PONTIFICIA UNIVERSIDAD CATÓLICA DE CHILE
Doctorado en Neurociencias

PONTIFICIA UNIVERSIDAD CATÓLICA DE CHILE
Doctorado en Neurociencias

Tesis Doctoral

The role of dentate gyrus somatostatin-expressing cells in pattern separation

Por

Cristian Morales

Julio 2020

PONTIFICIA UNIVERSIDAD CATÓLICA DE CHILE
Doctorado en Neurociencias

Tesis Doctoral

The role of dentate gyrus somatostatin-expressing cells in pattern separation

Tesis entregada a la Pontificia Universidad Católica de Chile en cumplimiento parcial de los requisitos para optar al Grado de Doctor en Neurociencias

Por

Cristian Enrique Morales Rojas

Director de Tesis: Pablo Fuentealba

Julio 2020

El Comité de Tesis, constituido por los Profesores abajo firmantes, aprueba la Defensa Publica de la Tesis Doctoral titulada:

PONTIFICIA UNIVERSIDAD CATÓLICA DE CHILE
Doctorado en Neurociencias

THE ROLE OF DENTATE GYRUS SOMATOSTATIN-EXPRESSING CELLS IN PATTERN SEPARATION

Aprobación Defensa:

CRISTIAN ENRIQUE MORALES ROJAS

Calificándose el trabajo realizado, el manuscrito sometido y la defensa oral, con nota

…..... (……..)

Dr. Mauricio Cuello
Director de Investigación y Doctorado
Escuela de Medicina
Pontificia Universidad Católica de Chile

Dr. Felipe Heusser R.
Decano
Facultad de Medicina
Pontificia Universidad Católica de Chile

Dra. Claudia Sáez
Sub-Directora
Dirección de Investigación y Doctorado
Escuela de Medicina
Pontificia Universidad Católica de Chile

Dr. Francisco Aboitiz D.
Jefe Programa Doctorado en Neurociencias
Facultad de Medicina
Pontificia Universidad Católica de Chile

Dr. Pablo Fuentealba
Director de Tesis
Escuela de Medicina
Pontificia Universidad Católica de Chile

Dra. Katia Gysling
Profesor Evaluador Interno
Facultad de Ciencias Biológicas
Pontificia Universidad Católica de Chile

Dr. José Fuentealba
Profesor Evaluador Interno
Facultad de Química
Pontificia Universidad Católica de Chile

Dr. Felipe Court
Profesor Evaluador
Universidad Mayor

Dr. Rómulo Fuentes
Profesor Evaluador Externo
F. Medicina. Universidad de Chile.

Santiago, 16 de septiembre de 2020

DEDICATORIA

Este logro no es solo mío, sino de todes aquelles que me ayudaron en el camino. Pues, para mí, esto no se trata solo de generar conocimientos sino de convivir en el asombro.

Quiero agradecer a mis amigues del colegio, Iván, Guille, Mario, Dafne, Claudio. Especialmente gracias, Claudio por ayudarme a seguir adelante. Gracias a mis compañeras de laboratorio, Rilda, Cote, Ale, Carol, hermosas personas que se conoce en el camino. Especialmente gracias, Carol por las tardes Lastarrinas. Gracias, a mis amigos de pregrado, Juan, Pedro, Miguel. Especialmente gracias, Juan, amigo, colega, profesor, gracias por tantas conversaciones. Gracias Debbie y Claudia siempre disponibles para ayudar, más allá de sus responsabilidades.

Gracias Gonzalo Marín, por permitirme no desencantarme de la ciencia, gracias por tantas enseñanzas. Gracias Facundo Morici, un gran amigo y colega que me dio la tesis. Gracias a mi madre y a su apoyo incondicional. Finalmente, agradecerte Natalia, amor de mi vida, gracias por tanto apoyo, gracias por esa bendición mirando el lago.

ABSTRACT

A critical step in the formation of a new episodic memory (memories about our personal experiences) is to ensure that it will be stored in a different way than similar episodic memories previously acquired. Pattern separation, which is a mechanism that takes place in Dentate Gyrus (DG), would allow the discrimination of similar episodic memories by increasing the differences of synaptic inputs that reach DG. Disruptions of this memory discrimination have been related to cognitive impairment in aging, neuropsychiatric disorders and epilepsy. Thus, understanding the neuronal mechanism of pattern separation, which until now is not well understood, has important benefits for public health.

The excitability regulation of DG is critical, as evidenced by deteriorated pattern separation when this excitability is experimentally enhanced. Somatostatin containing cells (SOM+), a type of GABAergic interneurons of DG are good candidates to control the excitatory inputs reaching DG, but its role in controlling the flow of information in the dentate gyrus circuitry remains elusive. Interestingly, recent evidences have shown an important role of SOM+ in the control of DG excitability and in the memory impairments, but its direct role in pattern separation have been not studied.

In this thesis I tested the hypothesis that functional SOM+ are required for contextual discrimination through the control of DG excitability. For that purpose, I studied the effect of inhibition of SOM+ in DG excitability in anesthetized mice. Then, in a second experiment, in

awake mice I studied the effect of inhibition of SOM+ in behavioural tasks that need the pattern separation mechanism. Since I wanted to control the activity of SOM+, without affecting the activity of other members of the neural network, I chose the optogenetic as experimental approach. This technique allows a selective and fast manipulation of SOM+ activity.

I found out that optogenetic suppression of SOM+ modulated the firing rate of putative excitatory cells and putative Parvalbumin containing cells (PV+), other type of GABAergic interneurons. Moreover, optogenetic stimulation impaired both contextual and spatial discrimination of overlapping recognition memories during task acquisition. These results suggest that SOM+ are required for successful pattern separation during episodic memory encoding. Indicating that SOM+ must be considered in future model of pattern separation mechanism. Specifically, I propose that SOM+ command a delayed feedback inhibition.

RESUMEN

Un paso crítico en la formación de una nueva memoria episódica (memorias sobre nuestras experiencias personales) es garantizar que se almacene de una manera diferente a los recuerdos episódicos similares, previamente adquiridos. La separación de patrones, que es un mecanismo que tiene lugar en el Giro dentado (GD), permitiría la discriminación de recuerdos episódicos similares al aumentar las diferencias de los input sinápticos que llegan a GD. La incapacidad de generar esta discriminación se ha relacionado con el deterioro cognitivo en el envejecimiento, los trastornos neuropsiquiátricos y la epilepsia. Por lo tanto, comprender el mecanismo neuronal de la separación de patrones, que hasta ahora no se conoce bien, tiene importantes beneficios para la salud pública.

La regulación de la excitabilidad del GD es crítica, como lo demuestra el deterioro que ocurre en la separación de patrones cuando se induce un aumento artificial de la excitabilidad de GD. Las células que contienen somatostatina (SOM +), un tipo de interneuronas GABAérgica de GD, son buenas candidatas para controlar los inputs excitatorios que alcanzan GD, pero su papel en el control del flujo de información en el circuito de GD sigue siendo difícil de entender. Curiosamente, las evidencias recientes han demostrado un papel importante de SOM + en el control de la excitabilidad DG y en las alteraciones de la memoria, pero su papel directo en la separación de patrones no se ha estudiado.

En esta tesis puse a prueba la hipótesis de que se requieren las SOM+ para la discriminación de contextos similares a través del control de la excitabilidad de las células granulares de GD. Para

ese propósito, estudié el efecto de la inhibición de SOM + en la excitabilidad de GD en ratones anestesiados. Luego, en un segundo experimento, en ratones despiertos estudié el efecto de la inhibición de SOM + en tareas conductuales que necesitan el mecanismo de separación de patrones. Como quería controlar la actividad de SOM +, sin afectar la actividad de otros miembros de la red neuronal, elegí la optogenética como herramienta metodológica. Esta técnica permite una manipulación selectiva y rápida de la actividad de las SOM +.

Descubrí que la supresión optogenética de la actividad de las SOM + modula la velocidad de disparo de un grupo de células que cumplen con los criterios fisiológicos de células glutaminérgicas del GD (cellas granulares y células mossy) y de un grupo de células que cumplen con los criterios fisiológicos de células que contienen Parvalbumin (PV +), un tipo de interneuronas GABAérgica del GD. Además, la estimulación optogenética perjudicó la discriminación contextual y espacial de memorias de reconocimiento similares. Estos resultados sugieren que se requieren las SOM + para una separación de patrones exitosa durante la codificación de memorias episódicas. Indicando que las SOM + deben ser consideradas en futuros modelos del mecanismo de separación de patrones. Específicamente, propongo que las SOM + dirigen una inhibición retrasada.

TABLE OF CONTENTS

DEDICATORIA 4

ACKNOWLEDGEMENT ..... **¡Error! Marcador no definido.**

ABSTRACT 5

RESUMEN 7

TABLE OF FIGURES 12

ABBREVIATIONS 13

1. INTRODUCTION 14

1.1 MEMORY SYSTEMS, EPISODIC MEMORY AND PATTERN SEPARATION 14

1.1.1 Episodic memory and patient HM 15

1.1.2 Medial temporal lobe 16

1.1.3 Pattern separation: stage of episodic memory that takes place in dentate gyrus 19

1.2 RECOGNITION MEMORY IN MICE: A MODEL FOR THE STUDY OF EPISODIC MEMORY AND PATTERN SEPARATION 20

1.2.1 Recognition memory and hippocampus: recollection and familiarity 21

1.2.2 Object in context and object in place. Two tests for the study of recollection memory 22

1.3 PATTERN SEPARATION TAKES PLACE IN DENTATE GYRUS 24

1.3.1 Dentate Gyrus network and neuronal types 27

1.4 PATTERN SEPARATION MECHANISM 31

1.4.1 The relevance of low excitability and orthogonalization 31

1.4.2 Evidence that low excitability and orthogonalization of the granule cells are mechanisms that happen in DG and are necessary for pattern separation 32

1.4.3 Does SOM+ interneurons participate in pattern separation? 33

2.REFERENCES OF INTRODUCTION 36

3. HYPOTHESIS AND OBJECTIVES 42

3.1 General hypothesis 42

3.2 Specific Hypothesis 42

3.2.1 Specific hypothesis 1: 42

3.2.2 Specific hypothesis 2: 42
3.3 Specific Objectives 42
3.3.1 Objective 1: 42
3.3.2 Objective 2: 42
4. RESULTS 43
4.1 Article that was sent to "Cerebral cortex" 44
4.1.1 Abstract....45
4.1.2 Introduction 46
4.1.3 Materials and Methods 49
4.1.4 Results....60
4.1.5 Discussion....67
4.1.7 References 73
4.1.8 Figures 79
4.2 Article that was sent to "Frontiers in Neural Circuits"....95
4.2.1 Abstract....96
4.2.2 Introduction 97
4.2.3 Implications of Memory Engram theory in Pattern separation process 100
4.2.3.1 Neurophotonics in the development of engrams theory....102
4.2.4 Dentate Gyrus Glutamatergic cells participation in pattern separation 107
4.2.4.1 Usage of Neurophotonics in the study of the spatial codification of granule and mossy cells....107
4.2.4.2 What can we say about the "irritable" hypothesis using neurophotonic techniques? 111
4.2.5 Neurophotonics applications in the study of the DG interneurons role in Pattern separation....113
4.2.6 Neurophotonics techniques to understand the role of adult-born granule cells in patter separation....117
4.2.7 Final Remarks 120
4.2.8 Funding....123
4.2.9 Figure....124
4.2.10 References 126

5. DISCUSSION.....137

5.1 **ORTHOGONALIZATION AND LOW EXCITABILITY: TWO MECHANISMS MEDIATED BY SOM+**.....137

5.1.1 SOM+ would mediate lateral feedback inhibition 137

5.1.2 SOM+ would mediate delayed feedback inhibition 139

5.1.3 SOM+ would participate in remapping 140

5.2 COMPUTATIONAL MODEL OF PATTERN SEPARATION.....141

5.2.1 SOM+ and computational models of pattern separation 141

5.2.2 Proposal of an integrative model for pattern separation that considers the activity of SOM interneurons. 142

5.3 DISEASE THAT ARE IN RELATION WITH LOSS OR BAD FUNCTION OF SOM+ CAN IMPLICATE PROBLEMS WITH PATTERN SEPARATION.....144

5.4 KIND OF TEST USED FOR STUDY OF PATTERN SEPARATION.....144

5.5 THE POSSIBLE ROLE OF SOMATOSTATIN NEUROPEPTIDE.....145

6. REFERENCES OF DISCUSSION.....145

TABLE OF FIGURES

1-Different kind of memory system.....14
2-Schematic that represent the connectivity of Medial temporal lobe......17
3-Schematic that show principal connectivity within hippocampal regions. 18
4-Novel object recognition task (NOR) 20
5-Recollection task: Object in place and Object in context task 23
6-Spatial pattern separation task.....24
7-Neuronal types of DG.....30
8-Optogenetic inhibition of hilar somatostatin cells disrupts local network activity patterns in the dentate gyrus......79
9-Encoding, but not retrieval, of overlapping contextual memories requires functional glutamatergic transmission the dentate gyrus. 81
10- Encoding of overlapping contextual memories requires somatostatin cells in the dentate gyrus. 83
11-Dentate gyrus somatostatin cells regulate the encoding of spatial recognition memory. 84
12-Neural spiking responses to optogenetic stimulation in the hippocampus. 85
13-Field potential changes in response to optogenetic stimulation in the hippocampus. .86
14-Arenas and objects used for similar (s-OIC) and dissimilar (d-OIC) object-in-context behavioural tests. 87
15-Exploratory behaviour during training sessions in the object-in-context test.....88
16-Dynamics of contextual discrimination during the retrieval phase of the object-in-context test......90
17-Dentate gyrus AMPA receptors do not regulate memory retrieval in the similar object-in-context behavioural test. 91
18-Dynamics of Discrimination Index during the spontaneous location recognition test. 92
19-Conceptual model for the role of dentate gyrus somatostatin neurons during pattern separation orthogonalization. 94
20-Representation of the DG-CA3 circuit 124

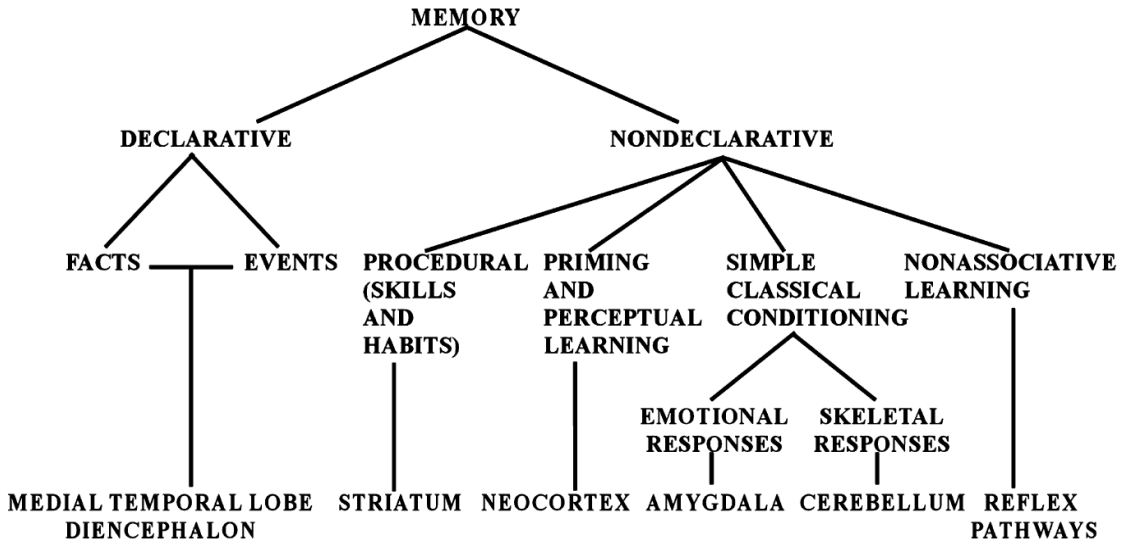
ABBREVIATIONS

abGCs	adult born granule cells
BC	Basket cells
CA1	Cornu Ammonis 1
CA2	Cornu Ammonis 2
CA3	Cornu Ammonis 3
CCK+	CCK-containing cells
DG	Dentate Gyrus
EC	Entorhinal cortex
EPSP	excitatory post sinaptic potential
GCs	Granule cells
HICAP	Hilar commissural-associational pathway
HIIL	Hilus associated interneurons
HIPP	Hilar perforant path-associated cell
HM	Henry Molaison
IPSP	Inhibitory post synaptic potential
LEC	Lateral entorhinal cortex
LFP	Local Field Potential
MCs	mossy cells
MEC	Medial entorhinal cortex
MML	medial molecular layer of DG
NOR	Novel object recognition
NpHR	Natronomonas pharaonis halorhodopsin
NpHR−	mice that do not express NpHR
NpHR+	mice that express NpHR
OML	outer molecular layer
PP	Perforant path
PV	Parvalbumin
PV+	Parvalbumin containing cells
SD	standard deviation
SOM	Somatostatin
SOM+	Somatostatin containing cells

1. INTRODUCTION

1.1 MEMORY SYSTEMS, EPISODIC MEMORY AND PATTERN SEPARATION

Studies (Tulving, 1993, 2002; Squire, 2004, 2009; Squire and Wixted, 2011) about memory function in patients with cerebral lesions suggest the existence of several types of memories (Figure1). The concept of memory system was used to distinguish each type of memory. This theoretical framework means that each memory system works with different kinds of information in different brain regions and uses different mechanisms (Tulving, 1993).



1-Different kind of memory system.

Figure 1: Different memory system that has been recognized. Note that each memory system take place in different brain regions. In this thesis will be important declarative memories (Extracted from Squire 2004).

The different memory systems were clustered in two major sets (Figure 1). One of them was called declarative memory that refers to the capacity for conscious recollection about facts and events that allows memories to be compared and contrasted; thus, it can guide performance under a wide range of test conditions (Squire, 2004b). The other group was called non-declarative memory that includes memory systems that support habit learning, skill learning, simple conditioning, and priming (Squire, 2004b, 2009b). Non-declarative memories are dispositional and are expressed through performance rather than recollection (Squire, 2004b; Squire and Wixted, 2011b).

1.1.1 Episodic memory and patient HM

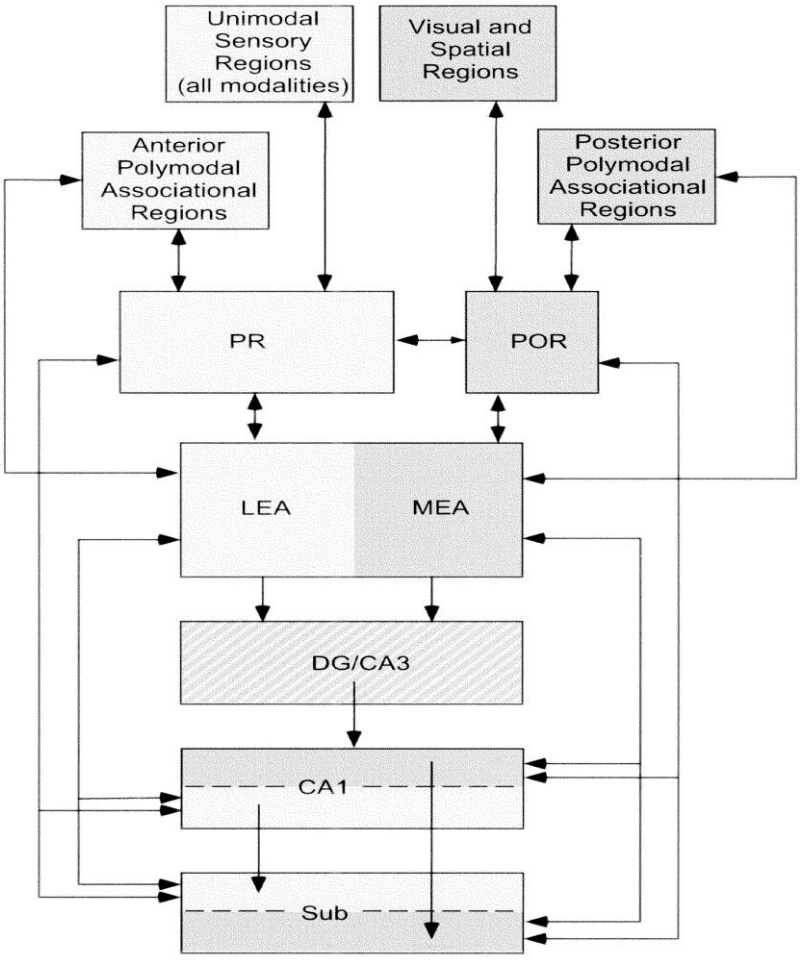
In 1972 Endel Tulving proposed the distinction between two types of declarative memories, mainly based, in the kind of information that can be remembered; episodic and semantic memory. In the episodic memory, the stored information is about a person's experience. In contrast, in semantic memories, the information is about words and verbal symbols and rules for manipulating these symbols. Episodic memories can be described in terms of its perceptible properties and its spatial and temporal relationship with other episodes (Tulving, 1972). Thus, episodic memory has an autobiographic component that depends on personal experience. On the other hand, semantic memory does not depend on experiences, and It is always the same because it is about concepts

A paradigmatic episodic memory study is about memory capacities of a patient denominated HM (Squire, 2009b). HM was a patient that in 1953 had a bilateral medial temporal lobe resection carried out to relieve epilepsy. In 1957 Brenda Milner reported that the bilateral brain lesion of HM produced a profound forgetfulness in him but in the absence of any general

intellectual loss or perceptual disorders (Squire and Wixted, 2011b). Specifically, HM had incapacity for conscious recollection about his life's facts and events while he could acquire new motor skills (Squire, 2004b, 2009b). This finding supports the idea that several types of memory systems work independently and depend on the different brain regions.

1.1.2 Medial temporal lobe

Medial temporal lobe is the structure that was damaged in HM (Squire, 2004b, 2009b) and other patients that had similar memory problems (Squire and Wixted, 2011b). Anatomical studies in human and non-human animals have allowed to understand its possible functional organization (Figure 2) (Burwell, 2000; Eichenbaum et al., 2007). The medial temporal lobe is integrated by the hippocampus, entorhinal, perirhinal, and parahippocampal cortices (Burwell, 2000). he afferents to the perirhinal cortex come from neocortical areas that are implicated with sensory information about qualities of objects, that have been referred as *"what information",* while inputs to the post rhinal cortex come from neocortical areas that process spatial information, which is referred as *"where information"* (Eichenbaum et al., 2007). Then, perirhinal and post rhinal cortex project to entorhinal cortex.



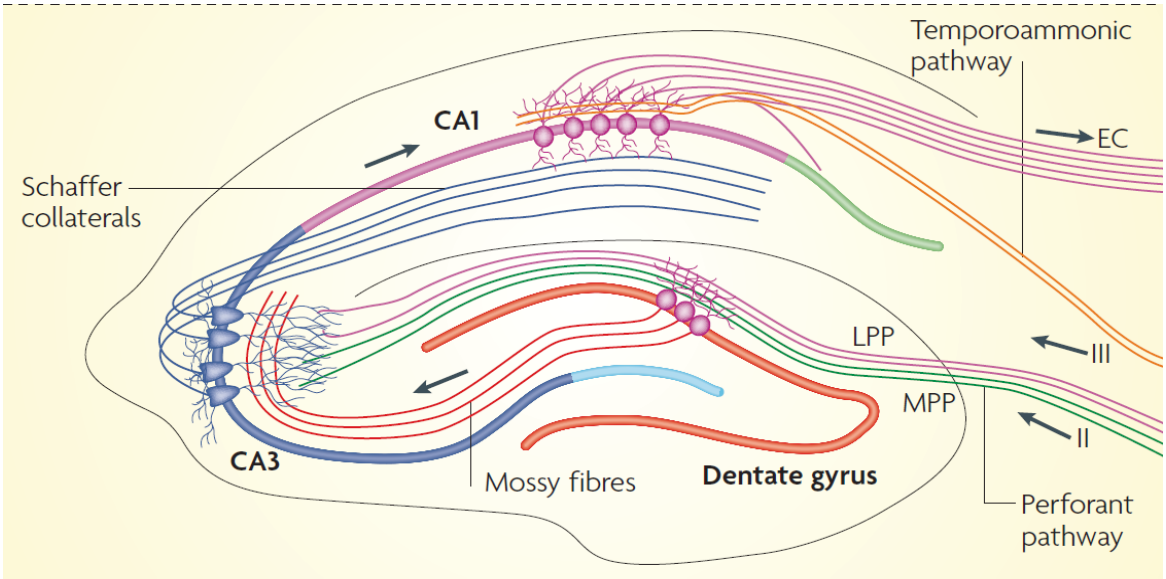
2-Schematic that represent the connectivity of Medial temporal lobe.

Figure 2: Arrow and colour indicate the influence of each regions. Note that spatial information arrives to post rhinal cortex that then send information to Medial entorhinal cortex. On the other hand, contextual information arrives to perirhinal cortex that sends information to Lateral entorhinal cortex. Finally, both information is integrated in DG. (Extracted from Burwell 2000)

Perirhinal cortex projects to the lateral entorhinal cortex (LEC). The parahippocampal cortex projects to the medial entorhinal cortex. Then the entorhinal information converges into Hippocampus (Burwell, 2000; Eichenbaum et al., 2007).

The Hippocampus is a cortical structure that establishes loop connectivity with other cortical networks (Andersen et al., 2007; Watson et al., 2012). There are three regions within

Hippocampus (Figure 3), which are Dentate Gyrus (DG), CA1, and CA3. All three hippocampal divisions share the three-layered appearance characteristic (Andersen et al., 2007; Watson et al., 2012). The principal cells of CA1 and CA3 are called pyramidal cells, while DG's principal cells are called granule cells (GCs). Projections from the entorhinal cortex reach the dendrites of DG's GCs, via the perforant path. The GCs project to CA3 pyramidal cells via the mossy fiber, then CA3 pyramidal cells do to CA1 pyramidal cells; finally, CA1 pyramidal cells project back into the entorhinal cortex.



3-Schematic that show principal connectivity within hippocampal regions.

Figure 3: Within the hippocampus three regions are recognized, Dentate Gyrus (DG), CA3 and CA1. DG is a region that receive axons from entorhinal cortex, specifically receive contextual information from Lateral perforant pathway and spatial information from Medial perforant pathway. Within DG, GCs receive this activity and send axons to CA3 regions, where form synapsis with Pyramidal neurons. Pyramidal neurons send its axons to CA1 region, where form synapsis with other pyramidal neurons that finally project out of Hippocampus. (Extracted from Deng, Aimone, and Gage 2010)

1.1.3 Pattern separation: stage of episodic memory that takes place in DG

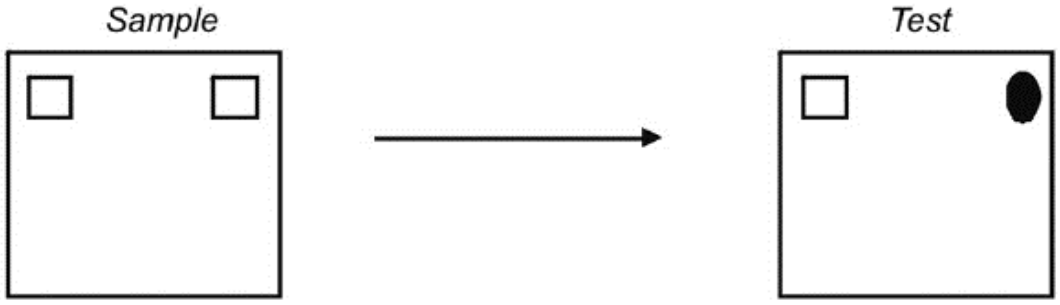
A critical aspect of episodic memory formation is to ensure that memories recently acquired and stored will not affect the previously learned and consolidated memories. If similar memories cannot be distinguished, then interference could occur, and recent memories could distort or replace previous memories (Mcclelland et al., 1995).

It has been suggested that our brain implements an algorithm that prevents catastrophic interference between similar episodes. This algorithm is called pattern separation, and this algorithm would increment the differences between the representations of similar episodes (O'Reilly and McClelland, 1994; Treves and Rolls, 1994; Yassa and Stark, 2011). There is evidence in rodents as well as in humans that show that the process of pattern separation takes place in the Hippocampus; specifically in a region of Hippocampus called Dentate Gyrus (DG) (Aimone et al., 2011; Yassa and Stark, 2011; Hunsaker and Kesner, 2013).

In this thesis, I am interested in understanding how similar episodic memories are stored as two different memories, i.e., how is the mechanism of pattern separation. For this purpose, I used mice as models for this study. In the following section, I will first explain how mice can be used as models for studying this kind of memory. Second, I will show evidence that supports that pattern separation takes place in DG. Finally, I will show evidence that suggests an essential role of somatostatin containing cells (SOM+) of DG in pattern separation.

1.2 RECOGNITION MEMORY IN MICE: A MODEL FOR THE STUDY OF EPISODIC MEMORY AND PATTERN SEPARATION

In 1956 Robert Frantz used a method to study visual recognition. He showed that when a chimpanzee see two objects, one familiar object (seen previously) and a new object, the chimpanzee has more visual fixation events for novel object indicating that information about the familiar object was successfully acquired and remembered. Later studies implemented this task (with some variants) in human infants, monkeys, and rats (Ennaceur;J.Delacour, 1988; Clark et al., 2000) suggesting that novel objects' preferences are preserved in mammals. In rats, this test was called "novel object recognition" (NOR) (Figure 4), and in monkeys was called "visual paired comparison" (VPC). Posterior studies showed that this test also depended on the medial temporal lobes structures, and then was accepted as a test for studying episodic memory (Clark et al., 2000).



4-Novel object recognition task (NOR)

Figure 4: In this test the time of exploration of each object during test phase is measured. Note that in test phase a new object replaces a familiar object.

1.2.1 Recognition memory and hippocampus: recollection and familiarity

When an episode is being remembered, it is possible to recognize that one item is more familiar than others, It is also possible to remember some aspects like “when” or “where” the episode happened. Thus, two elements of the recognition memory task were postulated: familiarity and recollection (Yonelinas, 2001). "Recollection" gives information about the episode in which an object was founded, and "familiarity" allows to recognize the object without information about the context (Squire et al., 2004; Eichenbaum et al., 2007).

In search of the neuronal substrate of recollection and familiarity, different models have been proposed (Rugg and Yonelinas, 2003; Eichenbaum et al., 2007). Some theories suggest that medial temporal lobe support recollection and familiarity, thus lesion in this region would affect both processes (Squire et al., 2004). On the other hand, other theories propose that different regions of medial temporal lobe support recollection and familiarity (Eichenbaum et al., 2007), , specifically it has been suggested that Hippocampus is critical for "recollection" whereas the perirhinal cortex supports "familiarity" of specific stimuli (Brown and Aggleton, 2001).

Experiments in rodents support the idea that different regions of medial temporal lobes have different functions. Lesion of perirhinal cortex consistently impaired the performance in object recognition task but no affect spatial memory task (Ennaceur;Neave;Aggleton, 1996; Bussey et al., 1999; Eichenbaum et al., 2007). Conversely, a lesion in Hippocampus does not always affect NOR's performance (Ennaceur;Neave;Aggleton, 1996; Bussey et al., 1999; Eichenbaum et al., 2007). These results indicate that Hippocampus and perirhinal cortex have different functions and that these two brain regions would contribute to different aspects of recognition memory. In fact, the test that changes the object's position or the context where the object is presented

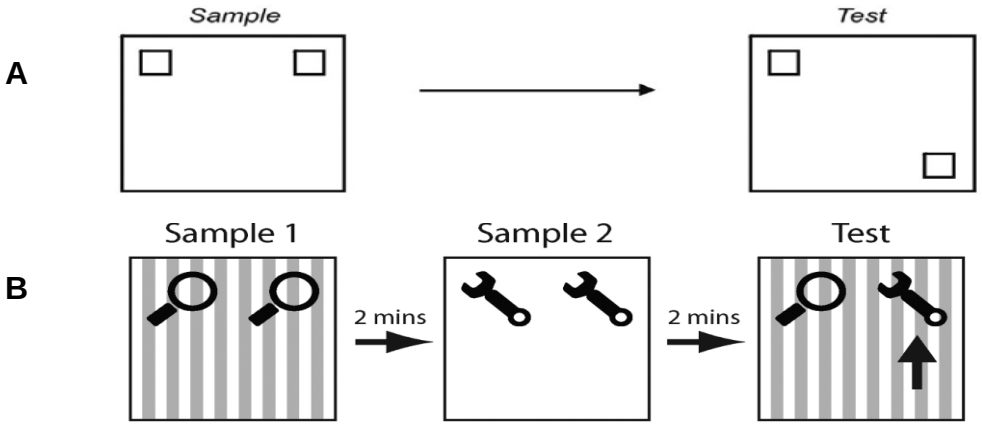
shows that selective hippocampal or parahippocampal lesions result in deficits in recognition memory (Eichenbaum, Yonelinas, and Ranganath 2007).Thus, Hippocampus and parahippocampal cortex may participate in spatial or contextual memory. In contrast, the perirhinal cortex would participate in object-recognition memory.

1.2.2 Object in context and object in place. Two tests for the study of recollection memory

It has been postulated that episodic memory is about happenings in particular places at particular times, or about "*what*" (what is remembered), "*where*" (where is remembered), and "*when*" (where is remembered), that are types of personal report in studies about episodic memory in humans (Tulving, 2002; Dere et al., 2006). This important observation allows studying episodic memory in non-human animals without the need for language. Then, in the case of non-human animals the test that explores some of the dimension of episodic memory in human ("what," "where," and "when") was called "episodic-like memory test" (Clayton et al., 2001; Dere et al., 2006).

Two are the principal tests used to study recollection memory; the *object in context* (OIC) and *object in place* test (OIP) (Figure 5). In the test phase of the object in context task, two objects are presented, one of them is in the same environment as in the sample phase, and the other object is in the different environment to the sample phase. In this test, rodents explore more time the object that is incongruent in the context (Dix and Aggleton, 1999), thus, although both objects were familiar, only one is congruent with the contextual background that was previously learned in sample phase. That indicates sensitivity to the association between a specific object and a particular background. In object in place, task rodents explore more time the object in a

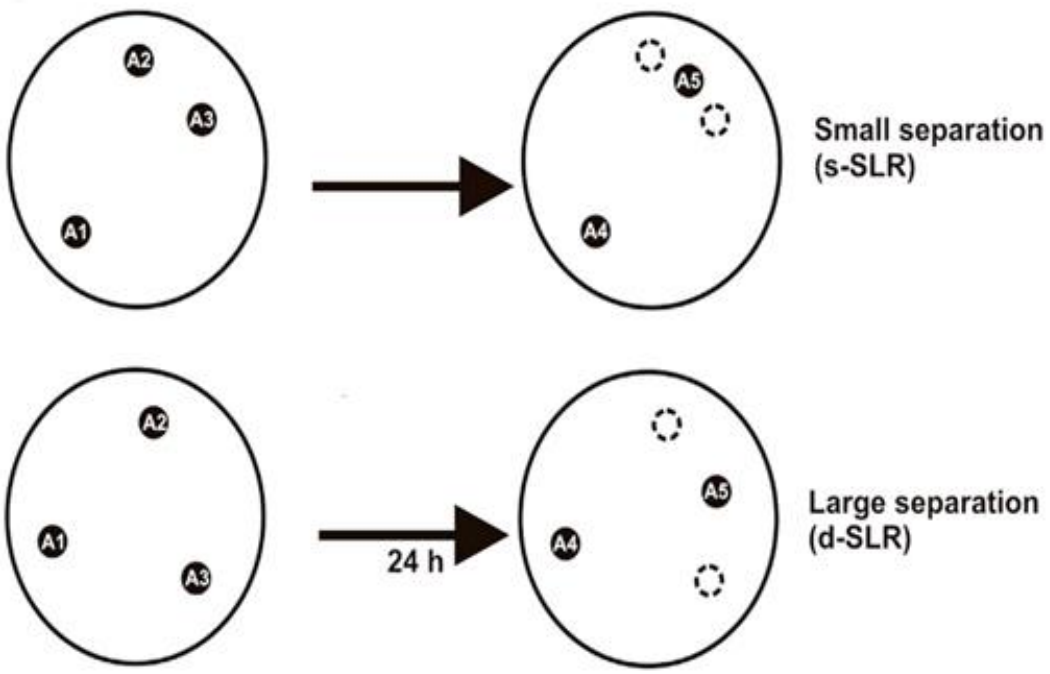
novel position (Nick, 1997; Murai et al., 2007). Because this test is dependent on external cues, i.e it needs allocentric information, this task is considered to be a spatial task (Murai et al., 2007).



5-Recollection task: Object in place and Object in context *task*

Figure 5: In each test the time of exploration of each object during test phase is measured. **A**: in object in place task the position of an object changes **B:** in object in context task on object is congruent with the context while the other is incongruent with the context.

In this thesis, I will adapt "object in context" and "object in place" task to study pattern separation in mice. In the case of "object in place", previous work has done this in rats (Bekinschtein et al., 2013). The authors compare the discrimination performance when the object's separation is small and large (Figure 6). The rationale is that in small separation is more difficult the discrimination, then pattern separation is necessary. In the case of "object in context", to date, no adaptation of this test has been published to study pattern separation. Thus, the adaptation that I did in my thesis will be the first.



6-Spatial pattern separation task

Figure 6: Schematic that show spatial configuration for small and large separation. Left side of cartoon is the sample phase while the right side is the test phase, note that in test phase the discrimination of new position is more difficult in small separation. (extracted from Bekinschtein et al., 2013)

1.3 PATTERN SEPARATION TAKES PLACE IN DG

I mentioned in 1.1.3 that the pattern separation algorithm would increment the differences between similar episodes' representations (O'Reilly and McClelland, 1994; Treves and Rolls, 1994; Yassa and Stark, 2011) Gilbert et.al (2001) studied pattern separation in rats. They studied spatial pattern separation, which happens when it is necessary to discriminate between a new spatial configuration and other similar spatial configuration, previously learned. In their

experiments, rats were trained to displace an object which covered food. Then, in a test phase, two objects were put. Rats had to choose between both objects; one object covered the place previously learned (correct choice), and other object covered another place (incorrect place). The incorrect object's election is interpreted, as that the spatial configuration of the incorrect object is confused with the spatial configuration of the correct object, i.e, the new and older spatial configuration are represented equally, they are not separated. Gilbert et.al studied the performance in rats, whose different regions of the Hippocampus were injured. Rats that were injured in DG showed a higher error percentage than the rats control without lesion or rats with injury in CA1, when two new objects were too close. These results indicate that rats with a lesion in DG do not discriminate the spatial change. The rats with lesion did not do spatial pattern separation.

In another study (Goodrich-hunsaker et al., 2005), which is an example of spatial pattern separation strategy, rats explored a board that contained two objects separated from some distance, after habituation, objects were moved to some distance. The time of exploration was measured in this new context. Rats, that were injured in DG showed shorter exploration time than the rats control without lesion. These results indicate that rats with a lesion in DG do not discriminate the spatial change; the rats with lesion did not do spatial pattern separation.

Another way to study pattern separation is to study context pattern separation. For that purpose, the fear conditioning test has been used. Fear condition is a test where rats or mice receive occasional electric shocks. This causes the rats to become immobile, states that are called “freezes”. This response has an advantage in natural conditions; motionless rodents are more difficult to attack than a moving rodent. It is important to note that this type of memory is not

episodic, rather the “freezes” response is a conditioning response where participate the amygdala and Hippocampus (Phillips and LeDoux, 1992; Maren et al., 1998). Specifically, Hippocampus participates in the codification of contextual information that is present when conditional Stimulus are presented (Phillips and LeDoux, 1992; Maren et al., 1998). Interestingly, when rodents are exposed to a similar context, they present “freezes” responses. This behavioural response is called generalization. McHugh et al studied the performance in the discrimination between a new context and another similar context previously memorized in a fear conditioning test. Rats were conditioned through “fear condition” in a particular context, then the “freezing” was measured in another context highly similar. Rats, in which the activity of DG was impaired after the elimination of the NMDA receptor, showed more “freezing” than rat control. These results indicate that DG is fundamental for discriminating between a new context and a similar context previously memorized (McHugh et al., 2007).Finally, there is evidence in human that recently have shown that patter separation takes place in DG. Some studies have used high-resolution functional magnetic resonance imaging (fMRI) to measure brain activity during incidental memory encoding (Bakker et al., 2008; Lacy et al., 2011). In such studies, subjects viewed a series of pictures, such that, on each trial, a presented object could be either (a) new, (b) repetition of a previously shown object, or (c) a slightly different version of a previously shown object. The results of the study showed that the activity of DG that was triggered for (a) and (c) was highly similar, and activity triggered for (b) was the smallest, i.e., objects that were highly similar were encoded as different objects. The interpretation is that DG amplifies the differences between highly similar objects, thus generates highly different and non-overlapping representations.

1.3.1 Dentate Gyrus network and neuronal types

The first stage in the Hippocampal network is the Dentate Gyrus (DG). There are three layers in DG. From outside to inside, these are the molecular layer, the granular layer, and the Hilus. While the molecular layer is relatively free of cells, in the granular layer the GCs are densely packaged (Amaral et al., 2007) .GCs is a type of glutamatergic cells (Amaral et al., 2007). They have a cone-shaped tree of apical dendrites (figure 7A), where GCs receive inputs from the entorhinal cortex mainly from layer II. On the other hand, GCs give rise to axons called mossy fibers that form synapses with mossy cells, CA3 pyramidal cells, and other GABA-ergic interneurons.

Mossy cell (Figure 7B) is another type of glutamatergic cells (Soriano and Frotscherf, 1994). Mossy cells form synapsis with GCs and with Basket cells, a kind of GABAergic interneurons (Scharfman and Myers, 2012; Scharfman, 2018). Thus, besides controlling only excitation o inhibition, mossy cells control the excitation/inhibition balance onto GCs (Hsu et al., 2016; Hashimotodani et al., 2017).

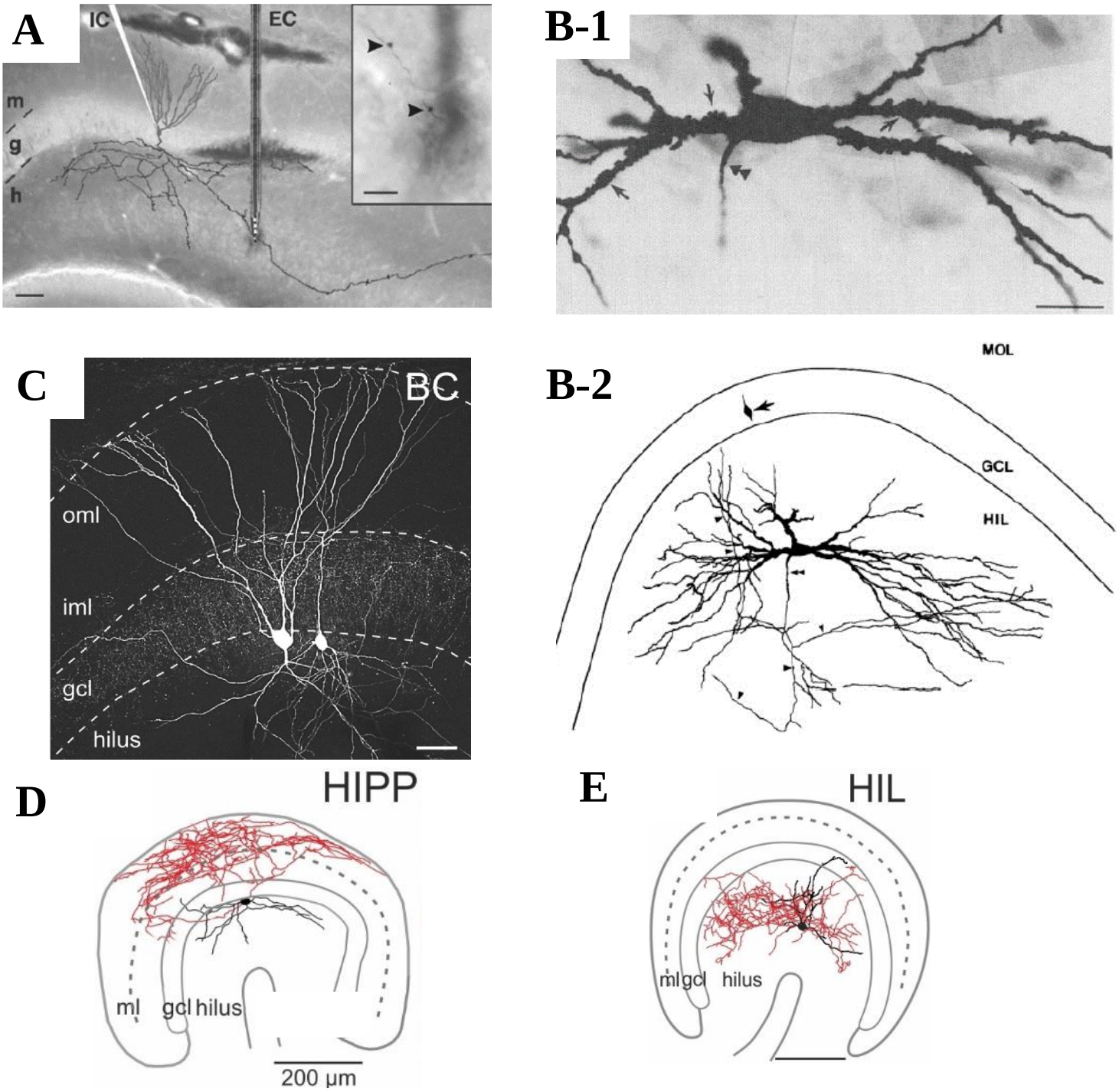
The third type of DG's glutamatergic cells is the adult born granule cells (abGCs) that have several properties that distinguish them from mature granule cells (Marín-burgin et al., 2012). This type of neurons forms synapsis with GABAergic interneurons. Thus it has been suggested that they can control the excitability of GCs (Groisman et al., 2020). A recent article also showed that abGCs could directly inhibit GCs (Luna et al., 2019), Thus abGCs would control the excitability of GCs directly and indirectly.

Other types of neurons are several types of GABAergic interneurons. Regarding this type of neurons, the classifications are not clear. Anatomical and neuro-histochemistry criterion has been used (Freund and Buzsáki, 1996). Considering anatomical characteristic, it is possible to recognize the following neurons: The "*Chandelier cells"* which are interneurons that have characteristic terminal axons within granular cell layer, its dendrites are in the molecular layer that indicates that its principal input originates from the perforant and the commissural-associational pathway. Thus these interneurons participate in feedforward inhibition of GCs (Freund and Buzsáki, 1996). "Basket cells" is a type of interneurons innervating predominantly the perisomatic region of GCs. This interneurons' soma is in deep granule cell layer, and its dendrites are in the hilus and molecular layer. Thus this type of interneurons can inhibit GCs with feedback and feedforward mechanism (Freund and Buzsáki, 1996). Other kinds of interneurons called *"Hilar perforant path-associated cell"* (HIPP) (Figure 7D) has a dendritic tree limited to the hilar region and its axons in the outer two-thirds of the molecular layer. Thus, HIPP interneurons produce feedback inhibition onto GCs. Hilar commissural-associational pathway (HICAP) are other interneurons that innervate the dendritic region of GCs, specifically innervate the inner molecular layer. Due to that, its dendrites are located in the Hilus and in the molecular layer. This type of interneurons can participate in feedback and feedforward inhibition (Freund and Buzsáki, 1996). The third interneurons that inhibit dendritic regions of GCs are the "molecular layer perforant path-associated cell (MOPP). This interneuron has its dendritic trees and axon in the outer molecular layer..

The expression of neuropeptides and Calcium-binding proteins has been used for discriminating between different types of GABA-ergic interneurons. However, this classification is not coincident with the anatomical classification. In Parvoalbumin(PV)-containing interneurons

(Figure 7C), their characteristic is similar to basket and chandelier cells. Thus, all PV-containing interneurons can be classified as basket or chandelier. However, it is not sure if all basket and chandelier contain PV. In fact, it has been shown that some basket cells do not express PV, but express cholecystokinin (CCK) and neuropeptide VIP (Freund and Buzsáki, 1996).

In the case of Somatostatin containing interneurons (SOM+), the first studies suggested that SOM containing interneurons are the HIPP interneurons, however recent article (Yuan et al., 2017) showed that within SOM containing interneurons there are two types of GABA-ergic interneurons. One of them corresponds with HIPP interneurons. The other is a type of GABAergic interneurons, that do not have axons in the molecular layer, have their axons in the hilus, in which they form synapsis with other interneurons like basket cells. For this reason, it was called "*Hilus associated interneurons*" (HIL) (Figure 7E) . HIL interneurons form long-range connections to the medial septum. That indicates that this interneuron communicates with extrahippocampal regions. Interestingly, the SOM+ interneurons do not receive inhibition of other interneurons (Savanthrapadian et al., 2014; Ramaswamy, 2015), this suggest that SOM interneurons can control the activity of all interneurons.



7-Neuronal types of DG

Figure 7: A: GCs (scale bar = 50 um; extracted from Acsady and Kali 2007). **B1:** Mossy cells, **B2:** reconstruction from B1. (scale bar= 12.5 um extracted from Scharfman 1995) **C**: PV-containing cells (scale bar= 20 um; extracted from Savanthrapadian et.al 2014) **D:** SOM+ HIPP interneurons (scale bar= 200 um; extracted from Yuan.et al 2017) **E:** SOM+ HIL interneurons (scale bar= 200 um; extracted from Yuan.et al 2017). Molecular layer (**m** in A, **mol** in B2, **oml and iml** in C, **ml** in D and E). Granular layer (**g** in A; **GCL** in B2; **gcl** in C,D and E). HILUS (**h** in A; **HIL** in B-2; **hilus** in C, D, E)

1.4 PATTERN SEPARATION MECHANISM

In 1970 D. Marr proposed (Marr.D, 1971) that episodic memory would be established in CA3, due to the recurrent collaterals of CA3 . The axons of pyramidal cells in CA3 that project to CA1, have extensive collaterals that contact at the same pyramidal cells of CA3, thus in CA3 there are extensive recurrent collaterals (Ishizuka et al., 1990). Marr proposed that multimodal activity arrives to DG from the entorhinal cortex. Then GCs of DG send its activity to CA3. Finally, the extensive recurrent collaterals of pyramidal cells would allow the formation of episodic memory, because recurrent collaterals would allow associations among multimodal activity.

1.4.1 The relevance of low excitability and orthogonalization

In the model of Marr (Marr.D, 1971), DG does not do a particular transformation of the activity that comes from the entorhinal cortex. However, several investigators (Treves and Rolls, 1992; Rolls and Kesner, 2006; Rolls, 2013) have proposed that Marr's model has problems in explaining the formation of episodic memory completely. The most evident problem is storage capacity. The pyramidal cell number is not infinite; therefore, if many pyramidal neurons represent an episode, few episodes could be represented. The investigators propose (Treves and Rolls, 1992; Rolls and Kesner, 2006; Rolls, 2013) that for a correct formation of episodic memory in CA3 DG must transform the activity that comes from the entorhinal cortex, in such a way that few pyramidal neurons represent an episode, i.e., sparse coding. This type of coding allows two things. On the one hand, it produces a large memory capacity and, on the other hand, allows not superimposed representations of different memories. If the activity of DG were high, many GCs would be activated. Then the sparse coding would be affected (Rolls, 2013). Thus,

keep low excitability of GCs in DG would be the mechanism that would allow that few pyramidal cells represent an episode.

The other problem in Marr's model is the possibility of a catastrophic interference (Mcclelland et al., 1995). Two similar episodes will be represented for a highly similar pattern of activity in the entorhinal cortex. If DG does not transform the activity that comes from the entorhinal cortex before that the activity arrives in CA3, then the representation of two episodic memory will be highly similar. Thus, the new memory may distort or remove the previous memory. The investigators propose (Treves and Rolls, 1992; Rolls and Kesner, 2006; Rolls, 2013) that for a correct formation of episodic memory in CA3 is necessary that DG transform the activity that comes from the entorhinal cortex. Specifically, they propose that DG increment the differences between the representation of similar episodes. Thus, the similar pattern activity of an episode previously memorized would be represented for a population of GCs completely different from the previously selected population. This mechanism is called the orthogonalization.

1.4.2 Evidence that low excitability and orthogonalization of the GCs are mechanisms that happen in DG and are necessary for pattern separation

Jung.M et.al in 1993 showed that GCs of DG of rats have lower firing than pyramidal neurons of CA1, which is almost independent if they are explored or not (Jung and Mcnaughton, 1993). Posterior studies (Nitz and McNaughton, 2004) have shown that DG interneurons have higher activity than other hippocampal interneurons. On the other hand, dendrites of GCs have several features that favour the low excitability (Krueppel et al., 2011). Interestingly, some studies have shown that there is a relation between high excitability and poor performance in pattern separation task. In one study (Jinde et al., 2012), a type of interneurons of DG was eliminated

in rats. Then, the excitability of the GCs, increased and deficits occurred in context pattern separation. Thus, the correct performance in context pattern separation is correlated with the GCs' lower excitability. Regarding the orthogonalization mechanism, there is evidence showing that minimal changes in the shapes of the environment in which rats are exploring can substantially affect the population of GCs that respond (Leutgeb et al., 2007; Neunuebel and Knierim, 2014). That evidence suggests that similar episodes would be represented for different GCs; however, its direct relation whit pattern separation task has not been shown.

1.4.3 Does SOM+ interneurons participate in pattern separation?

Interestingly, this role has not been investigated, until now, despite several shreds of evidence indicating that SOM+ have properties that agree with a role in pattern separation.

First, SOM+ extend its axon through the outer molecular layer (Freund and Buzsáki, 1996), precisely where inputs of the lateral entorhinal cortex (LEC) arrive. Interestingly, it is has been shown that lesion of the LEC impairs the performance in pattern separation tests (Vivar et al., 2012). Thus, SOM+ are ideally positioned to modulate the LEC's influence and, in this way, control pattern separation.

Second, the axons of the SOM+ interneurons spread over a distance of 2.0 mm in the molecular layer, i.e., approximately 2/3 of the longitudinal extent of the molecular layer (Han et al., 1993), thus the activity of the one SOM+ would affect several and distant GCs. This property would allow that SOM+ interneurons mediate a lateral inhibition that has been postulated how an essential mechanism for pattern separation.

Third, there is a direct relation between memory impairment and selective loss of somatostatin expression in DG; in fact, it has been postulated that memory deficit can be explained by problems in pattern separation (Spiegel et al., 2013). Thus, this evidence suggests that the presence of SOM+ interneurons is necessary for pattern separation.

Fourth, evidence has shown that epilepsy is related to selective loss of the somatostatin containing cells (SOM+), within the Hilus.(Goldberg and Coulter, 2013)One feature of the epilepsy is the high and abnormal activity in DG; thus, in normal conditions, SOM+ would control the system's high activity.

Fifth, the SOM+ interneurons may liberate somatostatin toward synaptic environmental. Studies have shown that somatostatin could control the excitability of the GCs (Baratta et al., 2002; Baraban and Tallent, 2004).

Sixth, in a recent paper (Hofmann et al., 2016), the authors stimulated electrically the perforant pathway (cortical axons that contact with GCs), while SOM+ were optogenetically activated. They showed that the field excitatory postsynaptic potential, which is related to the excitability of GCs, is reduced when SOM+ interneurons are stimulated immediately before electrical stimulation. Thus, this work showed in slice experiments that GCs' excitability is reduced when SOM+ were activated. However, until now, these results have not tested in vivo experiments.

Seventh, in other recent paper (Stefanelli et al., 2016), the authors decreased SOM+ interneurons' activity while mice were subjected to a fear condition test. They showed that when SOM+ were silenced, the number of GCs that are actives in fear condition test increases, which would allow an increase in the superposition between similar engrams.

Finally, in 1996, Mosser (Moser, 1996) showed that, when the rats are exploring a new environment, the peri-somatic inhibition is decreased, while dendritic inhibition is augmented. Liu et.al 2014 (Liu et al., 2014) have shown that interneurons' activity, that contact peri-somatic region of GCs, decreases its probability of discharge with successive stimulations of the terminal of the entorhinal cortex. While the interneurons that reach the dendritic region of granule cell increase its probability of discharging with the same protocol of stimulation. When the stimulation was in high frequency (like when rats explore), only interneurons that contact dendritic elicited high inhibitory post-synaptic potential. Thus, during exploratory behaviour, that is the moment when pattern separation happens, SOM+ interneurons would take control of inhibition in DG.

In this thesis, I investigated if SOM+ interneurons are necessary for pattern separation. For that purpose, I studied how SOM+ inhibition affects DG activity and determined if these effects can affect the performance in pattern separation task. Because SOM+ are not unique interneurons in Dentate Gyrus (DG), experimental approximation chosen was optogenetic. This technic allowed selective and fast manipulation of the SOM+ activity. Transgenic mice were used, which contains the halorhodopsin chloride pump, specifically in SOM+. The halorhodopsin allows chloride ingress within the neurons when these neurons are stimulated with laser light.

2.REFERENCES OF INTRODUCTION

Aimone, J. B., Deng, W., and Gage, F. H. (2011). Resolving New Memories: A Critical Look at the Dentate Gyrus, Adult Neurogenesis, and Pattern Separation. *Neuron* 70, 589–596. doi:10.1016/j.neuron.2011.05.010.

Amaral, D. G., Scharfman, H. E., and Lavenex, P. (2007). The dentate gyrus: fundamental neuroanatomical organization (dentate gyrus for dummies). *Prog Brain Res* 163, 3–22. doi:10.1016/S0079-6123(07)63001-5.

Andersen, P., Morris, R., Amaral, D., Bliss, T., and Okeefe, J. (2007). *the hippocampus book*.

Bakker, a., Kirwan, C. B., Miller, M., and Stark, C. E. L. (2008). Pattern Separation in the Human Hippocampal CA3 and Dentate Gyrus. *Science (80-)* 319, 1640–1642. doi:10.1126/science.1152882.

Baraban, S. C., and Tallent, M. K. (2004). Interneuron Diversity series: Interneuronal neuropeptides - Endogenous regulators of neuronal excitability. *Trends Neurosci* 27, 135–142. doi:10.1016/j.tins.2004.01.008.

Baratta, M. V, Lamp, T., and Tallent, M. K. (2002). Somatostatin depresses long-term potentiation and Ca2+ signaling in mouse dentate gyrus. *J Neurophysiol* 88, 3078–3086. doi:10.1152/jn.00398.2002.

Bekinschtein, P., Kent, B. a., Oomen, C. a., Clemenson, G. D., Gage, F. H., Saksida, L. M., et al. (2013). BDNF in the dentate gyrus is required for consolidation of "pattern-separated" memories_suplementary_information. *J Chem Inf Model* 53, 1689–1699. doi:10.1017/CBO9781107415324.004.

Brown, M. W., and Aggleton, J. P. (2001). Recognition memory: What are the roles of the perirhinal cortex and hippocampus? *Nat Rev Neurosci* 2, 51–61. doi:10.1038/35049064.

Burwell, R. D. (2000). The Parahippocampal Region : Corticocortical. *Ann York Acad Sci*, 25–42.

Bussey, T. J., Muir, J. L., and Aggleton, J. P. (1999). Functionally Dissociating Aspects of Event Memory : the Effects of Combined Perirhinal and Postrhinal Cortex Lesions on Object and Place Memory in the Rat. 19, 495–502.

Clark, R. E., Zola, S. M., and Squire, L. R. (2000). Impaired Recognition Memory in Rats after Damage to the Hippocampus. 20, 8853–8860.

Clayton, N. S., Gri, D. P., Emery, N. J., and Dickinson, A. (2001). Elements of episodic-like memory in animals. *R Soc*. doi:10.1098/rstb.2001.0947.

Dere, E., Huston, J. P. Ã., and Silva, M. A. D. S. (2006). The case for episodic memory in animals. 30, 1206–1224. doi:10.1016/j.neubiorev.2006.09.005.

Dix, S. L., and Aggleton, J. P. (1999). Extending the spontaneous preference test of recognition : evidence of object-location and object-context recognition. *Behav Brain Res* 99, 191–200.

Eichenbaum, H., Yonelinas, A. P., and Ranganath, C. (2007). The Medial Temporal Lobe and Recognition Memory. *Annu Rev Neurosci* 30, 123–152. doi:10.1146/annurev.neuro.30.051606.094328.

Ennaceur;J.Delacour, A. (1988). A new one-trial test for neurobiological studies of memory in rats . 1 " Behavioral data. 31, 47–59.

Ennaceur;Neave;Aggleton (1996). BEHAVIOURAL Neurotoxic lesions of the perirhinal cortex do not mimic the behavioural effects of fornix transection in the rat. 80, 9–25.

Freund, T. F., and Buzsáki, G. (1996). Interneurons of the hippocampus. *Hippocampus* 6, 347–470. doi:10.1002/(SICI)1098-1063(1996)6:4<347::AID-HIPO1>3.0.CO;2-I.

Goldberg, E. M., and Coulter, D. a (2013). Mechanisms of epileptogenesis: a convergence on neural circuit dysfunction. *Nat Rev Neurosci* 14, 337–49. doi:10.1038/nrn3482.

Goodrich-hunsaker, N. J., Hunsaker, M. R., and Kesner, R. P. (2005). Dissociating the role of the parietal cortex and dorsal hippocampus for spatial information. *Behav Neurosci* 119, 1307–1315. doi:10.1037/0735-7044.119.5.1307.

Groisman, A., Yang, S., and Schinder, A. (2020). Differential Coupling of Adult-Born Granule Cells to Article Differential Coupling of Adult-Born Granule Cells to Parvalbumin and Somatostatin Interneurons. *Cell Rep* 30, 202–214. doi:10.1016/j.celrep.2019.12.005.

Han, Z. S., Buhl, E. H., Lörinczi, Z., and Somogyi, P. (1993). A high degree of spatial selectivity in the axonal and dendritic domains of physiologically identified local-circuit neurons in the dentate gyrus of the rat hippocampus. *Eur J Neurosci* 5, 395–410. doi:10.1111/j.1460-9568.1993.tb00507.x.

Hashimotodani, Y., Nasrallah, K., Jensen, K. R., Carrera, D., and Castillo, P. E. (2017). LTP at Hilar Mossy Cell-Dentate Granule Cell Synapses Modulates Dentate Gyrus Output by Increasing Excitation / Inhibition Balance Article LTP at Hilar Mossy Cell-Dentate Granule Cell Synapses Modulates Dentate Gyrus Output by Increasing Excitation / Inhi. *Neuron* 95, 928–943. doi:10.1016/j.neuron.2017.07.028.

Hofmann, G., Balgooyen, L., Mattis, J., Deisseroth, K., and Buckmaster, P. S. (2016). Hilar somatostatin interneuron loss reduces dentate gyrus inhibition in a mouse model of temporal lobe epilepsy. *Epilepsia* 1, n/a-n/a. doi:10.1111/epi.13376.

Hsu, T., Lee, C., Tai, M., and Lien, C. (2016). Differential Recruitment of Dentate Gyrus Interneuron Types by Commissural Versus Perforant Pathways. *Cereb Cortex* 26, 2715–2727. doi:10.1093/cercor/bhv127.

Hunsaker, M. R., and Kesner, R. P. (2013). The operation of pattern separation and pattern completion processes associated with different attributes or domains of memory. *Neurosci Biobehav Rev* 37, 36–58. doi:10.1016/j.neubiorev.2012.09.014.

Ishizuka, N., Weber, J., and Amaral, D. G. (1990). Organization of intrahippocampal projections originating from CA3 pyramidal cells in the rat. *J Comp Neurol* 295, 580–623. doi:10.1002/cne.902950407.

Jinde, S., Zsiros, V., Jiang, Z., Nakao, K., Pickel, J., Kohno, K., et al. (2012). Hilar Mossy Cell Degeneration Causes Transient Dentate Granule Cell Hyperexcitability and Impaired Pattern Separation. *Neuron* 76, 1189–1200. doi:10.1016/j.neuron.2012.10.036.

Jung, M. W., and Mcnaughton, B. L. (1993). Spatial Selectivity of Unit Activity in the Hippocampal Granular Layer. *Hippocampus* 3, 165–182.

Krueppel, R., Remy, S., and Beck, H. (2011). Dendritic integration in hippocampal dentate granule cells. *Neuron* 71, 512–528. doi:10.1016/j.neuron.2011.05.043.

Lacy, J. W., Yassa, M. a, Stark, S. M., Muftuler, L. T., and Stark, C. E. L. (2011). Distinct pattern separation related transfer functions in human CA3/dentate and CA1 revealed using high-resolution fMRI and variable mnemonic similarity. *Learn Mem* 18, 15–18. doi:10.1101/lm.1971111.

Leutgeb, J. K., Leutgeb, S., Moser, M., and Moser, E. I. (2007). Pattern Separation in the Dentate Gyrus and CA3 of the Hippocampus. *Science (80-)* 315.

Liu, Y., Cheng, J., and Lien, C. (2014). Rapid Dynamic Changes of Dendritic Inhibition in the Dentate Gyrus by Presynaptic Activity Patterns. 34, 1344–1357. doi:10.1523/JNEUROSCI.2566-13.2014.

Luna, V. M., Anacker, C., Burghardt, N. S., Khandaker, H., Andreu, V., Millette, A., et al. (2019). Adult-born hippocampal neurons bidirectionally modulate entorhinal inputs into the dentate gyrus. *Science (80-)* 364, 578–583.

Maren, S., Anagnostaras, S. G., and Fanselow, M. S. (1998). The startled seahorse: Is the hippocampus necessary for contextual fear conditioning? *Trends Cogn Sci* 2, 39–42. doi:10.1016/S1364-6613(98)01123-1.

Marín-burgin, A., Mongiat, L. A., Pardi, M. B., and Schinder, A. F. (2012). Unique Processing During a Period of High Excitation/Inhibition Balance in Adult- Born Neurons. *Science (80-)*.

Marr.D (1971). Simple Memory : A Theory for archicortex. *R Soc* 262, 23–81.

Mcclelland, J. L., McNaughton, B. L., and O'Reilly, R. C. (1995). Why there are complementary learning systems in the hippocampus and neocortex: insights from the successes and failures of conectionist models of learning and memory. *Physiol Rev* 302, 419–457.

McHugh, T. J., Jones, M. W., Quinn, J. J., Balthasar, N., Coppari, R., Elmquist, J. K., et al. (2007). Dentate gyrus NMDA receptors mediate rapid pattern separation in the hippocampal network. *Science* 317, 94–99. doi:10.1126/science.1140263.

Moser, E. I. (1996). Altered Inhibition in an Exploration of Dentate Task Granule Cells during

Spatial Learning. *J Neurosci* 16, 1247–1259. Available at: http://www.jneurosci.org/cgi/content/short/16/3/1247.

Murai, T., Okuda, S., Tanaka, T., and Ohta, H. (2007). Characteristics of object location memory in mice: Behavioral and pharmacological studies. *Physiol Behav* 90, 116–124. doi:10.1016/j.physbeh.2006.09.013.

Neunuebel, J. P., and Knierim, J. J. (2014). CA3 retrieves coherent representations from degraded input: Direct evidence for CA3 pattern completion and dentate gyrus pattern separation. *Neuron* 81, 416–427. doi:10.1016/j.neuron.2013.11.017.

Nick, E. (1997). Spontaneous object recognition and object location memory in rats : the effects of lesions in the cingulate cortices , the medial prefrontal cortex , the cingulum bundle and the fornix. 509–519.

Nitz, D., and McNaughton, B. (2004). Differential modulation of CA1 and dentate gyrus interneurons during exploration of novel environments. *J Neurophysiol* 91, 863–872. doi:10.1152/jn.00614.2003.

O'Reilly, R. C., and McClelland, J. L. (1994). Hippocampal conjunctive encoding, storage, and recall: Avoiding a trade-off. *Hippocampus* 4, 661–682. doi:10.1002/hipo.450040605.

Phillips, R. G., and LeDoux, J. E. (1992). Differential Contribution of Amygdala and Hippocampus to Cued and Contextual Fear Conditioning. *Behav Neurosci* 106, 274–285. doi:10.1037/0735-7044.106.2.274.

Ramaswamy, S. (2015). Exciting times for inhibition: GABAergic synaptic transmission in dentate gyrus interneuron networks. *Front Neural Circuits* 9, 9–12. doi:10.3389/fncir.2015.00013.

Rolls, E. T. (2013). The mechanisms for pattern completion and pattern separation in the hippocampus. *Front Syst Neurosci* 7, 1–21. doi:10.3389/fnsys.2013.00074.

Rolls, E. T., and Kesner, R. P. (2006). A computational theory of hippocampal function, and empirical tests of the theory. *Prog Neurobiol* 79, 1–48. doi:10.1016/j.pneurobio.2006.04.005.

Rugg, M. D., and Yonelinas, A. P. (2003). Human recognition memory: A cognitive neuroscience perspective. *Trends Cogn Sci* 7, 313–319. doi:10.1016/S1364-6613(03)00131-1.

Savanthrapadian, S., Meyer, T., Elgueta, C., Booker, S. a, Vida, I., and Bartos, M. (2014). Synaptic properties of SOM- and CCK-expressing cells in dentate gyrus interneuron networks. *J Neurosci* 34, 8197–209. doi:10.1523/JNEUROSCI.5433-13.2014.

Scharfman, H. E. (2018). Advances in understanding hilar mossy cells of the dentate gyrus. *Cell Tissue Res* 373, 643–652.

Scharfman, H. E., and Myers, C. E. (2012). Hilar mossy cells of the dentate gyrus: a historical

perspective. *Front Neural Circuits* 6, 106. doi:10.3389/fncir.2012.00106.

Soriano, E., and Frotscherf, M. (1994). Mossy Cells of the Rat Fascia Dentata Are Glutamate-immunoreactive. *Hippocampus* 4, 65–70.

Spiegel, A. M., Koh, M. T., Vogt, N. M., Rapp, P. R., and Gallagher, M. (2013). Hilar interneuron vulnerability distinguishes aged rats with memory impairment. *J Comp Neurol* 521, 3508–3523. doi:10.1002/cne.23367.

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

Squire, L. R. (2004b). Memory systems of the brain : A brief history and current perspective. *Neurobiol Learn Mem* 82, 171–177. doi:10.1016/j.nlm.2004.06.005.

Squire, L. R. (2009a). The Legacy of Patient H.M. for Neuroscience Larry. 6–9. doi:10.1016/j.neuron.2008.12.023.

Squire, L. R. (2009b). The Legacy of Patient H.M. for Neuroscience Larry. *Neuron* 61, 6–9. doi:10.1016/j.neuron.2008.12.023.

Squire, L. R., Stark, C. E. L., and Clark, R. E. (2004). THE MEDIAL TEMPORAL LOBE. *Annu Rev Neurosci* 27, 279–306. doi:10.1146/annurev.neuro.27.070203.144130.

Squire, L. R., and Wixted, J. T. (2011a). *The Cognitive Neuroscience of Human Memory Since H . M* . doi:10.1146/annurev-neuro-061010-113720.

Squire, L. R., and Wixted, J. T. (2011b). *The Cognitive Neuroscience of Human Memory Since H . M* . doi:10.1146/annurev-neuro-061010-113720.

Stefanelli, T., Bertollini, C., Luscher, C., Muller, D., and Mendez, P. (2016). Hippocampal Somatostatin Interneurons Control the Size of Neuronal Memory Ensembles Article Hippocampal Somatostatin Interneurons Control the Size of Neuronal Memory Ensembles. *Neuron* 89, 1–12. doi:10.1016/j.neuron.2016.01.024.

Treves, a, and Rolls, E. T. (1992). Computational constraints suggest the need for two distinct input systems to the hippocampal CA3 network. *Hippocampus* 2, 189–199. doi:10.1002/hipo.450020209.

Treves, A., and Rolls, E. T. (1994). Computational analysis of the role of the hippocampus in memory. *Hippocampus* 4, 374–391.

Tulving, E. (1972). "Episodic and Semantic memory," in *organization of memory*, 382–402.

Tulving, E. (1993). What Is Episodic Memory ? 2, 67–70.

Tulving, E. (2002). EPISODIC MEMORY: FromMind to Brain. *Annu Rev Psychol* 53, 1–25.

Vivar, C., Potter, M. C., Choi, J., Lee, J. Y., Stringer, T. P., Callaway, E. M., et al. (2012). Monosynaptic inputs to new neurons in the dentate gyrus. *Nat Commun* 3, 1107–1111.

doi:10.1038/ncomms2101.

Watson, C., Paxino, G., and Puelles, L. (2012). *the mouse nervous system*.

Yassa, M. A., and Stark, C. E. L. (2011). Pattern separation in the hippocampus. *Trends Neurosci* 34, 515–525. doi:10.1016/j.tins.2011.06.006.

Yonelinas, A. P. (2001). Components of episodic memory : the contribution of recollection and familiarity. *R Soc*, 1363–1374. doi:10.1098/rstb.2001.0939.

Yuan, M., Meyer, T., Benkowitz, C., Savanthrapadian, S., Ansel-bollepalli, L., Foggetti, A., et al. (2017). Somatostatin-positive interneurons in the dentate gyrus of mice provide local- and long-range septal synaptic inhibition. *Elife* 6, 1–25. doi:10.7554/eLife.21105.

3. HYPOTHESIS AND OBJECTIVES

3.1 General hypothesis

The HIPP have a key role in the mechanisms that allow that pattern separation takes place in DG

3.2 Specific Hypothesis

3.2.1 Specific hypothesis 1:

"Inhibition of the HIPP will produce an increase in the excitability of the granule cells, which, will be noted in increase of firing rate of the granule cells or in the Local Field Potential"

3.2.2 Specific hypothesis 2:

"Inhibition of the HIPP will affect the mechanism that permit that the process of pattern separation takes place in DG. Thus a mouse will have problem in the discrimination between a new spatial configuration and other spatial related configuration, previously learned"

3.3 Specific Objectives

3.3.1 Objective 1:

Establish if optogenetic inhibition of HIPP cells affect the excitability of granule cells

3.3.2 Objective 2:

Establish if inhibition of HIPP cells affect discrimination between a new spatial configuration and other spatial related configuration, previously learned

4. RESULTS

How results of my thesis, in this section, I will show two writings that were sent for their publication. The first work that I will show contains the principal results of my thesis experiments. This work was sent to “Cerebral cortex”. The reviewers requested an immunohistochemical control for its publication. At present, due to the covid-19 pandemic, this experiment is retarded. If you want to see an online version of this works, you can visit.

https://www.biorxiv.org/content/10.1101/830182v1

DOI: 10.1101/830182

The second writing consists of a review that was recently published in “Frontiers in Neural Circuits”. In this review, I discuss how neurophotonics techniques have been used to explain how different neuronal types of Dentate gyrus participate in pattern separation. Specifically, I propose a model, supported by the results of my thesis that show how somatostatin-containing cells in coordination with other neuronal types of Dentate gyrus can participate in pattern separation.

If you want to see an online version of this works, you can visit.

https://www.frontiersin.org/articles/10.3389/fncir.2020.00026/full

DOI: 10.3389/fncir.2020.0002

4.1 Article that was sent to "Cerebral cortex"

Dentate gyrus somatostatin cells are required for contextual discrimination during episodic memory encoding

Cristian Morales*[a], Juan Facundo Morici*[b], Nelson Espinosa[a], Agostina Sacson[b], Ariel Lara-Vasquez[a], M. Alexandra Garcia-Perez[a], Pedro Bekinschtein[b], Noelia V. Weisstaub[b], Pablo Fuentealba[a,c,#]

* CM and JFM contributed equally to this work

[a] Departamento de Psiquiatria, Centro Interdisciplinario de Neurociencia, Pontificia Universidad Catolica de Chile, Santiago, Chile.

[b] Instituto de Neurociencia Cognitiva y Traslacional, Instituto de Neurologia Cognitiva, Consejo Nacional de Investigaciones Cientificas y Tecnicas, Universidad Favaloro, Buenos Aires, Argentina.

[c] Centro de Investigacion en Nanotecnologia y Materiales Avanzados, Pontificia Universidad Catolica de Chile, Santiago, Chile.

Email: pfuentealba@uc.cl

Address: Pontificia Universidad Catolica de Chile, Marcoleta 391, Santiago, Chile.

Telephone: +562 2354 3806

4.1.1 Abstract

Memory systems ought to store and discriminate representations of similar experiences in order to efficiently guide future decisions. This problem is solved by pattern separation, implemented in the dentate gyrus by granule cells to support episodic memory formation. Pattern separation is enabled by tonic inhibitory bombardment generated by multiple GABAergic cell populations that strictly maintain low activity levels in granule cells. Somatostatin-expressing cells are one of those interneuron populations, selectively targeting the distal dendrites of granule cells, where cortical multimodal information reaches the dentate gyrus. Nonetheless, somatostatin cells have very low connection probability and synaptic efficacy with both granule cells and other interneuron types. Hence, the role of somatostatin cells in dentate gyrus circuitry, particularly in the context of pattern separation, remains uncertain. Here, by using optogenetic stimulation and behavioural tasks in mice, we demonstrate that somatostatin cells are required for the acquisition of both contextual and spatial overlapping memories.

Keywords: Hippocampus, Dentate gyrus, Pattern separation, Interneurons, Memory.

4.1.2 Introduction

Episodic memory is made up of a collection of different events which may contain overlapping information. The capacity to discriminate similar memory episodes, which could otherwise be confused, is critical for correct encoding (Colgin et al. 2008), effective retrieval (Dickerson and Eichenbaum 2009; Hulbert and Norman 2015), and avoiding catastrophic interference (Mcclelland and Goddard 1997). Accordingly, disruptions of memory discrimination have been related to cognitive impairment in aging and neuropsychiatric disorders, such as depression or posttraumatic stress disorder (Holden and Gilbert 2012; Kheirbek et al. 2012; Leal and Yassa 2018). In addition, conditions such as aging and epilepsy exhibit episodic memory deficits as very common symptoms, also showing disrupted pattern separation (Lanerolle et al. 1989; Holden et al. 2012; Reyes et al. 2018). Computational models suggest that pattern separation ought to be implemented for efficient discrimination of overlapping memories, by the transformation of similar patterns of inputs into segregated synaptic outputs (McClelland et al. n.d.; Reilly and Mcclelland 1994; Treves and Rolls 1994). Abundant evidence from animal experiments support the hippocampus as the brain locus where pattern separation is implemented, in particular the dentate gyrus, which allows the discrimination of overlapping spatial representations (Gilbert et al. 1998; Clelland et al. 2009; Bekinschtein et al. 2013) and similar contextual fear conditioning memories (T McHugh et al. 2007).

Subsets of granule cells in the dentate gyrus represent memory episodes in their activation patterns (Ramirez et al. 2013, 2014), with similar episodes of experience being stored into distinct non-overlapping activation patterns in a process called orthogonalization (Leutgeb et al. 2007; Deng et al. 2013a; Neunuebel and Knierim 2013), that is essential for pattern separation. Granule cells conform a large neuronal population that remains sparsely active due to strong

inhibitory control provided by local GABAergic inputs (Jung and Mcnaughton 1993; Nitz and Mcnaughton 2004; Danielson, Kaifosh, Zaremba, Lovett-Barron, et al. 2016a). The tight regulation of granule cell excitability is critical (Rolls and Kesner 2006; Rolls 2013), as evidenced by deteriorated pattern separation when the spiking activity of granule cells is experimentally enhanced (Jinde et al. 2013) or when tonic inhibition is decreased. Moreover, tonic inhibition of dentate gyrus granule cells is important for the control of memory interference (Engin et al. 2015). The most significant inhibitory inputs to granule cells arise locally from parvalbumin-expressing (PV) cells that provide massive synaptic inhibition to the perisomatic region (Kepecs and Fishell 2014) and tightly control the spike timing of granule cells (Hu et al. 2014). In addition, somatostatin-expressing neurons (SOM), mostly located in the hilus (Freund and Buzsáki 1996; Yuan et al. 2017), densely innervate the distal dendritic arbor of granule cells (Halasy and Somogyi 1993; Yuan et al. 2017). The dendritic arbor of granule cells is largely distributed across the molecular layer and is impinged by entorhinal inputs massively carrying contextual information (Yuan et al. 2017). Thus, SOM are good candidates to control the excitatory inputs reaching distal dendrites, yet they provide slow, weak and unreliable inhibition upon postsynaptic targets, including granule cells (Espinoza et al. 2018) and other interneuron types (Savanthrapadian et al. 2014a). Thus, the role of SOM in controlling the flow of information in the dentate gyrus circuitry remains elusive. Recent experiments have started to change this view and established the relevance of SOM in the control of excitability of granule cells in vitro (Savanthrapadian et al. 2014a; Hofmann et al. 2016) and the size of neuronal ensembles during memory encoding in vivo (Stefanelli et al. 2016). Taken together, these findings suggest that SOM are ideally positioned to participate in contextual discrimination, possibly through the regulation of pattern separation by controlling the dendritic excitability of

granule cells (Goldberg and Coulter 2013). Hence, we tested the hypothesis that functional dentate gyrus SOM are required for contextual discrimination through the control of granule cells excitability. We found that optogenetic suppression of SOM in the dentate gyrus modulated the firing rate of putative excitatory cells and putative PV interneurons. Moreover, optogenetic stimulation impaired both contextual and spatial discrimination of overlapping recognition memories during task acquisition. Our results suggest that SOM are required for successful pattern separation during episodic memory encoding.

4.1.3 Materials and Methods

Mice were housed at 12 h light/dark cycle at 23°C with food and water ad libitum. Experiments took place during the light phase of the cycle (lights on at 8 AM) in a quiet room located inside the Animal facility with dim light. The experimental protocol for this study was approved by the National Animal Care and Use Committee of the Catholic University of Chile and Favaloro University of Argentina. All experiments were performed on adult (8–30 weeks old) mice.

All experimental procedures were in accordance with institutional regulations of Institutional Animal Care and Use Committee of the Favaloro University, ASP # 49527/15, Argentinian government regulations (SENASAARS617.2002) and in accordance with Comite Etico Cientifico para el Cuidado de Animales y Ambiente (ID 151223006 and CEBA 13-014) of the Pontificia Universidad Catolica de Chile. All efforts were made to minimize the number of animals used and their suffering.

Experimental Animals

For the electrophysiological and optogenetics experiments, three strains of mice were used, C57Bl/6, Ai39(RCL-eNpHR3.0/EYFP) and Sst-IRES-Cre. All transgenic lines were obtained from Jackson laboratories (www.jax.org). We used these strains as controls and refer to them as NpHR− animals throughout the text. Double transgenic animals were obtained from the breeding of SST-IRES-Cre+/+ and Ai39+/- mice, so that they expressed functional Natronomonas pharaonis halorhodopsin (NpHR) exclusively in somatostatin cells. We refer to such animals as NpHR+ throughout the text. For the pharmacological experiments, we use wild-type C57Bl/6 mice from the Pharmacy and Biochemistry School, University of Buenos Aires, Argentina. A total of 95 mice (70 male, 25 female) were used for experiments. We pooled data

between female and male mice as discrimination was not significantly different (Unpaired Student´s t test, t = 0.9290, P = 0.3712).

In vivo electrophysiological recordings under anesthesia

Anesthesia was induced with isoflurane 4% followed by an intraperitoneal injection of urethane (0.8 g/kg). Animals were left to rest for 20 minutes and placed in a homeothermic blanket that maintained the body temperature at 37° throughout the experiment. After 20 minutes, an intraperitoneal injection of ketamine/xylazine (40/4 mg/kg) was applied. When mice exhibited no reflexes were put into a stereotaxic frame. The skin above the skull was surgically removed and a small craniotomy (1 mm) was drilled above the cortex (coordinates 1.5 mm lateromedial, 2.0 anteroposterior (Paxinos and Watson 2007)). In addition, a customized support bar was glued to the skull to release pressure from the ears and mouth, and hold the animal's head in position for recordings. At this time, an intraperitoneal cannula was placed to deliver supplementary doses of urethane each 20 minutes (1/12 of the initial volume). After removing the dura over the cortex, the electrode array was placed on the cortex and carefully descended to the dentate gyrus. The electrode array consisted of a matrix of 32 microelectrodes, 50 um apart. An optical fiber (0.1 mm diameter) was associated with the electrodes. The tip of the fiber was 100 um above the most superficial electrode.

Surgery for chronic implantation and optogenetic stimulation

Mice were anaesthetized with isoflurane (4% induction, 1.5–2% maintenance) and placed on a stereotaxic frame. Temperature was kept at 37° throughout the procedure (1–2 h) using a homeothermic blanket. The skin was incised to expose the skull. Three or two craniotomies

were made with a dental drill for anchoring screws. Additionally, another craniotomy (~1mm) was made above the dentate gyrus bilaterally (anteroposterior -2 mm, mediolateral ± 1.5mm from Bregma). Two optic fibers (diameter 200 um) glued to ceramic ferrules (diameter 230 um) were descended through both craniotomies until reaching the dentate gyrus and fixed in position using dental cement. After surgery, mice received a daily dose of enrofloxacin for 3 days and supplementary analgesia with ketoprofen for 3 days. Animals were allowed 5 days to recover before behavioral training.

Surgery and drug infusions for pharmacological experiments

For pharmacological experiments, mice were deeply anesthetized with ketamine/xylazine (150/6.6 mg/kg) and placed in a stereotaxic frame. The skin was incised to expose the skull. Small craneotomy holes were then drilled and a set of 23 G guide cannulae (0.5 cm long) were implanted bilaterally over the dentate gyrus (anteroposterior -2 mm, mediolateral ±1.5 mm from bregma). Cannulae were fixed to the skull with dental acrylic. At the end of surgery, animals were injected with a single dose of meloxicam (0.33 mg/kg) as analgesic and gentamicin (5 mg/kg) as antibiotic. Behavioral procedures were started 5-7 days after surgery. Infusions were made using a 30 G injection cannula connected to a 10 ul Hamilton syringe. Infusions were made on the training day or test day. Cannulated mice received bilateral 0.3 ul infusions of DNQX or vehicle into the dentate gyrus 15 minutes before each training or test session. DNQX was diluted in physiologic solution to a final concentration of 1.89 ug/ul.

Optogenetic stimulation

For chronic implants, optogenetic stimulation of dentate gyrus somatostatin interneurons was achieved with a 200 um optic fiber (N.A. 0.37) coupled to a green laser (532 nm, Laserglow Techonologies) that provided a total light power of 0.1–60 mW at the fiber tip. Light stimuli consisted of 5 s light pulses each 15 s, and power at the tip of the fiber was set between 5-15 mW.

For acute recordings, optogenetic stimulation was achieved with an optrode , which consisted of an optic fiber (100 um, N.A. 0.22) attached to an array of electrodes, so electrical recordings and optical stimulation could be performed simultaneously on the same site. Light stimuli consisted of 5 s light pulses each 20 s and power at the tip of the fiber was set between in 5-12 mW.

Unit crosscorrelation analysis

Neural activity of dentate gyrus was crosscorrelated with the light pulse. A time window of ± 15 s was defined with point 0 assigned to the light onset. The timestamps of the spikes within the time window were considered as a template and were represented by a vector of spikes relative to t = 0 s, with a time bin of 200 ms and normalized to the basal firing rate of hippocampus neurons. Thus, the central bin of the vector contained the ratio between the number of neural spikes elicited between ± 100 ms and the total number of spikes within the template. Next, the window was shifted to successive light pulses throughout the recording session, and an array of templates was obtained. Then, we calculated the z-score of this crosscorrelogram using the mean and standard deviation obtained, bin-to-bin, from the distribution of 1000 shuffled crosscorrelograms. We classified as excited units, those that presents more than 4 bins

with Z-score larger than 3 during laser presentation. Similarly, we classified as inhibited units, those that presents more than 4 bins with z-score more negative than -3 during laser presentation.

Identification of putative neuron types

We defined laser-inhibited units as somatostatin cells. To identify different types of units within excited cells we adapted a previous analysis (Senzai and Buzsáki 2017). Specifically, the trough-to-peak latency and burst index allows to distinguish between glutamatergic cells and GABAergic interneurons, as glutamatergic neurons have longer trough-to-peak latency and higher burst index than GABAergic interneurons. We performed a similar analysis for units that were responsive to laser stimulation. To measure the trough-to-peak, we calculated, for the mean waveform of each detected unit, the temporal difference between the minimum voltage and the maximum voltage (between the minimum and the end of the waveform). To calculate the burst index, first, we calculated the autocorrelogram of the timestamps of each unit outside laser presentation (i.e.; baseline activity); second, we computed the ratio between the peak of the autocorrelogram in the central bins (-1 to 6 ms) and the mean value of the autocorrelogram (200-300 ms); finally, we calculated the logarithm of such ratio. This allowed us to construct a bidimensional vector for excited units. Then, we used hierarchical cluster analysis to discriminate different type of units. The hierarchical cluster analysis showed three types of cluster, two of them were merged in a new cluster because both had similar properties when were compared with somatostatin interneurons. Finally, we compared the trough-to-peak latency and burst index among somatostatin and two clusters were obtained.

Detection of dentate spikes

Based on LFP activity, we identified the electrodes located in the hilus and filtered activity between 100-249 Hz. Then, we calculated the z-score of the signal using the mean and the standard deviation of the entire LFP recording. Finally, we selected high-frequency events based on amplitude, with 5-7 SD threshold.

Arenas and objects used in behavioral tests

Identical copies of objects made from plastic, glass, or aluminum were used. The height of objects ranged from 4 to 6 cm. All objects were affixed to the floor of the apparatus with an odorless reusable adhesive to prevent them for being displaced during exploration. Objects had no natural relevance for mice as they were not associated with reinforcement. The objects, floor and walls were cleaned with ethanol 10% between sessions. Since no differences were observed in behavioral performance during the experiments between sexes, we pooled animals depending on the genotype or treatment received.

Object-in-context task. Four different contexts were used for these experiments. In the dissimilar condition a rectangular and triangular arena were used. Both had homogenous gray walls constructed from opaque foam board. The rectangular apparatus was 40 cm x 25 cm length x 30 cm high, while the triangular one was 40 cm x 25 cm length x 30 cm high. Both contexts had the same surface area to avoid differences due to the size of the arena. Contextual cues were geometric shapes of different colors. In the similar condition, we used two rectangular arenas made of white opaque foam board. The measures of these arenas were identical to those used for the rectangular arena of the dissimilar condition. Then, the cues presented in both contexts were different in shape but same sizes and colors.

Spontaneous location recognition task. A circular maze of 40 cm diameter with walls 40 cm high was used. Both floor and wall were gray. Three spatial clues were glued at 15 cm over the floor. A video camera and laser cable were mounted above the maze.

Object-in-context task

This behavioral test is composed of three phases that allows the evaluation of the congruency between pairs of context and object (Wilson et al. 2013). During the training phase, animals were exposed to two different object-context associations. These two training sessions were 1 hour apart. The test session was carried out twenty-four hours after the training 2. During this phase, a new copy of each of the objects used during the training phase was presented in one of the arenas. The context to be used during the test phase was randomly selected, preserving similar total proportions. Thus, one of the objects was presented in contextual miss-match, the incongruent object, whereas the other object was not, the congruent object. In this task, novelty arises from the novel combination of object and context. Then, exploratory behavior should be driven by retrieval of a particular conjunctive representation of object and context (Eacott and Norman 2004; Morici, Ciccia, et al. 2015).

Habituation sessions. These sessions were conducted to familiarize animals with the procedure of being exposed to an environment where they could encounter novel objects. On the first day mice were handled and placed in a circular context and allowed to explore for 10 minutes. Thirty minutes later, they were reintroduced in the arena, yet in this case two different objects were placed in the arena. Mice were allowed to freely explore for 5 minutes.

Training sessions. On the first training session, two identical objects (A1 and A2) were placed into one of the arenas (context 1). Animals were placed into the arena and allowed to freely

explore the environment for 10 minutes. At the end of the session, animals were returned to their home cage. After 1 hour, animals were exposed to a second object-context association, different from the first one. For that, mice were placed in a different context (context 2), in which a second pair of objects was present (B1 and B2). Arenas were pseudo-randomly assigned as context 1 or 2.

Test session. During this session animals were re-exposed to previously familiar context-object pairs for 5 minutes. Mice were reintroduced to context 1 or context 2 where they could explore one copy of object A and one copy of object B. Then depending of the context in which this phase takes place, one of the objects presented will be contextually congruent while the other will be contextually incongruent.

Spatial Location recognition text

This behavioral paradigm, as the object-in-context, was comprised by three phases that allowed for the evaluation of spatial location novelty detection (Ennaceur et al. 1997; Warburton et al. 2000). During training sessions animals were exposed to three identical objects placed into a circular arena and were allowed to explore them. The separation angle between two of those objects was manipulated in order to generate conditions with variable levels of spatial-location similarity. During the test session, one of the objects was placed in a familiar location, while another copy of the same object was placed in a new location at the middle point between the two previous objects' locations. The rationale behind the task was that if mice were able to discriminate the two similar spatial locations, their representations should be distinct and resilient to confusion. Thus, mice should show preference to explore the object presented in the novel position during the retrieval phase. However, if the representations of the two similar

locations were not sufficiently segregated, then mice should behave as if the new location was familiar.

Habituation sessions. These sessions were conducted to familiarize animals with the procedure of being exposed to an environment where they could encounter novel objects. During these sessions, animals had the opportunity to generate a spatial map of the environment. To that end, mice were repeatedly exposed to the environment for 10 minutes during four consecutive days.

Training session. During this session, animals were placed in the circular arena where three identical objects had been previously positioned. The angle between two of the objects was changed depending on the variation in use (large, 180 degrees; medium, 120 degrees; or small, 50 degrees). Mice were then allowed to explore the environment for 10 minutes.

Test session. In this session only 2 copies of the previously presented objects were placed in the arena. One of the objects was located in the same position as before, yet the other object was placed in the intermediate position occupied by the two objects in the previous session. This session lasted 5 minutes.

Behavioral Analysis

For each behavioural session we quantified the exploration time of each object. For the test phase, we analyzed the exploration time for every copy of the object using a Matlab plug-in (ID tracker). For the training phase, manual score was performed. For the object-in-context test session we calculated the Discrimination Index (DI) as $t_{incongruent} - t_{congruent} / t_{total\ exploration}$ of the session. For the spontaneous location recognition test, the DI was calculated as $t_{novel\text{-}position} - t_{familiar\text{-}position} / t_{total\ exploration}$. For all experiments, object exploration was defined as the mouse having its nose directed to the object and located at a distance of 2 cm or less. Climbing over or

sitting on the objects was not considered as exploration. Two persons scored independently the videos; one of them was blind to all conditions.

Statistical analysis

Statistical analyses were performed with GraphPad 6.01 and Matlab. We used parametric analysis depending when data distributed normally. Electrophysiological data were analyzed using Kruskal-Wallis followed by Tukey-Kramer multiple comparison test. Behavioral data were analyzed using two-tailed Student's t test or two-tailed Wilcoxon test. For comparisons between two repeated-measured groups two-tail paired Student's T test or two-tailed paired Wilcoxon test were used. For more than three groups, we performed One-way ANOVA followed by Tukey's post-hoc test or Kruskal-Wallis test followed by Dunn's post-hoc test. Two-way ANOVA followed by Tukey's post-test was used when three or more groups were involved. In all cases, P-values were considered statistically significant when smaller than 0.05. All data are presented as the mean ± s.e.m.

Histology and Immunocytochemistry

At the end of electrophysiological recordings and behavioural testing, mice were terminally anesthetized and intracardially perfused with saline followed by 20-min fixation with 4% paraformaldehyde. Brains were extracted and post-fixed in paraformaldehyde for a minimum of 24 h before being transferred to PBS azide and sectioned coronally (70–100 um thickness). Sections were further Nissl-stained. Location of electrode shanks and optical fibers were determined in reference to standard brain atlas coordinates (Paxinos and Watson 2007) under a light transmission microscope.

Spike Sorting

Semiautomatic clustering was performed by KlustaKwik (Harris et al. 2000). This method was applied over the 32 channels of the silicon probe, grouped in 8 pseudo-tetrodes of 4 nearby channels.

4.1.4 Results

Optogenetic suppression of somatostatin cells disrupts dentate gyrus neuronal firing patterns and trigger dentate spikes. In order to establish the effect of locally inhibiting SOM on the dentate gyrus network, we stereotaxically implanted an optrode covering the entire dorsoventral extension of the dentate gyrus (**Fig. S1**) in anesthetized double transgenic mice that selectively expressed the inhibitory halorhodopsin pump (NpHR+) in SOM (Espinosa et al. 2018, 2019). To ensure the simultaneous recording of all three dentate gyrus laminae and optical suppression of hilar SOM (Freund and Buzsáki 1996), the tip of the optical fibre was positioned close to the hippocampal fissure (**Fig. 1A**). Next, we delivered prolonged laser pulses to achieve maximal optogenetic inhibition, reproducing previous experimental protocols (Espinosa et al. 2018, 2019). Control experiments showed that effects were selective for transgenic NpHR+ animals, with little effect on control (NpHR-) mice (**Fig. S1**).

Overall, we found a small proportion of units changing their firing patterns upon laser stimulation in NpHR+ mice. A minor fraction of units (6.3%, n = 111) robustly decreased (median = 57%, IQR = 55%) their activity relatively fast (median = 400 ms, IQR = 200 ms). Hence, such neuronal population was defined as SOM (**Fig. 1C**). Another small group of cells (12 %, n = 203) showed a significant increase in firing rate (median = 87%, IQR 127%), presumably by synaptic disinhibition, with significantly slower kinetics than SOM (median = 2.4 s, IQR = 2 s, $P < 0.001$, **Fig. 1C, Fig. S1**). Dentate gyrus units have been previously classified as glutamatergic or GABAergic based on their in vivo spike duration and bursting patterns (Senzai and Buzsáki 2017). Indeed, excitatory cells, such as mossy cells and granule cells exhibit longer trough-to-peak latency and higher burst index than GABAergic interneurons (Senzai and Buzsáki 2017). Thus, we carried out a similar analysis. We first plotted all laser-

responsive units in a bidimensional space comprised by spike duration and burst index (**Fig. 1D**). By definition, SOM comprised one cluster given their common physiological characteristic of optogenetic inhibition (**Fig. 1D,E**). Within the optically excited units we recognized two different populations. We considered one group of excited units as putative PV cells because of their short spikes, significantly more rapid than the other groups (median = 0.4 ms, IQR = 0.1 ms, $P < 0.001$; **Fig. 1F**). Such short spike duration has been well documented in GABAergic PV cells (Frotscher et al. 1998; Yanagawa et al. 2014; Senzai and Buzsáki 2017) that are synaptically targeted by SOM (Savanthrapadian et al. 2014b). On the other hand, the other cluster of excited units was consistent with the presence of mossy cells and granule cells (M/G). Indeed, M/G units had slower spikes (median = 0.95 ms, IQR = 0.34 ms, $P < 0.001$; **Fig. 1F**) and higher burst index (median = 0.8, IQR = 0.6, $P < 0.001$; **Fig. 1G**) than either PV cells or SOM. Furthermore, there was no significant difference in response latency to optogenetic stimulation between PV cells and M/G units (**Fig. 1H**), but they were both significantly slower than SOM (median = 0.4 s, IRQ = 0.2 s, $P < 0.001$; **Fig. 1H**). These results suggest that a small proportion of both PV cells and M/G units was probably disinhibited upon optogenetic suppression of SOM.

Next, we aimed to evaluate the effect of the modulated synaptic output of SOM onto dentate gyrus oscillatory activity. We noted that at the end of laser pulses a prominent deflection was apparent in the dentate gyrus field potential (**Fig. 1B**) that was probably the result of rebound activity in SOM (**Fig. 1C**). A similar effect has been previously described in the dorsal CA1 area (Royer et al. 2012). We then quantified the spectral distribution of dentate gyrus activity. The LFP frequency spectrum showed a prominent shoulder in the gamma range (30-80 Hz) which then decayed with the characteristic 1/f distribution at higher frequencies ((Freeman et

al. 2000), **Fig. S2**). Interestingly, optogenetic suppression of SOM selectively decreased power in the high-frequency range (100-250 Hz, **Fig. S2**). Dentate spikes are hallmark population patterns exclusive of the dentate gyrus, which due to their large-amplitude, short-duration can be detected in the high-frequency activity range (Bragin et al. 1995). Indeed, high-pass filtering LFP recordings, particularly in the hilar region, evidenced the presence of prominent dentate spikes (**Fig. 1I,J**). Dentate spikes result from massive dendritic depolarization and perisomatic inhibition of granule cells (Bragin et al. 1995; Penttonen et al. 1997). Given that optogenetic suppression of SOM increased the activity of putative granule cells and PV interneurons, that inhibit the perisomatic region of granule cells (Freund and Buzsáki 1996), we reasoned that during optical stimulation the incidence of dentate spikes was likely to increase. Indeed, crosscorrelation analysis between laser pulses and dentate spikes showed a significant increase in their probability of occurrence (**Fig. 1K**). Similarly, their incidence increased when comparing the periods before and after the onset of laser stimulation (**Fig. S2**). Hence, the inhibition of SOM may disinhibit PV interneurons and granule cells, with the concomitant increase of network excitability, reflected in the elevated density of dentate spikes. Altogether, our results suggest that optogenetic inhibition of SOM disrupts local network activity patterns in the dentate gyrus.

Encoding of overlapping contextual memories requires functional glutamatergic transmission and somatostatin cells in the dentate gyrus. Fully functional granule cells are necessary for the discrimination of similar contexts (T McHugh et al. 2007; Danielson, Kaifosh, Zaremba, Losonczy, et al. 2016) as well as the acquisition of novel information. However, the role of other dentate gyrus cell-types is not so well established, particularly interneuron

populations that regulate the spike timing of granule cells. Hence, we investigated the contribution of hilar SOM to behavioural performance in context-dependent memory tasks. To test this idea, we developed a variation of the object-in-context task (Eacott and Norman 2004; Wilson et al. 2013; Morici, Bekinschtein, et al. 2015; Morici, Ciccia, et al. 2015; Morici et al. 2018). We reasoned that discrimination of the novel object-context pairing should recruit the dentate gyrus only when contextual configurations were overlapping. Accordingly, we manipulated contextual information by training animals in the object-in-context task using similar or dissimilar contexts (**Fig. 2A, Fig. S3**). First, we evaluated whether the dentate gyrus was engaged in contextual memory encoding in this test. For this, we blocked glutamatergic transmission during memory encoding by infusing DNQX into the dentate gyrus preceding every training session. Regardless of the object-context configuration, blockade of AMPA receptors did not affect exploratory behaviour during training sessions (**Fig. S4**). Consistent with our hypothesis, blocking dentate gyrus excitatory transmission obliterated discrimination during the test only when contexts were similar (mean = 0.013, SEM = 0.012, $P < 0.001$; **Fig. 2C,E, Fig. S5**). Importantly, the experimental manipulation did not affect total exploration time (**Fig. 2D,F**), suggesting that these results were not due to changes in motivation, awareness, or exploratory behaviour in general. Together, these results suggest that glutamatergic transmission in the dentate gyrus is necessary for the encoding of object-in-context memories only when the contextual information presented is similar, consistent with the observation that pattern separation is engaged only when overlapping spatial representations ought to be discriminated (Gilbert et al. 1998, 2001).

It has also been proposed that the dentate gyrus is recruited during the retrieval of similar representations (Denny et al. 2014; Krasne et al. 2015; Bernier et al. 2017). Therefore, we

manipulated glutamatergic transmission in the dentate gyrus during memory recall. For that purpose, we performed the object-in-context task in the similar condition and infused DNQX into the dentate gyrus 15 minutes before the test session, when memory is retrieved (**Fig. 2G**). In this condition, we did not detect any effect of the drug on discrimination. Interestingly, blocking AMPA receptors influenced behaviour as exploration times were higher for drug-infused animals (mean = 33.83 s, SEM = 1.95 s, $P < 0.001$; **Fig. 2H**). Hence, this result suggests that the dentate gyrus is actively engaged during encoding of similar contextual representations, but not during their retrieval.

Recent evidence suggests that SOM are recruited during fear memory formation (Lovett-Barron et al. 2014; Stefanelli et al. 2016) and may participate in pattern separation by controlling the size of engrams encoding spatial representations (Freund and Buzsáki 1996; Hargreaves et al. 2005; Stefanelli et al. 2016). Consequently, we conducted the object-in-context task in our transgenic mice expressing functional halorhodopsin (NpHR+) in SOM (Espinosa et al. 2018). We bilaterally implanted optic fibres into the dentate gyrus (**Fig. 3B**) in order to activate NpHR during the training sessions (**Fig. 3A**). Laser stimulation did not affect the distribution of object exploration times during training sessions for neither task version (**Fig. 3D,F, Fig. S4**). Nonetheless, optogenetic inhibition of SOM during the training session impaired discrimination during retrieval when contexts were similar (mean = 0.09, SEM = 0.098, $P < 0.01$; **Fig. 3C, Fig. S5**). This effect was specific, as it was not detected for the dissimilar context (**Fig. 3E, Fig. S5**), suggesting that dentate gyrus SOM regulate the encoding of object-in-context memory only when contextual information is similar. Overall, this result suggests that SOM regulate the encoding of contextual recognition memory by controlling excitatory activity in the dentate gyrus.

Discrimination of overlapping spatial configurations is regulated by somatostatin neurons during memory encoding. We showed that SOM can control the encoding of contextual recognition memory. Previous studies have shown the essential role played by granule cells in encoding distinct neural representations of overlapping spatial configurations (Leutgeb et al. 2007; Deng et al. 2013b; Neunuebel and Knierim 2013). Furthermore, inhibition of the lateral entorhinal input disrupts pattern separation in spatial tasks (Vivar et al. 2012). Therefore, we reasoned that hilar SOM may also regulate the encoding of overlapping spatial configurations. To test this, we conducted the spontaneous location recognition task that has been shown to be sensitive to the functional integrity of the dentate gyrus (Bekinschtein et al. 2013). In this task animals explore three identical objects placed in separate locations and are then tested with two objects, with one object placed in a familiar location and another object placed in between the previous two locations (**Fig. 4A**). As the two objects are placed closer together in the training session, it becomes more difficult for mice to discriminate the novel location in the test session (Bekinschtein et al. 2013; Miranda et al. 2017, 2018). Accordingly, we varied the angle between objects during the training sessions in small (50 degrees), medium (120 degrees), or large (180 degrees) separations; and compared the exploration times between the training and test session. We found that mice were able to discriminate correctly the medium and large separations, yet failed to distinguish the small separation (mean = -0.062, SEM = 0.068, $P < 0.05$; **Fig. 4B**). This effect was robustly expressed during the entire test session (**Fig. S7**). These results suggest that the small separation is too ambiguous for mice to be able to discriminate it. Thus, we assessed whether spatial discrimination of the medium configuration relied on the activity of hilar SOM. For that, we chronically implanted bilateral optic fibres on the hippocampal fissure of transgenic NpHR+ mice (**Fig. 4D**) to optogenetically inhibit SOM during memory encoding. Laser

stimulation had no effect on exploratory behaviour during the training sessions (**Fig. S7**). However, optogenetic inhibition of SOM during acquisition selectively impaired spatial discrimination in NpHR+ mice without affecting performance in control NpHR- mice (mean = 0.014, SEM = 0.012, $P < 0.05$; **Fig. 4E**). Importantly, exploratory behaviour of mice was not affected by laser stimulation (**Fig. 4F**). This result suggests that SOM regulate the encoding of spatial recognition memory by controlling neural activity in the dentate gyrus. Overall, our results suggest that SOM can regulate both the reactivity and excitability of granule cells in-vivo (**Fig. S8**).

4.1.5 Discussion

Here, we studied the physiological and behavioral effects of selectively suppressing dentate gyrus SOM during contextual memory acquisition. We found that inhibition of SOM increased the activity of two neuronal populations identified on the basis of their brief spike waveforms and firing patterns. Indeed, inhibitory interneurons, putative PV cells, were identified by their fast spiking pattern and short spikes (Frotscher et al. 1998; Yanagawa et al. 2014; Senzai and Buzsáki 2017); whereas, excitatory neurons, putative granule cells and mossy cells, were recognised by their bursting pattern and slow spikes (Senzai and Buzsáki 2017). Both neuronal populations increased their activity during laser stimulation, probably resulting from synaptic disinhibition (Yuan et al. 2017), which was also consistent with the enhanced incidence of dentate spikes, a hallmark of dentate gyrus synchronous activity (Bragin et al. 1995; Penttonen et al. 1997). Low excitability of granule cells has been proposed as a requirement for efficient orthogonalization of afferent spiking patterns arising in the entorhinal cortex (Rolls 2013). Thus, nonselective increments of activity in granule cells are predicted to alter efficient pattern separation. By performing two types of episodic-like memory tasks, one contextual and another spatial, we reveal that discrimination of similar memories is disrupted by optogenetic inhibition of dentate gyrus SOM.

The hippocampus has been proposed as a key structure for the control of memory expression using contextual information (Preston and Eichenbaum 2013). For this reason, the ability to differentiate overlapping contextual information during encoding should be important to facilitate posterior memory recall (Greene et al. 2013). In this regard, previous studies have typically used the fear conditioning paradigm to characterize the role of the dentate gyrus in encoding overlapping contextual information (T McHugh et al. 2007; Sahay et al. 2011). Given

the emotional valence of this kind of task, the amygdala is likely to be recruited (Zheng et al. 2019), and possibly activate the hippocampus. Indeed, the basolateral amygdala is densely interconnected with the ventral hippocampus (Felix-Ortiz and Tye 2014; Yang and Wang 2017; Ahlgrim and Manns 2019). Thus, engagement of the dentate gyrus is expected when the amygdala is massively activated during aversive memory formation. The dentate gyrus also participates in the differentiation of overlapping object-spatial representations in neutral conditions (Bekinschtein et al. 2013; Miranda et al. 2018). By experimentally manipulating object-context associations we establish here that excitatory activity in the dentate gyrus mediated by AMPA receptors is necessary to discriminate overlapping contextual associations. Our results suggest that the dentate gyrus is involved in the general process of discriminating overlapping spatial-contextual information independently of its emotional valence. Specifically, we show that inhibition of hilar SOM impaired the acquisition of similar contextual and spatial representations, thus suggesting that somatostatin-dependent inhibition of the dentate gyrus is at play in the discrimination of different types of similar episodic memories. During pattern separation, different neuronal engrams represent similar contexts (Deng et al. 2013b). It has been shown that some of the dentate gyrus cells are highly sensitive to small changes in contextual cues (Leutgeb et al. 2007; Danielson, Kaifosh, Zaremba, Lovett-Barron, et al. 2016b), suggesting that dentate gyrus ensembles recognize differences in contextual information with high sensitivity. Importantly, SOM regulate the size of such memory ensembles (Stefanelli et al. 2016), and thus inhibiting SOM is expected to dysregulate both the size and specificity of memory engrams. This hypothesis is supported by the anatomical distribution of SOM that selectively target the most external region of the dendritic trees of granule cells in the outer molecular layer (Freund and Buzsáki 1996), where contextual information is conveyed by

entorhinal inputs (Hargreaves et al. 2005), and damage to those afferents impairs spatial pattern separation (Vivar et al. 2012). Thus, the behavioral impairment resulting from the optogenetic inhibition of SOM could be attributed to deficits in the ability of the dentate gyrus to differentially encode contextual information resulting from impaired pattern separation.

Pattern separation is believed to be critical during contextual memory acquisition, but its contribution to memory recall remains debated. It has been proposed that inhibition and excitation of dentate gyrus circuits play different roles during encoding, consolidation, or recall of overlapping memories (Lee and Kesner 2004; Rolls 2018). This is supported by the blockade of dentate gyrus AMPA and kainite receptors, which impairs the expression of fear memory (Pierson et al. 2015), while the blockade of $GABA_A$ receptors impairs consolidation, yet not the acquisition or retrieval of fear memories (Shahidi et al. 2008). Some theoretical models propose that sparse patterns of neuronal activation in the dentate gyrus guide memory encoding in the CA3 region, whereas memory retrieval is mediated through direct entorhinal inputs to the CA3 region (Rolls and Kesner 2006). However, other studies suggest that the dentate gyrus contributes to both memory encoding and retrieval (Denny et al. 2014; Krasne et al. 2015; Bernier et al. 2017). This is supported by recent studies showing that recall cues can trigger reactivation of neural ensembles active in the dentate gyrus during memory encoding (Liu et al. 2012; Ramirez et al. 2013; Ryan et al. 2015). Interestingly, we found that blockade of dentate gyrus AMPA receptors did not affect the recall of contextual memories. The differences observed could be, at least partly, due to methodological reasons. For example, we pharmacologically blocked AMPA receptors before the test session whereas the above-mentioned studies selectively controlled very specific subsets of dentate gyrus neurons. Hence,

our results support the idea that the role of the dentate gyrus in the process of pattern separation is restricted to the encoding phase, at least, for emotionally-neutral memories.

Feedback inhibition in the dentate gyrus is necessary for appropriate orthogonalization of neuronal ensembles representing similar memory episodes (Mcavoy et al. 2015). SOM receive direct inputs from both mature (Freund and Buzsáki 1996) and newborn (Yang et al. 2019) granule cells. Both granule cell populations have been proposed as drivers of feedback inhibition in pattern separation (Drew et al. 2016; Stefanelli et al. 2016). Several studies proposed a leading role for abGCs (Mcavoy et al. 2015; Drew et al. 2016). Indeed, the activation of abGCs engages inhibitory feedback principally arising from PV interneurons that provide perisomatic inhibition, but not from SOM that provide dendritic inhibition (Temprana et al. 2015). Moreover, ablation of hippocampal neurogenesis induces high excitability in granule cells (Ikrar et al. 2013) and impairs pattern separation (Clelland et al. 2009), supporting the idea that feedback inhibition mediated by PV interneurons controls pattern separation. On the other hand, a recent study suggests that mature granule cells poorly engage PV interneurons and preferentially excite SOM in vivo (Stefanelli et al. 2016). Thus, it is plausible that in pattern separation two parallel circuits are engaged during feedback inhibition. One circuit in which abGCs preferentially recruit PV cells and another circuit where mature granule cells preferentially activate SOM that would control the inhibition of granule cells directly. Since newborn cells are more excitable that granule cells (Marín-burgin et al. 2012), it is possible that feedback activity of SOM is more delayed than feedback inhibition of PV cells. Interestingly, computational models of pattern separation predict little contribution for the inhibitory feedback provided by SOM (Mcavoy et al. 2015). Our results suggest that feedback inhibition provided

by SOM is necessary for pattern separation and it will be interesting that future computational studies consider this parameter when modelling hippocampal networks.

Furthermore, the regulation of orthogonalization of granule cells relies heavily on the perisomatic lateral inhibition provided by parvalbumin cells (Sambandan et al. 2010; Guzman et al. 2019). In turn, lateral inhibition might be controlled by SOM through direct dendritic inhibition of granule cells and perisomatic inhibition of parvalbumin cells (Yuan et al. 2017). When mice explore novel environments, thus forming new memories, or during intense presynaptic activity in the dentate gyrus in vitro, dendritic inhibition is significantly larger than perisomatic inhibition (Moser 1996; Liu et al. 2014). A population of SOM locally innervates fast-spiking PV cells in the dentate gyrus and distally several other cell-types in the septum (Yuan et al. 2017). Consistent with such anatomic connectivity, we found that inhibition of SOM increased the activity of putative PV neurons and granule cells. Moreover, we observed increased incidence of dentate spikes, which results from simultaneous, brief dendritic depolarization and perisomatic inhibition of granule cells (Bragin et al. 1995; Penttonen et al. 1997). Furthermore, SOM synaptically target PV interneurons unidirectionally, with no feedback projection described to date (Acsády et al. 2000; Savanthrapadian et al. 2014b). This synaptic projection regulates both the discharge probability and spike timing of PV neurons (Savanthrapadian et al. 2014b; Yuan et al. 2017). These changes in the dentate gyrus circuit are consistent with memory deficits and disrupted pattern separation taking place in cases where the population of hilar SOM is selectively decreased, such as epilepsy or aging (Holden and Gilbert 2012; Spiegel et al. 2013; Reyes et al. 2018). In summary, our results suggest that SOM regulate general excitability in the dentate gyrus and are required for pattern separation during episodic memory encoding.

4.1.6 Acknowledgments

We thank Maria José Díaz and David Alberto Jaime for their technical assistance. This work was supported by the National Commission for Scientific and Technological Research (CONICYT) scholarship, Chile (to C.M. 21140967); The School of Medicine, Pontificia Universidad Catolica de Chile (to C.M. PMD-02/17), The Programa de Investigacion Asociativa (PIA) Anillos de Ciencia y Tecnologia (to P.F. ACT 1414 and ACT 172121); and Fondecyt Regular grant (to P.F. 1190375); The National Agency of Scientific and Technological Promotion of Argentina (ANPCyT, to N.V.W. PICT 2015-2344) and 2016 LARC-IBRO PROLAB program (to N.V.W.) for Latin American labs cooperation.

4.1.7 References

Acsády L, Katona I, Martínez-Guijarro F, Buzsáki G, Freund T. 2000. Unusual Target Selectivity of Perisomatic Inhibitory Cells in the Hilar Region of the Rat Hippocampus. 20:6907–6919.

Ahlgrim NS, Manns JR. 2019. Optogenetic Stimulation of the Basolateral Amygdala Increased Theta-Modulated Gamma Oscillations in the Hippocampus. Frontiers in Behavioral Neuroscience. 13:1–13.

Bekinschtein P, Kent BA, Oomen CA, Clemenson GD, Gage FH, Saksida LM, Bussey TJ. 2013. BDNF in the Dentate Gyrus Is Required for Consolidation of "' Pattern-Separated "' Memories. CellReports. 5:759–768.

Bernier BE, Lacagnina AF, Ayoub A, Shue F, Zemelman B V, Krasne FB, Drew MR. 2017. Dentate Gyrus Contributes to Retrieval as well as Encoding : Evidence from Context Fear Conditioning , Recall , and Extinction. 37:6359–6371.

Bragin A, Jandó G, Nádasdy Z, Van Landeghem M, Buzsáki G. 1995. Dentate EEG Spikes and Associated Interneuronal Population Bursts in the Hippocampal Hilar Region of the Rat. 73.

Clelland CD, Choi M, Romberg C, Clemenson GD, Fragniere A, Tyers P, Jessberger S, Saksida LM, Barker RA, Gage FH, Bussey TJ. 2009. A functional role for adult hippocampal neurogenesis in spatial pattern separation. Science (New York, NY). 325:210–213.

Colgin LL, Moser EI, Moser M. 2008. Understanding memory through hippocampal remapping. 469–477.

Danielson NB, Kaifosh P, Zaremba JD, Losonczy A, Kheirbek MA, Danielson NB, Kaifosh P, Zaremba JD, Lovett-barron M, Tsai J. 2016. Distinct Contribution of Adult-Born Hippocampal Granule Cells to Context Encoding Article Distinct Contribution of Adult-Born Hippocampal Granule Cells to Context Encoding. 101–112.

Danielson NB, Kaifosh P, Zaremba JD, Lovett-Barron M, Tsai J, Denny CA, Balough EM, Goldberg AR, Drew LJ, Hen R, Losonczy A, Kheirbek MA. 2016a. Distinct Contribution of Adult-Born Hippocampal Granule Cells to Context Encoding. Neuron. 101–112.

Danielson NBB, Kaifosh P, Zaremba JDD, Lovett-Barron M, Tsai J, Denny CAA, Balough EMM, Goldberg ARR, Drew LJJ, Hen R, Losonczy A, Kheirbek MAA. 2016b. Distinct Contribution of Adult-Born Hippocampal Granule Cells to Context Encoding. Neuron. 90:101–112.

Deng W, Mayford M, Gage FH. 2013a. Selection of distinct populations of dentate granule cells in response to inputs as a mechanism for pattern separation in mice. 1–21.

Deng W, Mayford M, Gage FH. 2013b. Selection of distinct populations of dentate granule cells in response to inputs as a mechanism for pattern separation in mice. 1–21.

Denny CA, Kheirbek MA, Alba EL, Tanaka KF, Brachman RA, Laughman KB, Tomm NK, Turi GF, Losonczy A. 2014. Hippocampal Memory Traces Are Differentially Modulated by Experience , Time , and Adult Neurogenesis. 189–201.

Dickerson BC, Eichenbaum H. 2009. The Episodic Memory System : Neurocircuitry and Disorders. Neuropsychopharmacology. 35:86–104.

Drew LJ, Kheirbek MA, Luna VM, Denny CA, Cloidt MA, Wu M V., Jain S, Scharfman HE, Hen R. 2016. Activation of local inhibitory circuits in the dentate gyrus by adult-born neurons. Hippocampus. 26.

Eacott MJ, Norman G. 2004. Integrated Memory for Object , Place , and Context in Rats : A Possible Model of Episodic-Like Memory ? 24:1948–1953.

Engin E, Zarnowska ED, Benke D, Tsvetkov E, Sigal M, Keist R, Bolshakov VY, Pearce RA, Rudolph U. 2015. Tonic Inhibitory control of dentate gyrus granule cells by α5-containing GABAA receptors reduces memory interference. Journal of Neuroscience. 35:13698–13712.

Ennaceur A, Neave N, Aggleton JP. 1997. Spontaneous object recognition and object location memory in rats: The effects of lesions in the cingulate cortices, the medial prefrontal cortex, the cingulum bundle and the fornix. Experimental Brain Research. 113:509–519.

Espinosa N, Alonso A, Ariel L, Fuentealba P. 2019. Basal forebrain somatostatin cells differentially regulate local gamma oscillations and functionally segregate motor and cognitive circuits. 1–12.

Espinosa N, Alonso A, Morales C, Espinosa P, Chávez AE, Fuentealba P. 2018. Basal Forebrain Gating by Somatostatin Neurons Drives Prefrontal Cortical Activity. 1–12.

Espinoza C, Guzman SJ, Zhang X, Jonas P. 2018. Parvalbumin + interneurons obey unique connectivity rules and establish a powerful lateral-inhibition microcircuit in dentate gyrus. Nat Commun. 9:1–10.

Felix-Ortiz AC, Tye KM. 2014. Amygdala Inputs to the Ventral Hippocampus Bidirectionally Modulate Social Behavior. Journal of Neuroscience. 34:586–595.

Freeman WJ, Rogers LJ, Holmes MD, Silbergeld DL. 2000. Spatial spectral analysis of human electrocorticograms including the alpha and gamma bands. Journal of Neuroscience Methods. 95:111–121.

Freund TF, Buzsáki G. 1996. Interneurons of the Hippocampus. 470.

Frotscher M, Spruston N, Frotscher M, Spe- NS. 1998. Specialized Electrophysiological Properties of Anatomically Identified Neurons in the Hilar Region of the Rat Fascia Dentata.

Gilbert PE, Kesner RP, Decoteau WE. 1998. Memory for Spatial Location : Role of the Hippocampus in Mediating Spatial Pattern Separation. 18:804–810.

Gilbert PE, Kesner RP, Lee I. 2001. Dissociating Hippocampal Subregions: A Double Dissociation Between Dentate Gyrus and CA1. 636:626–636.

Goldberg EM, Coulter DA. 2013. Mechanisms of epileptogenesis : a convergence on neural circuit dysfunction. Nature Publishing Group. 14:337–349.

Greene P, Howard M, Bhattacharyya R, Fellous JM. 2013. Hippocampal anatomy supports the use of context in object recognition: A computational model. Computational Intelligence and Neuroscience. 2013.

Guzman S, Schlögl A, Espinoza C, Zhang X, Suter B, Jonas P. 2019. Fast signaling and focal connectivity of PV+ interneurons ensure efficient pattern separation by lateral inhibition in a full-scale dentate gyrus network model.

Halasy K, Somogyi P. 1993. Subdivisions in the multiple GABAergic innervation of granule cells in the dentate gyrus of the rat hippocampus. Eur J Neurosci. 5:411–429.

Hargreaves EL, Rao G, Lee I, Knierim JJ. 2005. Neuroscience: Major dissociation between medial and lateral entorhinal input to dorsal hippocampus. Science. 308:1792–1794.

Harris KD, Henze DA, Csicsvari J, Hirase H, Buzsáki G. 2000. Accuracy of tetrode spike separation as determined by simultaneous intracellular and extracellular measurements. Journal of Neurophysiology. 84:401–414.

Hofmann G, Balgooyen L, Mattis J, Deisseroth K, Buckmaster PS. 2016. Hilar somatostatin interneuron loss reduces dentate gyrus inhibition in a mouse model of temporal lobe epilepsy. 1:1–7.

Holden HM, Gilbert PE. 2012. Less efficient pattern separation may contribute to age-related spatial memory deficits. 4:1–6.

Holden HM, Hoebel C, Loftis K, Gilbert PE, Al HET. 2012. Spatial Pattern Separation in Cognitively Normal Young and Older Adults. 000.

Hu H, Gan J, Jonas P. 2014. Interneurons. Fast-spiking, parvalbumin^{+} GABAergic interneurons: from cellular design to microcircuit function. Science. 345:1255263.

Hulbert JC, Norman KA. 2015. Neural Differentiation Tracks Improved Recall of Competing Memories Following Interleaved Study and Retrieval Practice. 3994–4008.

Ikrar T, Guo N, He K, Besnard A, Levinson S, Hill A, Lee H-K, Hen R, Xu X, Sahay A. 2013. Adult neurogenesis modifies excitability of the dentate gyrus. Frontiers in Neural Circuits. 7:1–15.

Jinde S, Zsiros V, Jiang Z, Nakao K, Pickel J, Kohno K, Belforte JE, Nakazawa K. 2013. Hilar Mossy Cell Degeneration Causes Transient Dentate Granule Cell Hyperexcitability and Impaired Pattern Separation. 76:1189–1200.

Jung MW, Mcnaughton BL. 1993. Spatial Selectivity of Unit Activity in the Hippocampal Granular Layer. 3:165–182.

Kepecs A, Fishell G. 2014. Interneuron Cell Types: Fit to form and formed to fit. Nature. 505:318–326.

Kheirbek MA, Klemenhagen KC, Sahay A, Hen R. 2012. review Neurogenesis and generalization : a new approach to stratify and treat anxiety disorders. 15:1613–1620.

Krasne FB, Cushman JD, Fanselow MS. 2015. A Bayesian context fear learning algorithm / automaton. 9:1–22.

Lanerolle NC De, Kim JH, R RJ, Spencer DD. 1989. Hippocampal interneuron loss and plasticity in human temporal lobe epilepsy. 495:387–395.

Leal SL, Yassa MA. 2018. applications of pattern separation.

Lee I, Kesner RP. 2004. Differential Contributions of Dorsal Hippocampal Subregions to Memory Acquisition and Retrieval in Contextual Fear-Conditioning. 310:301–310.

Leutgeb JK, Leutgeb S, Moser M, Moser EI. 2007. Pattern Separation in the Dentate Gyrus and CA3 of the Hippocampus. 315.

Liu X, Ramirez S, Pang PT, Puryear CB, Govindarajan A, Tonegawa S, Sciences C, Sciences B. 2012. Optogenetic stimulation of a hippocampal engram activates fear memory recall. 484:381–385.

Liu Y, Cheng J, Lien C. 2014. Rapid Dynamic Changes of Dendritic Inhibition in the Dentate Gyrus by Presynaptic Activity Patterns. 34:1344–1357.

Lovett-Barron M, Kaifosh P, Kheirbek M, Danielson N, Zaremba J, Reardon T, Turi G, Hen R, Zemelman B, Losonczy A. 2014. Dendritic Inhibition in the Hippocampus Supports Fear Learning. 1:857–864.

Marín-burgin A, Mongiat LA, Pardi MB, Schinder AF. 2012. Unique Processing During a Period of High Excitation/Inhibition Balance in Adult- Born Neurons.

Mcavoy K, Besnard A, Sahay A. 2015. Adult hippocampal neurogenesis and pattern separation in DG : a role for feedback inhibition in modulating sparseness to govern population-based coding. 9:1–7.

McClelland J, McNaughton B, O´Reilly R. n.d. Why there are Complementary Learning Systems in the Hippocampus and Neocortex: Insights from the Successes and Failures of connectionist models of Learninf and Memory.

Mcclelland JL, Goddard NH. 1997. Considerations Arising From a Complementary Learning Systems Perspective on Hippocampus and Neocortex. 665:654–665.

Miranda M, Kent B, Facundo Morici J, Gallo F, Weisstaub N V., Saksida LM, Bussey TJ, Bekinschtein P. 2017. Molecular Mechanisms in Perirhinal Cortex Selectively Necessary for

Discrimination of Overlapping Memories, but Independent of Memory Persistence. Eneuro. 4:ENEURO.0293-17.2017.
Miranda M, Kent BA, Morici JF, Gallo F, Saksida LM, Bussey TJ, Weisstaub N, Bekinschtein P. 2018. NMDA receptors and BDNF are necessary for discrimination of overlapping spatial and non-spatial memories in perirhinal cortex and hippocampus. Neurobiology of Learning and Memory. 155:337–343.
Morici JF, Bekinschtein P, Weisstaub N V. 2015. Medial Prefrontal Cortex Role in Recognition Memory in Rodents. Elsevier BV.
Morici JF, Ciccia L, Malleret G, Gingrich JA, Bekinschtein P, Weisstaub N V. 2015. Serotonin 2a receptor and serotonin 1a receptor interact within the medial prefrontal cortex during recognition memory in mice. Frontiers in Pharmacology. 6:1–12.
Morici JF, Miranda M, Gallo FT, Zanoni B, Bekinschtein P, Weisstaub N V. 2018. 5-HT2a receptor in mPFC influences context-guided reconsolidation of object memory in perirhinal cortex. eLife. 7:e33746.
Moser E. 1996. Altered Inhibition in an Exploration of Dentate Task Granule Cells during Spatial Learning. 76.
Neunuebel JP, Knierim JJ. 2013. CA3 Retrieves Coherent Representations from Degraded Input : Direct Evidence for CA3 Pattern Completion and Dentate Gyrus Pattern Separation. Neuron. 81:416–427.
Nitz D, Mcnaughton B. 2004. Differential Modulation of CA1 and Dentate Gyrus Interneurons During Exploration of Novel Environments. 863–872.
Paxinos G, Watson C. 2007. The rat brain in Stereotaxic coordinates. 123Library.
Penttonen M, Kamondi A, Sik A. 1997. Feed-Forward and Feed-Back Activation of the Dentate Gyrus In Vivo During Dentate Spikes and Sharp Wave Bursts. 450:437–450.
Pierson JL, Pullins SE, Quinn JJ, Al PET. 2015. Dorsal Hippocampus Infusions of CNQX into the Dentate Gyrus Disrupt Expression of Trace Fear Conditioning. 785:779–785.
Preston AR, Eichenbaum H. 2013. Interplay of hippocampus and prefrontal cortex in memory. Current Biology. 23:1–21.
Ramirez S, Liu X, Lin P, Suh J, Pignatelli M, Redondo R, Ryan T, Tonegawa S. 2013. Creating a False Memory in the Hippocampus. 341:387–391.
Ramirez S, Tonegawa S, Liu X. 2014. Identification and optogenetic manipulation of memory engrams in the hippocampus. 7:1–9.
Reilly RCO, Mcclelland JL. 1994. Hippocampal Conjunctive Encoding , Storage , and Recall : Avoiding a Trade-off. 4.
Reyes A, Holden HM, Chang YA, Uttarwar VS, Sheppard DP, Deford NE, Yandall S, Kansal L, Gilbert PE, Mcdonald CR. 2018. Neuropsychologia Impaired spatial pattern separation performance in temporal lobe epilepsy is associated with visuospatial memory de fi cits and hippocampal volume loss. 111:209–215.
Rolls ET. 2013. The mechanisms for pattern completion and pattern separation in the hippocampus. Frontiers in systems neuroscience. 7:74.
Rolls ET. 2018. The storage and recall of memories in the hippocampo-cortical system. 577–604.
Rolls ET, Kesner RP. 2006. A computational theory of hippocampal function , and empirical tests of the theory. 79:1–48.
Royer S, Zemelman B V., Losonczy A, Kim J, Chance F, Magee JC, Buzsáki G. 2012. Control of timing, rate and bursts of hippocampal place cells by dendritic and somatic inhibition. Nature Neuroscience. 15:769–775.

Ryan TJ, Roy DS, Pignatell M, Arons A, Tonegawa S. 2015. Engram cells retain memory under retrograde amnesia. 348:1007–1014.

Sahay A, Scobie K, Hill A, O'Carroll C, Kheirbek M, Burghardt N, Fenton A, Dranovsky A, Hen R. 2011. Increasing adult hippocampal neurogenesis is sufficient to improve pattern separation. 3–10.

Sambandan S, Sauer J, Vida I, Bartos M. 2010. Associative Plasticity at Excitatory Synapses Facilitates Recruitment of Fast-Spiking Interneurons in the Dentate Gyrus. 30:11826–11837.

Savanthrapadian S, Meyer T, Elgueta C, Booker SA, Vida I, Bartos M. 2014a. Synaptic Properties of SOM- and CCK-Expressing Cells in Dentate Gyrus Interneuron Networks. J Neurosci. 34:8197–8209.

Savanthrapadian S, Meyer T, Elgueta C, Booker SA, Vida I, Bartos M. 2014b. Synaptic Properties of SOM- and CCK-Expressing Cells in Dentate Gyrus Interneuron Networks. 34:8197–8209.

Senzai Y, Buzsáki G. 2017. Physiological Properties and Behavioral Correlates of Hippocampal Granule Cells and Mossy Cells Article Physiological Properties and Behavioral Correlates of Hippocampal Granule Cells and Mossy Cells. 691–704.

Shahidi S, Komaki A, Mahmoodi M, Lashgari R. 2008. The role of GABAergic transmission in the dentate gyrus on acquisition , consolidation and retrieval of an inhibitory avoidance learning and memory task in the rat. 04:1–7.

Spiegel AM, Koh MT, Vogt NM, Rapp PR, Gallagher M. 2013. Hilar Interneuron Vulnerability Distinguishes Aged Rats With Memory Impairment. 3523:3508–3523.

Stefanelli T, Bertollini C, Muller D, Stefanelli T, Bertollini C, Lu C. 2016. Hippocampal Somatostatin Interneurons Control the Size of Neuronal Memory Ensembles Article Hippocampal Somatostatin Interneurons Control the Size of Neuronal Memory Ensembles. 1–12.

T McHugh, Jones M, Quinn J, Balthasar N, Coppari R, Elmquist J, Lowell B, Fanselow M, Wilson M, Tonegawa S. 2007. Dentate Gyrus NMDA Receptors Mediate Rapid Pattern Separation in the Hippocampal Network. 317:94–100.

Temprana SG, Mongiat LA, Lanuza GM, Schinder AF, Temprana SG, Mongiat LA, Yang SM, Trinchero MF, Alvarez DD, Kropff E. 2015. Delayed Coupling to Feedback Inhibition during a Critical Period for the Integration of Adult-Born Granule Cells Article Delayed Coupling to Feedback Inhibition during a Critical Period for the Integration of Adult-Born Granule Cells. Neuron. 85:116–130.

Treves A, Rolls ET. 1994. Computational Analysis of the Role of the Hippocampus in Memory. 4:374–391.

Vivar C, Potter MC, Choi J, Lee JY, Stringer TP, Callaway EM, Gage FH, Suh H, Van Praag H. 2012. Monosynaptic inputs to new neurons in the dentate gyrus. Nature Communications. 3:1107–1111.

Warburton EC, Baird AL, Morgan A, Muir JL, Aggleton JP. 2000. Disconnecting hippocampal projections to the anterior thalamus produces deficits on tests of spatial memory in rats. European Journal of Neuroscience. 12:1714–1726.

Wilson DIG, Langston RF, Schlesiger MI, Wagner M, Watanabe S, Ainge JA. 2013. Lateral Entorhinal Cortex is Critical for Novel Object-Context Recognition. 366:352–366.

Yanagawa Y, Obata K, Vida I, Hosp JA, Str M, Jonas P, Bartos M. 2014. Morpho-physiological Criteria Divide Dentate Gyrus Interneurons into Classes. 203:189–203.

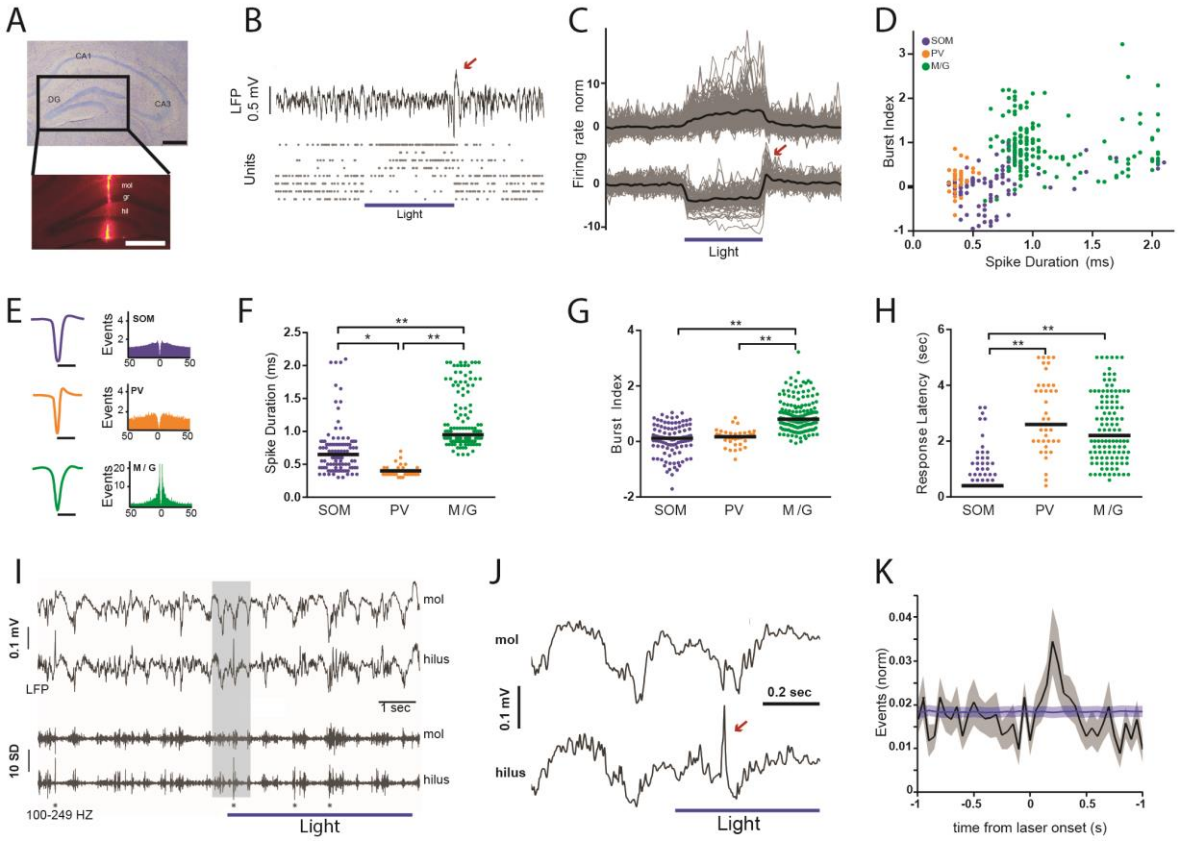
Yang S, Groisman A, Schinder A. 2019. Modulation of adult-born neuron excitability by coupling to GABAergic networks.

Yang Y, Wang J-Z. 2017. From Structure to Behavior in Basolateral Amygdala-Hippocampus Circuits. Frontiers in Neural Circuits. 11:1–8.

Yuan M, Meyer T, Benkowitz C, Savanthrapadian S, Ansel-bollepalli L, Foggetti A, Wulff P, Alcami P, Elgueta C, Bartos M. 2017. Somatostatin-positive interneurons in the dentate gyrus of mice provide local- and long-range septal synaptic inhibition. 1–25.

Zheng J, Stevenson RF, Mander BA, Knight RT, Yassa MA, Lin JJ, Zheng J, Stevenson RF, Mander BA, Mnatsakanyan L, Hsu FPK, Vadera S. 2019. Multiplexing of Theta and Alpha Rhythms in the Amygdala-Hippocampal Circuit Supports Pattern Separation of Emotional Information Article Multiplexing of Theta and Alpha Rhythms in the Amygdala-Hippocampal Circuit Supports Pattern Separation of Emotional I. Neuron. 1–12.

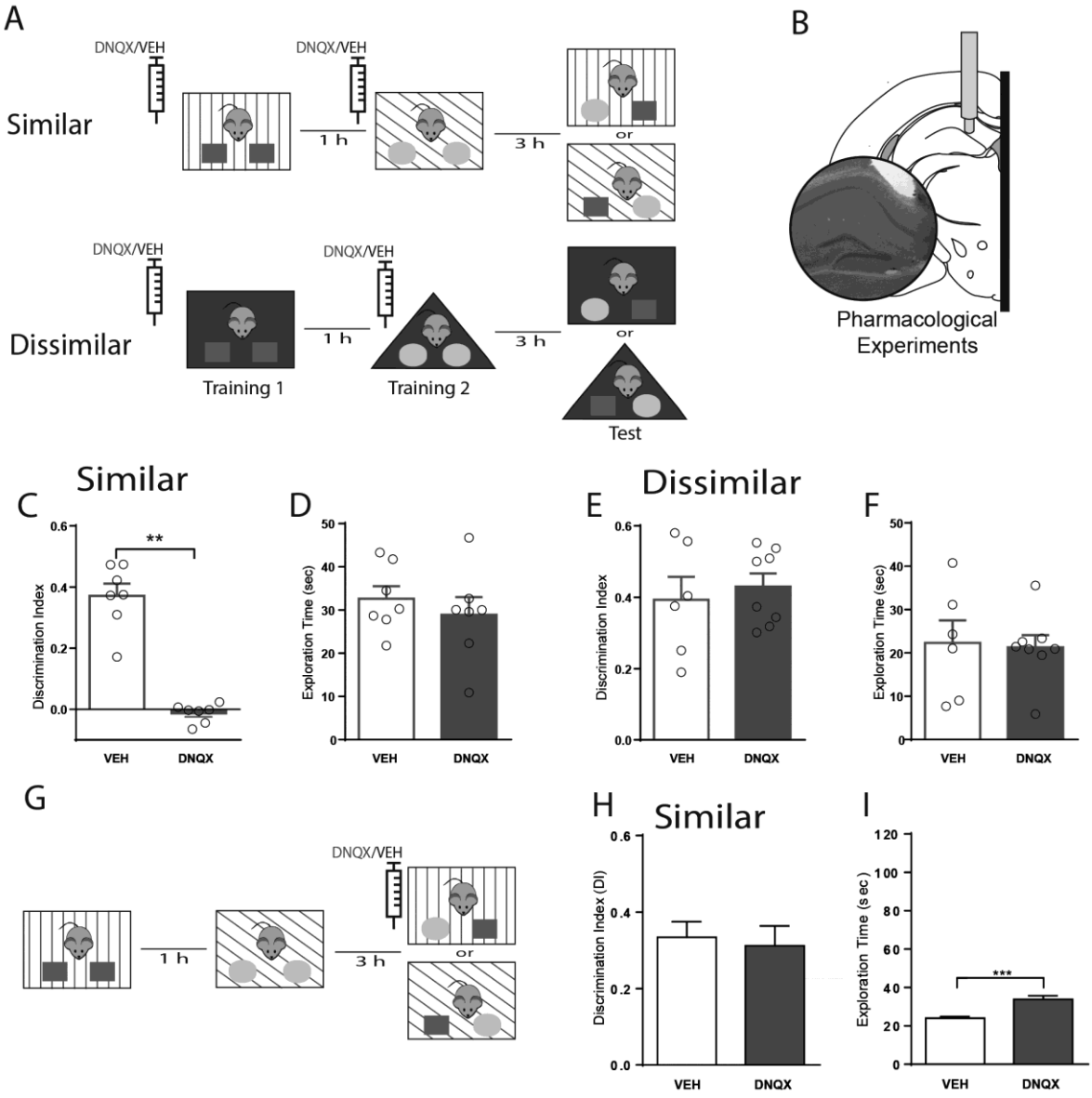
4.1.8 Figures



8-Optogenetic inhibition of hilar somatostatin cells disrupts local network activity patterns in the dentate gyrus.

Figure 1: A, Nissl-stained coronal brain section of dorsal hippocampus showing different regions, CA1, CA3 and dentate gyrus (DG). Scale bar, 500 µm. Inset, fluorescence micrography of the DG with optrode track stained with DiI. mol, molecular layer; gr., granular layer; hil., hilus. Scale bar, 500 µm. **B,** field potential and unit activity from the DG shown in (A). Horizontal bar depicts laser stimulation (5 sec-pulse delivered every 15 sec, 12 mW, fiber diameter 100 um). Top, LFP (filtered 1 Hz − 4 kHz); bottom, raster plots for eight simultaneously recorded cells. Note that during laser stimulation the four upper units increased their firing rate whereas the four lower units decreased thei firing rate. Red arrow in B and C depicts rebound activity at the end of the laser pulse. **C,** normalized discharge probability of responsive units during laser stimulation. Horizontal bar depicts laser stimulation (5–12 mW). Top, excited units (average increase = 12 %, n = 203). Bottom, inhibited units (average decrease = 6.3 %, n = 111). Black line, population average; gray lines, individual units. **D,** scatter plot showing the distribution of spike duration (trough-to-peak latency) and burst index for all laser-responsive units. SOM, putative somatostatin-expressing units; PV, putative parvalbumin-expressing units; M/G, putative mossy or granule cells. **E,** average waveforms and autocorrelograms for neuronal populations shown in (D). **F,** comparison of the spike duration

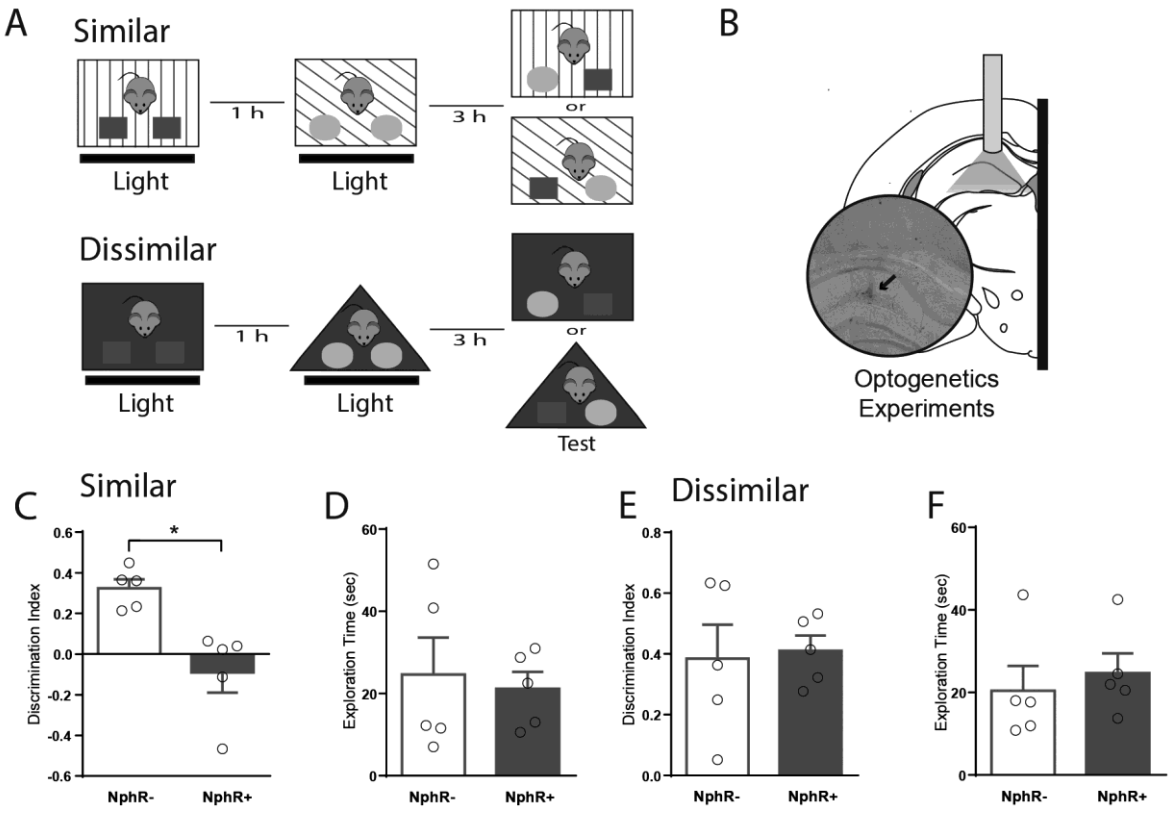
between unit clusters. Lines, population median; points, individual units. Kruskal-Wallis test, $P = 4.95x10^{-37}$; Tukey-Kramer multiple comparison test, *, $P = 3.48x10^{-5}$; **, $P = 9.56x10^{-10}$. **G,** comparison of burst index between unit clusters. Kruskal-Wallis test, $P = 2.82x10^{-32}$; Tukey-Kramer multiple comparison test, **, $P = 9.56x10^{-10}$. Lines, population median; points, individual units. **H,** comparison of response latency between unit clusters. Kruskal-Wallis test, $P = 1.12x10^{-37}$; Tukey-Kramer multiple comparison test, **, $P = 9.56x10^{-10}$. Lines, population median; points, individual units. **I,** top traces show examples of molecular and hilar LFP activity. Bottom traces show the same periods filtered for high-frequency activity (100-249 Hz). Asterisks depict dentate spikes. Shaded gray region is expanded in the next panel (J). **J,** arrow depicts an example dentate spike. **K,** crosscorrelogram between laser onset and dentate spikes. Black line, data average (n = 34 recording sites); gray area, data standard error; blue line, shuffling average (1000 iterations), blue area, shuffling standard error. Note significant increase in the relative density of dentate spikes during the first part of optical stimulation.



9-Encoding, but not retrieval, of overlapping contextual memories requires functional glutamatergic transmission the dentate gyrus.

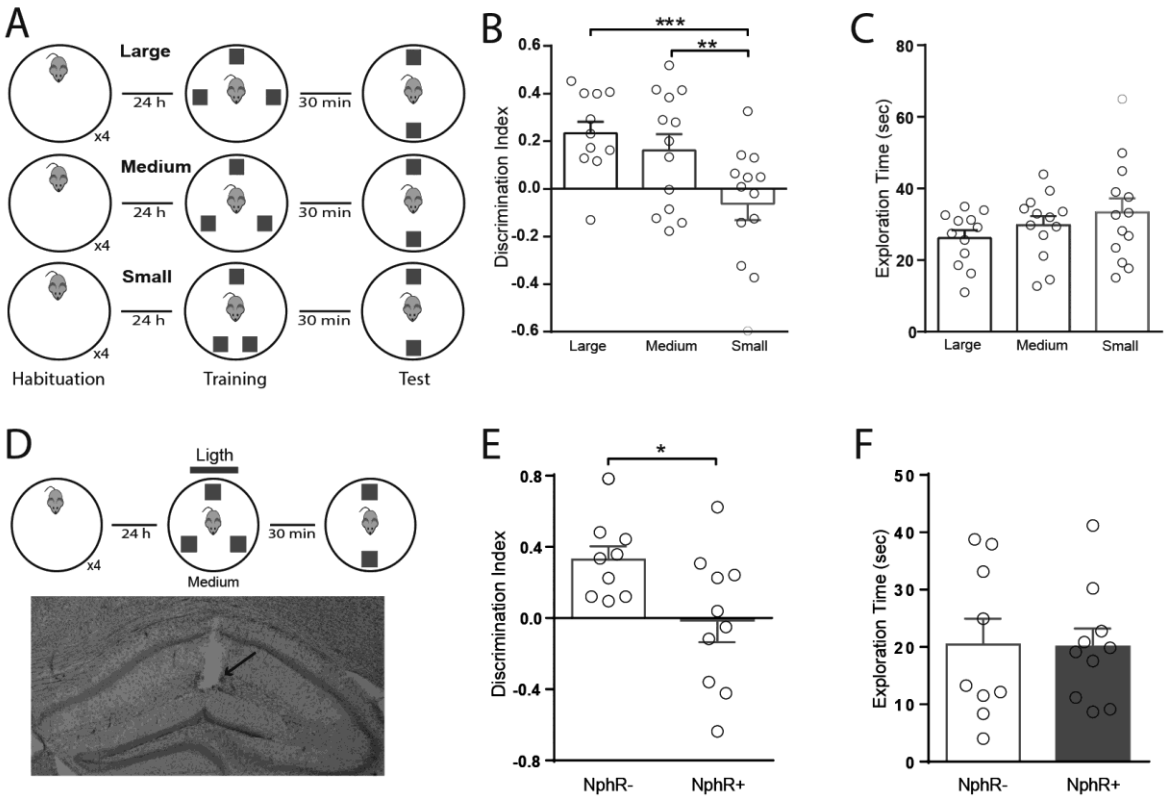
Figure 2.: A, schematic representation of the two versions of the object-in-context behavioural test used to assess context discrimination. Pharmacological experiments were performed by locally injecting either DNQX (1.89 µg/µl, 0.3 ul) or vehicle (veh., saline 0.3 µl) preceding every training session, when memory was encoded. **B,** anatomical representation of the hippocampus locations targeted during pharmacological experiments. Inset, histological verification was performed with cresyl violet staining. Discrimination Index (DI) accumulated over the entire behavioural test (5 minutes) for animals during pharmacological probing for both versions of the behavioural test, similar (**C**) and dissimilar (**E**). Note a significant effect only in the similar condition. Total exploration time accumulated over the entire behavioural test (5

minutes) for animals during pharmacological probing was not affected during either the similar (**D**) or dissimilar (**F**) condition. Data is represented as population mean ± SEM and individual animals (circles). Total exploration time during the training phase. **G,** schematic representation of the object-in-context behavioural test used in the similar condition to test memory retrieval. **H,** Discrimination Index during the retrieval phase. **I,** total exploration time during the retrieval phase. Unpaired Student´s t test; 2C, **, $P = 8.74x10^{-7}$; 2D, P = 0.56; 2E, P = 0.61; 2F, P = 0.43; 2H, P = 0.74; 2I, *, P = 0.0008.



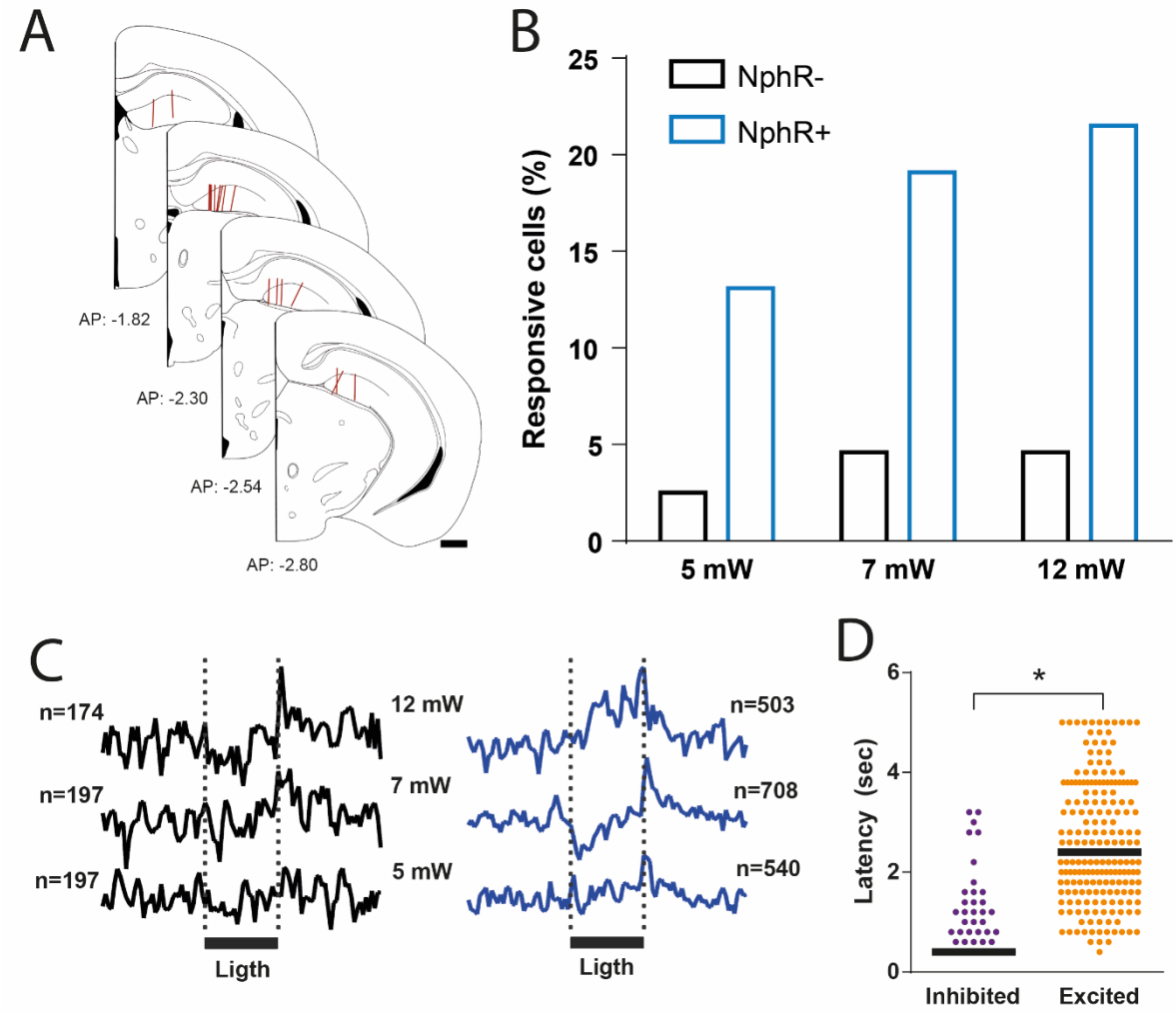
10- Encoding of overlapping contextual memories requires somatostatin cells in the dentate gyrus.

Figure 3. :A, schematic representation of the two versions of the object-in-context behavioural test used to assess context discrimination. **B,** anatomical representation of the hippocampus locations targeted during optogenetic experiments. Black arrow indicates the location of fibber tip. Histological verification was performed with cresyl violet staining. Optogenetic experiments were performed in either transgenic mice expressing functional halorhodopsin (NpHR+) or control mice lacking functional halorhodopsin (NpHR-) optically stimulated (5 sec-pulse every 15 sec, 15 mW) during both training sessions. Discrimination Index (DI) accumulated over the entire behavioural test (5 minutes) for animals during optogenetic probing for both versions of the behavioural test, similar (**C**) and dissimilar (**E**). Note a significant effect only in the similar condition. Total exploration time accumulated over the entire behavioural test (5 minutes) for both versions of the behavioural test was not affected (**D, F**). Data is represented as population mean ± SEM and individual animals (circles). Unpaired Student´s t test; 2C, *, P = 0.005; 2D, P = 0.75; 2E, P = 0.84; 2F, P = 0.63



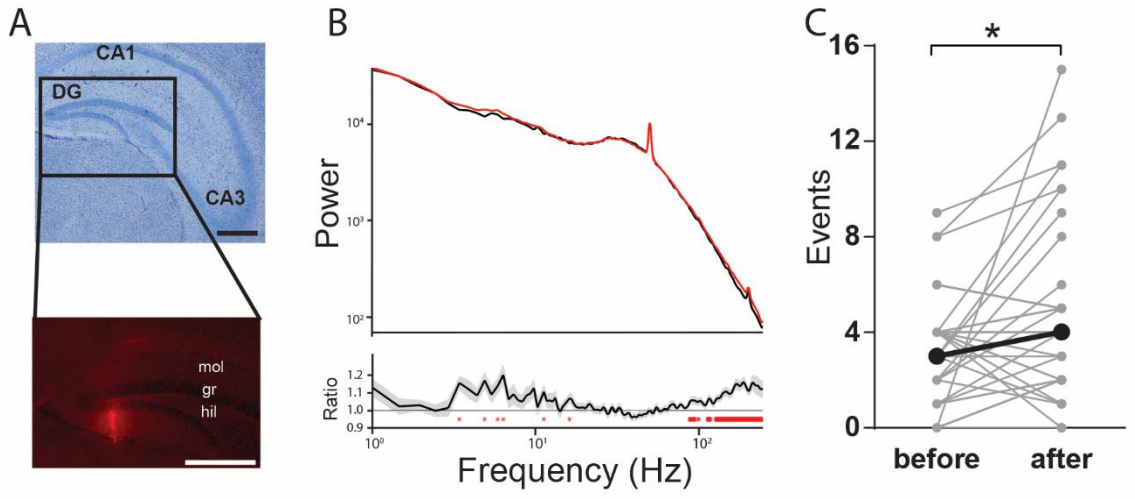
11-Dentate gyrus somatostatin cells regulate the encoding of spatial recognition memory.

Figure 4. : A, schematic representation of the spontaneous location recognition test used in three versions (large = 180°, medium = 120°, and small = 50° separation) to assess spatial memory. Note that angular distance between B and C objects change in training. **B,** discrimination Index (DI) accumulated over the entire behavioural test (5 minutes) for animals performing the three different versions. Note that only the small separation is not discriminated. One-way ANOVA, P = 0.0057; Tukey post-hoc test; **, P = 0.039; ***, P = 0.006. **C,** total exploration time of test objects accumulated over the entire session for every version of the test, One-way ANOVA, P = 0.26. **D,** schematic representation of the medium version of the spontaneous location recognition test combined with optogenetic stimulations (blue line; 5 sec-pulse every 15 sec, 15 mW) delivered during training. Bottom, coronal Nissl-stained coronal hippocampus section showing the track of the chronically implanted optic fibber (arrow). **E,** discrimination Index (DI) accumulated over the entire behavioural test for animals performing the intermediate version of the spontaneous location recognition test combined with optogenetic stimulation. Unpaired Student´s t test; *, P= 0.03. **F,** total exploration time of test objects accumulated over the complete session. Unpaired Student´s t test; P = 0.94.



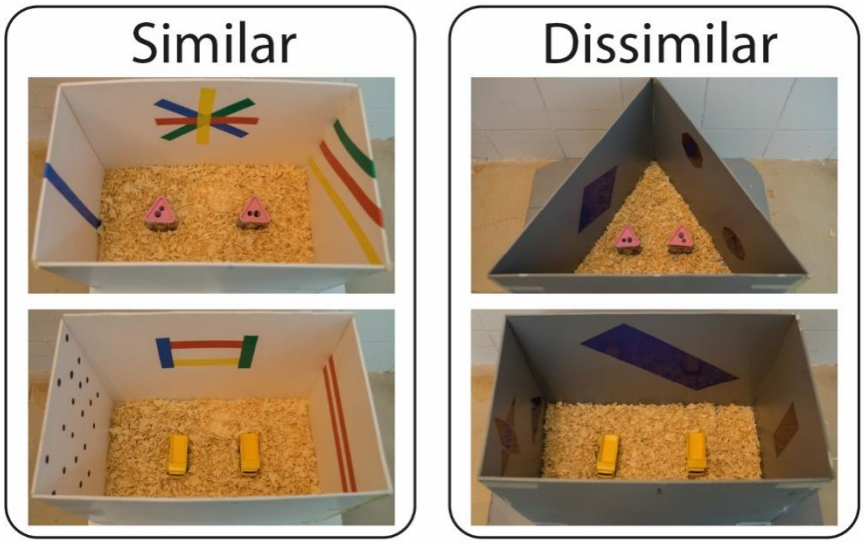
12-Neural spiking responses to optogenetic stimulation in the hippocampus.

Supplementary Figure 1. A, Anatomical tracks (red lines) of optrodes positioned in the dentate gyrus. Scale bar, 500 µm. **B,** Proportion of units responsive to optogenetic stimulation at different power intensities in transgenic mice expressing halorhodopsin (NphR+) or control mice (NphR-). Proportions were significantly different between NphR+ or NphR- mice. X^2 test; 5 mW, $P < 10^{-4}$; 7 mW, $P < 10^{-6}$; 12 mW, $P < 10^{-6}$. **C,** Normalized average discharge probability for all recorded units in NphR- mice (black lines) and NphR+ mice (blue line). Black horizontal bar depicts laser stimulation (5 seconds, fiber diameter 100 um). **D,** Response latency of hippocampus units to laser stimulation. Latency was significantly larger in excited units than inhibited units (Wilcoxon rank sum test, * $p = 7{,}8\ 10^{-39}$), thus suggesting different synaptic connectivity.



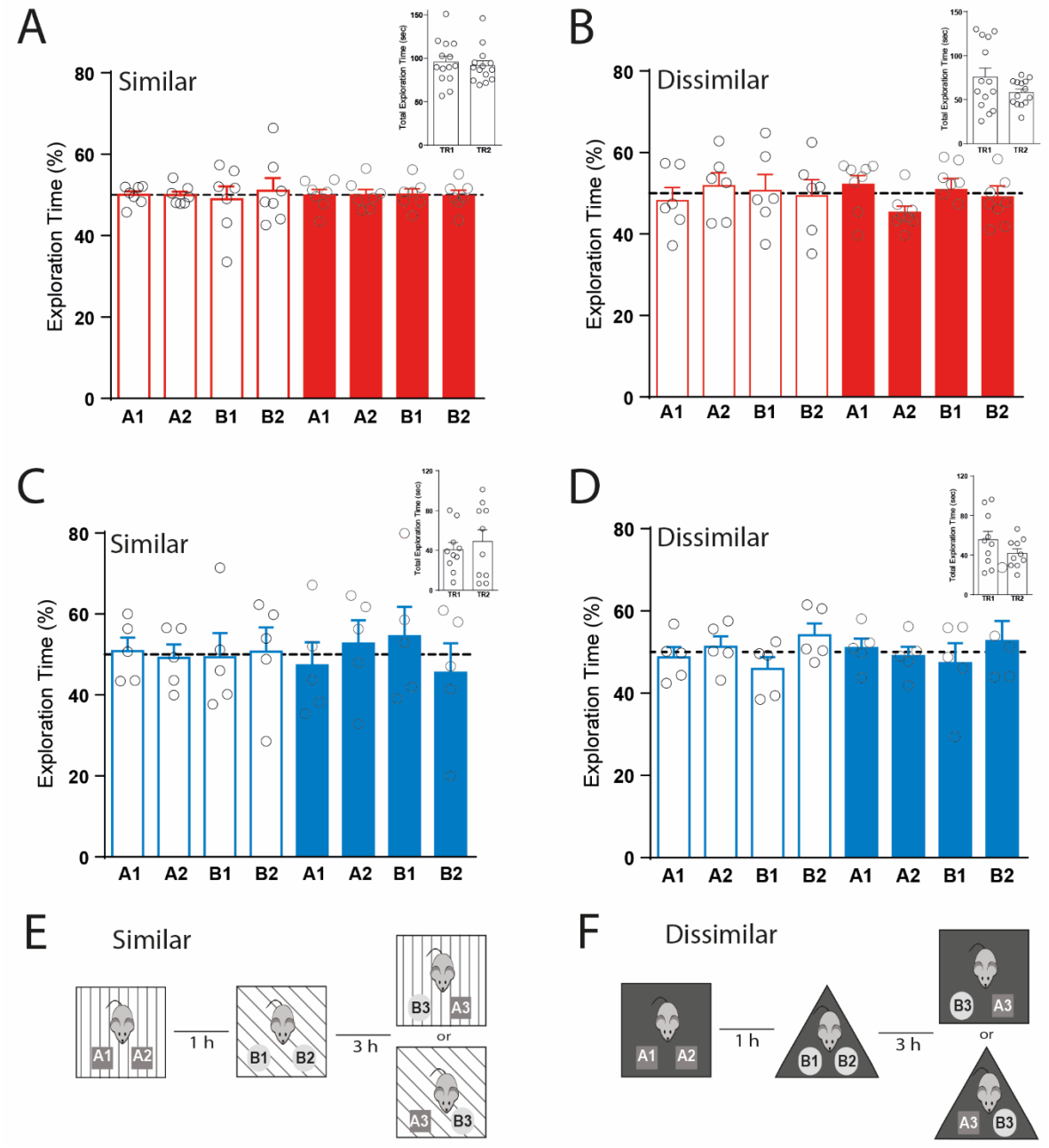
13-Field potential changes in response to optogenetic stimulation in the hippocampus.

Supplementary Figure 2. A, Nissl-stained brain section of dorsal hippocampus showing different regions, CA1, CA3 and dentate gyrus (DG). Scale bar, 500 µm. Inset: fluorescence micrography of the DG with optrode track stained with DiI. mol, molecular layer; gr., granular layer; hil., hilus. Scale bar, 500 µm. **B,** Power spectral distribution of granular layer activity during light-on periods (red line) contrasted with light-off periods (black line). Bottom, ratio between light-on periods (red line) and light-off periods (black line) shows significant differences in high-frequency activity (100-250 Hz). $p < 0.05$, false discovery rate; red asterisks. **C,** Dentate spike counts during light-on and light-off periods of optogenetic stimulation. Wilcoxon paired test, * p = 0,017.



14-Arenas and objects used for similar (s-OIC) and dissimilar (d-OIC) object-in-context behavioural tests.

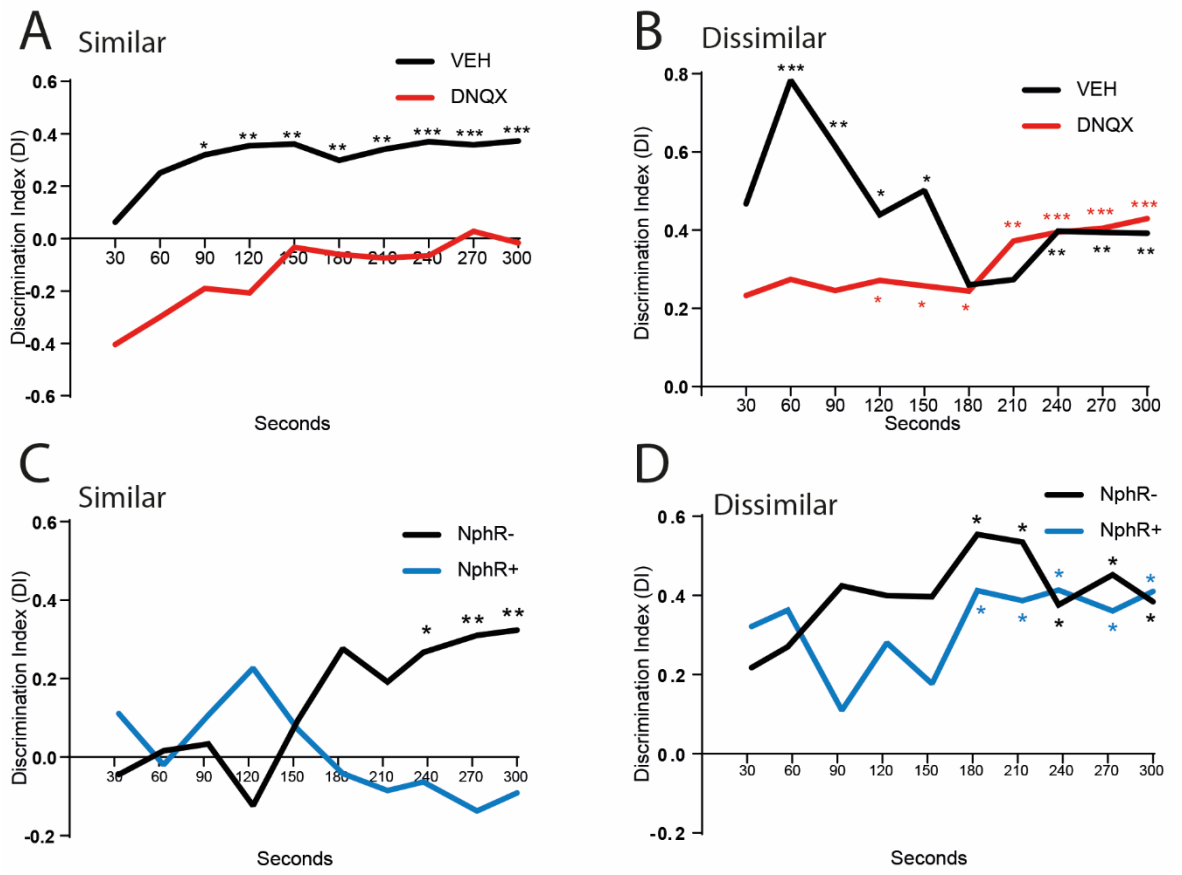
Supplementary Figure 3. The rectangular apparatus was 40 cm x 25 cm length x 30 cm high, while the triangular one was 40 cm x 25 cm length x 30 cm high



15-Exploratory behaviour during training sessions in the object-in-context test.

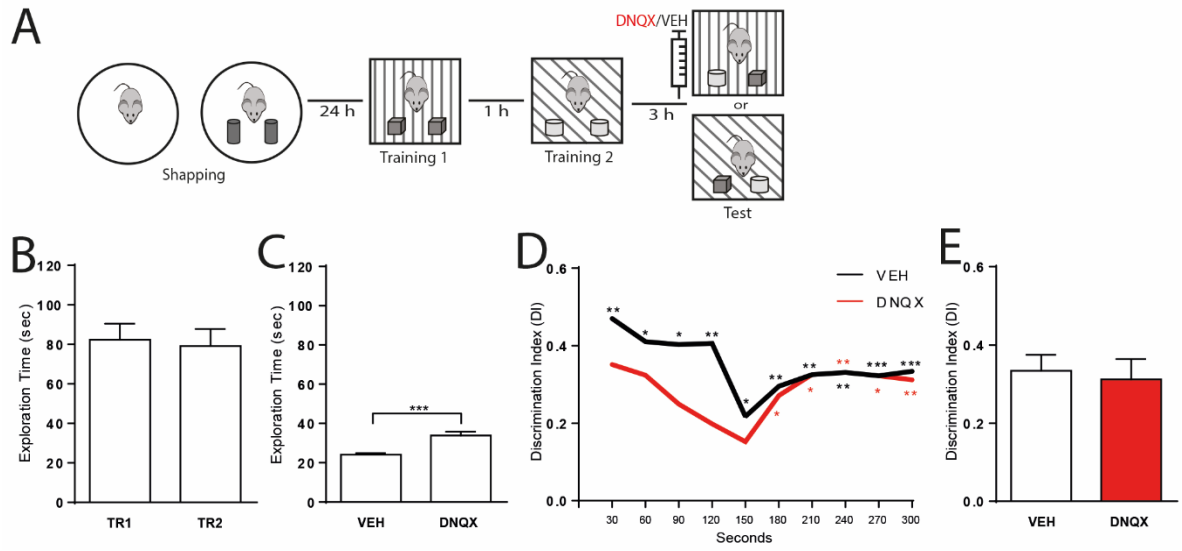
Supplementary Figure 4. Fraction of time spent in the exploration of each object during training sessions in pharmacological (red) and optogenetic (blue) experiments. Empty bars represent control groups (vehicle in red, NphR- in blue), while solid bars represent experimental groups (DNQX, red; NphR+, blue). **A** and **C** show similar object-in-context tests, and **B** and **D** show dissimilar object-in-context tests. Paired two-way ANOVA; A $p > 0,05$; B $p > 0,05$; C p

> 0,05; D p > 0,05. Insets show the total exploration times of objects during the first (TR1) and the second (TR2) training sessions. Inset : Paired Student´s t test; A p = 0,45; B p = 0,16; C p = 0,49; D p = 0,18. **E** and **F** show schematic representation of the two versions of the object-in-context behavioural test used to assess context discrimination



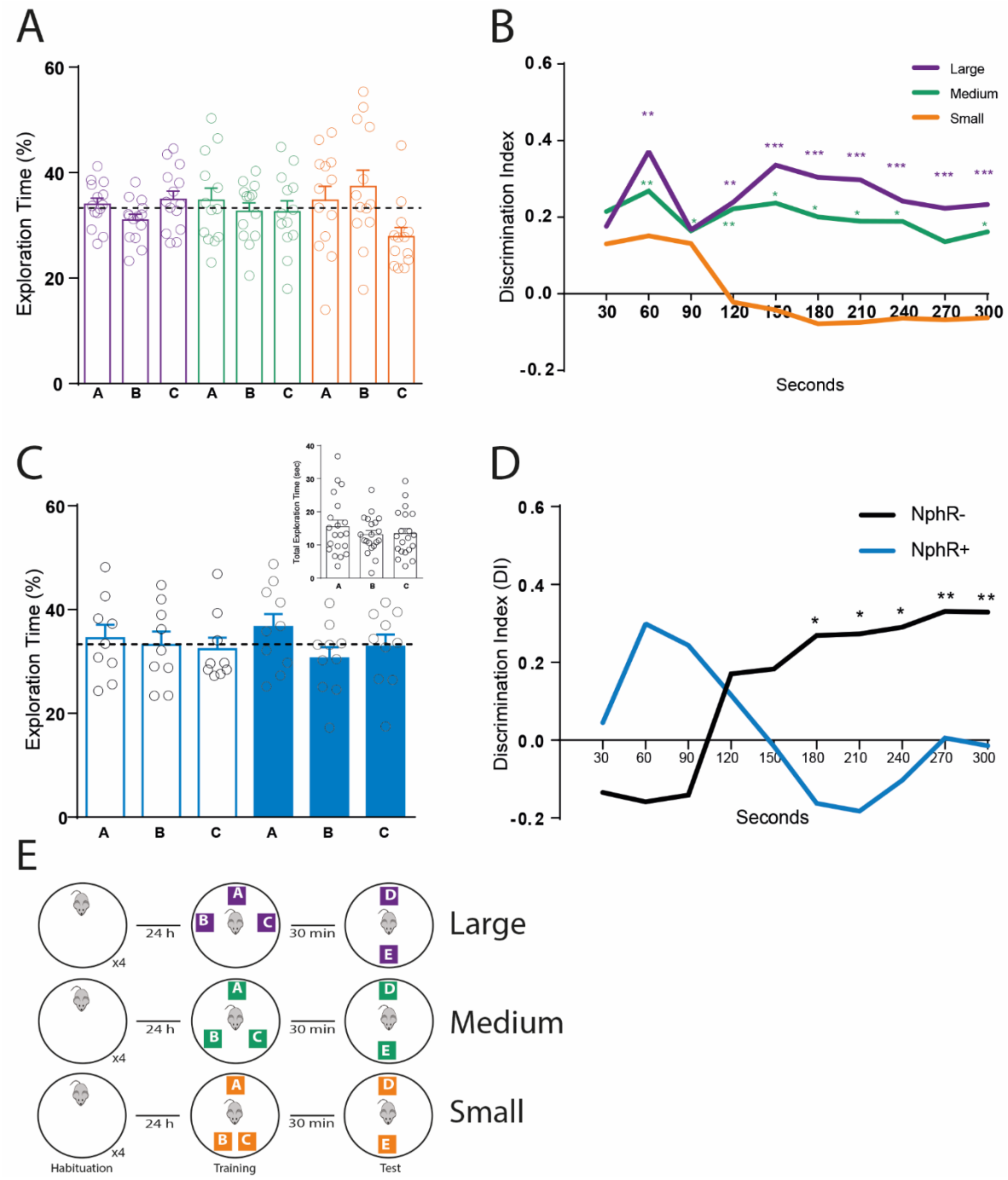
16-Dynamics of contextual discrimination during the retrieval phase of the object-in-context test.

Supplementary Figure 5: Black lines represent control groups (vehicle, **A** and **B**; NphR-, **C** and **D**), whereas red and blue lines represent DNQX and NphR+ groups; respectively. Panels **A** and **C** show the similar object-in-context condition, whereas panels **B** and **D** show the dissimilar object-in-context condition. Student´s t test performed point-by-point against zero; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$. **DNXQ affects the DI of similar but not dissimilar contexts 2way- ANOVA results: (A)** ****p_{drug}<0.0001, $F(1,12)=46.46$, ****p_{time}=0.0001, $F(9,108)=4.127$; **(B)** p_{drug}=0,1405, $F(1,12)=2.491$, p_{time}=0.4136, $F(9,108)=1.04$; **(C)** p_{geno}=0.1788, $F(1,80)=1.84$, p_{time}>0.999, $F(9,80)=0.0659$; **(D)** p_{geno}=0.2838, $F(1,80)=1.64$, p_{time}=0.8921, $F(9,80)=0.4677$.



17-Dentate gyrus AMPA receptors do not regulate memory retrieval in the similar object-in-context behavioural test.

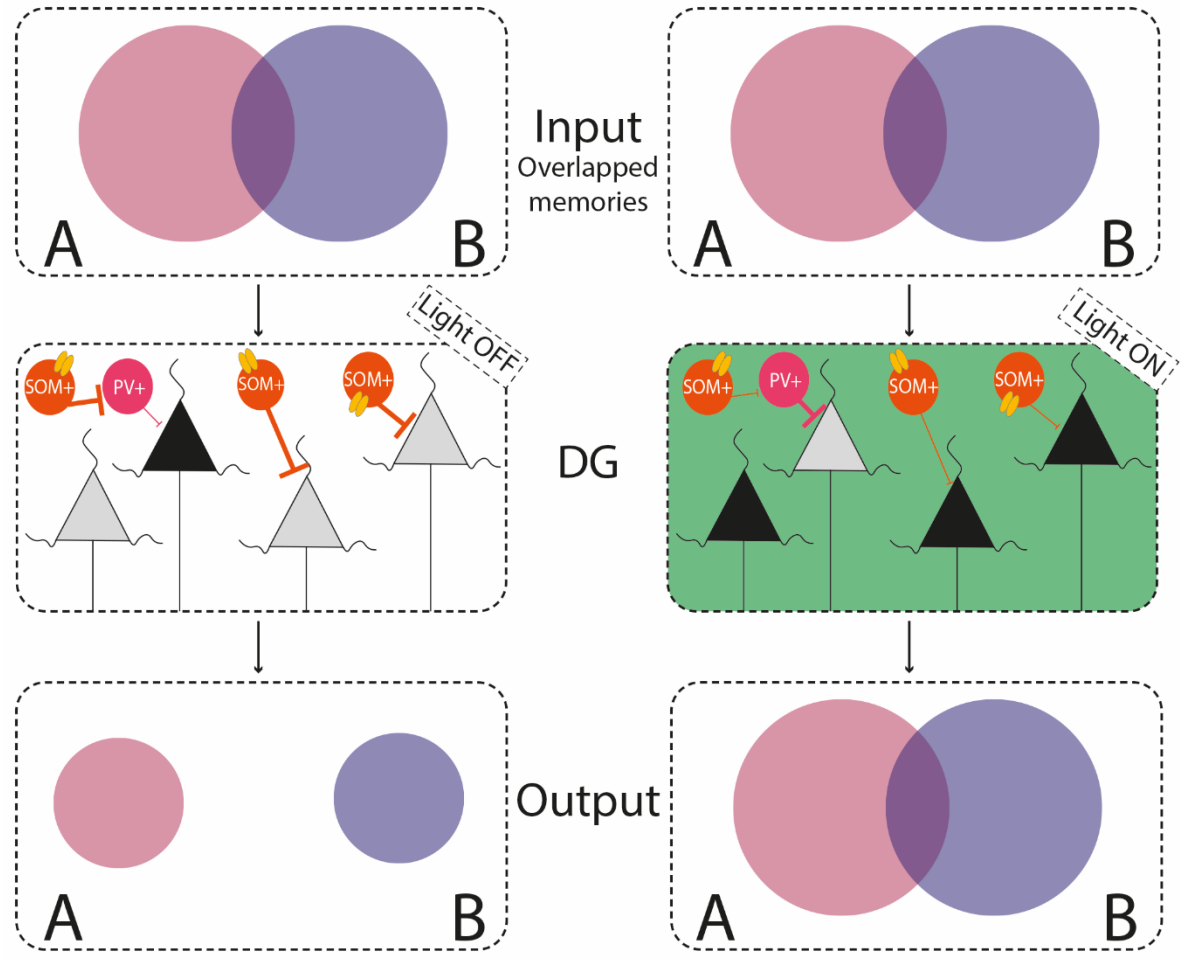
Supplementary Figure 6. :A, Schematic representation of drug administrations and similar object-in-context behavioural test paradigm. Animals were locally injected in the dentate gyrus with either DNQX or vehicle 15 minutes preceding the retrieval phase. **B,** Total exploration time during the training phase. Student´s t test, p = 0,8. **C,** Total exploration time during the retrieval phase. Student´s t test, *** p =0,0008. **D,** Discrimination Index during the retrieval phase for DNQX (red) and vehicle (black) groups. Student´s t test performed point-by-point against zero; *, P < 0.05; **, P < 0.01; ***, P < 0.001. **E,** Cumulative Discrimination Index during the entire retrieval phase. Student´s t test, p = 0,74.



18-Dynamics of Discrimination Index during the spontaneous location recognition test.

Supplementary Figure 7. **A,** Fraction of time spent in the exploration of each of the three objects (A, B, C) in the different test variations (large, medium, small). Paired two-way ANOVA p> 0,05 **B,** Dynamics of the Discrimination Index during the retrieval phase in the different variations. Student´s t test performed point-by-point against zero; *, P < 0.05; **, P < 0.01; ***,

P < 0.001. **C,** Fraction of time spent in the exploration of each of the three objects (A, B, C) in the different test variations. Empty bars represent NphR- animals, while solid bars represent NphR+ animals. Paired two-way ANOVA p> 0,05 Inset: Total exploration time combining all animals. One-way ANOVA p= 0,2**D,** Dynamics of Discrimination Index during the retrieval phase for NphR- and NphR+ groups. Student´s t test performed point-by-point against zero, *, P < 0.05; **, P < 0.01. **E**, schematic representation of the spontaneous location recognition test used in three versions (large =180°, medium=120°, and small=50° separation) to assess spatial memory. Note that angular distance between B and C objects change in training



19-Conceptual model for the role of dentate gyrus somatostatin neurons during pattern separation orthogonalization.

Supplementary Figure 8. : Top, overlapped contextual information recruits the dentate gyrus (DG). Middle, we propose that somatostatin cells (SOM) regulate orthogonalization by directly controlling excitability of granular cells or indirectly by inhibiting parvalbumin cells. Bottom, during successful orthogonalization (left) overlapping input patterns are separated, whereas during SOM suppression by optogenetic stimulation, more granular cells are activated (black cells), thus overlapping inputs are not properly separated.

4.2 Article that was sent to "Frontiers in Neural Circuits"

Neurophotonics approaches for the study of pattern separation

Morales Cristian[1]*, Morici Juan Facundo[2]*†, Miranda Magdalena[2], Gallo Francisco Tomás[2], Bekinschtein Pedro[2,] and Weisstaub Noelia V[2]

* MC and MJF contribute equally to this work.

† corresponding author: faq.morici@gmail.com

[1]Departamento de Psiquiatria, Centro Interdisciplinario de Neurociencia, Pontificia Universidad Católica de Chile, Santiago, Chile.

[2] Instituto de Neurociencias Cognitiva y Traslacional, Concejo Nacional de Investigaciones Científicas y tecnológicas (CONICET), Universidad Favaloro, Instituto de Neurología Cognitiva (INECO), Pacheco de Melo 1860, C1126AAB, Buenos Aires, Argentina.

4.2.1 Abstract

Successful memory involves not only remembering over time but also keeping memories distinct. Computational models suggest that pattern separation appears as a highly efficient process to discriminate between overlapping memories. Furthermore, lesion studies have shown that the dentate gyrus (DG) participates in pattern separation. However, these manipulations did not allow identifying the neuronal mechanism underlying pattern separation. The development of different neurophotonics techniques, together with other genetic tools, have been useful for the study of the micro-circuit involved in this process. It has been shown that less-overlapped information would generate distinct neuronal representations within the granule cells. However, since glutamatergic nor GABAergic cells in the DG are not functionally or structurally homogeneous, identifying the specific role of the different subpopulations remains elusive. Then, understanding pattern separation requires the ability to manipulate a temporal and spatially specific subset of cells in the DG and ideally to analyze DG cells activity in individuals performing a pattern separation dependent behavioral task. Thus, neurophotonics and calcium imaging techniques in conjunction with activity-dependent promoters and high-resolution microscopy appear as important tools for this endeavor. In this work, we review how different neurophotonics techniques have been implemented in the elucidation of a neuronal network that supports pattern separation alone or in combination with traditional techniques. We discuss the limitation of these techniques, and how other neurophotonic techniques could be used to complement the advances presented up to this date.

4.2.2 Introduction

Research in the memory field has been interested not only in the ability to remember over time but also in the capacity to keep memories differentiated and resistant to confusion. To evoke a memory our brain needs to integrate the information it receives from the environment. This integration is important for coding the general structure of the environment and abstracting it from the specificities of individual events, which allows us to generalize to novel situations. This ability to separate memory components into unique representations was postulated to rely on a computational process known as “pattern separation” (Mcclelland et al., 1995; Norman and Reilly, 2003). Computational models define this process as a transformation of the correlated input information into an orthogonal output(Marr.D, 1971; Treves and Rolls, 1994; Ranganath, 2010). According to these theories, the correct storage and retrieval of memories requires the stored of the information in non-overlapping representations. Since episodic memory implies learning about unique events and avoid interference, being able to differentiate them is particularly important for this kind of memories so that storing new information does not lead to overwriting previously-stored ones. For this reason, pattern separation is proposed as an essential component for the storage of differentiated representations of episodic memories and as such has been mainly studied in the hippocampus(Ranganath, 2010) (HP).

The HP is one of the structures that constitute the medial temporal lobe and it has been associated with the pattern separation process. Classically, four regions have been identified in the HP that have distinct anatomical, physiological and genetic characteristics (see Figure 1):

the regions Cornu Ammonis 1, 2 and 3 (CA1, CA2, and CA3) and the dentate gyrus (DG). Computational models first suggested the potential importance of the DG for this cognitive function. The attractor system present in CA3 would be favored by a previous decorrelating process in the DG that could increase the storage capacity of the CA3 system(Amaral et al., 1990; Rolls et al., 1998). The presence of a highly inhibited DG structure or subregion, with a 5-time greater number of cells than the upstream entorhinal cortex (EC), and divergent connectivity towards the CA3 region appears as the perfect structure to be able to achieve this randomizing function(Amaral et al., 1990; Jung and McNaughton, 1993; Chawla et al., 2005; Leutgeb et al., 2007). The potential adaptive role of this putative function was immediately appreciated since very similar events could lead to different outcomes and being able to judge this is crucial for our cognitive versatility.

Many tasks have been developed to show the relevance of the pattern separation process for cognition (Gilbert et al., 1998, 2001; Clelland et al., 2009; Toner et al., 2009; Creer et al., 2010; Bekinschtein et al., 2013). Gilbert et al. (2001) found that the DG ablation leads to a deficit in the discrimination of two similar positions based on distal cues(Gilbert et al., 2001). This deficit was not observed if the separation between the positions was greater. These results were confirmed in subsequent studies (Goodrich-hunsaker et al., 2005) strongly supporting the role of the DG in pattern separation. The gradually of the observed impairment indicates a failure in pattern separation at the behavioral level. Consistently, McHugh (2007) found, using a genetic approach, that mice lacking the essential NR1 subunit of the NMDA receptors (rNMDA) in granule cells (GCs) of the DG could not distinguish two similar contexts during a fear conditioning task although their performance in a regular task of contextual fear conditioning was normal(McHugh et al., 2007). Thus, the results indicate that dentate gyrus

participates in the discrimination of spatial or contextual information. The experiments commented above allowed to postulate the existence of a pattern separation process which it can be deduced from the behavioral performance (e.g. good execution on the pattern separation task) have correctly occurred, and can only hypothesize about the existence of an underlying circuit-level process that supports this kind of cognitive discrimination.

Human studies indicate that patter separation takes place in the DG. In studies using high-resolution functional magnetic resonance imaging (fMRI) to measure brain activity during incidental memory encoding (Bakker et al., 2008; Lacy et al., 2011), authors found that CA3/DG activity was highly sensitive to small changes in the input. In such studies, the interpretation is that DG amplifies the differences between highly similar objects, thus generating highly dissimilar and non-overlapping representations. Then, the evidence accumulated from animal and human studies support the theoretical models proposed for the DG to be involved in pattern separation. As from the mechanism underlying this process, theoretical models proposed that the correct occurrence of the pattern separation process requires low excitability of GCs to induce the orthogonalization of memory representations (Treves and Rolls, 1992; Rolls and Kesner, 2006; Rolls, 2013). The low excitability would permit a small number of GCs to represent an episode, then decreasing the possibility of superposition between similar representations (Rolls, 2013). Also, orthogonalization could be a mechanism that forces distinct GCs to be active in the codification of similar episodes (Rolls, 2013). Lesion studies by electrolytic or histochemical techniques (Gilbert et al., 1998, 2001; Goodrich-hunsaker et al., 2005; Hunsaker et al., 2008) and traditional electrophysiological techniques (Leutgeb et al., 2007; Neunuebel and Knierim, 2014) support this theoretical model. However, the exact mechanism by which pattern separation occurs

remains unresolved mainly for technical limitations. Over the last decades, the implementation of new technologies in the study of biologically relevant questions open a new window of opportunity to tackle in undertaking the complexity of the DG circuitry. Particularly, photonic techniques are one of the most used in neuroscience research (Torricelli et al., 1997). Their popularity comes from their characteristics, such us their adaptability to different settings and their versatility to study different problems, from the cellular to the behavioral level, as well as their high temporal and structural accuracy for cell-specific activity measurement and activity intervention (Cho et al., 2016). In this scenario, the usage of neurophotonics has shown some advantages over previous techniques in the study of the DG microcircuit involved in pattern separation.Regarding the complexity of this issue due to the cell-type variability proposed to be involved during the pattern separation process, it is important to try and contrast the theoretical models with the empirical evidence. In this review, we focus primordially on the evidence obtained by neurophotonics techniques.

4.2.3 Implications of Memory Engram theory in Pattern separation process

Pattern separation computational models (Rolls and Kesner, 2006; Rolls, 2013) propose that orthogonalization implicates the activation of different GCs within the total population in different contextual experiences. Empirical experiments have supported this statement. Using the expression of an activity-dependent gene, like the activity-regulated cytoskeleton-associated protein (ARC), Chawla and collaborators found that when mice were exposed to two different environments, ARC was expressed in two different sets of neurons (Chawla et al., 2005). Similar results were obtained for ZIF268 in an experiment where mice were allowed to explore the same environments but with two different motivations (Satvat et al., 2011). Both results indicate that

in the DG an orthogonalization process occurs not only to encode different contextual features but also to encode differences between the experiences *per se*. This activation of different cell populations appears to be specific to situations that require differentiation because when the mice explored the same environment with the same motivation, the sets of zif268 positive cells obtained was significantly overlapped (Satvat et al., 2011). This suggests a correspondence between the behavioral experience and the subset of cells that encode it. Interestingly, the representational differentiation also occurs in the CA3 region but not in the CA1 region (Leutgeb et al., 2005, 2007) of the hippocampus, This differential recruitment highlight the differentiation process as a DG and/or CA3 region property.

The existence of the neural substrate of memories that make us unique and unrepeatable individuals has been a matter of discussion for over a century. Richard Semon proposed the existence of physical changes in the brain generated by the encoding of new information and called them “engrams” (Tonegawa et al., 2015). Engrams are commonly defined as a set of cells that are synchronously activated during the encoding of a particular experience resulting in the storage of this new information(Wang, 2019). Semon’s idea was too progressive for his time to experimentally contrast it., though it has changed in the last decades, when several publications supporting the engrams theory have appeared (for review,(Bocchio et al., 2017; Tanaka and Mchugh, 2018; Josselyn and Tonegawa, 2020) as predicted by Martin and Morris (2002): "In our view, the final test of any hypothesis concerning memory encoding and storage must be a mimicry experiment, in which apparent memory is generated artificially without the usual requirement for sensory experience, or indeed any form of experience, during learning. […] In another sense, such an experiment would constitute a critical test that changes in synaptic efficacy are sufficient for memory, rather than merely necessary" (Martin and Morris, 2002).

Neurophotonics give us, for the first time, the opportunity to test the engram hypothesis. In the next section, we will try to address some of the advances made in the engram research area by focusing on the use of neurophotonics techniques.

4.2.3.1 Neurophotonics in the development of engrams theory

It was not until a few years ago that huge steps were taken in the quest to identify memory engrams thanks to the generation of different behavioral, molecular, genetic and optic tools (Josselyn and Tonegawa, 2020). Particularly, optogenetics was the first neurophotonic technique used to tackle this question. Liu et al (2012) injected an adenoassociated virus (AAV) AAV9-TRE-ChR2-EYFP in the DG of a c-fos-tTA mice. The main idea of this strategy was to express channelrhodopsin-2 (ChR2, excitatory opsin) in the DG cells that were active during the training phase of a fear conditioning task (Liu et al., 2012) (c-fos will be recruited and drive the expression of tTA) They showed that activating this population of DG cells was enough to trigger the reactivation of the fear response. These results indicated that reactivating the cells recruited during the encoding of a fear memory was sufficient to retrieve and express this memory. This finding was supported and expanded when (Ramirez et al., 2013) were able to create a false memory. By reactivating the DG cells recruited during the encoding of a neutral context (context A) while the animal received a shock at a different one(context B), the authors were able to create a false association between context A and the delivery of the electrical shock (Ramirez et al., 2013). Interestingly, this phenomenon was observed in other parts of the brain, such as the olfactory bulb (Vetere et al., 2019) and the amygdala (Redondo et al., 2014), suggesting an underlying common neural mechanism. To study the stability of the contextual component of a fear memory within the neuronal representation in the HP, Ghandour, et al

(2019) performed *in vivo* Ca2+ imaging of putative engram and non-engram cells in the CA1 region of the HP at post-training sessions (Ghandour et al., 2019). To do this, they injected a TRE-KikGR lentivirus into the CA1 (to label engram cells) of a Thy1-G-CaMP7 × c-fos-tTA double transgenic mice. KikGR is a high effective fluorescent protein that could photoconvert from green to red upon the exposure to 365 nm light without affecting the Ca^{2+} imaging signal. Its expression was controlled in an activity and time-dependent manner (by the c-fos-tTA construct). G-Camp7 is a very sensitive calcium sensor. Then, by combining both tools they could identify the activated engram cells during the recordings. They observed that the total activity pattern of the engram cells during learning were more stable across post-learning memory processing than the activity of the non-engram cells. However, as far as we know, this kind of experiments were not yet performed in the DG.. Nevertheless, these results suggest that neurophotonics is a powerful instrument in the quest and identification of memory engrams and their role in the different memory stages (Roy et al., 2016; Denny, Christine A; Lebois, Evans;Ramirez, 2017)(Roy et al., 2016; Denny, Christine A; Lebois, Evans;Ramirez, 2017)of normal and pathological conditions (Roy et al., 2016; Denny, Christine A; Lebois, Evans;Ramirez, 2017).

The capacity of the brain to maintain differentiated engrams could be really useful for the storage of overlapping memories (Deng et al., 2013). Then, understanding how a particular subset of cells is recruited to be part of an engram is indeed important. It has been shown that neurons excitability could be enhanced by CREB expression (Han, Jin-Hee ; Kushner, Steven A; P. Yiu, Adelaide ; Hsiang , Hwa-Lin (Liz) ;Buch, Thorsten; Waisman, Ari ;Bontempi, Bruno ;L. Neve, Rachael ; Frankland, Paul W; Josselyn, 2009; Rao-ruiz et al., 2019) helping with the engram allocation into that particular subset of neurons (Zhou et al., 2009; Kim et al.,

2013; Yiu et al., 2014). With this background in mind, Rashid et al (2016) asked if two fear-conditioning episodes closely in time could recruit similar neuronal populations in the lateral amygdala (Rashid et al., 2016). They observed that the overlapping between *arc* and *homer1,* two immediate early genes, mRNA increased. Moreover, there were interested in dissecting if the excitability of a particular neuronal ensemble was sufficient to direct the memory allocation to those neurons. To do this, they guide the expression of halorhodopsin (NpHR3.0, inhibitory opsin) and ChR2 to inhibit or excite the activity of the same neuronal population before the first fear conditioning. The main objective behind this experimental design was to, bidirectionally modulate the excitability of the transfected neurons to enhance or decrease the degree of overlapping between memory engrams. They observed that the optogenetics manipulations had effects over the engram overlapping outcomes only when the two fear-conditioning episodes were generated within a limited time frame. This result suggests that depending on the temporal proximity between two slightly different experiences the neuronal ensemble recruited by both of them could be similar. This experiment suggest that, at least in the amygdala, the time interval between two similar experiences is crucial in the ability to generate distinct memory engrams and implies that this structure might not have the computational ability to use pattern separation as a disambiguating process. This kind of experimental setting would be really useful to dissect if pattern separation happening in the DG requires the allocation of two similar experiences into different neuronal ensembles. However, to tackle this idea, future experiments should parameterize the similarity of the contexts used during training.

More recently, it has been shown that the enhancement of the engram cells excitability after reactivation is mediated by the internalization of Kir2.1 inward-rectifier potassium channels and the activation of NMDA receptors (rNMDA) (Pignatelli et al., 2019). It was shown that K2+

inward rectifier currents are negatively modulated by the activation of AMPA receptors (Houzen et al., 1998; Jones et al., 2000; Schroder et al., 2002). Using optogenetics to tag DG engram cells associated with a fear memory, Ryan et al (2015) have shown that engram cells present an enhancement of the spine density and rAMPA/rNMDA ratios compared with non-engram cells (Ryan et al., 2015). Interestingly, this phenotype was depleted when the animals received a protein synthesis inhibitor, anisomycin, classically used to impair memory consolidation. To study more in detail this aspect, a technique called dual-eGRASP has been developed (Kim et al., 2012). The conventional GRASP technique requires two complementary mutant GFP fragments, which are expressed separately on presynaptic and postsynaptic membranes. When the complementary GFP fragments interact within each other at the synaptic cleft, a functional GFP appears. Then, the GFP signal indicates a formed synapse between presynaptic and postsynaptic neurons. Using this approach, it was shown that the CA3-CA1 spine density is enhanced after the training phase (Choi et al., 2018), suggesting that the excitability changes observed happens in the entire engram cells ensemble. the increase of spine density observed in the engram cells was related to the enhancement of the rAMPA/rNMDA ratio and subsequently with the Kir2.1 inward-rectifier potassium channel internalization. Then, these results suggested that changes in the excitability of neurons (Park et al., 2016) that are part of an engram could be key in the mechanism of pattern separation of overlapping memories.

It has been proposed that engram cells form distinctive ensembles spreads all over the brain(Kastellakis and Poirazi, 2019). This particularity it is associated with the capacity of integrating different features of the encoding experience(Guan et al., 2016). According to this idea, activation of DG engram cells, as a hub in the pattern separation mechanism, could trigger the expression of aversive or appetitive responses that are commonly located in different down-

stream structures (Redondo et al., 2014; Ramirez et al., 2015; Roy et al., 2017; Chen et al., 2019). Ramirez et al. (2015) shown that chronic optogenetic reactivation of rewarded experiences reverts the depressive-behavior in a mouse model (Ramirez et al., 2015). Moreover, the chronic reactivation of the dorsal DG engram cells associated with an aversive experience generates extinction response, whereas the reactivation of the ventral DG engram cells generates an enhancement of the fear response (Chen et al., 2019). However, how memory function emerges from the coordinated activity between all the engram nodes remains a mystery. It has been shown that distinct neuronal populations of the basolateral amygdala participate in giving positive or negative valence (Redondo et al., 2014; Gore et al., 2015) to a particular experience. Then, it was proposed that the reactivation of contextual engram cells located in the DG could guide the reactivation of the valence engram cells associated with guiding the behavioral outcome of a certain experience (Tonegawa et al., 2015). These results are in line with the postulation that reactivation of one of the nodes guides the reactivation of the entire engram. Understanding the mechanisms underlying this feature could be useful for the treatment of different anxiety and mood disorders.

Then, the accumulated evidence proposes that the information in the brain could be stored at specifics cells ensembles. In this sense, the differentiation of similar information by generating non-overlapping engrams is proposed as the material outcome of a pattern separation process. Most of the studies focus their attention on the interaction between excitatory neurons at the time of characterizing the intrinsic properties of engram cells. However, the GCs are not the only glutamatergic cells within the DG neither the only population within the structure. Then, it is plausible that other cell populations might also be engram cells or at least play important modulatory roles to the main cells. In this regard, inhibitory engrams have been proposed to be

important for specific memory reactivation (for review (Barron et al., 2017)). In the following sections, we discuss the role of different cell types in the DG circuit involved in pattern separation.

4.2.4 Dentate Gyrus Glutamatergic cells participation in pattern separation

4.2.4.1 Usage of Neurophotonics in the study of the spatial codification of granule and mossy cells

One of the most studied hippocampal function is its involvement in spatial memory encoding. Lesions or pharmacological interventions on the DG impairs the performance in different spatial memory tasks (Gilbert et al., 1998, 2001; Goodrich-hunsaker et al., 2005; Goodrich-Hunsaker et al., 2008), suggesting a role of this structure during the storage or recall of this kind of memory. Moreover, electrophysiological recordings have shown the existence of place cells within the dentate gyrus (Jung and McNaughton, 1993; Leutgeb et al., 2007), neurons that are selectively activated when rodents moved into a specific location within a maze (O'Keefe and Dostrovsky, 1971). A closer analysis has shown that the DG place cells present multiple place fields (Jung and McNaughton, 1993; Leutgeb et al., 2007) and, like CA1 place cells, can remap (Leutgeb et al., 2007). Remapping is a property consisting of the change of the place cell firing pattern in response to a small change in the sensory (Muller and Kubie, 1987; Colgin et al., 2008) or behavioral context (Colgin et al., 2008). In this way, it has been suggested that this remapping property allows the encoding of information emerging from similar experiences into distinct neuronal representations, which in turn is important for pattern separation. However, one of the disadvantages of the data obtained with electrophysiological recordings is the difficulty in distinguishing between other neurons that are also glutamatergic like mossy cells (MCs)(Soriano and Frotscherf, 1994). In this scenario, neurophotonic

techniques have allowed researchers to separate the contribution of GCs and MCs to pattern separation.

The DG is composed primarily of GCs, whose dendrites are arranged within the molecular layer, and their cell bodies form the adjacent granule cell layer(Amaral et al., 2007). Between the molecular layer and the CA3 region, there is a polymorphic layer called the hilus. MCs are located only in the hilus region (see Figure 1). However, since GCs fibers and MCs co-exist in this region (Scharfman, 2016)(Scharfman, 2016)(Scharfman, 2016), their differential contribution to the circuitry functionality has been difficult to be dissected. One possible, and elegant, setting used for the study of the differential contribution from GCs and MCs has been to perform simultaneous optical stimulation and electrophysiological recordings (Senzai and Buzsáki, 2017; Jung et al., 2019). In these studies, an optrode was used. This array allows the simultaneous recording of the voltage field while the light is delivered to the tissue making possible to see the instantaneous effect of light in the firing rate of units recorded(Royer et al., 2010; Anikeeva et al., 2011). Sensay et.al 2017 used dopamine receptor D2 (DRD2) promoter to drives the expression of a chloride pump called archaerhodopsin specifically to MCs. Using this strategy, neurons that suppressed their activity during the optical stimulation were classified as putative MCs (Senzai and Buzsáki, 2017). Surprisingly, this study found that MCs from the DG present one or more than one place fields, like GCs, making it necessary to reinterpret previous works (Jung and McNaughton, 1993; Leutgeb et al., 2007), but also proving the strength of the new optical techniques.

With this system, the accuracy of the study of the MCs electrophysiological characteristics can be enhanced. However, this form of identification presents some limitations that depend on

neuronal connectivity. For example, in some cases, neurons that express archaerhodopsin can activate other neurons. Then, optical stimulation can induce suppression of the activity of neurons that express archaerhodopsin but excitation of neurons that are subsequently activated by them (Senzai and Buzsáki, 2017; Morales et al., 2019). This method does not allow the identification of GCs (that excite directly the MCs) but is useful for the identification of MCs. To tackle this issue, Danielson et.al 2017 used in vivo two-photon calcium imaging in awake behaving mice to differentiate the role of MCs and GCs. To achieve selective manipulation of MCs, they took advantage of the anatomical properties of MCs(Danielson et al., 2017). Specifically, they injected an adeno-associated virus expressing Cre-recombinase into the Dentate gyrus. Then they injected a Cre-dependent rAAV expressing GCaMP6f, a sensitive fluorescent protein used for imaging of neuronal activity, in one of the Dentate gyrus. Since MCs project to contralateral Dentate gyrus, this technique allows the expression of fluorescent markers only in MCs of the contralateral dentate gyrus. Then a chronic imaging window was implanted above the DG to visualize Ca2+ activity from MCs in head-fixed mice that have to run on a treadmill in different linear environments to receive a reward. They found that MCs have place fields, as has been previously described, but while GCs have high tuning specificity, MCs have low tuning specificity indicating that MCs have multiple firing fields. This supports the findingthat place cells with multiple place fields that were founded in electrophysiological experiments (Jung and McNaughton, 1993; Leutgeb et al., 2007) are principally MCs. Danielson et.al 2017 showed that the fraction of place coding was bigger in MCs than in GCs, supporting the idea that, in electrophysiological recording, MCs represent an important number of place cells. Though a powerful approach, a disadvantage of this setting is that the cell-specificity is given by anatomical properties. Some studies show that MCs are not the unique cells that project

to contralateral DG (Scharfman, 2018). If this is confirmed, the results reviewed above required to be interpreted with caution.

The results of a recent study (Jung et al., 2019) suggest a possible driver role of MCs in remapping. They showed that when there are changes in the environment, like those used to induce remapping, MCs response precedes the activity change on GCs. It is important to note, that to differentiate between GCs and MCs, Jung et.al 2019 injected a CRE inducible AAV to drive the expression of Chronos, an excitatory opsin that is faster and more light-sensitive than the conventional channelrhodopsin (Klapoetke et al., 2014), in two different transgenic mice line, DRD2-Cre and POMC-Cre mice, that allowed them to excite, MCs and GCs respectively. Although the use of this setting to differentiate the role of MCs and GCs in the DG circuit was previously used, they argued that excitation is better than inhibition to discriminate between neuronal types.

In summary, the evidence described above suggests that both CGs and MCs may be deferentially involved in the functionality of the DG circuitry. In particular, MCs showed more sensibility to contextual changes than GCs suggesting that MCs could be part of the circuits that detect and encode the non-overlapped information while the GCs could be encoding the overlapped information. Though further research would be needed to completely dissect their specific function, the evidence accumulated until now propose to MCs as important players in the mechanism of pattern separation.

4.2.4.2 What can we say about the "irritable" hypothesis using neurophotonic techniques?

One of the most exciting topics on the study of the DG is related to the role of each cell-type in terms of which node orchestrate the activity of the other nodes. It has been shown that MCs can excite GCs directly and can inhibit GCs indirectly through the recruitment of feedback inhibition (Scharfman, 1995; Larimer and Strowbridge, 2008). However, due to the complexity of the DG circuit, the neuronal mechanisms underlying the net effect on GCs activity is still a controversial matter (Scharfman, 2018).

In the early 80's some seminal studies showed that the stimulation of commissural (fibber of MCs) just before stimulating the perforant path (PP) produced an inhibition over the GCs spikes population (Buzsaki and Eidelberg, 1981; Douglas et al., 1983) suggesting that the activation of MCs principally inhibits the activity of GCs. Interestingly, this conclusion was confirmed by subsequent experiments (Sloviter, 1983, 1991; Scharfman, 1995), allowing the establishment of the "dormant basket cells" hypothesis. This hypothesis proposed that the net effect of MCs on GCs activity was mediated by the activation of parvalbumin (PV+) GABA-ergic interneurons within the DG that then inhibit GCs (Sloviter, 1991). However, in contrast to this theory, other experiments suggested that the net effect was mediated by the excitation of the GCs by the MCs (Buckmaster et al., 1996; Ratzliff et al., 2004). This alternative hypothesis called the "irritable mossy cells" hypothesis, proposed that the MCs hyperexcitability increase the activity of the GCs affecting in this way the net effect onto the DG. (Santhakumar et al., 2000). Despite that the last hypothesis was described in pathological conditions like epilepsy, it has been extrapolated to memory function. Then, based on these two perspectives, MCs could modulate GCs response by indirect inhibition or direct activation.

One of the problems with electrical stimulation is how to selectively and specifically stimulate MCs (Amaral et al., 2007; Leranth and Hajszan, 2007). Then, identification of MCs by other parameters than their electrophysiological properties is required. Jackson et al 1996 used in hippocampal slice the voltage-sensitive dyes technique (Ebner and Chen, 1995; Chemla and Chavane, 2009; Tsytsarev et al., 2014)This technique has a better spatial resolution than traditional electrophysiological approaches and permits to study the spread of activity within DG after stimulation of PP. They showed that the spread of activity depends specifically on the hilar activation, while the PP damage was not related to this outcome (Jackson and Scharfman, 1996) and that electrical stimulation of the hilus induce depolarization at the Inner molecular layer. Thus, they suggested that the spread of activity delivered by PP stimulation depends on positive feedback between GCs and MCs. Despite this results, other investigation that combine a laser-scanning photostimulation with a voltage-sensitive dye (Xu et al., 2010) have shown that photostimulation of the hilus does not increment the activity in GCs (Sun et al., 2017). Hsu et al 2016 performed a series of experiments to resolve the discrepancy between these results. They injected unilaterally a CRE inducible AAV carrying the ChR2 gene in the hilus of Grik4-cre hemizygous mice to direct the expression of ChR2 to the commissural fibber of contralateral DG. The authors found that inhibition/excitation balance in GCs was increased when commissural fibbers were photostimulated. Moreover, they showed that while concurrent activation of commissural and perforant pathways increased the response of GCs, the delayed activation of PP compared with the commissural pathway decreased the percentage of responding GCs (Hsu et al., 2016). These results proposed that the optogenetic stimulation of the PP at 10 Hz, the “dormant basket cells” hypothesis seems to apply. Interestingly, the

frequency of the Opto-stimulation is quite similar to the one observed in the hippocampus of animals during active exploration periods.

Contrasting with what it was described above, another study (Hashimotodani et al., 2017) decided to address the effect of fast (30 Hz) optical stimulation of MCs fibber, because this kind of stimulation can induce long term potentiation (LTP). To generate an optical fast stimulation, they used ChIEF, a faster version of ChR2 (Lin et al., 2009). They found that fast optical stimulation induces LTP between MCs-GCs synapses, but not in mossy-interneurons synapses. This facilitation increases the excitation/inhibition balance thereby inducing an increment in GCs activity. These results obtained using a fast opto-stimulation protocol, support the "irritable" hypothesis. Taking all this evidence together, the Opto-stimulation frequency performed at the PP seems to be critical in defining the role of the MCs in the modulation of the GCs activity. If the input is low frequency, the indirect inhibition mechanism seems to modulate the GCs net effect, while the direct MCs-GCs excitation seems to be more preponderant when higher frequency inputs impact into the circuit.

4.2.5 Neurophotonics applications in the study of the DG interneurons role in Pattern separation

Several models have proposed that GABA-ergic DG interneurons mediate the control of GCs excitability and the orthogonalization of engrams that represents similar contexts (Rolls, 2013). Electrophysiological experiments have shown that GABA-ergic interneuron activity in the DG, unlike other hippocampal regions, is higher in a novel than in a familiar context (Nitz and McNaughton, 2004), suggesting its role in encoding novel information (see Figure 1). Some of the GABA-ergic interneurons that contact GCs at the perisomatic region are basket cells

(BCs), whose inputs come from other GCs, the perforant pathway (Freund and Buzsáki, 1996) and mossy cells (Scharfman and Myers, 2012; Hsu et al., 2016; Scharfman, 2016; Danielson et al., 2017)(Scharfman and Myers, 2012; Hsu et al., 2016; Scharfman, 2016; Danielson et al., 2017)(Scharfman and Myers, 2012; Hsu et al., 2016; Scharfman, 2016; Danielson et al., 2017). Thus, in this way, these interneurons could control both feedback and feedforward inhibition onto GCs (Freund and Buzsáki, 1996; Savanthrapadian et al., 2014). On the other hand, within interneurons that contact the dendritic region of GCs are the hilar perforant path associated interneurons (HIPP), that correspond to a type of interneurons that have their soma in the Hilus, where contact with GC axons take place(Freund and Buzsáki, 1996; Savanthrapadian et al., 2014; Yuan et al., 2017). It has been proposed that HIPP could control GCs activity through feedback mechanisms (Houser, 2007). There has been an established relationship between the anatomical characteristics of these subpopulations and the presence of specific neuronal markers(Freund and Buzsáki, 1996; Savanthrapadian et al., 2014). The expression of neuronal markers associated with distinct GABA-ergic cells has allowed the use of optogenetics techniques to analyze the role of each of these GABA-ergic interneurons in DG networks and pattern separation. Specially, BCs interneurons are PV+ while HIPP interneurons are SOM+(Freund and Buzsáki, 1996; Savanthrapadian et al., 2014; Yuan et al., 2017).

The stronger inhibition mediated by the recruitment of PV+ interneurons counterbalance excitation of DG networks. In this way, stronger excited cells recruit GCs more effectively than less excited cells, allowing a “winner-takes-all” situation that would allow a good pattern separation mechanism(Sambandan et al., 2010b; Guzman et al., 2019). Electrophysiological experiments have shown that this property depends on the coactivation of the perforant pathway and mossy fibers(Sambandan et al., 2010b)(Sambandan et al., 2010a)(Sambandan et al., 2010a).

Although there are other mechanisms capable of regulating the activity of PV+ interneurons. Hu et al (2010), using confocal imaging and patch-clamp simultaneously, showed that some of the intrinsic properties of PV+ interneurons dendrites, like the presence of Kv3 channels, are implicated in the rapid and precise time inhibition mediated by PV+(Hu et al., 2010). On the other hand, several studies (Savanthrapadian et al., 2014; Yuan et al., 2017) showed that SOM+ also contributes to the precision of the discharge of PV+. In this line of evidence, Savanthrapadian et al (2014) injected a Cre-inducible rAAV vector containing ChR2-tdT into the DG of SOM-Cre mice. They studied the PV+ interneurons while paired optical stimulation of SOM+ interneurons with electrical stimulation of PP. They showed that the optical stimulation of the outer molecular layer, where the axons of HIPP are present, increase the precision of action potential generation in PV+ interneurons (Savanthrapadian et al., 2014). Yuan et al(2017) showed that there are two types of SOM+ interneurons within the DG, the HIPP interneurons, that were studied by Savanthrapadian (2014), and SOM+ interneurons that have their axons in the hilus and contact other interneurons like PV+ interneurons. This last group is called hilus-associated interneuron (HIL; Yuan et al., 2017). In this study, the scientists used a similar injection protocol as described by Savanthrapadian (2014), but besides stimulating the outer molecular layers for the recruitment of HIPP, they stimulated the perisomatic region of PV+ interneurons. Using this approach, they showed that the activity of the HIL determines the activity of PV+ interneurons (Yuan et al., 2017). Thus, the activity of PV+ interneurons is regulated by SOM+ interneurons through dendritic inhibition by HIPP and perisomatic inhibition by HIL (see Figure 1). This complex array of inhibitory control seems to indicate a complementary role between PV+ and SOM+ interneurons and could be instrumental for pattern separation.

The entorhinal cortex (EC) is generally characterized as the main input to the DG (Rolls, 2013). Understanding how cortical inputs modulate DG inhibitory microcircuits is crucial to understand the processing of information in the HP. To this end, Lee et. al (2016) studied how PV+ and SOM+ interneurons affect the activity of GCs in response to cortical stimulation. They injected an adeno-associated virus (AAV5) expressing Cre-dependent enhanced halorhodopsin (eNpHR3.0). Using this experimental approach, they selectively inhibit each of these interneurons, PV+ and SOM+. They showed that inhibition of PV+ interneurons suppresses GCs responses to single cortical stimulation. When cortical stimulation was in theta (θ) or gamma (γ) frequencies (Lee et al., 2016), i.e. frequencies present during exploration (Bragin et al., 1995), they found that both types of interneurons differentially regulate GCs responses. Interestingly they found that PV+ regulates the onset of the spike series, while SOM+ interneurons regulate principally late spikes. Overall, these results are in agreement with the view that PV+ and SOM+ interneurons play complemenetary roles in pattern separation.

Besides regulating GCs excitability, is possible that GABA-ergic interneurons also participate in the orthogonalization of engrams that represents similar contexts through a lateral inhibition mechanism. By coupling the expression of td-Tomato or EGFP reports with the expression of neurochemical marker for its identification in slice experiments, Espinoza et al (2018) found that in the case of GCs-PV+ connection, the ratio of lateral inhibition regarding recurrent inhibition was higher, suggesting an important role of this interneurons in lateral inhibition (Espinoza et al., 2018). On the other hand, Stefanelli et al (2016) were interested in the size of the ensemble recruited during the encoding of contextual information and how it modulates the specificity during recall. To tackle this question, they expressed ChR2 in GCs, SOM+ and PV+ interneurons to opto-stimulate these cells during the encoding of a contextual

fear memory paradigm. They showed that the rise time of GABA-ergic current response induced by PV+ stimulation was the shortest. While the rise time of GABA-ergic current response induced by SOM+ and GCs did not have significant differences. In this way, the authors conclude that, due to the similarity between GABA-ergic current response induced by SOM+ and GCs, the lateral inhibition induced by GCs corresponds to the recruitment of SOM+ interneurons(Stefanelli et al., 2016). Thus, in the case of orthogonalization, experimental evidence suggests that PV+ and SOM+ participate in a complementary way. These results provide evidence that integrates the role of different DG cell-types in the memory allocation and how it could contribute to the pattern separation process. From this perspective, DG interneurons are recruited during the encoding of contextual fear memory. Their role during this process seems to be circumscribed to the control of the size of the ensemble. If this process is affected by blocking the activity PV+ or SOM+ cells, the number of recruited GCs would increase. This outcome could affect the selectivity of the storage and/or recall of the information since the probability of overlap with other neuronal ensembles coding other memories is enhanced.

4.2.6 Neurophotonics techniques to understand the role of adult-born granule cells in patter separation

The DG circuit, as well as the olfactory bulb, is continuously changing because of the integration of adult-born GCs (abGCs) (Sahay et al., 2011b). A growing body of studies are currently focused on finding if abGCs play a particular role in pattern separation. Clelland et al. (2009) found that blocking hippocampal adult neurogenesis by X-ray irradiation altered the animal's ability to distinguish small changes in spatial discrimination, but not unmistakable changes(Clelland et al., 2009). Consistently, Sahay et al. (2011) observed that animals with genetically increased levels of adult neurogenesis were better at discriminating between two

similar contexts (Sahay et al., 2011a). Moreover, many studies suggested that these new neurons could be a preferential substrate for remapping the place cells in presence of subtle changes in the environment. This is because the immature granular neurons have higher excitability and plasticity that distinguishes them from the population of old and relatively silent neurons (Esposito et al., 2005; Marín-burgin et al., 2012). In addition to this, it has been proposed that mature neurons could be specialized for certain, more stable characteristics of their environment since they would respond preferentially to the inputs they received during their development(Aimone et al., 2011). On the other hand, immature GCs showed a low threshold for the induction of LTP (Schmidt-Hieber et al., 2004; Ge et al., 2007). Then, the particular properties of immature GCs confer them the characteristics required to be involved in pattern separation. Consistently with this idea, Nakashiba et al. (2012) suggested that neither the larger number nor the more dispersed activity of the DG are sufficient to separate similar contexts and that young aGCs would be necessary to allow this process(Nakashiba et al., 2012).

Ikrar et al (2013) studied the DG response to electrical stimulation with a Voltage Sensitive Dye Technique (Ebner and Chen, 1995; Chemla and Chavane, 2009; Tsytsarev et al., 2014) using an iBax-nestin mice, a model mice in which neurogenesis can be enhanced with tamoxifen administration. They showed that photo or electrical stimulation of DG induced a smaller and less-spread neuronal excitability in mice with increase adult neurogenesis compared to the controls (Ikrar et al., 2013). These results suggest that adult neurogenesis is an important factor in the control of the DG neurons' excitability. This result was supported by a different studies (Temprana et al., 2015; Drew et al., 2016). In this case, a retrovirus expressing a light-activated channel channelrhodopsin-2ChR2 was delivered to the DG of adult mice for its selective transduction in neural progenitor cells of the adult DG. Then, acute slices were

prepared some weeks post-injection for studying the effect of photostimulation of abGCs generated at different time points. They showed that abGCs activate hilar GABAergic interneurons that in turn inhibit mature GCs (see Figure 1). Specifically, Temprana et al (2015) showed that recruitment of feedback inhibition is higher in abGCs of 7 weeks than in abGCs of 4 weeks. This result suggests that as time passes abGCs tend to be more integrated into inhibitory circuits that facilitate their role in controlling the excitability of surrounding neurons

A recent study (Luna et al., 2019)howed that besides the recruitment of feedback inhibition by abGCs (Temprana et al., 2015; Drew et al., 2016) these newborn neurons can directly inhibit mature GCs. Specifically, Luna et al (2019) selectively expressed Archaerhodopsin T in abGCs. They showed that optical inhibition of abGCs produced an increment in the DG LTP response to electrical stimulation, even when GABA antagonists were used. This could indicate that inhibition is independent of GABAergic interneurons activation in the hilus. They also studied the effect of abGCs activation in mature GCs, by selectively expressing channelrhodopsin2 in abGCs. They showed that low intensities of light, that produce low levels of glutamate release from abGCs, induce IPSPs in mature GCs. Moreover, high intensities of light, that produce high levels of glutamate liberation, induce EPSP in mature GCs. Finally, they showed that low glutamate liberation, i.e. IPSP in mature GCs, is due to the preferential activation of the lateral entorhinal cortex that carries contextual information (Hargreaves et al., 2005; Wilson et al., 2013).Keeping low levels of mature granule cells excitability is important for pattern separation(Jinde et al., 2012), Luna et. al (2019) suggested that contextual information ─beside spatial information─ is relevant to promote a sparse coding in DG. Consistently, using calcium imaging Danielson et al (2016) differentiate the activity of abGCs from other populations that present a low spatial tuning but are good novelty detectors,

supporting a fundamental role of abGCs in disambiguating contextual information through the process of pattern separation(Danielson et al., 2016).

All the evidence described in this section proposed that abGCs could play a key role in the formation of orthogonal representations from similar inputs. This is because of their high excitability during the early stages of their development is critical to determine which inputs will recruit them subsequently. Since neurogenesis is a continuous process, there are always abGCs at different stages of development. Therefore, the probability that two different experiences recruit the same subset of abGCs at the same developing time is low. This characteristic gives them a potential role in orchestrating the rest of the cells that potentially form the differentiated engrams in the DG.

4.2.7 Final Remarks

Neurophotonic techniques allowed the study of the role of different neuronal types in the DG networks functionality. Specifically, the evidence described above suggests a differential role of each neuronal type in the mechanisms underlying pattern separation. As we have described, neurophotonic studies led to propose models that go beyond unique neuronal types for information processing, and where several elements of DG network share complementary roles in the differentiation of overlapping information. Based on the body of evidence presented above, we are proposing a possible way in which all these different cell types might interact and contribute to pattern separation

Neurophotonics have contributed to differentiate the role of three types of DG glutamatergic cells, mossy cells (MCs), newborn granule cells (abGCs) and mature granule cells

(GCs). Thus, it has been shown that MCs and abGCs present more remapping than mature GCs(Danielson et al., 2016, 2017; Senzai and Buzsáki, 2017), which suggests that MCs and abGCs are more sensible than GCs to detect small environmental changes, i.e. when differences must be detected in similar episodes. Besides, neurophotonics experiments suggest that these neuronal types would respond before than GCs (Marín-burgin et al., 2012; Jung et al., 2019) suggesting that both neuronal types could initiate the process of pattern separation. Besides, both neuronal types are more sensitive to contextual changes (Danielson et al., 2016, 2017; Senzai and Buzsáki, 2017; Luna et al., 2019) which means that contextual information would be more relevant than other type of information. Thus, MCs and abGCs could initiate pattern separation through the detection of environmental changes, especially changes in contextual information. After activation of MCs and abGCs, they can initiate an inhibitory network. While abGCs can inhibit directly GCs (Luna et al., 2019), MC and abGCs would activate PV (Scharfman, 2018; Groisman et al., 2020), which in turn produces a lateral inhibition proposed to be important for pattern separation (Espinoza et al., 2018). Interestingly, the activity of SOM modulates the activity of PV(Savanthrapadian et al., 2014; Yuan et al., 2017) and can produce, itself a lateral inhibition (Stefanelli et al., 2016). On the other hand, the evidence indicates that the activity of SOM is delayed when compared with the activity of PV (Hsu et al., 2016; Stefanelli et al., 2016). Thus, both PV and SOM interneurons control the activity of GCs, but likely in a different time with PV activity preceding SOM activity. Some models propose pattern separation mechanisms that take into account and emphasize the role of PV interneurons (Guzman et al., 2019), abGCs (Sahay et al., 2011b) and MCS (Nakazawa, 2017). In this work we make a complementary interpretation to all these models, paying special attention to the interaction between the different cell types present in the DG (see Figure 1). Still, more work is

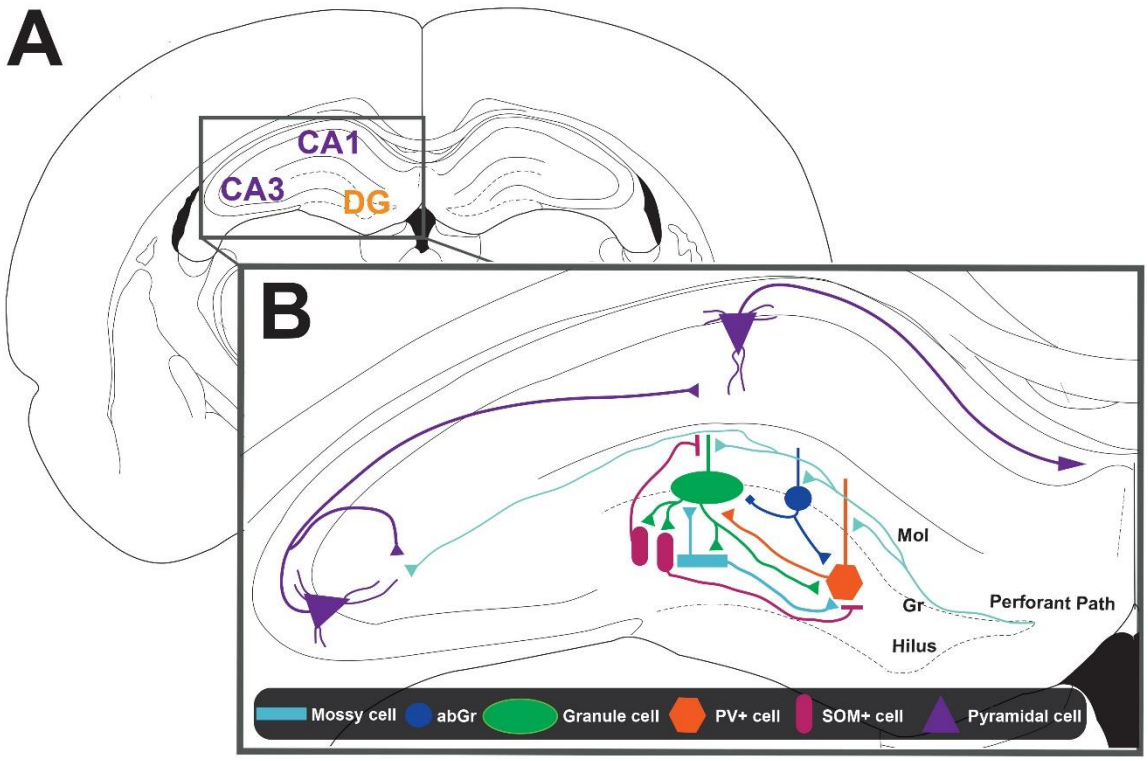
required to better understand the mechanism, dynamics, and constraints of pattern separation in the DG.

However, it is important to highlight that in the last years our understanding of this process advanced enormously thanks to the development of neurophotonic techniques. We believe that the continuous advancement in this field in combination with genetic tools will prove to be a powerful strategy for ll for modeling pattern separation where a complementary role of different types could be studied.

4.2.8 Funding

This work was supported by grants from the National Agency of Scientific and Technological Promotion of Argentina (Agencia Nacional de Promoción Científica y Tecnológica, ANPCyT, Argentina) PICT2015-2344 (NVW).

4.2.9 Figure



20-Representation of the DG-CA3 circuit

Figure 1:. *(A) Schematic representation of a coronal slice from rat brain*. The hippocampus (HIP) is constituted by the Cornu Ammonis regions 1 and 3 (CA1 and CA3, in violet) and the Dentate Gyrus region (DG, in orange). *(B) Zoom inset from the HIP*. The main inputs to the HIP come from the layers 2/3 of the Entorhinal Cortex (EC) that constitute the perforant path (PP, light blue lines). The information coming from the EC could project to the CA3 Pyramidal layer directly or indirectly by making and intermediate synapse on the Granule cells (GCs, green ellipse) located in the Granular Layer (Gr) of the DG. Mossy cells (MCs, ligth blue rectangle) and adult-born Granule cells (abGC, blue circle) would be the first neurons activated, and could initiate pattern separation. The abGCs can modulate the activation of GCs through a direct connection, which is excitatory or inhibitory depending on the activity pattern of entorhinal input. On the other hand, abGCs and MCs can inhibit GCs by recruitment of parvalbumin containing interneurons (PV+, orange hexagon). Also, the activity of PV+ interneurons is modulated by somatostatin containing interneurons (SOM+, pink ellipse) and by GCs itself. SOM+ interneurons can directly inhibit GCs, specifically distal dendrites, where contextual information arrives. Thus, the interaction between the different cell types present in the DG

defines the activity of GCs, which project to CA3. Finally, the CA3 pyramidal cells project through the Schaffer collaterals to the CA1 pyramidal cells forming the main hippocampal output into Layer 5 of the EC.

4.2.10 References

Aimone, J. B., Deng, W., and Gage, F. H. (2011). Resolving New Memories: A Critical Look at the Dentate Gyrus, Adult Neurogenesis, and Pattern Separation. *Neuron* 70, 589–596. doi:10.1016/j.neuron.2011.05.010.

Amaral, D. G., Ishizuka, N., and Claiborne, B. (1990). Neurons, numbers and the hippocampal network. *Prog Brain Res* 83, 1–11.

Amaral, D. G., Scharfman, H. E., and Lavenex, P. (2007). The dentate gyrus: fundamental neuroanatomical organization (dentate gyrus for dummies). *Prog Brain Res* 163, 3–22. doi:10.1016/S0079-6123(07)63001-5.

Anikeeva, P., Andalman, A. S., Witten, I., Warden, M., Goshen, I., Grosenick, L., et al. (2011). Optetrode: a multichannel readout for optogenetic control in freely moving mice. *Nat Neurosci* 15, 163–170. doi:10.1038/nn.2992.

Bakker, a., Kirwan, C. B., Miller, M., and Stark, C. E. L. (2008). Pattern Separation in the Human Hippocampal CA3 and Dentate Gyrus. *Science (80-)* 319, 1640–1642. doi:10.1126/science.1152882.

Barron, H. C., Vogels, T. P., Behrens, T. E., and Ramaswami, M. (2017). Inhibitory engrams in perception and memory. *PNAS* 114, 6666–6674. doi:10.1073/pnas.1701812114.

Bekinschtein, P., Kent, B. a., Oomen, C. a., Clemenson, G. D., Gage, F. H., Saksida, L. M., et al. (2013). BDNF in the dentate gyrus is required for consolidation of "pattern-separated" memories_suplementary_information. *J Chem Inf Model* 53, 1689–1699. doi:10.1017/CBO9781107415324.004.

Bocchio, M., Nabavi, S., and Capogna, M. (2017). Review Synaptic Plasticity , Engrams , and Network Oscillations in Amygdala Circuits for Storage and Retrieval of Emotional Memories. *Neuron* 94, 731–743. doi:10.1016/j.neuron.2017.03.022.

Bragin, A., Jandó, G., Nádasdy, Z., Hetke, J., Wise, K., and Buzsáki, G. (1995). Gamma (40-100 Hz) oscillation in the hippocampus of the behaving rat. *J Neurosci* 15, 47–60.

Buckmaster, P. S., Wenzel, H. jurge., Kunkel, D. D., and Schwartzkroin, P. A. (1996). Axon Arbors and Synaptic Connections of Hippocampal Mossy Cells in the Rat In Vivo. *J Comp Neurol* 366, 270–292.

Buzsaki, G., and Eidelberg, E. (1981). Commiasural projection to the dentate gyrus of the rat : evidence for feed- forward inhibition. *Brain Res* 230, 346–350.

Chawla, M. K., Guzowski, J. F., Ramirez-Amaya, V., Lipa, P., Hoffman, K. L., Marriott, L. K., et al. (2005). Sparse, environmentally selective expression of Arc RNA in the upper blade of the

rodent fascia dentata by brief spatial experience. *Hippocampus* 15, 579–586. doi:10.1002/hipo.20091.

Chemla, S., and Chavane, F. (2009). Voltage-sensitive dye imaging : Technique review and models. *J Physiol*. doi:10.1016/j.jphysparis.2009.11.009.

Chen, B. K., Murawski, N. J., Cincotta, C., Zaki, Y., Fortin, A., Ramirez, S., et al. (2019). Artificially Enhancing and Suppressing Report Artificially Enhancing and Suppressing Hippocampus-Mediated Memories. *Curr Biol* 29, 1–10. doi:10.1016/j.cub.2019.04.065.

Cho, Y. K., Zheng, G., Augustine, G. J., Hochbaum, D., Cohen, A., Knopfel, G., et al. (2016). Roadmap on neurophotonics. *J Opt*.

Choi, J., Sim, S., Kim, J., Choi, D. Il, Oh, J., Ye, S., et al. (2018). Interregional synaptic maps among engram cells underlie memory formation. *Science (80-)* 360, 430–435.

Clelland, C. D., Choi, M., Romberg, C., Clemenson, G. D., Fragniere, a, Tyers, P., et al. (2009). A functional role for adult hippocampal neurogenesis in spatial pattern separation. *Science* 325, 210–213. doi:10.1126/science.1173215.

Colgin, L. L., Moser, E. I., and Moser, M. B. (2008). Understanding memory through hippocampal remapping. *Trends Neurosci* 31, 469–477. doi:10.1016/j.tins.2008.06.008.

Creer, D. J., Romberg, C., Saksida, L. M., van Praag, H., and Bussey, T. J. (2010). Running enhances spatial pattern separation in mice. *Proc Natl Acad Sci U S A* 107, 2367–2372. doi:10.1073/pnas.0911725107.

Danielson, N. B. ., Kaifosh, P., Zaremba, J. D. D., Lovett-Barron, M., Tsai, J., Denny, C. A. A., et al. (2016). Distinct Contribution of Adult-Born Hippocampal Granule Cells to Context Encoding. *Neuron* 90, 101–112. doi:10.1016/j.neuron.2016.02.019.

Danielson, N. B., Turi, G. F., Ladow, M., Chavlis, S., Petrantonakis, P. C., Danielson, N. B., et al. (2017). In Vivo Imaging of Dentate Gyrus Mossy Cells in Behaving Mice. *Neuron* 93, 552–559. doi:10.1016/j.neuron.2016.12.019.

Deng, W., Mayford, M., and Gage, F. H. (2013). Selection of distinct populations of dentate granule cells in response to inputs as a mechanism for pattern separation in mice. 1–21. doi:10.7554/eLife.00312.

Denny, Christine A; Lebois, Evans;Ramirez, S. (2017). From Engrams to Pathologies of the Brain. *Front Neural Circuits* 11, 1–20. doi:10.3389/fncir.2017.00023.

Douglas, R. M., Mcnaughton, B. L., and Goddard, G. V (1983). Commissural Inhibition and Facilitation of Granule Cell Discharge i n Fascia Dentata. *J Comp Neurol* 219, 285–294.

Drew, L. J., Kheirbek, M. A., Luna, V. M., Denny, C. A., Cloidt, M. A., Wu, M. V., et al. (2016). Activation of local inhibitory circuits in the dentate gyrus by adult-born neurons. *Hippocampus* 26. doi:10.1002/hipo.22557.

Ebner, T., and Chen, G. (1995). Use of Voltage-sensitive dyes ans optical recordings in the central nervous system. *Prog Neurobiol* 46, 463–506.

Espinoza, C., Guzman, S. J., Zhang, X., and Jonas, P. (2018). Parvalbumin+ interneurons obey unique connectivity rules and establish a powerful lateral-inhibition microcircuit in dentate gyrus. *Nat Commun*, 1–10. doi:10.1038/s41467-018-06899-3.

Esposito, M. S., Piatti, C., Laplagne, D. A., Morgenstern, A., Ferrari, C. C., Pitossi, F. J., et al. (2005). Neuronal Differentiation in the Adult Hippocampus Recapitulates Embryonic Development. *J Neurosci* 25, 10074–10086. doi:10.1523/JNEUROSCI.3114-05.2005.

Freund, T. F., and Buzsáki, G. (1996). Interneurons of the hippocampus. *Hippocampus* 6, 347–470. doi:10.1002/(SICI)1098-1063(1996)6:4<347::AID-HIPO1>3.0.CO;2-I.

Ge, S., Yang, C., Hsu, K., Ming, G., and Song, H. (2007). A Critical Period for Enhanced Synaptic Plasticity in Newly Generated Neurons of the Adult Brain. *Neuron* 54, 559–566. doi:10.1016/j.neuron.2007.05.002.

Ghandour, K., Ohkawa, N., Chung, C., Fung, A., Asai, H., Hayashi, Y., et al. (2019). Orchestrated ensemble activities constitute a hippocampal memory engram. *Nat Commun* 10, 1–14. doi:10.1038/s41467-019-10683-2.

Gilbert, P. E., Kesner, R. P., and DeCoteau, W. E. (1998). Memory for spatial location: role of the hippocampus in mediating spatial pattern separation. *J Neurosci* 18, 804–810.

Gilbert, P. E., Kesner, R. P., and Lee, I. (2001). Dissociating Hippocampal Subregions: A Double Dissociation Between Dentate Gyrus and CA1. 636, 626–636. doi:10.1002/hipo.1077.

Goodrich-hunsaker, N. J., Hunsaker, M. R., and Kesner, R. P. (2005). Dissociating the role of the parietal cortex and dorsal hippocampus for spatial information. *Behav Neurosci* 119, 1307–1315. doi:10.1037/0735-7044.119.5.1307.

Goodrich-Hunsaker, N. J., Hunsaker, M. R., and Kesner, R. P. (2008). The interactions and dissociations of the dorsal hippocampus subregions: how the dentate gyrus, CA3, and CA1 process spatial information. *Behav Neurosci* 122, 16–26. doi:10.1037/0735-7044.122.1.16.

Gore, F., Schwartz, E. C., Brangers, B. C., Aladi, S., Stujenske, J. M., Likhtik, E., et al. (2015). Neural Representations of Unconditioned Stimuli in Basolateral Amygdala Mediate Innate and Learned Responses. *Cell* 162, 134–145. doi:10.1016/j.cell.2015.06.027.

Groisman, A., Yang, S., and Schinder, A. (2020). Differential Coupling of Adult-Born Granule Cells

to Article Differential Coupling of Adult-Born Granule Cells to Parvalbumin and Somatostatin Interneurons. *Cell Rep* 30, 202–214. doi:10.1016/j.celrep.2019.12.005.

Guan, J., Jiang, J., Xie, H., and Liu, K. (2016). How Does the Sparse Memory " Engram " Neurons Encode the Memory of a Spatial – Temporal Event ? *Front Neural Circuits* 10, 1–10. doi:10.3389/fncir.2016.00061.

Guzman, S. J., Schlogl, A., Espinoza, C., Zhang, X., Suter, B., and Jonas, P. (2019). Fast signaling and focal connectivity of PV+ interneurons ensure efficient pattern separation by lateral inhibition in a full-scale dentate gyrus network model. *biorxiv*.

Han, Jin-Hee ; Kushner, Steven A; P. Yiu, Adelaide ; Hsiang , Hwa-Lin (Liz) ;Buch, Thorsten; Waisman, Ari ;Bontempi, Bruno ;L. Neve, Rachael ; Frankland, Paul W; Josselyn, S. A. (2009). Selective Erasure of a Fear Memory. *Science (80-)* 323, 1492–1496.

Hargreaves, E. L., Rao, G., Lee, I., and Knierim, J. J. (2005). Major dissociation between medial and lateral entorhinal input to dorsal hippocampus. *Science (80-)* 308, 1792–1794. doi:10.1126/science.1110449.

Hashimotodani, Y., Nasrallah, K., Jensen, K. R., Carrera, D., and Castillo, P. E. (2017). LTP at Hilar Mossy Cell-Dentate Granule Cell Synapses Modulates Dentate Gyrus Output by Increasing Excitation / Inhibition Balance Article LTP at Hilar Mossy Cell-Dentate Granule Cell Synapses Modulates Dentate Gyrus Output by Increasing Excitation / Inhi. *Neuron* 95, 928–943. doi:10.1016/j.neuron.2017.07.028.

Houser, C. R. (2007). Interneurons of the dentate gyrus: an overview of cell types, terminal fields and neurochemical identity. *Prog Brain Res* 163, 217–233. doi:10.1016/S0079-6123(07)63013-1.

Houzen, H., Kanno, M., and Kikuchi, S. (1998). AMPA / Kainate Receptor Activation Inhibits Neuronal Delayed Rectifier K / Current via Na / Entry in Rat Cortical Neurons. *Biochem Biophys Res Commun* 243, 617–621.

Hsu, T., Lee, C., Tai, M., and Lien, C. (2016). Differential Recruitment of Dentate Gyrus Interneuron Types by Commissural Versus Perforant Pathways. *Cereb Cortex* 26, 2715–2727. doi:10.1093/cercor/bhv127.

Hu, H., Martina, M., and Jonas, P. (2010). Dendritic Mechanisms Underlying Rapid Synaptic Activation of Fast-Spiking Hippocampal Interneurons. *Science (80-)* 327, 52–58. doi:10.1126/science.1177876.

Hunsaker, M. R., Rosenberg, J. S., and Kesner, R. P. (2008). The Role of the Dentate Gyrus , CA3a , b , and CA3c for Detecting Spatial and Environmental Novelty. *Hippocampus* 18, 1064–1073. doi:10.1002/hipo.20464.

Ikrar, T., Guo, N., He, K., Besnard, A., Levinson, S., and Hill, A. (2013). Adult neurogenesis modifies excitability of the dentate gyrus. 7, 1–15. doi:10.3389/fncir.2013.00204.

Jackson, M. B., and Scharfman, H. E. (1996). Positive Feedback From Hilar Mossy Cells to Granule Cells in the Dentate Gym Revealed by Voltage-Sensitive Dye and Microelectrode Recording. *J Neurophysiol* 76, 601–616. doi:10.1152/jn.1996.76.1.601.

Jinde, S., Zsiros, V., Jiang, Z., Nakao, K., Pickel, J., Kohno, K., et al. (2012). Hilar Mossy Cell Degeneration Causes Transient Dentate Granule Cell Hyperexcitability and Impaired Pattern Separation. *Neuron* 76, 1189–1200. doi:10.1016/j.neuron.2012.10.036.

Jones, G., Boyd, D. F., Yeung, S. Y., and Mathie, A. (2000). Inhibition of delayed recti ® er K + conductance in cultured rat cerebellar granule neurons by activation of calcium- permeable AMPA receptors. *Eur J Neurosci* 12.

Josselyn, S., and Tonegawa, S. (2020). Memory engrams: Recalling the past and imagining the future. *Science (80-)* 367, 1–14. doi:10.1126/science.aaw4325.

Jung, D., Kim, S., Sariev, A., Sharif, F., and Kim, D. (2019). Dentate granule and mossy cells exhibit distinct spatiotemporal responses to local change in a one- dimensional landscape of visual-tactile cues. *Sci Rep*, 1–15. doi:10.1038/s41598-019-45983-6.

Jung, M. W., and McNaughton, B. L. (1993). Spatial selectivity of unit activity in the hippocampal granular layer. *Hippocampus* 3, 165–182. doi:10.1002/hipo.450030209.

Kastellakis, G., and Poirazi, P. (2019). Synaptic Clustering and Memory Formation. *Front Mol Neurosci* 12, 1–13. doi:10.3389/fnmol.2019.00300.

Kim, D., Pare, D., and Nair, S. S. (2013). Assignment of Model Amygdala Neurons to the Fear Memory Trace Depends on Competitive Synaptic Interactions. *J Neurosci* 33, 14354–14358. doi:10.1523/JNEUROSCI.2430-13.2013.

Kim, J., Zhao, T., Petralia, R. S., Yu, Y., Peng, H., Myers, E., et al. (2012). mGRASP enables mapping mammalian synaptic connectivity with light microscopy. *Nat Methods* 9. doi:10.1038/nmeth.1784.

Klapoetke, N. C., Murata, Y., Kim, S. S., Pulver, S. R., Birdsey-benson, A., Cho, Y. K., et al. (2014). Independent optical excitation of distinct neural populations. *Nat Methods* 11, 338–346. doi:10.1038/nmeth.2836.

Lacy, J. W., Yassa, M. a, Stark, S. M., Muftuler, L. T., and Stark, C. E. L. (2011). Distinct pattern separation related transfer functions in human CA3/dentate and CA1 revealed using high-resolution fMRI and variable mnemonic similarity. *Learn Mem* 18, 15–18. doi:10.1101/lm.1971111.

Larimer, P., and Strowbridge, B. W. (2008). Nonrandom Local Circuits in the Dentate Gyrus. *J Neurosci* 28, 12212–12223. doi:10.1523/JNEUROSCI.3612-08.2008.

Lee, C., Kao, M., Hou, W., Wei, Y., Chen, C., and Lien, C.-C. (2016). Causal Evidence for the Role of Specific GABAergic Interneuron Types in Entorhinal Recruitment of Dentate Granule Cells. *Sci Rep*, 1–13. doi:10.1038/srep36885.

Leranth, C., and Hajszan, T. (2007). Extrinsic afferent systems to the dentate gyrus. *Prog Brain Res* 163, 63–85. doi:10.1016/S0079-6123(07)63004-0.

Leutgeb, J. K., Leutgeb, S., Moser, M.-B., and Moser, E. I. (2007). Pattern separation in the dentate gyrus and CA3 of the hippocampus. *Science* 315, 961–966. doi:10.1126/science.1135801.

Leutgeb, S., Leutgeb, J. K., Barnes, C. a, Moser, E. I., McNaughton, B. L., and Moser, M.-B. (2005). Independent codes for spatial and episodic memory in hippocampal neuronal ensembles. *Science* 309, 619–623. doi:10.1126/science.1114037.

Lin, J. Y., Lin, M. Z., Steinbach, P., and Tsien, R. Y. (2009). Characterization of Engineered Channelrhodopsin Variants with Improved Properties and Kinetics. *Biophys J* 96, 1803–1814. doi:10.1016/j.bpj.2008.11.034.

Liu, X., Ramirez, S., Pang, P. T., Puryear, C. B., Govindarajan, A., Deisseroth, K., et al. (2012). activates fear memory recall. *Nature* 484, 381–385. doi:10.1038/nature11028.

Luna, V. M., Anacker, C., Burghardt, N. S., Khandaker, H., Andreu, V., Millette, A., et al. (2019). Adult-born hippocampal neurons bidirectionally modulate entorhinal inputs into the dentate gyrus. *Science (80-)* 364, 578–583.

Marín-burgin, A., Mongiat, L. A., Pardi, M. B., and Schinder, A. F. (2012). Unique Processing During a Period of High Excitation/Inhibition Balance in Adult- Born Neurons. *Science (80-)*.

Marr.D (1971). Simple memory: a theory for archicortex. *R Soc* 262, 23–81.

Martin, S. J., and Morris, R. G. M. (2002). New Life in an Old Idea : The Synaptic Plasticity and Memory Hypothesis Revisited. *Hippocampus* 12, 609–636. doi:10.1002/hipo.10107.

Mcclelland, J. L., O'Reilly, R. C., and McNaughton, B. L. (1995). Why there are complementary learning System in the Hippocampus and Neocortex: Insights from the successes and failures of connectionist models of learning and memory. *Psychol Rev* 102, 419–457.

McHugh, T. J., Jones, M. W., Quinn, J. J., Balthasar, N., Coppari, R., Elmquist, J. K., et al. (2007). Dentate gyrus NMDA receptors mediate rapid pattern separation in the hippocampal network. *Science* 317, 94–99. doi:10.1126/science.1140263.

Morales, C., Facundo, J. F., Espinosa, N., Sacson, A., Lara-Vasquez, A., Garcia-Perez, M. A., et al.

(2019). Dentate gyrus somatostatin cells are required for contextual discrimination during episodic memory encoding. *biorxiv*.

Muller, R. U., and Kubie, J. L. (1987). The Effects of Changes in the Environment Hippocampal Cells on the Spatial Firing of Hippocampal Complex-Spike Cells. *J Neurosci* 7, 1951–1968.

Nakashiba, T., Cushman, J. D., Pelkey, K. a., Renaudineau, S., Buhl, D. L., McHugh, T. J., et al. (2012). Young dentate granule cells mediate pattern separation, whereas old granule cells facilitate pattern completion. *Cell* 149, 188–201. doi:10.1016/j.cell.2012.01.046.

Nakazawa, K. (2017). Dentate Mossy Cell and Pattern Separation. *Neuron*, 465–467. doi:10.1016/j.neuron.2017.01.021.

Neunuebel, J. P., and Knierim, J. J. (2014). CA3 retrieves coherent representations from degraded input: Direct evidence for CA3 pattern completion and dentate gyrus pattern separation. *Neuron* 81, 416–427. doi:10.1016/j.neuron.2013.11.017.

Nitz, D., and McNaughton, B. (2004). Differential modulation of CA1 and dentate gyrus interneurons during exploration of novel environments. *J Neurophysiol* 91, 863–872. doi:10.1152/jn.00614.2003.

Norman, K. A., and Reilly, R. C. O. (2003). Modeling Hippocampal and Neocortical Contributions to Recognition Memory : A Complementary-Learning-Systems Approach. *Psychol Rev* 110, 611–646. doi:10.1037/0033-295X.110.4.611.

O'Keefe, J., and Dostrovsky, J. (1971). The hippocampus as a spatial map. Preliminary evidence from unit activity in the freely-moving rat. *Brain Res* 34, 171–175.

Park, S., Kramer, E. E., Mercaldo, V., Rashid, A. J., Insel, N., Frankland, P. W., et al. (2016). Neuronal Allocation to a Hippocampal Engram. *Neuropsychopharmacology* 41, 2987–2993. doi:10.1038/npp.2016.73.

Pignatelli, M., Roy, D. S., Lovett, C., Smith, L. M., Muralidhar, S., Ryan, J., et al. (2019). Engram Cell Excitability State Determines the Efficacy of Memory Retrieval Article Engram Cell Excitability State Determines the Efficacy of Memory Retrieval. *Neuron* 101, 274–284. doi:10.1016/j.neuron.2018.11.029.

Ramirez, S., Liu, X., MacDonald, C. J., Moffa, A., Zhou, J., Redondo, R. L., et al. (2015). Activating positive memory engrams suppresses depression-like behaviour. *Nature* 522, 335–339. doi:10.1038/nature14514.

Ramirez, S., Tonegawa, S., and Liu, X. (2013). Identification and optogenetic manipulation of memory engrams in the hippocampus. *Front Behav Neurosci* 7, 226. doi:10.3389/fnbeh.2013.00226.

Ranganath, C. (2010). A Unified Framework for the Functional Organization of the Medial Temporal Lobes and the Phenomenology of Episodic Memory. *Hippocampus* 20, 1263–1290. doi:10.1002/hipo.20852.

Rao-ruiz, P., Couey, J. J., Marcelo, I. M., Bouwkamp, C. G., Slump, D. E., Matos, M. R., et al. (2019). Engram-specific transcriptome profiling of contextual memory consolidation. *Nat Commun* 10, 1–14. doi:10.1038/s41467-019-09960-x.

Rashid, A. J., Yan, C., Mercaldo, V., Hsiang, H. L., Park, S., Cole, C. J., et al. (2016). Competition between engrams influences fear memory formation and recall. *Science (80-)* 353, 383–388.

Ratzliff, A. H., Howard, A. L., Santhakumar, V., Osapay, I., and Soltesz, I. (2004). Rapid Deletion of Mossy Cells Does Not Result in a Hyperexcitable Dentate Gyrus : Implications for Epileptogenesis. *J Neurosci* 24, 2259–2269. doi:10.1523/JNEUROSCI.5191-03.2004.

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

Rolls, E. T. (2013). The mechanisms for pattern completion and pattern separation in the hippocampus. *Front Syst Neurosci* 7, 1–21. doi:10.3389/fnsys.2013.00074.

Rolls, E. T., and Kesner, R. P. (2006). A computational theory of hippocampal function, and empirical tests of the theory. *Prog Neurobiol* 79, 1–48. doi:10.1016/j.pneurobio.2006.04.005.

Rolls, E. T., Treves, A., Robertson, R. G., Georges-franc, P., Edmund, T., Treves, A., et al. (1998). Information About Spatial View in an Ensemble of Primate Hippocampal Cells. *J Neurophysiol* 79, 1797–1813.

Roy, D. S., Arons, A., Mitchell, T. I., Pignatelli, M., and Tonegawa, S. (2016). Memory retrieval by activating engram cells in mouse models of early Alzheimer's disease. *Nature* 531, 508–512. doi:10.1038/nature17172.

Roy, D. S., Muralidhar, S., Smith, L. M., and Tonegawa, S. (2017). Silent memory engrams as the basis for retrograde amnesia. doi:10.1073/pnas.1714248114.

Royer, S., Zemelman, B. V., Barbic, M., Losonczy, A., Buzsáki, G., and Magee, J. C. (2010). Multi-array silicon probes with integrated optical fibers: Light-assisted perturbation and recording of local neural circuits in the behaving animal. *Eur J Neurosci* 31, 2279–2291. doi:10.1111/j.1460-9568.2010.07250.x.

Ryan, T., Roy, D. S., Pignatelli, M., Arons, A., and Tonegawa, S. (2015). Engram cells retain memory under retrograde amnesia. *Science (80-)* 348, 1007–1013.

Sahay, A., Scobie, K. N., Hill, A. S., O'Carroll, C. M., Kheirbek, M. a, Burghardt, N. S., et al.

(2011a). Increasing adult hippocampal neurogenesis is sufficient to improve pattern separation. *Nature* 472, 466–470. doi:10.1038/nature09817.

Sahay, A., Wilson, D. a., and Hen, R. (2011b). Pattern Separation: A Common Function for New Neurons in Hippocampus and Olfactory Bulb. *Neuron* 70, 582–588. doi:10.1016/j.neuron.2011.05.012.

Sambandan, S., Sauer, J.-F., Vida, I., and Bartos, M. (2010a). Associative plasticity at excitatory synapses facilitates recruitment of fast-spiking interneurons in the dentate gyrus. *J Neurosci* 30, 11826–11837. doi:10.1523/JNEUROSCI.2012-10.2010.

Sambandan, S., Sauer, J., Vida, I., and Bartos, M. (2010b). Associative Plasticity at Excitatory Synapses Facilitates Recruitment of Fast-Spiking Interneurons in the Dentate Gyrus. 30, 11826–11837. doi:10.1523/JNEUROSCI.2012-10.2010.

Santhakumar, V., Bender, R., Frotscher, M., Ross, S. T., Hollrigel, G. S., Toth, Z., et al. (2000). Granule cell hyperexcitability in the early post-traumatic rat dentate gyrus : the ‘ irritable mossy cell ’ hypothesis. *J Physiol* 524, 117–134.

Satvat, E., Schmidt, B., Argraves, M., Marrone, D. F., and Markus, E. J. (2011). Changes in Task Demands Alter the Pattern of z if268 Expression in the Dentate Gyrus. *J Neurosci* 31, 7163–7167. doi:10.1523/JNEUROSCI.0094-11.2011.

Savanthrapadian, S., Meyer, T., Elgueta, C., Booker, S. a, Vida, I., and Bartos, M. (2014). Synaptic properties of SOM- and CCK-expressing cells in dentate gyrus interneuron networks. *J Neurosci* 34, 8197–209. doi:10.1523/JNEUROSCI.5433-13.2014.

Scharfman, H. E. (1995). Electrophysiological Evidence That Dentate Hilar Mossy Cells Are Excitatory and Innervate Both Granule Cells and Interneurons. *J Neurophysiol* 74, 179–194. doi:10.1152/jn.1995.74.1.179.

Scharfman, H. E. (2016). The enigmatic mossy cell of the dentate gyrus. *Nat Rev Neurosci* 17. doi:10.1038/nrn.2016.87.

Scharfman, H. E. (2018). Advances in understanding hilar mossy cells of the dentate gyrus. *Cell Tissue Res* 373, 643–652.

Scharfman, H. E., and Myers, C. E. (2012). Hilar mossy cells of the dentate gyrus: a historical perspective. *Front Neural Circuits* 6, 106. doi:10.3389/fncir.2012.00106.

Schmidt-Hieber, C., Jonas, P., and Bischofberger, J. (2004). Enhanced synaptic plasticity in newly generated granule cells of the adult hippocampus. *Nature* 429, 184–187. doi:10.1038/nature02553.

Schroder, W., Seifert, G., Hu, K., Hinterkeuser, S., and Steinha, C. (2002). MCN AMPA Receptor-

Mediated Modulation of Inward Rectifier K + Channels in Astrocytes of Mouse Hippocampus. *Mol Cell Neurosci* 19, 447–458. doi:10.1006/mcne.2001.1080.

Senzai, Y., and Buzsáki, G. (2017). Physiological Properties and Behavioral Correlates of Hippocampal Granule Cells and Mossy Cells. *Neuron* 93, 691-704.e5. doi:10.1016/j.neuron.2016.12.011.

Sloviter, R. S. (1983). " Epileptic " Brain Damage in Rats Induced by Sustained Electrical Stimulation of the Perforant Path . I . Acute Electrophysiological and Light Microscopic Studies. *Brain Res Bull* 10, 675–697.

Sloviter, R. S. (1991). Permanently Altered Hippocampal Structure , Excitability , and Inhibition After Experimental Status Epilepticus in the Rat : The " Dormant Basket Cell " Hypothesis and its Possible Relevance to Temporal Lobe Epilepsy. *Hippocampus* 1, 41–66.

Soriano, E., and Frotscherf, M. (1994). Mossy Cells of the Rat Fascia Dentata Are Glutamate-immunoreactive. *Hippocampus* 4, 65–70.

Stefanelli, T., Bertollini, C., Muller, D., Stefanelli, T., Bertollini, C., and Lu, C. (2016). Hippocampal Somatostatin Interneurons Control the Size of Neuronal Memory Ensembles Article Hippocampal Somatostatin Interneurons Control the Size of Neuronal Memory Ensembles. 1074–1085. doi:10.1016/j.neuron.2016.01.024.

Sun, Y., Grieco, S. F., Holmes, T. C., and Xu, X. (2017). Local and Long-Range Circuit Connections to Hilar Mossy Cells in the Dentate Gyrus. *eNeuro* 4, 1–21.

Tanaka, K. Z., and Mchugh, T. J. (2018). The Hippocampal Engram as a Memory Index. *J Exp Neurosci* 12, 1–4. doi:10.1177/1179069518815942.

Temprana, S. G., Mongiat, L. A., Lanuza, G. M., Schinder, A. F., Temprana, S. G., Mongiat, L. A., et al. (2015). Delayed Coupling to Feedback Inhibition during a Critical Period for the Integration of Adult-Born Granule Cells Article Delayed Coupling to Feedback Inhibition during a Critical Period for the Integration of Adult-Born Granule Cells. *Neuron* 85, 116–130. doi:10.1016/j.neuron.2014.11.023.

Tonegawa, S., Liu, X., Ramirez, S., and Redondo, R. (2015). Memory Engram Cells Have Come of Age. *Neuron* 87, 918–931. doi:10.1016/j.neuron.2015.08.002.

Toner, C. K., Pirogovsky, E., Kirwan, C. B., and Gilbert, P. E. (2009). Visual object pattern separation deficits in nondemented older adults. *Learn Mem* 16, 338–342. doi:10.1101/lm.1315109.

Torricelli, A., Contini, D., Mora, A. D., Pifferi, A., Re, R., Zucchelli, L., et al. (1997). Neurophotonics : non-invasive optical techniques for monitoring brain functions. 223–230.

Treves, a, and Rolls, E. T. (1992). Computational constraints suggest the need for two distinct input systems to the hippocampal CA3 network. *Hippocampus* 2, 189–199. doi:10.1002/hipo.450020209.

Treves, A., and Rolls, E. T. (1994). Computational Analysis of the Role of the Hippocampus in Memory. 4, 374–391.

Tsytsarev, V., Liao, L., Kong, K. V., Liu, Y., Erzurumlu, R. S., Olivo, M., et al. (2014). Recent Progress in Voltage-Sensitive Dye Imaging for Neuroscience Recent Progress in Voltage-Sensitive Dye Imaging for Neuroscience. *J Nanosci Nanotechnol* 14, 4733–4744. doi:10.1166/jnn.2014.9531.

Vetere, G., Tran, L. M., Moberg, S., Steadman, P. E., Restivo, L., Morrison, F. G., et al. (2019). Memory formation in the absence of experience. *Nat Neurosci* 22, 940. doi:10.1038/s41593-019-0389-0.

Wang, J. (2019). Searching basic units in memory traces : associative memory cells. *F1000Research* 8, 1–32.

Wilson, D. I. G., Langston, R. F., Schlesiger, M. I., Wagner, M., Watanabe, S., and Ainge, J. A. (2013). Lateral Entorhinal Cortex is Critical for Novel Object-Context Recognition. *Hippocampus* 23, 352–366. doi:10.1002/hipo.22095.

Xu, X., Olivas, N. D., Levi, R., Ikrar, T., and Nenadic, Z. (2010). High Precision and Fast Functional Mapping of Cortical Circuitry Through a Novel Combination of Voltage Sensitive Dye Imaging and Laser Scanning Photostimulation. *J Neurophysiol* 103, 2301–2312. doi:10.1152/jn.00992.2009.

Yiu, A. P., Mercaldo, V., Yan, C., Richards, B., Rashid, A. J., Hsiang, H. L., et al. (2014). Article Neurons Are Recruited to a Memory Trace Based on Relative Neuronal Excitability Immediately before Training. *Neuron* 83, 722–735. doi:10.1016/j.neuron.2014.07.017.

Yuan, M., Meyer, T., Benkowitz, C., Savanthrapadian, S., Ansel-bollepalli, L., Foggetti, A., et al. (2017). Somatostatin-positive interneurons in the dentate gyrus of mice provide local- and long-range septal synaptic inhibition. *Elife* 6, 1–25. doi:10.7554/eLife.21105.

Zhou, Y., Won, J., Karlsson, M. G., Zhou, M., Balaji, J., Neve, R., et al. (2009). CREB regulates excitability and the allocation of memory to subsets of neurons in the amygdala. 12, 1438–1443. doi:10.1038/nn.2405.CREB.

5. DISCUSSION

The results of this thesis suggest that SOM+ are necessary for pattern separation. How I mentioned in the introduction, low excitability and orthogonalization are two mechanisms that allow pattern separation. In the firsts part of this discussion, I will explain how SOM+ would participate in both processes. Then I will discuss the collaborative participation between SOM+ and other neuronal types of DG in pattern separation computational models. Finally, I will discuss some points that offer changes in the traditional view of pattern separation.

5.1 ORTHOGONALIZATION AND LOW EXCITABILITY: TWO MECHANISMS MEDIATED BY SOM+

Figure 8 shows that inhibition of SOM+ increases the firing rate in GCs and PV+. That suggests that, in pattern separation, SOM+ would control the excitability of GCs directly through feedback inhibition and indirectly through the control of the activity of PV+. In the following sections, I will discuss both possible mechanisms.

5.1.1 SOM+ would mediate lateral feedback inhibition

Theoretical models have proposed that lateral feedback inhibition of GCs in DG is necessary for appropriate ortogonalización of ensembles of GCs representing similar episodes (Rolls, 2013). Studies have proposed a fundamental role of PV+ in perisomatic lateral inhibition (Sambandan et al., 2010; Guzman et al., 2019). Recent research has suggested that HIL would

control this process through dendritic inhibition of GCs and perisomatic inhibition of PV+ (Yuan et al., 2017). In this line, studies have shown that when mice explore novel environments or during intense inputs activity, dendritic inhibition of GCs is significantly larger than perisomatic inhibition (Moser, 1996; Liu et al., 2014). This control involves not only reducing the discharge probability, but also controlling the temporal precision of action potential generation (Savanthrapadian et al., 2014; Yuan et al., 2017). In fact, for adequate recruitment of PV+ is necessary temporal coordination of input received (Sambandan et al., 2010). The PV+ activity generates a "winner takes all" mechanism in DG that would be used in pattern separation (Sambandan et al., 2010; Guzman et al., 2019; Morales et al., 2019). Thus, SOM+ would control orthogonalization of ensembles of GCs trough modulation of recruitment of "winner takes all" mechanism of PV interneuron (Yuan et al., 2017). For this temporal coordination, the intrinsic synaptic properties of HIL+ would be important and the projection of HIL onto the septum (Yuan et al., 2017) as well, which would allow the establishment of a coordinated theta rhythm between DG and septum, coordination that can be relevant during exploration of a novel environmental.

On the other hand, recent studies have shown that SOM+ also contribute with lateral inhibition of granule cell trough HIPP that would operate as other "winner takes all" mechanism (Yuan et al., 2017). But in this case, this lateral inhibition is probably more posterior at lateral inhibition of PV+ (Stefanelli,et.al 2016). Thus, inhibition of PV+ activity, as a recent theoretical model suggests (Guzman et al., 2019), and inhibition of SOM+, as I report here, implicates a bad performance in the pattern separation test. That could be explained for a bad modulation of "fast winner takes all " of PV+ trough HIL or a bad "delayed winner takes all " through HIPP. Future researches must study the role of both populations of SOM+ in the orthogonalization process.

5.1.2 SOM+ would mediate delayed feedback inhibition

Theoretical models have proposed that feedback inhibition of GCs in DG is necessary to control the excitability of GCs (Rolls, 2013). Mature and abGCs have been proposed as feedback inhibition drivers in DG (Drew et al., 2016; Stefanelli et al., 2016). Several studies suggested a leading role for abGCs (McAvoy et al., 2015b; Drew et al., 2016). In fact, the activation of abGCs engages inhibitory feedback principally emerging from PV+ , but not from SOM+ (Temprana et al., 2015). Moreover, the ablation of hippocampal neurogenesis induces high excitability in GCs (Ikrar et al., 2013) and impairs pattern separations (Clelland et al., 2009), supporting the idea that feedback inhibition mediated PV+ controls pattern separation. On the other hand, a recent study suggests that mature GCs poorly engage PV+ preferentially exciting SOM+ (Stefanelli et al., 2016). In this line, slice studies have shown HIPP's role in controlling the excitability of GCs (Hofmann et al., 2016; Stefanelli et al., 2016). Here I show that this control happens in vivo experiments and is necessary for pattern separation. Thus, it is likely that in pattern separation, two parallel circuits can be engaged during feedback inhibition. One in which abGCs preferentially recruit PV+ and another in which mature GCs preferentially activate SOM+ that would control the inhibition of GCs directly through HIPP. Due to that, newborn cells are more excitable that GCs (Marín-burgin et al., 2012). Feedback activity of SOM+ may be more delayed than feedback inhibition of PV+ cells; in fact, slice experiments show this (Stefanelli et al., 2016).

5.1.3 SOM+ would participate in remapping

Figure 10 suggests that SOM+ are necessary for contextual pattern separations. Additionally, figure 11 indicates that SOM+ are required for spatial memory. These results indicate that SOM+ could participate in the remapping of place cells. Specifically, suggest that "low excitability" and "orthogonalization" could induce a remapping mechanism.

Place cells are neurons of the Hippocampus that are selectively activated when rodents move in a maze (O'Keefe and Dostrovsky, 1971; O'Keefe, 1976). Place cells are part of a neural network that creates a map of an environment and where each place cells represent a part of this environment. This map would be useful to navigate based on previous experience (O'Keefe and Dostrovsky, 1971; O'Keefe, 1976). In this line, it has been shown that the set of place cells that are activated in exploration are reactivated in sleep (Wilson and Mcnaughton, 1993), and this information is useful for further navigations (Jarosiewicz and Skaggs, 2004; Leutgeb et al., 2005).

Remapping is a Hippocampus mechanism in which "place cells" change their firing pattern in response to a small change in the sensory inputs. (Muller and Kubie, 1987; Colgin et al., 2008) or behavioral context (Moita et al., 2004; Colgin et al., 2008). Thus, the remapping could be a mechanism for pattern separation.

HIPP project toward the outer molecular layer (OML) of the dentate DG where the LEC afferents carry contextual information (Hargreaves et al., 2005). On the other hand, there is evidence that shows that the inactivation of LEC afferents affects pattern separation (Vivar et al., 2012). Thus, HIPP are good candidates to control the excitatory inputs reaching distal

dendrites of GCs, i.e., contextual information from LEC. Therefore, the behavioral impairment resulting from the optogenetic inhibition of SOM+ could be attributed to deficits in the ability of the DG to differentially encode contextual information resulting from impaired in remapping and subsequent pattern separation

5.2 COMPUTATIONAL MODEL OF PATTERN SEPARATION

The results of my thesis suggest that SOM+ participates in pattern separation. However, its possible participation must consider the other neuronal types of DG, which has been related to pattern separation. An adequate model must consider SOM+ interaction with abGCs, mossy cells, and PV+.In the following sections, I will discuss these interactions.

5.2.1 SOM+ and computational models of pattern separation

The first pattern separation models contain four neuronal types: mossy cells, GCs, HIPP, and BC interneurons (Myers and Scharfman, 2009). One of the controversial results of this model was that they showed that the elimination of GABAergic interneurons improved pattern separation, which contradicts my thesis's results. A possible explication is that HIPP receives direct afferents from the perforant pathway in this model and does not receive input from GCs, i.e, according to this models, HIPP mediates a feedforward inhibition. However, abundant evidence shows that HIPP mediates feedback inhibition (Freund and Buzsáki, 1996; Yuan et al., 2017).

Other models considered the existence of neurogenesis in DG (Wiskott et al., 2006; Finnegan and Becker, 2015; Hofmann et al., 2016; Chavlis and Poirazi, 2017). However, these models do not show, with biological precision, how newborn cells interact with other neurons of DG. Other

models have suggested an interaction between abGCs and GABAergic interneurons (McAvoy et al., 2015a). Specifically, propose that abGCs activate a feedback inhibition. In this line, some studies show that abGCs can contact GABAergic interneurons like SOM+ (Groisman et al., 2020). However, these connections, in the context of pattern separation, has not been studied.

Other types of models have studied how mossy cells mediate GCs' inhibition through activation of BC interneurons (Danielson et al., 2017). They show that the elimination of mossy cells increases GCs' activity, which impairs pattern separation, which is explained by the loss of BC activity. However, some investigations (Larimer and Strowbridge, 2008; Yuan et al., 2017) suggest that mossy cells excite HIL. This new connection that has not been studied would obligate to postulate a new mechanism by which the mossy cells participate in pattern separation, besides the control of the excitability of GCs (Nakazawa, 2017)

5.2.2 Proposal of an integrative model for pattern separation that considers the activity of SOM+.

In this section, I propose a possible way in which different cell types of DG might interact and contribute to pattern separation.

It has been shown that mossy cells and abGCs present more remapping than mature granule cells (Danielson et al., 2016, 2017; Senzai and Buzsaki, 2017). That indicates that mossy cells and abGCs are more sensitive than GCs to detect small environmental changes. Besides, experiments suggest that these neuronal types would respond before than GCs (Marín-burgin et al., 2012; Jung et al., 2019), suggesting that both neuronal types could initiate pattern separation. On the other hand, both neuronal types are more sensitive to contextual changes (Danielson et

al., 2016, 2017; Senzai and Buzsaki, 2017; Luna et al., 2019), which means that contextual information would be more relevant than another type of information.

After activation of mossy cells and abGCs, they can initiate an inhibitory network. While abGCs can inhibit directly GCs (Luna et al., 2019), mossy cells and abGCs would activate PV (Scharfman, 2018; Groisman et al., 2020), which in turn produces a lateral inhibition (Espinoza et al., 2018).

On the other hand, SOM+ activity modulates the activity of PV+ (Savanthrapadian et al., 2014; Yuan et al., 2017) and can produce a lateral inhibition itself (Stefanelli et al., 2016). However, the evidence indicates that SOM+ activity is delayed when compared with the activity of PV+ (Hsu et al., 2016; Stefanelli et al., 2016). Thus, both PV+ and SOM+ interneurons control the activity of GCs, but likely in a different time with PV+ activity preceding SOM+ activity.

Some models propose pattern separation mechanisms that take into account and emphasize the role of PV interneurons (Guzman et al., 2019), abGCs (Sahay et al., 2011) and mossy cells (Nakazawa, 2017). Now, the results of my thesis indicate that there is another element, the SOM+. Thus, future models must investigate the participation of all elements mentioned.

5.3 DISEASE THAT ARE IN RELATION WITH LOSS OR BAD FUNCTION OF SOM+ CAN IMPLICATE PROBLEMS WITH PATTERN SEPARATION

An interesting prediction of my thesis is that diseases associated with a decline in SOM+ could also be related to pattern separation problems.

Evidence shows that in epilepsy, the SOM+ are significantly affected (Goldberg and Coulter, 2013; Hofmann et al., 2016). In agreement with my work, studies have shown that pattern separation is one of the cognitive abilities affected in epilepsy (Yim et al., 2015; Madar, 2018; Reyes et al., 2018).On the other hand, SOM+ loss is related to memory problems in the aging population, and it has been suggested that these memory problems result from problems in pattern separation (Spiegel et al., 2013).

Thus, a pattern separation test could be used to detect if DG is affected in these pathologies. The study of the neuronal circuit of DG could help develop appropriate therapy.

5.4 KIND OF TEST USED FOR STUDY OF PATTERN SEPARATION

Studies typically use the fear conditioning paradigm to characterize the DG' role in the encoding of overlapping contextual information (McHugh et al., 2007; Sahay et al., 2011; Deng et al., 2013). The results of my thesis (Figure 10) show that SOM+ are necessary for discrimination of overlapping contextual information in the episodic memory task. Then, SOM+ may participate in discrimination of overlapping contextual information in the fear conditioning task. However, it is important to note that both test do not activate the same neuronal networks. Given the fear conditioning task's emotional valence, the amygdala is likely to be recruited (Zheng et al., 2019). On the other hand, in the test that I used, the amygdala should not be recruited.

Thus, future research must study if SOM+ also participate in discrimination of context with emotional valence. Therefore, understand if there are several pattern separations mechanisms in Hippocampus or a unique pattern separation process.

5.5 THE POSSIBLE ROLE OF SOMATOSTATIN NEUROPEPTIDE

GABAergic interneurons release neuropeptides that are different in size and in action mechanisms than traditional neurotransmitters. An important characteristic is that they are released slower than traditional neurotransmitters, suggesting that they do not participate in rapid integrative functions (Baraban and Tallent, 2004). On the other hand, the release of neuropeptides could be significant when interneurons discharge a high frequency. In fact, in CA1, somatostatin containing interneurons discharge at high frequency in response to Achetilcoline (Pitler and Alger, 1992). This suggests that a possible role of somatostatin in pattern separation would happen when SOM+ firing rate is high. Probably, when mice explore a new enriched context, the activity of SOM+ increases. Interestingly, studies have shown that somatostatin receptors are principally in the molecular layer (Tallent, 2007) where arrives perforant inputs. That supports the idea that somatostatin participates in the control of perforant pathway inputs.

In the, CA1 of hippocampus somatostatin hyperpolarize the postsynaptic neurons through its effect in the current of calcio and potassium (Pittman and Siggins, 1981; Moore et al., 1988; Ishibashi and Akaike, 1995). Additionally, also in CA1, it has been shown that somatostatin have direct effect in inhibition of excitatory transmission (Tallent, 2007) and important role in

counteract the epileptic activity (Tallent and Siggins, 1999). Specifically, somatostatin have an important function in the control of neuronal activity of Dentate Gyrus activity. The induction of long-term potentiation (LTP) in Dentate Gyrus was impaired when application of high-frequency trains happens during the application of somatostatin (Baratta et al., 2002). That suggest a possible roles o somatostatin in memory formation. In this line, there are evidences that show that, somatostatin decrease with age and there is a relation between this decrease and the impaired in memory function (Tallent, 2007). In addition, there are evidences that show that the activity of somatostatin is relevant to control of the epileptic activity. That suggest that the possible cognitive role of somatostatin include the control of dentate gyrus excitability.

6. REFERENCES OF DISCUSSION

Acsády, L., Katona, I., Martínez-Guijarro, F. J., Buzsáki, G., and Freund, T. F. (2000). Unusual target selectivity of perisomatic inhibitory cells in the hilar region of the rat hippocampus. *J Neurosci* 20, 6907–6919. doi:20/18/6907 [pii].

Baraban, S. C., and Tallent, M. K. (2004). Interneuron Diversity series: Interneuronal neuropeptides - Endogenous regulators of neuronal excitability. *Trends Neurosci* 27, 135–142. doi:10.1016/j.tins.2004.01.008.

Baratta, M. V, Lamp, T., and Tallent, M. K. (2002). Somatostatin depresses long-term potentiation and Ca2+ signaling in mouse dentate gyrus. *J Neurophysiol* 88, 3078–3086. doi:10.1152/jn.00398.2002.

Bekinschtein, P., Kent, B. a., Oomen, C. a., Clemenson, G. D., Gage, F. H., Saksida, L. M., et al. (2013). BDNF in the dentate gyrus is required for consolidation of "pattern-separated" memories_suplementary_information. *J Chem Inf Model* 53, 1689–1699. doi:10.1017/CBO9781107415324.004.

Burke, S. N., Wallace, J. L., Nematollahi, S., Uprety, A. R., and Barnes, C. A. (2010). Pattern Separation Deficits May Contribute to Age-Associated Recognition Impairments. *Behav Neurosci* 124, 559–573. doi:10.1037/a0020893.

Chavlis, S., and Poirazi, P. (2017). Pattern separation in the hippocampus through the eyes of computational modeling. doi:10.1002/syn.21972.

Clelland, C. D., Choi, M., Romberg, C., Clemenson, G. D., Fragniere, A., Tyers, P., et al. (2009). A functional role for adult hippocampal neurogenesis in spatial pattern separation. *Science* 325, 210–3. doi:10.1126/science.1173215.

Danielson, N. B. ., Kaifosh, P., Zaremba, J. D. D., Lovett-Barron, M., Tsai, J., Denny, C. A. A., et al. (2016). Distinct Contribution of Adult-Born Hippocampal Granule Cells to Context Encoding. *Neuron* 90, 101–112. doi:10.1016/j.neuron.2016.02.019.

Danielson, N. B., Turi, G. F., Ladow, M., Chavlis, S., Petrantonakis, P. C., Danielson, N. B., et al. (2017). In Vivo Imaging of Dentate Gyrus Mossy Cells in Behaving Mice. *Neuron* 93, 552–559. doi:10.1016/j.neuron.2016.12.019.

Deng, W., Mayford, M., and Gage, F. H. (2013). Selection of distinct populations of dentate granule cells in response to inputs as a mechanism for pattern separation in mice. *Elife* 2013, 1–21. doi:10.7554/eLife.00312.

Drew, L. J., Kheirbek, M. A., Luna, V. M., Denny, C. A., Cloidt, M. A., Wu, M. V., et al. (2016). Activation of local inhibitory circuits in the dentate gyrus by adult-born neurons. *Hippocampus* 26. doi:10.1002/hipo.22557.

Espinoza, C., Guzman, S. J., Zhang, X., and Jonas, P. (2018). Parvalbumin+ interneurons obey unique connectivity rules and establish a powerful lateral-inhibition microcircuit in dentate gyrus. *Nat Commun*, 1–10. doi:10.1038/s41467-018-06899-3.

Felix-Ortiz, a. C., and Tye, K. M. (2014). Amygdala Inputs to the Ventral Hippocampus Bidirectionally Modulate Social Behavior. *J Neurosci* 34, 586–595. doi:10.1523/JNEUROSCI.4257-13.2014.

Finnegan, R., and Becker, S. (2015). Neurogenesis paradoxically decreases both pattern separation and memory interference. *Front Syst Neurosci* 9, 1–12. doi:10.3389/fnsys.2015.00136.

Freund, T. F., and Buzsáki, G. (1996). Interneurons of the hippocampus. *Hippocampus* 6, 347–470. doi:10.1002/(SICI)1098-1063(1996)6:4<347::AID-HIPO1>3.0.CO;2-I.

Goldberg, E. M., and Coulter, D. a (2013). Mechanisms of epileptogenesis: a convergence on neural circuit dysfunction. *Nat Rev Neurosci* 14, 337–49. doi:10.1038/nrn3482.

Groisman, A., Yang, S., and Schinder, A. (2020). Differential Coupling of Adult-Born Granule Cells to Article Differential Coupling of Adult-Born Granule Cells to Parvalbumin and Somatostatin Interneurons. *Cell Rep* 30, 202–214. doi:10.1016/j.celrep.2019.12.005.

Guzman, S. J., Schlogl, A., Espinoza, C., Zhang, X., Suter, B., and Jonas, P. (2019). Fast signaling and focal connectivity of PV+ interneurons ensure efficient pattern separation by lateral inhibition in a full-scale dentate gyrus network model. *biorxiv*.

Hofmann, G., Balgooyen, L., Mattis, J., Deisseroth, K., and Buckmaster, P. S. (2016). Hilar somatostatin interneuron loss reduces dentate gyrus inhibition in a mouse model of temporal lobe epilepsy. *Epilepsia* 1, n/a-n/a. doi:10.1111/epi.13376.

Holden, H. M., and Gilbert, P. E. (2012). Less efficient pattern separation may contribute to age-related spatial memory deficits. *Front Aging Neurosci* 4, 1–6. doi:10.3389/fnagi.2012.00009.

Holden, H. M., Hoebel, C., Loftis, K., and Gilbert, P. E. (2012). Spatial pattern separation in cognitively normal young and older adults. *Hippocampus* 22, 1826–1832. doi:10.1002/hipo.22017.

Hsu, T., Lee, C., Tai, M., and Lien, C. (2016). Differential Recruitment of Dentate Gyrus Interneuron Types by Commissural Versus Perforant Pathways. *Cereb Cortex* 26, 2715–2727. doi:10.1093/cercor/bhv127.

Ikrar, T., Guo, N., He, K., Besnard, A., Levinson, S., Hill, A., et al. (2013). Adult neurogenesis modifies excitability of the dentate gyrus. *Front Neural Circuits* 7, 1–15. doi:10.3389/fncir.2013.00204.

Ishibashi, H., and Akaike, N. (1995). Somatostatin modulates high-voltage-activated Ca2+ channels in freshly dissociated rat hippocampal neurons. *J Neurophysiol* 74, 1028–1036. doi:10.1152/jn.1995.74.3.1028.

Jung, D., Kim, S., Sariev, A., Sharif, F., and Kim, D. (2019). Dentate granule and mossy cells exhibit distinct spatiotemporal responses to local change in a one- dimensional landscape of visual- tactile cues. *Sci Rep*, 1–15. doi:10.1038/s41598-019-45983-6.

Leal, S. L., and Yassa, M. A. (2018). Integrating new findings and examining clinical applications of pattern separation. *Nat Neurosci* 21, 163–173. doi:10.1038/s41593-017-0065-1.

Liu, Y., Cheng, J., and Lien, C. (2014). Rapid Dynamic Changes of Dendritic Inhibition in the Dentate Gyrus by Presynaptic Activity Patterns. 34, 1344–1357. doi:10.1523/JNEUROSCI.2566-13.2014.

Luna, V. M., Anacker, C., Burghardt, N. S., Khandaker, H., Andreu, V., Millette, A., et al. (2019). Adult-born hippocampal neurons bidirectionally modulate entorhinal inputs into the dentate gyrus. *Science (80-)* 364, 578–583.

Madar, A. D. (2018). Pattern separation in the hippocampus, in health and epilepsy. *thesis Phd* 34, 515–525. doi:10.1016/j.tins.2011.06.006.

Marín-burgin, A., Mongiat, L. A., Pardi, M. B., and Schinder, A. F. (2012). Unique Processing During a Period of High Excitation/Inhibition Balance in Adult- Born Neurons. *Science (80-)*.

McAvoy, K., Besnard, A., and Sahay, A. (2015a). Adult hippocampal neurogenesis and pattern separation in DG: a role for feedback inhibition in modulating sparseness to govern population-based coding. *Front Syst Neurosci* 9, 1–7. doi:10.3389/fnsys.2015.00120.

McAvoy, K., Besnard, A., and Sahay, A. (2015b). Adult hippocampal neurogenesis and pattern separation in DG: A role for feedback inhibition in modulating sparseness to govern population-based coding. *Front Syst Neurosci* 9, 1–7. doi:10.3389/fnsys.2015.00120.

McHugh, T. J., Jones, M. W., Quinn, J. J., Balthasar, N., Coppari, R., Elmquist, J. K., et al. (2007). Dentate gyrus NMDA receptors mediate rapid pattern separation in the hippocampal network. *Science* 317, 94–99. doi:10.1126/science.1140263.

Miranda, M., Kent, B. A., Morici, J. F., Gallo, F., Saksida, L. M., Bussey, T. J., et al. (2018). NMDA receptors and BDNF are necessary for discrimination of overlapping spatial and non-spatial memories in perirhinal cortex and hippocampus. *Neurobiol Learn Mem* 155, 337–343. doi:10.1016/j.nlm.2018.08.019.

Moore, S. D., Madamba, S. G., Joëls, M., and Siggins, G. R. (1988). Somatostatin augments the M-current in hippocampal neurons. *Science (80-)* 239, 278–280. doi:10.1126/science.2892268.

Morales, C., Facundo, J. F., Espinosa, N., Sacson, A., Lara-Vasquez, A., Garcia-Perez, M. A., et al. (2019). Dentate gyrus somatostatin cells are required for contextual discrimination during episodic memory encoding. *biorxiv*.

Moser, E. I. (1996). Altered Inhibition in an Exploration of Dentate Task Granule Cells during

Spatial Learning. *J Neurosci* 16, 1247–1259. Available at: http://www.jneurosci.org/cgi/content/short/16/3/1247.

Myers, C. E., and Scharfman, H. E. (2009). A Role for hilar cells in pattern separation in the dentate gyrus: A computational approach. *Hippocampus* 19, 321–337. doi:10.1002/hipo.20516.

Nakazawa, K. (2017). Dentate Mossy Cell and Pattern Separation. *Neuron*, 465–467. doi:10.1016/j.neuron.2017.01.021.

Pitler, B. Y. T. A., and Alger, B. E. (1992). Cholinergic excitation of GABAergic interneurons in the rat hippocampal slice. *J Physiol*, 127–142.

Pittman, Q. J., and Siggins, G. R. (1981). Somatostatin hyperpolarizes hippocampal pyramidal cells in vitro. *Brain Res* 221, 402–408. doi:10.1016/0006-8993(81)90791-5.

Reyes, A., Holden, H. M., Chang, Y. H. A., Uttarwar, V. S., Sheppard, D. P., DeFord, N. E., et al. (2018). Impaired spatial pattern separation performance in temporal lobe epilepsy is associated with visuospatial memory deficits and hippocampal volume loss. *Neuropsychologia* 111, 209–215. doi:10.1016/j.neuropsychologia.2018.02.009.

Rolls, E. T. (2013). The mechanisms for pattern completion and pattern separation in the hippocampus. *Front Syst Neurosci* 7, 1–21. doi:10.3389/fnsys.2013.00074.

Sahay, A., Wilson, D. a., and Hen, R. (2011). Pattern Separation: A Common Function for New Neurons in Hippocampus and Olfactory Bulb. *Neuron* 70, 582–588. doi:10.1016/j.neuron.2011.05.012.

Sambandan, S., Sauer, J.-F., Vida, I., and Bartos, M. (2010). Associative plasticity at excitatory synapses facilitates recruitment of fast-spiking interneurons in the dentate gyrus. *J Neurosci* 30, 11826–11837. doi:10.1523/JNEUROSCI.2012-10.2010.

Savanthrapadian, S., Meyer, T., Elgueta, C., Booker, S. a, Vida, I., and Bartos, M. (2014). Synaptic properties of SOM- and CCK-expressing cells in dentate gyrus interneuron networks. *J Neurosci* 34, 8197–209. doi:10.1523/JNEUROSCI.5433-13.2014.

Scharfman, H. E. (2018). Advances in understanding hilar mossy cells of the dentate gyrus. *Cell Tissue Res* 373, 643–652.

Senzai, Y., and Buzsaki, G. (2017). Physiological Properties and Behavioral Correlates of Hippocampal Granule Cells and Mossy Cells. 691–704. doi:10.1016/j.neuron.2016.12.011.

Spiegel, A. M., Koh, M. T., Vogt, N. M., Rapp, P. R., and Gallagher, M. (2013). Hilar interneuron vulnerability distinguishes aged rats with memory impairment. *J Comp Neurol* 521, 3508–3523. doi:10.1002/cne.23367.

Stefanelli, T., Bertollini, C., Luscher, C., Muller, D., and Mendez, P. (2016). Hippocampal Somatostatin Interneurons Control the Size of Neuronal Memory Ensembles Article

Hippocampal Somatostatin Interneurons Control the Size of Neuronal Memory Ensembles. *Neuron* 89, 1–12. doi:10.1016/j.neuron.2016.01.024.

Tallent, M. K. (2007). Somatostatin in the dentate gyrus. *Prog Brain Res* 163, 265–284. doi:10.1016/S0079-6123(07)63016-7.

Tallent, M. K., and Siggins, G. R. (1999). Somatostatin acts in CA1 and CA3 to reduce hippocampal epileptiform activity. *J Neurophysiol* 81, 1626–1635. doi:10.1152/jn.1999.81.4.1626.

Temprana, S. G., Mongiat, L. A., Lanuza, G. M., Schinder, A. F., Temprana, S. G., Mongiat, L. A., et al. (2015). Delayed Coupling to Feedback Inhibition during a Critical Period for the Integration of Adult-Born Granule Cells Article Delayed Coupling to Feedback Inhibition during a Critical Period for the Integration of Adult-Born Granule Cells. *Neuron* 85, 116–130. doi:10.1016/j.neuron.2014.11.023.

Vivar, C., Potter, M. C., Choi, J., Lee, J. Y., Stringer, T. P., Callaway, E. M., et al. (2012). Monosynaptic inputs to new neurons in the dentate gyrus. *Nat Commun* 3, 1107–1111. doi:10.1038/ncomms2101.

Wiskott, L., Rasch, M. J., and Kempermann, G. (2006). A functional hypothesis for adult hippocampal neurogenesis: Avoidance of catastrophic interference in the dentate gyrus. *Hippocampus* 16, 329–343. doi:10.1002/hipo.20167.

Yim, M. Y., Hanuschkin, A., and Wolfart, J. (2015). Intrinsic rescaling of granule cells restores pattern separation ability of a dentate gyrus network model during epileptic hyperexcitability. *Hippocampus* 25, 297–308. doi:10.1002/hipo.22373.

Yuan, M., Meyer, T., Benkowitz, C., Savanthrapadian, S., Ansel-bollepalli, L., Foggetti, A., et al. (2017). Somatostatin-positive interneurons in the dentate gyrus of mice provide local- and long-range septal synaptic inhibition. *Elife* 6, 1–25. doi:10.7554/eLife.21105.

Zheng, J., Stevenson, R. F., Mander, B. A., Knight, R. T., Yassa, M. A., Lin, J. J., et al. (2019). Multiplexing of Theta and Alpha Rhythms in the Amygdala-Hippocampal Circuit Supports Pattern Separation of Emotional Information. *Neuron*, 1–12. doi:10.1016/j.neuron.2019.03.025.