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# Triggering hippocampal LTP and learning by dopamine: a teaching signal

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# THÈSE

En vue de l'obtention du  
**DOCTORAT DE L'UNIVERSITÉ DE TOULOUSE**  
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Présentée et soutenue par  
**Fares SAYEGH**

Le 21 février 2022

**Déclenchement de la LTP hippocampique et de l'apprentissage  
par la dopamine : un signal d'apprentissage.**

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مَنْ أَنَا لِأَقُولَ لَكُمْ  
مَا أَقُولَ لَكُمْ؟  
وَأَنَا لَمْ أَكُنْ حَجْرًا صَقَلَتْهُ الْمِيَاهُ  
فَأَصْبَحَ وَجْهًا...  
وَلَا قَصَبًا ثَقَبَتْهُ الرِّيحُ  
فَأَصْبَحَ نَائِيًا...  
أَنَا لَاعِبُ النَّزْدِ  
أُرِيحُ حِينًا وَأُخْسِرُ حِينًا  
أَنَا مِثْلَكُمْ...  
أَوْ أَقَلُّ قَلِيلًا...

محمود درويش - شاعر فلسطيني 1941 - 2008

Qui serais-je, pour prétendre de pouvoir parler  
de ce que je m'apprête à vous raconter ?  
Et moi, qui n'étais jamais une pierre affinée par l'eau  
pour créer un visage...  
Et jamais ne serais-je une canne forgée par le vent  
pour devenir une flûte...  
Je suis un joueur de dés,  
J'en gagne et j'en perds  
Je suis comme vous...  
Ou bien un peu moins...  
Mahmoud Darwish – Poète Palestinien 1941-2008

Who am I to be telling you  
what I am about to tell you?  
And me, who was never a stone refined by water  
to create a face...  
Nor was I ever a cane forged by wind  
to become a flute...  
I am a dice player,  
I win some and I lose some  
I am like you...  
Or perhaps a bit less...  
Mahmoud Darwish – Palestinian poet 1941-2008

**Dedicate to**

**Kinan, you were the most human amongst us,  
you were snatched too soon for they were afraid you'd  
spoil the meaning for us ...**

**My family, the endless source of love, motivation and  
inspiration.**



## **English abstract:**

The hippocampus is the main brain structure involved in episodic memory formation. The role of the hippocampus in learning, memory and their underlying mechanisms has been studied extensively in rodents, in particular by using contextual learning.

Long-Term Potentiation (LTP) is an increase in synaptic transmission of glutamatergic afferents that lasts for hours, days or months and is thought to underlie hippocampal memory formation. It can be triggered in the hippocampus by an artificial High frequency Stimulation (HFS). This phenomenon helped in deciphering memory mechanisms, showing that both memory and LTP rely firstly on phosphorylation and later on *de novo* protein synthesis. The link between memory and LTP was confirmed by showing that blocking LTP mechanisms hinders memory formation, and that contextual learning induces LTP in the CA1 of the hippocampus.

Since LTP, just like memory, can be saturated, the nervous system cannot store every sensory input that the animal encounters. Moreover, HFS is not compatible with neuronal activity. Hence, there must be a teaching signal that would be the natural molecular trigger of LTP during learning, acting as a filter choosing the pertinent inputs to store.

Dopamine is a neuromodulator that has historically been thought of as a value signal, for dopamine gets released during rewarding events. However, dopamine has later been shown to be released whenever a salient unrewarding, or even punishing, event occurs. Dopamine receptors can trigger both phosphorylation and *de novo* protein formation in most brain structures showing plasticity, and D1/5 dopaminergic receptors are necessary for LTP maintenance and long-term memory. Moreover, dopaminergic stimulation *in vitro* can modulate synaptic transmission in CA1. Thus, we hypothesized that dopamine could act as a teaching signal.

In this work, we use behavior and electrophysiology coupled with optogenetic manipulations of midbrain dopamine afferents and pharmacology inhibition of D1/5 dopaminergic receptors in order to study the role of dopamine as a teaching signal triggering LTP so that pertinent sensory inputs get stored. Using electrophysiology, we show that coupling optogenetic stimulations of midbrain dopamine with glutamatergic inputs in CA1 induces a progressive LTP that reaches its plateau 90 minutes after the pairing. This LTP endures at least 5 hours, is dependent on D1/5 receptors and partially occludes HFS-triggered LTP.

Then, using contextual fear conditioning coupled with auditory cue conditioning we show that intraperitoneal injection of D1/5 receptor inhibitor, SHC23390, hinders both contextual and cue fear memories. Alternatively, intra-hippocampal infusion of SCH23390 blocks contextual memory but preserves cue fear memory intact. These results allowed us to conclude that hippocampal D1/5 receptors are necessary for contextual fear memories and in another brain structure for associative fear memories. Finally, we use a variation of contextual fear conditioning called contextual pre-exposure facilitation effect, which separates contextual learning from fear conditioning since the animal in this task learns each of them on two consecutive days. This allows studying dopamine as a teaching signal without the interference of any value inputs. We show that mice require between 2-8 minutes to encode contextual information. Furthermore, we show that D1/5 receptors are necessary for contextual and fear learning. Finally, we show that optogenetic stimulation of dopaminergic axons in the hippocampus promotes contextual learning and, conversely, their inhibition hinders contextual learning.

This work allows us to conclude that the dopaminergic pathway from the midbrain to the hippocampus has all the characteristics of a teaching signal, namely, triggering LTP on co-activated sensory inputs promoting the storage of contextual information in the hippocampus without the need for any value information.

## **Abstract Français :**

L'hippocampe est la principale structure cérébrale impliquée dans la formation de la mémoire épisodique. Les mécanismes sous-jacents la mémoire hippocampique ont été étudiés en détail chez les rongeurs, en particulier grâce à l'utilisation de tests de mémoire contextuelle.

La potentialisation à long terme (PLT) est une augmentation de la transmission synaptique des afférences glutamatergiques ; elle sous-tend la formation des mémoires hippocampiques. Elle peut être déclenchée par une stimulation à haute fréquence (SHF). Ce phénomène a permis de déchiffrer les mécanismes de la mémoire, montrant que la PLT, tout comme la mémoire, repose dans sa phase précoce sur des mécanismes de phosphorylation, ensuite, elle nécessite la formation de protéines *de novo*. Le lien entre la mémoire et la PLT est démontré par le fait que le blocage des différentes étapes de la PLT empêche la formation de la mémoire contextuelle et que celle-ci déclenche la PLT dans le CA1 de l'hippocampe.

Étant donné que la PLT, tout comme la mémoire, est saturable, le système nerveux ne peut pas enregistrer tous les événements vécus par l'animal. De plus, la SHF n'est pas compatible avec l'activité neuronale. Cela implique l'existence d'un signal d'apprentissage qui choisirait les entrées pertinentes à sauvegarder, et qui serait le déclencheur moléculaire de la PLT lors de l'apprentissage.

La dopamine est un neuro-modulateur longtemps considéré comme indiquant la récompense. Cependant, la dopamine est libérée en réponse à tous les événements saillants, y compris aversifs. Les récepteurs dopaminergiques peuvent déclencher la phosphorylation et la formation *de novo* des protéines, et les récepteurs dopaminergiques D1/5 sont nécessaires pour la PLT tardive et la mémoire à long terme. De plus, la stimulation dopaminergique *in vitro* peut moduler la transmission synaptique du CA1.

Dans ce travail, nous avons utilisé le comportement et l'électrophysiologie couplés aux manipulations optogénétiques des afférences dopaminergiques du mésencéphale et à l'inhibition pharmacologique des récepteurs dopaminergiques D1/5 pour étudier le rôle de la dopamine en tant que signal d'apprentissage déclenchant la PLT et l'apprentissage.

En utilisant l'électrophysiologie, nous montrons que le couplage de stimulations optogénétiques des afférences dopaminergiques du mésencéphalique avec des entrées glutamatergiques du CA1 induit une PLT progressive de ces dernières, qui atteint un plateau 90 minutes après la dernière stimulation dopaminergique. Cette PLT dure au moins 5 heures, dépend des récepteurs D1/5 et occlue partiellement la PLT déclenchée par SHF.

Ensuite, en utilisant le conditionnement de peur au contexte, nous montrons que l'infusion intra-hippocampique de l'inhibiteur des récepteurs D1/5, SCH23390, bloque l'apprentissage du conditionnement de peur au contextuel mais pas à un indice auditif. Nous concluons que les récepteurs D1/5 hippocampiques sont nécessaires pour la mémoire de peur au contexte.

Enfin, nous avons utilisé une variante du conditionnement de peur au contexte appelée effet de facilitation par la préexposition contextuelle. Dans ce test, le conditionnement de peur a lieu le lendemain de l'apprentissage contextuel. Il permet ainsi d'étudier indépendamment chacune de ces deux étapes. Nous montrons que les récepteurs D1/5 sont nécessaires à l'apprentissage du contexte et à celui de la peur. Enfin, nous montrons que la stimulation optogénétique des axones dopaminergiques dans l'hippocampe favorise l'apprentissage contextuel et que leur inhibition empêche l'apprentissage contextuel.

Ce travail nous permet de conclure que la voie dopaminergique du mésencéphale vers l'hippocampe a toutes les caractéristiques d'un signal d'apprentissage : elle déclenche la PLT sur les entrées sensorielles co-activées favorisant l'enregistrement d'informations contextuelles dans l'hippocampe indépendamment de toute information de valeur positive ou négative.

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## **Abbreviations**

7TM	Seven-Transmembrane
AAV	Adeno-Associated Virus
AC	Adenylate Cyclase
AMPA	$\alpha$ -Amino-3-hydroxy-5-Methyl-4-isoxazolePropionic Acid
ATP	Adenosine Triphosphate
CA	Cornu Ammonis
CaM	CalModulin
CAMKII	Calcium/CalModulin-dependent protein Kinase II
cAMP	cyclic Adenosine monophosphate
ChR2	ChannelRhodopsin-2
COMT	Catechol-O-MethylTransferase
CPFE	Contextual Pre-exposure Facilitation Effect
CREB	C-AMP Response Element-Binding protein
CS	Conditioned Stimulus
D1/5	Dopamine 1/5
DAG	DiAcylGlycerol
DARPP-32	Dopamine- and cAMP-regulated phosphoprotein, Mr 32 kDa
DAT	Dopamine Transporter
DG	Dentate Gyrus
eF1a	elongation Factor-1 alpha
E-LTP	Early-LTP
ER	Endoplasmic Reticulum
ERK	Extracellular signal-Regulated Kinases
eYFP	enhanced Yellow Fluorescent Protein
fEPSP	field Excitatory Postsynaptic Potentials
GABA	Gamma-Aminobutyric Acid
GIRK	G-protein-regulated Inwardly Rectifying K <sup>+</sup> channel
GPCR	G-Protein Coupled Receptors
HFS	High Frequency Stimulation
IP3	Inositol (1,4,5) trisphosphate
ITR	Inverted Terminal Repeat
LC	Locus Coeruleus
L-LTP	Late-LTP
LTD	Long-Term Depression
LTP	Long Term Potentiation
MAO	Monoamine Oxidase
MAPK	Mitogen-Activated Protein Kinases
MF	Mossy Fibers
NMDA	N-Methyl-D-Aspartate
PIP2	Phosphatidylinositol 4,5-bisPhosphate
PKA	Protein Kinase A
PKC	Protein Kinase C

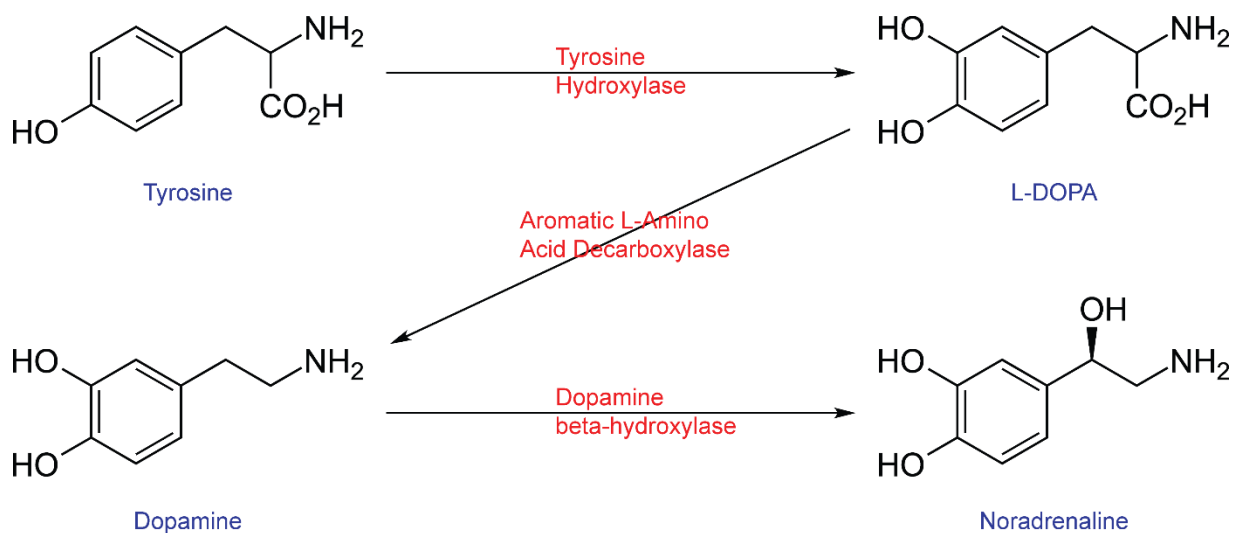
PLC	Phospholipase C
PP1	Protein phosphatase 1
PRPs	Plasticity Related Products
PTSD	Post-traumatic stress disorder
REM	Rapid Eye Movement
SC	Schaeffer Collaterals
SNC	Substantia Nigra pars compacta
STEP	Striatal-enriched protein tyrosine phosphatase
TBS	Theta Burst Stimulation
US	Unconditioned Stimulus
vHC	ventral Hippocampal Commissure
VTA	Ventral Tegmental Area
WPRE	Woodchuck Hepatitis Virus (WHP) Posttranscriptional Regulatory Element

# Chapter 1 General introduction

## 1.1 Dopamine

### 1.1.1 Biochemistry of dopamine

Dopamine is a neuromodulator, first described by Arvid Carlsson in 1957, it is chemically named 3,4-dihydroxytyramine and produced by dopaminergic neurons situated mainly in the Ventral Tegmental Area (VTA) and the Substantia Nigra pars compacta (SNc) in the midbrain. Dopaminergic neurons synthesize it from tyrosine, by first adding a hydroxyl group, which transforms tyrosine into L-DOPA; the enzyme responsible for this reaction is Tyrosine Hydroxylase (TH) (Figure 1.1). Aromatic-L-amino-acid decarboxylase turns L-DOPA into dopamine through decarboxylation. When released, dopamine binds to five different receptors: D1 – D5 receptors.



**Figure 1.1 Dopamine biochemical synthesis pathway**

In red are the necessary enzymes and in blue the names of the molecules

After exercising its action, dopamine signal gets terminated either by its reuptake into its axon terminal through dopamine transporter (DAT) to be repackaged in vesicles and released again when needed, or metabolized by monoamine oxidase (MAO) and further by catechol-O-Methyl Transferase (COMT).

### 1.1.2 How is the dopaminergic system activated?

Dopamine neurons have two profile of activity; they either show singular action potentials, firing at around 3 Hz, this activity is called tonic, or they switch to a bursting activity of bursts 3-10 spikes long with an inter spike interval of <50 ms (Grace and Bunney 1983), this activity is called phasic. This switch in dopamine cell firing rate correlates with a non-linear increase in dopamine release in the target structures (Gonon 1988; Venton et al. 2003).

Dopamine neurons show a complex heterogeneity in terms of response to different stimuli. At first, it was thought that only reward was able to trigger its phasic activity. Indeed, dopamine is released after pleasurable stimuli such as sucrose intake (Avena et al. 2006), feeding (Dahan et al. 2007), or drugs of abuse (Di Chiara and Imperato 1988). Activating midbrain dopamine neurons or their axons in the olfactory tubercle induces place preference (Tsai et al. 2009; Zhang et al. 2017).

On the one hand, dopamine bursting-activity gets habituated after repeated exposure to these rewarding stimuli (Ljungberg et al. 1992). Another important observation was that this dopaminergic population shows a decrease in their response in the face of reward omission (Waelti et al. 2001). The response observed in these dopaminergic cells start resembling a signal that codes for reward prediction error; in this model, dopamine codes for the level of difference between the predicted outcome and the real outcome and gets triggered when high unpredicted reward is received (Schultz 2007).

On the other hand, other dopamine cell populations responded to non-rewarding, even aversive, stimuli as long as they are salient and novel (Ljungberg et al. 1992; Horvitz 2000; Tang et al. 2020; Matsumoto and Hikosaka 2009; Bromberg-Martin et al. 2010; Matsumoto et al. 2016). These results suggest that dopamine is not merely a reward-computing signal or a reward prediction error signal.

Some dopamine cells gets activated by merely salient and novel stimuli that are not associated, nor are they themselves, reward or punishments (Hyland et al. 2002). In this work, we are interested in the role of this pattern of activity of dopamine cell that can be generally viewed as a signal coding for novelty and a possibility for learning to occur (Lisman and Grace 2005).

### 1.1.3 Dopamine receptors signaling

Dopaminergic receptors are Seven-Transmembrane 7TM receptors, meaning they are metabotropic receptors, that, when activated, they are coupled to a G protein, activating or

inhibiting it. In certain receptors, G proteins are already coupled to the receptor and only get activated when the receptor is (Qin et al. 2011).

G proteins are trimeric proteins composed of  $\alpha$ ,  $\beta$  and  $\gamma$  subunits and are categorized into 3 general types of G proteins depending on the  $\alpha$  subunit,  $G_s$ ,  $G_{q/11}$  and  $G_{i/o}$  (with a fourth type called  $G_{12/13}$  that is important mostly in development and will not be developed in this work).  $\beta$  and  $\gamma$  subunits form a dimer after G protein activation, and showed less versatile effects compared to  $\alpha$  cascades, and therefore, no further subdivision concerning these subunits was proposed (Beaulieu and Gainetdinov 2011).

D1 like receptors (D1 and D5) receptors are coupled with  $G_s$  with a possibility for D5 receptors to be coupled with  $G_{q/11}$ . D2 like receptors (D2 D3 D4) are coupled with  $G_{i/o}$ .

$G_s$  and  $G_{i/o}$  regulate adenylate cyclase (AC). D1 like receptors stimulates AC activity through the action of  $G_s$ ; on the contrary, D2 like receptors inhibits it through  $G_{i/o}$ . AC when activated, converts adenosine triphosphate (ATP) to cyclic adenosine monophosphate (cAMP), a secondary messenger, which activates enzymes of the family protein kinase A (PKA), secondary effector, PKA then phosphorylates different substrates such as Extracellular signal-Regulated Kinases (ERK) and cAMP Response Element-binding protein (CREB) (Girault et al. 2007; Zaccolo et al. 2021; Esteban et al. 2003).

$G_{q/11}$  pathway is different passing through phospholipase C (PLC), which cleaves a lipidic molecule in the membrane called phosphatidylinositol 4,5-bisphosphate (PIP<sub>2</sub>) into two second messengers, namely, inositol (1,4,5) trisphosphate (IP<sub>3</sub>) and diacylglycerol (DAG). The first interacts with IP<sub>3</sub> receptors found in the membrane of the endoplasmic reticulum (ER) inducing  $Ca^{2+}$  release from the ER or its entry from the extracellular fluid. DAG on the other hand activates protein kinase C (PKC).  $Ca^{2+}$  increases can also activate PKC, therefore, the convergence of both these pathways leads to the activation of this secondary effector (Xu et al. 2015).

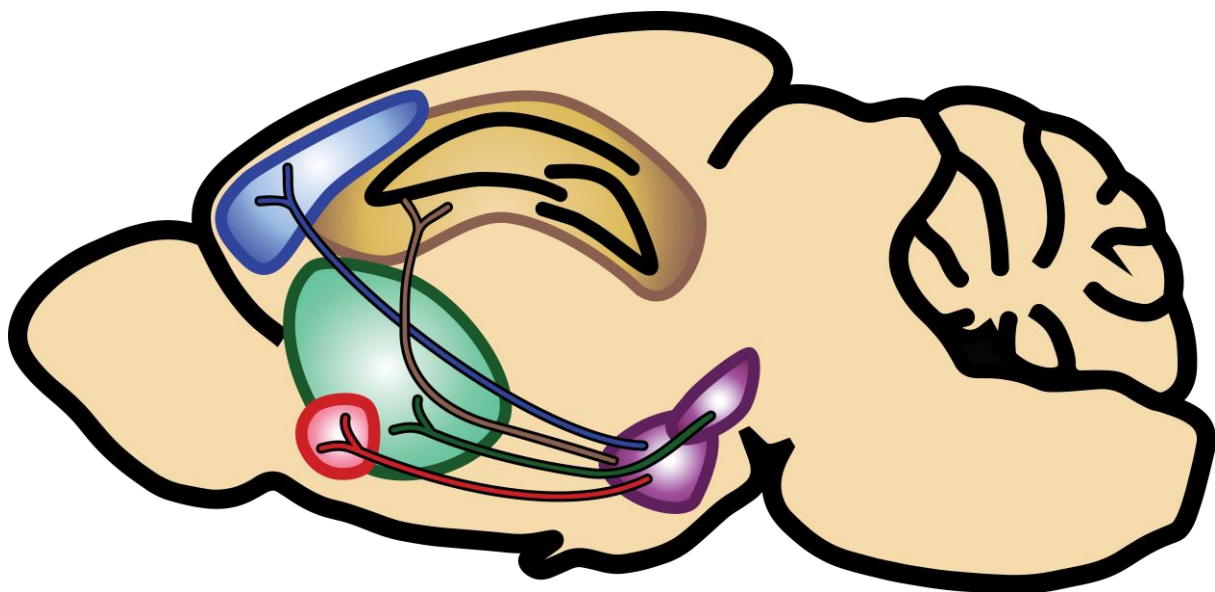
As mentioned before, there is also  $G_{\beta\gamma}$  signaling in response to 7TM signaling. Their signaling is especially important in  $G_{i/o}$ -coupled 7TMs. They primarily control G-protein-regulated inwardly rectifying  $K^+$  channels (GIRKs) alongside P/Q- and N-type voltage-gated  $Ca^{2+}$  channels (Dupré et al. 2009).

7TM signaling also have G-protein-independent pathways. These pathways include  $\beta$ -Arrestin, which is generally implicated in internalization of long activated receptors. Nevertheless, it was shown that  $\beta$ -Arrestin does not only induce internalization, but also activate different secondary

effectors that converge onto the Mitogen-Activated Protein Kinases (MAPK) and ERK (Eishingdrelo et al. 2015). Based on these findings, the name G-Protein Coupled Receptors (GPCR) was considered outdated, and the community started naming them 7TM.

### 1.1.4 Dopamine pathways

Dopamine has four major pathways in the brain: mesocortical, mesolimbic, nigrostriatal and tuberoinfundibular (Figure 1.2). The mesocortical pathway refers to dopaminergic projections from the VTA to the Prefrontal Cortex and is involved in the motivation (Hauser et al. 2017). The mesolimbic pathway is the one that delivers dopamine from the VTA to the Nucleus Accumbens and is involved in reward and its malfunction might be the basis of addictive behaviors (Di Chiara and Imperato 1988). The nigrostriatal pathway connects the SNc with the striatum and is a key pathway in movement and the progressive decrease of dopaminergic neurons in this pathway underlies Parkinson's disease (Hefti et al. 1980). Finally, the tuberoinfundibular pathway describes a small population of dopamine cells that project from a substructure in the hypothalamus to the median eminence; these neurons inhibit Prolactin secretion from the anterior pituitary through D2 receptors (Gudelsky 1981).



**Figure 1.2 Dopaminergic pathways**

Blue: Mesocortical pathway projecting from the VTA to the prefrontal cortex  
Red: Mesolimbic pathway projecting from the VTA to the Nucleus Accumbens  
Green: Nigrostriatal pathway projecting from the SNc to the Striatum  
Yellow: a less studied pathway projecting dopamine from the VTA to the hippocampus  
The Tuberoinfundibular Pathway is not represented here.

In addition to these pathways, one was less studied in the early years of dopamine research. In this pathway, dopamine sparsely projects from a population of dopamine cells in the midbrain to the hippocampus (Gasbarri et al. 1994a, 1994b, 1997). In contrast to this low innervation, the hippocampus shows high dopamine receptor expression of both D1 and D2 like receptors (Wei et al. 2018), which was shown to have a direct effect on memory functions and plasticity in the hippocampus (Du et al. 2016; Rosen et al. 2015). Different cells in the hippocampus show specific distributions of dopamine receptors, while interneurons mostly express D2 like receptors, pyramidal cells express mostly D5 receptors all along the neurons and D1 receptors around axonal arborizations (Edelmann and Lessmann 2018 for review).

The role of this last pathway is the main topic of this work. Dopamine receptors in the hippocampus were shown to have a role in memory through different approaches. Actually, while behavioral experiments suggest that dopamine is involved in learning and memory, electrophysiological experiments show its implication in the neurobiological mechanisms underlying memory such as synaptic plasticity (Bethus et al. 2010; McNamara et al. 2014; Rosen et al. 2015; Kempadoo et al. 2016; Takeuchi et al. 2016; Tsetsenis et al. 2021). In these studies, we find controversies concerning the source of dopamine in the hippocampus, the timing of when it should be released in relationship to learning and its exact psychophysical role.

In this work, we investigated the role of midbrain dopamine released in the hippocampus in response to novelty.

## 1.2 The hippocampus

### 1.2.1 The anatomy of the hippocampus and the trisynaptic loop

The hippocampus is a structure in the temporal lobe of the human brain. It is composed of many subfields: The dentate Gyrus (DG) that contains mostly granular glutamatergic neurons, and the main three subfields of the *Cornu Ammonis* (CA) - CA1, CA2 and CA3 - containing mostly pyramidal glutamatergic neurons. The Entorhinal Cortex is a region that assembles inputs from many sensory cortices. From the entorhinal cortex, two types of projections to the hippocampus arise: one that starts from the second layer of the entorhinal cortex and projects to the DG and the CA3 is called the perforant path and the second type, arising from the entorhinal third layer and projecting directly to the CA1, is called the Temporoammonic path.

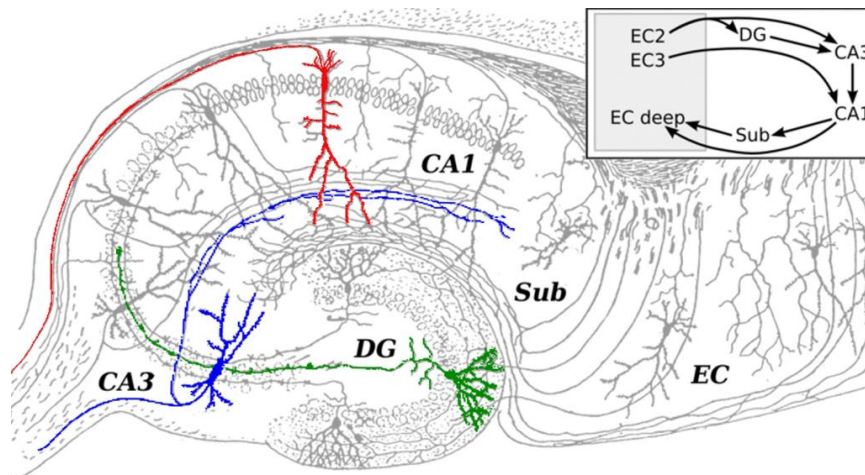
The DG sends projections, called the Mossy Fibers (MF), to the CA3 region. The granular cells in the DG have a high firing threshold that was shown to induce the activation of many downstream neurons. Therefore, the DG was proposed as a pattern separation zone, which will encode specific elementary inputs allowing their identification separately (Treves et al. 2008). CA3 pyramidal neurons have high auto-associative projections called the recurrent collaterals where pyramidal neurons from this sub-region project to one another. In computational models of these projection is hypothesized to allow for pattern completion where a distinct stimulus allows for the reactivation of a vast pattern of activity in this region (Cheu et al. 2012).

The CA1 region receives two distinct inputs, those from the Entorhinal Cortex called the temporoammonic, and those from the CA3 called Schaffer collaterals (SC). Since this region receives this dual input, it was hypothesized that it has a role in sequentially analyzing sensory inputs, giving rise to what could be called an episode. The CA1 region, which is considered as the main output region of the hippocampus, sends projections back to the fifth layer of the Entorhinal Cortex either indirectly through the subiculum or directly. Moreover, the CA1 region projects and receives projections to and from other cortices in the brain such as the insular cortex, olfactory and somatosensory cortices (Cenquizca and Swanson 2007).

The CA2 region, which was neglected until recent data identified it as a major actor of social memory in rodent (Hitti and Siegelbaum 2014) is usually not included in computational models of hippocampo-dependant memory. It actually receives inputs from DG, CA3 and extra hippocampal areas, for example, paraventricular nucleus, median raphe and medial septum. Many different types of inhibitory interneurons are found in area CA2 and form synapses on CA2 pyramidal neurons (Dudek et al. 2016). It was shown to have high level of interneuron showing a diverse heterogeneity and projects to CA1 (Chevalyere and Siegelbaum 2010; Chevalyere and Piskorowski 2016).

The hippocampus is structured in layers: the external layer, named Stratum Oriens contains dendrites of pyramidal neurons, where they receive local input from other adjacent pyramidal cells. Stratum pyramidale comes next, which contains mostly the pyramidal neuron bodies alongside different types of interneurons, most of them are inhibitory and use gamma-Aminobutyric acid (GABA) as a neurotransmitter. Then, Stratum radiatum that connects different forward projections in the trisynaptic loop. Finally, Stratum lacunosum, which is often grouped together with stratum moleculare into a single stratum called stratum lacunosum-moleculare where the perforant path fibers form synapses onto the distal dendrites of pyramidal cells (Figure 1.3).





**Figure 1.3 Ramon y Cajal depiction of the hippocampus (1911)**

Rodent hippocampus with key adjacent structures; Sub for subiculum, EC for Entorhinal cortex.

In green a granular DG cell projecting MS to CA3, in Blue a pyramidal projecting SC to CA1 and in red CA1 pyramidal cell.

### 1.2.2 The role of the hippocampus in memory; the case of H.M.

The hippocampus was considered the structure of memory formation ever since the ablation of the temporal lobe of H. M. (Scoville and Milner 1957). In fact, H. M. was suffering for years from debilitating epileptic episodes that started after a head injury and that originated from the medial temporal lobe. Lacking the pharmaceutical approaches necessary for the management of this type of cases, H. M. received a bilateral ablation of the source of his epileptic episodes. The surgery was a success, meaning H. M. no longer suffered from epilepsy. However, he woke up lacking the ability to form new autobiographical memories. However, he was still able to learn a visual-motor coordination task in which he was asked to trace a shape by only looking at his hand through a mirror; H.M. had a learning curve that was similar to that of non-operated subjects (Milner 1965). These findings not only confirmed the role of the hippocampus and its adjacent structures in memory formation, but also confirmed the specificity of different structures of the brain for different types of memories.

### 1.3 Memory; in biology, different types ... different substrates

Memory can be defined as the processes allowing the encoding, storage and retrieval of information to adapt behavior (Stuchlik 2014). This broad definition forces research to delve into the classification of different types of memory in biological systems. For example, even the blob (*Physarum polycephalum*) can be considered to have memory under this definition;

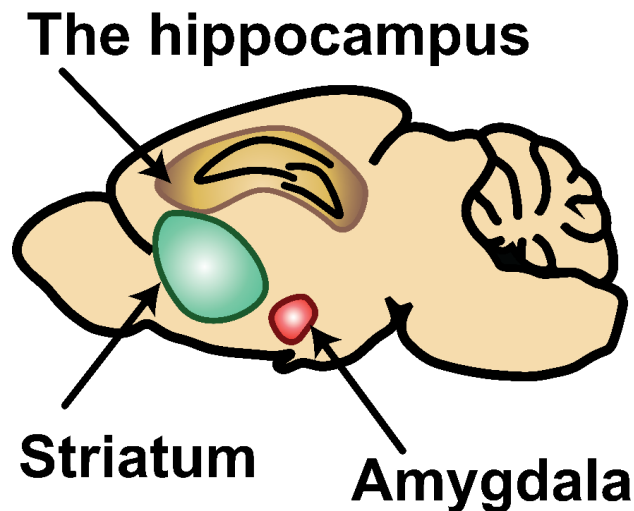
since chemical sensors in its body allow it to modulate the extension of its foraging behavior depending on already encountered chemical stimuli, even after going dormant (Vogel and Dussutour 2016).

For purpose of clarity, in this thesis, we consider **encoding** as the general processes by which a biological organism recognizes external and internal inputs. **Storage** would then encapsulate all the long-lasting changes that would occur in order to “save” the processed inputs by the biological organism. Finally, **retrieval** is the ensemble of mechanisms allowing the recollection of already “saved” inputs, which would be detected by an outside observer through the modified behavioral output.

Therefore, in mammals at least, and more precisely in humans, memory is separated into processes that do not require conscious recollection of information, called non-declarative memories, and memories that do, called declarative memories.

### 1.3.1 Non-declarative memories:

Another name for these memories is implicit memories, for in humans the retrieval of this type information is indeed implicit. Many subtypes of memory fall under this category. Firstly, procedural learning, such as learning a sequence of button presses to get a reward (Miyachi et al. 2002), this type of memory involves the striatum. Secondly, priming, an example of which is a task where people are asked to complete truncated words that they had already seen (Nielsen-Bohlman et al. 1997); this subtype involves the neocortex. Thirdly, Pavlovian classical conditioning. In his famous experiments, Ivan Pavlov, while working on the digestive system, showed the first instance of this conditioning, hence the name. He noticed that exposing a dog to food caused salivation; food in this case is called Unconditioned Stimulus (US). Then, he repeated the exposition to food with a Conditioned Stimulus (CS), such as the same experimenter who gives the food or a specific frequency on a metronome, which does not induce salivation by itself. This repetition started inducing salivation in response to the CS alone (Windholz 1997). Another example is training mice to associate simple sound cue with an electric shock, which involves the amygdala (Fanselow and Kim 1994). These are a few examples of a whole plethora of sub-types of non-declarative memories (Figure 1.4 for review Squire, 2004).



**Figure 1.4 Examples of structures involved in memory in the mouse brain**

In this schema we can observe the locations of the striatum (in green) involved in procedural memory, the amygdala (in red) involved in Pavlovian associative memory and the Hippocampus (outlined dark yellow) involved in Episodic-like memories in the mouse brain (Konsman et al. 2003).

### 1.3.2 Declarative memories

These are also called explicit memories. They resemble more what one might intuitively call memories. Consciously, one can recall when the information was acquired (**encoding**), will consciously make the effort to **retrieve** it, and will modify one's **behavior** accordingly.

Declarative memories are further sub-categorized into two main sub-types, Semantic and Episodic memories. Semantic are those declarative memories about facts that are normally not autobiographical, such as the earth is round (Mainwaring and Hanley 2019). Episodic memories, which are the main topic of this thesis, are declarative memories about one's life, one's own experiences and the events one encounters. They are the memories that allow us to recall what we did on our 26<sup>th</sup> birthday and separate it from the next one. In some sense, psychologists and philosophers base the idea of the self on these memories, going as far as considering the self as this stream of conscious recollections of what happened (Nimbalkar 2011). The brain region allowing for the formation of both subtypes of declarative memories was identified in the late fifties to be the hippocampus (Scoville and Milner 1957) through the series of experiments that helped in classifying clearly the types of memories H. M. was unable to form.

### 1.3.3 Episodic memory and episodic-like memory

The distinction between episodic memory and semantic memory is mostly based on theoretical and conceptual attributes. One delves with general knowledge of the world, semantic memory,

and the second with three intertwined questions “What?” “When?” and “Where?” and their relation to the self of the observer. Therefore, in order to understand episodic memory, researchers needed to ask explicit questions to the subjects, which limited research in the field on human subjects, who are for the moment the only subjects capable to talk to researchers, and caused a lot of criticism on even the existence of a real distinction between the two. Thusly, only accidental ablations or necessary surgical cases could be studied, and episodic memory remained a theoretical attribute to the human mind (Tulving 2002).

However, the brain substrate that is closely connected to both semantic and episodic memory is the hippocampus (Scoville and Milner 1957). In addition, since we can test for “What?” “When?” and “Where?” in animals a new concept emerged called episodic-like memory. This attribute was first described in food-hoarding birds (Clayton and Dickinson 1999, 2019).

In order to model this concept in mammals, one interesting paper that delves with all three questions was performed on rats. They were able to learn the taste of food they encountered “What?”, in which arm of an 8-arm-maze they encountered it “Where?”, and how much they had to wait to re-visit that arm and find the food “When?” (Babb and Crystal 2006).

In every day lab setting, simpler and more mainstream tasks are used to evaluate memory in rodents. For example, novel object recognition is a task that is based on the neophilia of rodents. In this task, the animal is presented two objects and when tested, one of them is replaced, if it remembers well which object was already encountered, it will pass more time exploring the novel object. This task involves the DG and medial prefrontal cortex (Chao et al. 2016; Jessberger et al. 2009). Another example that studied very closely was the morris water maze; this task is a spatial one and relies on the whole hippocampus. In this task rodents must learn to find a platform submerged under opaque water, so relying on spatial indices alone (Gasbarri et al. 1996). Finally, a simple task to assess hippocampal memory is contextual fear conditioning during which the animal learns to associate a context with a negative experience like an electric shock (Fanselow and Kim 1994). This task was used to evaluate hippocampal dependent memory in this work.

### 1.3.4 Cognitive map, latent learning and the role of hippocampus

Some of the most interesting experiments that tried to understand explicit memory came even earlier than the discovery of the role of the hippocampus as a key structure in memory. A review beautifully written by Tolman in 1948 describes a series of experiments performed on rats in

order to how they were able to build a cognitive map allowing for the acquisition of clues in order to find necessary rewards to survive.

In most of these experiments rats had to find their way in mazes to get to food or water rewards. One observation that cannot be accounted for if rats did not form a cognitive map was that most rats would follow a radial path directly towards a light cue even after changing the configuration of the maze omitting the original path learnt by the rat. The interesting thing about this experiment was that it was designed to test for a formerly incidental observation of rats jumping above the maze in order to get to the reward without actually solving it after having it learnt, a rule that was never meant to be taught to the rats in the first place. Therefore the author proposed that animals do not only learn the direct routes they take in these arenas, or the rule that we think we base our test on, but a more holistic representation.

Another interesting experiment demonstrated that rats can learn spatial mazes even if they are void of rewards. In this experiment, rats that freely explore a maze without any goal learn very quickly how to get to newly added rewards in later sessions. They actually not only outperform rats that had never formerly seen the maze before rewards were to be found in the maze, but also rats that were trained with rewards already put therein. This result suggests that when the rat is not involved in learning the strict places of rewards, it can form a fully functional cognitive map of its surroundings that it can later use when rewards are added. Learning the surroundings without a clear goal was named latent learning and another way to show it came from research published a couple of years after that review. In Spence et al. 1950, the authors taught satiated and hydrated rats the place of either food or water each on one side of a Y maze (the rat starts from the third side). Rats did not eat or drink during training sessions. On the day of the test the rats were either left without water or without food and were left to make a choice in the Y maze and a majority of the rats made the right choice going directly towards the side containing the “reward” they were seeking. These results suggest that, even though rats did not receive rewards during the training sessions nor were they motivated to search for food and water, these animals were able to form cognitive representations of this Y maze through latent learning that they were able to use when reward was sought after.

Pyramidal cells assemblies in CA1, called place cells, develop patterns of activity as the animal explores a new arena and cells start firing specifically in one location, named place field. the activity of these cells is thought to be the basis of the cognitive map role of the hippocampus (Rotenberg et al. 1996).

## 1.4 Molecular and cellular bases of hippocampal memory

We defined memory as a phenomenon allowing the behavior to change in response to experience. In this model, it is proposed that a stimulus triggers a certain response before learning that is different from the response it provokes thereafter; since the stimulus did not change in between, then, there must be a change occurring in the organism treating this information allowing for the modified behavior.

This implies that the brain structure is not fixed, but adaptive and changes to accommodate new information. This capacity is called plasticity, a term borrowed from physics and defined as the ability of a body to change form without breaking, and has different forms in neuroscience. The first form of brain plasticity with empirical evidence comes from a study about rats placed in enriched environments. The brains of a group of rats placed in rich environments with engaging toys and many play mates were compared to those of two groups of rats; one living in situations resembling modern lab caged rats and another group of isolated rats. They showed that the rats experiencing enriched environment had bigger cortices and stronger acetylcholinesterase activity compared to the other groups, showing for the first time that brain structure changes in response to living conditions (Bennett et al. 1964).

Many different plasticity mechanisms were discovered over the years; here we present some of the most pertinent ones in the field of learning and memory.

### 1.4.1 Adult neurogenesis

One change that might be the basis of learning is adult neurogenesis. This phenomenon is defined as the incorporation of adult newborn neurons that are produced in certain zones in the adult brain such as the DG in adulthood. Adult neurogenesis was shown important in the acquisition of precise learning tasks (Dupret et al. 2008; Goodman et al. 2010; Truche et al. 2009). Neurogenesis is enhanced by learning (Gould et al. 1999) and enriched environment (Bruel-Jungerman et al. 2005). Here we see that there is some sort of a synergic relationship between behavior and neurogenesis. Whereas neurogenesis plays a role in learning, learning also enhances neurogenesis in return as we see in enriched environments. However, in the adult mammalian brain, neurogenesis is restricted to the DG and the olfactory bulb and cannot account for all learning and memory processes.

## 1.4.2 Synaptic plasticity of the glutamatergic synapse

In the early seventies, a revolutionary new discovery came about showing that the glutamatergic synapse did not have a fixed efficiency, but adapts in response to its past activity by increasing or decreasing the efficiency of its transmission (Bliss and Lømo 1973; Bliss and Gardner-Medwin 1973; Ito and Kano 1982).

Decreasing synaptic transmission was named Long-Term Depression (LTD); and was shown to play different roles in different structures such as fine movement control in the cerebellum (Hansel et al. 2006) and an important role in active forgetting in the hippocampus that is necessary for reversal of already learned information (Dong et al. 2013).

The main scope of this thesis is Long-Term Potentiation (LTP), which is a long-term increase of synaptic transmission. The mechanisms and the role of this phenomenon will be detailed in the following paragraphs.

## 1.4.3 LTP mechanisms

Glutamate is the main excitatory neurotransmitter in the central nervous system. It binds to three types of ionotropic receptors:  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), N-methyl-D-aspartate (NMDA) and Kainate (Traynelis et al. 2010). Basic glutamate neurotransmission passes through AMPA receptors, which allow sodium ions to enter into the neuron after glutamate binding causing the depolarization of the neuron. If the sum of AMPA receptor depolarization is strong enough, this will induce a postsynaptic action potential. Kainate receptors are less studied, but are considered to allow for similar effects as AMPA receptors. Finally, NMDA receptors are particular for in some situations they allow for calcium ions to get into the postsynaptic element, calcium in turn activate molecular actors that can induce long lasting modifications through phosphorylation and *de novo* protein synthesis (Traynelis et al. 2010). Glutamate could also bind to different metabotropic receptors, in particular, mGluR1 were shown to be necessary for spatial learning (Balschun et al. 1999).

In 1973, Timothy Bliss and Terje Lømo showed for the first time a modulation of synaptic transmission driven by neuronal activity. They started by monitoring the stable transmission of a synapse to a single stimulation of a glutamatergic excitatory presynaptic element through electrophysiological recording of the postsynaptic response called field excitatory postsynaptic potentials (fEPSP). Afterwards, delivering a high frequency stimulation (HFS) to the presynaptic element induced a durable increase in the response of the postsynaptic element to

the same single stimulation used earlier (Bliss and Lømo 1973; Bliss and Gardner-Medwin 1973). This phenomenon was later called LTP, and defined as a stable increase in synaptic transmission caused by neuronal activity.

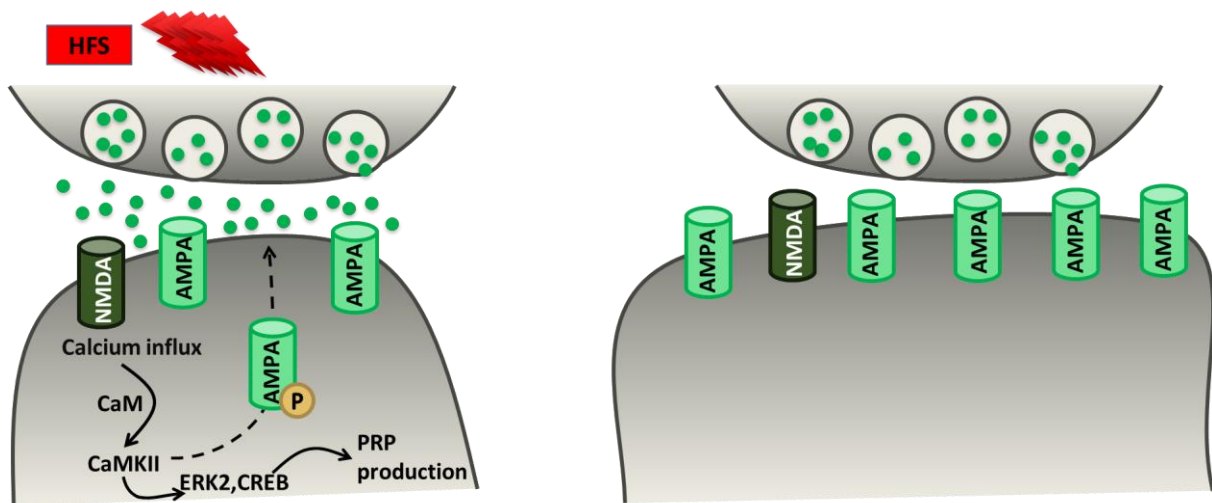
The molecular cascade of LTP was described in details in the following years, showing that both the presynaptic and the postsynaptic elements must be active at the same time for LTP to take place (Nowak et al. 1984). The detection of this coincidence is a property of NMDA receptors; these receptors get open by glutamate fixation, but if the postsynaptic element is not already depolarized, a magnesium ion blocks the entry point, preventing the inward current of calcium into the cell. However, if the postsynaptic element is already depolarized when glutamate binds the receptor, the magnesium ion is expelled and the receptor is open, allowing calcium ions to enter the cell (Nowak et al. 1984).

Following calcium influx, a protein called Calmodulin (CaM) captures these ions, which in turn activates Calcium/calmodulin-dependent protein kinases, especially Calcium/calmodulin-dependent protein kinase II (CAMKII). Through a special configuration, When activated by calcium influx, CAMKII can stay in an autonomously active state due to auto-phosphorylation (Malenka et al. 1989). When CAMKII is active, it translocates and physically interacts with active NMDAR (Bayer et al. 2001; Strack et al. 2000) allowing for synaptic specific LTP (Lisman et al. 2012). Moreover, CAMKII phosphorylates synaptic AMPA receptors, which increases their activity and might cause additional calcium influx (Liu and Cull-Candy 2002). Moreover, extra synaptic AMPA receptors get phosphorylated too causing their translocation towards the synapse (Perkinton et al. 1999). These phenomena induce an increase in synaptic transmission that lasts for a duration of an hour and a half to three hours, depending on the technique and the studied synapse (Shires et al. 2012). This cascade is called early LTP (E-LTP) and is traditionally characterized by the fact that it does not require protein synthesis, but only intact cellular phosphorylation in the synaptic elements.

The second phase of LTP is late LTP (L-LTP). LTP is a phenomenon that lasts for hours, or even days (Shires et al. 2012; Whitlock et al. 2006). Phosphorylation cannot account solely for this duration. Therefore, structural changes in proteins in the postsynaptic elements were shown to be necessary for the long-term maintenance of LTP induced by HFS (Krug et al. 1984). AMPA receptor need scaffolding proteins, such as Stargazin, PSD-95 and others, in order to get incorporated into the membrane (Schnell et al. 2002). These proteins are called Plasticity-related Products (PRPs) (Ballarini et al. 2009). Viewed this way, the scaffolding of postsynaptic proteins provides a certain number of places for AMPA receptors to fill, some that would



already be filled but others that are empty. Some synapses were shown to only contain NMDA receptors, and were called silent synapses, they would get activated by LTP (Isaac et al. 1995). When LTP is triggered, phosphorylation addresses AMPA receptors to these empty places. Then, in order to stabilize the new conformation of the synapse, PRPs are necessary and get, in their turn, addressed to potentiated synapses using phosphorylation as a tag to find their way. The cascade necessary to induce PRPS production, and long-term LTP maintenance, is less straight forward than that of AMPA phosphorylation, and E-LTP induction. However, we know that it involves phosphorylation of ERK and CREB and the increase in Zif268 production (Davis et al. 2000). This cascade is thought to induce the necessary protein production for long-term LTP maintenance. Baltaci and collaborators reviewed the role of these different molecular actors in E-LTP and L-LTP in 2018.



**Figure 1.5 LTP mechanisms**

A schema representing the major actors in the cascade necessary for LTP trigger and maintenance, glutamate and its receptors are depicted in green (on the left). Enlarged dendritic spines containing more AMPA receptors following LTP induction (on the right).

The LTP described in this paragraph (Figure 1.5) is the canonical postsynaptic LTP. This form is observed on Schaeffer Collaterals (SC); these are the axons used in this work as a model to study the role of dopamine in synaptic plasticity. However, other forms of LTP do exist with different molecular cascades. For example, MF can undergo a presynaptic form of LTP induced by an increase in neurotransmitter release (Schmitz et al. 2003) (Calixto et al. 2003), contrary to the increase in receptors on the postsynaptic element described earlier. Interestingly, this type of LTP necessitates *de novo* protein production even during its early stages (Barea-Rodríguez

et al. 2000) that was hypothesized to rely on mRNA particles already stored in the presynaptic element (Job and Eberwine 2001).

Another form of plasticity is a long-term depression of inhibitory transmission (iLTD) in CA2 of the hippocampus. This unique form of plasticity is triggered in response to high frequency stimulation that causes a decrease in current response in postsynaptic Parvalbumin interneurons (one of many subtypes of interneurons found in the brain, especially in the hippocampus). This novel form of plasticity was shown important in social learning (Domínguez et al. 2019) and similar mechanisms were reported in CA1 and shown important to remodeling of spatial representations (Udakis et al. 2020).

#### 1.4.4 Synaptic tagging and capture

LTP under the above-described definition is specific to the synapse on which it is triggered and is named homosynaptic LTP, where only the synapse that receives HFS is modified following the triggering protocol. It should be noted that when synaptic plasticity is triggered, whether LTP or LTD, it is generally specific to the pathway on which it is triggered and does not directly affect transmission over other pathways.

An interesting observation was later documented in which a weak HFS protocol on some synapses is able to trigger only E-LTP but is not sufficient to trigger L-LTP. However, if another synapse is stimulated with a strong HFS protocol that is able to trigger L-LTP projecting to the same neurons as the earlier pathway; this strong protocol can turn that former E-LTP into a long lasting one (Shires et al. 2012; Frey and Morris 1998).

This phenomenon was named synaptic tagging and capture (Frey and Frey 2008). In this model, both synapses are glutamatergic and are modified following their respective stimuli. The mechanism behind this effect suggest that weak HFS induces phosphorylation of the synapses needing potentiation, but cannot induce PRP production. The strong HFS on the other pathway projecting to the same cells would provoke PRP production that would be addressed to both synapses from the first and the second pathways.

#### 1.4.5 Heterosynaptic plasticity

Heterosynaptic LTP is triggered when the activity of one synapse modifies the efficiency of another (Ishikawa et al. 2013). In the cited example, the authors describe the plasticity that VTA-Nucleus Accumbens pathway show over its GABAergic transmission. They demonstrate that dopaminergic transmission over its parallel pathway is necessary and sufficient in

triggering the LTD they recorded over its GABAergic counterpart. They conclude that dopamine is not only implicated in neuromodulatory effects but in the triggering plasticity as well. Heterosynaptic LTP was also observed during a behavioral task in the aplysia. In this experiment, researchers associate mantle or tail stimulation with stimulation of the siphon of the aplysia. When the siphon is stimulated the gill and siphon are retracted, no such reflex is obtained by mantle or tail stimulation. When the stimulation of the siphon (US) is coupled with the stimulation of either the mantle or the tail (CS), the aplysia starts retracting the siphon in response to mantle or tail stimulation alone (Hawkins et al. 1989). This effect passes through serotonin and a heterosynaptic plasticity that recruits CREB in the process (Fiumara et al. 2015). In these examples, we can see that synaptic plasticity seems to be specific to the excitatory glutamatergic, or inhibitory GABAergic, pathway. However, what triggers it could be the activity of the synapse itself (as seen in the homosynaptic model) or another neuromodulatory actor could be what triggers it.

## **1.5 LTP as a mechanism for memory**

### **1.5.1 Learning induces LTP**

LTP in CA1 of brain slices from mice recently trained with a strong fear conditioning protocol is lower than control (Li et al. 2005); this is hypothesized to be due to the saturability of LTP. The understanding of this lower LTP is that fear conditioning triggers an undetectable LTP in the brains, which in turn occludes in part the LTP that would later be inducible in these slices taken from these brains.

Fear conditioning was shown to induce LTP in the CA1 of the hippocampus (Trifilieff et al. 2006). Moreover, this work showed that this LTP passes through two phases of ERK and CREB activation.

Many studies afterwards showed that learning induces LTP. Inhibitory avoidance induces LTP in CA1 of rats (Whitlock et al. 2006) results that were later replicated in mice (Broussard et al. 2016). Learning object location does that in the DG (Yang et al. 2017). Moreover, contextual fear conditioning induces LTP in CA1 over the SC commissural pathway (Subramaniyan et al. 2021), results that were also shown by work done in our lab (Remaud 2014).

### 1.5.2 Similar molecular actors behind both phenomena

Contextual fear conditioning is a task during which the animal learns a context and associate it with a fear-inducing stimulus. This task requires intact hippocampal activity (Daumas et al. 2005) and causes transcriptional modifications in CA1, which can be detected up to a week after training (Mizuno et al. 2020). Examples of these modifications, an increase in AMPA currents when tested in vitro after training (Zhou et al. 2009), Spines in the hippocampus are longer after retention of Fear conditioning (Giachero et al. 2013), Arc mRNA (an Immediate Early Gene) is higher in CA1 pyramidal cells in dorsal hippocampus during the first 30 minutes post learning (Inoue et al. 2005) and an increase in Ca<sup>2+</sup>-triggered phosphorylation in CA1 (Sindreu et al. 2007). Contextual fear conditioning recall activates preferentially CA1 pyramidal neurons as demonstrated in rats by the increase in Zif268 expression in these cells (Hall et al. 2001), which implies that the activity of these cells is essential for memory. The number of Cells expressing CREB in CA1 increases directly after learning and again three to four hours later (Stanciu et al. 2001). Mice with CREB over expression show better spatial and contextual memory accompanied with structural complexification of CA1 pyramidal neurons (Serita et al. 2017). Looking globally at these results, we can arguably propose that the molecular actors that form a cognitive map in the hippocampus of a context necessitates the same molecular actors that underlie LTP.

On the one hand, blocking NMDA receptors blocks learning and memory formation in the Morris Water Maze task (Morris et al. 1986) and in fear conditioning (Fanselow and Kim 1994). Moreover, a Knockout of a subunit of NMDA receptors, NR1, showed a phenotype that lacks both LTP and spatial memory (Shimizu et al. 2000). Furthermore, transgenic mice expressing a mutant version of CAMKII, with a mutation of the auto phosphorylation site necessary for its activation, show disturbed spatial memory (Bach et al. 1995).

On the other hand, activating certain molecular actors of the LTP pathway can promote learning. Transgenic mouse models were developed with reinforced expression of proteins involved in LTP triggering. An overexpression of NR2B subunit of the NMDA receptors facilitates LTP and novel object recognition (Tang et al. 1999). Moreover, overexpression of CAMKII facilitates spatial memory evaluated using the Morris water maze (Poulsen et al. 2007).

These two complimentary approaches, limiting and favoring plasticity, suggest a causal link between hippocampal LTP and spatial memory.

In our lab, former work showed that on the one hand, blocking *de novo* protein synthesis in CA1, where it is only necessary for L-LTP, blocks long-term memory retention but spares the short-term. On the other hand, the same blockade in CA3, where LTP is necessary for both E-LTP and L-LTP (Barea-Rodríguez et al. 2000), blocks both short term and long-term memory (Remaud et al. 2014) in the Contextual Fear Conditioning. These results suggest a causal link between E-LTP and short-term memory, and L-LTP and long-term memory.

### 1.5.3 Plasticity of place cells

As mentioned earlier, place cells are hippocampal cells that are activated in specific areas in an arena. The more the animal learns the arena the more place fields are precise and reliable (Cacucci et al. 2007). These place cells form in both DG and CA1, but different mechanisms rule their formation in each zone. While in DG it does not rely on NMDA receptors, in the CA1 they do (Wilson and Tonegawa 1997), following the same mechanisms of LTP in these zones.

Disrupting LTP mechanisms such as CAMKII and CREB disrupts both place cells formation and spatial representation (Rotenberg et al. 1996; Cho et al. 1998). The formation of these patterns of activity seen in place cells are probably the basis of cognitive map formations during latent learning.

## 1.6 Dopamine as a teaching signal

### 1.6.1 Learning in the Hebbian framework does not frame the whole picture

Research in the field of the neurobiology of memory has long been driven by a hypothesis proposed by the psychologist Donald Hebb. He famously put forward the proposition: “When an axon of cell A is near enough to excite a cell B and repeatedly or persistently takes part in firing it, some growth process or metabolic change takes place in one or both cells such that A's efficiency, as one of the cells firing B, is increased” in 1949 (Morris 1999). This concept was oversimplified by using the catchphrase “Neurons that fire together wire together”.

When LTP was discovered using HFS, LTP was considered a proof of this framework of looking into memory mechanisms, showing that when the activity of one neurons drives the activity of the next, a stronger connection between these two is constructed.

However, in its normal life an animal encounters an unquantifiable quantity of sensory input. Trying to encode and save every part of it would be an impossible task that would render biological memory inefficient. Very early in LTP studies, Bliss and collaborators showed that

this phenomenon could be saturated. Moreover, Moser and collaborators showed in 1998 that saturating LTP in the dorsal hippocampus impairs spatial learning. Thus, one can argue that in a given time memory can be saturated if all plasticity capacity were to be saturated. Therefore, ignoring unimportant input is crucial for the important ones to have a plastic brain that would allow for its encoding and storage.

Therefore, a NeoHebbian framework could be suggested in which a filtering signal arrives solely when novel, vitally important, events in the environment of the animal take place. In this vision, the sole activity of presynaptic and postsynaptic elements is not sufficient for learning, but there is a need for a distant modulatory signal that would trigger LTP in order for learning to take place (Lisman et al. 2011).

This modulatory signal was named a teaching signal (Harley 2004). This signal should be present whenever important stimuli around the animal occur, it should get habituated should the input repeat itself, because in that case, the input is either already learned or unimportant, and it should be involved in the molecular mechanisms of learning.

Although Dopamine cells show a wide heterogeneity in terms of projection targets and activity patterns (Poulin et al. 2018), the pattern of activity of some dopamine cells in the midbrain (see 1.1.2 How is the dopaminergic system activated?) follows the description of a teaching signal; active when stimuli are new and relevant for the survival of the animal (Ljungberg et al. 1992; Schultz et al. 2017).

### **1.6.2 Role of D1/5 receptors in hippocampal dependent learning**

Dopamine is released in the hippocampus in response to novelty (Ihalainen et al. 1999). Blocking D1/5 receptors hinders memory retention as shown in many associative tasks such as fear conditioning (Heath et al. 2015; Tsetsenis et al. 2021), classical eyelid conditioning (Ortiz et al. 2010). The same effect was found also in spatial tasks like Morris Water maze (da Silva et al. 2012), spatial learning of new goal locations (McNamara et al. 2014) and learning a new pair of odor/location in the Event Arena (Bethus et al. 2010). Systemic D1/5 receptors inhibition blocked cue fear conditioning (Inoue et al. 2000). Genetic manipulation of dopaminergic receptors genes showed that more specifically D1 receptors are the one involved in dopamine's role in memory (Granado et al. 2008) and further confirmed the involvement of these processes in spatial learning.

Moreover, dopaminergic transmission was shown necessary for the formation of place cells (Mouri et al. 2007) and systemic interference with dopaminergic transmission during latent learning of a spatial arena hinders the ability of mice to find a reward when tested the next day (Ichihara et al. 1993).

Reviewing these data, we see an important role of dopaminergic transmission in all mechanisms underlying memory.

### 1.6.3 Role of D1/5 receptors in LTP

Most studies suggest that the role of dopamine in LTP is only in L-LTP. D1/5 receptors are necessary for hippocampal LTP maintenance for the long-term in CA1 (Frey et al. 1990). *In vitro* recording showed that pharmacological activation of dopamine receptors using SKF38393 while stimulating glutamatergic afferents induces LTP that lasts for at least 7 hours, but if glutamatergic stimulation is stopped during SKF38393 application no LTP takes place (Navakkode et al. 2007). These results suggest a central role for dopamine in LTP triggering not only its maintenance on the long term.

Broussard and collaborators used inhibitory avoidance in 2016 to show learning induced LTP in CA1 and showed that SCH23390, a D1/5 receptors antagonist, blocks this LTP and learning. Soon after, Yang and collaborators in 2017, from the same team, showed that novel object recognition showed increases in AMPA currents in hippocampal slices taken from these mice, this time in the DG.

These results demonstrate clearly the role of dopamine in LTP. However, whether it is implicated in its initiation or its long term maintenance as a L-LTP remains controversial.

### 1.6.4 Hippocampus-VTA loop

The final piece of the puzzle comes from looking at the other side of the cyclic pathway between the hippocampus and the VTA reviewed by Lisman and Grace in 2005. In their review, they describe how it is not only the VTA that controls hippocampus plasticity, but also the hippocampus controls VTA dopamine neurons activity.

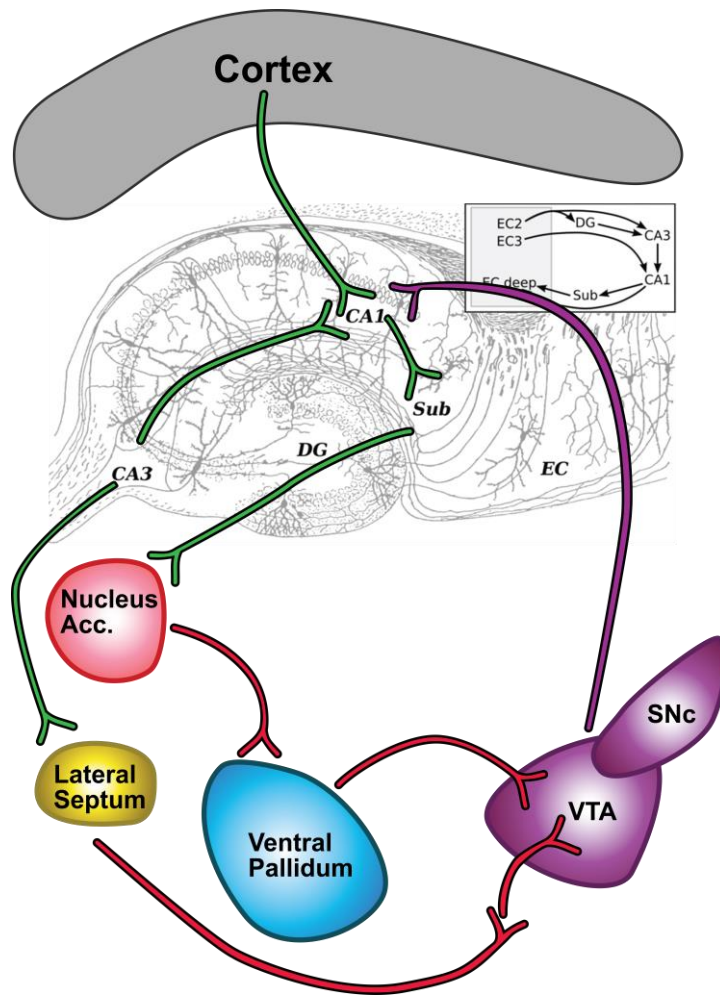
In CA1, predicted entries from the CA3 are compared with entries from the cortex (Otmakhova and Lisman 1999; Lisman 1999), giving rise to novelty detection. Indeed, they argue that since the hippocampus activity is modulated so quickly (<100ms), the hippocampus is not only affected by novelty, but rather it has an active role in detecting it.

This novelty signal then travels through a multi-synaptic pathway down to activate the VTA. First, CA1 notifies the subiculum, which was shown to be necessary for novelty triggered dopamine release from the VTA (Legault and Wise 2001; Legault et al. 2000). The subiculum then send glutamatergic afferents activating the Nucleus Accumbens (Floresco et al. 2001). Furthermore, the Nucleus Accumbens projects inhibitory GABAergic projections to the ventral pallidum, which in turn sends GABAergic projections to the VTA (Floresco et al. 2003) (Figure 1.6).

They argue that the complexity of the circuitry is due to the necessity of assembling many different signals that issue information saliency such as motivation, novelty and value in order to trigger the teaching signal correctly when needed (Lisman and Grace 2005).

Another functional pathway was discovered later showing that the CA3 can also indirectly activate dopamine neurons in the VTA through lifting the inhibition of GABA transmission over them by stimulating GABA neurons in the lateral septum, a pathway that was shown behaviorally functional in cocaine seeking re-instatement test (Luo et al. 2011).





**Figure 1.6 Hippocampus-VTA Loop**

In this model, the hippocampus can detect novelty through the CA1 (Glutamatergic green afferents), stimulate the subiculum (Glutamatergic green afferent), which stimulates GABA neurons in the Nucleus Accumbens (Glutamatergic green afferent) that in turn inhibits the inhibitory projections from the ventral pallidum to the VTA (GABAergic red afferent), leading to dopamine release (dopaminergic purple afferent) in the hippocampus allowing for plasticity to occur and to update information accordingly.

## **1.7 Hypothesis: Dopamine could play the role of a teaching signal**

LTP is the mechanism that allows hippocampal memories to be recorded. Both learning and HFS can trigger LTP, but the pattern of activity that triggers LTP experimentally does not occur during learning and the biological trigger of learning induced LTP remains unknown.

D1/5 receptors were shown to be involved in LTP. They are mainly considered to be necessary for L-LTP but some data also suggest their activation might be able to trigger LTP.

Moreover, the manipulation of VTA dopamine cells could facilitate context learning in the contextual fear conditioning test. However, in contextual fear conditioning we do not know whether dopamine facilitated contextual learning or the negative value of the electric shock.

In our work, we hypothesized that dopamine could work as a teaching signal in the hippocampus independently of its role in coding reward or punishment.

In order to verify this global hypothesis, we tested three working hypothesis:

- Dopamine should be able trigger LTP on co-activated glutamatergic afferent.
- Dopamine receptors in the hippocampus should be involved in contextual fear conditioning, but not cue mediated fear conditioning.
- Dopamine transmission from the midbrain to the hippocampus should be involved in contextual learning separately from value learning.

## Chapter 2 Methods

### 2.1 Optogenetics

#### 2.1.1 History of optogenetics

Historically, electrical stimulations were used to study the role of neural pathways in the brain. This technic has a very important caveat, namely, an electrical stimulation does not discriminate between neuron types and will activate all the around the electrode tip, even inhibitory ones which could produces to misleading conclusions. Francis Crick was the first to predict that the solution to this problem will include light, which could have the same temporal precision of electric stimulation. However, at the time he could not predict how this could be achieved (Crick 1979).

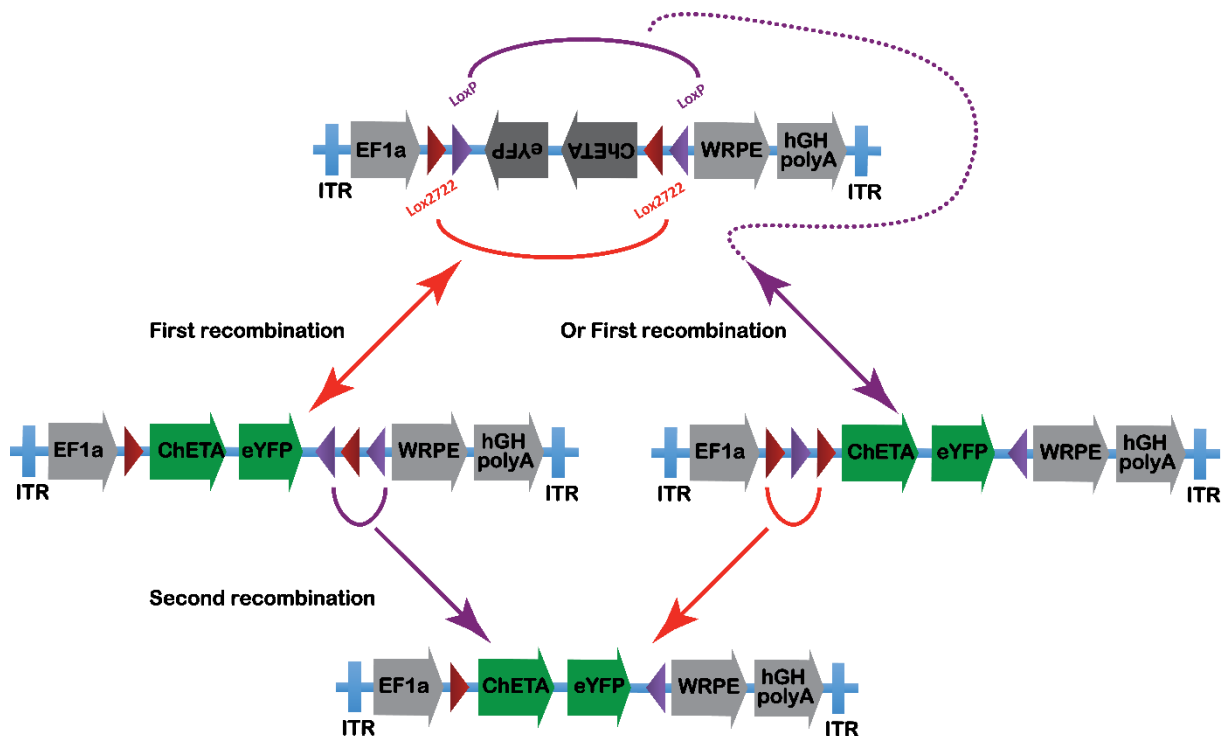
In an unrelated field of research, bacteriorhodopsin, a bacterial light-sensitive ion pump, was discovered (Oesterhelt and Stoeckenius 1973). Over the years many other similar channels were discovered, one such protein was the Channelrhodopsin-2 (ChR2), a light-sensitive channel selective for positively charged ions (Nagel et al. 2003). A couple of years later, these two fields collided which lead to the emergence of a new technology, later named optogenetics, in a paper showing electrophysiological responses to blue light (480 nm) stimulation of hippocampal cell cultures transfected with lentiviruses coding for ChR2 (Boyden et al. 2005). Another rhodopsin called halorhodopsin, and dubbed eNpHR, was discovered, this particular rhodopsin actively pumps negatively charged  $\text{Cl}^-$  ions inside cells in response to yellow light (590 nm) (Gradinaru et al. 2008). This manipulation, contrary to the former, hyperpolarizes the cells and inhibit them from inducing action potentials when the light is delivered.

#### 2.1.2 Cre-Recombinase

Afterwards, this technic was combined with further genetic modifications allowing the specific expression of these channels in certain populations of neurons taking advantage of the Cre recombination system. Cre-recombinase is an enzyme that can modify DNA sequences containing Lox sites following two strict rules (Figure 2.1).

- It inverses the sequence of DNA held between two Lox sites with inverse sequences.
- It excises the sequence of DNA held between two Lox sites with the same direction.

With these rules, and two distinct Lox sequences, LoxP and Lox2722, we can transfect a transgenic mouse that has Cre-recombinase expressed under a specific promoter of the targeted cells with a viral vector with DNA coding for the optogenetics protein in inverted sequence and between the two pairs of those distinct Lox sites. The transfected cells that do not have the Cre-recombinase will not produce the optogenetics proteins. On the other hand, the target cells that do have Cre-recombinase will be able to do the necessary modifications, first invert the DNA sequence, then excising one Lox sequence of each type, these modification will make it possible for the transfected cells to transcribe the mRNA and then produce the proteins, which in turn make them light-sensitive.



**Figure 2.1 Cre-Recombinase activation of double floxed transgenes**

Woodchuck Hepatitis Virus (WHP) Posttranscriptional Regulatory Element (WPRE) is a DNA sequence that enhances expression by creating a tertiary structure when transcribed. hGH polyA signal is necessary for adding of the poly(A) tail, which is important for the stability of the mRNA, and is necessary for nuclear export in order for the translation to occur. Inverted Terminal Repeat (ITR) sequences are important for circularization of adeno-associated virus genomes, which increases stability of the transgene. They contain also the origins of replication where DNA synthesis is initiated. As described in the body, Double Floxed DNA submit to two recombination episodes in cells containing Cre-Recombinase in order to get activated. For the eNpHR3.0, the same path applies by swapping the ChETA code with th eNpHR3.0 code.

### 2.1.3 Optogenetics used in this work

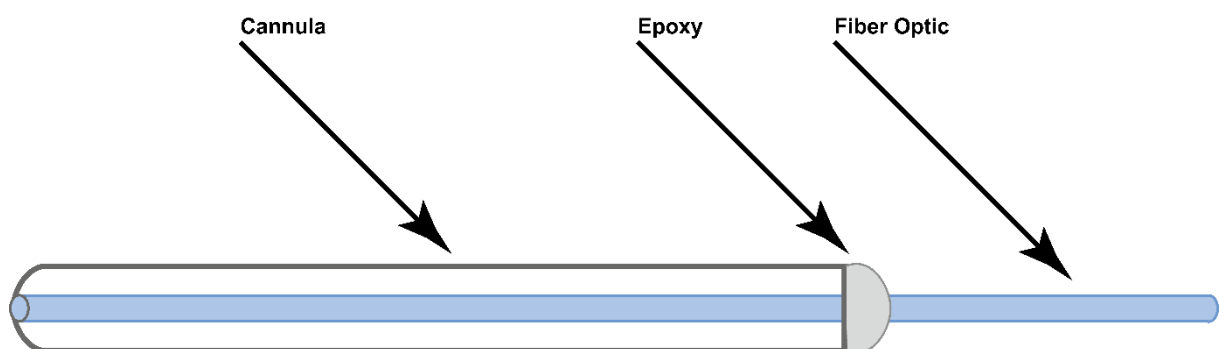
In our work, we used a modified version of the ChR2 called ChETA that allows for faster opening and closing of the rhodopsin cation channel, allowing for more precise control of the

targeted neurons with blue light (Gunaydin et al. 2010). In another experiment, eNpHR3.0 that opens in response to green light and allows negatively charged  $\text{Cl}^-$  ions to hyperpolarize the neurons, inhibiting them. The genes coding for these proteins were coupled with the enhanced Yellow Fluorescent Protein (eYFP) as a reporter gene and both were inversely coded after the ectopic promotor Elongation Factor-1 alpha (EF1a) and between two pairs of Lox sequences (Figure 2.1). Control vectors coded for eYFP alone. In order to control dopaminergic neurons specifically, we used a transgenic mouse line called DAT::Cre that expresses the Cre-recombinase under the control of Dopamine Transporter (DAT) promotor (Turiault et al. 2007). The viruses used in this work were bought from the UNC Vecto Core. All vector were Adeno-Associated Virus serotype 2 (AAV2) and were used at their original concentrations at purchase ( $3.5\text{-}5 \times 10^{12}$  molecule/mL).

#### 2.1.4 Implantable optic fibers manufacture

We used Thorlabs cannulas CFX230-10 ( $\text{\O}1.25$  mm, 6.4 mm Long Ceramic Ferrule for MM Fiber,  $\text{\O}230$   $\mu\text{m}$  Bore Size). The Optic fiber used was FT200UMT (0.39 NA,  $\text{\O}200$   $\mu\text{m}$  Core Multimode Optical Fiber, High OH for 300 - 1200 nm).

9mm long optic fibers were cut, with a ruby cutter placed inside the cannulas at their edge (Figure 2.2). Then they were glued in place using Loctite epoxy adhesive EA 3430 and left to dry for at least 72 hours.



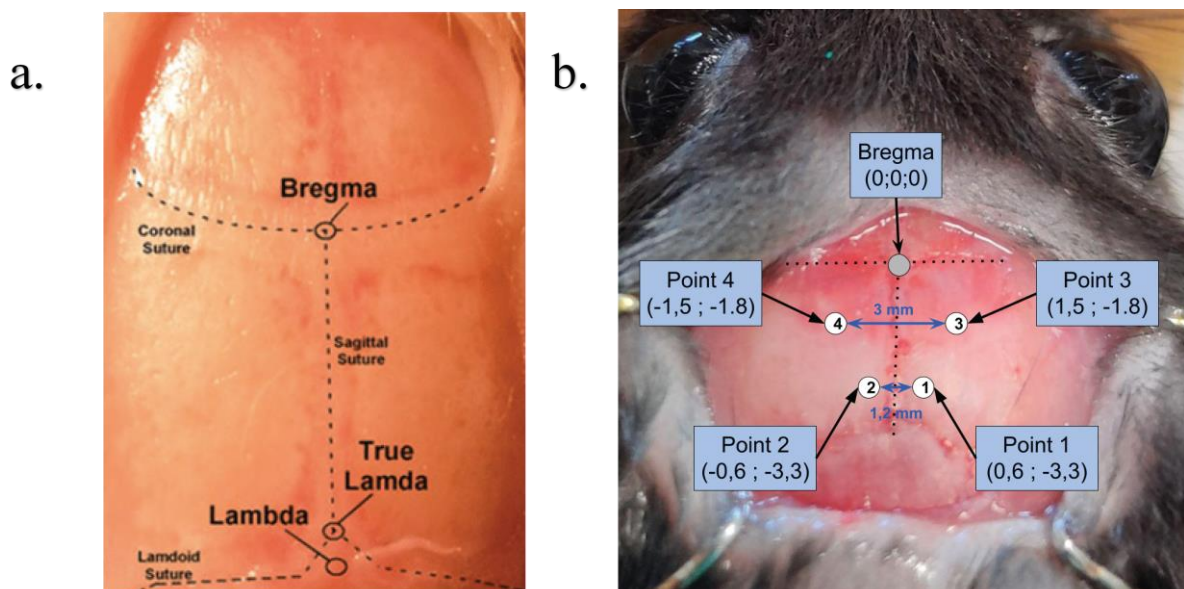
**Figure 2.2 Representative schema of cannula manufacture**

Cannulas with fibers in them were tested using PM100D Thorlabs Digital Optical Power and Energy Meter before implantation and cannulas showing at least 80% light conductance were used.

### 2.1.5 Vector injection and Optic fiber implantation

“Tem Sega” gas anesthesia machine was used to anesthetize mice with isoflurane. First, the mouse is put in the anesthesia box without anesthetic for a few minutes, so that it gets habituated to the environment which reduces novelty stress. After that, 3% isoflurane is sent, this is the rate necessary to induce gas anesthesia. After a few minutes, the mouse starts to breathe slowly and deeply, and can be put on the mask in the stereotaxic apparatus to keep a steady anesthesia and perform the surgery on 1-2% isoflurane. The mouse is put on a heating pad, NaCl 0.9% is added on its eyes, lidocaine is injected subcutaneously for local anesthesia at the location of the incision, and the hair on its head is shaved.

We performed a 1-1.5 cm incision to uncover the skull from the Bregma (anatomical point at which the coronal suture is intersected perpendicularly by the sagittal suture) to the Lambda (anatomical point of the sagittal suture and the lambdoid suture) (Figure 2.3 a.). All coordinates used in this work were done in relation to the Bregma and the locations of the structures were found in the “The mouse brain in stereotaxic coordinates” (Konsman et al. 2003). Four small craniotomy were performed for behavior two for injections and two for implantation. Only one craniotomy was performed at the right side injection point for the mice that underwent electrophysiology.

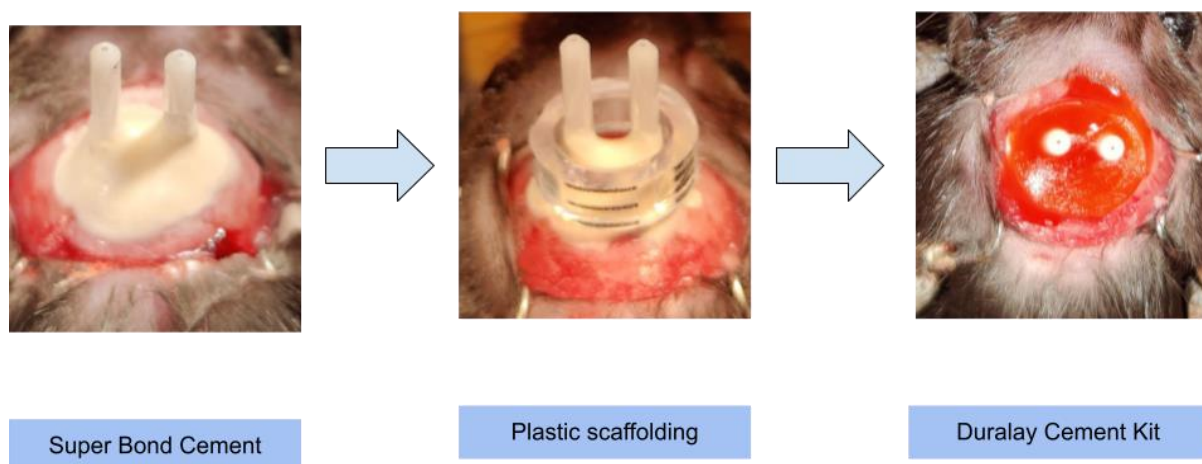


**Figure 2.3 Lambda, Bragma and coordinates for injections and implantations**

a. How to find the Bregma and the Lambda b. Four craniotomy points, 1 and 2 for viral injections at VTA, 3 and 4 for optic fiber implantation at CA1 of the hippocampus.

Viral injections were performed at (-3.3mm AP and  $\pm 0.6$ mm ML) (Figure 2.3 b.) for behavioral experiments, two injections were performed at each side (-4.0 and -4.6mm DV) 0.3  $\mu$ L injection at each site (1.2 in total for each mouse) at 0.1  $\mu$ L/min. We waited 10 minutes after the injections of each side to allow for diffusion. For mice that underwent electrophysiology, injections were performed only at +0.6mm, to the right from the midline (-4.0 and -4.6mm DV) 0.5 $\mu$ L at each site (1  $\mu$ L in total for each mouse) at the same rate.

This next part concerns behavioral studies only, optic fiber cannulas were implanted at CA1 (-1.8mm AP,  $\pm 1.5$ mm ML and -1.25 DV). They were cemented in place using one small scoop of Super Bond dental cement Bulk-mix Radiopaque mixed with 4 drops of Super Bond Quick Monomer and 1 drop of Super Bond Catalyst V. The implant is finalized using a round plastic scaffolding and Duralay red cement kit (Figure 2.4).



**Figure 2.4 Cementing of cannulas and optic fibers**

The incision around the head-implant was cleaned with NaCl 0.9% and disinfected with betadine and was then closed with two suture points one Anterior and one posterior to the implant. Mice without implant received 4 suture points that close up the whole incision. Mice then received 5 mg/kg Meloxicam for analgesia and their weight and behavior was monitored for the next week to make sure for the best recovery. Any mouse showing signs of long lasting pain or suffering (face signs, isolation of 10% body weight loss in 24 hours) was euthanized.



### 2.1.6 Lasers

Two lasers were used for this work 473 nm DPSS Laserglow (blue light for ChETA activation) and 532 nm DPSS Laserglow (green light for eNpHR3.0 activation). Light was then connected to the head of the mouse either through 200  $\mu\text{m}$  patch chords, then through 200  $\mu\text{m}$  nude optic fibers and finally, through the aforementioned implanted homemade cannulas (for behavioral studies). For electrophysiology, longer homemade cannulas were placed inside the recording glass micro-pipette that led the light to the recording site. The intensity was set to 10 mW at the implantable tip (optic fiber or micropipette tip). For inhibition, mice with eNpHR3.0 vectors received one continuous pulse (160 seconds long) covering 20 seconds before context pre-exposure the 2 minutes of pre-exposure and 20 seconds thereafter. For stimulation, mice received either 200 ms or 400 ms bursts (4 ms pulses, 50 Hz) depending on the protocol. These pulses were driven using Model 2100 Isolated Pulse Stimulator from A-M Systems and Spike2 connected through Micro1401-4 from CED.

## 2.2 In vivo LTP

### 2.2.1 Mice

Mice 2-6.5 month old male DAT::Cre mice were bred in the CRCA animal facility. To obtain these mice, a transgene allowing expression of Cre-recombinase under the control of the dopamine transporter promoter (DAT) was inserted into a bacterial artificial chromosome (BAC) in FVB/N mice. DAT is specifically expressed in these neurons. Therefore, in these mice, Cre-recombinase is expressed only in dopaminergic neurons. These mice were backcrossed more than 15 times on the C57BL/6J line. These animals are placed in stalls in groups of 3 to 5 individuals per cage, with food and water available *ad libitum*, in a room comprising a 12h/12h day/night cycle (day from 8h to 20h) at  $22 \pm 1$  °C.

All experiments were performed during the daylight period. First, surgery was performed on each mouse to inject a viral vector, allowing expression of the ChETA protein (for details, please read 2.1.5 Vector injection and Optic fiber implantation). After a waiting period of at least 1.5 weeks, the expression of the vector proteins is sufficient. Finally, the transfected mice underwent a second surgery to perform the electrophysiology experiments to measure LTP.



### 2.2.2 Simulation electrodes

The stimulation electrodes were made from tungsten wire (W5584 Advent research material 50µm diameter 12.5 µm Teflon coated). First, 8cm wire was twisted around itself (about ten turns) and held in this vertical and rectilinear configuration using clamps. They were then covered with a primer (Loctite 770) then with superglue (Loctite 406) then left to dry in this position for at least 24 hours.

This makes for two electrodes, the twisted glued wire was cut in half, then stripped at both ends for a few millimeters to be able to be soldered on 2-way connectors (electrode length would be around 2cm). At the place where the wires were cut, the wires were stripped (of the glue and the Teflon) with a scalpel over approximately 250-350 µm under a magnifying glass in order to obtain an optimal impedance which was measured in NaCl 0.9%, at 100Hz, only electrodes showing 100 and 150kΩ impedance were used.

### 2.2.3 Recording Micropipette

Recording was performed using glass micropipettes made by stretching (narishige PE-21 stretcher, no magnetic stretch, temperature configuration 53.4) glass tubes (Harvard Apparatus GCL50F-10). The tips of the pipettes were then broken manually under the microscope to obtain a tip with a 4-micron diameter. The micropipettes were filled with a 2M NaCl solution to conduct the electric current. Pipettes with impedance between 0.8 and 1.1 @100Hz Megohm were used.

### 2.2.4 Electrophysiology post

The equipment used for electrophysiology recording included an A-M Systems model 1800 amplifier (gain = 100) set to a bandwidth of 0.1Hz to 10kHz. This amplifier was connected to a mains noise eliminator (Humbug 50 Hz Noise Eliminator, QuestScientific) then the recording was transmitted to an analog-digital converter CED micro 1401 which allowed sampling at 10kHz analyzed using Spike 2 software (version 7.18). Two AM-Systems stimulators were used, the first to deliver biphasic, 100µs stimulations at SC through the aforementioned electrodes placed at the ventral Hippocampal Commissure (vHC) (Coordinates: -0.3mm AP, -0.5mm ML and -2.3 DV). The second stimulator was used to drive the stimulating, blue, laser activity.

## 2.2.5 Electrophysiology recording surgery

Anesthesia was induced using Isoflurane as described before, then maintained with an injection of urethane ( $1.5 \pm 0.2$  mg/g) during the recording period.

In the stereotaxic apparatus we did an incision and a craniotomy, then we placed the stimulation electrode at SC. This stimulation causes a response in the CA1 area that can be recorded as field excitatory Postsynaptic Potential (fEPSP) through recording micropipettes placed at the level of the stratum radiatum of area CA1.

The raw electrophysiological signal was first analyzed by averaging fEPSP waveforms every 5 minutes (10 fEPSP). We call these mean fEPSP. The slope of the initial phase of each mean fEPSP is measured. A baseline was obtained by stimulating at 70% of the maximal response recorded at the strongest stimulation (0.05-1mA). We considered 25min of recording (i.e. five mean fEPSPs) with a slope between 95 and 105% of the average as a stable baseline and the evolution of the fEPSP slope should not follow a linear change.

After baseline determination, we delivered the coupling of glutamatergic stimulation and dopaminergic optic stimulation. Afterwards, a follow up took place. In some cases, after 95 minutes of follow up an HFS protocol of LTP. This protocol followed Theta Burst Stimulation (TBS) pattern, which is 4 trains (30s inter-train interval) of 4 bursts (200ms inter-burst interval) of 5 stimulations at 100Hz.

At the end of the recording, the glass pipette is replaced with one filled with Chicago blue 2% in acetate buffer 0.5M. We searched for the same recorded fEPSP and the recording site was marked using electric expulsion of the dye with negative current of 20  $\mu$ A, cycles 10 sec "on" / 10 sec "off" for 10 minutes.

Finally, the mice were euthanized with a lethal injection of pentobarbital, and then, received an intracardial infusion of 0.9% NaCl solution and the brains were collected for immunohistochemical verification.

## 2.3 Contextual Fear Conditioning coupled to cue fear conditioning

### 2.3.1 Mice

Mice used in these experiments were bought from Charles River labs. These were female C57BL/6J mice. The animals were 3 to 4 months old at the start of the manipulations. They were placed in a stall in the laboratory in groups of 4 to 5 individuals per cage, with food and

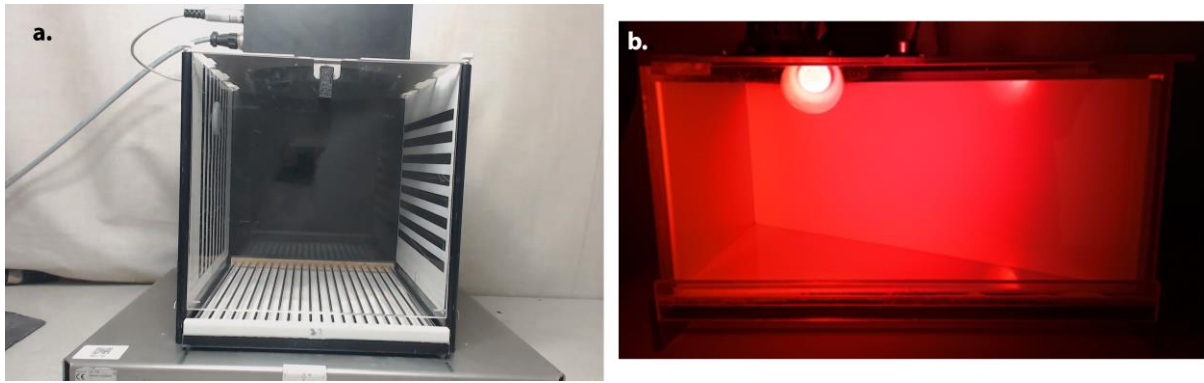
water available *ad libitum*, in a room comprising a 12h/12h day/night cycle (day from 8h to 20h) at  $22 \pm 1$  ° C. All experiments were performed during the daylight period.

### 2.3.2 Surgery for intra-hippocampal infusion

Mice were anesthetized with Isoflurane as described before. The animal was then placed in a stereotaxic device and the same steps described for optic fiber implantation were performed (2.1.5 Vector injection and Optic fiber implantation), but this time for stainless steel guide cannulas (Phymep 7mm long 24 Gauge), therefore, only two craniotomies for bilateral cannula implantations aimed at CA1 were necessary. The coordinates were (-1.8mm AP,  $\pm 1.5$ mm ML and -1.0 DV), the injectors used were 7.25 mm long and 0.25 mm diameter. Mice were left for a week to recuperate after the implantation.

### 2.3.3 Behavior apparatus

The context used was of a cubic box 27cm square. The front wall was made of transparent Plexiglas in order to film the mouse during the procedure. This wall doubles as a door, and allows placing the mouse in the context. The walls on either side included proximal visual cues in the form of white and black bands. The back wall was opaque and black Plexiglas. A loudspeaker was attached to the ceiling, allowing sound cues to be emitted. The floor of the box consisted of 22 metal bars ( $\text{Ø} = 4\text{mm}$ ) spaced one centimeter apart, through which the electric shock was delivered. White curtains surround the device. We placed two panels with distal visual cues clearly identifiable by the animal, namely, a black square on a white background on the left and vertical black and white stripes on the right. The entire device was illuminated by a white light source. The cage was cleaned with 70% ethanol before placing an animal in it (Figure 2.5 a.). This was considered the conditioned context and used for learning as well as contextual recall. In order to test for generalized fear, and to test for the sound cue recall, the animals were placed in a new context; a triangular Plexiglas box with transparent front and top walls with a loudspeaker attached to the ceiling. The animal was placed from the top of the box. The other walls (including the floor) are white and opaque. The curtains and distal cues of the learning context were removed, so the mouse has visibility over the entire room with red light illuminating this alternative context and the cage was cleaned with acetic acid (7%) before each mouse (Figure 2.5 b.).



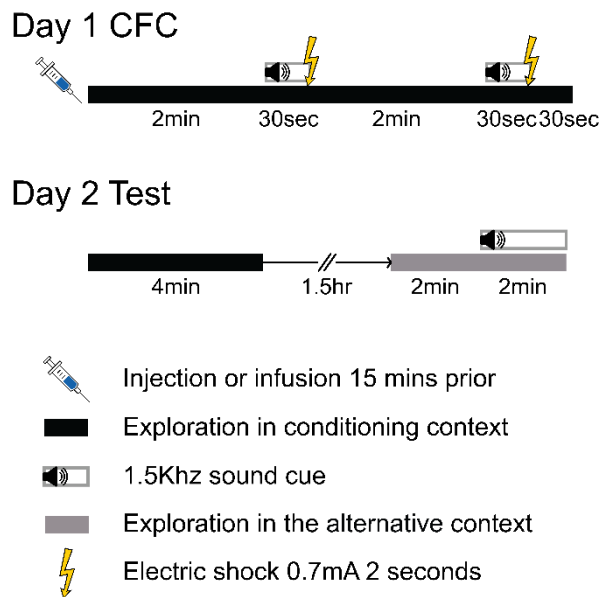
**Figure 2.5 Contextual Fear Conditioning contexts**

**a.** fear conditioning context **b.** alternative context

### 2.3.4 Contextual fear conditioning procedure

One week after the recuperation of the mice from the implantation surgery, we habituated the mice to the handling by the experimenter (30 seconds per mouse 3 times over one week).

The day of the experiment, mouse cages were placed in a room adjacent to that of the fear conditioning apparatus. One by one, non-implanted animals received an injection of NaCl 0.9% or SCH23390 (0.05mg/kg), and implanted animals received intra-hippocampal infusions of either NaCl 0.9% (0.35 $\mu$ L/side 0.11 $\mu$ L/min), Lidocaine (5 $\mu$ g/side) or SCH23390 (0.5 $\mu$ g/side). They were left 15 minutes to recuperate then placed in the conditioned context. The conditioning was performed using Panlab system s.l. Startfear 1.06. Mice explore for 2 minutes, then, a sound cue (1.5 KHz, 85 dB, 30 s) was emitted, ending with an electric shock (0.7 mA, 2 s) delivered by a grid floor. This sequence was repeated and followed by 30 seconds of exploration before taking the mouse back to its home cage (Figure 2.6).



**Figure 2.6 Contextual Fear Conditioning Protocol**

24 hours after learning, the mouse was placed in the learning cage, without sound nor electric shock, for 4 minutes (context test) in order to measure the time spent in freezing behavior, defined as periods of complete lack of movement except for respiratory ones. This behavior reflects the animal's fear and by extension, giving us a clue of the animal's level of memorization of the context. About 1.5 hours after the context test, the mouse underwent the alternative context test. It was placed in this device for 4 minutes. The time spent in freezing in the new context is measured for 2 minutes without sound to assess the generalization of fear. During the next two minutes, sound was presented, to test for simple cue memory. Infusion site locations were verified by infusing Chicago blue (2% in 0.5M acetate) 3 to 5 days after the last behavioral session.

Freezing was scored each 5 seconds by two independent experimenters blind to the experimental condition and expressed as a percentage of the sampled time spent freezing. In order to respect parametric requirements for ANOVA tests, percentages (P) were transformed using the equation  $Q = \text{Asin}(\sqrt{P/100})$  (ZAR 2014).

## 2.4 Contextual Pre-exposure Facilitation Effect

### 2.4.1 Procedure to test for pre-exposure time and pharmacology

Contextual Pre-exposure Facilitation Effect is a variation of the contextual fear conditioning introduced in the early 2000s (Fanselow 2000; Barrientos et al. 2002). It allows for the

separation of contextual learning from associative learning that take place at the same time in contextual fear conditioning.

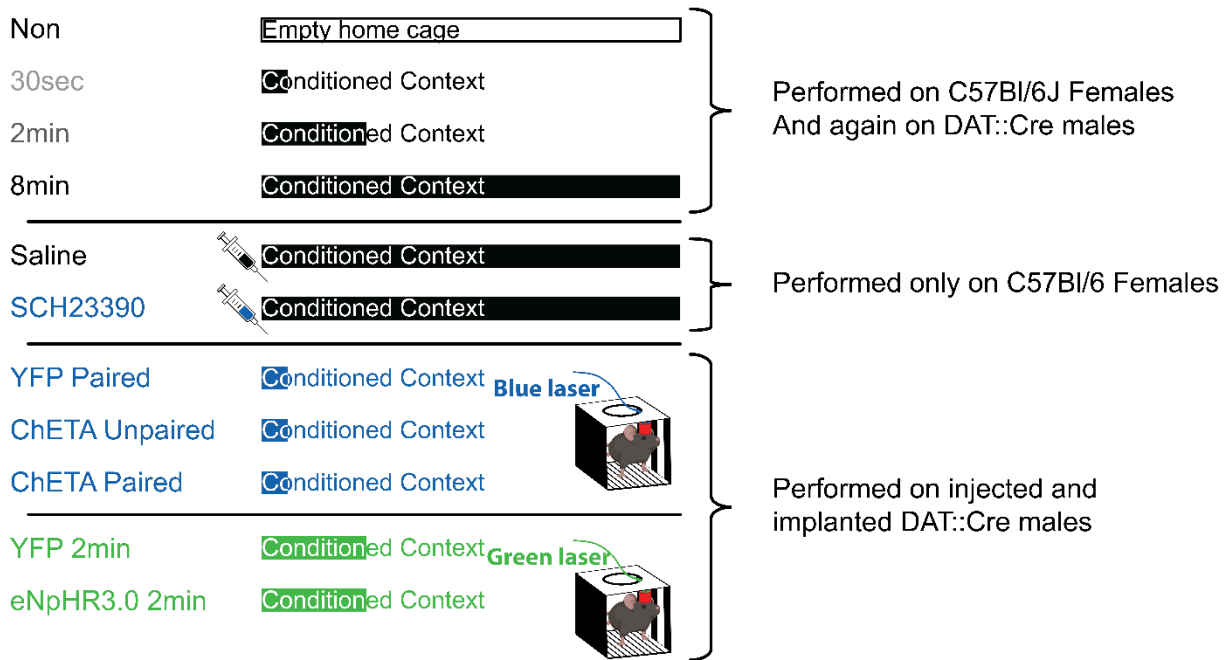
In this variation, mice learn the context on day one during a session of exposure to it more or less long. On day two, mice are very briefly shocked in the conditioned context during a session of conditioning that is less than 10 seconds long. On day three, the memory of the context is tested by measuring freezing in the context just as the test performed for the contextual fear conditioning. Generalized fear was tested in the alternative context and mice that froze more than 33% of the time in the alternative context were considered as generalizers and their freezing data was excluded from any further analysis.

The first four groups were tested to check for the least amount of time necessary for contextual learning; we tested non pre-exposed mice (put for 8minutes in an empty home cage), 30sec, 2min, and 8min pre-exposed mice. In order to test for D1/5 receptors involvement in contextual learning, the next two groups were injected with either NaCl 0.9% or SCH23390 (0.05mg/kg). One group that is not represented in this schema was C57Bl/6J females that received SCH23390 (0.05mg/kg) before the conditioning session (after 8 minutes of pre-exposure on day one) in order to test for the role of D1/5 receptors in the associative memory (Figure 2.7).

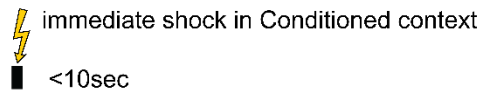
#### 2.4.2 Procedure to test pathway implication using optogenetics

We then replicated the data obtained with the first four groups, this time in male DAT::Cre. We had to take the amplifier out of the conditioning context to make room for the optic fibers to pass through (Figure 2.8). We then tested for the role of midbrain-hippocampus dopamine pathway in contextual learning using 3 groups. They received 30 seconds of pre-exposure, one of the groups received dopamine supplementation through optogenetics stimulation of the pathway (ChETA Paired vs. two necessary control groups, one receiving the light with no ChETA, YFP Paired, and the other receiving the stimulating light the day before). Finally, Two more groups received 2 minutes of pre-exposure, and dopamine axons were blocked using optogenetic inhibition (eNpHR3.0 vs YFP 2min that received the light but not the eNpHR3.0) (Figure 2.7).

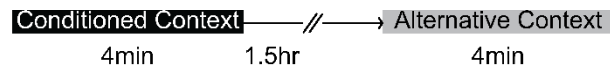
## Day 1 Context Pre-exposure



## Day 2 Conditioning



## Day 3 Test



**Figure 2.7** Representatif schema of the different procedures used for contextual pre-exposure facilitatin effect



**Figure 2.8** CPFE Context without the attached amplifier

## **2.5 Locomotion in response to novelty and cocaine**

Male DAT::Cre mice used in electrophysiology showed some hyperactivity (subjective observation), in order to characterize this phenomenon, and study whether it had a link to dopaminergic activity, we studied their locomotion in an open field after which we administered cocaine and compared that between wild type, DAT::Cre heterozygotes and DAT::Cre homozygotes.

To do this, we tested locomotion in a new environment, namely a circular open field (40cm) in low light. We then evaluated hyperactive induced by cocaine, a specific DAT blocker.

We measured the distance traveled every 5 minutes (using EthoVision) for 30 minutes (basal). Mice then received intraperitoneal injections of cocaine (10 mg/kg); locomotion was then measured for an hour and a half.

## **2.6 Immunohistochemical verification of transfection**

To measure the efficiency of the transfection, we carried out a double immunohistochemical labeling directed against the reporter protein eYFP, and TH the enzyme necessary for dopamine production, to visualize the cells infected and dopaminergic cells, respectively.

The animals are anesthetized (pentobarbital) before performing an intracardiac infusion with 0.9% NaCl solution (20-30s, 20mL/min). The brains were then removed and placed in 4% PFA solution for 24-72hr, then rinsed with 0.1M PBS. Finally, the brains are stored in a 30% sucrose solution containing 0.1% azide.

These brains were later sectioned into several serial 40 $\mu$ m sections with a freezer microtome and then stored in a cryoprotectant solution. Sections were put through two-day-immunostaining protocol.

On day one, sections were washed in 0.1 M phosphate buffered saline containing 0.25% triton (PBST), placed for 15 min in a solution containing 10% H<sub>2</sub>O<sub>2</sub> and 10% methanol (in PBST), to block the endogenous peroxidase. Then two rinses of 10 min with PBST, after that sections were placed for 1 hour in a solution saturating non-specific bonds (BlockNsp: 5% donkey serum in PBST). Finally, they were incubated overnight at room temperature in a solution of BlockNsp containing the primary antibodies (goat anti-YFP 1: 2500 (Rockland, 600101215) and rabbit anti-TH 1: 1000 (Millipore, AB152)).



The next day, the sections were twice rinsed in PBST before being placed for 1 hour and a half in a solution containing the fluorescent secondary antibodies (donkey anti-goat A488 1: 250 (Thermofisher, A11055) and donkey anti-rabbit A555 1: 250 (Thermofisher, A31572)).

Finally, the sections were twice rinsed in PBST before mounting them on slides. Slide covers were glued with a Mowiol solution containing Hoechst (1: 10,000) in order to mark the nuclei. Once dry, the slides were be observed using a Leica fluorescence microscope, a sampled transfection zone was counted for each mouse (using Mercator software). Sections were photographed using the same software and these photos were retouched using ImageJ. Slides were then stored at 4 ° C.

## **Chapter 3 Midbrain Dopamine triggers LTP**

In this chapter we explored the role dopamine could play in triggering LTP coupling the optogenetic stimulation of the dopaminergic midbrain-CA1 pathway with the electric stimulation of the glutamatergic SC-CA1 pathway.

### **3.1 Introduction: Dopamine and synaptic plasticity**

We pose our hypothesis on the model proposed in (Girault et al. 2007) concerning dopamine projections in the striatum. We adapt it to the hippocampus, dopamine can play the role of an “AND Gate” triggering plasticity of glutamatergic synapses allowing the hippocampus to keep a memory record of relevant events.

Experimentally triggered LTP requires HFS that activates NMDA glutamatergic receptors while the post synaptic element is depolarized, which allows for a massive calcium influx inducing phosphorylation events that allow for the trigger and maintenance of LTP (for details please refer to 1.4.3 LTP mechanisms in the introduction). We hypothesize that, if dopamine plays the role of a learning signal, it should be able to trigger LTP only on co-activated glutamatergic synapses.

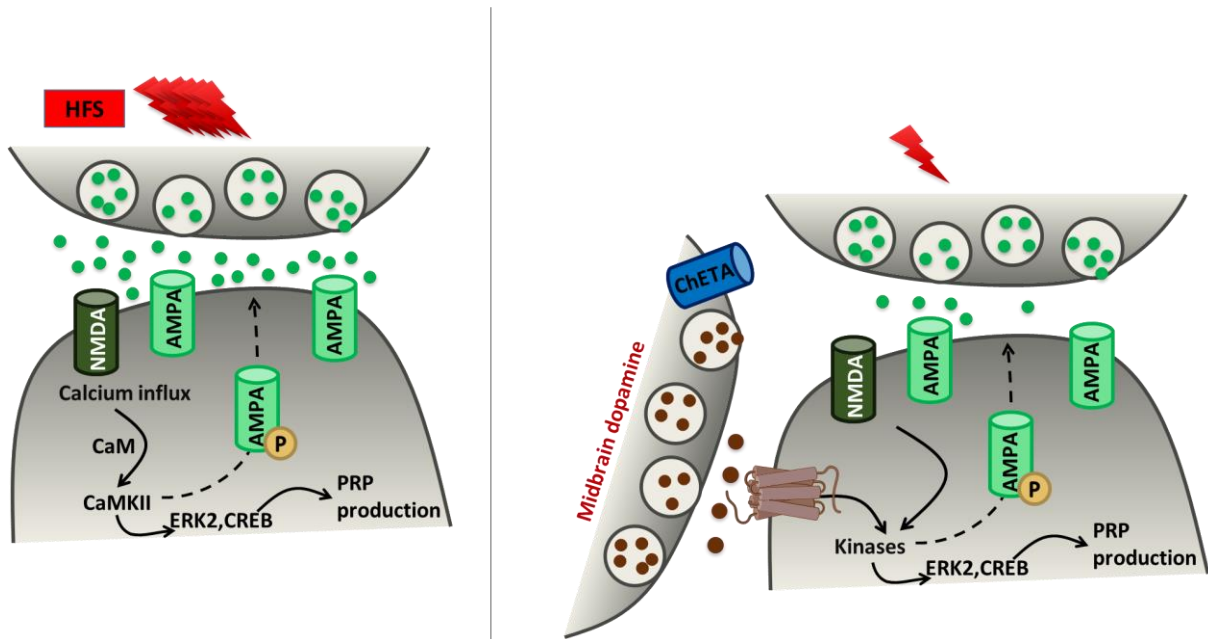
In *in vitro* work, Rosen showed in 2015 that the activation of dopaminergic neurons can bidirectionally modulated SC glutamatergic transmission. Tonic activation of the dopaminergic afferents of the midbrain causes a decrease in this latter transmission while phasic stimulation has the opposite effect. These effects involve the D4 and D1 receptors, respectively. However, these authors have not tested the effect of coupling dopamine and glutamatergic transmissions nor did they assess the long lasting effect of dopamine, monitoring synaptic transmission for a maximum of 35 minutes after stimulation.

Navakkode et al. showed in 2007 that stimulating dopamine receptors using SKF38393 while monitoring glutamatergic transmission did induce a long lasting increase in synaptic transmission, and that no such increase was observed if the monitoring is stopped during SKF38393 application. D1/5 receptors were shown to be implicated in dopamine role in learning and LTP (Broussard et al. 2016).

Our standing hypothesis is that we can use dopaminergic coupling in order to trigger LTP without needing HFS where the activation of dopaminergic afferents will trigger LTP on co-activated synapses, while separating the coupling will not induce this LTP (Figure 3.1).

This LTP should involve D1/5 receptors; therefore, their inhibition during coupling should be sufficient to block, at least partially, this LTP.

If HFS-triggered LTP and dopamine-triggered LTP are the manifestation of the same phenomenon, then triggering the latter should saturate the former, therefore, HFS-trigger LTP should be smaller in mice that already received dopamine-triggered LTP.



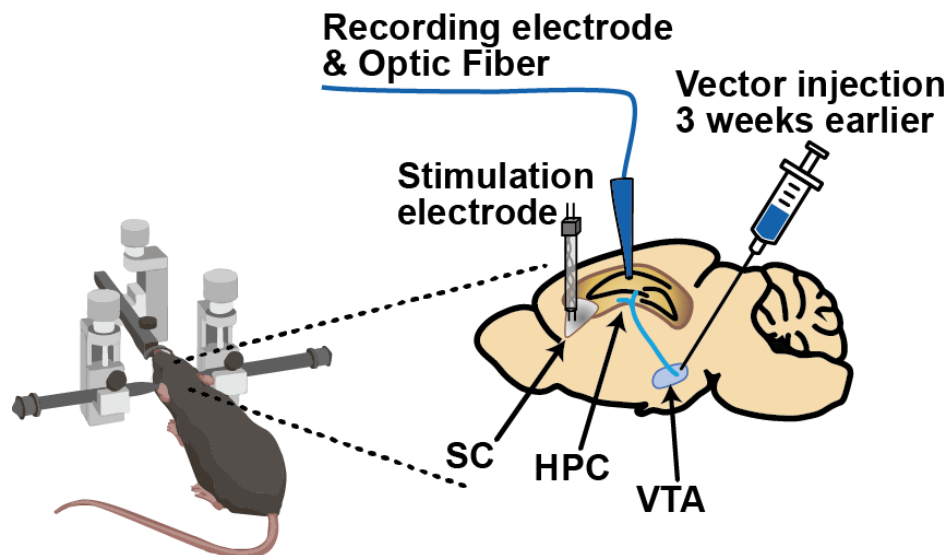
**Figure 3.1 Standing hypothesis of dopamine triggered LTP**

Two models of cellular mechanisms that may be the basis of LTP. On the left LTP induced by HFS: The high frequency stimulation causes release of glutamate while the postsynaptic element is depolarized, which in turn induces  $Ca^{2+}$  influx, followed by the activation of kinases, phosphorylation of AMPA receptors, and finally their targeting to the membrane. This will cause increased synaptic transmission. LTP is stabilized by PRP production. On the right, LTP induced by dopamine: The activation of kinases is not caused by NMDA but by the joint activation of the postsynaptic element and dopamine receptors, which will in turn activate the phosphorylation cascade that induces LTP.

## 3.2 Experimental approach

We used optogenetic stimulation of dopamine afferents in the hippocampus by transfecting DAT::Cre mice with double floxed ChETA expressing vectors (for more details 2.1.3 Optogenetics used in this work). This approach induces the expression of a light sensitive protein specifically in dopaminergic cells. Laser light was delivered through the glass micropipette to the recording site in order to specifically activate the local dopaminergic release from dopaminergic axons innervating the recorded zone (Figure 3.2).

Electrical stimulations to Schaeffer collaterals were delivered once every 30 seconds to the ventral hippocampal commissure in order to evoke fEPSP to monitor synaptic transmission and deliver glutamatergic stimulation.



### Figure 3.2 Electrophysiology methods coupled to optogenetics

Transfected mice were recorded under anesthesia. Electrical stimulations were delivered to Schaeffer Collaterals (SC) in the ventral hippocampal commissure. Recordings were acquired from the Stratum Radiatum of the CA1 where also light was delivered during coupling.

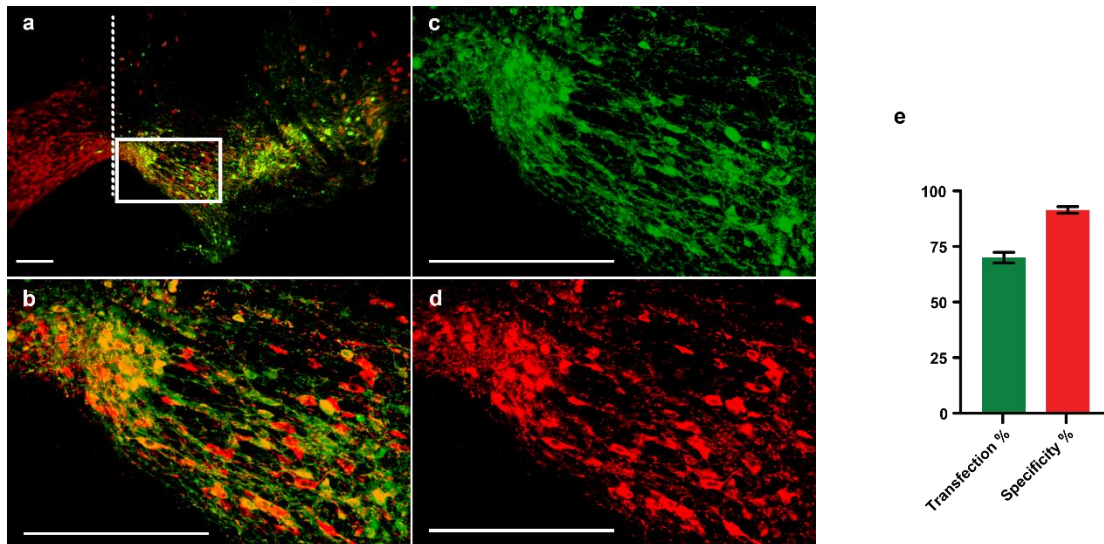
## 3.3 Results

### 3.3.1 Satisfactory transfection profiles for both vectors

DAT::Cre mice transfected with double floxed vectors expressing either ChETA and YFP or only the reporter gene YFP show satisfactory profiles of transfection.

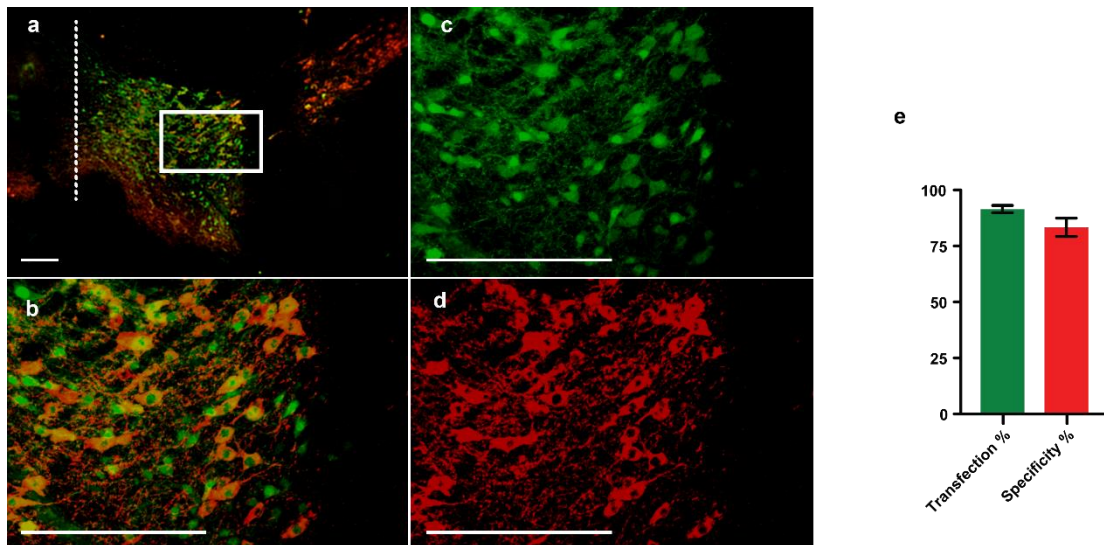
In these analyses, we studied two indices in order to judge this profile. The first was the transfection percentage (transfection%), this index shows how efficacious our vector was in expressing the vector genes in TH expressing cells (dopaminergic neurons). This was estimated by dividing the number of cells expressing both TH and YFP ([TH+,YFP+]) by the total number of cells expressing TH ([TH+]) in a sampled area of the VTA ( $\text{transfection\%} = \frac{[\text{TH+}, \text{YFP+}]}{[\text{TH+}]} * 100$ ). The second was the specificity percentage (specificity%). It shows how specific our genetic manipulation was in targeting dopaminergic neurons. This estimation was done by dividing the number of [TH+,YFP+] cells by the number of all cells expressing YFP ([YFP+]) ( $\text{specificity\%} = \frac{[\text{TH+}, \text{YFP+}]}{[\text{YFP+}]} * 100$ ) in the same sampled area.

ChETA,YFP coding vectors showed  $70 \pm 2.4\%$  transfection% and  $91.5 \pm 1.5\%$  specificity% (Figure 3.3) and YFP only coding vectors showed  $91.5 \pm 1.6\%$  transfection% and  $83.4 \pm 4.1\%$  specificity% (Figure 3.4). While both parameter show statistically significant difference between vectors (Mann-Whitney  $p < 0.05$  for specificity% and  $p < 0.01$  for transfection%), this difference can be explained by the fact that YFP only vectors express YFP in the cytosolic space, the same space where the TH resides. However, ChETA,YFP coding vectors address the expressed YFP to the membrane beside ChETA channels, which makes it harder to insure co-localization on the level of cells.



**Figure 3.3 Satisfactory expression of YFP in TH+ expressing cells of the VTA with ChETA, YFP coding vector.**

**a** Shows TH+ cells (in red) in the right VTA (x5 zoom) transfected, expressing YFP (in green), yellow shows merge. Rectangle represents the sampled area for analysis (at x20 zoom). Dashed line represents the midline. **b** Merge in the sampled rectangle captured at x20 (**c** and **d** show green and red color channels for YFP and TH, respectively). White horizontal bars represents 100µm **e** the quantification of cells show transfection%=70±2.4% (green) and specificity%=91.5±1.5% (red).



**Figure 3.4 Satisfactory expression of YFP in TH+ expressing cells of the VTA with YFP only coding vector.**

**a** Shows TH+ cells (in red) in the right VTA (x5 zoom) transfected, expressing YFP (in green), yellow shows merge. Rectangle represents the sampled area for analysis (at x20 zoom). Dashed line represents the midline. **b** Merge in the sampled rectangle captured at x20 (**c** and **d** show green and red color channels for YFP and TH, respectively). White horizontal bars represents 100µm **e** the quantification of cells show transfection%=91.5±1.6% (green) and specificity%=83.4±4.1% (red).

### 3.3.2 Concomitant dopamine/glutamate release induce long lasting increase in synaptic transmission

We first evaluated the long lasting effects of coupling dopamine and glutamate release on the glutamatergic transmission at Schaeffer Collaterals. In order to monitor the synaptic transmission of SC *in vivo*, we recorded field excitatory postsynaptic potentials (fEPSP) elicited in CA1 in response to electrical stimulations of the ventral hippocampal commissure (vHC) delivered every 30 seconds in mice anesthetized with urethane. Once a stable baseline of 25 minutes was established, we coupled the next 50 electrical stimulations of the Schaeffer collaterals (still delivered every 30 seconds) to an optogenetic stimulation of dopaminergic fibers innervating CA1, before resuming the monitoring of fEPSP, without optogenetic stimulation, for 5 hours.

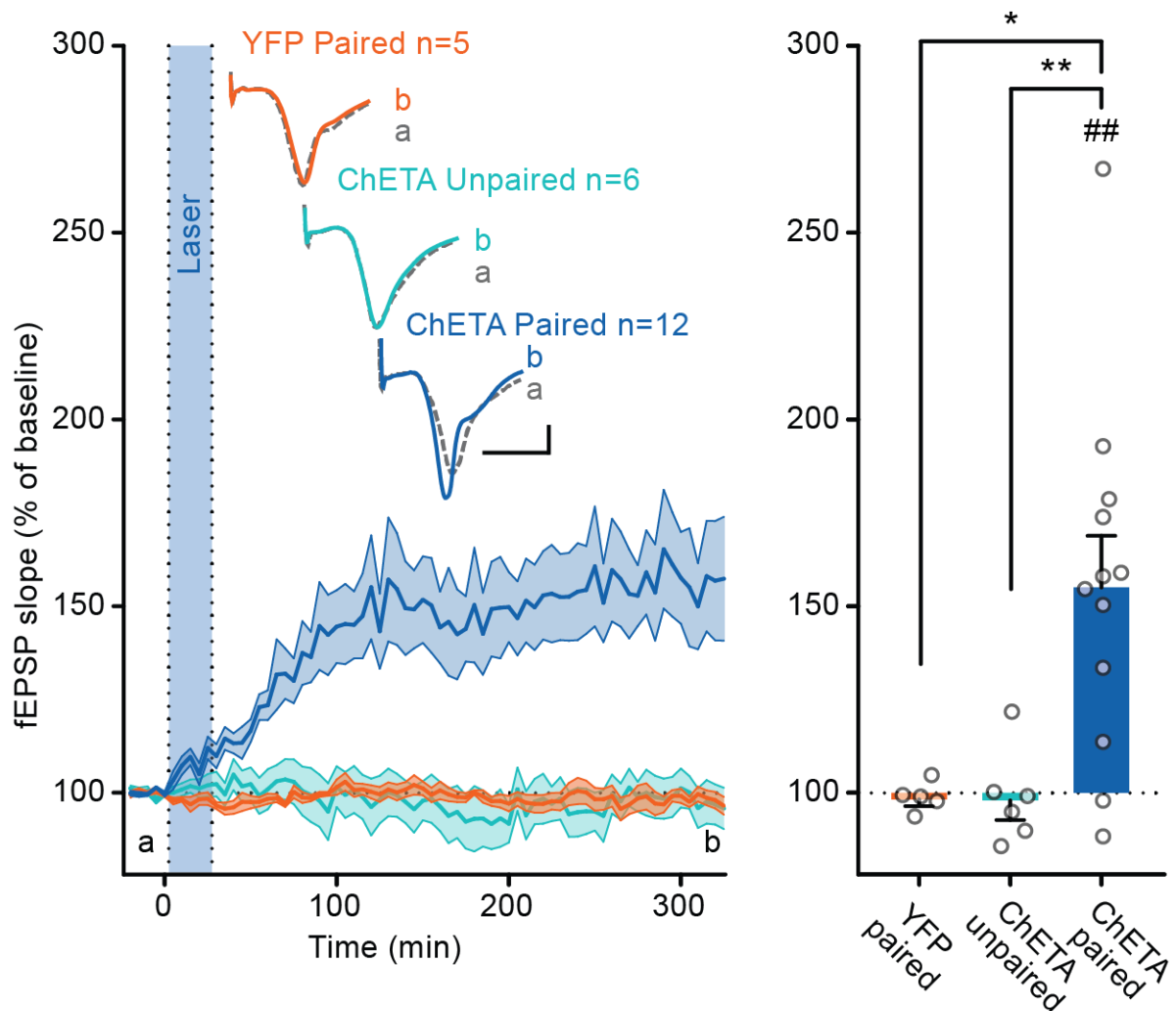
Stimulating the axons of dopamine neurons in the recorded area was achieved by delivering pulses of blue light through an optic fiber placed within the recording glass pipette in DAT::Cre mice expressing ChETA in midbrain dopaminergic neurons. This light stimulation mimicked typical burst firing of dopamine neurons (4ms pulses with inter pulse interval of 20ms). In order to get a pairing of dopamine and glutamate release, the light stimulation started 200ms before the electrical stimulation for a total duration of 400ms, so that the glutamatergic release occurred right in the middle of the stimulation of dopaminergic terminals (for details please visit 2.2 In vivo LTP paragraph in the methods).

This protocol triggered a progressive increase of the fEPSP of Schaeffer Collaterals that seems to start during the pairing protocol, steadily increases for 90 min after the end of the pairings and then stabilizes for at least 3.5 hours ( $+55\pm 14\%$  of baseline  $n=12$ ; Figure 3.5, group ChETA Paired). The same protocol did not influence the fEPSP of Schaeffer Collaterals in mice that received an injection of the control vector expressing only YFP but not the ChETA opsin ( $-2\pm 4\%$  of baseline  $n=5$ ; Figure 3.5, group YFP Paired). In another control group, light pulses were delivered 15 seconds after the electrical stimulation of SC. This “unpaired” protocol did not influence glutamatergic transmission neither ( $-2\pm 12\%$  of baseline  $n=6$ ; Figure 3.5, group ChETA Unpaired).

The increase observed for the ChETA Paired group was statistically significant compared to both other groups ( $p<0.01$  vs ChETA Unpaired,  $p<0.05$  vs YFP Paired. Mann Whitney test) and was statistically significant compared to baseline ( $p<0.01$ . t-test) (Figure 3.5).



These results show that dopamine, when stimulated concomitantly with glutamate, is able to trigger a long lasting form of synaptic plasticity resembling LTP.



**Figure 3.5 Dopamine release concomitant with glutamate synaptic transmission increases glutamatergic transmission for hours.**

Effect of 50 couplings of dopamine axons photo stimulations (400ms bursts of 4ms pulses 50Hz) to 50 electrical stimulations of Shaffer Collaterals (blue shaded part of the timeline). When stimulations were simultaneously coupled in ChETA injected mice, these couplings induced 55% increase in fEPSP slopes (Dark Blue, ChETA), we call this phenomenon DA-LTP. No such increase was observed when electrical and optogenetic stimulations were separated by 15 seconds (Light Blue, ChETA Unpaired), neither in mice injected with control vectors and simultaneously stimulated (Orange, YFP). Timelines with representative fEPSP of each group on the left, mean changes quantified by averaging the last 25 minutes of the recording for each mouse (on the right). \*  $p < 0.05$  Mann-Whitney, \*\*  $p < 0.01$  Mann-Whitney (after significant Kruskal Wallis), ##  $p < 0.01$  t-test vs. 100%.

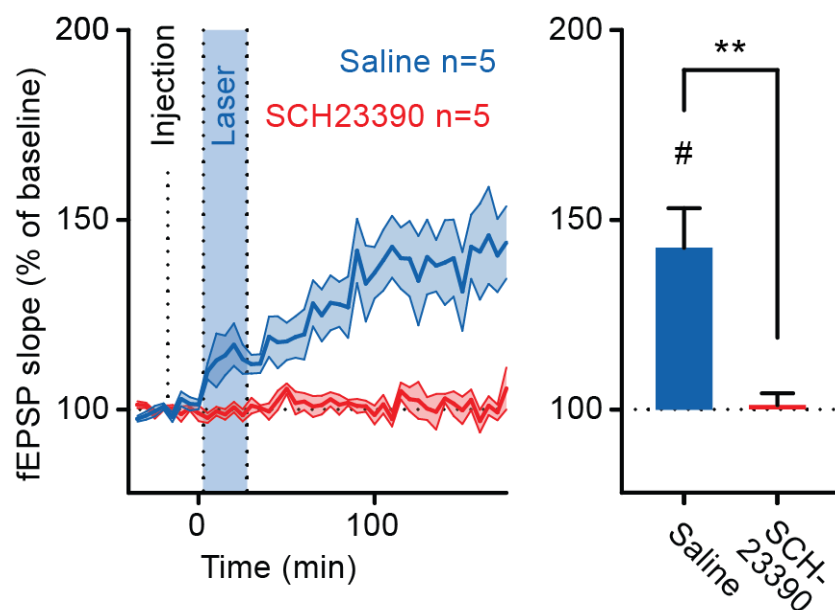
Dopamine transmission requires two families of receptors, D1 and D2. In order to discriminate between the two we used a pharmacological approach using SCH23390, a selective D1 like receptor antagonist.



We did the same monitoring of transmission in mice injected with the ChETA coding vector. After establishing 20 minutes of stable baseline, intraperitoneal injections of SCH23390 (0.05mg/Kg), or saline as control, were administered. The effect of the injection on the transmission was monitored for additional 20 minutes allowing the molecule to take effect. We performed the same light delivery as described as ChETA Paired earlier to both groups. This protocol triggered in the saline injected group the same progressive increase previously observed in ChETA Paired group which stabilizes 90 minute after the end of the pairings ( $+42\pm 10\%$  of baseline  $n=5$ ; Figure 3.6, group saline). However, the increase was completely blocked by SCH23390 ( $+1\pm 3\%$  of baseline  $n=5$ ; Figure 3.6, group SCH23390).

The increase observed for the saline injected group was statistically significant compared to SCH23390 group ( $p<0.01$  Mann Whitney test) and compared to baseline ( $p<0.05$  t-test) (Figure 3.6).

These results show that dopamine triggered plasticity is mediated by the activation of D1 like receptors.



**Figure 3.6 Dopamine-triggered plasticity is mediated by D1 like receptors.**

SCH23390 injected 20 minutes prior to the coupling; EPSP slope increase was no longer observed (red, SCH23390). NaCl 0.9% injections did not affect the effect of the couplings. DA-LTP involves D1/5 receptors (blue, saline). Timelines on the left (dashed line; SCH23390 0.05mg/Kg or NaCl 20 minutes before coupling), mean changes quantified by averaging the last 25 minutes of the recording for each mouse (on the right). \*\*  $p<0.01$  Mann-Whitney, #  $p<0.05$  t-test vs 100%

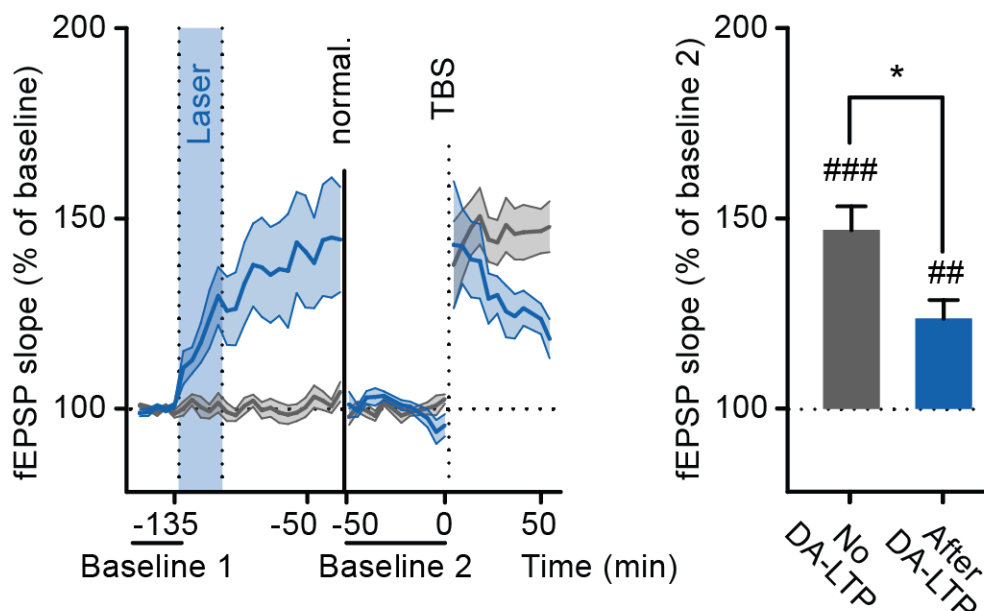
We hypothesized that this increased transmission triggered by dopamine pairing could use the very same mechanisms of the well-described classical LTP triggered by HFS. In order to test

this idea we tested if dopamine induced plasticity can partially occlude LTP triggered using a train of theta bursts stimulation (TBS).

We stimulated seven mice in the same way mice in the group ChETA Paired were stimulated, we named this group “After DA-LTP”. Four mice were stimulated as in the group ChETA Unpaired, and three additional mice were treated like the mice in the group YFP paired. We grouped these last two into one group and named it “No DA-LTP”. The amplitude of fEPSP was re-normalized and 90 minutes after the last light/electric stimulation coupling, a train of TBS was administered. We compared the LTP obtained in response to this last stimulation between After DA-LTP and No DA-LTP groups.

TBS triggered the same initial potentiation for the first fifteen minutes (Figure 3.7). The LTP obtained for the group No DA-LTP remained stable for at least 70 minutes after TBS at around 47% n=7 in average. However, for the After DA-LTP group, the TBS-LTP showed significant decay down to 23% n=7 (Figure 3.7).

These results show that dopamine-triggered plasticity partially occludes TBS induced LTP, suggesting that, although triggered differently, they might share similar mechanisms of expression and maintenance.



**Figure 3.7 Dopamine-triggered plasticity shares maintenance mechanisms with TBS-LTP**

We induced TBS LTP 90 minutes after the end of the couplings in 7 ChETA injected with simultaneous stimulations (Dark Blue, DA-LTP), 4 ChETA injected mice treated with unpaired stimulation of dopaminergic afferents and 3 YFP-injected simultaneously stimulated mice. No difference was observed between the two control groups (data not shown) therefore they were combined into one group (Grey, No DA-LTP). Both groups, DA-LTP and No DA LTP, showed similar TBS induction of LTP. However, LTP in DA-LTP group degraded quickly (47% for No DA-LTP vs 23% DA-LTP). Timelines on the left

(normalization to the mean slope 50 minutes before TBS), mean changes quantified by averaging the last 25 minutes of the recording for each mouse to baseline 2 (on the right).

\*  $p < 0.05$  Mann-Whitney, ##  $p < 0.01$  t-test vs. 0, ###  $p < 0.001$  t-test vs 100.

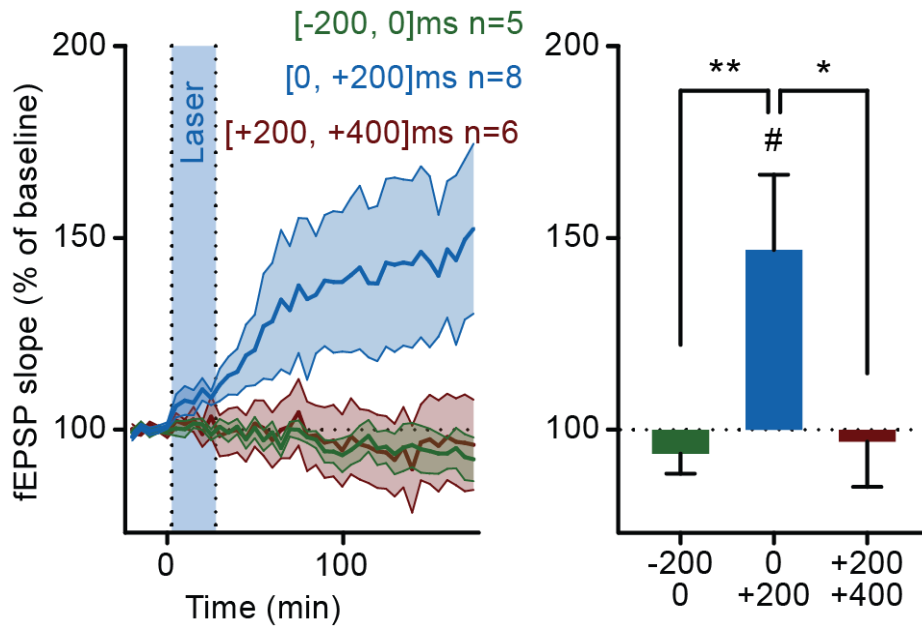
In the above-described protocols, couplings were performed with 400 millisecond-bursts of dopamine spanning from 200 milliseconds before the electrical stimulation of SC to 200 milliseconds after. However, dopamine bursts last for 100-200 milliseconds. Thus, we wanted to study the temporal relationship between dopamine and glutamate in order to determine the window during which this teaching signal must occur in order for this plasticity to take place.

All mice received ChETA expressing vectors. We either delivered bursts of 200 milliseconds of light bursts during the 200 milliseconds before electrical stimulation until the electrical stimulation itself ( $[-200, 0]$ ms, green, Figure 3.8), or just as the electrical stimulation takes place until 200 milliseconds thereafter ( $[0, +200]$ ms, blue, Figure 3.8). A third group received light 200 milliseconds after the electrical stimulation to 400 milliseconds thereafter ( $[+200, +400]$ ms, dark red, Figure 3.8).

Only the  $[0, +200]$ ms protocol triggered a synaptic plasticity ( $+46.8 \pm 20\%$  of baseline  $n=8$ ; Figure 3.8, group  $[0, +200]$ ms) that was similar to what was observed previously with the 400ms ChETA Paired protocol. Stimulating dopamine 200 milliseconds before or 200 milliseconds after electrical stimulation of that of SC did not induce similar effects ( $-6.3 \pm 5\%$  of baseline  $n=5$ , group  $[-200, 0]$ ms and  $-3.2 \pm 11.7\%$   $n=6$ , group  $[+200, +400]$ ms; Figure 3.8).

The increase observed for the  $[0, +200]$ ms group was statistically significant compared to both other groups ( $p < 0.01$  vs  $[-200, 0]$ ms,  $p < 0.05$  vs  $[+200, +400]$ ms Mann-Whitney test) and was statistically significant compared to baseline ( $p < 0.05$  t-test) (Figure 3.8).

The importance of these results stems from showing that dopamine induced plasticity is able to be triggered relying on a pattern of activity consistent with the physiology of dopaminergic neurons (Hyland et al. 2002) and that is specific to directly co-activated glutamatergic afferents which is consistent with Lisman and Grace model of 2005, the hippocampal-VTA loop.



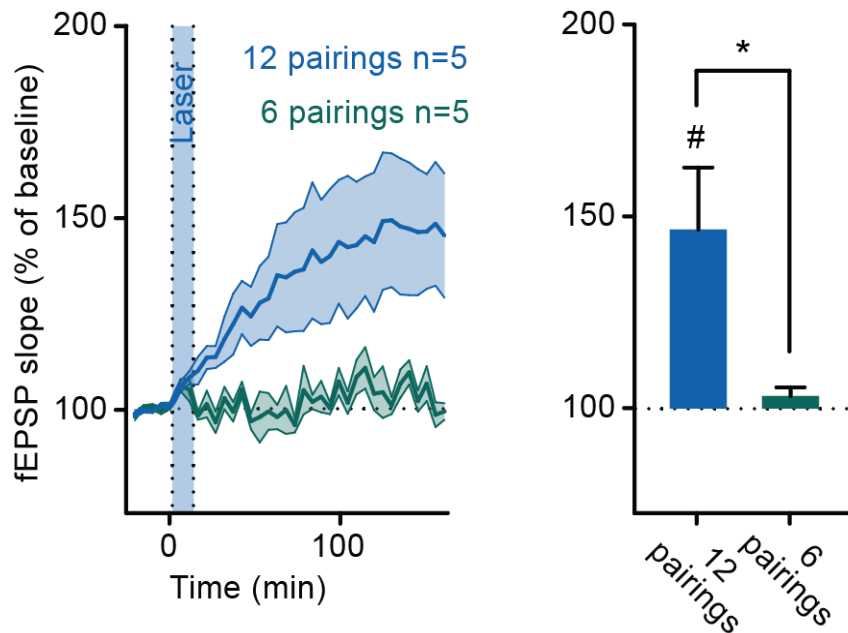
**Figure 3.8 Dopamine need to be released during the 200 ms following glutamate stimulation in order to triggered synaptic plasticity**

We used shorter Laser bursts (200ms) to determine the time window for DA-LTP induction. DA-LTP was induced when photo stimulations were delivered in the time window (0 to 200 ms) in relation to the electrical stimulation of SC (Dark Blue, +46%). No such increase was observed neither when photo stimulations were delivered (-200 to 0 ms) (Green, -6%) nor when photo stimulations were delivered (+200 to +400 ms) (Dark red, -3%) in relation to electrical stimulation of SC.

Timelines are shown on the left, mean changes quantified by averaging the last 25 minutes of the recording for each mouse (on the right). \*  $p < 0.05$  Mann-Whitney, \*\*  $p < 0.01$  Mann-Whitney (after significant Kruskal Wallis), #  $p < 0.05$  t-test vs. 100

Finally, we wanted to explore the minimal number of couplings necessary to trigger this form of plasticity. In our model this plasticity should not necessitate too many couplings for it should explain one shot learning that does not follow too many repetitions, in other words, it should explain everyday memories as described in Morris lab (Takeuchi et al. 2016).

We have tested the effect 12 and 6 dopamine/glutamate couplings on SC transmission. Since only the first 200ms after electrical stimulation of SC seemed necessary for DA-LTP, we used this protocol to try to induce DA-LTP with the minimal number of couplings.



### Figure 3.9 7-12 dopamine/glutamate pairings are necessary for DA-LTP

12 pairings of photo stimulations (0 to 200 ms in relation to SC electrical stimulations) were sufficient to induce DA-LTP, but not 6 pairings. Timelines on the left, mean changes quantified by averaging the last 25 minutes of the recording for each mouse on the right. \*  $p < 0.05$  Mann-Whitney, #  $p < 0.05$  t-test vs. 100

12 pairings were enough to show a significant LTP ( $+46.5 \pm 16\%$  of baseline  $n=5$ ; Figure 3.9) compared to 6 pairings ( $+3.3 \pm 5\%$  of baseline  $n=5$ ) ( $p < 0.05$  vs 6 pairings Mann-Whitney test) and to baseline ( $p < 0.05$  t-test) (Figure 3.9).

## 3.4 Discussion

The main goal of this chapter was to investigate the possibility that dopamine could trigger LTP in the hippocampus *in vivo*; if yes; we wanted to investigate further this plasticity. We have used optogenetics in order to stimulate these neurons specifically and observed that simultaneous activation of the dopaminergic midbrain-hippocampus pathway was sufficient to trigger LTP on co-activated CA1 glutamatergic afferents.

Our interpretation that only co-activated glutamatergic were potentiated is based on the fact that activating glutamate 15 seconds, or even 200ms, before or after optogenetic activation of dopaminergic afferents did not induce LTP.

This effect was shown to pass through D1/5 receptors, that were shown to be necessary in learning (McNamara et al. 2014; Bethus et al. 2010; Broussard et al. 2016) and in long term LTP maintenance (Manahan-Vaughan and Kulla 2003). Moreover, they were shown

responsible of the increase in synaptic transmission over the SC *in vitro* (Rosen et al. 2015) and their activation was able to trigger an increase in glutamatergic transmission when glutamate was stimulated concomitantly *in vitro* (Navakkode et al. 2007).

We have chosen to start with 400ms of dopaminergic activation with 50 couplings, we then further refined the protocol and based it on natural bursts observed to the response of a stimulus, which is about 100-200 milliseconds (Hyland et al. 2002; Dahan et al. 2007; Matsumoto and Hikosaka 2009). We showed that the first 200 milliseconds after glutamate release is the time window necessary for this teaching signal to be released in order for this LTP to occur. Moreover, only 7-12 couplings were sufficient to induce this form of LTP. These results are consistent with the teaching signal model proposed by (Harley 2004). Moreover, it fits quite nicely with the time window proposed by (Lisman and Grace 2005) where the mismatch detection occurring in the CA1 has the time to activate the release of dopamine from the VTA through the hippocampal-VTA loop (Figure 1.6).

We show that DA-LTP shares mechanisms with HFS-induced LTP in a similar way to studies that show that learning induced LTP occludes HFS-triggered LTP (Whitlock et al. 2006; Huber et al. 1995). Notice that dopamine induced LTP took around 90 minutes in anesthetized preparation, a similar time course observed in (Navakkode et al. 2007). Learning induced LTP also develop over time and does not show the high increase HFS-induced LTP show (Trifilieff et al. 2006; Whitlock et al. 2006; Shires et al. 2012)

We interpret these result as dopamine being the trigger of naturally occurring, learning-induced, LTP that develops over a long period. However, dopamine did not trigger early phase of LTP, which could only be an artefact of HFS-induced LTP.

### **3.5 Conclusion**

Dopamine trigger a long lasting LTP on co-activated glutamatergic afferents. This LTP necessitates a dozen of co-activations during the first 200 milliseconds after glutamatergic transmission, occludes HFS-triggered LTP and involve D1/5 receptors.

We name this increase in synaptic transmission: dopamine-induced LTP (DA-LTP).

## **Chapter 4 Role of D1/5 receptors in fear conditioning**

In this chapter, we explored the role of hippocampal dopaminergic receptors in cue fear conditioning and in contextual fear conditioning. The difference between these two tasks is that cue fear conditioning is a simple associative task where the CS is a simple stimulus that does not have to be learnt by itself, while in contextual fear conditioning the CS is the whole context, that by itself needs to be learnt, a process that involves the hippocampus.

### **4.1 Introduction**

#### **4.1.1 Contextual fear conditioning**

Fear conditioning is a behavior test in which animals learn to associate cues with aversive events (Maren 2001). In these tasks, different animals show an ability to associate neutral cues with negative cues. Neutral cues (odors, lights or sounds) are called Conditioned stimuli (CS) for they need to be conditioned to induce a response and do not induce it otherwise. Negative cues (predators, their odors or electric shocks) are called Unconditioned Stimuli (US) for, on the contrary, these can induce an innate response in the animal. After repeated exposure to this combination of stimuli, animals start producing the innate response to the CS alone. For example, honeybees are able to show odor discrimination using this process of associating a neutral odor - CS - with and electric shock –US - by extending their stingers (Laska et al. 1999). Rats show an ability to associate a light and/or a sound cue - CS - with an electric foot shock - US - triggering a fear response characterized as complete lack of movement other than respiratory and cardiac related. Using this task, the amygdala was shown necessary for fear association (Goosens and Maren 2001). These examples show how fear conditioning allow us to study underlying mechanisms and structures of neurobiological memory.

Contextual fear conditioning is an associative memory task that is a little more complex. The animal has to learn a context by assimilating the ensemble of different stimuli such as odors, visual cues and sensory inputs from the whiskers and paws to form one specific context. This representation is then associated with a negative stimulus, such as an electric shock (Daumas et al. 2005), the exposition to a predator (Ribeiro-Barbosa et al. 2005) or to a predator odor (Takahashi et al. 2008). This task is very interesting for it involves the hippocampus in forming this contextual representation, contrary to simple sound-shock association (Daumas et al. 2005). Therefore, it allows the study of mechanisms underlying hippocampal memory formation in a

task that takes one-shot to be learned. This later feature is useful since, as we know the exact time the acquisition occurs, we can directly interfere during this period.

#### **4.1.2 Role of D1/5 receptors in fear conditioning**

Dopamine transmission in the hippocampus is required for learning and memory. Indeed, D1/5 receptors antagonists infusion in the hippocampus prevents long term memory in the Morris Water maze in rats (da Silva et al. 2012), learning a new pair of odor/location in the Event Arena in rats (Bethus et al. 2010) and inhibitory avoidance in mice (Broussard et al. 2016).

This effect is also found in contextual and cued fear conditioning. Systemic D1/5 receptors inhibition blocks cue fear conditioning (Inoue et al. 2000) and contextual fear conditioning (Heath et al. 2015). Hippocampal dopamine receptors were shown to be necessary for contextual fear conditioning in rats (Heath et al. 2015).

Although a very recent article showed that D1/5 receptors antagonist infusion in the hippocampus prevents contextual fear conditioning in mice (Tsetsenis et al. 2021), these data were not available at the time when we planned and performed the experiments described in this chapter.

### **4.2 Aim and hypothesis**

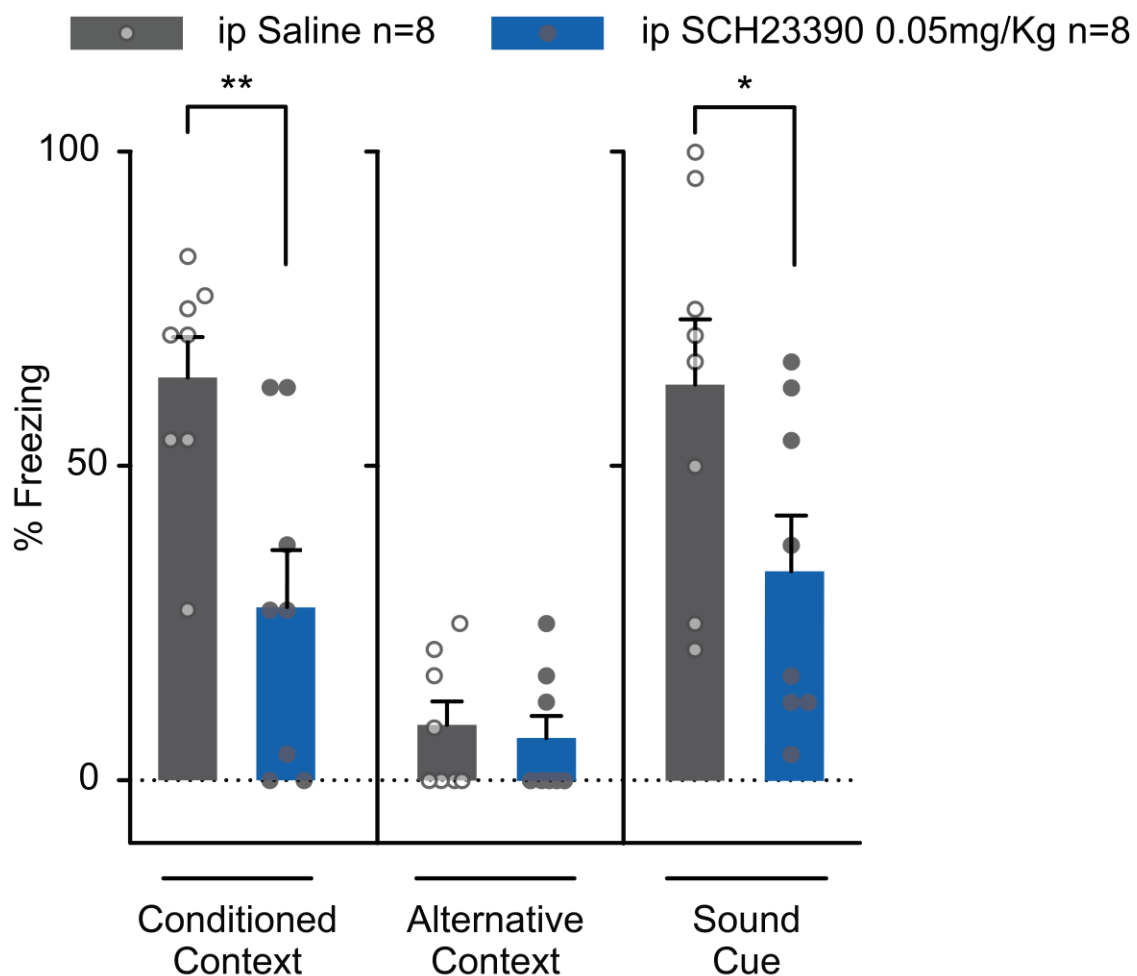
In this work, we wanted to study the role D1/5 receptors in the hippocampus on both contextual fear conditioning and cue fear conditioning. Considering our hypothesis of hippocampal dopamine as a teaching signal and available literature, intra-hippocampal D1/5 receptors antagonists should block contextual fear conditioning but spare cue fear conditioning, while systemic injection should block both.

This experiment was designed as a preliminary study, using the behavioral test well mastered in our lab. Which will help us establish the experiments presented in the next chapter, which directly test our main hypothesis in a variation of the contextual fear conditioning not already set in the lab.



### 4.3 Results

In experiment 1, mice (female, C57Bl/6J) received an intraperitoneal injection of either SCH23390 (0.05 mg/Kg) or saline (0.9% NaCl) 15 minutes before the conditioning session. Freezing in the conditioned context dropped from  $64 \pm 6\%$   $n=8$  for the saline group to  $27 \pm 9\%$   $n=8$  for the SCH23390 mice (t-test:  $p < 0.01$ ) (Figure 4.1). Similarly, freezing dropped in response to the cue from  $63 \pm 10\%$  to  $33 \pm 9\%$  (t-test:  $p < 0.05$ ) (Figure 4.1) ( $n=8$  per group). Freezing behavior in the alternative context was lower than 15% and comparable between both experimental groups (t-test:  $p=0.67$ ), indicating that freezing behavior was specific to acquired memories and that the dopamine D1/5 receptors antagonist only affected memory driven freezing.

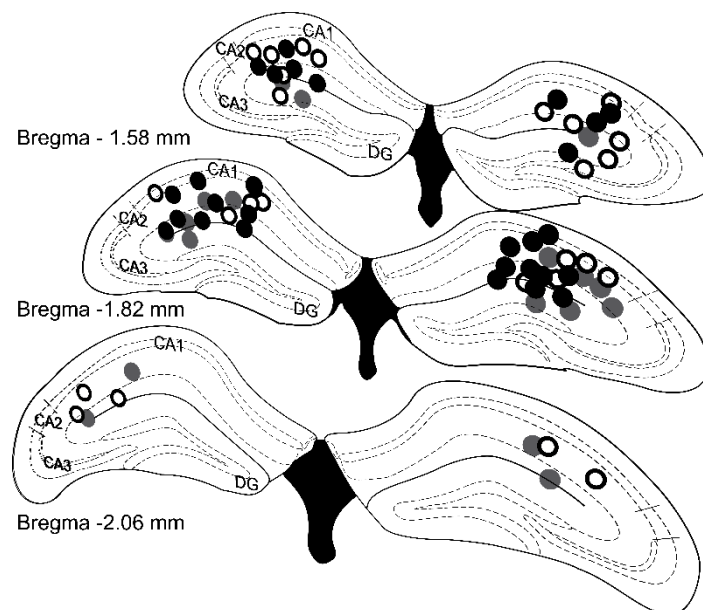


**Figure 4.1 Intraperitoneal injection of D1/5 receptors antagonist 15 minutes before conditioning prevents both cue-fear conditioning and contextual fear conditioning**

24 hours after training, freezing to the context is lower for mice treated with intraperitoneal injection of SCH23390 15 minutes before training (blue bar, black disks) compared to those injected with saline (grey bar, white disks). Both groups show comparable low levels of freezing behavior in the alternative

context. Cue fear memory is also reduced by SCH23390 intraperitoneal injection. (\*)  $p < 0.05$  t test, (\*\*)  $p < 0.01$  t-test.

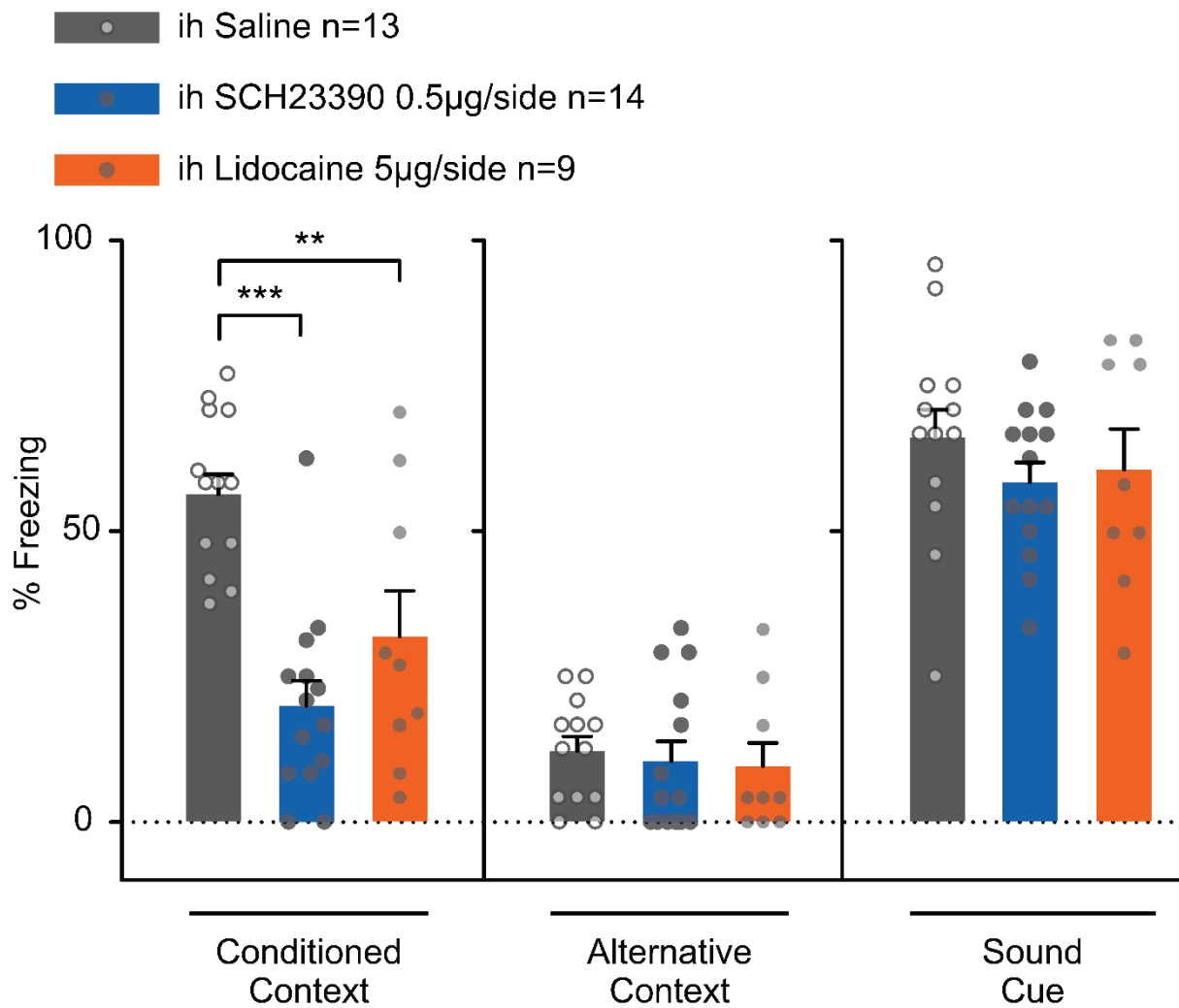
In experiment 2, we used the same fear conditioning protocol, but this time SCH23390 was delivered through intra-hippocampal infusion ( $0.5\mu\text{g}/\text{side}$ ,  $0.35\mu\text{l}/\text{side}$ ); the control group received infusions of the same volume of saline, a third group was injected with lidocaine ( $5\mu\text{g}/\text{side}$ ) as a positive control. Marking infusion sites showed that all infusions were all correctly centered in the hippocampus (Figure 4.2).



**Figure 4.2 Infusion site markings with Chicago Blue after behavior sessions**

These markings show that all 13 mice injected with saline (white disks), 14 mice injected with SCH23390 (black disks) and 9 mice injected with Lidocaine (grey disks) had their injections correctly centered in the hippocampus, with most targeting the CA1.

Freezing in the conditioned context dropped from  $57\pm 4\%$   $n=13$  for the saline group to  $20\pm 4\%$   $n=14$  for the SCH23390 group and  $31\pm 8\%$   $n=9$  for the lidocaine group (Dunnett's multiple comparisons  $p < 0.001$  NaCl Vs. SCH23390  $p < 0.01$  NaCl Vs. Lidocaine after ANOVA  $p < 0.001$ ) (Figure 4.3). Conversely, freezing in response to the auditory cue was preserved at  $66\pm 5\%$ ,  $58\pm 3\%$  and  $62\pm 7\%$  for saline, SCH23390 and lidocaine infusions, respectively. This difference was not significant (ANOVA  $p > 0.4$ ) (Figure 4.3). Freezing behavior in the alternative context was lower than 15% and comparable between both experimental groups (ANOVA  $p > 0.6$ ), showing that freezing behavior was specific to acquired memories and that SCH23390 and lidocaine only affected memory driven freezing.



**Figure 4.3 local infusion of D1/5 receptors antagonist or lidocaine into the hippocampus hinders contextual fear memory but not cue driven fear memory.**

24 hours after training, freezing to the context is lower for mice treated with intra-hippocampal infusion of SCH23390 (blue bar, black disks) or of lidocaine (orange bars, grey disks) 10 minutes before training, compared to those injected with saline (grey bar, white disks). All groups show comparable low levels of freezing behavior in the alternative context. Cue fear memory is preserved even after SCH23390 infusion or lidocaine infusion. (\*\*)  $p < 0.01$ , (\*\*\*)  $p < 0.001$  Dunnett's test following one-way ANOVA.

## 4.4 Discussion

Here we confirm that hippocampal D1/5 receptors are required for encoding contextual fear conditioning in mice, replicating previous data obtained in rats (Heath et al. 2015) and very recent data in mice (Tsetsenis et al. 2021). The effect we observed with intra hippocampal infusions of SCH23390 was comparable to that obtained with lidocaine with our results and formerly reported results (Daumas et al. 2005). This suggest a central role for hippocampal D1/5 in this task.

With cue fear conditioning, we replicate data already obtained with rats (Inoue et al. 2000), showing that SCH23390 intraperitoneal injections block cue fear conditioning. One novel observation that we demonstrated is that hippocampal D1/5 receptors are not necessary for cue induced fear conditioning. This effect seems to be specific to the amygdala (Guarraci et al. 1999).

An interesting result found both in (Heath et al. 2015) and (Inoue et al. 2000) is that administering SCH23390, whether through infusions or injections, after conditioning sessions did not affect learning. Since pharmacological manipulations induce long lasting effects, this observation confirms the role of D1/5 in learning the task and not only in the consolidation of learning.

Recent reports suggest that dopamine in the hippocampus comes from the Locus Coeruleus and not the midbrain (Takeuchi et al. 2016; Kempadoo et al. 2016), pharmacological approaches cannot help us learn more about the debate concerning the source of dopamine necessary for hippocampal learning. Moreover, during contextual fear conditioning the animal learns the context and learns to associate it with the shock during the same session, therefore, we cannot know whether dopamine in the hippocampus is necessary for value and associative learning or learning the context itself.

Both these issues will be addressed in the next chapter, by using optogenetic stimulation or inhibition of midbrain dopamine neurons specifically during contextual learning in contextual pre-exposure facilitation effect paradigm.

## 4.5 Conclusion

Consistent with previous data, we show that D1/5 receptors in the dorsal hippocampus are essential for contextual fear conditioning, whereas D1/5 receptors located outside the hippocampus, most likely in the amygdala, are required for cue fear conditioning.

## **Chapter 5 Midbrain Dopamine controls context learning**

In this chapter, we explore the role of midbrain-dopamine to hippocampus pathway specifically in contextual learning, regardless of any value information. In order to do that, we use contextual pre-exposure facilitation effect, a variation of contextual fear conditioning.

We used both pharmacological and optogenetic approaches to explore this question.

### **5.1 Introduction: VTA dopamine activity during novelty and exploration**

As proposed in the general introduction, animals encounter a great deal of sensory inputs that cannot all be stored in memory systems; else, these systems would be saturated. Therefore, we put forward the hypothesis that only a small relevant portion are needed to be stored, and there must be a teaching signal in order to trigger memory storage mechanisms.

In Chapter 3, we showed that dopamine triggers LTP in the hippocampus, a form of neural plasticity necessary for hippocampal-dependent memories (Whitlock et al. 2006; Broussard et al. 2016; Tsetsenis et al. 2021). In Chapter 4, we showed that dopamine receptors in the hippocampus are necessary for contextual fear conditioning, a hippocampal-dependent task as opposed to cue fear conditioning, a hippocampal-independent memory task.

Dopamine neurons are activated by either reward, aversive events or novelty. In contextual fear conditioning, mice learn the context at the same time they learn its association with the electric shock. Therefore, the requirement for hippocampal dopamine transmission for contextual fear conditioning acquisition could mean two things: either it demonstrates that dopamine provide a signal indicating that value of the US to the hippocampus or it provides a teaching signal indicating that something new or unpredicted occurred, as proposed by the hippocampus-VTA-loop model. This is the first issue we address with the next series of experiments.

The second issue we addressed was the source of dopamine in the hippocampus that is implicated in plasticity and learning due to the fact that the activity of TH neurons in both the midbrain and the LC can trigger dopamine facilitated learning and plasticity (Takeuchi et al. 2016; Tsetsenis et al. 2021; Broussard et al. 2016).

We try to palliate these issues using a slightly different variation of this task called contextual pre-exposure facilitation effect (CPFE). In this test, the animal learns the context on the first

day while its association to the electric shock takes place on the second day, during a very brief training session (5 to 10 seconds). The test for contextual fear memory takes place on the third day.

If the animal receives only the immediate shock in the context without having enough time to learn it, the animal does not freeze to the context completely when tested the next day. However, if the animal is pre-exposed to the context a day before conditioning, in order to learn it, animals show complete freezing on the third test day.

This paradigm was proposed in the early 2000s (Barrientos et al. 2002; Fanselow 2000) and allowed to show that the context in contextual fear conditioning is not a traditional CS but a very complex one, necessitating the assimilation of many subtle stimuli (visual cues, olfactory cues and somatosensory ones). The set of features must be integrated into a contextual representation also called “*Gestalt*” (Fanselow 2000; Brown et al. 2011) and this cognitive process takes time because it requires exploration and assimilation of these features.

Using this paradigm, CA1 pyramidal cells were shown to be solicited in both phases of this learning; c-Fos marked cells in the CA1 increase after pre-exposure to the novel context and after training (Murawski et al. 2012) which shows that pyramidal cell activity is involved during both sessions.

In unpublished work from the lab, J. Remaud showed that simply the exposition to the context used in contextual fear conditioning for 7 minutes even without shock triggers LTP in CA1. Results that are disputed by recent published reports (Subramaniyan et al. 2021). Thus, we propose that dopaminergic transmission from the midbrain to the hippocampus, which arises from novelty, triggers learning new contexts.

## 5.2 Hypothesis

We evaluated the role of midbrain dopamine to hippocampus pathway has in latent learning using CPFE. We first evaluate whether pre-exposure facilitates learning or causes latent inhibition in contextual fear conditioning in C57Bl/6J using different pre-exposure durations. We then test whether these results can be replicated in DAT::Cre mice.

We then test two hypotheses:

- Using pharmacological inhibition, we tested the implication of D1/5 receptors in learning a new context and/or associating it with the US separately.
- Using optogenetic manipulation, we tested the implication of midbrain dopamine to hippocampus pathway in controlling contextual learning by stimulating these axons during a short pre-exposure, and by inhibiting them during a long one.



## 5.3 Results

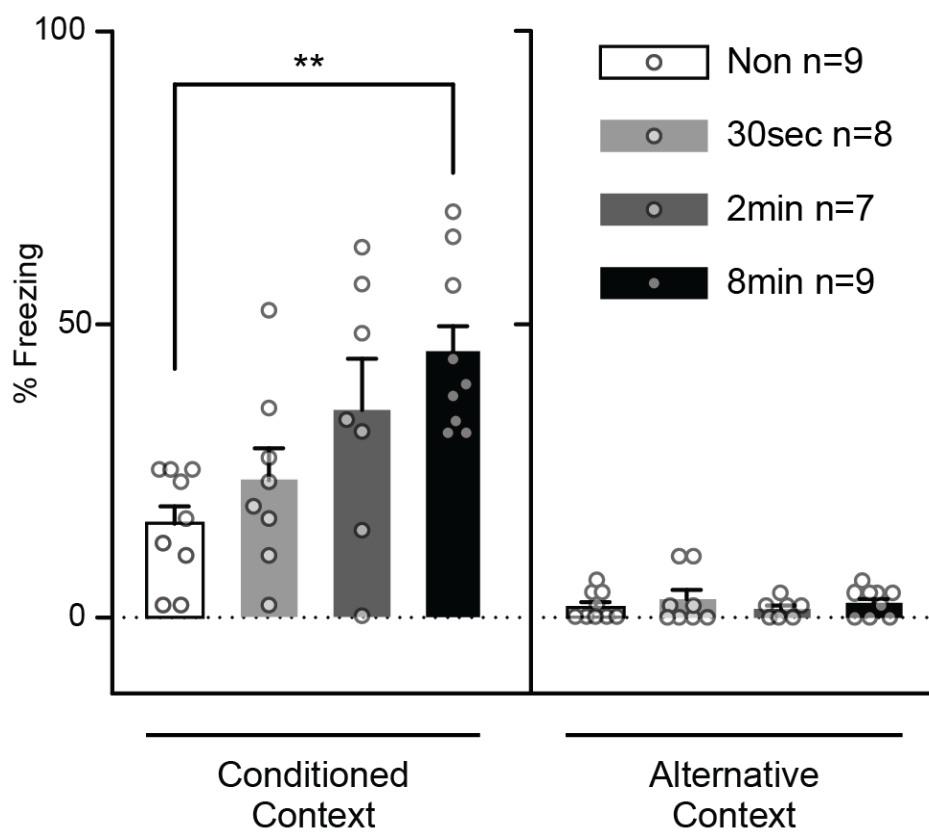
### 5.3.1 CPFE validation and dopaminergic pharmacology thereof

First we needed to validate the CPFE protocol in our lab, to do so we performed this modified version of contextual fear conditioning on four groups of mice, on day 1, mice were allowed to explore the context during 30 seconds, 2 minutes or 8 minutes. We used as control a non-pre-exposed group that explored an empty home cage for 8 minutes. On day 2, all animals received a very brief conditioning session (<10 seconds) during which they received one electric shock (0.7 mA, 2s) in the conditioning context, without any sound cue. Tests took place on day 3. Mice were placed 4 minutes in the context associated with the shock in order to test contextual memory. Ninety minutes later, they were placed during 4 minutes in the alternative context in order to test for generalized fear (for details please visit 2.4 Contextual Pre-exposure Facilitation Effect).

As expected, the non-pre-exposed animals exhibited very limited freezing behavior to the associated context ( $15\pm 3\%$   $n=9$ ). However, freezing behavior gradually increased with longer pre-exposure time; indeed, mice froze for  $23\pm 5\%$   $n=8$ ,  $35\pm 9\%$   $n=7$  and  $45\pm 5\%$   $n=9$  of the time in the conditioned context when pre-exposed for 30 seconds, 2 minutes or 8 minutes, respectively (One-way ANOVA  $p<0.01$ ). The group pre-exposed for 8 minutes was the only one to freeze significantly more than the control group (Dunnett's multiple comparison for non-pre-exposed vs. 30 seconds:  $p= 0.68$ ; vs. 2 minutes:  $p= 0.15$ ; vs. 8 minutes:  $p<0.01$ ) (Figure 5.1). Freezing behavior in the alternative context remained below 10% for all groups, showing that freezing was related to a specific memory of the context, even for the non-pre-exposed group. Altogether, we validate the CPFE experiment and identify that mice need 2 to 8 minutes to encode a new context without any aversive or appetitive stimuli.

We show that pre-exposure to the conditioning context induces more freezing, that we interpret, according to (Fanselow 2000; Barrientos et al. 2002), as mice were able to latently learn the context that they were able to associate it during the second day with the electric shock.

## C57Bl/6J Female



**Figure 5.1 Pre-exposure facilitate contextual learning in C57Bl/6J mice**

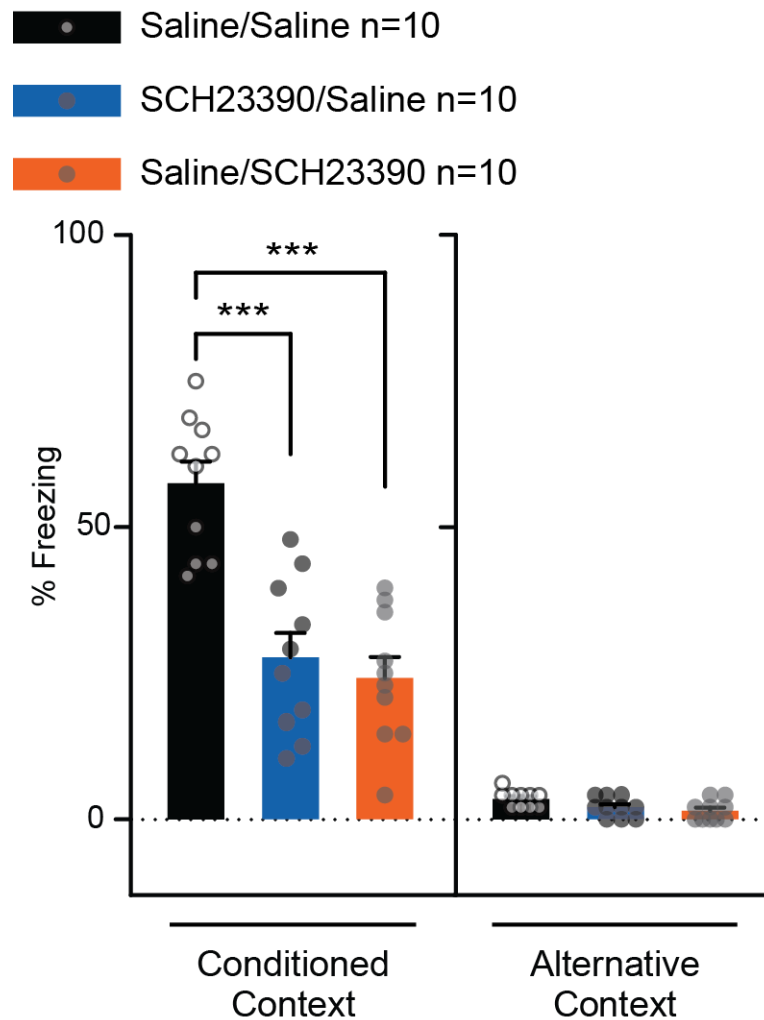
C57Bl/6J (female) mice increase their freezing behavior in the conditioned context with increased pre-exposure time. Mice pre-exposed for 8 minutes (black bar, white disks) freeze significantly more than non-pre-exposed mice.

(\*\*)  $p < 0.01$  Dunnett's test following one-way ANOVA ( $p < 0.01$ )

In the next experiment, mice underwent the CPFE experiment with a pre-exposure lasting 8 minutes and received an intraperitoneal injection of either SCH23390 (0.05mg/Kg) or saline 15 minutes prior to day 1 or day 2 (3 groups; Saline/Saline, SCH23390/Saline or Saline/SCH23390). Mice injected with SCH23390, before either day 1 or day 2, froze significantly less than mice injected with saline both days (Dunnett's multiple comparison after one-way ANOVA,  $p < 0.001$  Saline/Saline  $57.5 \pm 4\%$   $n = 10$  vs. SCH23390/Saline  $27.7 \pm 4\%$   $n = 10$  and vs. Saline/SCH23390  $24.2 \pm 4\%$   $n = 10$ ). In this case, also, freezing in the alternative context remained below 10%, showing that freezing was elicited by contextual memory.

These results do not suggest any localization of the effect of the inhibitor. However, based on the effects of local SCH23390 infusions presented in Chapter 4, we hypothesize that contextual

learning taking place in the hippocampus implicate hippocampal D1/5 receptors and associative learning takes place elsewhere, probably in the amygdala (Heath et al. 2015).



**Figure 5.2 D1/5 receptors involved separately in contextual learning and in associative learning**

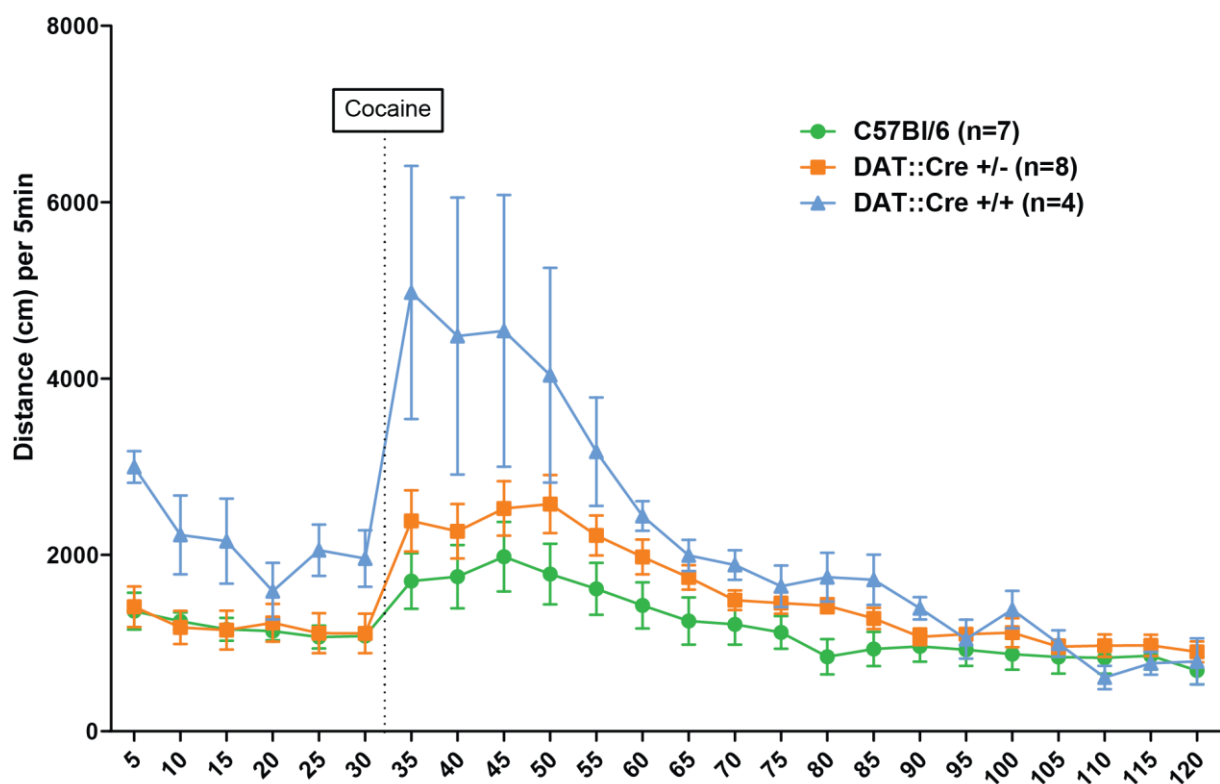
Decreased freezing behavior in the conditioned context after SCH23390 (0.05mg/kg) injection either before pre-exposure or before conditioning.

(\*\*\*)  $p < 0.001$  Dunnett's test following one-way ANOVA ( $p < 0.001$ )

To investigate further the role of dopamine specifically in contextual learning, we chose to go optogenetics. This technic allows us to explore not only the role of the dopaminergic pathway as a teaching signal, but also allows the verification of the source of this dopamine, VTA or LC.

### 5.3.2 Characterization of DAT::Cre behavior in novelty and CPFE

DAT::Cre mice have an accentuated response to startling stimuli (subjective observation) suggesting an enhanced dopaminergic transmission. In order to test this hypothesis, we measured their locomotion in a novel open field and in response to cocaine injection. Both were shown to be correlated to dopamine transmission (Lovinger 2010). Homozygote DAT::Cre mice show increased baseline locomotion in the open field and an increased locomotor response to cocaine injections therein as compared to C57Bl/6 mice. Heterozygotes, used in optogenetics experiments, do not show any baseline hyperactivity but a tendency toward an increased locomotion in response to cocaine injections (Figure 5.3).

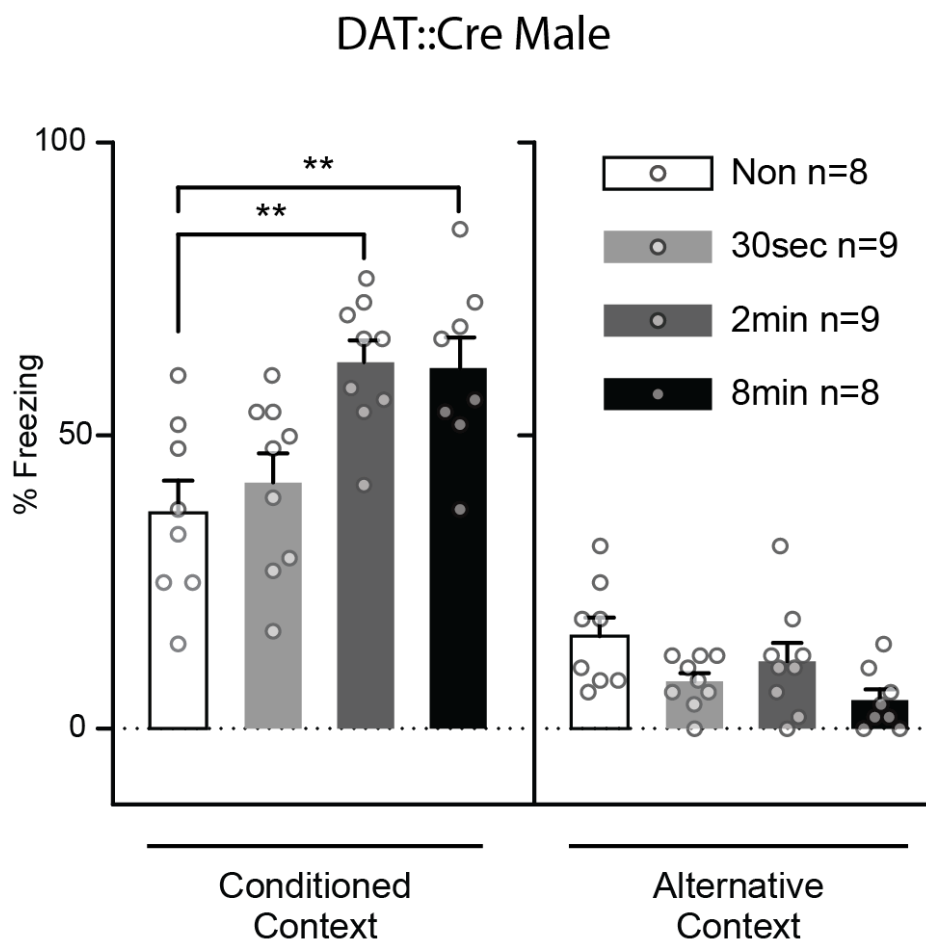


**Figure 5.3 Hyperactivity in homozygotes DAT::Cre mice**

We measure locomotion each 5 minutes in an open field for 30 minutes before and an hour and a half after cocaine injections in wild type, DAT::Cre heterozygote and homozygote. Homozygote DAT::Cre mice show increased locomotion in novel open field and in response to cocaine.

These results suggest that our DAT::Cre heterozygote mice, that we will use for optogenetics experiments, might have a slightly increased dopamine transmission that could modify their performances in the CPFE. We thus decided to calibrate our CPFE protocol by testing male heterozygote DAT::Cre mice pre-exposed for different time on the first day of the protocole, just like we previously did with female C57Bl/6J mice.

We observed two main differences. First, male DAT::Cre mice had higher freezing time than female C57Bl/6J mice whatever the experimental condition. This difference was observed in the conditioned context and also in the alternative context (up to 16% for non-pre-exposed mice). The second difference was that, contrary female C57Bl/6J mice, male DAT::Cre mice do not show a progressive increase of their freezing time according to the pre-exposure time. A pre-exposure of 30 seconds had barely any effect compared to the control non pre exposed group ( $42.1 \pm 5\%$   $n=8$  vs  $37 \pm 5\%$   $n=9$ ), while 2 minutes of pre-exposure were sufficient to trigger a significant increase reaching levels comparable to those seen in the group pre-exposed for 8 minutes ( $62.7 \pm 4\%$   $n=9$  and  $61.7 \pm 5\%$   $n=8$ , respectively) (One-way ANOVA  $p < 0.001$ ; Multiple comparisons  $p = 0.87$  Non vs. 30sec,  $p = 0.9$  2min vs. 8min,  $p < 0.01$  Non vs. 2min and vs. 8min,  $p < 0.05$  30sec vs. 2min and vs. 8min).



**Figure 5.4 CPFE in DAT::Cre mice**

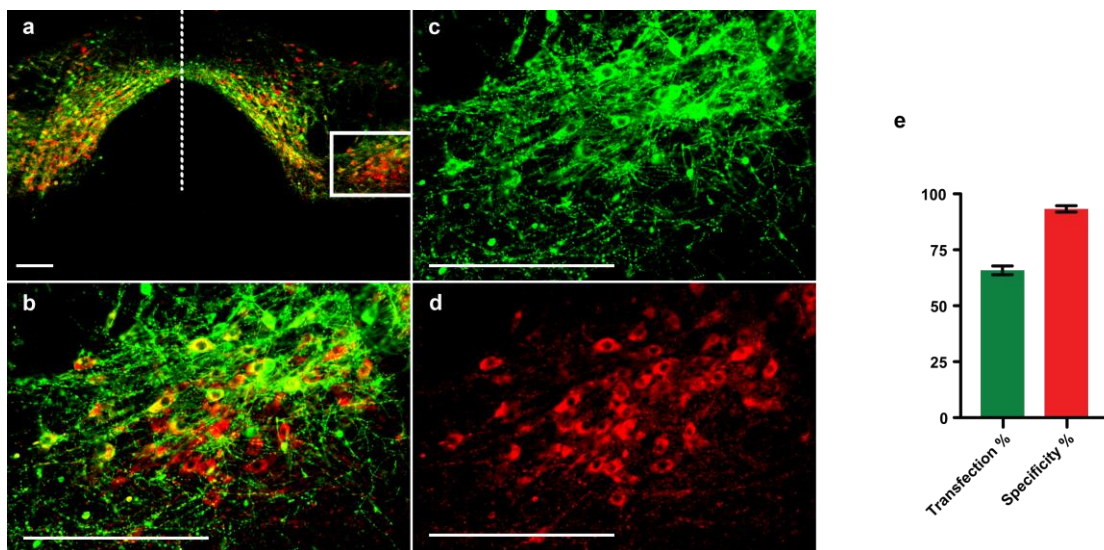
Male DAT::Cre mice show clear facilitation effect after 2 minutes of pre-exposure comparable to 8 minutes of pre-exposure. Male DAT::Cre mice show higher levels of generalized freezing compared to female C57Bl/6J.

(\*\*)  $p < 0.01$  Multiple comparisons following one-way ANOVA ( $p < 0.001$ )

### 5.3.3 Transfection profiles for control, ChETA and eNpHR3.0 vectors with bilateral injections

In order to investigate directly the role of midbrain dopamine transmission in the hippocampus during contextual learning, we bilaterally injected DAT::Cre mice with either control vectors, coding for double floxed YFP, or vectors Coding for double floxed ChETA, YFP or vectors coding for double floxed eNpHR3.0, YFP. These mice were also bilaterally implanted with optic fibers directed towards the CA1 of the hippocampus (for details please visit 2.1.5 Vector injection and Optic fiber implantation).

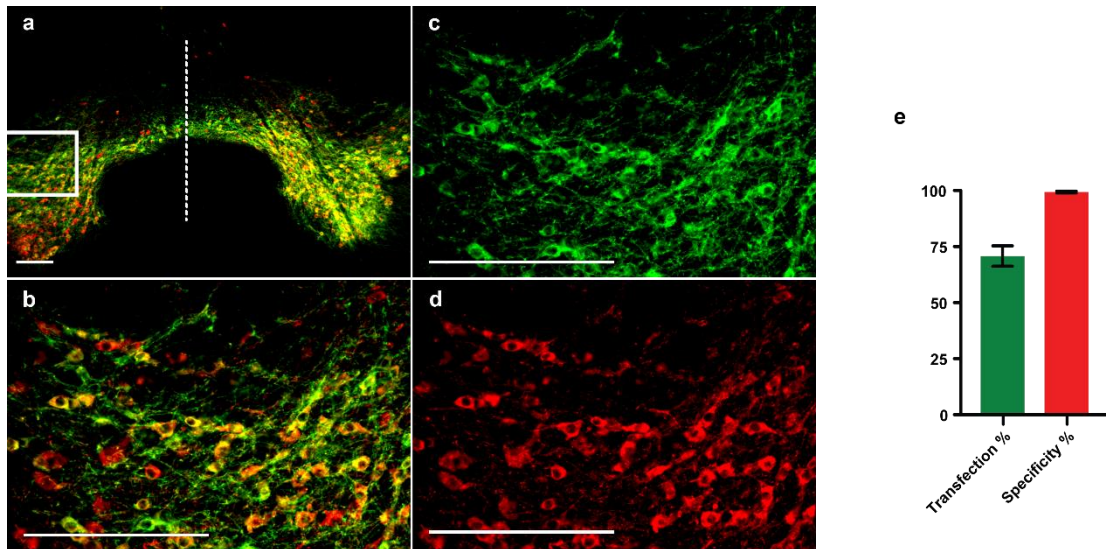
The same transfection verification was also performed on cerebral tissue after behavioral studies (for details please visit 3.3.1 Satisfactory transfection profiles for both vectors). ChETA, YFP coding vectors showed  $66\pm 2\%$  transfection% and  $93.3\pm 1.4\%$  specificity% (Figure 5.5) and eNpHR3.0, YFP coding vectors showed  $70.1\pm 4.6\%$  transfection% and  $99.4\pm 0.4\%$  specificity% (Figure 5.6). Finally, YFP only coding vectors showed  $84\pm 1.9\%$  transfection% and  $94.7\pm 1\%$  specificity% (Figure 5.7). These quantifications show satisfactory pattern of transfection of the midbrain dopamine cells and excellent specificity with all three vectors.



**Figure 5.5 Satisfactory bilateral expression of YFP in TH+ expressing cells of the VTA with ChETA, YFP coding vector.**

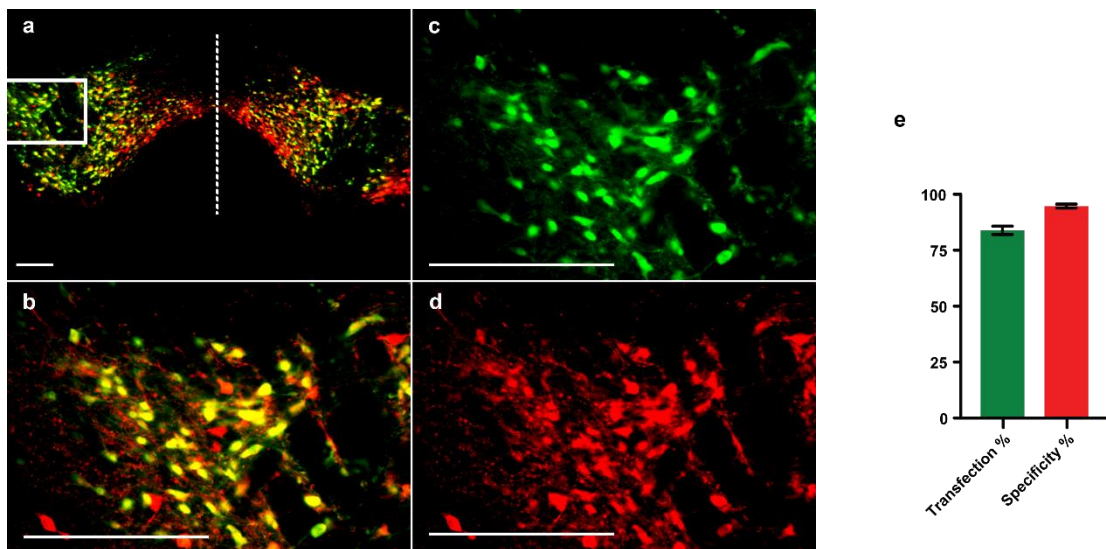
**a** Shows TH+ cells (in red) in the right VTA (x5 zoom) transfected, expressing YFP (in green), yellow shows merge. Rectangle represents the sampled area for analysis (at x20 zoom). Dashed line represents the midline. **b** Merge in the sampled rectangle captured at x20 (**c** and **d** show green and red color channels for YFP and TH, respectively). White horizontal bars represents  $100\mu\text{m}$  **e** the quantification of cells show transfection%= $66\pm 2\%$  (green) and specificity%= $93.3\pm 1.4\%$  (red).





**Figure 5.6 Satisfactory bilateral expression of YFP in TH+ expressing cells of the VTA with eNpHR3.0, YFP coding vector.**

**a** Shows TH+ cells (in red) in the right VTA (x5 zoom) transfected, expressing YFP (in green), yellow shows merge. Rectangle represents the sampled area for analysis (at x20 zoom). Dashed line represents the midline. **b** Merge in the sampled rectangle captured at x20 (**c** and **d** show green and red color channels for YFP and TH, respectively). White horizontal bars represents 100µm **e** the quantification of cells show transfection%=70.1±4.6% (green) and specificity%=99.4±0.4% (red).



**Figure 5.7 Satisfactory bilateral expression of YFP in TH+ expressing cells of the VTA with YFP only coding vector.**

**a** Shows TH+ cells (in red) in the right VTA (x5 zoom) transfected, expressing YFP (in green), yellow shows merge. Rectangle represents the sampled area for analysis (at x20 zoom). Dashed line represents the midline. **b** Merge in the sampled rectangle captured at x20 (**c** and **d** show green and red color channels for YFP and TH, respectively). White horizontal bars represents 100µm **e** the quantification of cells show transfection%=84±1.9% (green) and specificity%=94.7±1% (red).

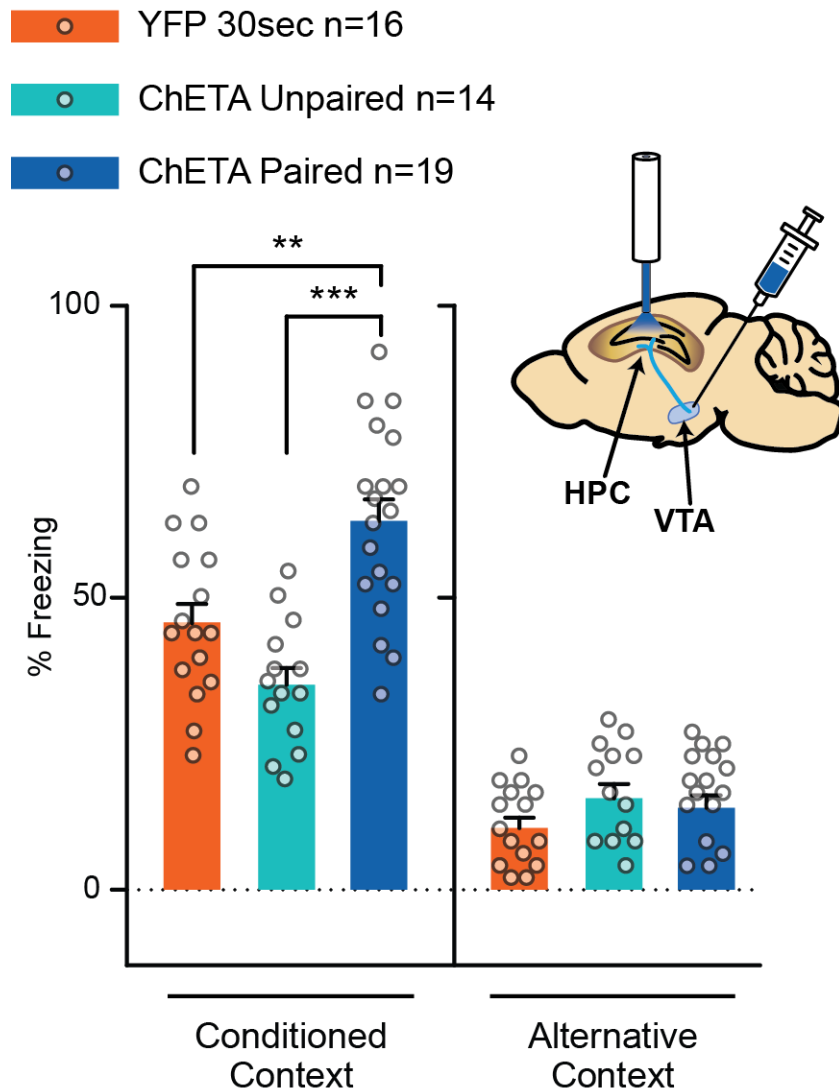
### 5.3.4 Optogenetic manipulation of VTA dopamine to hippocampus in contextual learning

In the first experiment, we pre-exposed three groups of mice the context for 30 seconds. The first group (YFP 30sec) was injected during surgery with the control vector and received blue light during pre-exposure, the second (ChETA Unpaired) was injected with ChETA, YFP coding vector and received blue light the day before pre-exposure (in an empty home cage). The third group (ChETA Paired) was injected with ChETA, YFP coding vector and blue light during pre-exposure (for details please visit 2.4.2 Procedure to test pathway implication using optogenetics).

The freezing levels observed during the test on day 3 were similar for the YFP 30sec ( $45.6 \pm 3.3\%$   $n=16$ ) and the ChETA Unpaired mice ( $35 \pm 2.8\%$   $n=14$ ) and increased in the ChETA Paired mice ( $62.8 \pm 3.7\%$   $n=19$ ) (One-way ANOVA  $p < 0.001$ ; Multiple comparisons YFP 30sec vs. ChETA Unpaired  $p=0.11$ , YFP 30sec vs ChETA Paired  $p < 0.01$ , ChETA Unpaired vs ChETA Paired  $p < 0.001$ ) (Figure 5.8).

Freezing in the alternative context was considerably lower than in the conditioned context, and was not significantly changed due to dopaminergic activation during pre-exposure (Figure 5.8). These results show that dopamine activation during pre-exposure facilitates contextual learning.





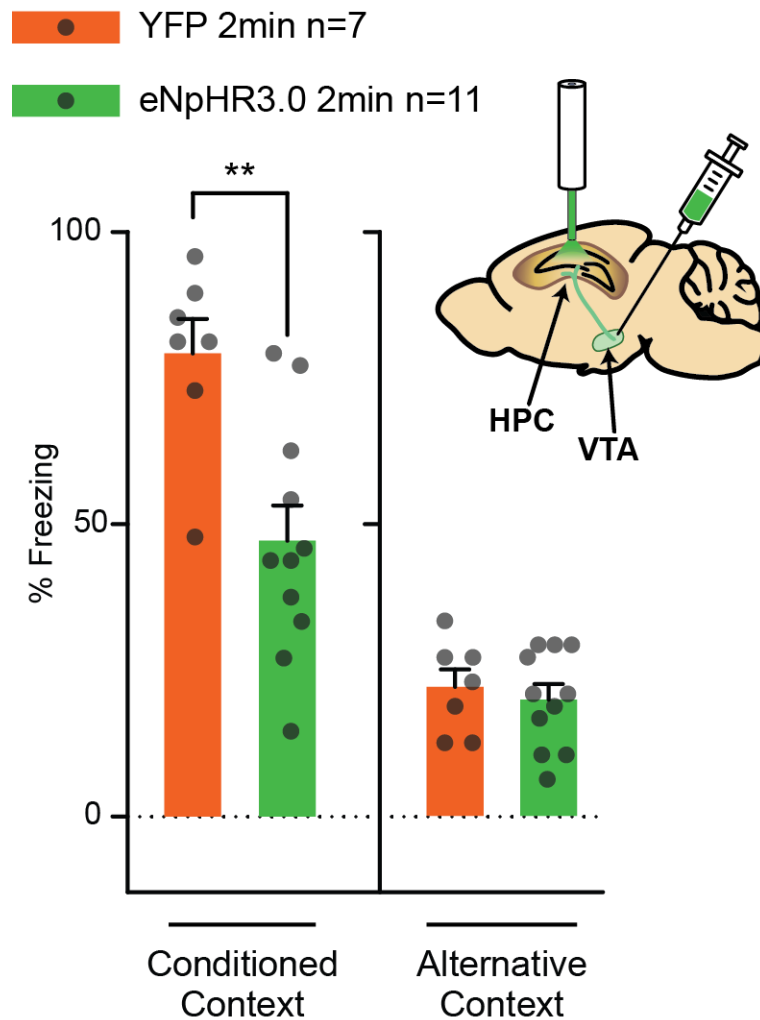
**Figure 5.8 Stimulation of VTA dopamine axons in the hippocampus promotes context learning**  
 Increased freezing behavior in the conditioned context after the activation of dopamine axons in the hippocampus specifically during pre-exposure.  
 (\*\*)  $p < 0.01$ , (\*\*\*)  $p < 0.001$  multiple comparisons test following One-way ANOVA ( $p < 0.001$ )

In the second experiment, two groups of mice, one injected with a vector coding for eNpHR3.0,Y7FP (eNpHR3.0 2min) and the other group injected with coding for YFP only (YFP 2min). Both groups were pre-exposed for 2 minutes to the conditioning context, receiving green laser directed at the CA1. eNpHR3.0 is a halorhodopsin that responds to green light inducing an active influx of  $Cl^-$  ions into dopaminergic cells causing their inhibition.

In the conditioned context, eNpHR3.0 2min mice exhibited significant lower freezing level than the control YFP 2min mice ( $47.2 \pm 6\%$   $n=11$  vs.  $79.2 \pm 5.9\%$   $n=7$ ;  $p < 0.01$  t-test). Although freezing in the alternative context was slightly higher in both groups than in previous

experiments, it was still considerably lower than freezing in the conditioned context ( $22\pm 3\%$  and  $19.9\pm 2.5\%$  for YFP 2min and eNpHR3.0 2min, respectively).

These results show the need of a fully functional VTA dopamine pathway to the hippocampus in order to form a full representation of a novel context so that mice would be able to associate it the next day with an electric shock.



**Figure 5.9 inhibition of VTA dopamine axons in the hippocampus hinders context learning**  
Decreased freezing behavior in the conditioned context after the inhibition of dopamine axons in the hippocampus specifically during pre-exposure.  
(\*\*)  $p < 0.01$  t-test.

## 5.4 Discussion

### 5.4.1 C57Bl/6J females vs DAT::Cre males

If we look closely to the results in Figure 5.1 and Figure 5.4, these experiments were performed with the same protocol. However, we can observe two main differences in the results obtained.

The first main difference is the fact that, in general, male DAT::Cre mice froze more than female C57Bl/6J. Whether in the conditioned context (37%, 42.1%, 62.7% and 61.7% vs 15%, 23%, 35% and 45%) or in the alternative context (15.9%, 8,1%, 11,6% and 5% vs 1,9% 3,1% 1,5% and 2,8%). This observation may be due to having done these experiments on different periods. However, each experiment was performed in two batches processed on different weeks and results were always comparable between batches. In the literature, lower freezing percentages were observed in female mice in fear conditioning (Markus and Zecevic 1997) which is one of the more noticeable variations between the two experiments. The second would be the genotype and the associated hyperdopaminergic phenotype revealed by the increased locomotion and reactivity to cocaine (Figure 5.3). Noteworthy, DAT::Cre males were aggressive and more likely to bite the experimenter (no quantification available nor necessary). However, the link between these phenotypes and freezing behavior are, to our knowledge, unclear. Altogether, although we cannot rule out an effect of the genotype, sex difference is the most likely explanation for the increase baseline freezing between DAT::Cre males and C57Bl/6J mice.

The second main difference was the ability of DAT::Cre males to encode the context during a 2 minutes long pre exposure. This result suggests that DAT::Cre males learned the context faster than C57Bl/6J females. Again, and due to having mice coming from different sexes and genotypes and experiments performed on different periods, it is hard to be certain about the explanation. Here again, the hyperdopaminergic phenotype might explain the better learning of DAT::Cre males. However, male rat need less time to encode a context before they can associated it with an electric shock (Wiltgen et al. 2001). Therefore, the more compelling argument would be sex differences in the necessary time to encode a new context.

Nevertheless, these differences merit an investigation by themselves that was outside the scope of this project and were not studied further due to the lack of time and resources.

### 5.4.2 D1/5 receptors implicated in learning the context

Hippocampal dopamine is required for learning a spatial labyrinth (McNamara et al. 2014), food localization (Bethus et al. 2010) or contextual fear conditioning (Tsetsenis et al. 2021). However, learning a spatial labyrinth needs a reward, learning food localization requires food value detection and fear conditioning involved evaluating the aversive stimulus. Even though all these studies show a role of dopamine in learning, knowing that dopamine is closely associated with value computation, we cannot distinguish a putative role of dopamine in encoding the context from its role in encoding the US value. Our results showing that intraperitoneal injection of SCH23390 before pre-exposure was able to block latent contextual learning offers a more compelling argument to indicate that dopamine does not only play the role in value computation, but also triggers latent learning.

The results obtained with pharmacological manipulation of D1/5 receptors lack the identification of their localization because mice received intraperitoneal injections. Therefore, we cannot conclude about the localization of the D1/5 receptors involved in context learning and in its association to the shock. However, given the results obtained in the contextual fear conditioning in Chapter 4 we can speculate that encoding the context might rely on hippocampal D1/5 receptors. Experiments involving intra hippocampal infusions had been planned. However, there is a controversy about the source (LC or midbrain) of the hippocampal dopamine involved in learning and memory and Tsetsenis et al. 2021 showed that optogenetic activation of dopamine neurons facilitates contextual fear conditioning while our experiments were in progress. Since pharmacological tools cannot differentiate between dopamine coming from the midbrain or from the LC, we found it more urgent to investigate the role of dopamine in context learning using the optogenetic tools. Therefore, pharmacological manipulation experiments of the receptors were put on hold.

### 5.4.3 Involvement of midbrain dopamine inputs to the hippocampus in contextual learning

We show that stimulating midbrain dopamine axons in the hippocampus facilitated contextual learning and that their inhibition hindered it.

These data might seem inconsistent with data obtained by Takeuchi et al. and Kampadoo et al. in 2016. These papers focused on stimulating the LC and not the midbrain because they show that a very high level of TH+ axons in the hippocampus originate from the LC, which dwarfs the quantity of TH+ axons coming from the midbrain. They also show that optogenetic

stimulation of the LC increases dopamine levels in the hippocampus alongside noradrenaline. They then show that stimulating the LC TH<sup>+</sup> cells can facilitate memory formation and that these effect pass through, counterintuitively, D1/5 receptors. They thus showed that LC inputs to the hippocampus are involved in memory through dopamine release and D1/5 receptors. However, Kempadoo et al. did not assess the role midbrain dopamine pathway to the hippocampus and their observation are thus compatible with ours. Conversely Takeuchi et al. concluded that midbrain dopamine did not facilitate learning. However, their data show a clear but non-significant increase in learning the location a reward in the event arena. Noteworthy, since they base their studies on the synaptic tagging and capture model, where dopamine would merely be involved in triggering PRP production in order to capture a short lasting memory into a long lasting one, they did not activate the dopamine neurons during learning, but 30 minutes afterward. In our framework, this is closer to our “unpaired group” that does not show any facilitation of learning rather than to our paired group, in which midbrain dopamine has to be modulated during the exposure to the context in order to modulate contextual learning.

Du et al. 2016 show that dopamine activation one hour before learning through optogenetic manipulation hinders contextual fear conditioning afterwards. A similar effect might be observed if we look at ChETA Unpaired group that show less freezing on the test day compared to YFP 30sec, although this decrease was not significant. Further investigation might be warranted by testing for the effects different delays between learning and dopaminergic stimulation that could be delivered before or after pre-exposure.

Our results are in line and complete those recently published by (Tsetsenis et al. 2021), who show that the activation of a subpopulation of midbrain dopamine cells, located at the border between lateral VTA and the SNc, facilitates contextual fear conditioning. In their work, they also elegantly show that the genetic depletion of catecholamines in the LC did not affect the performance of mice in their task. Our results, taken together with theirs, paint a fuller picture of the role of midbrain dopamine pathway to the hippocampus as a teaching signal and demonstrate that dopamine in the hippocampus originating from the midbrain acts as a teaching signal. In particular, our data in CPFE demonstrates its ability triggers latent contextual learning in the absence of any reward of punishment.

## 5.5 Conclusion

In this chapter, we show CPFE as a useful tool in the study on hippocampal contextual memory separately from the interference of value association. We then show that D1/5 receptors are implicated in both contextual memory and associative memory separately. Finally, we show a direct link between midbrain dopamine transmission to the hippocampus and contextual learning, confirming a crucial role for this pathway in triggering hippocampal learning.

## **Chapter 6 General Discussion**

### **6.1 Temporality of coincidence in LTP triggering**

In the first chapter, we demonstrated that the coincidence of a midbrain dopaminergic signal with a glutamatergic input in CA1 of hippocampus induces an increase of transmission of the glutamatergic input.

DA-LTP necessitate dopamine to arrive to the hippocampus in a time window that is between 0 and 200 milliseconds after glutamate stimulation. This time window is biologically significant, dopamine are activated 70 milliseconds after detection of visual saliency (Schultz 1998), 150-250 milliseconds after visual signals that predict reward or punishments in monkeys (Matsumoto and Hikosaka 2009) and a lower latency for predictive odor cues in mice (Matsumoto et al. 2016). Lisman and Grace suggested in their model that CA1 could be one origin of low latency (100ms) novelty signals to the VTA (Lisman and Grace 2005). The authors then revised their model, taking into account that the Superior Colliculus could also play this role (Redgrave and Gurney 2006), and propose that either inputs to midbrain dopamine cells could be the origin of their activation by unpredicted salient signals (Lisman et al. 2011). In either case, our data suggest that, regardless of its origin, this low latency dopamine signal in response to novel saliency could be the trigger of naturally occurring LTP during learning.

The VTA hippocampal loop involves different structures, such as the nucleus accumbens the ventral pallidum, or the alternative pathway that originated from the CA3 through the lateral septum (Luo et al. 2011; Otmakhova et al. 2013). Moreover, as discussed before, midbrain dopamine neurons could be activated through the Superior Colliculus. Further investigations of the role of these structures is needed to understand how this functional loop is able to activate dopamine neurons in response to novelty and in turn trigger hippocampal plasticity. An example would be to stimulate neurons projecting from the ventral pallidum to the midbrain, study the delay of the dopaminergic signal to reach the hippocampus and see if this stimulation could trigger LTP, the same applies to the other structures on this loop.

In our results, we report that the phasic activation of dopaminergic axons triggers LTP on co-activated glutamatergic inputs. Some reports showed that long-term dopaminergic activation in the hippocampus through pharmacological interventions (Otmakhova and Lisman 1999; Navakkode et al. 2017) or through optogenetic activation (Du et al. 2016) could induce a decrease in glutamatergic synaptic transmission that is mostly driven by D4 receptors. It was

later shown that dopamine could bidirectionally regulate glutamatergic transmission over the SC in CA1. They showed that tonic stimulation of midbrain dopamine axons causes a decrease in glutamatergic transmission through an indirect pathway involving interneurons and this effect was driven by D4 receptors and phasic dopaminergic activation increases glutamatergic transmission and this effect is direct on pyramidal neurons and involve D1/5 receptors (Rosen et al. 2015).

Du and collaborators argue that dopamine actually filters the interesting inputs by showing that when dopamine is stimulated before contextual fear conditioning, whether through optogenetics or through rewards, mice are less capable of learning this task. They explain their effect by showing an LTD that was triggered in the DG following long-term dopaminergic activation (5 minutes) of midbrain dopamine inputs.

Based on our results, we show that short dopaminergic phasic activation works to increase the saliency of glutamatergic inputs that occur right before and based on the literature we see that long-term dopamine transmission lowers the encoding of glutamatergic inputs that arrive after (Du et al. 2016). Thus, we argue that phasic dopamine cell activity opens a time window in response to novelty that increase the encoding and the storage of sensory inputs arriving before it, and filters what happens after it. In order to verify this claim and further understand these effects, we need to pass by a stage of modeling of these different time scales that would allow knowing which inputs are encoded and over which synapses. Then we can test these models to know under which circumstances dopamine triggers LTP or LTD on different synapses in the hippocampus and in response to which different time scales. This would help link observations on the level of the synapse with those on the level of learning,

## **6.2 Mechanisms of coincidence detection**

Our results show one of the first direct evidences demonstrating that midbrain dopamine can directly trigger LTP in the hippocampus. An earlier paper showed a similar effect (Navakkode et al. 2007) using bath applied D1/5 agonist, SKF38393, in this paper they show that activating D1/5 receptors induces LTP only if the monitoring of glutamatergic transmission is maintained when the drug is applied.

We demonstrated that dopamine-triggered LTP depended on D1/5 receptors using SCH23390. Based on genetic modification of D1 genes specifically it was shown that these receptors are the ones implicated in LTP and hippocampal dependent memory processes (Granado et al.



2008) which suggest as well that the phenomenon we observed probably depends on D1 and not D5 receptors.

While our study was able to elucidate the source and the temporality of dopamine-triggered LTP and its specificity to D1/5 receptors, we failed to address the other side of this coincidence, glutamatergic inputs. We tried to study the effect of blocking NMDA receptor to see whether their blockade could block DA-LTP. This experiment failed to show any conclusive result because low doses of MK-801, an NMDA non-competitive inhibitor, did not block neither classical LTP triggered by theta burst stimulations nor DA-LTP. Higher doses were lethal to our anesthetized mice. MK-801 is one of the few NMDA blocking agents that were shown to have an effect after systemic injections because it is able to cross the blood brain barrier, inducing lower levels of learning in the Morris water maze and lower LTP (Coan et al. 1987; Morgan and Teyler 1999; Wylie et al. 1994). AP5 is the more traditional choice as an NMDA antagonist, but this molecule has to be infused locally to perform its effect (Morris et al. 1986). Performing intra hippocampal infusions affects the electrophysiological fEPSP recordings and is thus not adapted to our experimental set up. Therefore, *in vivo* electrophysiology does not allow us to conclude on the role of NMDA receptors in detecting glutamate release over the potentiated synapse. The better way to assess this question would probably be *in vitro* experiments on hippocampus slices.

Three possible hypotheses come to mind in order to explain the detection of this coincidence:

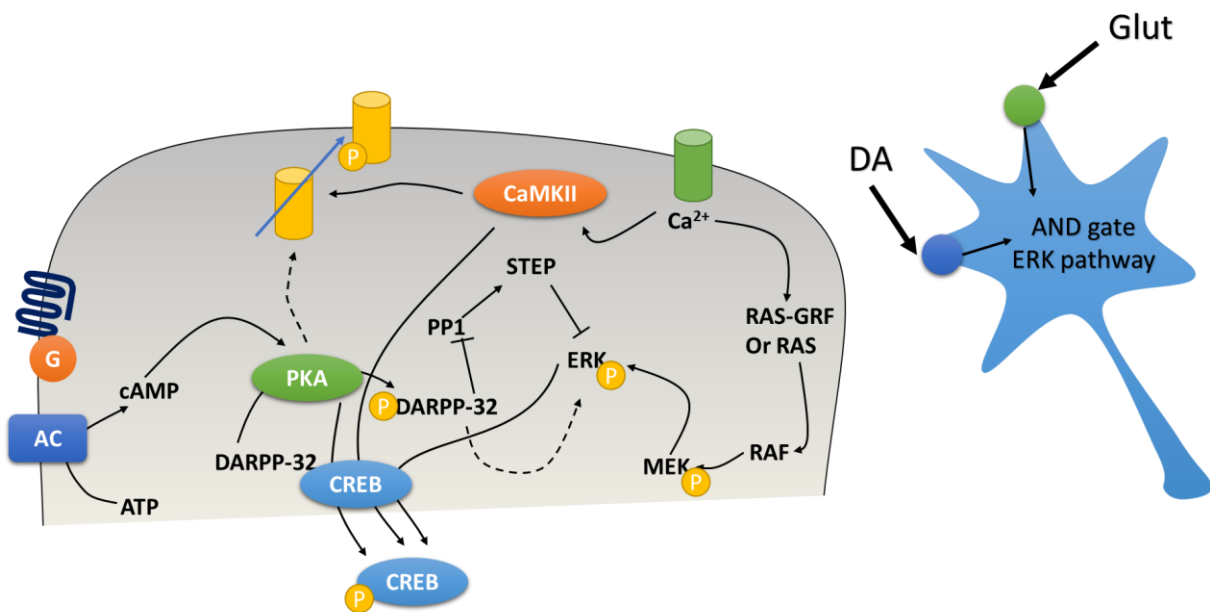
- The sole effects of depolarization detects the coincidence:

Electrical stimulation of the SC leads to activation of AMPA receptors that depolarizes the postsynaptic site. This depolarization, by itself, modulates different molecular actors implicated in synaptic plasticity. For instance, depolarizing cells by applying high concentration of  $K^+$  ions or by glutamate application on hippocampal slices or cultured neurons increases the levels of CAMKII activation and activated synapses show thickening of the post synaptic densities (Dosemeci et al. 2001). Voltage-gated channels might as well interact with dopamine transmission and could play a role in detecting the coincidence. For instance D1R interacts with  $Na^+$  channels through cAMP signaling (Cantrell et al. 1999).

However, this hypothesis does not account for the many studies that show a direct role of NMDA receptors in the learning and LTP (Zhu et al. 2011; Fanselow and Kim 1994; Place et al. 2012; Nguyen and Kandel 1997; Cooke et al. 2006).

- Convergent and synergistic signaling of NMDA-R and D1-R :

This model is based on the “AND Gate” model proposed by (Girault et al. 2007). In this model, ERK2 could play a role of coincidence detector. NMDA receptors induce ERK2 phosphorylation, when activated with single stimulation and not an HFS, through RAS,RAF,MEK pathway in a manner not capable to fully activate it. Dopamine would come to the rescue by activating the PKA phosphorylation pathway, which in turn activates Dopamine- and cAMP-regulated phosphoprotein, Mr 32 kDa (DARPP-32), which could directly, or indirectly through Protein phosphatase 1 (PP1) and Striatal-enriched protein tyrosine phosphatase (STEP), increase ERK2 phosphorylation on a second site allowing inducing its activation. These pathways have been deeply studied in the striatum (Hein et al. 2007; Greengard et al. 1999; Girault et al. 2007) and recently in the hippocampus showing a direct role in plasticity and memory (Haege et al. 2010; Kramar et al. 2014) (Figure 6.1).



**Figure 6.1 Different molecular actors to explore in order to explain coincidence detection.**

AMPA receptors (in yellow) might be phosphorylated in order to trigger dopamine LTP. A short calcium influx from NMDA receptors (in green) could be able to activate RAS signaling pathway alongside the activation of DARPP-32 which could lead to the phosphorylation of CREB and AMPA receptors which triggers LTP adopted from (Girault et al. 2007).

- NMDA D1 receptors coupling

Recent investigation of the relationship between dopamine D1 receptors and NMDA receptors show that these two might be coupled in their basal state, in which NMDA receptors cannot perform their calcium influx postsynaptic effect. Stimulating D1 receptors liberates NMDA

receptors and allows them to go into the postsynaptic membrane. If glutamate is present, in the synapse, NMDA receptors would be activated and could induce  $\text{Ca}^{2+}$  influx triggering LTP. This is a very compelling model that could explain how the co-release of dopamine and glutamate could trigger LTP (Ladepêche et al. 2013a; Ladépêche et al. 2014; Ladepêche et al. 2013b). We however still do not know if this mechanism has a role in synaptic plasticity nor in learning and memory, nor if its kinetics are compatible with DA-LTP.

Further investigations based on these hypotheses is needed to understand how dopamine plays its role in triggering learning and plasticity. These studies would probably be easier and more conclusive if performed *in vitro*, which originally was part of a planned collaboration that did not flourish yet.

## 6.3 VTA or LC

### 6.3.1 Novelty, plasticity and learning

Novelty causes dopamine transmission in many structures in the brain including the prefrontal cortex, hippocampus and nucleus accumbens. Dopamine released during novelty can facilitate the induction of LTP induced by HFS in CA1 (Li et al. 2003) and render a short lasting weakly triggered E-LTP into a long lasting L-LTP through D1/5 receptors (Lemon and Manahan-Vaughan 2006), a result that was replicated in different hippocampal structures and using different techniques (Hagena and Manahan-Vaughan 2012, 2016; Takeuchi et al. 2016; Wagatsuma et al. 2017).

More recently, it was shown that novelty activates TH<sup>+</sup> cells in the VTA and the LC (Takeuchi et al. 2016). Stimulating the LC TH<sup>+</sup> cells with optogenetics increases dopamine levels in the DG (Kempadoo et al. 2016). Stimulating dopamine in these studies was able to make memories last for 24hr and facilitated LTP.

This effect is reminiscent of the synaptic tagging and capture effect, showing that dopamine could have a key role in heterosynaptic plasticity and learning in a NeoHebbian framework (Lisman et al. 2011; Lisman and Grace 2005). Less elaborated literature, suggests that similar effects can be observed in the DG over the perforant path that implicate beta-noradrenergic receptors (Schimanski et al. 2007; Straube et al. 2003; Hansen and Manahan-Vaughan 2014; Manahan-Vaughan and Kulla 2003).

Even the raphe nucleus was shown able to increase the synaptic transmission of the SC (Teixeira et al. 2018) in a similar way midbrain dopamine does seen in (Rosen et al. 2015). Moreover, the authors show a facilitation of spatial learning when raphe serotonin neurons are stimulated, an effect that involves 5-HT4 receptors. A subpopulation of neurons in the raphe nucleus were shown to be dopaminergic and to be implicated in attention and arousal in response to salient stimuli (Cho et al. 2017).

This complicated, intertwined triple monoaminergic system involving dopamine, noradrenaline and serotonin is a fascinating topic that does not show a clear-cut role for each monoamine from each structure in one particular mechanism, but shows complimentary roles in attention, motivation and memory. Our optogenetics results from Chapter 3 and Chapter 5 allows us to place two pieces of the puzzle showing that midbrain dopamine is actually able to trigger LTP and learning in a non-value motivated manner. Further *in vitro* investigations of the effect of each of these monoamines on synaptic transmission over each of the synapses of the hippocampus can help us build a clearer model of how monoamines play with the trisynaptic loop in order to organize episodic and episodic-like memories.

### 6.3.2 Insight from REM and memory consolidation

The link between sleep and memory consolidation is a very old idea, many mechanisms were proposed to explain it, a very prominent one is the sorting of inputs during Rapid Eye Movement (REM) sleep. REM sleep is a distinct phase of sleep found in both mammals and birds during which random rapid movement of the eyes take place, hence the name; on the contrary, all other muscles show complete atony. Waking subjects during REM sleep showed that they recall their dreams more vividly. These observations suggested that during REM the brain can reprocess offline inputs that were acquired during wakefulness without the interference of new inputs (Eichenlaub et al. 2018).

Some elements suggesting a causal role between REM sleep and memory consolidation comes from the fact that mice showed higher levels of REM sleep following learning (Smith et al. 1980). REM sleep deprivation hinders memory (Harris et al. 1982) which can be countered by nicotine administration, a molecule known to increase dopaminergic transmission through its receptors that are directly expressed on midbrain dopamine cells (Aleisa et al. 2011). And the definitive proof of a causal role of REM sleep in memory processing is the fact that the specific inhibition of GABA neurons in the medial septum during REM sleep blocks contextual memory without interfering with sleep itself (Boyce et al. 2016).

LC dopamine cells become completely silent during REM sleep (Fenik et al. 2002), but VTA dopamine cells fire during this period (Dahan et al. 2007).

Learning induces two waves of dopamine related activity in the hippocampus necessary for memory retention, one that occurs during learning and the second 3-6 hours later (Bernabeu et al. 1997), the period during which REM sleep is increased post learning. Interfering with dopamine transmission late after learning blocks memory consolidation (Rossato et al. 2009).

These results suggest that dopamine has two waves during which it acts to store encoded sensory inputs, one during acquisition and a second during REM sleep, during which arbitrary associations could take place.

It would be interesting to study the role dopamine transmission has specifically during REM sleep by optogenetically inhibiting them during post learning elongated REM sleep periods.

## **6.4 Cell firing and plasticity, when and where in CPFE?**

CPFE pharmacology studies were performed with intraperitoneal injections of SCH23390 due to lack of time and resources. The conclusions about the location of D1/5 receptors in learning each part of this task were indirect and hypotheses based on results obtained with contextual fear conditioning performed with intra-hippocampal infusion experiments we performed and found in the literature.

However, it would be very interesting to carry on with the pharmacology project either with classic pharmacology intra-hippocampal manipulation using SCH23390, lidocaine or even muscimol (GABA<sub>A</sub> receptor agonist, this molecule would shut down pyramidal neuron firing without inhibiting the whole area, as lidocaine does).

In a recent paper, direct proof of a silent learning possibility, based on LTP, was shown using intra hippocampal infusions of muscimol (Rossato et al. 2018). In this work, the authors show, using a series of Morris water maze learning that blocking AMPA receptors inhibited both the recall of earlier spatial location of the platform and learning the new one. Blocking NMDA transmission only blocked learning the new configuration however spared the recall. Interestingly, blocking the firing (with muscimol) but sparing AMPA and NMDA transmission blocked recall but spared learning. Moreover, the authors show coherent LTP results under these drugs.

Based on this concept, we would propose to explore the question: which of these actors is recruited and when using CPFE?

Our hypothesis, hippocampal plasticity, dictated in our model by D1/5 receptor and probably NMDA, would be necessary during contextual acquisition on day one. Pyramidal cell firing would be an open question that can be answered either using muscimol local infusions or by optogenetic inhibition of hippocampal pyramidal neurons. The amygdala in this model would be unnecessary for the acquisition on day one, neither plasticity nor activity.

On day two, mice should be able to recognize their surround context to be able to learn the task. Therefore, we propose that cell firing in the hippocampus should be intact during day two, verifiable again through muscimol or optogenetic manipulations. Plasticity, on the other hand, could be either unnecessary if memory in CPFE paradigm is purely contextual on day one and purely associative on day two. Another possibility is that memory in this task is more episodic-like than purely separated. In that case, hippocampal plasticity could play a role in adding a new information to the already learned context and blocking hippocampal plasticity hinders learning this task.

Activity in the amygdala, and plasticity there, are probably both important on day two. Because either way, episodic-like or contextual and then associative, mice should have their amygdala intact to assess the value of the shock and associate it with the context, processes that necessitate an intact amygdala.

On day three, plasticity is not necessary in either structures, but firing probably is necessary in both.

Moreover, studying the role dopaminergic transmission plays in other structures, such as the nucleus accumbens and the prefrontal cortex (for dopamine's role in value and motivation) in this task would be interesting as well.

## **6.5 Other mechanisms behind dopamine-facilitated leaning**

Even though we showed that dopamine triggers hippocampal LTP and contextual learning, that does not provide a causal link between the two. Local SCH23390 injections in the hippocampus lowers exploration in an open field in rats (Heath et al. 2015). Most articles do not show higher exploratory behavior if dopamine was stimulated locally in the hippocampus (Takeuchi et al. 2016; Yamasaki and Takeuchi 2017; Kempadoo et al. 2016). However, the results observed with our CPFE protocol could still be due to a network mediated effect either with better

perception (Cho et al. 2017) or even by indirectly stimulating the activity of LC (Smith and Greene 2012) rather than triggering hippocampal plasticity.

Since we are able to trigger LTP by 7 minutes of contextual pre-exposure (Remaud 2014), it would be interesting to study whether 2 minutes of pre-exposure could induce similar effects compared to 30 seconds should not since no facilitation was observed. Then a way to directly prove that learning a context rely directly on dopamine triggered plasticity would be to assess if blocking dopamine transmission during 2 minutes of pre-exposure blocks this learning induced LTP, and whether dopamine activation during 30 seconds of pre-exposure induce LTP.

Different LTP marker following 30 seconds of pre-exposure and 2 minutes, should also be studied at different durations following pre-exposure in the hippocampus such as CREB and ERK phosphorylation, Zif268 and Arc mRNA expression. Similar approaches could be interesting as well in the amygdala and on day two.

Another approach would be to study the effect of dopaminergic transmission manipulation in other memory tasks and in other brain structures. For example, Hitti and Siegelbaum 2014 showed a central role for CA2 in social memory, Kempadoo et al. 2016 for DG in novel object recognition, Sariñana and Tonegawa 2016 a role for dopamine receptors in the prefrontal cortex using Morris water maze and in our lab Remaud et al. 2014 showed a role for protein production in CA3 in contextual fear conditioning. Studying the effect of dopamine stimulation or inhibition in these structures during these tasks would help paint a fuller picture of the role of dopamine in learning.

## **6.6 Implication in traumatic memory formation**

Data from the very first batch of mice that were used for the CPFE protocol with optogenetic stimulation of dopamine axons in the hippocampus were not included. These mice were never habituated to being connected to optic fibers, and the experimenter was naïve and learned how to connect them on the day the experiment was performed. Therefore, mice in this first batch were very stressed when they received the light stimulation of dopamine axons in their hippocampi, then on day two they received an electric shock in the same context as day one. These mice showed similar freezing behavior as mice included in the CPFE experiment, but afterwards they were used for another project that included observing their behavior in an open field. 50% of mice optogenetically stimulated showed a very high profile of fear generalization. More specifically, these mice would behave completely normally in their home cage, did not

show any alteration in their motor coordination during the tight rope test, nor deficits in muscle tone in grip test. However, these mice showed complete freezing behavior in the open field.

In order to solve this issue for later experiments we habituated mice to be connected to optic fibers 3 times during the week leading to the behavior experiments. Moreover, the experimenter had learned to connect the mice quickly and with a technic that induced less constraint on mice. Indeed, we saw less resistance from mice that were habituated, and we did not see fear generalization behavior.

The stress endured by the mice in the aforementioned first batch might have caused their fear of the context to be generalized. Post-traumatic stress disorder (PTSD) is a type of chronic anxiety disorder that manifests itself as a result of a traumatic episode either experienced or perceived. The main symptom is reliving the internal state of these situations in "Flashbacks" or in nightmares. The efficacy of noradrenergic blockers in the treatment of this disease is currently debated (Giustino et al. 2016). In a fear conditioning model, which attempts to study PTSD, dopamine appears to be involved in synaptic plasticity in the amygdala, and in the persistence of fear memory (Kwon et al. 2015). In this study, dopamine inhibition in the amygdala through different pharmacological, genetic and optogenetic approaches hindered a form of LTD that was necessary in the extinction of fear conditioning.

Mice that partially learn a context show a higher generalization profile that can be countered with relearning and recontextualizing the fear memory (Al Abed et al. 2020). Stress was shown to enhance fear learning and generalization (Rajbhandari et al. 2018). However, the role of dopamine transmission in the hippocampus in the pathological persistence of fear memories or in their generalization is not clear.

We could therefore hope that a better understanding of the roles of the different dopaminergic pathways could lead to new therapeutic approaches. For example, we could develop a protocol of acute stress (stress induced by constraint for example) before different length of pre-exposure and study the effect it has on fear generalization. Moreover, we could study the effect activating, or inhibiting, dopamine during learning has on generalization. It would be interesting to assess these effects in the hippocampus and in the amygdala as well.



## 6.7 Conclusion

In this work, we were able to show a novel method to trigger LTP that relies on midbrain dopamine coupling with glutamate entry over Schaeffer Collaterals. We call this phenomenon DA-LTP. We show that D1/5 receptors, most probably in the hippocampus, are necessary for storing the memory of a new context. We also demonstrate a central role for this pathway in learning a new context separately from any value.

These results, although novel and interesting, are only a starting seed for many more studies that aim at understanding how dopamine interact with glutamate to store encoded information. First on the level of intra cellular pathways to understand the underlying molecular signaling of coincidence detection, then on the synaptic and cellular levels to understand the effects of different timing of dopamine and glutamate release. Moreover, studies should be led on the level of structures to understand the role of dopamine plasticity in learning, and finally we need to assure a direct causal role for dopamine in learning induced plasticity.

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## RESUME

I am a neuroscience PhD and a pharmacist. I studied during my PhD research in *Université Paul Sabatier* the role of dopamine in hippocampal synaptic plasticity at the “*Centre de Recherches sur la Cognition Animale*” (CRCA) under Dr. Lionel DAHAN’s supervision. I had previously obtained my Pharmacist's degree in "Pharmacy and pharmaceutical chemistry" at Aleppo university-Syria in 2015. Then, after two years of my stay in France, I obtained my Master's degree in "Pharmacological Innovation" at *Université Paul Sabatier* in 2018 ranked first and with honors, which allowed me to claim and obtain a ministerial scholarship to do my doctorate.

## Education

- 2018-2022 PhD in Neurosciences. *Université Paul Sabatier Toulouse-3*
- 2017-2018 Master 2 Pharmacological Innovation, *Université Paul Sabatier Toulouse-3*  
*Average result: 16,47/20 (final ranking: 1/15)*
- 2016-2017 Master 1 Health Biology, *Université Paul Sabatier Toulouse-3*  
*Average result: 13.57/20 (final ranking: 6/89)*
- 2010-2015 Pharmacist Diploma, *Aleppo University, Syria*. To obtain the diploma, the student must validate 5 years of university education providing more than 60 different subjects, and a bachelor thesis, for which I wrote a summary review on “insomnia in the elderly”  
*Average result: 79.11/100 (final ranking: 31/145)*

## Professional and Associative Experience

- 2018-2022 PhD Student *CRCA Team REMEMBeR*. Research topic: “Triggering hippocampal LTP and learning by dopamine: a teaching signal”
- 2021 Temporary teaching contract at *Toulouse faculty of science and engineering*, statics practical sessions, master level (20hrs)
- 2021 University teaching contract for PhD students at *Toulouse faculty of science and engineering*, introduction to the scientific method, bachelor level (32hrs/year)
- 2019 University teaching contract for PhD students at *Toulouse faculty of pharmacy*, ligand-receptor interaction pharmacodynamics, bachelor level (32hrs/year)
- 2018 (5 months) Master 2 internship *CRCA Team REMEMBeR*. Research topic: “Triggering hippocampal LTP by dopamine”
- 2017 (2 months) Master 1 internship *Centre de Biologie du Développement (CBD)*. Research topic “The effect of altering metabolic aspects on Neural Stem Cells Fate”
- 2015-2016 Pharmacist, *JRS (Jesuits-Refugee-Service)*  
Distribution of medication in the *JRS* free clinic in Aleppo.
- 2010-2015 Chef scout, *Scoutes Jesuites*.  
In charge of activities aimed at supporting the physical and personal development of adolescents aged 12-15 year-old.
- 2010-2011 Physics and chemistry tutor, *JRS (Jesuits-Refugee-Service)*.  
Supported seven students preparing for the baccalaureate (Iraqi refugees) in these two subjects

## Skills

Neurobiology:	Electrophysiology	Performed <i>in vivo</i> electrophysiology in anesthetized mice, recordings of evoked field potentials coupled with optogenetic stimulation in order to assess the role of dopamine in triggering LTP
	Optogenetics	Used to activate or inhibit dopamine afferents in the hippocampus coupled with electrophysiology and behavior
	Immunohistochemistry	Used to evaluate transfection efficacy and specificity, and to trace dopamine axons in the hippocampus
	Behavior	Contextual fear conditioning coupled with optogenetics or local pharmacological infusions
Languages:	Arabic:	Mother tongue
	English:	Fluent (TOEFL-iBT certificate. Score : 102/120)
	French:	Fluent (TCF B2 certificate in 2016)
	German:	Satisfactory (Goethe-Institut certificate B1 in 2014)
Computer skills:	Microsoft Office, Photoshop and Illustrator	
	Spike2 for signal acquisition and treatment	
	GraphPad Prism 8 and Minitab 19 for statistical analyses	

## Communications

**SAYEGH FJP.**, MACRI C., HERRAIZ L., PI MACEDO J., LEJARDS C., VERRET L., RAMPON C., DAHAN L. Dopamine: a Hippocampal teaching signal. Poster presented at: *1er Colloque Interdisciplinaire du GDR Mémoire*. 12-15 Oct 2021; Cahors, France. (Best poster communication: 1<sup>st</sup> prize)

**SAYEGH FJP.**, LEJARDS C., PECH S., RAMPON C., DAHAN L. Dopamine triggers Long Term Potentiation. Poster presented at: *Neurofrance 2019*. 22-24 May 2019; Marseille, France.

**SAYEGH FJP.** La dopamine, un signal d'apprentissage. Oral presentation at: *Qui-Quoi-Où de la recherche sur les apprentissages et la mémoire à Toulouse – 2ème édition*. 6 Dec 2019; Toulouse, France.

**SAYEGH FJP.** Triggering long-term potentiation by dopamine: synaptic mechanisms of a teaching signal. Oral presentation at: 4th CRCA PhD Students Symposium. 9 Oct 2019; Toulouse, France.

## Publications

**SAYEGH FJP.**, HERRAIZ L., COLOM M., LOPEZ S., RAMPON C., DAHAN L. D1/5 dopamine receptors are necessary for learning a novel context. *Learning and Memory* (under revision)

**SAYEGH FJP.**, MACRI C., PI MACEDO J., LEJARDS C., VERRET L., RAMPON C., DAHAN L. Dopamine triggers hippocampal LTP and contextual learning (in prep.)

CATTAUD V., B. SZABO A., BEZZINA C., DARD R., **SAYEGH FJP.**, LOPEZ S., LEJARDS C., VALTON L., RAMPON C., VERRET L., DAHAN L. Neuronal hyperexcitability in the Tg2576 mouse model of Alzheimer's disease – The influence of sleep and monoaminergic transmission (in prep.)

## References

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