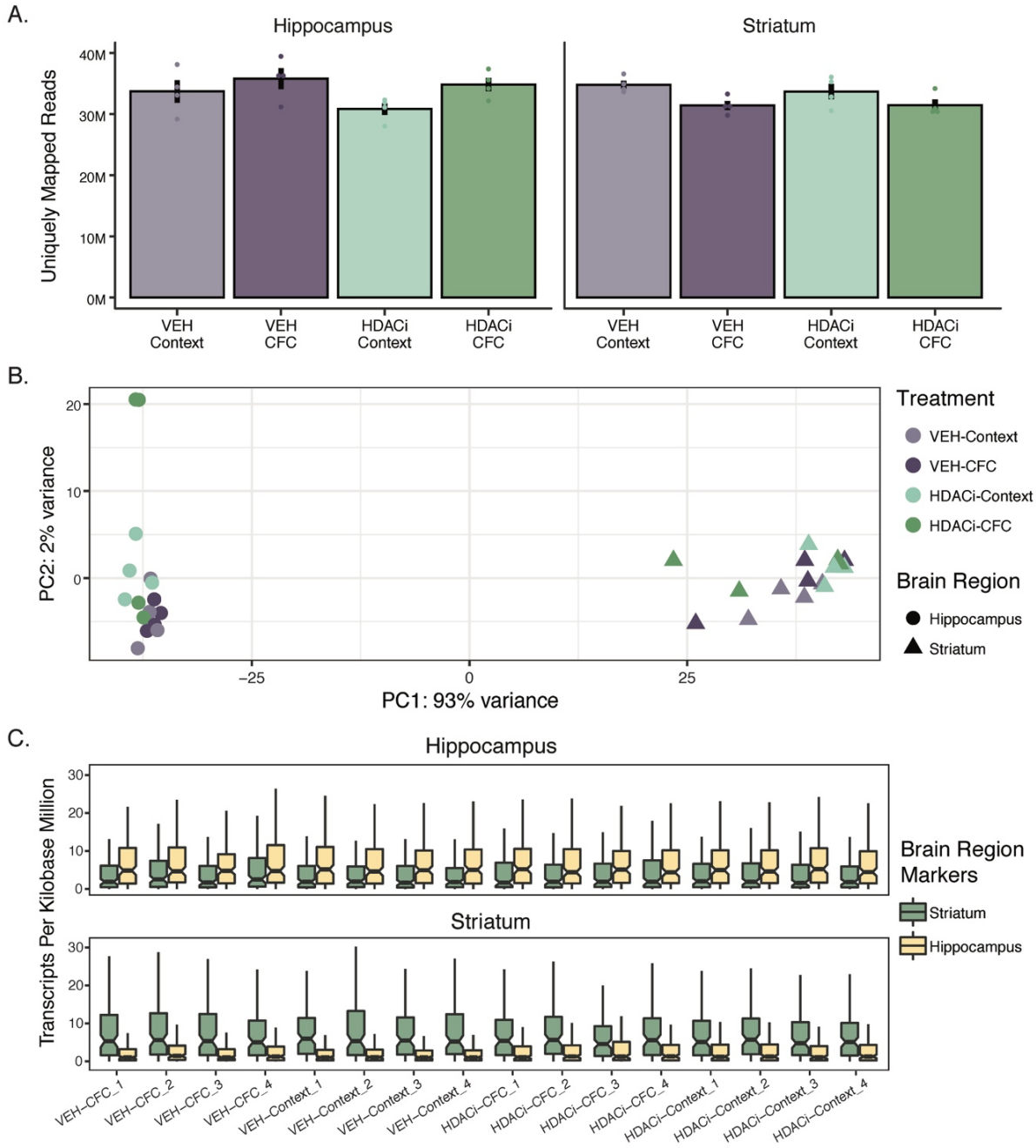
Supplementary Figure 2.4 HDACi combined with strong CFC enhances LTP in CA1 but not in striatum.

(A and B) HDACi combined with strong CFC (3x 0.8mA – 1s) enhanced LTP in response to 3 trains of high frequency stimulation (HFS – arrows) in the Schaffer Collaterals of the hippocampal CA1 **(A)** but not in the cortical-striatal pathway **(B)** one hour after initial behavioral conditioning. Statistical differences were calculated for the 30 minutes (end of short-term-potentiation) to 90 minutes (end of recording) for each mouse. **(C and D)** There are no treatment induced differences in paired pulse facilitation (PPF) in either the CA1 **(C)** or the striatum **(D)** 1 hour after strong CFC. **(E and F)** There are also no treatment induced differences in the input/output (I/O) relationship in either the CA1 **(C)** or the striatum **(D)** 1 hour after strong CFC. Graphs represent mean ± SEM. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns = not significant. n = 6-10 animals/group.

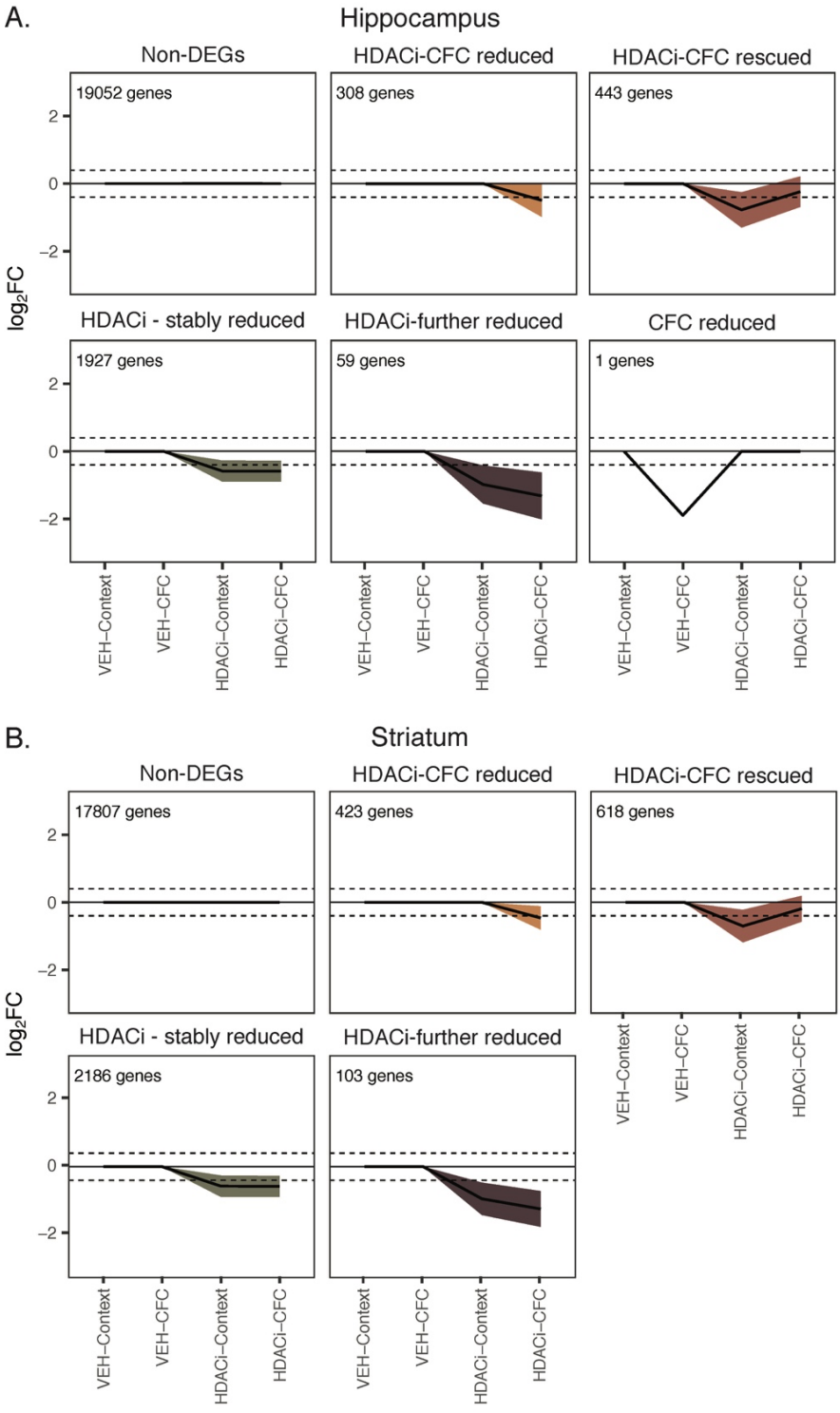


Supplementary Figure 2.5 HDACi enhances PPF in the cortico-striatal pathway after motor learning.

(A) Schematic representation of the striatal specific behavioral paradigm, rotarod training. Animals were i.p. injected with either vehicle or CI-994 (30mg/kg) one hour prior to rotarod training. Rotarod training began by placing each mouse on the rod and accelerating the rod from 4-40rpm in 5 minutes or until the mouse fell off. Mice were tested for 4 trials with 1-minute inter-trial intervals. **(B)** HDACi combined with CFC increases the time to fall off the rotarod after repeated training. **(C and D)** HDACi combined with rotarod training does not alter LTP in response to 3 trains of high frequency stimulation (HFS – arrows) in the Schaffer Collaterals of the hippocampal CA1 **(C)** or in the cortical-striatal pathway **(D)** one hour after final rotarod trials. Statistical differences were calculated for the 30 minutes (end of short-term-potentiation) to 90 minutes (end of recording) for each mouse. **(E and F)** HDACi paired with rotarod training does not lead to any differences in PPF in the Schaffer Collaterals of the CA1 **(E)** but it enhances PPF in the striatum **(F)** after a delay of 100 or 150ms between pulses. **(G and H)** I/O relationships were overall similar in response to HDACi and rotarod training in the Schaffer Collaterals of the CA1 **(G)** or the striatum **(H)**. Graphs represent mean ± SEM. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns = not significant. n = 6-10 animals/group.

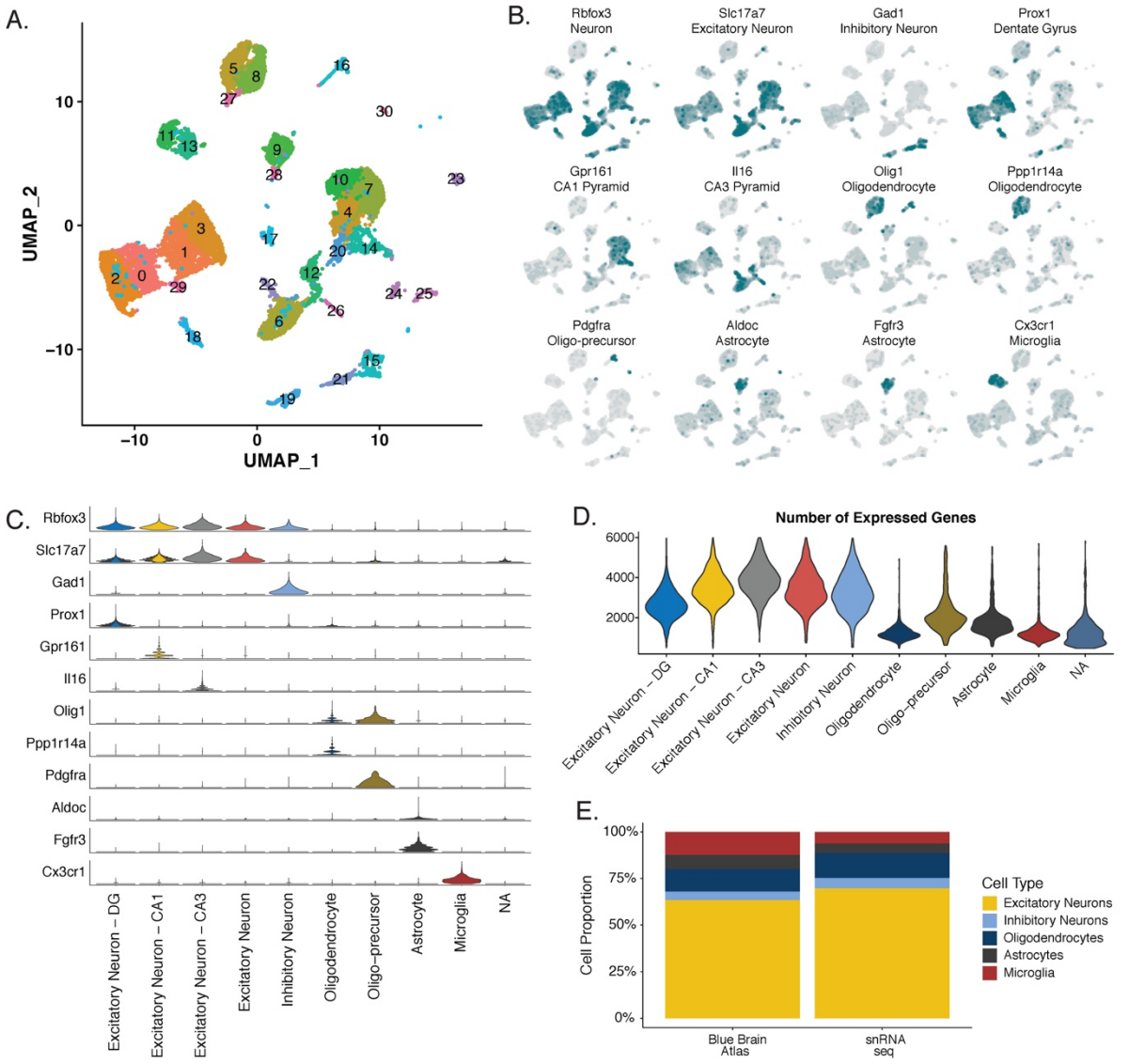


Supplementary Figure 2.6 Alignment statistics for bulk RNA-sequencing.
(A) All libraries contained 28-40M uniquely mapping reads with no treatment group having significantly more reads than any other (One-way ANOVA, $F(7,24) = 2.34$, $P = 0.057$). Graphs represent mean ± SEM. **(B)** Principal component analysis (PCA) showing that 93% of the variance of the top 1000 genes within all 8 libraries comes from differences between brain regions. Only 2% comes from within brain regions. Points are colored by treatment and shape represents brain region. **(C)** Brain region specificity was confirmed by comparing transcripts per kilobase million (TPM) expression of hippocampal and striatal libraries to marker genes from the hippocampus and striatum. The top 250 marker genes for each region compared to all other regions were downloaded from the Allen Brain Atlas. Hippocampal marker genes were mostly highly expressed in libraries from the hippocampus (top), while libraries that were created from striatal tissue were enriched for striatal marker genes (bottom). n = 4 biologically independent samples.

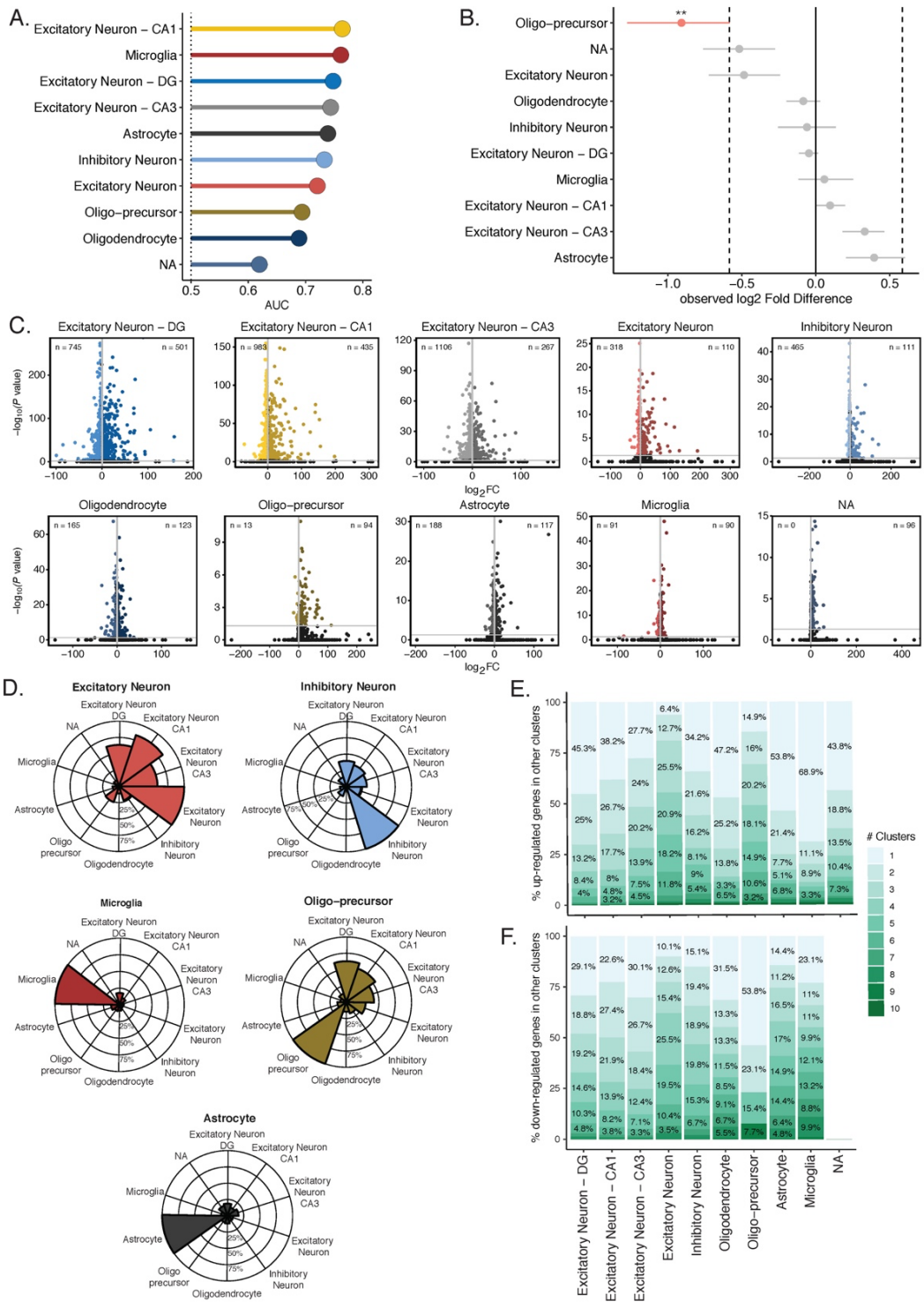


Supplementary Figure 2.7 Clusters representing down-regulated genes in both the hippocampus and the striatum.

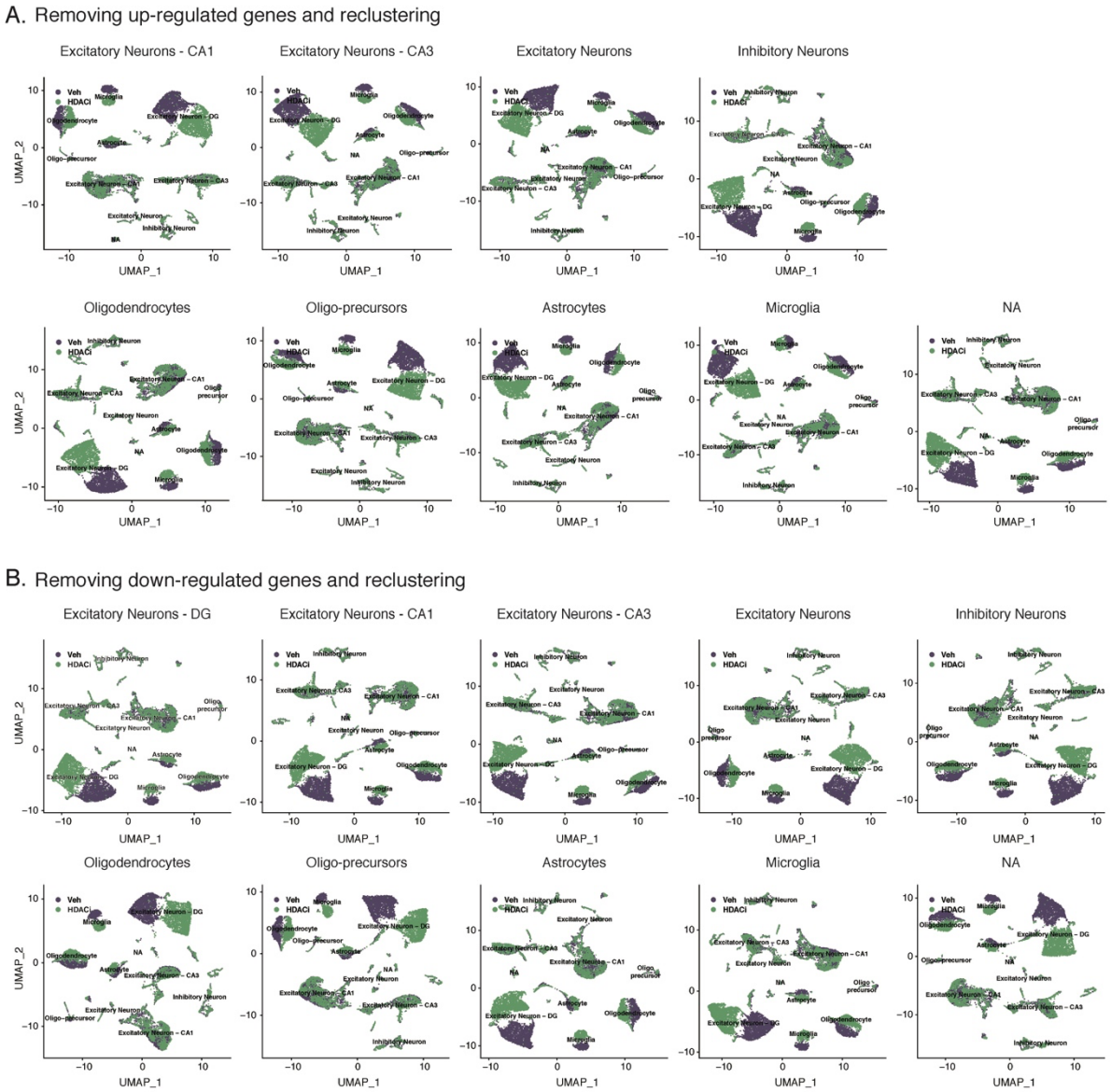
Clusters representing non-changed and down-regulated genes in both the hippocampus **(A)** and the striatum **(B)**. Most expressed genes in both brain regions were not differentially expressed in any comparison (top left cluster). Both brain regions included similar numbers of genes that were only down-regulated after combined HDACi-CFC (top middle) and genes that were reduced by HDACi-Context treatment and rescued by combined HDACi-CFC (top right). Both brain regions also included genes that were stably reduced by HDACi treatment, regardless of behavioral paradigm (bottom left) and genes that were reduced by HDACi-Context and further reduced by HDACi-CFC (bottom middle). Only the hippocampus contained one gene that was reduced in the VEH-CFC group and rescued by HDACi (bottom right). Line plots represent mean ± SEM



Supplementary Figure 2.8 snRNA-Seq cell type assignments.
(A) snRNA-seq gene count matrices were merged, filtered and normalized using SCTransform and underwent uniform manifold approximation and projection (UMAP) mapping. All nuclei were then clustered based on the k-nearest neighbors. Clusters with fewer than 50 nuclei were removed, yielding 15,339 total nuclei in 30 distinct clusters. **(B)** Clusters were assigned to cell types by overlaying UMI expression for known cell type markers over each cluster. **(C)** Clusters were assigned to cell types (Rbfox3, Slc17a7, Gad1, Olig1, Ppp1r14a, Pdgfra, Aldoc, Fgfr3 and Cx3cr1) and cell locations (Prox1, Gpr161, Il16) based on the markers with the highest UMI expression in each cluster. This analysis yielded 10 distinct cell type and location clusters. **(D)** On average, nuclei assigned to neuronal cell types had more expressed genes than glial cell types (Oligodendrocytes, oligo-precursors, astrocytes, microglia and NA). **(E)** Cell type assignments (right column) were similar to known cell proportions in the hippocampus, taken from the Blue Brain Atlas (left column), irrespective of drug and behavioral treatment. n = 2 biological replicates per group (HDACi-CFC and VEH-CFC).

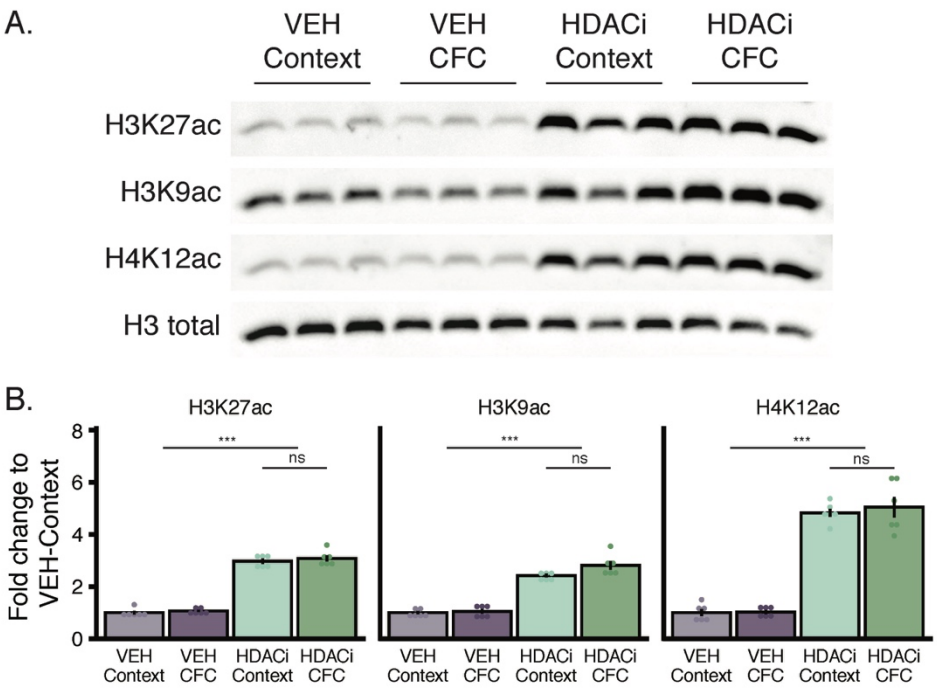


Supplementary Figure 2.9 HDACi perturbs distinct gene sets in different cell types.
(A) Augur analysis. Area under the receiver operating characteristic curve (AUC) is above random chance (0.5) for all cell types. **(B)** Cell type composition permutation test (#permutations = 1000). HDACi only altered relative abundance of cell type by more than 1.5-fold for oligo-precursors. **(C)** Volcano plots showing magnitude of differential expression (log_2FC; $|log_2FC| \geq 1$; adjusted p-value ≤ 0.05) versus statistical significance ($-log_{10}$ P-value) for HDACi-CFC compared to Veh-CFC in each cell type. n-values in corners represent the genes that are up-regulated (right corner) or down-regulated (right corner). **(D)** Overlap of up-regulated genes across cell types not shown in main figure. **(E and F)** Percent of genes found in other clusters for each cell type. Genes that were found in only one cluster are unique to that cell type for up-regulated **(E)** and down-regulated **(F)** genes in each cluster. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, n = 2 biological replicates per group.

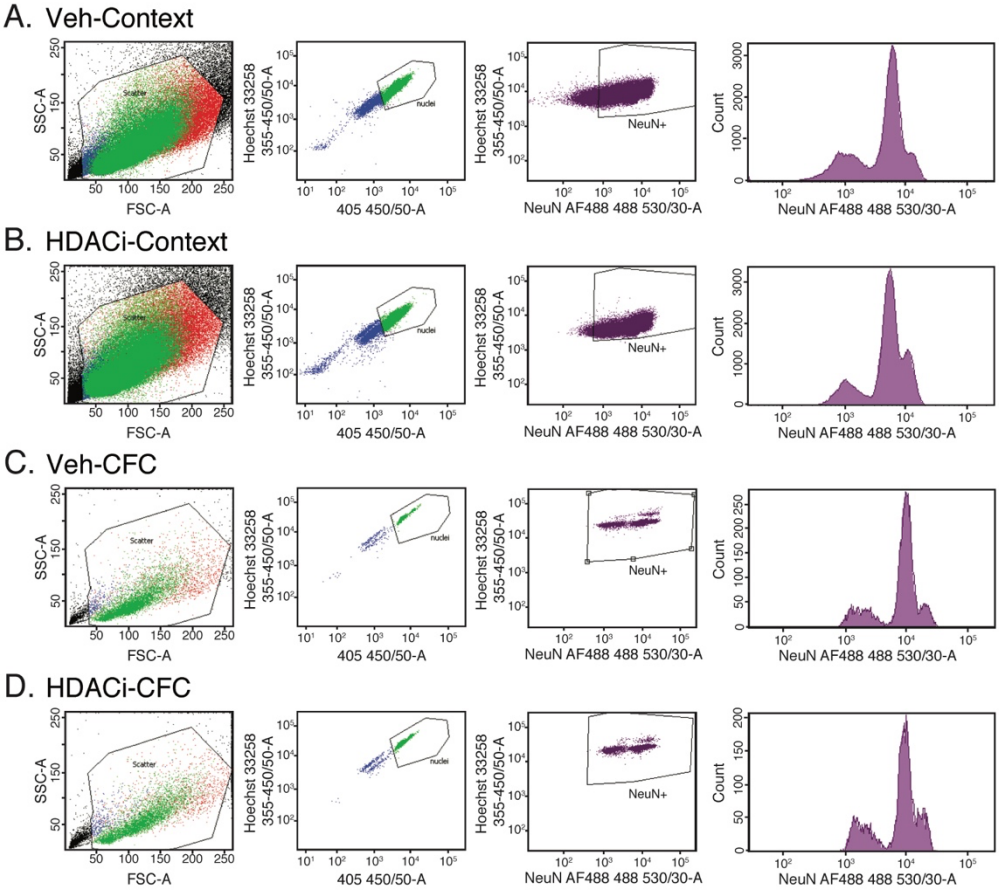


Supplementary Figure 2.10 Removing up- and down-regulated genes and re-running UMAP to determine unique gene sets.

Removing up- **(A)** and down-regulated **(B)** genes and re-running UMAP clustering revealed that HDACi-induced differential expression calculated for each cell type was unique for that cell type. For example, removing up-regulated genes from astrocytes re-merged the astrocyte cluster in the UMAP. Whereas removing down-regulated genes from astrocyte clusters did not.



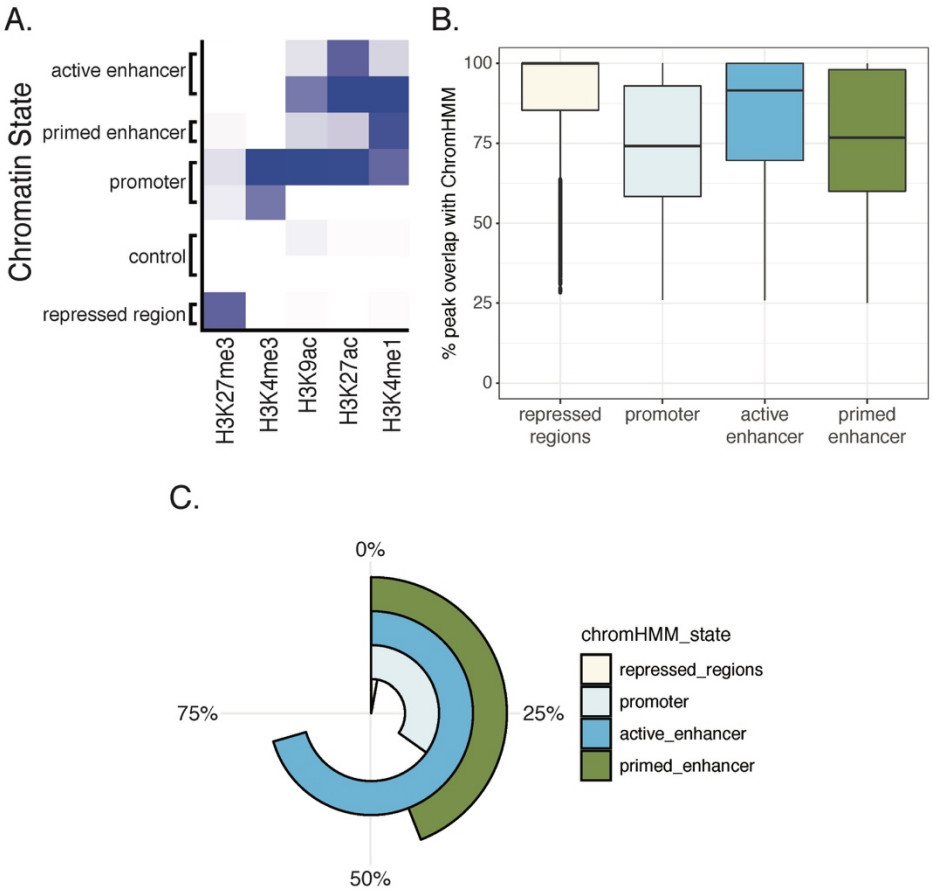
Supplementary Figure 2.11 HDACi enriches H3K27ac, H3K9ac and H4K12ac protein levels regardless of behavioral condition.
(A) Representative Western blot for hippocampal H3K27ac, H3K9ac and H4K27ac including 3 biological replicates for all four treatments and compared to total H3 presence (control). **(B)** Western blot quantification for H3K27ac, H3K9ac and H4K12ac plotted as fold change to the average VEH-Context luminescence indicating that HDACi enriched histone acetylation for all 3 marks shown, regardless of behavioral conditioning. Graphs represent mean ± SEM. n = 6 biological replicates per group, each with 2 technical replicates. Two-way ANOVA, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns = not significant.



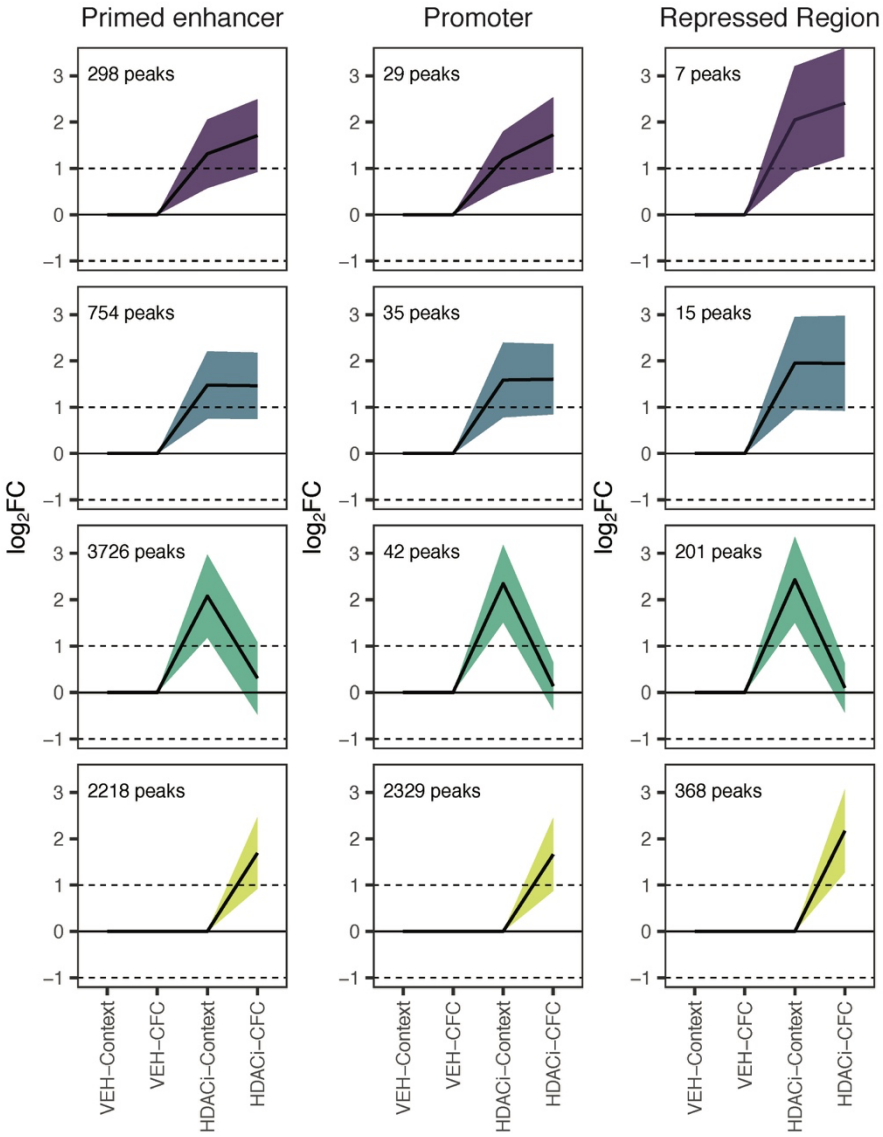
E. Sort Report Values

Treatment Group	Replicate	% Total Input	# Cells Output
Vehicle - Context	1	60.9%	434,578
	2	64.9%	40,693
	3	62.4%	436,261
HDACi - Context	1	60.3%	116,931
	2	59.6%	441,491
	3	66.4%	331,570
Vehicle - CFC	1	65.7%	400,927
	2	61.4%	400,983
	3	64.3%	287,840
HDACi - CFC	1	55.4%	226,345
	2	69.7%	401,243
	3	57.1%	364,140

Supplementary Figure 2.12 FANS gating strategy and report
(A-D) Example FANS gating strategies for each treatment group. **(E)** FANS sort report for all treatment groups and replicates performed for ChIP-sequencing.

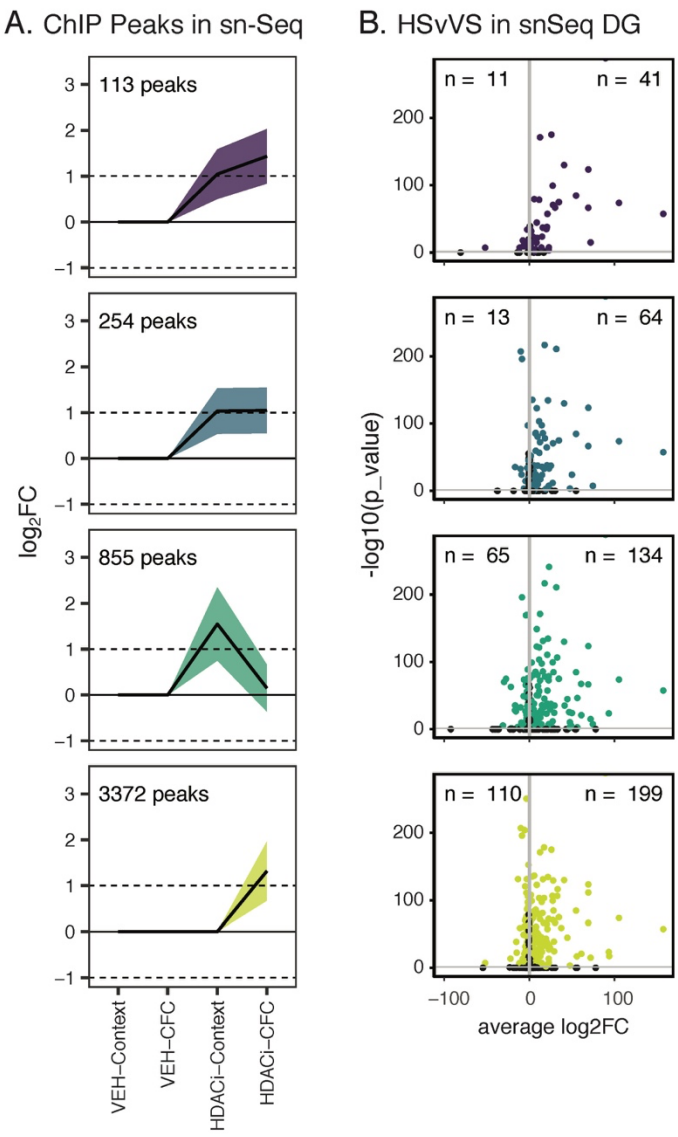


Supplementary Figure 2.13 H3K27ac peaks at enhancer and promoter regions.
(A) Five distinct chromatin states were assigned to the full mm10 genome using previously published histone PTMs and ChromHMM to assign chromatin states. H3K27me3 acts as a repressive marker, H3K4me3 and H3K9ac are promoter markers, H3K27ac is a marker of active enhancers and H3K4me1 is enriched at primed-enhancers. **(B)** Percent of peak overlap with assigned chromatin states shows that most peaks overlap with their assigned state by more than 50%. Graphs shown as box and whisker plots with outliers plotted as points. **(C)** Proportion of each chromatin state (for full genome) enriched by H3K27ac peaks in all four treatments indicates that H3K27ac peaks are enriched at active and primed enhancers and not repressed regions.



Supplementary Figure 2.14 Trajectory analysis for H3K27ac peaks not associated with active enhancers.

Line graphs in trajectory plots represent significant log_2FC values for each group in clusters of interest for primed enhancers (left), promoters (middle) and repressed regions (right). Count in upper left corner indicates the number of genes in each cluster. Line plots shown as mean ± SEM.



Supplementary Figure 2.15 ChIP to sn-seq comparison.
(A) Line graphs in trajectory plots represent H3K27ac active enhancer peaks that are associated with genes also found in the snRNA-seq analysis. These graphs compare log_2FC values for each group in clusters of interest. Count in lower left corner indicates the number of genes in each cluster. *P*-values for differential expression were calculated using the Wald test in DEseq2 followed by Benjamini-Hochberg multiple comparisons test. Line plots shown as mean ± SEM. **(B)** Volcano plots for genes in the snRNA-seq analysis show that most genes that have increased H3K27ac at active enhancer peaks are also up-regulated in the excitatory neurons of the DG. n-values in corners represent the genes that are up-regulated ($log_2FC \geq 1$; adjusted *P*-value ≤ 0.05).

Chapter 3 Conclusion

3.1 Discussion of results

In brief, we show that systemic inhibition of HDAC activity does have a priming effect as it enhances behavioral responses to both CFC and rotarod training, while uniquely altering synaptic communication in the two brain regions associated with these training paradigms. In addition, HDACi differentially alters transcriptional pathways after CFC in the hippocampus and striatum, as well as between cell types within the hippocampus, thus indicating that HDACi can specifically alter cellular processes based on their underlying activity patterns. We then show that HDACi increases H3K27ac at enhancers associated with synaptic communication in hippocampal neurons. Many of the transcriptionally activated genes were not associated with enhancers that were enriched for H3K27ac, suggesting that a subset of HDACi and CFC induced DEGs is regulated by other mechanisms at this time point. Overall, we show that HDACi epigenetically and transcriptionally targets the MAPK signaling pathway and that this likely plays an important role in enhancing memory formation via epigenetic priming (**Figure. 3.1**).

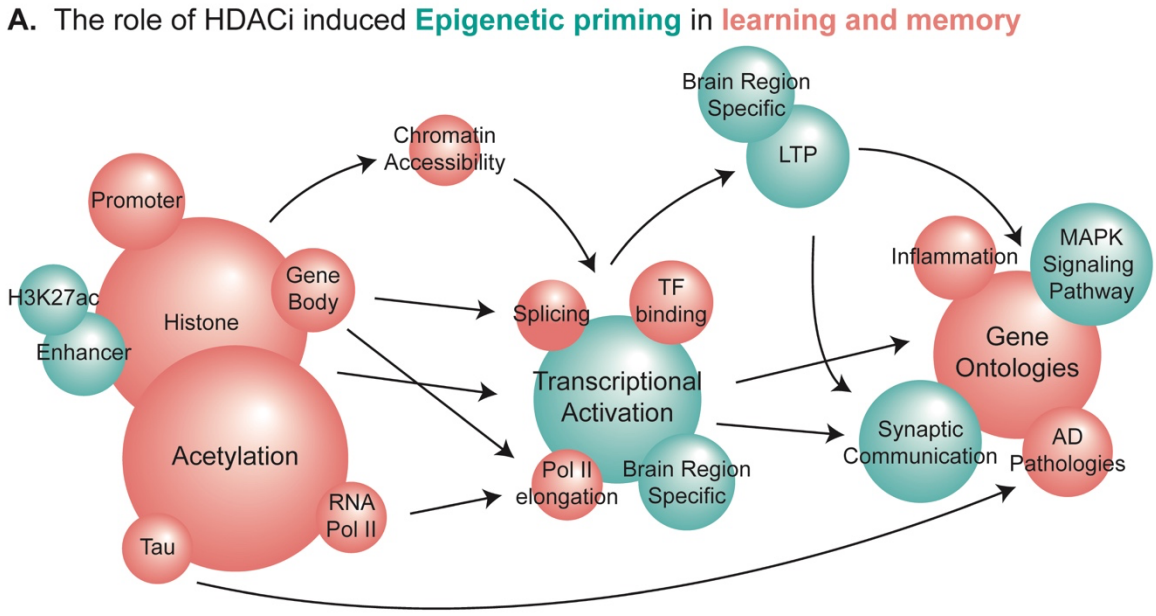


Figure 3.1 Role of HDACi mediated epigenetic priming in the context of what is currently known.

3.1.1 Behavior and LTP

Our results showing that systemic HDACi treatment enhances both subthreshold CFC and rotarod training are in agreement with other behavioral studies using HDACis. HDACi has previously been shown to enhance other weak training paradigms for novel object recognition (NOR), Morris Water Maze (MWM) and CFC in both non-impaired (Stefanko et al. 2009; Villain, Florian, and Roullet 2016; Levenson et al. 2004) and cognitively impaired animals (Eva Benito et al. 2015; Alarcón et al. 2004). In addition, treatment with HDACis improved rotarod performance in two models of Huntington's disease, R6/2 and N171-82Q (Thomas et al. 2008; Jia et al. 2012), showing that HDACi can rescue disease-related impairments that are involved in motor regulation. In the hippocampus, there is evidence that HDACi improves memory performance by transforming the transient transcription-independent form of early-LTP into the longer lasting transcription-dependent form of late-LTP (Stefanko et al. 2009; Vecsey et al. 2007).

Our results support these finding as LTP was increased in two regions within the hippocampus, the CA1 and the DG, after combined HDACi-CFC but not after either treatment or conditioning alone. This is particularly relevant because LTP has specifically been shown to underlie learning and memory in both of these hippocampal regions (Bliss and Collingridge 1993; Sacchetti et al. 2002; Saxe et al. 2006). On the other hand, in the striatum, HDACi paired with rotarod training increased PPF, a form of short-term pre-synaptic enhancement. This is in line with a previous study showing that reduced PPF in the cortico-striatal synapses corresponds with impaired rotarod training (Assous et al. 2019). We further showed that the systemic HDACi treatment reduced HDAC activity in both brain regions by about 50%, suggesting that these results are not a function of differential HDAC inhibition. The findings, that systemic HDACi paired with different methods of training uniquely enhances synaptic communication in their respective brain regions, indicate that HDACi may function by enhancing the specific underlying processes of each activity its target location.

3.1.2 Transcriptional activation

We further illustrate HDACi-mediated process specificity, by comparing transcriptional activation in the hippocampus and striatum after CFC. In both brain regions, we found that transcriptional activation was primarily driven by HDACi treatment and not CFC. This is in agreement with previous hippocampal studies showing that there is little transcriptional activation when comparing context and CFC-exposed animals one hour after conditioning (Halder et al. 2016) or after HDACi alone (Lopez-Atalaya et al. 2013) but that pairing HDACi with neuronal activity leads to increased differential expression globally (Gräff et al. 2014;

Villain, Florian, and Roullet 2016; Takuma et al. 2014; Sewal et al. 2015). This, combined with the data showing similar amounts of differential expression in the striatum after CFC, indicates that HDACi is driving the transcriptional activation in both brain regions one hour after CFC.

Interestingly, there was a noteworthy family of genes that was activated by HDACi, regardless of brain region or treatment. In both the hippocampus and the striatum, our *primed-stable* cluster was enriched for genes that were members of the "MAPK cascade", "positive regulation of ERK1 and ERK2 cascade" and "positive regulation of Ras protein signaling" pathways, indicating that HDACi enhances the classical MAPK signaling pathway either in disregard to the underlying cellular processes or because MAPK is basally active in both regions during CFC. The MAPK cascade is a mitogen-activated protein kinase cascade that amplifies and integrates a diverse range of signals in order to activate an appropriate response. It is particularly known to drive processes that include cellular proliferation, differentiation and inflammatory response (Zhang Wei and Liu 2002). Many studies have also shown that in the hippocampus, the classic ERK-mediated MAPK pathway is necessary for memory consolidation (Atkins et al. 1998; Blum et al. 1999) and that once activated, ERK phosphorylates protein targets that are implicated in gene transcription, protein synthesis and synaptic plasticity (Davis et al. 2000), as well as histone acetylation (Levenson et al. 2004). This pathway is also important for motor performance as inhibiting ERK activation in the dorsal striatum impairs rotarod performance (Bureau et al. 2010). It is possible that HDACi treatment alone enhances expression of this pathway globally so that it can respond faster to a downstream stimulation, such as neuronal activation.

This is supported by our decision tree cluster analysis in which many of the genes and pathways in our clusters were unique to each brain region. For example, the ontology, "cognition" was specific the hippocampus in the *primed-stable* cluster and included the IEGs *Bdnf* and *Jun*, both of which are known to be activated during learning and memory (Liu et al. 2004; Kyriakis et al. 1994). In addition, the "ERK1 and ERK2 cascade" is enriched in the *primed-active* cluster in the hippocampus but not the striatum, suggesting that the MAPK signaling pathway could be induced in both brain regions after HDACi and could then be further enriched in the hippocampus in response to CFC stimulation. It is also of interest that in the *non-primed active* cluster genes activated in the hippocampus are uniquely enriched for ion transport whereas this cluster in the striatum is enriched for genes involved in the "negative regulation of response to stimulus". This could indicate that after CFC, HDACi mediates synaptic communication in the hippocampus while trying to repress activation in the striatum.

These unique and relevant gene subsets further indicate a process specificity response after HDACi treatment.

To further show that HDACi enhances the underlying processes activated by CFC, we performed snRNA-seq to compare the cell type specific responses within the hippocampus. Our results indicate that differential expression was induced in all cell types and that there was very little overlap between the DEGs between cell types. Of note, we found up-regulation of many genes in the MAPK pathway in excitatory neurons of the DG, providing further evidence that HDACi may be mediating learning and memory through this pathway. While some of these genes were also differentially expressed in excitatory neurons of the CA1 and CA3, they were not differentially expressed in glial cell types. These exploratory results are in line with previous work pointing towards cell type-specific roles of the MAPK pathway in learning and memory (Ryu and Lee 2016), however, further analysis would be needed to better understand the role of MAPK in neurons and glia after CFC.

Altogether, the results of the transcriptional analysis show HDACi paired with CFC activates different gene sets in the hippocampus and striatum, as well as within different cell types of the hippocampus. This specialized activation likely builds upon the process specific transcription and activation that occurs in these brain regions in response to CFC. They also indicate that HDACi may be enhancing these process specific pathways by activating the MAPK pathway. Further experiments would be needed to better understand the interplay between HDACi and MAPK activation.

3.1.3 Epigenetic response

Our final aim was to characterize histone acetylation changes in neurons of the hippocampal DG in order to determine the extent to which CFC and HDACi mediated epigenetic changes are driving transcriptional activation. To support the theory of epigenetic priming, we expected that HDACi would uniformly enhance H3K27ac across the entire genome and that locations that are associated with genes up-regulated by HDACi-CFC would see additional acetylation after CFC (Burns and Gräff 2021). As expected, HDACi treatment enhanced H3K27ac at defined peaks, regardless of behavioral conditioning, and the combination of HDACi and CFC produced the largest H3K27ac enrichment. Many of the genes associated with H3K27ac enriched enhancers after combined treatment were members of the RAS/MAPK complex, lending more evidence to our theory that, in the hippocampus, HDACi activates the MAPK pathway as a means of strengthening memory. Initially, we were surprised to find that synaptic and learning specific genes were also enriched after HDACi treatment, regardless of learning paradigm. However, upon further consideration, we ascertained that HDACi may not uniformly

enhance genome acetylation, but that it can only allow for the maintenance of histone acetylation at locations that have previously been activated by HATs (Wang et al. 2009), likely in a process-specific manner. Past results have already demonstrated that both HDACi (Lopez-Atalaya et al. 2013) and CFC (Halder et al. 2016) primarily enrich acetylation at regions that were previously acetylated in the basal states. This would suggest that, by definition, HDACi primes in a targeting fashion, regardless of neuronal activation, as it can only maintain previously acetylated regions that may be region and/or cell-type specific. In this case, enrichment of H3K27ac at regions associated with synaptic function in our comparisons are likely to be expected.

Finally, our analysis of H3K27ac demonstrates that epigenetic changes are strongly driven by HDACi treatment combined with CFC (*non-primed active*) – a pattern that more closely resembles the behavioral and electrophysiological response than the transcriptional response. This suggests that there is an uncoupling of H3K27ac enrichment at active enhancers and transcriptional activation of their respective genes one hour after CFC. This was similarly observed in other studies in which an uncoupling of histone acetylation and transcriptional activation occurs both 75 minutes after HDACi treatment alone (Lopez-Atalaya et al. 2013) and one hour after CFC alone (Halder et al. 2016). This uncoupling could be driven by an independence of acetylation for a subset of genes. This is supported by our bulk RNA-seq, where genes in the primed-stable group are independent of H3K27ac priming. Specifically, it is possible that the genes in the *primed-active* group in the RNA-seq analysis are maximally activated and cannot be further enhanced by H3K27ac, while the genes in the *primed-stable* group are susceptible to H3K27ac priming, allowing them to potentially be further expressed at a later time point. This is in line with a recent study in which enhancer accessibility following CFC was only matched by transcriptional responses that occurred 5 days later (Marco et al. 2020). This could suggest that HDACi induced epigenetic priming is more important at later stages of consolidation than encoding. Taken together, this implies that, while HDACi induced priming does partially rely on underlying molecular changes, the H3K27ac measured at enhancers one hour after CFC is not the only factor driving transcriptional activation at this time point.

3.2 Future development

Given the complex physiological and molecular interplays involved in both learning and the underlying epigenetics, there are several factors that would need to be further explored in order to better understand the mechanisms by which HDACi selectively enhances learning and memory. In particular, the decoupling of the enhancer H3K27ac enrichment and the

transcriptional activation still needs to be conclusively explained. Here, we describe a few possible factors that may be contributing to this phenomenon.

3.2.1 Learning and HDACi mediated differences between males and females

To reduce sample variability and focus primarily on HDACi responses, all experiments in this study were performed in male animals. However, many memory-related diseases are more common in women than men, suggesting that biological sex does play a role in learning and memory (Beam et al. 2018) as well as diseases that could be treated with an HDACi (Burns and Gräff 2021). Gender specific differences in CFC are still uncertain, as some studies indicate that males show higher levels of fear after conditioning than females (Maren, De Oca, and Fanselow 1994; Barker and Galea 2010) and others show that females exhibit stronger fear responses (Keiser et al. 2017). Nonetheless, there are sex-dependent epigenetic differences as females exhibit oestrous cycle-dependent changes in 3D chromatin organization (Rocks et al. 2021). Many of these female specific-changes in chromatin organization are associated with changes in transcription of genes involved in neuronal function and behavior and can be linked to anxiety related behaviors (Jaric et al. 2019). This could mean that females experience a different response to HDACi depending on where they are in the oestrus cycle. Despite the prevalent use of HDACis in clinical trials to treat cancers and brain-related disorders, few studies have compared sex-specific responses to HDACi. One group, studying a mouse model of neonatal hypoxic-ischemic brain injury, found that TSA increased H4 acetylation in the brain and reduced grey matter injury in females only (Fleiss et al. 2012), suggesting that HDACi may also have a sex-specific effect. To better understand the sex-specific responses to HDACi it would be important for future studies to explore how HDACi effects change throughout the oestrus cycle.

3.2.2 Contributions of other brain regions and hippocampal subregions

While we only considered histone acetylation changes in neurons of the DG, we cannot exclude the contribution of other cell types or hippocampal subregions involved in memory formation. For example, our electrophysiology analysis, demonstrating that the CA1 subregion also has an increased LTP response after combined HDACi and CFC, is supported by other work illustrating the relevance of the CA1 in memory formation and consolidation (Sacchetti et al. 2002; Whitlock et al. 2006). Furthermore, the transcriptional differences between the excitatory neurons of the DG and the CA1 after combined HDACi-CFC suggest that they have slightly different contributions during learning (Alkadhi 2019). Given this information and the fact that the ChIP analysis was carried out in DG neurons while the bulk RNA-seq analysis was carried out in the full hippocampus, it is possible that the decoupling seen between our

experiments could be minimally driven by differences of cell populations. We initially decided to perform the ChIP-sequencing only in DG neurons to better understand the strong and specific transcriptional response seen in the snRNA-seq. However, given our results, this analysis should be repeated in nuclei coming from the full hippocampus. Nonetheless, since other studies also allude to decoupling at similar time points (Halder et al. 2016; Lopez-Atalaya et al. 2013), this likely does not entirely explain the transcriptional and epigenetic decoupling. For future experiments, it would be important to also determine the HDACi mediated priming events in these other hippocampal subregions and more specifically compare the histone acetylation and transcriptional activation coming from similar populations.

Considering that our HDACi treatment is systemic, it is likely that its effects on other brain regions also contribute to the enhanced fear learning seen 24 hours after conditioning. Although the hippocampus is known to be imperative for CFC, the amygdala – typically associated with emotion and motivation – may also play a role (W. Bin Kim and Cho 2020). For example, selectively inhibiting HDAC3 in either the hippocampus or the basal lateral amygdala (BLA) enhances CFC (Kwapis et al. 2017), suggesting that HDACi in both regions contributes to learning and memory. Furthermore, direct HDACi treatment in the BLA enhances consolidation of inhibitory avoidance (IA) learning, a fear-motivated conditioning, while increasing levels of BDNF in the hippocampus, but not the amygdala itself (Valiati et al. 2017). This suggests that the interplay between the two brain regions is likely also affected by HDACi and that other brain region communication could be altered by systemic HDACi treatment. Considering that memory related diseases, such as AD, typically affect many different brain regions, a system-wide HDACi treatment may be able to amend neuronal acetylation and functioning in many affected regions at once. Therefore, it would be important to further study the brain-region specific responses the HDACi, both in healthy and impaired animals.

3.2.3 Experimental timing

It is also possible that the 1-hour time point after memory formation could be outside of the time resolution for the histone acetylation or transcriptional activation responses. For example, the transcriptional activation that is mediated by HDACi and learning-mediated acetylation could be occurring at later time points, as was described in previous work (Marco et al. 2020). Alternatively, it is possible that measuring mRNA and histone acetylation changes 1 hour after CFC might be more representative of second-wave effects of HDACi and CFC training. Previous work has shown that many IEGs - some of which act as transcription factors themselves - are already up-regulated 30 minutes after CFC and some secondary response

genes begin to be expressed by one hour after CFC (Tyssowski et al. 2018; Saha et al. 2011). This indicates that CFC induced transcriptional activation would already be showing secondary effects at our one-hour time point.

The lapse in time after CFC could also partially explain the substantial transcriptional down-regulation, which is surprising given that HDACs are members of protein complexes involved in transcriptional silencing (Nakayama and Hayakawa 2011; Hoffmann and Spengler 2019). However, this is also demonstrated in other studies in which an hours long treatment with the pan-HDACi, TSA, leads to gene down-regulation basally in the hippocampus (Lopez-Atalaya et al. 2013) and in both cancerous and non-cancerous cell lines (Greer et al. 2015). They suggest that the global acetylation changes induced by these inhibitors redistributes the binding of the elongation factor, Brd4, to other acetylated sites, thus reducing its occupancy at promoters and enhancers of silenced genes. Our data also supports a reallocation or transcriptional machinery as we found the *primed-silenced* cluster of the hippocampal bulk RNA-seq analysis to be enriched for only broad ontologies such as “system processes”, and thus decoupled from the same cluster in the H3K27ac dataset. This suggests that the HDACi alone may prime genes involved in cell function and maintenance, but these processes are then reset by neuronal activity since they are not required.

Altogether, our results, along with other studies of HDACi, indicate that we are not yet able to conclusively explain the temporal response of HDACi treatment paired with CFC. In the future, it is important to profile both histone acetylation and transcriptional activation at alternative time points to further understand the variation of the HDACi and CFC response over time.

3.2.4 Epigenetic cross-talk

Additionally, the apparent decoupling between H3K27ac and transcriptional activation implies that these transcriptional changes may also rely on the involvement of other epigenetic modifications during memory formation. For example, DNA methylation is also necessary for protein synthesis and downstream memory formation (C. A. Miller, Campbell, and Sweatt 2008; Pearce et al. 2017) and correlates with transcription of plasticity genes one hour after CFC (Halder et al. 2016; Duke et al. 2017). While little work has been done to explore the DNA methylation changes that are induced by HDACi during motor learning, one study indicates that the HDACi, 4b, can alter DNA methylation in a mouse model of Huntington’s Disease (Jia et al. 2015). Further studies would be needed to confirm the HDACi-mediated effects on DNA methylation after CFC.

In addition to DNA methylation, other histone PTMs are also regulated during learning. Histone phosphorylation is rapidly increased by neuronal stimulation both *in vitro* and *in vivo* and these changes are blocked by ERK inhibition (Crosio et al. 2003; Chwang et al. 2006). Furthermore, inhibiting the histone phosphatase, PP1, in the hippocampus and forebrain improves long-term object recognition and spatial memory (Koshibu et al. 2009; Gräff, Woldemichael, et al. 2012). Interestingly, PP1 inhibition increases histone acetylation and methylation in addition to histone phosphorylation (Koshibu et al. 2009), suggesting that enhancing histone phosphorylation may be a complementary method to HDACi for enhancing memory formation. Indeed, the classic yeast HAT, *Gcn5*, has been shown to have a high binding affinity to histone phosphorylation in mammalian cell lines (Cheung et al. 2000), suggesting that changes in histone phosphorylation could further localize histone acetylation one hour after learning and memory, though this has yet to be shown for mammalian HATs. The role of HDACi treatment on histone phosphorylation has not yet been explored in the context of hippocampal learning and memory, and further investigation would be needed to better understand this interplay.

3.2.5 Direct HDACi mediation of transcriptional processes

Another limitation of this analysis could be our understanding of the interplay between histone acetylation and transcriptional activation. Although many studies indicate that histone acetylation regulates transcription by increasing chromatin accessibility (Klose and Bird 2006; Hoffmann and Spengler 2019; Schübeler et al. 2000), recent evidence suggests that it may also play a more direct role in stimulation transcription. A recent study shows that, despite an initial increase in histone acetylation, chromatin accessibility actually changes more slowly than transcription in response to HDAC inhibition (Vaid, Wen, and Mannervik 2020). They suggest that HDACi treatment doesn't increase transcriptional initiation globally by opening induced by increased histone acetylation but instead stimulates RNA Polymerase II (PolII) elongation at genes containing a poised PolII molecule, possibly by directly acetylating the PolII C-terminal domain. Considering that many of the IEGs that are associated with learning and memory have been shown to be in the poised state (Saha et al. 2011), it's possible that HDACi can aid in the activation of those genes by stimulating PolII elongation (Schröder et al. 2013).

Interestingly, HDACi may also regulate memory consolidation by blocking transcription induced de-acetylation. During transcription, the PolII CTD recruits HDACs to curtail PolII elongation and remove histone acetylation (Ali et al. 2019). HDACi treatment blocks this recruitment, potentially allowing for genes that had been acetylated during transcription to remain acetylated, thus preserving the long-term maintenance of those genes. This would

likely play a role in learning and memory as neuronal activity drives chromatin opening that can remain for up to 48 hours and underlie plasticity in the adult brain (Fernandez-Albert et al. 2019). In the case of HDACi, these maintained-open sites could then be more likely to remain open, possibly contributing to the enhanced memory recall that we observed 24 hours post-CFC. In effect, this would mean that HDACi improves memory consolidation, not by enriching histone acetylation before transcription but by maintaining acetylation at regions that were transcriptionally activated. To better understand whether or not these processes are contributing to epigenetic priming and memory consolidation, we would need to better understand how HDACs interact with PolII during memory formation.

3.3 Final thoughts

In effect, our results provide important evidence that, when paired with a targeting stimulus, HDACi initiates a specific response that has a unique behavioral, electrophysiological and transcriptional output. It is likely that HDACi, in part, initiates these specific responses by increasing transcription of genes involved in MAPK signaling, which then further promotes processes based on the underlying activity patterns. Given the decoupling of histone acetylation and transcriptional changes, it is likely that the HDACi induced H3K27ac measured at enhancers one hour after CFC is not the only factor driving transcriptional activation at this time point.

To conclusively understand the role of HDACi in epigenetic priming, future studies are needed to analyze the role of other hippocampal subregions in learning and memory and the temporal interactions of histone acetylation and other epigenetic marks. In addition, the direct interactions of histone acetylation, transcription factors and PolII during memory formation and consolidation will also need to be better studied. These open questions notwithstanding, our findings shed light on the mechanisms by which a systemic HDACi treatment can lead to specific memory promotion. Understanding the mode of action of HDACis is important in light of their preclinical utility as cognitive enhancers for brain disorders. Whether class I HDACis similarly trigger enrichment of the ERK/MAPK pathway in conditions characterized by impaired cognition, such as AD or age-related cognitive decline, remains to be determined.

Finally, although many HDACis are already approved for use in cancer treatments and are currently in clinical trials to treat memory disorders (Terranova-Barberio et al. 2017; Burns and Gräff 2021), whether they are a viable option for memory enhancement in healthy individuals remains to be seen. While our results indicate that HDACi enhances underlying processes, secondary effects in response to long-term treatment remain unknown. In addition, we cannot

ensure that systemically enriching underlying processes always has a beneficial outcome. There are indeed examples of negative HDACi-mediated priming events, particularly when paired with drugs of abuse. For instance, HDACi treatment in brain reward regions, such as the Nucleus accumbens, enhanced place conditioning to cocaine and withdrawal related anxiety (Kumar et al. 2005; Mews, Walker, and Nestler 2018), suggesting that HDACi treatment can also lead to greater drug dependency. Based on this, further studies would be needed to determine the safety of HDACi for use in the general population. In the meantime, there are healthier methods of altering our epigenetic states that also led to cognitive enhancement. Environmental enrichment, or modifications that enhance the physical and social stimulation in one's environment, also increases histone acetylation, increased synaptic numbers and improved memory in impaired animals (Fischer et al. 2007). For otherwise healthy individuals, it is probably best to maintain a physically active and socially enriched lifestyle as a way to maintain cognitive health.

In all, our results show that systemic HDACi improves neuronal communication and activates transcription in two different brain regions in an activity dependent manner, thus giving credence to a broad treatment having a specific effect. This presents HDACis as a potential pharmacological intervention to improve cognitive function in those suffering from an array of neurological disorders.

References

Abate, Georgia, Sandra Colazingari, Alessandra Accoto, David Conversi, and Arturo Bevilacqua. 2018. “Dendritic Spine Density and EphrinB2 Levels of Hippocampal and Anterior Cingulate Cortex Neurons Increase Sequentially during Formation of Recent and Remote Fear Memory in the Mouse.” *Behavioural Brain Research* 344 (August 2017): 120–31. https://doi.org/10.1016/j.bbr.2018.02.011.

Adams, J. Paige, and J. David Sweatt. 2002. “Molecular Psychology: Roles for the ERK MAP Kinase Cascade in Memory.” *Annual Review of Pharmacology and Toxicology* 42 (1): 135–63. https://doi.org/10.1146/annurev.pharmtox.42.082701.145401.

Adamsky, Adar, and Inbal Goshen. 2018. “Astrocytes in Memory Function: Pioneering Findings and Future Directions.” *Neuroscience* 370: 14–26. https://doi.org/10.1016/j.neuroscience.2017.05.033.

Agís-Balboa, Roberto Carlos, Paulo S Pinheiro, Nelson Rebola, Cemil Kerimoglu, Eva Benito, Michael Gertig, Sanaz Bahari-Javan, et al. 2017. “Formin 2 Links Neuropsychiatric Phenotypes at Young Age to an Increased Risk for Dementia.” *The EMBO Journal* 36 (19): 2815–28. https://doi.org/10.15252/embj.201796821.

Alarcón, Juan M., Gaël Malleret, Khalid Touzani, Svetlana Vronskaya, Shunsuke Ishii, Eric R. Kandel, and Angel Barco. 2004. “Chromatin Acetylation, Memory, and LTP Are Impaired in CBP+/- Mice: A Model for the Cognitive Deficit in Rubinstein-Taybi Syndrome and Its Amelioration.” *Neuron* 42 (6): 947–59. https://doi.org/10.1016/j.neuron.2004.05.021.

Ali, Ibraheem, Diego Garrido Ruiz, Zuyao Ni, Jeffrey R. Johnson, Heng Zhang, Pao Chen Li, Mir M. Khalid, et al. 2019. “Crosstalk between RNA Pol II C-Terminal Domain Acetylation and Phosphorylation via RPRD Proteins.” *Molecular Cell* 74 (6): 1164–74. https://doi.org/10.1016/j.molcel.2019.04.008.

Alkadhi, Karim A. 2019. “Cellular and Molecular Differences Between Area CA1 and the Dentate Gyrus of the Hippocampus.” *Molecular Neurobiology* 56 (9): 6566–80. https://doi.org/10.1007/s12035-019-1541-2.

Alvarez, Veronica A., and Bernardo L. Sabatini. 2007. “Anatomical and Physiological Plasticity of Dendritic Spines.” *Annual Review of Neuroscience* 30: 79–97. https://doi.org/10.1146/annurev.neuro.30.051606.094222.

An, Juan Ji, Kusumika Gharami, Guey Ying Liao, Newton H. Woo, Anthony G. Lau, Filip Vanevski, Enrique R. Torre, et al. 2008. “Distinct Role of Long 3′ UTR BDNF MRNA in Spine Morphology and Synaptic Plasticity in Hippocampal Neurons.” *Cell* 134 (1): 175–87. https://doi.org/10.1016/j.cell.2008.05.045.

Assous, Maxime, Edward Martinez, Carol Eisenberg, Fulva Shah, Aleksandra Kosc, Kristie Varghese, Diego Espinoza, et al. 2019. “Neuropilin 2 Signaling Mediates Corticostriatal Transmission, Spine Maintenance, and Goal-Directed Learning in Mice.” *The Journal of Neuroscience* 39 (45): 8845–59. https://doi.org/10.1523/JNEUROSCI.1006-19.2019.

Atkins, Coleen M., Joel C. Selcher, Joseph J. Petraitis, James M. Trzaskos, and J. David Sweatt. 1998. “The MAPK Cascade Is Required for Mammalian Associative Learning.” *Nature Neuroscience* 1 (7): 602–9. https://doi.org/10.1038/2836.

Baker-Andresen, Danay, Vikram S. Ratnu, and Timothy W. Bredy. 2013. “Dynamic DNA

Methylation: A Prime Candidate for Genomic Metaplasticity and Behavioral Adaptation." *Trends in Neurosciences* 36 (1): 3–13. https://doi.org/10.1016/j.tins.2012.09.003.

Barker, Jennifer M., and Liisa A.M. Galea. 2010. "Males Show Stronger Contextual Fear Conditioning than Females after Context Pre-Exposure." *Physiology and Behavior* 99 (1): 82–90. https://doi.org/10.1016/j.physbeh.2009.10.014.

Beam, Christopher R., Cody Kaneshiro, Jung Yun Jang, Chandra A. Reynolds, Nancy L. Pedersen, and Margaret Gatz. 2018. "Differences between Women and Men in Incidence Rates of Dementia and Alzheimer's Disease." *Journal of Alzheimer's Disease* 64 (4): 1077–83. https://doi.org/10.3233/JAD-180141.

Benito, E., B. Ramachandran, H. Schroeder, G. Schmidt, H. Urbanke, S. Burkhardt, V. Capece, C. Dean, and A. Fischer. 2017. "The BET/BRD Inhibitor JQ1 Improves Brain Plasticity in WT and APP Mice." *Translational Psychiatry* 7 (9): e1239. https://doi.org/10.1038/tp.2017.202.

Benito, Eva, Camin Dean, André Fischer, Eva Benito, Hendrik Urbanke, Binu Ramachandran, Jonas Barth, et al. 2015. "HDAC Inhibitor – Dependent Transcriptome and Memory Reinstatement in Cognitive Decline Models." *Journal of Clinical Investigation* 125 (9): 3572–84. https://doi.org/10.1172/JCI79942.This.

Bird, Chris M., and Neil Burgess. 2008. "The Hippocampus and Memory: Insights from Spatial Processing." *Nature Reviews Neuroscience* 9 (3): 182–94. https://doi.org/10.1038/nrn2335.

Bliss, T. V.P., and G. L. Collingridge. 1993. "A Synaptic Model of Memory: Long-Term Potentiation in the Hippocampus." *Nature* 361 (6407): 31–39. https://doi.org/10.1038/361031a0.

Blum, Sonja, Anthony N. Moore, Frank Adams, and Pramod K. Dash. 1999. "A Mitogen-Activated Protein Kinase Cascade in the CA1/CA2 Subfield of the Dorsal Hippocampus Is Essential for Long-Term Spatial Memory." *Journal of Neuroscience* 19 (9): 3535–44. https://doi.org/10.1523/jneurosci.19-09-03535.1999.

Bolger, Anthony M., Marc Lohse, and Bjoern Usadel. 2014. "Trimmomatic: A Flexible Trimmer for Illumina Sequence Data." *Bioinformatics* 30 (15): 2114–20. https://doi.org/10.1093/bioinformatics/btu170.

Bonifer, Constanze, and Peter N. Cockerill. 2017. "Chromatin Priming of Genes in Development: Concepts, Mechanisms and Consequences." *Experimental Hematology* 49: 1–8. https://doi.org/10.1016/j.exphem.2017.01.003.

Bousiges, Olivier, Anne Pereira De Vasconcelos, Romain Neidl, Brigitte Cosquer, Karine Herbeaux, Irina Panteleeva, Jean Philippe Loeffler, Jean Christophe Cassel, and Anne Laurence Boutillier. 2010. "Spatial Memory Consolidation Is Associated with Induction of Several Lysine-Acetyltransferase (Histone Acetyltransferase) Expression Levels and H2B/H4 Acetylation-Dependent Transcriptional Events in the Rat Hippocampus." *Neuropsychopharmacology* 35 (13): 2521–37. https://doi.org/10.1038/npp.2010.117.

Bowers, Mallory E., Bing Xia, Samantha Carreiro, and Kerry J. Ressler. 2015. "The Class I HDAC Inhibitor RGFP963 Enhances Consolidation of Cued Fear Extinction." *Learning and Memory* 22 (4): 225–31. https://doi.org/10.1101/lm.036699.114.

Bradner, James E., Raymond Mak, Shyam K. Tanguturi, Ralph Mazitschek, Stephen J.

Haggarty, Kenneth Ross, Cindy Y. Chang, et al. 2010. “Chemical Genetic Strategy Identifies Histone Deacetylase 1 (HDAC1) and HDAC2 as Therapeutic Targets in Sickle Cell Disease.” *Proceedings of the National Academy of Sciences of the United States of America* 107 (28): 12617–22. https://doi.org/10.1073/pnas.1006774107.

Bredy, Timothy W., and Mark Barad. 2008. “The Histone Deacetylase Inhibitor Valproic Acid Enhances Acquisition, Extinction, and Reconsolidation of Conditioned Fear.” *Learning and Memory* 15 (1): 39–45. https://doi.org/10.1101/lm.801108.

Brouillette, Jonathan, and Rémi Quirion. 2008. “Transthyretin: A Key Gene Involved in the Maintenance of Memory Capacities during Aging.” *Neurobiology of Aging* 29 (11): 1721–32. https://doi.org/10.1016/j.neurobiolaging.2007.04.007.

Bureau, Geneviève, Mélanie Carrier, Manon Lebel, and Michel Cyr. 2010. “Intrastriatal Inhibition of Extracellular Signal-Regulated Kinases Impaired the Consolidation Phase of Motor Skill Learning.” *Neurobiology of Learning and Memory* 94 (1): 107–15. https://doi.org/10.1016/j.nlm.2010.04.008.

Burns, Allison M., and Johannes Gräff. 2021. “Cognitive Epigenetic Priming: Leveraging Histone Acetylation for Memory Amelioration.” *Current Opinion in Neurobiology* 67: 75–84. https://doi.org/10.1016/j.conb.2020.08.011.

Campbell, Rianne R., and Marcelo A. Wood. 2019. “How the Epigenome Integrates Information and Reshapes the Synapse.” *Nature Reviews Neuroscience* 20 (3): 133–47. https://doi.org/10.1038/s41583-019-0121-9.

Cao, Ting, Xiaojuan Zhou, Xianjie Zheng, Yue Cui, Joe Z. Tsien, Chunxia Li, and Huimin Wang. 2018. “Histone Deacetylase Inhibitor Alleviates the Neurodegenerative Phenotypes and Histone Dysregulation in Presenilins-Deficient Mice.” *Frontiers in Aging Neuroscience* 10 (MAY): 1–14. https://doi.org/10.3389/fnagi.2018.00137.

Carew, Thomas J., Vincent F. Castellucci, and Eric R. Kandel. 1971. “An Analysis of Dishabituation and Sensitization of the Gill-Withdrawal Reflex in Aplysia.” *International Journal of Neuroscience* 2 (2). https://doi.org/10.3109/00207457109146995.

Cataldi, Stefano, Adrien T. Stanley, Maria Concetta Miniaci, and David Sulzer. 2021. “Interpreting the Role of the Striatum during Multiple Phases of Motor Learning.” *The FEBS Journal*, May. https://onlinelibrary.wiley.com/doi/10.1111/febs.15908.

Chatterjee, Snehajyoti, Raphaelle Cassel, Anne Schneider-Anthony, Karine Merienne, Brigitte Cosquer, Laura Tzeplaeff, Sarmistha Halder Sinha, et al. 2018. “Reinstating Plasticity and Memory in a Tauopathy Mouse Model with an Acetyltransferase Activator.” *EMBO Molecular Medicine* 10 (11): 1–20. https://doi.org/10.15252/emmm.201708587.

Chen, Liang Fu, Yen Ting Lin, David A. Gallegos, Mariah F. Hazlett, Mariana Gómez-Schiavon, Marty G. Yang, Breanna Kalmeta, et al. 2019. “Enhancer Histone Acetylation Modulates Transcriptional Bursting Dynamics of Neuronal Activity-Inducible Genes.” *Cell Reports* 26 (5): 1174-1188.e5. https://doi.org/10.1016/j.celrep.2019.01.032.

Cheung, Peter, Kirk G. Tanner, Wang L. Cheung, Paolo Sassone-Corsi, John M. Denu, and C. David Allis. 2000. “Synergistic Coupling of Histone H3 Phosphorylation and Acetylation in Response to Epidermal Growth Factor Stimulation.” *Molecular Cell* 5 (6): 905–15. https://doi.org/10.1016/S1097-2765(00)80256-7.

Chwang, Wilson B., J. Simon Arthur, Armin Schumacher, and J. David Sweatt. 2007. “The

Nuclear Kinase Mitogen- and Stress-Activated Protein Kinase 1 Regulates Hippocampal Chromatin Remodeling in Memory Formation." *Journal of Neuroscience* 27 (46): 12732–42. https://doi.org/10.1523/JNEUROSCI.2522-07.2007.

Chwang, Wilson B., Kenneth J. O'Riordan, Jonathan M. Levenson, and J. David Sweatt. 2006. "ERK/MAPK Regulates Hippocampal Histone Phosphorylation Following Contextual Fear Conditioning." *Learning and Memory* 13 (3): 322–28. https://doi.org/10.1101/lm.152906.

Coda, Davide M., Tessa Gaarenstroom, Philip East, Harshil Patel, Daniel S.J. Miller, Anna Lobley, Nik Matthews, Aengus Stewart, and Caroline S. Hill. 2017. "Distinct Modes of SMAD2 Chromatin Binding and Remodeling Shape the Transcriptional Response to NODAL/Activin Signaling." *ELife* 6: 1–31. https://doi.org/10.7554/eLife.22474.

Collins, Bridget E., Celeste B. Greer, Benjamin C. Coleman, and J. David Sweatt. 2019. "Histone H3 Lysine K4 Methylation and Its Role in Learning and Memory 06 Biological Sciences 0604 Genetics 11 Medical and Health Sciences 1109 Neurosciences." *Epigenetics and Chromatin* 12 (1): 1–16. https://doi.org/10.1186/s13072-018-0251-8.

Cooper, Alexis, Tamer Butto, Niklas Hammer, Somanath Jagannath, Desiree Lucia Fend-Guella, Junaid Akhtar, Konstantin Radyushkin, et al. 2020. "Inhibition of Histone Deacetylation Rescues Phenotype in a Mouse Model of Birk-Barel Intellectual Disability Syndrome." *Nature Communications* 11 (1): 1–14. https://doi.org/10.1038/s41467-019-13918-4.

Costa, Rui M., Dana Cohen, and Miguel A.L. Nicolelis. 2004. "Differential Corticostriatal Plasticity during Fast and Slow Motor Skill Learning in Mice." *Current Biology* 14 (13): 1124–34. https://doi.org/10.1016/j.cub.2004.06.053.

Creyghton, Menno P., Albert W. Cheng, G. Grant Welstead, Tristan Kooistra, Bryce W. Carey, Eveline J. Steine, Jacob Hanna, et al. 2010. "Histone H3K27ac Separates Active from Poised Enhancers and Predicts Developmental State." *Proceedings of the National Academy of Sciences of the United States of America* 107 (50): 21931–36. https://doi.org/10.1073/pnas.1016071107.

Crick, Francis. 1984. "Memory and Molecular Turnover." *Nature*.

Crosio, Claudia, Estelle Heitz, C. Davis Allis, Emiliana Borrelli, and Paolo Sassone-Corsi. 2003. "Chromatin Remodeling and Neuronal Response: Multiple Signaling Pathways Induce Specific Histone H3 Modifications and Early Gene Expression in Hippocampal Neurons." *Journal of Cell Science* 116 (24): 4905–14. https://doi.org/10.1242/jcs.00804.

Davis, Sabrina, Peter Vanhoutte, Christiane Pagès, Jocelyne Caboche, and Serge Laroche. 2000. "The MAPK/ERK Cascade Targets Both Elk-1 and CAMP Response Element-Binding Protein to Control Long-Term Potentiation-Dependent Gene Expression in the Dentate Gyrus in Vivo." *Journal of Neuroscience* 20 (12): 4563–72. https://doi.org/10.1523/jneurosci.20-12-04563.2000.

Day, Jeremy J., and J. David Sweatt. 2012. "Epigenetic Treatments for Cognitive Impairments." *Neuropsychopharmacology* 37 (1): 247–60. https://doi.org/10.1038/npp.2011.85.

Denny, Christine A., Mazen A. Kheirbek, Eva L. Alba, Kenji F. Tanaka, Rebecca A. Brachman, Kimberly B. Laughman, Nicole K. Tomm, Gergely F. Turi, Attila Losonczy, and René Hen. 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.

Dobin, Alexander, Carrie A. Davis, Felix Schlesinger, Jorg Drenkow, Chris Zaleski, Sonali Jha, Philippe Batut, Mark Chaisson, and Thomas R. Gingeras. 2013. "STAR: Ultrafast Universal RNA-Seq Aligner." *Bioinformatics* 29 (1): 15–21. https://doi.org/10.1093/bioinformatics/bts635.

Dolgachev, Vladislav, Molly Thomas, Aaron Berlin, and Nicholas W. Lukacs. 2007. "Stem Cell Factor-Mediated Activation Pathways Promote Murine Eosinophil CCL6 Production and Survival." *Journal of Leukocyte Biology* 81 (4): 1111–19. https://doi.org/10.1189/jlb.0906595.

Duke, Corey G., Andrew J. Kennedy, Cristin F. Gavin, Jeremy J. Day, and J. David Sweatt. 2017. "Experience-Dependent Epigenomic Reorganization in the Hippocampus." *Learning and Memory* 24 (7): 278–88. https://doi.org/10.1101/lm.045112.117.

Durieux, Pierre F., Serge N. Schiffmann, and Alban De Kerchove D'Exaerde. 2012. "Differential Regulation of Motor Control and Response to Dopaminergic Drugs by D1R and D2R Neurons in Distinct Dorsal Striatum Subregions." *EMBO Journal* 31 (3): 640–53. https://doi.org/10.1038/emboj.2011.400.

Ernst, Jason, and Manolis Kellis. 2012. "ChromHMM: Automating Chromatin-State Discovery and Characterization." *Nature Methods* 9 (3): 215–16. https://doi.org/10.1038/nmeth.1906.

Erö, Csaba, Marc Oliver Gewaltig, Daniel Keller, and Henry Markram. 2018. "A Cell Atlas for the Mouse Brain." *Frontiers in Neuroinformatics* 12 (November): 1–16. https://doi.org/10.3389/fninf.2018.00084.

Falkenberg, Katrina J., and Ricky W. Johnstone. 2014. "Histone Deacetylases and Their Inhibitors in Cancer, Neurological Diseases and Immune Disorders." *Nature Reviews Drug Discovery* 13 (9): 673–91. https://doi.org/10.1038/nrd4360.

Fernandez-Albert, Jordi, Michal Lipinski, María T. Lopez-Cascales, M. Jordan Rowley, Ana M. Martin-Gonzalez, Beatriz del Blanco, Victor G. Corces, and Angel Barco. 2019. "Immediate and Deferred Epigenomic Signatures of in Vivo Neuronal Activation in Mouse Hippocampus." *Nature Neuroscience* 22 (10): 1718–30. https://doi.org/10.1038/s41593-019-0476-2.

Ferreira, Tatiana L., Karin M. Moreira, Daniela C. Ikeda, Orlando F.A. Bueno, and Maria Gabriela M. Oliveira. 2003. "Effects of Dorsal Striatum Lesions in Tone Fear Conditioning and Contextual Fear Conditioning." *Brain Research* 987 (1): 17–24. https://doi.org/10.1016/S0006-8993(03)03217-7.

Fiala, John C., Josef Spacek, and Kristen M. Harris. 2002. "Dendritic Spine Pathology: Cause or Consequence of Neurological Disorders?" *Brain Research Reviews* 39 (1): 29–54. https://doi.org/10.1016/S0165-0173(02)00158-3.

Fischer, Andre, Farahnaz Sananbenesi, Xinyu Wang, Matthew Dobbin, and Li Huei Tsai. 2007. "Recovery of Learning and Memory Is Associated with Chromatin Remodelling." *Nature* 447 (7141): 178–82. https://doi.org/10.1038/nature05772.

Fleiss, Bobbi, Marie K.L. Nilsson, Klas Blomgren, and Carina Mallard. 2012. "Neuroprotection by the Histone Deacetylase Inhibitor Trichostatin A in a Model of Lipopolysaccharide-

Sensitised Neonatal Hypoxic-Ischaemic Brain Injury." *Journal of Neuroinflammation* 9: 1–15. https://doi.org/10.1186/1742-2094-9-70.

Gardner, Tina M., Rehan Aziz, Sunanda Muralee, and Rajesh R. Tampi. 2009. "Valproic Acid-Induced Myoclonus in a Demented Patient: A Case Report." *Case Reports in Medicine* 2009: 1–3. https://doi.org/10.1155/2009/392091.

Goldberg, Aaron D., C. David Allis, and Emily Bernstein. 2007. "Epigenetics: A Landscape Takes Shape." *Cell* 128 (4): 635–38. https://doi.org/10.1016/j.cell.2007.02.006.

Gräff, Johannes, Nadine F. Joseph, Meryl E. Horn, Alireza Samiei, Jia Meng, Jinsoo Seo, Damien Rei, et al. 2014. "Epigenetic Priming of Memory Updating during Reconsolidation to Attenuate Remote Fear Memories." *Cell* 156 (1–2): 261–76. https://doi.org/10.1016/j.cell.2013.12.020.

Gräff, Johannes, Damien Rei, Ji Song Guan, Wen Yuan Wang, Jinsoo Seo, Krista M. Hennig, Thomas J.F. Nieland, et al. 2012. "An Epigenetic Blockade of Cognitive Functions in the Neurodegenerating Brain." *Nature* 483 (7388): 222–26. https://doi.org/10.1038/nature10849.

Gräff, Johannes, and Li Huei Tsai. 2013. "Histone Acetylation: Molecular Mnemonics on the Chromatin." *Nature Reviews Neuroscience* 14 (2): 97–111. https://doi.org/10.1038/nrn3427.

Gräff, Johannes, Bisrat T. Woldemichael, Dominik Berchtold, Grégoire Dewarrat, and Isabelle M. Mansuy. 2012. "Dynamic Histone Marks in the Hippocampus and Cortex Facilitate Memory Consolidation." *Nature Communications* 3. https://doi.org/10.1038/ncomms1997.

Greer, Celeste B., Yoshiaki Tanaka, Yoon Jung Kim, Peng Xie, Michael Q. Zhang, In Hyun Park, and Tae Hoon Kim. 2015. "Histone Deacetylases Positively Regulate Transcription through the Elongation Machinery." *Cell Reports* 13 (7): 1444–55. https://doi.org/10.1016/j.celrep.2015.10.013.

Groenewegen, Henk J. 2003. "The Basal Ganglia and Motor Control." *Neural Plasticity* 10 (1–2): 107–20. https://doi.org/10.1155/NP.2003.107.

Guan, Ji Song, Stephen J. Haggarty, Emanuela Giacometti, Jan Hermen Dannenberg, Nadine Joseph, Jun Gao, Thomas J.F. Nieland, et al. 2009. "HDAC2 Negatively Regulates Memory Formation and Synaptic Plasticity." *Nature* 459 (7243): 55–60. https://doi.org/10.1038/nature07925.

Gulmez Karaca, Kubra, Janina Kupke, David V.C. Brito, Benjamin Zeuch, Christian Thome, Dieter Weichenhan, Pavlo Lutsik, Christoph Plass, and Ana M.M. Oliveira. 2020. "Neuronal Ensemble-Specific DNA Methylation Strengthens Engram Stability." *Nature Communications* 11 (1): 1–13. https://doi.org/10.1038/s41467-020-14498-4.

Gupta, Swati, Se Y. Kim, Sonja Artis, David L. Molfese, Armin Schumacher, J. David Sweatt, Richard E. Paylor, and Farah D. Lubin. 2010. "Histone Methylation Regulates Memory Formation." *Journal of Neuroscience* 30 (10): 3589–99. https://doi.org/10.1523/JNEUROSCI.3732-09.2010.

Haber, Suzanne N. 2016. "Corticostriatal Circuitry." *Dialogues in Clinical Neuroscience* 18 (1): 7–21. https://doi.org/10.1007/978-1-4614-6434-1_135-1.

Haberland, Michael, Rusty L. Montgomery, and Eric N. Olson. 2009. “The Many Roles of Histone Deacetylases in Development and Physiology: Implications for Disease and Therapy.” *Nature Reviews Genetics* 10 (1): 32–42. https://doi.org/10.1038/nrg2485.

Hafemeister, Christoph, and Rahul Satija. 2019. “Normalization and Variance Stabilization of Single-Cell RNA-Seq Data Using Regularized Negative Binomial Regression.” *Genome Biology* 20 (1): 296. https://doi.org/10.1186/s13059-019-1874-1.

Halder, Rashi, Magali Hennion, Ramon O. Vidal, Orr Shomroni, Raza Ur Rahman, Ashish Rajput, Tonatiuh Pena Centeno, et al. 2016. “DNA Methylation Changes in Plasticity Genes Accompany the Formation and Maintenance of Memory.” *Nature Neuroscience* 19 (1): 102–10. https://doi.org/10.1038/nn.4194.

Han, Jin-hee, Steven a Kushner, Adelaide P Yiu, Christy J Cole, Anna Matynia, Robert a Brown, Rachael L Neve, John F Guzowski, Alcino J Silva, and Sheena a Josselyn. 2007. “Neuronal Competition and Selection During Memory Formation.” *Science* 316 (April): 457–60. http://www.ncbi.nlm.nih.gov/pubmed/17446403%5Cnhttp://www.ncbi.nlm.nih.gov/sites/entrez?Db=pubmed&DbFrom=pubmed&Cmd=Link&LinkName=pubmed_pubmed&LinkReadableName=Related Articles&IdsFromResult=17446403&ordinalpos=3&itool=EntrezSystem2.PEntrez.Pubmed.Pubmed.

Han, Seungrie, Soontaek Hong, Dongmin Lee, Myeong Hoe Lee, June Seek Choi, Min Jung Koh, Woong Sun, Hyun Kim, and Hyun Woo Lee. 2012. “Altered Expression of Synaptotagmin 13 MRNA in Adult Mouse Brain after Contextual Fear Conditioning.” *Biochemical and Biophysical Research Communications* 425 (4): 880–85. https://doi.org/10.1016/j.bbrc.2012.07.166.

Hanson, Jesse E., Hank La, Emile Plise, Yung Hsiang Chen, Xiao Ding, Taleen Hanania, Emily V. Sabath, et al. 2013. “SAHA Enhances Synaptic Function and Plasticity In Vitro but Has Limited Brain Availability In Vivo and Does Not Impact Cognition.” *PLoS ONE* 8 (7). https://doi.org/10.1371/journal.pone.0069964.

Hao, Yuhan, Stephanie Hao, Erica Andersen-Nissen, William M. Mauck, Shiwei Zheng, Andrew Butler, Maddie J. Lee, et al. 2021. “Integrated Analysis of Multimodal Single-Cell Data.” *Cell* 184 (13): 3573–87. https://doi.org/10.1016/j.cell.2021.04.048.

Heinz, Sven, Christopher Benner, Nathanael Spann, Eric Bertolino, Yin C. Lin, Peter Laslo, Jason X. Cheng, Cornelis Murre, Harinder Singh, and Christopher K. Glass. 2010. “Simple Combinations of Lineage-Determining Transcription Factors Prime Cis-Regulatory Elements Required for Macrophage and B Cell Identities.” *Molecular Cell* 38 (4): 576–89. https://doi.org/10.1016/j.molcel.2010.05.004.

Herrmann, Nathan, Krista L. Lanctôt, Lana S. Rothenburg, and Goran Eryavec. 2007. “A Placebo-Controlled Trial of Valproate for Agitation and Aggression in Alzheimer’s Disease.” *Dementia and Geriatric Cognitive Disorders* 23 (2): 116–19. https://doi.org/10.1159/000097757.

Hinds, Heather L., Ivan Goussakov, Kazu Nakazawa, Susumu Tonegawa, and Vadim Y. Bolshakov. 2003. “Essential Function of α-Calcium/Calmodulin-Dependent Protein Kinase II in Neurotransmitter Release at a Glutamatergic Central Synapse.” *Proceedings of the National Academy of Sciences of the United States of America* 100 (7): 4275–80. https://doi.org/10.1073/pnas.0530202100.

Hoffmann, Anke, and Dietmar Spengler. 2019. “Chromatin Remodeling Complex NuRD in Neurodevelopment and Neurodevelopmental Disorders.” *Frontiers in Genetics* 10 (JUL). https://doi.org/10.3389/fgene.2019.00682.

Hong, L., G. P. Schroth, H. R. Matthews, P. Yau, and E. M. Bradbury. 1993. “Studies of the DNA Binding Properties of Histone H4 Amino Terminus. Thermal Denaturation Studies Reveal That Acetylation Markedly Reduces the Binding Constant of the H4 ‘tail’ to DNA.” *Journal of Biological Chemistry* 268 (1): 305–14.

Hori, Kei, Taku Nagai, Wei Shan, Asami Sakamoto, Manabu Abe, Maya Yamazaki, Kenji Sakimura, Kiyofumi Yamada, and Mikio Hoshino. 2015. “Heterozygous Disruption of Autism Susceptibility Candidate 2 Causes Impaired Emotional Control and Cognitive Memory.” *PLoS ONE* 10 (12): 1–13. https://doi.org/10.1371/journal.pone.0145979.

Hrvatin, Sinisa, Daniel R. Hochbaum, M. Aurel Nagy, Marcelo Cicconet, Keiramarie Robertson, Lucas Cheadle, Rapolas Zilionis, et al. 2017. “Transcriptomic States in the Mouse Visual Cortex.” *Nature Neuroscience* 21 (January): 1–19. https://doi.org/10.1038/s41593-017-0029-5.

Hsu, Chin Jung, Min Huan Wu, Chin Yuan Chen, Chun Hao Tsai, Horng Chaung Hsu, and Chih Hsin Tang. 2013. “AMP-Activated Protein Kinase Activation Mediates CCL3-Induced Cell Migration and Matrix Metalloproteinase-2 Expression in Human Chondrosarcoma.” *Cell Communication and Signaling* 11 (1): 1–15. https://doi.org/10.1186/1478-811X-11-68.

Huang, Yan You, Amir Levine, Denise B. Kandel, Deqi Yin, Luca Colnaghi, Bettina Drisaldi, and Eric R. Kandel. 2014. “D1/D5 Receptors and Histone Deacetylation Mediate the Gateway Effect of LTP in Hippocampal Dentate Gyrus.” *Learning and Memory* 21 (3): 153–60. https://doi.org/10.1101/lm.032292.113.

Jaeger, Baptiste N., Sara B. Linker, Sarah L. Parylak, Jerika J. Barron, Iryna S. Gallina, Christian D. Saavedra, Conor Fitzpatrick, et al. 2018. “A Novel Environment-Evoked Transcriptional Signature Predicts Reactivity in Single Dentate Granule Neurons.” *Nature Communications* 9 (1): 3084. https://doi.org/10.1038/s41467-018-05418-8.

Janczura, Karolina J., Claude Henry Volmar, Gregory C. Sartor, Sunil J. Rao, Natalie R. Ricciardi, Guerline Lambert, Shaun P. Brothers, and Claes Wahlestedt. 2018. “Inhibition of HDAC3 Reverses Alzheimer’s Disease-Related Pathologies in Vitro and in the 3xTg-AD Mouse Model.” *Proceedings of the National Academy of Sciences of the United States of America* 115 (47): E11148–57. https://doi.org/10.1073/pnas.1805436115.

Jaric, Ivana, Devin Rocks, John M. Greally, Masako Suzuki, and Marija Kundakovic. 2019. “Chromatin Organization in the Female Mouse Brain Fluctuates across the Oestrous Cycle.” *Nature Communications* 10 (1). https://doi.org/10.1038/s41467-019-10704-0.

Jeong, Hyeanjeong, Seulgi Shin, Jun Seok Lee, Soo Hyun Lee, Ja Hyun Baik, Sungsu Lim, and Yun Kyung Kim. 2019. “Pan-HDAC Inhibitors Promote Tau Aggregation by Increasing the Level of Acetylated TAU.” *International Journal of Molecular Sciences* 20 (17). https://doi.org/10.3390/ijms20174283.

Jia, Haiqun, Ryan J. Kast, Joan S. Steffan, and Elizabeth A. Thomas. 2012. “Selective Histone Deacetylase (HDAC) Inhibition Imparts Beneficial Effects in Huntington’s Disease Mice: Implications for the Ubiquitin-Proteasomal and Autophagy Systems.” *Human Molecular Genetics* 21 (24): 5280–93. https://doi.org/10.1093/hmg/dds379.

Jia, Haiqun, Charles D. Morris, Roy M. Williams, Jeanne F. Loring, and Elizabeth A. Thomas. 2015. “HDAC Inhibition Imparts Beneficial Transgenerational Effects in Huntington’s Disease Mice via Altered DNA and Histone Methylation.” *Proceedings of the National Academy of Sciences of the United States of America* 112 (1): E56–64. https://doi.org/10.1073/pnas.1415195112.

Jin, Long, Qing Guo, Guang Lei Zhang, Xiao Xu Xing, Mei Fu Xuan, Qi Rong Luo, Zhao Bo Luo, Jun Xia Wang, Xi Jun Yin, and Jin Dan Kang. 2018. “The Histone Deacetylase Inhibitor, CI994, Improves Nuclear Reprogramming and in Vitro Developmental Potential of Cloned Pig Embryos.” *Cellular Reprogramming* 20 (3): 205–13. https://doi.org/10.1089/cell.2018.0001.

Josselyn, Sheena A., Stefan Köhler, and Paul W. Frankland. 2015. “Finding the Engram.” *Nature Reviews Neuroscience* 16 (9): 521–34. https://doi.org/10.1038/nrn4000.

Josselyn, Sheena A., Chanjun Shi, William A. Carlezon, Rachael L. Neve, Eric J. Nestler, and Michael Davis. 2001. “Long-Term Memory Is Facilitated by CAMP Response Element-Binding Protein Overexpression in the Amygdala.” *Journal of Neuroscience* 21 (7): 2404–12. https://doi.org/10.1523/jneurosci.21-07-02404.2001.

Josselyn, Sheena A., and Susumu Tonegawa. 2020. “Memory Engrams: Recalling the Past and Imagining the Future.” *Science*. https://doi.org/10.1126/science.aaw4325.

Kandel, Eric R. 2001. “The Molecular Biology of Memory Storage: A Dialogue between Genes and Synapses.” *Science (New York, N.Y.)* 294 (5544): 1030–38. https://doi.org/10.1126/science.1067020.

Kang, HJ J., and EM M. Schuman. 1995. “Neurotrophin-Induced Modulation of Synaptic Transmission in the Adult Hippocampus.” *Journal of Physiology - Paris* 89 (1): 11–22. https://doi.org/10.1016/0928-4257(96)80547-X.

Keiser, Ashley A., Lacie M. Turnbull, Mara A. Darian, Dana E. Feldman, Iris Song, and Natalie C. Tronson. 2017. “Sex Differences in Context Fear Generalization and Recruitment of Hippocampus and Amygdala during Retrieval.” *Neuropsychopharmacology* 42 (2): 397–407. https://doi.org/10.1038/npp.2016.174.

Kheirbek, Mazen A., Jon P. Britt, Jeff A. Beeler, Yoshihiro Ishikawa, Daniel S. McGehee, and Xiaoxi Zhuang. 2009. “Adenylyl Cyclase Type 5 Contributes to Corticostriatal Plasticity and Striatum-Dependent Learning.” *Journal of Neuroscience* 29 (39): 12115–24. https://doi.org/10.1523/JNEUROSCI.3343-09.2009.

Kim, J., and M. Fanselow. 1992. “Modality-Specific Retrograde Amnesia of Fear.” *Science* 256 (5057): 675–77. https://doi.org/10.1126/science.1585183.

Kim, Woong Bin, and Jun Hyeong Cho. 2020. “Encoding of Contextual Fear Memory in Hippocampal–Amygdala Circuit.” *Nature Communications* 11 (1): 1–22. https://doi.org/10.1038/s41467-020-15121-2.

Klemm, Sandy L., Zohar Shipony, and William J. Greenleaf. 2019. “Chromatin Accessibility and the Regulatory Epigenome.” *Nature Reviews Genetics* 20 (4): 207–20. https://doi.org/10.1038/s41576-018-0089-8.

Klose, Robert J., and Adrian P. Bird. 2006. “Genomic DNA Methylation: The Mark and Its Mediators.” *Trends in Biochemical Sciences* 31 (2): 89–97. https://doi.org/10.1016/j.tibs.2005.12.008.

Koshibu, Kyoko, Johannes Gräff, Monique Beullens, Fabrice D. Heitz, Dominik Berchtold, Holger Russig, Mélissa Farinelli, Mathieu Bollen, and Isabelle M. Mansuy. 2009. “Protein Phosphatase 1 Regulates the Histone Code for Long-Term Memory.” *Journal of Neuroscience* 29 (41): 13079–89. https://doi.org/10.1523/JNEUROSCI.3610-09.2009.

Koul, Hari K., Mantu Pal, and Sweaty Koul. 2013. “Role of P38 MAP Kinase Signal Transduction in Solid Tumors.” *Genes and Cancer* 4 (9–10): 342–59. https://doi.org/10.1177/1947601913507951.

Krishna, Kumar, Thomas Behnisch, and Sreedharan Sajikumar. 2016. “Inhibition of Histone Deacetylase 3 Restores Amyloid-β Oligomer-Induced Plasticity Deficit in Hippocampal CA1 Pyramidal Neurons.” *Journal of Alzheimer’s Disease* 51 (3): 783–91. https://doi.org/10.3233/JAD-150838.

Kumar, Arvind, Kwang Ho Choi, William Renthal, Nadia M. Tsankova, David E.H. Theobald, Hoang Trang Truong, Scott J. Russo, et al. 2005. “Chromatin Remodeling Is a Key Mechanism Underlying Cocaine-Induced Plasticity in Striatum.” *Neuron* 48 (2): 303–14. https://doi.org/10.1016/j.neuron.2005.09.023.

Kwapis, Janine L., Yasaman Alaghband, Alberto J. López, André O. White, Rianne R. Campbell, Richard T. Dang, DIane Rhee, et al. 2017. “Context and Auditory Fear Are Differentially Regulated by HDAC3 Activity in the Lateral and Basal Subnuclei of the Amygdala.” *Neuropsychopharmacology* 42 (6): 1284–94. https://doi.org/10.1038/npp.2016.274.

Kyriakis, John M., Papia Banerjee, Eleni Nikolakaki, Tianang Dai, Elizabeth A. Rubie, Mir F. Ahmad, Joseph Avruch, and James R. Woodgett. 1994. “The Stress-Activated Protein Kinase Subfamily of c-Jun Kinases.” *Nature* 369 (6476): 156–60. https://doi.org/10.1038/369156a0.

Landeira-Fernandez, J., Joseph P. DeCola, Jeansok J. Kim, and Michael S. Fanselow. 2006. “Immediate Shock Deficit in Fear Conditioning: Effects of Shock Manipulations.” *Behavioral Neuroscience* 120 (4): 873–79. https://doi.org/10.1037/0735-7044.120.4.873.

Langmead, Ben, and Steven L. Salzberg. 2012. “Fast Gapped-Read Alignment with Bowtie 2.” *Nature Methods* 9 (4): 357–59. https://doi.org/10.1038/nmeth.1923.

Lee, Yong Seok, and Alcino J. Silva. 2009. “The Molecular and Cellular Biology of Enhanced Cognition.” *Nature Reviews Neuroscience* 10 (2): 126–40. https://doi.org/10.1038/nrn2572.

Lein, Ed S., Michael J. Hawrylycz, Nancy Ao, Mikael Ayres, Amy Bensinger, Amy Bernard, Andrew F. Boe, et al. 2007. “Genome-Wide Atlas of Gene Expression in the Adult Mouse Brain.” *Nature* 445 (7124): 168–76. https://doi.org/10.1038/nature05453.

Levenson, Jonathan M., Kenneth J. O’Riordan, Karen D. Brown, Mimi A. Trinh, David L. Molfese, and J. David Sweatt. 2004. “Regulation of Histone Acetylation during Memory Formation in the Hippocampus.” *Journal of Biological Chemistry* 279 (39): 40545–59. https://doi.org/10.1074/jbc.M402229200.

Levenson, Jonathan M., and J. David Sweatt. 2005. “Epigenetic Mechanisms in Memory Formation.” *Nature Reviews Neuroscience* 6 (2): 108–18. https://doi.org/10.1038/nrn1604.

Li, Heng, Bob Handsaker, Alec Wysoker, Tim Fennell, Jue Ruan, Nils Homer, Gabor Marth,

Goncalo Abecasis, and Richard Durbin. 2009. “The Sequence Alignment/Map Format and SAMtools.” *Bioinformatics* 25 (16): 2078–79. https://doi.org/10.1093/bioinformatics/btp352.

Li, Jie, Rena Y. Jiang, Kristin L. Arendt, Yu Tien Hsu, Sophia R. Zhai, and Lu Chen. 2020. “Defective Memory Engram Reactivation Underlies Impaired Fear Memory Recall in Fragile x Syndrome.” *ELife* 9: 1–20. https://doi.org/10.7554/eLife.61882.

Li, Shuyuan, Xiaoshuang Lu, Qian Shao, Zixin Chen, Qiong Huang, Zinan Jiao, Xiaodi Huang, et al. 2019. “Early Histone Deacetylase Inhibition Mitigates Ischemia/Reperfusion Brain Injury by Reducing Microglia Activation and Modulating Their Phenotype.” *Frontiers in Neurology* 10 (AUG): 1–17. https://doi.org/10.3389/fneur.2019.00893.

Lin, Chin Hsing, Stacey Hansen, Zhenshan Wang, Daniel R. Storm, Stephen J. Tapscott, and James M. Olson. 2005. “The Dosage of the NeuroD2 Transcription Factor Regulates Amygdala Development and Emotional Learning.” *Proceedings of the National Academy of Sciences of the United States of America* 102 (41): 14877–82. https://doi.org/10.1073/pnas.0506785102.

Liu, Ingrid Y.C., W. Ernest Lyons, Laura A. Mamounas, and Richard F. Thompson. 2004. “Brain-Derived Neurotrophic Factor Plays a Critical Role in Contextual Fear Conditioning.” *Journal of Neuroscience* 24 (36): 7958–63. https://doi.org/10.1523/JNEUROSCI.1948-04.2004.

Liu, Jiancheng, Xiwei Wu, Heying Zhang, Gerd P. Pfeifer, and Qiang Lu. 2017. “Dynamics of RNA Polymerase II Pausing and Bivalent Histone H3 Methylation during Neuronal Differentiation in Brain Development.” *Cell Reports* 20 (6): 1307–18. https://doi.org/10.1016/j.celrep.2017.07.046.

Liu, Xu, Steve Ramirez, Petti T. Pang, Corey B. Puryear, Arvind Govindarajan, Karl Deisseroth, and Susumu Tonegawa. 2012. “Optogenetic Stimulation of a Hippocampal Engram Activates Fear Memory Recall.” *Nature* 484 (7394): 381–85. https://doi.org/10.1038/nature11028.

Liz, Marcia Almeida, Teresa Coelho, Vittorio Bellotti, Maria Isabel Fernandez-Arias, Pablo Mallaina, and Laura Obici. 2020. “A Narrative Review of the Role of Transthyretin in Health and Disease.” *Neurology and Therapy* 9 (2): 395–402. https://doi.org/10.1007/s40120-020-00217-0.

Lopez-Atalaya, Jose P., Satomi Ito, Luis M. Valor, Eva Benito, and Angel Barco. 2013. “Genomic Targets, and Histone Acetylation and Gene Expression Profiling of Neural HDAC Inhibition.” *Nucleic Acids Research* 41 (17): 8072–84. https://doi.org/10.1093/nar/gkt590.

Love, Michael I., Wolfgang Huber, and Simon Anders. 2014. “Moderated Estimation of Fold Change and Dispersion for RNA-Seq Data with DESeq2.” *Genome Biology* 15 (12): 1–21. https://doi.org/10.1186/s13059-014-0550-8.

Luo, Weijun, and Cory Brouwer. 2013. “Pathview : An R / Bioconductor Package for Pathway-Based Data Integration and Visualization” 29 (14): 1830–31. https://doi.org/10.1093/bioinformatics/btt285.

Malik, Athar N., Thomas Vierbuchen, Martin Hemberg, Alex A. Rubin, Emi Ling, Cameron H. Couch, Hume Stroud, et al. 2014. “Genome-Wide Identification and Characterization of Functional Neuronal Activity-Dependent Enhancers.” *Nature Neuroscience* 17 (10):

1330–39. https://doi.org/10.1038/nn.3808.

Marco, Asaf, Hiruy S. Meharena, Vishnu Dileep, Ravikiran M. Raju, Jose Davila-Velderrain, Amy Letao Zhang, Chinnakkaruppan Adaikkan, et al. 2020. “Mapping the Epigenomic and Transcriptomic Interplay during Memory Formation and Recall in the Hippocampal Engram Ensemble.” *Nature Neuroscience* 23 (12): 1606–17. https://doi.org/10.1038/s41593-020-00717-0.

Maren, Stephen, Beatrice De Oca, and Michael S. Fanselow. 1994. “Sex Differences in Hippocampal Long-Term Potentiation (LTP) and Pavlovian Fear Conditioning in Rats: Positive Correlation between LTP and Contextual Learning.” *Brain Research* 661 (1–2): 25–34. https://doi.org/10.1016/0006-8993(94)91176-2.

Mayford, Mark, Steven A. Siegelbaum, and Eric R. Kandel. 2012. “Synapses and Memory Storage.” *Cold Spring Harbor Perspectives in Biology* 4 (6): 1–18. https://doi.org/10.1101/cshperspect.a005751.

Maze, Ian, Wendy Wenderski, Kyung Min Noh, Rosemary C. Bagot, Nikos Tzavaras, Immanuel Purushothaman, Simon J. Elsässer, et al. 2015. “Critical Role of Histone Turnover in Neuronal Transcription and Plasticity.” *Neuron* 87 (1): 77–94. https://doi.org/10.1016/j.neuron.2015.06.014.

McGinnis, Christopher S., Lyndsay M. Murrow, and Zev J. Gartner. 2019. “DoubletFinder: Doublet Detection in Single-Cell RNA Sequencing Data Using Artificial Nearest Neighbors.” *Cell Systems* 8 (4): 329–37. https://doi.org/10.1016/j.cels.2019.03.003.

McQuown, Susan C., Ruth M. Barrett, Dina P. Matheos, Rebecca J. Post, George A. Rogge, Theresa Alenghat, Shannon E. Mullican, et al. 2011. “HDAC3 Is a Critical Negative Regulator of Long-Term Memory Formation.” *Journal of Neuroscience* 31 (2): 764–74. https://doi.org/10.1523/JNEUROSCI.5052-10.2011.

Mews, Philipp, Deena M. Walker, and Eric J. Nestler. 2018. “Epigenetic Priming in Drug Addiction.” *Cold Spring Harbor Symposia on Quantitative Biology* 83: 131–39. https://doi.org/10.1101/sqb.2018.83.037663.

Miller, Courtney A., Susan L. Campbell, and J. David Sweatt. 2008. “DNA Methylation and Histone Acetylation Work in Concert to Regulate Memory Formation and Synaptic Plasticity.” *Neurobiology of Learning and Memory* 89 (4): 599–603. https://doi.org/10.1016/j.nlm.2007.07.016.

Miller, Courtney A., Cristin F. Gavin, Jason A. White, R. Ryley Parrish, Avinash Honasoge, Christopher R. Yancey, Ivonne M. Rivera, María D. Rubio, Gavin Rumbaugh, and J. David Sweatt. 2010. “Cortical DNA Methylation Maintains Remote Memory.” *Nature Neuroscience* 13 (6): 664–66. https://doi.org/10.1038/nn.2560.

Miller, Sam A, and Bob Policastro. 2020. “ScProportionTest.” https://github.com/rpolicastro/scProportionTest.

Miry, Omid, Jie Li, and Lu Chen. 2021. “The Quest for the Hippocampal Memory Engram: From Theories to Experimental Evidence.” *Frontiers in Behavioral Neuroscience* 14 (January): 1–11. https://doi.org/10.3389/fnbeh.2020.632019.

Nabavi, Sadegh, Rocky Fox, Christophe D. Proulx, John Y. Lin, Roger Y. Tsien, and Roberto Malinow. 2014. “Engineering a Memory with LTD and LTP.” *Nature* 511 (7509): 348–52. https://doi.org/10.1038/nature13294.

Nakayama, Jun Ichi, and Tomohiro Hayakawa. 2011. “Physiological Roles of Class i HDAC Complex and Histone Demethylase.” *Journal of Biomedicine and Biotechnology* 2011. https://doi.org/10.1155/2011/129383.

Narita, Takeo, Brian T. Weinert, and Chunaram Choudhary. 2019. “Functions and Mechanisms of Non-Histone Protein Acetylation.” *Nature Reviews Molecular Cell Biology* 20 (3): 156–74. https://doi.org/10.1038/s41580-018-0081-3.

Panikker, Priyalakshmi, Song Jun Xu, Haolin Zhang, Jessica Sarthi, Mariah Beaver, Avni Sheth, Sunya Akhter, and Felice Elefant. 2018. “Restoring Tip60 HAT/HDAC2 Balance in the Neurodegenerative Brain Relieves Epigenetic Transcriptional Repression and Reinstates Cognition.” *Journal of Neuroscience* 38 (19): 4569–83. https://doi.org/10.1523/JNEUROSCI.2840-17.2018.

Parsons, Ryan G., and Michael Davis. 2012. “A Metaplasticity-like Mechanism Supports the Selection of Fear Memories: Role of Protein Kinase A in the Amygdala.” *Journal of Neuroscience* 32 (23): 7843–51. https://doi.org/10.1523/JNEUROSCI.0939-12.2012.

Pavlov, Ivan Petrovitch. 2006. *Lectures on Conditioned Reflexes: Twenty-Five Years of Objective Study of the Higher Nervous Activity (Behaviour) of Animals. Lectures on Conditioned Reflexes: Twenty-Five Years of Objective Study of the Higher Nervous Activity (Behaviour) of Animals.* https://doi.org/10.1037/11081-000.

Pearce, Kaycey, Diancai Cai, Adam C. Roberts, and David L. Glanzman. 2017. “Role of Protein Synthesis and DNA Methylation in the Consolidation and Maintenance of Long-Term Memory in Aplysia.” *ELife* 6: 1–20. https://doi.org/10.7554/eLife.18299.

Peixoto, Lucia, and Ted Abel. 2013. “The Role of Histone Acetylation in Memory Formation and Cognitive Impairments.” *Neuropsychopharmacology* 38 (1): 62–76. https://doi.org/10.1038/npp.2012.86.

Peleg, Shahaf, Farahnaz Sananbenesi, Athanasios Zovoilis, Susanne Burkhardt, Sanaz Bahari-Javan, Roberto Carlos Agis-Balboa, Perla Cota, et al. 2010. “Altered Histone Acetylation Is Associated with Age-Dependent Memory Impairment in Mice.” *Science (New York, N.Y.)* 328 (May): 753–56. https://doi.org/10.1126/science.1186088.

Peng, Sheng, Yan Zhang, Jiannan Zhang, Hua Wang, and Bingxu Ren. 2010. “ERK in Learning and Memory: A Review of Recent Research.” *International Journal of Molecular Sciences* 11 (1): 222–32. https://doi.org/10.3390/ijms11010222.

Phillips, R. G., and J. E. LeDoux. 1992. “Differential Contribution of Amygdala and Hippocampus to Cued and Contextual Fear Conditioning.” *Behavioral Neuroscience* 106 (2): 274–85. https://doi.org/10.1037//0735-7044.106.2.274.

Restivo, Leonardo, Gisella Vetere, Bruno Bontempi, and Martine Ammassari-Teule. 2009. “The Formation of Recent and Remote Memory Is Associated with Time-Dependent Formation of Dendritic Spines in the Hippocampus and Anterior Cingulate Cortex.” *Journal of Neuroscience* 29 (25): 8206–14. https://doi.org/10.1523/JNEUROSCI.0966-09.2009.

Revest, J. M., A. Le Roux, V. Roullot-Lacarrière, N. Kaouane, M. Vallée, F. Kasanetz, F. Rougé-Pont, F. Tronche, A. Desmedt, and P. V. Piazza. 2014. “BDNF-TrkB Signaling through Erk1/2MAPK Phosphorylation Mediates the Enhancement of Fear Memory Induced by Glucocorticoids.” *Molecular Psychiatry* 19 (9): 1001–9. https://doi.org/10.1038/mp.2013.134.

Robison, Alfred J., and Eric J. Nestler. 2011. “Transcriptional and Epigenetic Mechanisms of Addiction.” *Nature Reviews Neuroscience* 12 (11): 623–37. https://doi.org/10.1038/nrn3111.

Rocks, Devin, Mamta Shukla, Silvia C. Finnemann, Achyuth Kalluchi, M. Jordan Rowley, and Marija Kundakovic. 2021. “Sex-Specific Multi-Level 3D Genome Dynamics in the Mouse Brain.” *BioRxiv*, 2021.05.03.442383. https://www.biorxiv.org/content/10.1101/2021.05.03.442383v1%0Ahttps://www.biorxiv.org/content/10.1101/2021.05.03.442383v1.abstract.

Ryu, Hyun Hee, and Yong Seok Lee. 2016. “Cell Type-Specific Roles of RAS-MAPK Signaling in Learning and Memory: Implications in Neurodevelopmental Disorders.” *Neurobiology of Learning and Memory* 135: 13–21. https://doi.org/10.1016/j.nlm.2016.06.006.

Sacchetti, Benedetto, Carlo Ambrogi Lorenzini, Elisabetta Baldi, Corrado Bucherelli, Marisa Roberto, Giovanna Tassoni, and Marcello Brunelli. 2002. “Time-Dependent Inhibition of Hippocampal LTP in Vitro Following Contextual Fear Conditioning in the Rat.” *European Journal of Neuroscience* 15 (1): 143–50. https://doi.org/10.1046/j.0953-816x.2001.01844.x.

Sada, Naoki, Yuki Fujita, Nanano Mizuta, Masaki Ueno, Takahisa Furukawa, and Toshihide Yamashita. 2020. “Inhibition of HDAC Increases BDNF Expression and Promotes Neuronal Rewiring and Functional Recovery after Brain Injury.” *Cell Death & Disease* 11 (8): 655. https://doi.org/10.1038/s41419-020-02897-w.

Saha, Ramendra N., Erin M. Wissink, Emma R. Bailey, Meilan Zhao, David C. Fargo, Ji Yeon Hwang, Kelly R. Daigle, J. Daniel Fenn, Karen Adelman, and Serena M. Dudek. 2011. “Rapid Activity-Induced Transcription of Arc and Other IEGs Relies on Poised RNA Polymerase II.” *Nature Neuroscience* 14 (7): 848–56. https://doi.org/10.1038/nn.2839.

Sartor, Gregory C., Andrea M. Malvezzi, Ashok Kumar, Nadja S. Andrade, Hannah J. Wiedner, Samantha J. Vilca, Karolina J. Janczura, et al. 2019. “Enhancement of BDNF Expression and Memory by HDAC Inhibition Requires BET Bromodomain Reader Proteins.” *Journal of Neuroscience* 39 (4): 612–26. https://doi.org/10.1523/JNEUROSCI.1604-18.2018.

Saxe, Michael D., Fortunato Battaglia, Jing Wen Wang, Gael Malleret, Denis J. David, James E. Monckton, A. Denise R. Garcia, et al. 2006. “Ablation of Hippocampal Neurogenesis Impairs Contextual Fear Conditioning and Synaptic Plasticity in the Dentate Gyrus.” *Proceedings of the National Academy of Sciences of the United States of America* 103 (46): 17501–6. https://doi.org/10.1073/pnas.0607207103.

Scandura, Joseph M., Gail J. Roboz, Michelle Moh, Ewelina Morawa, Fabienne Brenet, J. Robi Bose, Luis Villegas, et al. 2011. “Phase 1 Study of Epigenetic Priming with Decitabine Prior to Standard Induction Chemotherapy for Patients with AML.” *Blood* 118 (6): 1472–80. https://doi.org/10.1182/blood-2010-11-320093.

Schaefer, Anne, Srihari C. Sampath, Adam Intrator, Alice Min, Tracy S. Gertler, D. James Surmeier, Alexander Tarakhovsky, and Paul Greengard. 2009. “Control of Cognition and Adaptive Behavior by the GLP/G9a Epigenetic Suppressor Complex.” *Neuron* 64 (5): 678–91. https://doi.org/10.1016/j.neuron.2009.11.019.

Schröder, Sebastian, Eva Herker, Friederike Itzen, Daniel He, Sean Thomas, Daniel A. Gilchrist, Katrin Kaehlcke, et al. 2013. “Acetylation of RNA Polymerase II Regulates Growth-Factor-Induced Gene Transcription in Mammalian Cells.” *Molecular Cell* 52 (3): 314–24. https://doi.org/10.1016/j.molcel.2013.10.009.

Schübeler, Dirk, Claire Francastel, Daniel M. Cimbora, Andreas Reik, David I.K. Martin, and Mark Groudine. 2000. “Nuclear Localization and Histone Acetylation: A Pathway for Chromatin Opening and Transcriptional Activation of the Human β-Globin Locus.” *Genes and Development* 14 (8): 940–50. https://doi.org/10.1101/gad.14.8.940.

Sewal, Angila S., Holger Patzke, Evelyn J. Perez, Pul Park, Elin Lehrmann, Yongqing Zhang, Kevin G. Becker, Bonnie R. Fletcher, Jeffrey M. Long, and Peter R. Rapp. 2015. “Experience Modulates the Effects of Histone Deacetylase Inhibitors on Gene and Protein Expression in the Hippocampus: Impaired Plasticity in Aging.” *Journal of Neuroscience* 35 (33): 11729–42. https://doi.org/10.1523/JNEUROSCI.4339-14.2015.

Shih, Pu Yun, Bing Yuan Hsieh, Ching Yen Tsai, Chiu An Lo, Brian E. Chen, and Yi Ping Hsueh. 2020. “Autism-Linked Mutations of CTTNBP2 Reduce Social Interaction and Impair Dendritic Spine Formation via Diverse Mechanisms.” *Acta Neuropathologica Communications* 8 (1): 185. https://doi.org/10.1186/s40478-020-01053-x.

Skinnider, Michael A., Jordan W. Squair, Claudia Kathe, Mark A. Anderson, Matthieu Gautier, Kaya J. E. Matson, Marco Milano, et al. 2021. “Cell Type Prioritization in Single-Cell Data.” *Nature Biotechnology* 39 (1): 30–34. https://doi.org/10.1038/s41587-020-0605-1.

Squair, Jordan W., Michael A. Skinnider, Matthieu Gautier, Leonard J. Foster, and Grégoire Courtine. 2021. “Prioritization of Cell Types Responsive to Biological Perturbations in Single-Cell Data with Augur.” *Nature Protocols*, 1–42. https://doi.org/10.1038/s41596-021-00561-x.

Squire, Larry R. 2009. “Memory and Brain Systems: 1969-2009.” *Journal of Neuroscience* 29 (41): 12711–16. https://doi.org/10.1523/JNEUROSCI.3575-09.2009.

Squire, Larry R. 1984. “Memory” 232 (1983).

Stark, Rory, and Gord Brown. 2011. “DiffBind: Differential Binding Analysis of ChIP-Seq Peak Data.” Bioconductor. http://bioconductor.org/packages/release/bioc/vignettes/DiffBind/inst/doc/DiffBind.pdf.

Stefanko, Daniel P., Ruth M. Barrett, Alexandra R. Ly, Gustavo K. Reolon, and Marcelo A. Wood. 2009. “Modulation of Long-Term Memory for Object Recognition via HDAC Inhibition.” *Proceedings of the National Academy of Sciences of the United States of America* 106 (23): 9447–52. https://doi.org/10.1073/pnas.0903964106.

Su, Yijing, Jaehoon Shin, Chun Zhong, Sabrina Wang, Prith Roychowdhury, Jongseuk Lim, David Kim, Guo Li Ming, and Hongjun Song. 2017. “Neuronal Activity Modifies the Chromatin Accessibility Landscape in the Adult Brain.” *Nature Neuroscience* 20 (3): 476–83. https://doi.org/10.1038/nn.4494.

Sütterlin, Philipp, Emma J. Williams, David Chambers, Kathryn Saraf, David von Schack, Melina Reisenberg, Patrick Doherty, and Gareth Williams. 2013. “The Molecular Basis of the Cooperation between EGF, FGF and ECB Receptors in the Regulation of Neural Stem Cell Function.” *Molecular and Cellular Neuroscience* 52: 20–30. https://doi.org/10.1016/j.mcn.2012.10.006.

Takuma, Kazuhiro, Yuta Hara, Shunsuke Kataoka, Takuya Kawanai, Yuko Maeda, Ryo Watanabe, Erika Takano, et al. 2014. “Chronic Treatment with Valproic Acid or Sodium Butyrate Attenuates Novel Object Recognition Deficits and Hippocampal Dendritic Spine Loss in a Mouse Model of Autism.” *Pharmacology Biochemistry and Behavior* 126: 43–49. https://doi.org/10.1016/j.pbb.2014.08.013.

Terranova-Barberio, Manuela, Scott Thomas, Niwa Ali, Nela Pawlowska, Jeenah Park, Gregor Krings, Michael D. Rosenblum, Alfredo Budillon, and Pamela N. Munster. 2017. “HDAC Inhibition Potentiates Immunotherapy in Triple Negative Breast Cancer.” *Oncotarget* 8 (69): 114156–72. https://doi.org/10.18632/oncotarget.23169.

Thomas, Elizabeth A., Giovanni Coppola, Paula A. Desplats, Bin Tang, Elisabetta Soragni, Ryan Burnett, Fuying Gao, et al. 2008. “The HDAC Inhibitor 4b Ameliorates the Disease Phenotype and Transcriptional Abnormalities in Huntington’s Disease Transgenic Mice.” *Proceedings of the National Academy of Sciences of the United States of America* 105 (40): 15564–69. https://doi.org/10.1073/pnas.0804249105.

Tonegawa, Susumu, Xu Liu, Steve Ramirez, and Roger Redondo. 2015. “Memory Engram Cells Have Come of Age.” *Neuron* 87 (5): 918–31. https://doi.org/10.1016/j.neuron.2015.08.002.

Tyssowski, Kelsey M., Nicholas R. DeStefino, Jin Hyung Cho, Carissa J. Dunn, Robert G. Poston, Crista E. Carty, Richard D. Jones, et al. 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.

Vaid, Roshan, Jiayu Wen, and Mattias Mannervik. 2020. “Release of Promoter–Proximal Paused Pol II in Response to Histone Deacetylase Inhibition.” *Nucleic Acids Research* 48: 4877–90. https://doi.org/10.1093/nar/gkaa234.

Valiati, Fernanda E., Mailton Vasconcelos, Martina Lichtenfels, Fernanda S. Petry, Rosa M.M. de Almeida, Gilberto Schwartsmann, Nadja Schr-der, Caroline B. de Farias, and Rafael Roesler. 2017. “Administration of a Histone Deacetylase Inhibitor into the Basolateral Amygdala Enhances Memory Consolidation, Delays Extinction, and Increases Hippocampal BDNF Levels.” *Frontiers in Pharmacology* 8 (JUN): 1–8. https://doi.org/10.3389/fphar.2017.00415.

Vecsey, Christopher G., Joshua D. Hawk, K. Matthew Lattal, Joel M. Stein, Sara A. Fabian, Michelle A. Attner, Sara M. Cabrera, et al. 2007. “Histone Deacetylase Inhibitors Enhance Memory and Synaptic Plasticity via CREB: CBP-Dependent Transcriptional Activation.” *Journal of Neuroscience* 27 (23): 6128–40. https://doi.org/10.1523/JNEUROSCI.0296-07.2007.

Villain, Hélène, Cédrick Florian, and Pascal Roullet. 2016. “HDAC Inhibition Promotes Both Initial Consolidation and Reconsolidation of Spatial Memory in Mice.” *Scientific Reports* 6 (May): 1–9. https://doi.org/10.1038/srep27015.

Volmar, Claude Henry, Hasib Salah-Uddin, Karolina J. Janczura, Paul Halley, Guerline Lambert, Andrew Wodrich, Sivan Manoah, et al. 2017. “M344 Promotes Nonamyloidogenic Amyloid Precursor Protein Processing While Normalizing Alzheimer’s Disease Genes and Improving Memory.” *Proceedings of the National Academy of Sciences of the United States of America* 114 (43): E9135–44. https://doi.org/10.1073/pnas.1707544114.

Waddington, C H. 1957. *The Strategy of the Genes*. George Allen & Unwin.

Wang, Allen, Feng Yue, Yan Li, Ruiyu Xie, Thomas Harper, Nisha A. Patel, Kayla Muth, et al. 2015. “Epigenetic Priming of Enhancers Predicts Developmental Competence of HESC-Derived Endodermal Lineage Intermediates.” *Cell Stem Cell* 16 (4): 386–99. https://doi.org/10.1016/j.stem.2015.02.013.

Wang, Zhibin, Chongzhi Zang, Kairong Cui, Dustin E. Schones, Artem Barski, Weiqun Peng, and Keji Zhao. 2009. “Genome-Wide Mapping of HATs and HDACs Reveals Distinct Functions in Active and Inactive Genes.” *Cell* 138 (5): 1019–31. https://doi.org/10.1016/j.cell.2009.06.049.

Wei, Zhang, and Hui Tu Liu. 2002. “MAPK Signal Pathways in the Regulation of Cell Proliferation in Mammalian Cells.” *Cell Research* 12 (1): 9–18. https://doi.org/10.1038/sj.cr.7290105.

Wei, Zhiyun, Xingjun Meng, Rachid El Fatimy, Bowen Sun, Dongmei Mai, Junfang Zhang, Ramil Arora, et al. 2020. “Environmental Enrichment Prevents Aβ Oligomer-Induced Synaptic Dysfunction through Mirna-132 and Hdac3 Signaling Pathways.” *Neurobiology of Disease* 134 (September 2019). https://doi.org/10.1016/j.nbd.2019.104617.

Whitlock, Jonathan R., Arnold J. Heynen, Marshall G. Shuler, and Mark F. Bear. 2006. “Learning Induces Long-Term Potentiation in the Hippocampus.” *Science* 313 (5790): 1093–97. https://doi.org/10.1126/science.1128134.

Whittle, N., V. Maurer, C. Murphy, J. Rainer, D. Bindreither, M. Hauschild, A. Scharinger, et al. 2016. “Enhancing Dopaminergic Signaling and Histone Acetylation Promotes Long-Term Rescue of Deficient Fear Extinction.” *Translational Psychiatry* 6 (12). https://doi.org/10.1038/tp.2016.231.

Wilson, C. Brad, Leslie D. McLaughlin, Philip J. Ebenezer, Anand R. Nair, and Joseph Francis. 2014. “Valproic Acid Effects in the Hippocampus and Prefrontal Cortex in an Animal Model of Post-Traumatic Stress Disorder.” *Behavioural Brain Research* 268: 72–80. https://doi.org/10.1016/j.bbr.2014.03.029.

Yuan, Robin K., Jenna C. Hebert, Arthur S. Thomas, Ellen G. Wann, and Isabel A. Muzzio. 2015. “HDAC I Inhibition in the Dorsal and Ventral Hippocampus Differentially Modulates Predator-Odor Fear Learning and Generalization.” *Frontiers in Neuroscience* 9 (SEP): 1–11. https://doi.org/10.3389/fnins.2015.00319.

Zalocusky, Kelly A., Ramsey Najm, Alice L. Taubes, Yanxia Hao, Seo Yeon Yoon, Nicole Koutsodendris, Maxine R. Nelson, et al. 2021. “Neuronal ApoE Upregulates MHC-I Expression to Drive Selective Neurodegeneration in Alzheimer’s Disease.” *Nature Neuroscience* 24 (6): 786–98. https://doi.org/10.1038/s41593-021-00851-3.

Zeisel, Amit, A. B. Munoz-Manchado, Simone Codeluppi, P. Lonnerberg, G. La Manno, A. Jureus, Sueli Marques, et al. 2015. “Cell Types in the Mouse Cortex and Hippocampus Revealed by Single-Cell RNA-Seq.” *Science (New York, N.Y.)* 347 (6226): 1138–42. https://doi.org/10.1126/science.aaa1934.

Zerbino, Daniel R., Premanand Achuthan, Wasiu Akanni, M. Ridwan Amode, Daniel Barrell, Jyothish Bhai, Konstantinos Billis, et al. 2018. “Ensembl 2018.” *Nucleic Acids Research* 46 (D1): D754–61. https://doi.org/10.1093/nar/gkx1098.

Zhang, Ye, Kenian Chen, Steven A. Sloan, Mariko L. Bennett, Anja R. Scholze, Sean O’Keeffe, Hemali P. Phatnani, et al. 2014. “An RNA-Sequencing Transcriptome and Splicing Database of Glia, Neurons, and Vascular Cells of the Cerebral Cortex.” *Journal of Neuroscience* 34 (36): 11929–47. https://doi.org/10.1523/JNEUROSCI.1860-14.2014.

Zhang, Yong, Tao Liu, Clifford A. Meyer, Jérôme Eeckhoute, David S. Johnson, Bradley E. Bernstein, Chad Nussbaum, et al. 2008. “Model-Based Analysis of ChIP-Seq (MACS).” *Genome Biology* 9 (9): R137. https://doi.org/10.1186/gb-2008-9-9-r137.

Zhao, Wen Ning, Balaram Ghosh, Marshall Tyler, Jasmin Lalonde, Nadine F. Joseph, Nina Kosaric, Daniel M. Fass, Li Huei Tsai, Ralph Mazitschek, and Stephen J. Haggarty. 2018. “Class i Histone Deacetylase Inhibition by Tianeptinaline Modulates Neuroplasticity and Enhances Memory.” *ACS Chemical Neuroscience* 9 (9): 2262–73. https://doi.org/10.1021/acschemneuro.8b00116.

Zhou, Hengyu, Ying Cai, Dina Liu, Menghui Li, Yu Sha, Wenlu Zhang, Kai Wang, et al. 2018. “Pharmacological or Transcriptional Inhibition of Both HDAC1 and 2 Leads to Cell Cycle Blockage and Apoptosis via P21Waf1/Cip1 and P19INK4d Upregulation in Hepatocellular Carcinoma.” *Cell Proliferation* 51 (3): 1–14. https://doi.org/10.1111/cpr.12447.

Zovkic, Iva B., Brynna S. Paulukaitis, Jeremy J. Day, Deepa M. Etikala, and J. David Sweatt. 2014. “Histone H2A.Z Subunit Exchange Controls Consolidation of Recent and Remote Memory.” *Nature* 515 (7528): 582–86. https://doi.org/10.1038/nature13707.

ALLISON MARIE BURNS

Doctoral Student in Neuroscience

EDUCATION

current | 2016

Ph.D. in Neuroscience
École Polytechnique Fédérale de Lausanne — Lausanne, Switzerland

Title: Epigenetic memory aids: Synaptic and molecular effects of HDAC inhibition that support memory formation

2013 | 2012

M.Sc. in Bioinformatics
University of Oregon — Eugene, Oregon, USA

Title: Effects of the splicing inhibitor, Isoginkgetin, on human Telomerase RNA

2012 | 2008

B.Sc. in Biology
University of Oregon — Eugene, Oregon, USA

Minor: Chemistry
Study Abroad: Uppsala Universitet, Sweden (Spring 2011)
Project: Evolutionary genetics of geographical variation, seasonal development and circadian timing in the North American mosquito species, Wyeomyia smithii

RESEARCH EXPERIENCE

current | 2016

Graduate Research Assistant
Laboratory of Neuroepigenetics - Gräff Lab — EPFL

- Designed, performed and analyzed experiments for thesis
- Advised on best methods for NGS sample and library preparation
- Maintained and organized computational needs for the lab
- Oversaw students for both bench and computational techniques

2016 | 2013

Bioinformatician / Research Technician
Baumann Laboratory — Stowers Institute for Medical Research

- Analyzed omics data in collaboration with colleagues in the lab
- Taught analysis in R to colleagues in the lab
- Performed basic tissue culture and molecular experiments

2012 | 2010

Undergraduate Researcher
Bradshaw-Holzapfel Laboratory — University of Oregon

- Measured animal fitness in extreme light environments
- Used the program “Geneious” to study the evolutionary divergence of circadian clock genes in mosquitos

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PERSONAL DATA

Nationality: USA
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TECHNICAL SKILLS

R, Bash, Python
Molecular biology and omics techniques
Data cleaning, processing & visualization
Statistical analysis

LANGUAGES

English (Native)
French (A2)
German (A1)

DISCLAIMER

This resume was made with the R package **pagedown**.

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PRESENTATIONS

2020 **FENS 2020 (online poster)**
Testing the theory of Epigenetic Priming in Fear Memory Conditioning — Edinburgh, Scotland

2019 **Molecular and Cellular Cognition Society 18th Annual Meeting (talk)**
Testing the theory of Epigenetic Priming in Fear Memory Conditioning — Chicago, Illinois, USA

2019 **Single Cell Biology Symposium (talk)**
Variations of epigenetic priming across hippocampal cell types during memory formation — Lausanne, Switzerland

2019 **SFN 2019 (poster)**
Testing the theory of Epigenetic Priming in Fear Memory Conditioning — Chicago, Illinois, USA

2019 **LNAM (poster)**
Testing the theory of Epigenetic Priming in Fear Memory Conditioning — Diableret, Switzerland

2019 **FENS-Hertie Winter School 2019 (poster)**
Testing the theory of Epigenetic Priming in Fear Memory Conditioning — Obergurgl, Austria

2018 **Neural Circuits and Behavior (talk)**
HDAC inhibitors that prime in fear memory conditioning — Lausanne, Switzerland

2018 **Functional Genomics (talk)**
Epigenetic priming in fear memory conditioning — Lausanne, Switzerland

2018 **FENS 2018 (poster)**
Testing the theory of Epigenetic Priming in Fear Memory Conditioning — Berlin, Germany

PUBLICATIONS

2021 **The HDAC inhibitor CI-994 acts as a molecular memory aid by enhancing synaptic and intra-cellular communication after learning**
Allison M Burns, Mélissa Farinelli-Scharly, Sandrine Hugues-Ascery, Jose Vicente Sanchez-Mut, Giulia Santoni,Johannes Gräff
BioRxiv

2021 **A thalamo-amygdalar circuit underlying the extinction of remote fear memories**
Bianca A. Silva, Simone Astori, Allison M. Burns, Hedrik Heiser, Lukas van den Heuvel, Giulia Santoni, MariaFernanda Martinez-Reza, Carmen Sandi, Johannes Gräff
https://doi.org/10.1038/s41593-021-00856-y
Nature Neuroscience

2020 **Cognitive epigenetic priming: Leveraging histone acetylation for memory amelioration**
Allison M. Burns, Johannes Gräff
https://doi.org/10.1016/j.conb.2020.08.011
Current Opinion in Neurobiology

2018 **A cFos activation map of remote fear memory attenuation**
Bianca A. Silva, Allison M. Burns, Johannes Gräff
https://doi.org/10.1007/s00213-018-5000-y
Psychopharmacology

2015 **Human Telomerase RNA Processing and Quality Control**
Chi-KangTseng, Hui-Fang Wang, Allison M. Burns, Morgan R Schroeder, Martina Gaspari, Peter Baumann
https://doi.org/10.1016/j.celrep.2015.10.075
Cell Reports

COURSES AND TRAINING

2020 **Advanced topics in single-cell transcriptomics**
Swiss Institute of Bioinformatics

2019 **FENS-Hertie Winter School 2019**
Genetic and epigenetic mechanisms underlying brain disorders — Obergurgl, Austria

2016 **Image Processing for Life Scientists**
Lausanne, Switzerland

2016 **High Performance Computing (HPC) in Life Sciences**
Swiss Institute of Bioinformatics

TEACHING EXPERIENCE

2019 **Robust data analysis: an introduction to R**
Design and teaching of course in afternoon lecture — Open Science in Practice Summer School

2017-21 **Short Project Supervisor for Bachelor Students**
Mentor students for short projects pertaining to neuroepigenetics — EPFL

2017-19 **Laboratoire Intégré en Sciences de la Vie I/II**
TA and demonstrate lab techniques and analysis — EPFL

LEADERSHIP AND OUTREACH

2019-21 **Pint of Science**
Webmaster

2019 **EDNE Neuroscience Student Association**
Event Organizer

2018-21 **R-Ladies Lausanne**
Member

2018-21 **Letters to a Pre-scientist**
STEM Professional

2018-20 **Association of Doctoral Students in Life Sciences (ADSV)**
Webmaster & Head of Coaches