THE INVOLVEMENT OF THE GABAERGIC HIPPOCAMPAL SYSTEM DURING AGING AND ITS RELATIONSHIP WITH COGNITIVE PERFORMANCE IN *OCTODON DEGUS*

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θ: Firing rate threshold in the network model

τ_{mem}: Time membrane constant

Aβ: Amyloid-β

aCSF: Artificial cerebrospinal fluid

ADL: Activity of daily living

AD: Alzheimer disease

APOE: Apolipoprotein E

BB: Bad burrower

BT: Burrowing task

CA1, CA3: Cornu Ammonis

CGE: Caudal ganglionic eminence

C_{mem}: Membrane conductance

DG: Dentate gyrus

EC: Entorhinal cortex

E/I: Excitation/Inhibition balance

EP: Episodic memory

EPSP: Excitatory postsynaptic potential

FR: Firing rate

g: Inhibition/excitation ratio

GABA: Gamma-aminobutyric acid

$GABA_AR$: GABA ionotropic receptor

$GABA_BR$: GABA metabotropic receptor

GABA-T: GABA-transaminase enzyme

GAD: Glutamate decarboxylase enzyme

GAT1: GABA transporter 1

GB: Good burrower

IN: Integrate and fire model

ISI: inter-spike interval

J: EPSP amplitude in the network model

LTD: Long-term depression

LTP: Long-term potentiation

MCI: Mild cognitive impairment

MEA: Multielectrode arrays

MGE: Medial ganglionic eminence

MPFC: Medial prefrontal cortex

MTL: Medial temporal lobe

NOR: Novel object recognition

OF: Open field

PFC: Prefrontal Cortex

PP: Perforant pathway

PTX: Picrotoxin

SA: Spontaneous activity

SEM: Semantic memory

SP: Synaptic Plasticity

SPM: Spatial memory

V_0: Network activity

V_{ext}: External activity

vGAT: Vesicular GABA transporter

V_{thr}: Threshold frequency

WHO: World of health Organization

ABSTRACT

Si bien es conocido que el envejecimiento se acompaña de deterioro cognitivo severo, desconocemos por qué ciertos individuos mantienen excelentes capacidades cognitivas hasta muy avanzada edad. El objetivo de esta tesis fue estudiar las capacidades cognitivas del *Octodon degus*, un roedor que en condiciones de laboratorio vive hasta 10 años, y el cual ha demostrado ser un excelente modelo para el estudio natural del envejecimiento y neurodegeneración. En este trabajo de tesis nos interesó identificar posibles mecanismos biológicos para mantener una buena capacidad cognitiva durante el envejecimiento. Para responder a esta pregunta utilizamos un enfoque conductual y el estudio fisiológico de los circuitos neuronales del hipocampo. Las pruebas conductuales fueron: i) Reconocimiento de Objetos Nuevos (NOR), ii) Campo Abierto (OF) y iii) y la prueba de escarbar (BT). La BT se encuentra relacionada con la Actividad de la Vida Diaria (ADL), constituye una actividad natural y espontánea en la vida de los degus y se ha visto asociada a la presencia de biomarcadores de neurodegeneración en el hipocampo. En los estudios fisiológicos evaluamos la actividad eléctrica de neuronas del hipocampo para determinar la integridad funcional del sistema GABAérgico. Nuestros resultados sugieren que los degus envejecidos que mantienen un buen rendimiento en BT, muestran también una buena actividad hipocampal. De particular interés, fueron los resultados de la región CA3 del hipocampo, la cual es considerada un núcleo de procesamiento y codificación de señales, que muestra un sistema GABAergico mejor preservado. Interesantemente, los degus con bajo rendimiento cognitivo en BT muestran baja actividad en cuanto a su actividad espontanea hipocampal, lo cual sugiere un control sináptico a través de una vía alternativa a GABA. Finalmente, el uso de un modelo computacional para la región de CA3, una red neuronal de equilibrio aleatorio no fue concluyente en cuanto a poder diferenciar nuestras dos poblaciones de degus envejecidos. En resumen, nuestros resultados sugieren que el buen rendimiento cognitivo en BT para ciertos degus se explica gracias a un sistema GABAérgico más saludable.

SUMMARY

Although it is known that aging is accompanied by severe cognitive impairment, we do not understand why specific individuals maintain excellent cognitive abilities until very old age. The objective of this thesis was to study the cognitive skills of *Octodon degus*, a long-lived rodent that in laboratory conditions lives up to 10 years and which has proven to be an excellent model for the natural study of aging and neurodegeneration. In this thesis work, we identified possible biological mechanisms for maintaining good cognitive capacity during aging. To examine this question, we used a behavioral approach and physiological studies of hippocampal neural circuits. The behavioral tests were: i) Novel Object Recognition (NOR), ii) Open Field (OF), and iii) and the Burrowing Test (BT), all related to hippocampal functioning.

Interestingly, BT is related to Activity of Daily Living (ADL), constitutes a natural and spontaneous activity in the life of degus, and is associated with biomarkers of neurodegeneration in the hippocampus. In physiological studies, we evaluated the electrical activity of hippocampal neurons to determine the functional integrity of the GABAergic system. Our results suggest that aged degus that maintain good BT performance also show good hippocampal activity. Of particular interest, results from the CA3 region of the hippocampus, which is considered a signal processing and coding nucleus, showed a better-preserved GABAergic system. Interestingly, degus with low cognitive performance in BT show low activity in terms of their spontaneous hippocampal activity, suggesting a synaptic control through an alternative pathway to GABA. Finally, the use of a computational model for CA3, a random equilibrium neural network, was inconclusive in terms of differentiating our two populations of aged degus, those that exhibit good cognitive performance, from those that do not occur during aging. In summary, our results suggest that a healthier GABAergic system explains the good cognitive performance in BT for certain degus.

INTRODUCTION

According to the World Health Organization (WHO), the impact of aging on cognitive capabilities is highly associated with the dementia process affecting around 50 million people. More than 900 million of the world's population are aged 60 years or more (elderly population) and 2 billion by 2050 (WHO, 2018). The rate of dementia will be 23.5% in 2050, and the number of elderly people will increase by 140% in the next 50 years (Eshkoor et al., 2015).

According to Chilean Instituto Nacional de Estadística (INE 2019) projections, elderly people corresponding to 11.9% of the Chilean population in 2019 and will rise to 18.9% in 2035, almost duplicating the percentage in 16 years. In 2017 Chilean government heralded dementia and aging as a public health problem, developing a unique program (Plan de Dementia, MINSAL 2017).

In biological terms, a decline in cognitive function during aging has been associated with molecular and cellular alterations, affecting neuronal plasticity mechanisms, brain network circuits, and therefore the integrity of cognition capacities (Ball 1977; Landfield et al., 1978; Markowska et al., 1989; Bliss & Collingridge 1993; Foster & Norris 1997). Although several age-related neurological changes have been identified during healthy aging, the alterations observed in age-associated disorders, such as dementia as Alzheimer disease (AD) or Parkinson's disease (Burke & Barnes, 2006) are more dominant. Nevertheless, it is very interesting to note that certain individuals age while maintaining good cognitive conditions and even at very advanced ages. Here we were interested in identifying, in a long-lived rodent model, *Octodon degus* (degus), possible biological mechanisms associated with healthy cognitive states in an aged degus population.

COGNITION, LEARNING, AND MEMORY

The study of cognition includes learning, memory, mental or motor actions (Okano et al., 2000). There are different types of memories, such as **semantic memory** (SEM) related to living things, objects, facts, and events without temporal context; **declarative or episodic memory** (EM), ability to recall facts and circumstances consciously; and **spatial memory** (SPM) required for identification and navigation of space, among others (Buzsáki & Moser, 2012).

Aging introduces a progressive impairment for cognition, where a mild cognitive impairment (MCI) is characterized by a progressive and irreversible reduction of EM, eliciting a lower ability to think and decide (Riepe 2005; Eschkoor et al., 2015). Moreover, a decrease in cognitive skills during aging is accompanied by neurodegeneration occurring in multiples brain areas (Squire et al., 2007).

The integrity of EM depends on the medial temporal lobe (MTL), where the hippocampus is located (Fig 1A) (Ulanovsky & Moss 2007), and its deterioration in humans induces mostly EM impairment, not affecting classic conditioning and non-associative learning (Squire & Zola 1996). In rats, lesions on different MTL structures produce impairments in learning for complex spatial discrimination (related with SPM) and in working memory involving non-spatial information (Jarrard et al., 2004).

HIPPOCAMPAL STRUCTURE

The hippocampus is a convex elevation of gray matter inside the inferior temporal horn of the lateral ventricle floor, forming part of the limbic system in MTL (Fig 1A) (John, 2006) and comprising the hippocampal network (CA1, CA3, dentate gyrus, and subiculum) (Giap et al., 2000)

Figure 1B, illustrated the hippocampus localization and its associated neural circuits. The MTL complex is shown in the left and its connectivity with the prefrontal cortex (PF) in the right (Simons & Spiers 2003). The interaction between these areas plays a key role supporting long-term memory across the following steps: a) Synaptic processing and action potential generation in the PFC is sent

to the hippocampus through MTL structures, such as the perirhinal cortex, para-hippocampus, and entorhinal cortex (EC) (Simons & Spiers 2003). b) After processing in the hippocampus, the information is sent back to the PFC, to the medial prefrontal cortex (MPFC), through the Fornix and subcortical nuclei (Simons & Spiers 2003). Figure 1B also shows the hippocampus inputs from high processing areas as PFC and subcortical nuclei to neocortical association areas and external sensory inputs. In this interaction, the hippocampus represents a nucleus of concurrent processing.

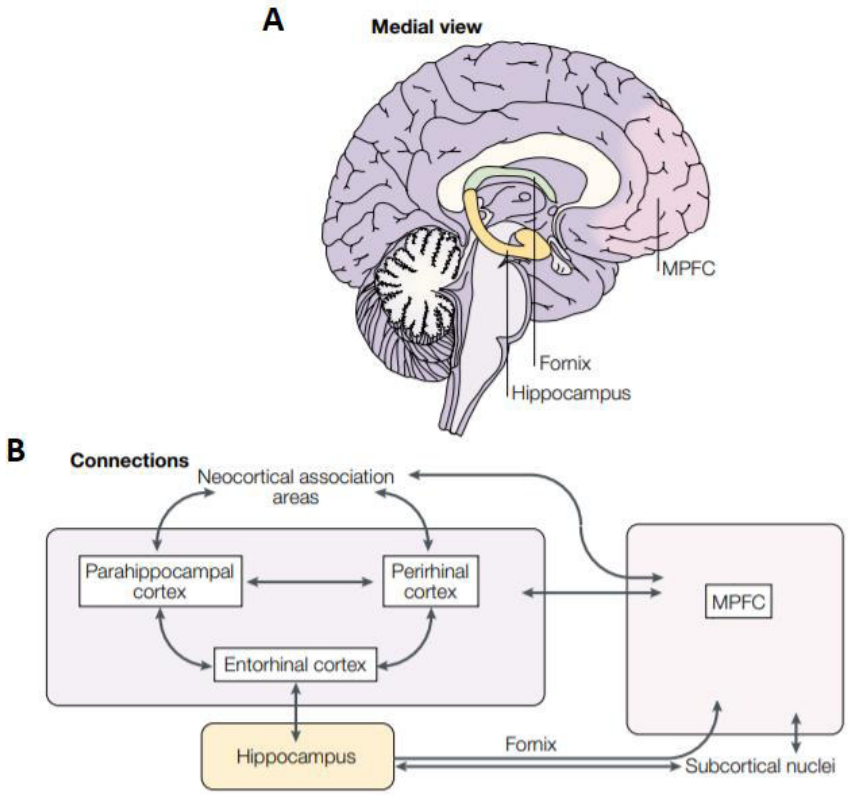

Figure 1. Interactions between PFC and MTL in the long-term memory process in humans. A Medial view of the brain where are located Hippocampus (orange), Fornix (green), and medial prefrontal cortex (MPFC). **B** Connections between medial temporal lobe (MTL) structure (left) and prefrontal cortex (PFC, right). The MTL comprises the hippocampus and amygdala and the entorhinal cortex, perirhinal cortex, and parahippocampal cortex. There are large cortico-cortical direct reciprocal connections between the PFC and the MTL. Hippocampus is connected to PFC through unidirectional projections from CA1 to caudal MPFC and subicular complex but with reciprocal connections. In addition, the medial temporal lobe receives information from a range of unimodal and polymodal sensory association areas. This information predominantly enters through the perirhinal and para-hippocampal cortices, which project back to these regions. Modified from Simons & Spiers 2003.

The internal structure and circuitry of the hippocampus consist of three main areas: Dentate Gyrus (DG), CA1, and CA3. These three areas are highly connected, forming the internal hippocampal network, and consist of an excitatory three synaptic pathway: 1) the EC projecting its axons to the DG through the perforant pathway (PP) (synapse 1); 2) the DG (granular cells) projecting its axons to pyramidal cells in CA3 through mossy fibers (synapse 2); 3) CA3 pyramidal neurons relay their signals to CA1 pyramidal neurons through Schaffer collaterals (synapse 3), where CA3 is the core of the hippocampal network in processing and coding information. Besides, CA3 pyramidal neurons send projection over the same CA3 neurons, known as recurrent excitatory synapses, and receive direct projections from EC layer II neurons through the PP (Witter, 2007). Finally, CA1 pyramidal neurons back-projected into deep-layer neurons of the EC (Deng et al., 2010) (Fig 2A). Moreover, CA2 seems to play its computational function, and it is not a simple transition zone between CA3 and CA1 (Knierim 2015).

Despite these three zones working together as part of the limbic system, each separated zone has its features and specific tasks.

1.- **The CA1 region** has few associational connections, focused on smaller modules of cells, and decodes the output of CA3 (Yang et al., 2014). Place cells were discovered in CA1, and some of these do not require input from CA3 and DG in some cases, only receiving spatial information from layer III of EC (Moser et al., 2008). Synaptic plasticity (SP) has been studied quite deeply in CA1 with different protocols to induce it (Citri & Malenka, 2008).

2.- **The CA3 region** is related to memory processes, encoding spatial localization and EM, being susceptible to seizures and neurodegeneration processes (Cherubini & Miles 2015). One of the main features of CA3 is the internal connectivity due to recurrent connections. The axon collaterals of CA3 pyramidal cells ramify extensively in the same region, allowing to make many excitatory contacts with

both excitatory and inhibitory neurons, typically called associational connections (Witter, 2007). CA3 relates to every hippocampal zone, even with himself, receiving and communicating signal as the hippocampal network's computational core (Knierim 2015). This region is also implicated in pattern completion task, being capable of using partial cues to recover stored representations from DG (O'Reilly & McClelland 1994)

3.- **DG** comprises granular cells connected with EC (presynaptic) and CA3 (postsynaptic). Granular cells have meager firing rates (0.01 – 0.1Hz), allowing them and the DG network to produce sparse coding activity, that means the network minimize overlap neural pattern among neurons using fewer small subsets of the available neurons (Jung & McNaughton 1993; Neunuebel & Knierim 2012; Diamantaki et al., 2016). Its characteristic has been related to pattern separation tasks, being the DG crucial for preprocessing information, changing in a sparse representation from EC, and then sending to CA3 (McHugh et al., 2007; Kassab & Alexandre, 2018). Although, neurogenesis in the adult brain is very uncommon (Piatti et al., 2013), the adult hippocampus conserves its capacity to produce new neurons (Gage et al., 1995; Toda et al.,2018). This process adds flexibility to the hippocampal network related to the new acquisition of memories and knowledge (Deng et al., 2010).

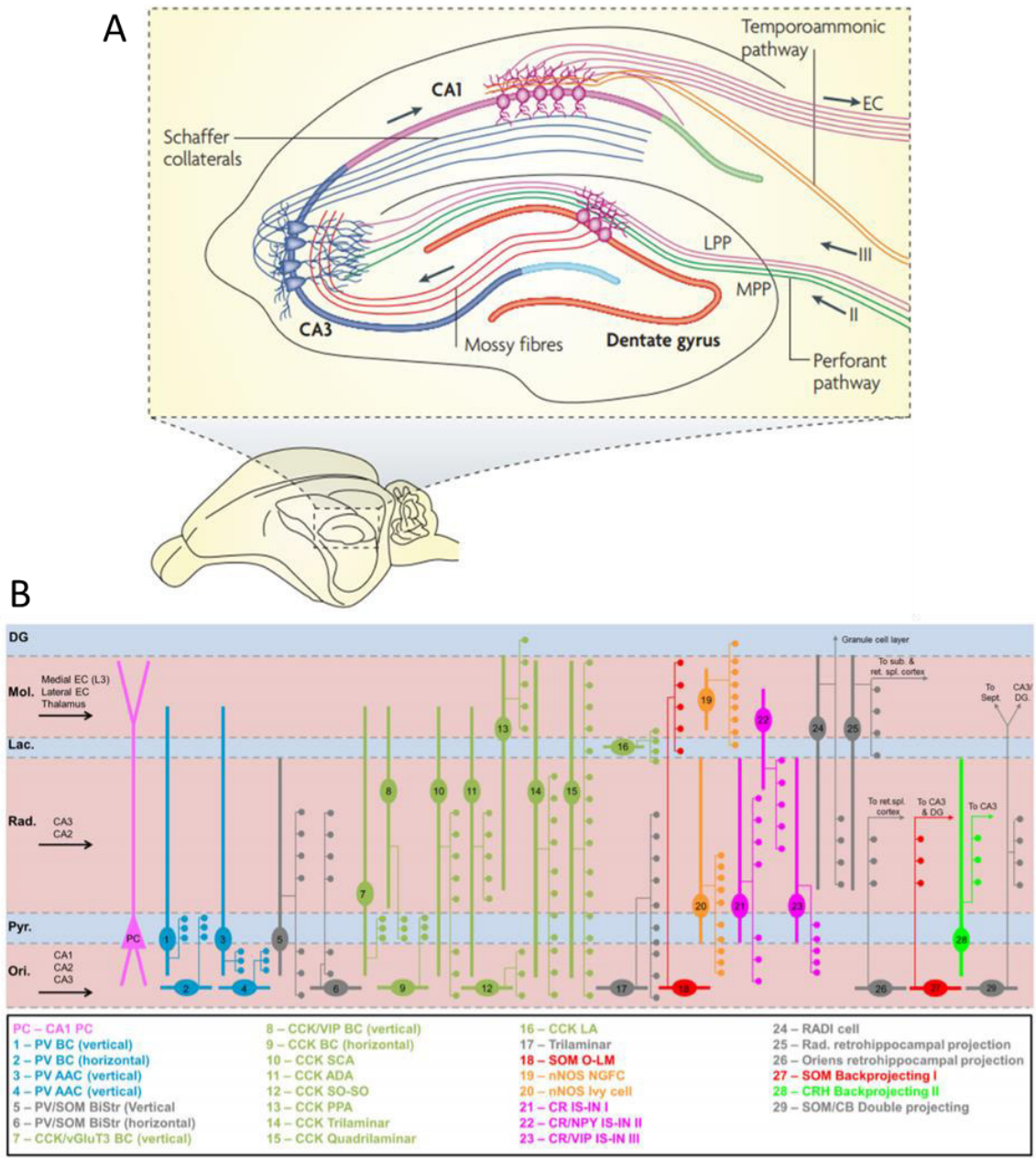

Figure 2. Hippocampal structure. A Hippocampal structure and pathways of excitatory neurons. Dentate gyrus (DG), CA3, and CA1 (Deng et al., 2010). **B** Hippocampal Interneuron network and their subtypes depending on molecular markers (Parvalbumin (PV), Somatostatin (SOM), Cholecystokinin (CCK)) (Booker and Vida 2018).

As mentioned before, the hippocampus relates to brain associative areas through the EC, its anatomic structure is helpful for post-processing and comparison. The hippocampus is assimilated to a hub, integrating multiple sources in particular for the spatial navigation (O'keefe & Nadel 1978). O'Keefe & Dostrovsky, in 1971, recording CA1 neurons, in freely moving mice, established the hippocampus as a spatial reference map (O'Keefe & Dostrovsky 1971), since specific cells (place cells) fire for an exclusive place in the environment. According to their place field, the entire environment was represented by neuronal population activity or cell assemblies (O'Keefe 1976, Wilson & McNaughton 1993). Later, place cells have been reported for different zones of the hippocampus CA1, CA3, and DG (Moser et al., 2008).

Cell assemblies was a term introduced by Donald Hebb in 1949 *"that hypothesized a discrete and strongly interconnected group of neurons that represent a cognitive entity, such as thought, concept, or object"* (Buzsaky 2010). Place cells encoding an environment are being stored as SMP and recalling in two manners: being exposed again to the same environment or evoking the trajectory done (Buzsaky 2013).

In summary, the hippocampus encodes the environment using spatial representation and SPM through place cells reinforcing the idea that the primary function of the hippocampal system is supporting spatial navigation (Nadel 1991, Rolls 1999, Ekstrom et al. 2003, Ulanovsky & Moss 2007).

HIPPOCAMPUS AT AGING

Aging corresponds to a natural biological process in which our primary biological functions are gradually and progressively altered. Our sensory and cognitive abilities are no exception, and great attention has been paid to the deterioration of our learning and memory capacities. The hippocampus has been one of the principal structures of studies related to the aging process and loss of cognitive skills. Moreover, a series of behavioral tasks (e.g., Morris water maze, novel object recognition, eight-arm maze) have been developed to measure the dependency of cognitive abilities and the

hippocampal function. For example, SP, well studied in the hippocampus, corresponds to the modification of synaptic transmission efficacy in creating, modifying, or erasing memories, and its biological integrity plays a central role in incorporating new experiences into persistent memory traces (Citri & Malenka 2008). In classical terms, two types of SP (among others) have been described at the synaptic level, long-term potentiation (LTP) and long-term depression (LTD), both corresponding to critical mechanisms for learning and memory and has been study during aging and neurodegenerative processes (Burke & Barnes 2006). For example, alterations in LTP and LTD mediated by Schaffer collaterals to CA1 neurons have been associated with age-related cognitive decline (Rosenzweig et al., 1997 (Landfield & Lynch 1977; Burke and Barnes, 2006; Boric et al., 2008). Decreased LTP in the hippocampus leads to memory dysfunction, while increased LTP leads to improved SPM. (Tang et al., 1999; Morris et al., 2003). Partial or complete loss of LTP and LTD in CA1, CA3, and DG are consistent with cognitive impairment during aging (Deupree et al., 1991; Burke & Barnes, 2006). **Moreover, the hippocampus and its normal SP state are critical for the aging process and conservation of healthy memories and learning processes.**

According to cognitive maps theory (Tolman 1948), place cells are responsible for an individual's mental neural representation in an environment (allocentric map). Hence EM (in humans) relates to spatial memory (in animals) (Cacucci et al., 2008). Furthermore, hippocampal place cells failure impairs spatial codification during aging, precluding animals for an adequate representation of the environment and, therefore, an EM y impairment (Cacucci et al., 2008).

GABAERGIC SYSTEM

The GABAergic interneurons synthesize and release the gamma-aminobutyric acid (GABA), an inhibitory neurotransmitter (Fig. 3). Interneurons are essential to determine and control the Excitation/Inhibition balance (E/I) in neuronal networks (Rozycka & Liguz-Lecznar, 2017). The GABA synthesis occurs into the presynaptic neuron by glutamate decarboxylase enzyme dimer (GAD65 and GAD67), which decarboxylate the glutamate amino acid. Vesicle uptake synthesized GABA by

vesicular GABA transporter (vGAT). Action potential increases Ca^{2+} inward to the presynaptic terminal of the GABA nerve by voltage-dependent Ca^{2+} channels that trigger GABA vesicles release to synaptic cleft (Gonzalez-Burgos et al., 2011). GABA can activate GABA ionotropic receptors ($GABA_AR$) or GABA metabotropic receptors ($GABA_BR$). Postsynaptic GABAR induces Cl^- inward to the neuron by $GABA_AR$, decreasing membrane potential and hyperpolarizing the neuron. In a hyperpolarization state, it is more difficult for the neuron to reach a threshold to trigger an action potential, producing postsynaptic inhibition (Sigel & Steinmann., 2012). GABA degradation is mediated by the GABA-transaminase enzyme (GABA-T), generating succinic semialdehyde (Rowley et al., 2012). At the same time, GABA transporter 1 (GAT1), localized at the extra synaptic neuronal membrane, takes up GABA inside the presynaptic terminal after activating $GABA_AR$ (Gonzalez-Burgos et al., 2011). Glial cells also support GABA intake, expressing GAT3, limited spread of GABA from the synapsis (Minelli et al., 1996).

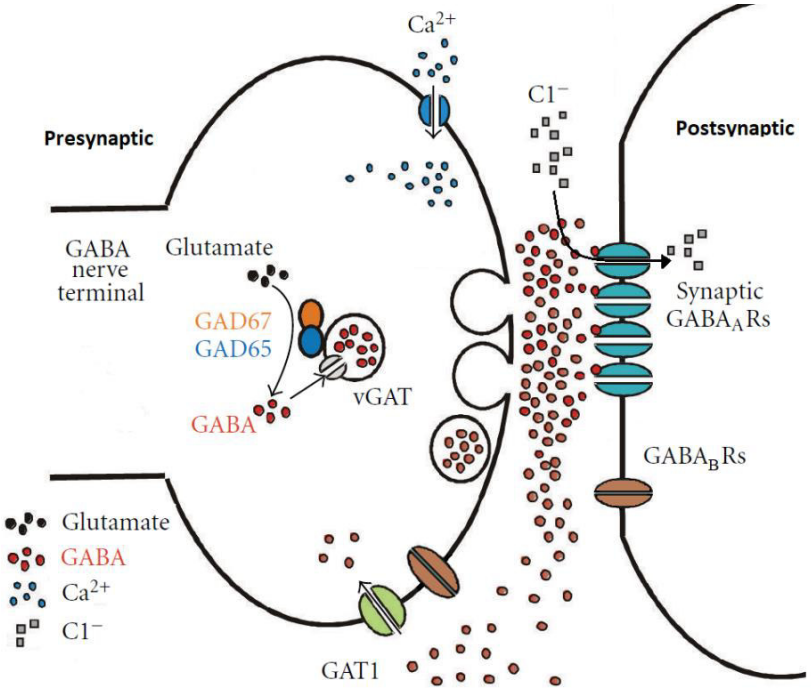

Figure 3. Scheme of GABA in an inhibitory terminal. Presynaptic terminal of GABA nerve where GABA (red circle) is synthesized from glutamate amino acid (black circle) by enzyme dimer GAD67-GAD65 (orange and blue, respectively). New GABA synthesized is uptake to vesicle by vesicular GABA transporter (vGAT, gray). After an action potential, voltage-dependent Ca^{2+} channels (blue) inward Ca^{2+} triggering the vesicular release of GABA to the synaptic cleft. GABA activates $GABA_AR$ (cyan), located in the postsynaptic neuron, promoting Cl^- (gray square) inward and hyperpolarizing postsynaptic terminal (Modified from Gonzalez-Burgos et al., 2011).

GABAERGIC SYSTEM AT AGING

During aging, neuronal chemical changes affect the efficiency of synaptic transmission and thus dysfunction of neuronal circuits, SP, learning, memory, and finally behavior (Sibille 2013). Studying neuronal networks and their dynamic response in a time and space manner during aging and neurodegeneration is possible experimentally by inducing molecular changes into the synaptic system, the basic unit of a neural networks (Pelkey et al., 2017). For example, a decrease in GABA levels can be related to a decline of GAD enzyme in their two isoforms GAD65 and GAD67, as well the GABA receptors distribution (Rozycka & Liguz-Lecznar 2017). The receptor number and its distribution are critical in preserve the functional strength of GABAergic synapses. $GABA_A$ receptor, mediates fast inhibition and express in the postsynaptic neuron, has been shown to decrease aging (between 5% to 35%), reducing inhibition (Shen et al, 2010). The $GABA_B$ receptor, which mediates tonic inhibition, can be expressed into pre- and postsynaptic manners (Fig. 3) (Emson 2007). The decrease of $GABA_B$ and $GABA_A$ receptor in postsynaptic neurons decreases inhibition. Nevertheless, reducing the $GABA_B$ receptor at the presynaptic level strengthens inhibition due to losing inhibition that promotes GABA vesicles release (Rozycka & Liguz-Lecznar, 2017). Hence, the impact of $GABA_B$ receptors in the aged population still unclear since it can lead to opposite effects on the control of neuronal activity (Wang et al., 2010; Bañuelos et al., 2014).

During aging, neuronal hyperactivity has been describe in mouse AD brains with cognitive impairments (Palop et al., 2007; Haberman, Koh, et al., 2017). Specifically, the increase of spontaneous activity in pyramidal neurons is due to a decrease of spontaneous activity of postsynaptic inhibitory potential, as well as a reduction of amplitude and frequency of the inhibitory current, altering the E/I imbalance, and is mediated by GABA receptors and interneurons (McQuail et al. 2015). Furthermore, a mouse AD-like showed a reduction of gamma oscillatory activity due to impaired parvalbumin interneurons, where restoration of its functions recovered gamma oscillations, reduced memory deficits, and premature mortality of mice (Verret et al., 2012). Also in an AD mouse, a transition of neuronal activity from a hyperactive state in early stages of the disease to a decreased

activity in late stages, due to the effect of amyloid peptides on the glutamatergic system, has been observed (Palop et al., 2007).

Moreover, aged rats conserving cognitive performances increased CA1 inhibitory synaptic strength, through tonic inhibition, where DG exhibited fast inhibitory transmission (Tran et al., 2018). The later represent an alternative compensatory mechanism to restore E/I balance, a mechanism that is different to young rats because they did not show a similar inhibition state. In a related work, rats without cognitive impairment had identical values of E/I ratio in DG compared to young rats through DG interneurons recruitment (feedforward inhibition) (Tran et al., 2019). The E/I balance corresponds to a critical mechanism to preserve cognitive skills during aging, where GABAergic function in the hippocampus seems to be crucial.

HIPPOCCAMPAL CONNECTIVITY

The interneurons play essential functions, as gain control and dynamic range to modulate neuronal circuits. The later helping the selectivity of sensorial features; SP; accuracy and temporal regulation of neuronal firing of excitatory cells; synchronization and generation of cortical rhythms; maintenance the E/I balance (Tremblay et al., 2016). Anatomically, excitatory cells follow a structured distribution (Fig. 2A) where interneurons have their neuronal bodies scattered into principals subareas, positioning their somatodendritic tree to achieve an effective integration and control towards excitatory cells (Pelkey et al., 2017)(Fig. 2B).

During development, interneurons need to be precisely integrated to coordinate and control neuronal activity (Batista-Brito & Fishell 2009). In this process, activation and expression of different genes confer their characteristics: Parvalbumin corresponds to 40% of interneurons in the cortex and hippocampus; Somatostatin (30%); and Serotonin receptor 5HT3a (10%)(Tremblay et al., 2016). Germinal proliferative zones, medial ganglionic eminence (MGE), and caudal ganglionic eminence (CGE), both in the posterior zone, produce around 90% of interneurons in the cortex and hippocampus (60% MGE and 30% CGE) (Pelkey et al., 2017).

The principal function of interneurons is the excitability control of pyramidal cells (CA1 and CA3) and granular cells (DG), thus controlling hippocampus activity from action potential level to network connectivity (Pelkey et al., 2017). A network with strong inhibition improves synaptic transmission (Rozycka & Liguz-Lecznar, 2017). Although interneurons represent only 10-15% of the hippocampus cells, they regulate the neuronal synaptic activity, both afferent and efferent, including cortical circuits synchronization across a broad frequency spectrum for the brain critical functions (O'Keefe & Recce 1993; Steriade et al., 1993; Csicsvari et al., 2003; Pelkey et al., 2017). Brain oscillations (0.5 Hz to 500 Hz) are related to different brain states (Buzsaki & Watson 2012). Hippocampal gamma oscillations (30-80 Hz) are associate with cell assembly synchrony, triggered by both external stimuli (spatial) or internal stimuli (non-spatial stimuli), and are essential for propagating and storing information in neuronal circuits (Harris et al., 2003). Brain oscillatory activity are based on repetitive trains of inhibitory postsynaptic potentials (Buzsaki & Watson 2012). Furthermore, inhibitory activity reduced and enhanced excitability in alternance and a temporally coordinated manner, synchronized a group of neurons or cell assembly (Buzsaki 2010).

BEHAVIORAL TESTS TO MEASURE COGNITIVE STATE OF ANIMALS

Behavior corresponds to a series of coordinated brain actions, based on learning and memory, in response to internal or external stimuli (Levitis et al., 2009). Currently, there is a wide variety of behavioral tests designed to assess the cognitive state of animals (Byrne & Bates 2006). In addition, these tests are related to specific brain areas, e.g., the water maze assess the function of the SPM and hippocampus (Vorhees & Williams 2006). There is clear advantage to use spontaneous or natural behaviors to test for learning and memory capacities in animals. Burrowing, climbing, or standing upright, are examples of behaviors that naturally involve exploration, motivation, learning, and memory (Makowska & Weary 2016) and can be used to test for the integrity of the brain function under less stress levels or overtraining (Hanell and Marklund 2014). Moreover, natural, or spontaneous behaviors are assimilated with Activities of Daily Living (ADL) (Merceron-Martınez et

al., 2021). ADL is defined as the necessary personal care activities as dressing, grooming, bathing, toileting, eating, and ambulation or more complex activities as meal preparation, shopping, telephone use. In humans, ADL are the first behaviors been affected during neurodegenerative diseases, such as Alzheimer's disease (AD) (Reisberg et al., 2001).

Here we introduce a burrowing task (BT) to measure animals' cognitive state related to spatial exploration (Deacon 2009). Burrowing, which can be assimilate with ADL, correspond to a natural behavior carried by various rodents, allowing them to take advantage of their natural environment, to protect them against depredators or climate conditions, food storage, or protection (Dudek et al. 1983). Under laboratory conditions, the cytotoxic injury of the hippocampus decreases the burrowing performance (Deacon R et al., 2002). In a previous study in degus the BT performance was correlated with the presence of AD biomarkers (APP, ApoE, beta-amyloid), including neuroinflammation marker (Interleukin 1-beta, Interleukin 6, Interferon alfa, tumor necrosis factor alfa) (Deacon et al. 2015).

DEGUS A NATURAL MODEL OF AGING

Natural animal models that show neurodegenerative manifestations during aging are clear alternative to transgenic models, where gene overexpression is a problem. Degus, an endemic rodent from Chile, belong to the Octodontidae family, are diurnal crepuscular, medium-sized rodents and live-in friendships with high social interaction (Hurley et al., 2018). Under natural conditions, about 90% of degus are depredated before turning two years old (Ebensperger et al., 2009). However, under laboratory conditions degus can live up to 8–10 years old and they are useful in the search of biomarkers for neurodegeneration (Ardiles et al., 2013). It has been reported in older degus a decrease in their cognitive performances associated with accumulation of amyloid-β (Aβ) and tau deposition (Inestrosa et al. 2005). Degus and humans share high peptide homology for Aβ sequence, differing in only one amino acid. Where rat and mouse show three amino acids substitution (Inestrosa et al. 2005) (Salazar et al., 2016). Moreover, ApoE correspond to a gene risk associated with AD and its polymorphism (three variants) in humans increase AD risk (Yin et al., 2018). In degus, the ApoE

shows high homology with human ApoE (Salazar et al., 2016). Deacon et al. 2015 shows a good correlation between poor BT performance and the increase of ApoE and Aβ-peptide expression (Tai et al., 2014) (Deacon et al. 2015). Moreover, degus aged from 24-60 months old presented cognitive impairment in a T-maze and novel object recognition (NOR), with a decline in long-term potentiation (LTP) and long-term depression (LTD) (Ardiles et al. 2012).

Here we were interested in understanding why some degus are more cognitively affected than others during aging. To quantify the cognitive deficit, we will use NOR, OF, and BT tests. To assess the physiological status of hippocampal E/I GABAergic mechanisms, we will use a multi-electrode and pharmacological methodology.

Experimental design

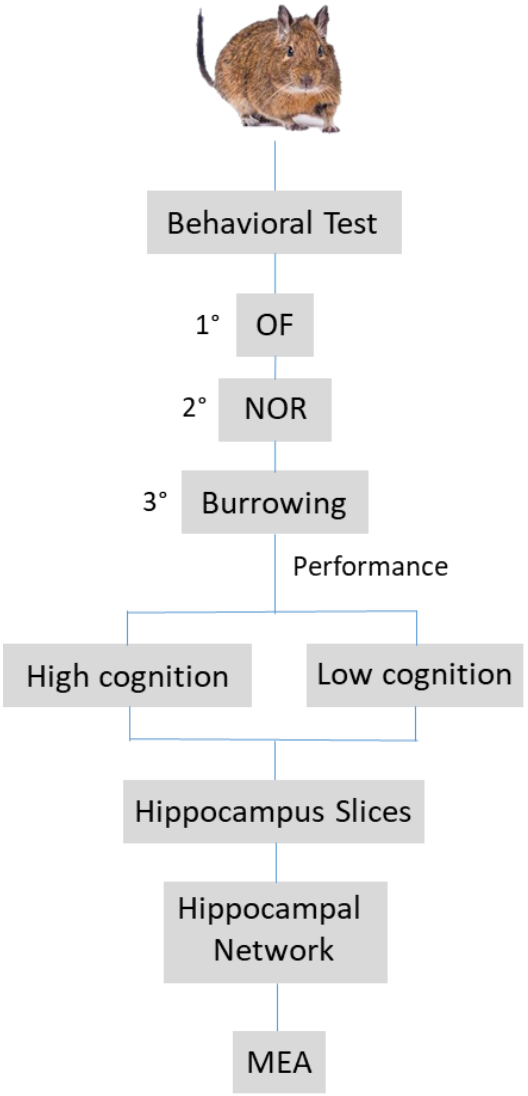

Figure 4. Experimental design: 35 aged degus (between 24 and 94 months) were submitted to behavioral tests: Open Field (OF), Novel Object Recognition (NOR), and Burrowing Task (BT). Based on their respective cognitive performances in BT, degus were classified as performing with "high" or "low" cognition. Hippocampal slices were used to study their functional properties using a multielectrode device along a pharmacological approach. Finally, a computational model approach was used to approach the hippocampal neural networks proprieties during aging.

Animals

Degus were maintained in the animal facility at the Universidad de Valparaiso, at 25 °C with 12/12hr light and dark cycle, with *ad-libitum* access to food and water. Before a behavioral procedure, degus were maintained for 45 min in habituation, next to the behavioral room, to avoid possible stress associated with the transfer from the animal facility to the lab. All procedures were made during the morning (9 am to 11 am) to avoid behavioral variation due to circadian rhythms.

For the physiological experiments, degus were sacrificed by decapitation but previously anesthetized with Isoflurane (3.5% over 600 mL/min of O_2) (RWD, China) using an anesthesia chamber (RWD 510, China). Hippocampal slices (300 µm) were obtained by cutting in a vibratome (1000 Plus, Vibratome) in a cold-dissection buffer (4°C). It contains 2.6 mM KCl, 1.23 mM NaH_2PO_4, 26 mM $NaHCO_3$, 212.7 mM Sucrose, 10 mM Glucose, 3 mM $MgCl_2$, and 1 mM $CaCl_2$, bubbled with mix O_2 (95%) and CO_2 (5%). The slices before recording were recovered for 1 hr in a stabilization chamber at 32°C in artificial cerebrospinal fluid (aCSF) containing 2.6 mM KCl, 1.23 mM NaH_2PO_4, 26 mM $NaHCO_3$, 124 mM NaCl, 10 mM Glucose, 1 mM $MgCl_2$ (3mM to MEA recording), and 2 mM $CaCl_2$, bubbled with mix O_2 (95%) and CO_2 (5%). All procedures met the Universidad de Valparaíso protocols for bioethics #BEA 141-19 and international and ANID bioethics and biosecurity standards.

Behavior test

Open field (OF)

To ensures mobility, health, and maze habituation degus were evaluated for their locomotor activity using a OF consisting of 5 min free exploration in a white Plexiglass circle (180 cm. in diameter and 80 cm in higher). Before an experiment, the surface of the OF was cleaned with ethanol 70%. The total time exploration and the total distance traveled was analyzed using the video recording of the session and an ANY-maze software. The exploration of the center and periphery of the OF was calculated as the ratio: (center time) or (periphery time) /(center time + periphery time).

Novel object recognition memory (NOR)

To evaluate recognition memory, we use a novel object NOR test consisting of three parts. (i) Familiarization, where degus explore a pair of identical objects by 180 s; (ii) retention, where degus are transferred to its cage for 2 hrs. and objects are cleaned and changed; (iii) recognition, where degus explore a pair of different objects during 180 s consisting in a familiar object (FO) (extra copy of a previous now familiar object) and a novel object (NO). A preference index (PI) was calculated based on object selective exploration as PI = NO / NO + FO in seconds. Objects were select of similar size in metal, glass, or plastic with similar size.

Burrowing test

We adapted the method for BT described in (Decon 2006). The Burrowing Test uses a burrow apparatus (gray plastic tube with 30 cm long and 10.5 cm diameter) filled with 1.300 g rabbit food pellet. Degus did not eat rabbit food. The test consisted in keeping the degus during one hour in the burrowing cage, where the food displaced (weight in grams) from the apparatus was measured. We fix a threshold value of 130 g (10% of total food in the device) of food expelled out as a criterion to characterize bad burrower (BB) degus for values less than the 10% or good burrower (GB) degus with values equal or superior to the 10% (Deacon et al., 2002; Deacon et al., 2015).

Electrophysiological recordings

Hippocampal slices were mounted into a multi-electrode recording device (MEA, 252 recording electrodes, Multichannel Systems, Germany), fitting on a plastic O-ring cylinder with a dialysis membrane (Spectra MWCO 25,000) for contact. The diameter of each electrode was 30 µm, separated by 200 µm between them and covering a surface of 3.2 cm. Temperature was maintained at 33 °C (TCO2, Multichannel Systems, Germany) with a constant ACSF at 5mL/min perfusion (PPS2, Multichannel Systems, Germany), bubble with O_2 (95%) and CO_2 (5%) by 2 hrs previous to electrophysiological recording in order to stabilize the slices. Data were recorded using MC/Rack software (MultichannelSystem, Germany) with a sampling rate of 20000 Hz. Once mounted into the

MEA matrix, the hippocampal slices stay for 10 min for stabilization before an experiment. Video pictures of the hippocampal slice into the recording chamber were saved for control (Pco pixelfly, Germany, into an inverted microscope (ECLIPSE TE200, Nikon, Japan), with 5x ocular)

Each recording consists of 10 min of spontaneous activity, follow of pharmacological activation by picrotoxin (PTX) 100 µM by 20 min. 50 µL of PTX (100 mM main stock) diluted in 45 mL of aCSF under a constant O_2/CO_2 mix bubble.

Spike Sorting and analysis

After completion of an electrophysiological experiment a spike sorting procedure was performed to separate neurons as described in (Yger et al. 2018). Briefly, spike sorting consists of five main steps:

- Filtering: The raw extracellular signals are high pass filtered with a Butterworth filter of order three (cutoff of 300 Hz).
- Whitening: Spurious spatial correlations between nearby recordings electrodes are removed in this step.
- Clustering is a spike sorting core, where action potentials are given to a single unit (template) and separated from other units. The clustering algorithm is based on classifying spikes according to their similarity and separating them.
- Fitting: Once the template dictionary is done, the fitting process is used to match the template in recording and thus reconstruct the signal along the whole recording.
- Merging: Finally, duplicate templates merge to get valid units or neurons.

The spiking-circus software was ran using default parameters, only changing the waveform time detection (from 5 ms to 3 ms). The configuration file is divided into five main steps: the frequency and filter to use; number of clusters and template per electrode; threshold to detect spikes; or set-up experiments condition (number of electrodes and geometric distribution in MEA matrix, the distance among electrodes, etc.). Once cell sorting was done, a manual supervisor (GUI) was required, using

a Matlab version GUI. We selected units that accomplished the following criterion: More than 1000 spikes during the complete recording and less than 1% of spikes-pair violate the refractory period (3 ms).

Firing, maximum firing rate, Inter-spike Interval (ISI), and Burst Rate: We compare our experimental recording groups using: a) Firing rate (FR) computed as the number of spikes divided by the recording length during SA (10 min) and PTX activity (20 min) conditions; b) ISI measured as the time difference between two consecutives spikes; c) the burst rate calculated, according to their ISI distribution (Chen et al., 2009), with a time window whereby three spikes must be found to consider a burst event and burst length defined as the mean length of the burst. The spikes in burst corresponds to the number of spikes belonging at burst, compared with all neuron spikes. All computations were done using Matlab software (The MathWorks, Inc.).

Neuronal classification: Interneuron or putative pyramidal cells were classified according to their spike waveform and firing rate (Csicsvari et al. 1998). Supplementary S1-A shows the representative waveform to putative interneuron and putative pyramidal cell (Csicsvari et al. 1998) and S1-B shows an example of the waveform to both neuronal types, like S21-A.

Hippocampal zones: Neurons were classified according to their location during the recording after their hippocampal zones (CA1, CA3, and DG) using a picture of the hippocampus over the MEA matrix, taken on experiment day. A hippocampal map was built with the recorded neurons (using spike sorting information, specifically the electrode where the neuron was detected and sorted).

Network connectivity: A network connectivity was estimated using pair Pearson correlation analysis between two neurons. However, since the data are discrete, Pearson values were relatively small; so we introduced a relevant correlations method. Briefly, it consists of taking the spikes of neurons and shuffling, disrupting the time influences, and computing only a random correlation, repeating that process 1000 times. All correlation values higher than "random correlation" were considered as 1.

Finally, we measured relevant connections possible among all neurons in the network as percentage values.

Computational Model

Random balanced network model

A "random balanced network" model was selected after (Brunel 2000) due to its simplicity, fast implementation, low computational cost, and Excitation/Inhibition balance consideration. It was mounted on the NEST platform using Python. The software used was Oracle VM VirtualBox, which virtualized the NEST 2.20 version for Ubuntu.

Briefly, the model comprises a network of N neurons, represented by the Integrate and Fire (IN) model, for both excitatory and inhibitory neurons. The ratio of excitatory and inhibitory neurons was 4:1, respectively, according to model data and biological proportion (Pelkey et al., 2017). Total neurons of the model network were 12500, being 10000 excitatory and 2500 inhibitory. Each neuron receives a random number of connections from other neurons and from excitatory neurons outside the network. An independent Poisson process gave external stimulation of the neuron. The inhibition/excitation balance of the network was represented by g. Physiological properties of neurons were taking from neocortex values, such as firing rate threshold (θ), membrane time constant (τ_{mem}), synaptic delay, and membrane conductance (C_{mem}).

We set the network with CA3 parameters, as shown in table 1. These parameters were chosen based on *in vivo* recording of CA3 works (patch-clamp). In these works, the authors also built a model using its physiological parameters.

Model Parameters	Neocortex	CA3	Reference
θ (firing threshold)	20	80	Kali et al., 2000
J (EPSP amplitude)	0.1	0.56	Guzman et al, 2016
τ_{mem}	20	80	Guzman et al, 2016
synaptic delay	1.5	2.3	Guzman et al., 2016
C_{mem}	250	1000	Guzman et al., 2016

Table 1. Model parameters of the network model to NeoCortex (default) and CA3 (using in our work)

Statistical analysis

A Shapiro-Wilk test was performed to evaluate parametric and non-parametric data distribution to determine the statistical significance difference. Parametric data were applied to a test-T to compare the two groups. A Kolmogorov-Smirnov test was applied to compare two groups and the Klustal-Wallis test to compare multi-groups, both test to nonparametric data. A Fisher's exact test was used for contingency data, classified on two types. Statistical analysis was carried using Prism software (Graphpad Software Inc) and Matlab.

RESULTS

I] BEHAVIORAL RESULTS

In this thesis, we used the BT and the NOR to evaluate the cognitive status and the OF to evaluate the locomotive state and the exploration capacity of degus during aging.

Open Field (OF)

The OF is a simple, fast, spontaneous, and untrained test where the exploratory capacity of an animal can be determined in terms of distance traveled and pattern exploration (Walsh & Cummins 1976), as well animals' anxiety or stress according to the time they spend in the center and periphery areas. Rodent in general try to avoid open unsecure spaces and expend more time in closed or protected spaces and this behavior has been related to animal anxiety or stress level (Seibenhener & Wooten, 2015). The test, mostly used in mice and rats (Seibenhener & Wooten 2015), has also been used in degus (Ardile et al., 2012; Lindsay et al., 2019; River et al., 2021).

Figure 5A shows the OF results for thirty-five degus. The results did not show a relationship between distance traveled and degus age (r^2=-0.05) (Fig 5B). Interestingly, degus tended to increase the traveled distance with age. This result could be since aged degus are quite accustomed to behavioral tests and to the experimenter, showing a low level of anxiety that allows them to explore without problems. The BT performance (r^2=0.002) (Fig 9B-C, respectively) was independent of locomotor activity.

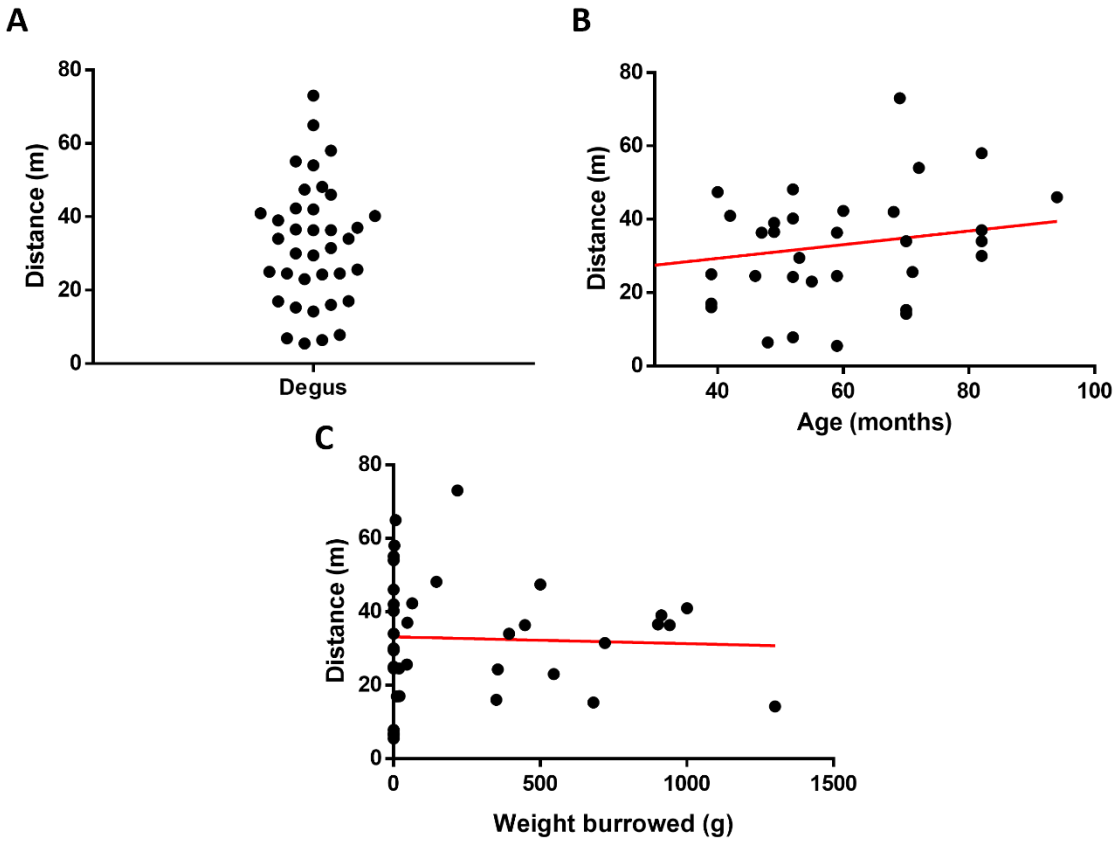

Figure 5. Locomotor activity for BB and GB degus in OF. A Traveled distance by degus in OF maze (same maze where we carried BT) (n=35). **B** Distance travelled by degus according to their age. **C** Distance traveled by degus according to their BT performance.

Novel Object Recognition (NOR)

To measure memory, we used a novel object recognition test (NOR) (Ennaceur & Delacour 1988; Ardiles et al. 2012; Rivera et al., 2021), characterized by not requiring external motivation or reward or punishment. The test's is base in the spontaneous or natural preference of rodents to select or to explore new object or places (Antunes & Biala 2012).

We carried first, a pilot screening using OF, NOR and BT with seventeen degus from 39 to 82 months old (Fig 6). For the NOR we select a 70% preference index (IP) as threshold criteria performance according to previous reports on degus (Ardiles et al., 2012). Only four degus of seventeen satisfied

the criterion (Fig 6A, left). These results are consistent with previous reports where degus over 36 months failed to perform NOR successfully compared to young degus (Ardiles et al., 2012).

The results also indicated a slight decrease in IP concerning age (Fig. 6C). The NOR test did not show a relationship between BT or the OF exploration (Fig, 6D-E). Interestingly, degus with poor performance for both behavioral tests (NOR and BT) were present, but more affected by aging. On the other hand, we also found animals with good performance in both tests, indicating a lesser effect of aging.

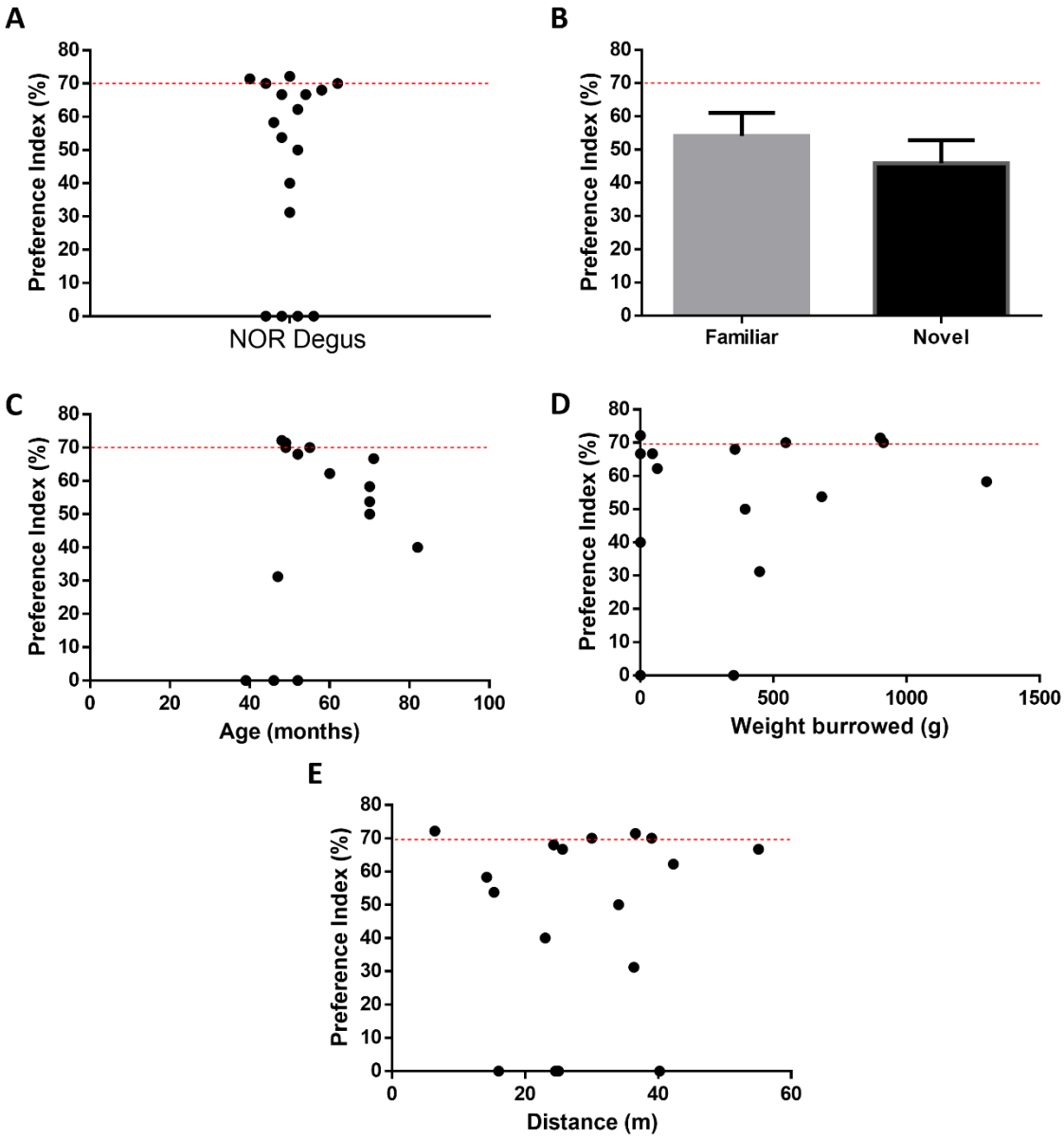

Figure 6. Unsuccessful NOR test performance in aged degus. A NOR test performance as preference index percentage (n=17). The redline represents the threshold value criterion (70% of preference index). **B** Comparison between familiar (gray bar) and novel object (black bar) preference index. **C** NOR performance (percentage of preference index) according to degus age (in months). **D** NOR performance (ratio of preference index (PI)) according to BT performance (in grams). **E** NOR performance (rate of preference index) according to OF performance (distance traveled in meter). Data are shown as mean ± SEM. Statistical analysis using T-test.

Burrowing Test (BT)

For the BT we first follow Deacon's recommendation (Deacon et al., 2015) and place the BT device into a smaller area into the maintenance cages, however it did not work well in our hands. Then we move to us OF maze (180 cm diameter) (Fig 6A-B) to place the BT device. All degus started the BT located in the same position and orientation into the OF arena (the head of degus in front of burrowing apparatus). Figure 6C shows a representative example of a degu burrowing, with rabbit food outside of the burrowing apparatus. Degus that did not make BT successfully remained quiescent in the OF area until finish BT time.

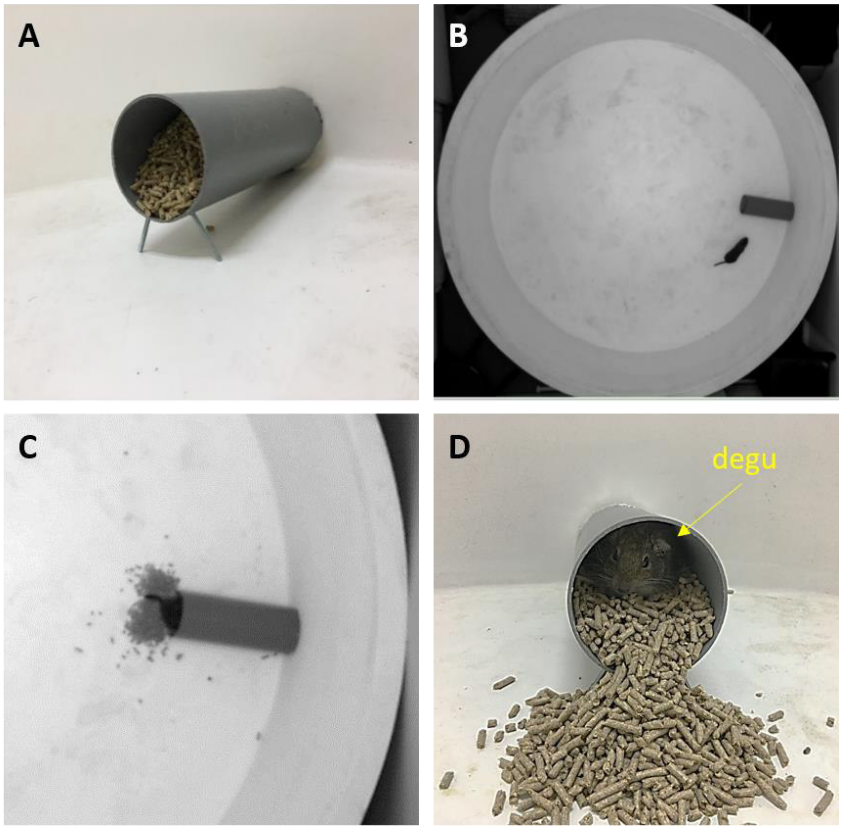

Figure 7. Burrowing set-up. **A** Burrowing apparatus consists of a gray plastic tube with two screws at the entrance for support, filled with rabbit food pellets (1300 g). **B** Localized the set-up against the wall of a circular OF (diameter 180 cm). The degus were put in the OF for free exploration and BT. **C** Degus burrows rabbit food and displaces out the content of the burrowing apparatus. The food carried out was measured as BT performance (weight burrowed in grams). After finishing BT, **D** Representative photo of a good burrower degus.

The BT was carried out with thirty-five degus, between 24-94 months old, from our colony. After completing a BT, the amount of food scrubbed by a degus were weighed (weight burrowed) a become its BT performance. Figure 8A shows the BT degus distribution were a 10% of burrowed pellet was set as a threshold value (see methods) to separate GB from BB degus, see Figure 8B (red line).

The results showed that 13 degus of 35 or the 37.4% exceeded the 10% threshold and were classified as GB. The remained 22 (62.6%) did not exceed the 10% value and were classified as BB. Both groups being statistically different (Fig 8A; BB = 15.7 $\pm$ 33.6 g, GB = 661.9 $\pm$ 312.4 g; $p<0.0001$). Our results did not showed bias by sex, where 21 degus were female and 14 males, being statistically similar both groups, according to their BT performance (Fig 8B; females (n=21) = 293.9 $\pm$ 405.8 g, males (n=14) = 211.6 $\pm$ 316.6 g; $p = 0.49$).

The OF exploring traveled distance during the first 5 min of the BT was measured to assess bias by locomotor activity or anxiety behavior showing in the burrowing apparatus. The results did not show differences in terms of exploring distance between BB and GB (Fig 8C; BB = 32.2 $\pm$ 17.5 m, GB = 33.4 $\pm$ 15.4 m; $p = 0.84$), neither between the time of exploration from the central vs periphery zone (Fig 8D; BB = -0.46 $\pm$ 0.41, GB = -0.41 $\pm$ 0.34; $p = 0.72$). The exploration time ratio between center and periphery has a negative value when the periphery was preferred. Previous report has shown that peripheral zones over central zones are preferred in degus (Ardiles et al. 2012; Rivera et al., 2021), therefore our results are consistent.

Interestingly, to understand the ontogeny of the burrowing capacity in degus, we attempted to measure BT in young degus (6 months; n=6). However, they fail to do the task, they show freezing, not exploring the maze and showing anxiety behaviors (jump maze wall, fast movement, and then freeze) (data not shown). Unfortunately, we couldn't sufficiently enough to habituated previously those young degus to our experimental conditions. Unpublished data from Deacon lab shows that young degus are capable of burrowing.

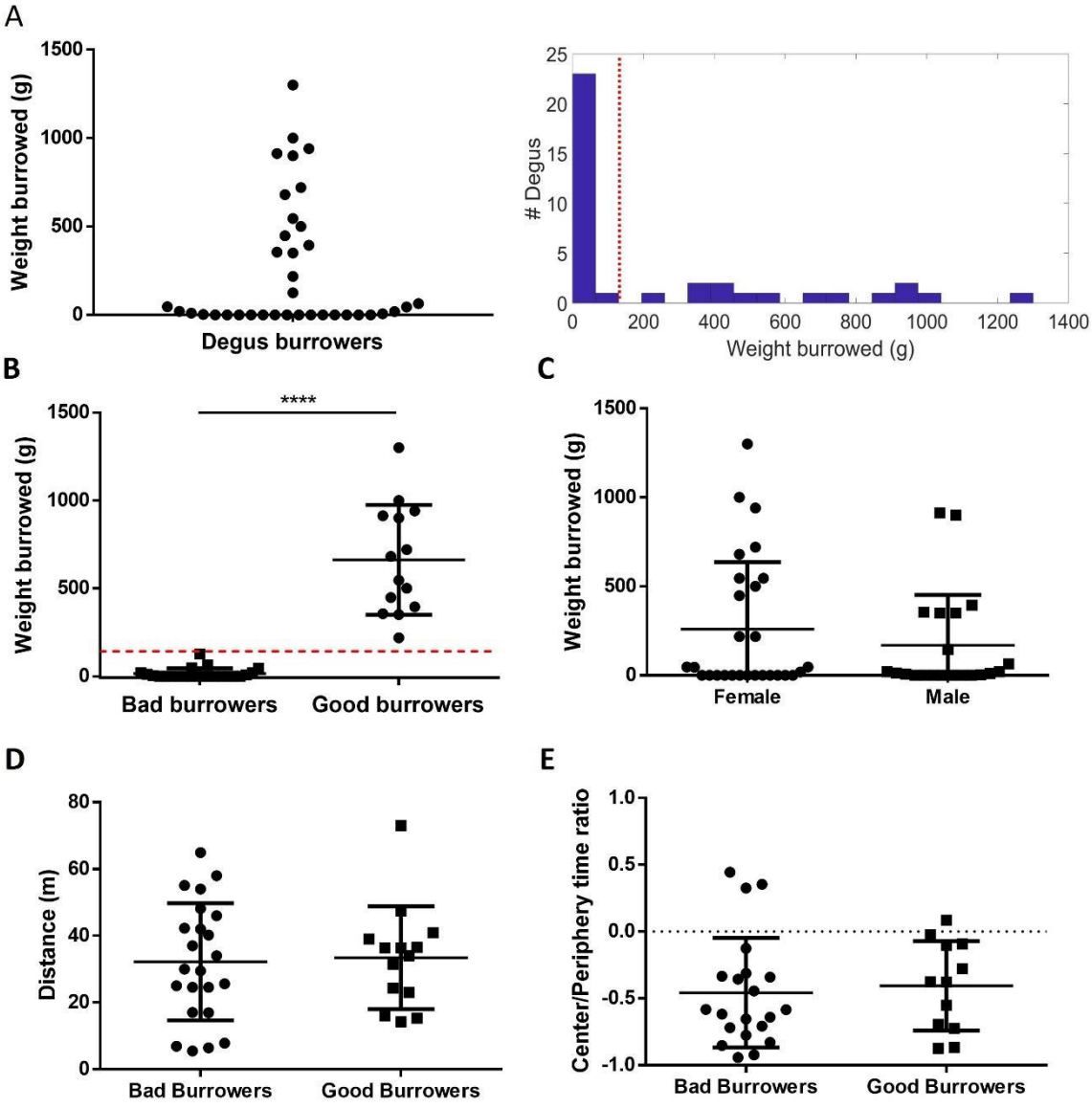

Figure 8. BT measure in aged degus. **A** Burrowing performance of degus in terms of weight of pellet burrowed (n=35) (left) and histogram for select a threshold (red line) to split aged degus population (left). Degus were classifying according to their performance into good or bad burrowers. A threshold (red line) value of 10% of total pellet burrow was determined (130g) to separate GB from BB according to the histogram in A left. **B** Burrowing performance according to degus sex. **C** Traveled distance by degus in OF maze (a same maze of BT) and separated by Bad or Good burrowers. **D** Center-periphery time ratio where degus spend the time (5 min) in the maze, dividing this into two areas, proportionally equal. Data are shown as mean ± SD. Statistical analysis using T-test. ****=p<0.0001

To quantify any possible effect of age within our tested degus population, we took the approach of first establishing for our animal facility the average age of life of our degus. In wild conditions, degus can live in average up to two years old since the high risk of depredation. However, they can live as long as 8-9 years in captivity (Ebensperger et al., 2009), however each animal facility needs to determinate its own rate. The Figure 9A shows the distribution of the age of natural death of degus in our animal facility and since the origin of the colony around year 2000 (n=188). The observed living expectative distribution was stablished with a mean of 55 months and median of 53 months, were degus with less than 55 months represents 53.19% of the population. We fitted a gaussian function over our data (Fig 9A, red line). So, taking 55 months as the separation point of our population, we distribute our experimental degus here in aging categories. GB degus with less than 55 months represents 59%, while GB degus higher than that age were 31.2% (Fig 9B).

These results suggest that GB degus decrease through the time, perhaps evidencing a relationship between BT and age. Moreover, if we divided the oldest group symmetrically, we obtained two new groups: 56-75 months and 76-94 months. With this separation, we see a strong age dependency in the BT, where in the group 56-75 months a 45.5% of degus were GB, while for the 76-94 months group, we did not find GB degus (Fig 9C).

These results suggest, like all behavioral tests that measure an animal's cognitive status, a decline with age. Especially when BT is related to ADLs, the first tasks lost in the aging process and AD (Reisberg et al., 2001; Deacon 2009). This is an important new result on aging and BT, since for degus catch from wild aging determination is not possible, condition for previous BT work.

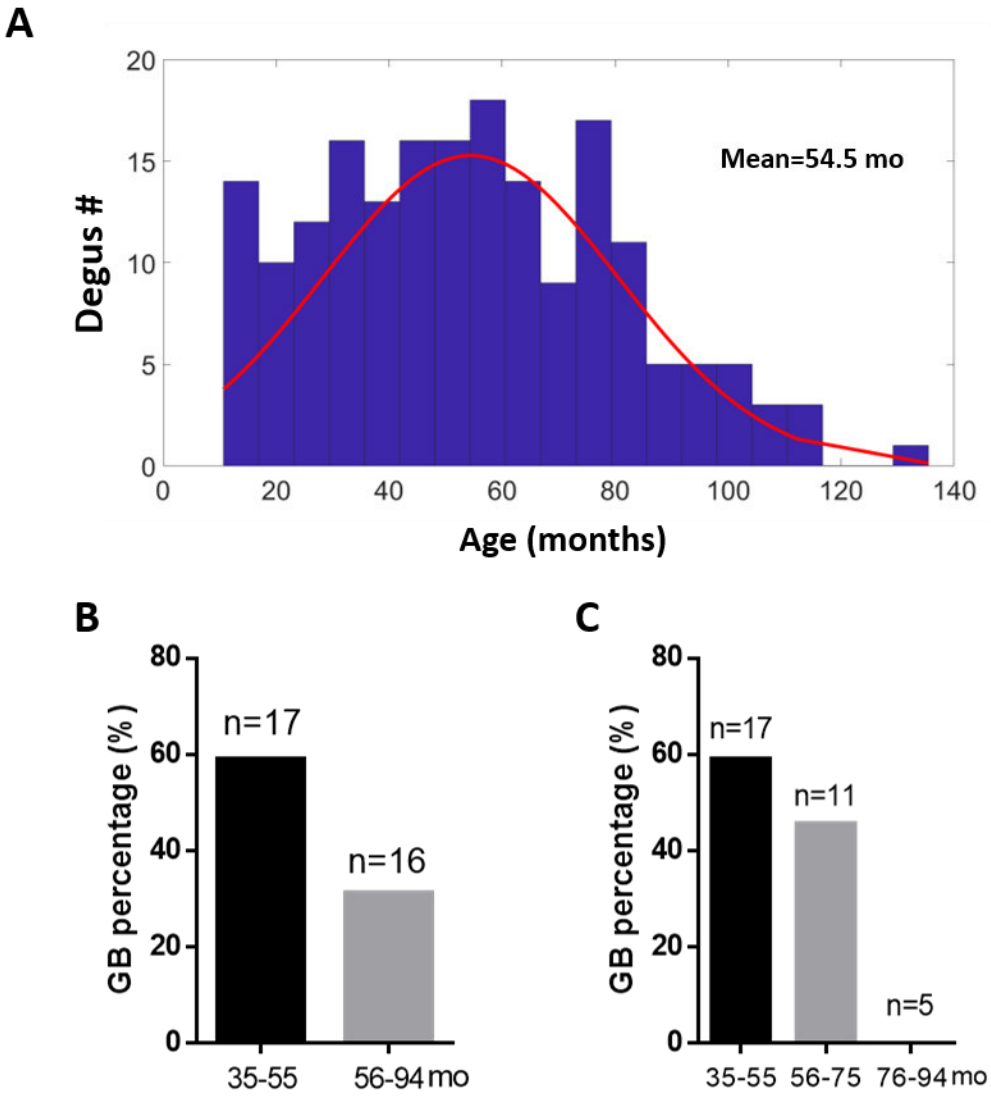

Figure 9. GB decrease as increase degus life. **A** Death age histogram distribution of all degu's naturally dead in our colony (n=188) in months. The redline represents a Gaussian fit with mean at 54.5 months. **B** GB performance dividing our colony into two main groups: 35-55 months (black bar) and 56-94 months (gray bar). **C** GB performance dividing our colony into three groups with the same time interval (20 months): 35-55 months (black bar), 56-75 months (gray bar), and 76-94 months (no bar).

Behavioral tasks through the time

To accomplish further measurement including electrophysiological experiment we carried a second screen for BT using eight degus already tested ten months before. We use the same battery of behavioral tests. Later, a third screening was carried with five degus after two years to assess the aging effect on our experimental tests.

Ten degus carried OF, NOR and BT one day per session respectively. After behavioral completion, degus were euthanatized following the bioethical protocols, and electrophysiological experiments carried as described in the methods. Figure 10A shows BT performance and degus classification on BB or GB (BB = 0 $\pm$ 0 g; GB = 841.7 $\pm$ 338.5 g; $p<0.0001$). Similar as shown in Figure 8, distance traveled did not correlate with BT performance (Fig 10B and 10C), both exploring distance BB = 25.1 $\pm$ 18.9 m, GB = 35.4 $\pm$ 11.6 m; $p = 0.97$) and exploration zones (Fig 8D; BB = -0.71 $\pm$ 0.22, GB = -0.82 $\pm$ 0.07; $p = 0.26$). Hence, our test showed similar results and tendency as our first screening (Fig 5, 6 and 8)

For NOR, one of ten degus reached the threshold criterion of 70% of the PI (Fig 10D-F), reinforcing the idea that the NOR is a test that requires different cognitive ability compared to BT.

For the analysis of the effect of aging, the number of animals varied, because more degus were tested for BT and OF (n=13) than NOR (n=5). We found a decrease in both task performance (Fig 10E-F) in most degus (53.5% (n=7) and 80% (n=4) of degus in BT and NOR respectively). Moreover, all degus (n=5) classified as BB in the first screening maintained this condition (Fig 10F), which corresponded to 38.5% (Fig 10E).

These results suggest that NOR could be considered a standard task to measure a certain type of memory in degus (Ardiles et al. 2012; Lindsay et al., 2019; Rivera et al., 2021), however different from the one measured using BT (Deacon R, 2009).

Burrowing is an essential behavior in the wildlife protecting animals from depredators, food storage, or build burrows (Dudek et al. 1983). The burrowing capacity has been associated with the hippocampal function (Deacon et al., 2002) and showed to decreases through age see (Fig 9 and Fig 10E-F).

The highest decrease during aging on NOR compared with BT represent a clear difference of cognitive needed between both tasks. NOR is susceptible to aging (Antunes & Biala 2012) but we know little about BT.

The locomotor activity measure in OF shows more variation compared to the other test, and we observe an increase of mobility for 75% of degus (Fig 10E), further experiment will be needed to address this observation, including an increase time for the exploration, since 5 minutes could be too short to capture a complete picture of locomotor capacities in aged degus.

In brief, these results show the reproducibility of the tests over time, especially in the case of BT, and allow us to make a reasonable interpretation with other results including the electrophysiological data.

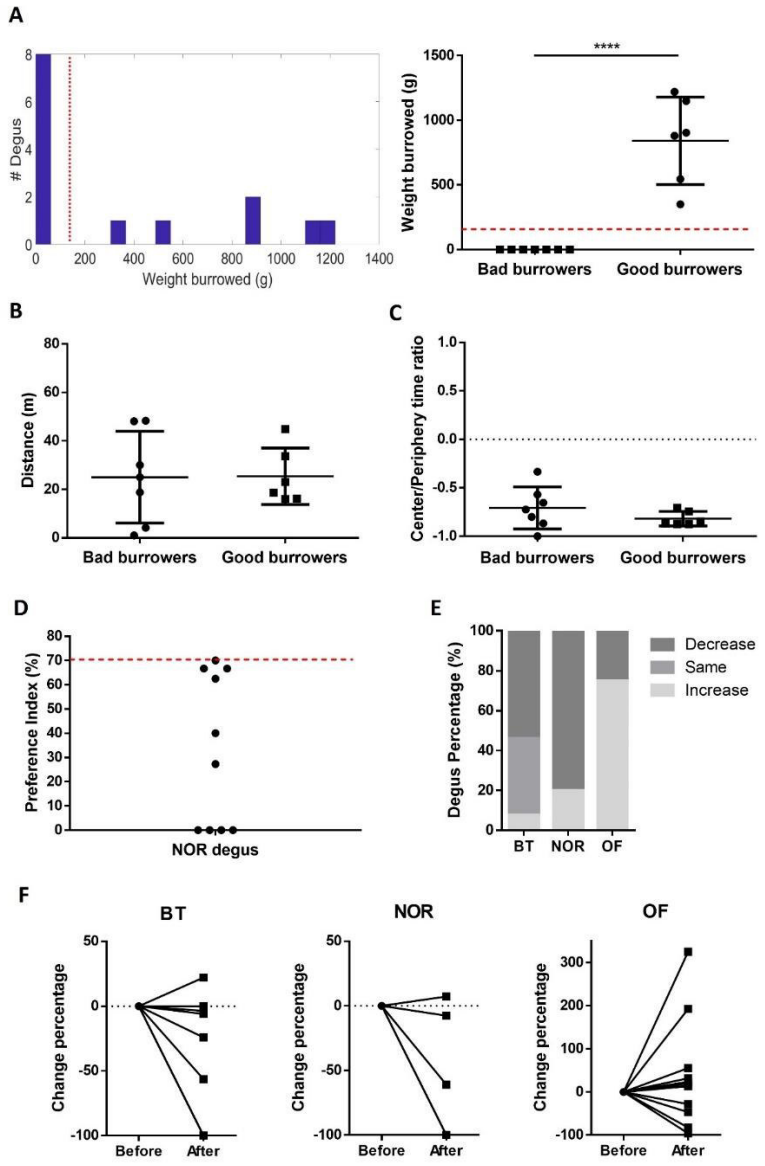

Figure 10. BT performance during aging. A Burrowing performance of degus and histogram distribution (left) in terms of pellet burrowed weight (n=13)**.** Degus classification according to their performance into GB (n=6) or BB (n=7) (right). A red segmented line represented the threshold. **B** Traveled distance by degus in OF maze (a same maze of BT) and separated by Bad or Good burrowers. **C** Center-periphery time ratio where degus spend the time (5min) in the maze, dividing this into two areas, proportionally equal. **D** NOR performance in terms of preference index. The redline represents the threshold value criterion (70% of preference index). **E** Performance comparison for three behavioral tasks (BT, NOR, and OF) before and after ten months. Degus performance decreases (dark gray), maintains (gray), or decreases (light gray). **F** Performance comparison between the first-time degus did the task (after) and after at least ten months (after) to BT (n=13), NOR (n=5), and OF (n=13), in terms of change percentage. Data are shown as mean ± SD. Statistical analysis using T-test. ****=p<0.0001

II] PHYSIOLOGY RESULTS

Once behavioral tasks were done, we evaluated the hippocampal network state of BB and GB through electrophysiology technique, specifically interested in the GABAergic transmission. As mentioned before, the inhibitory system plays a fundamental role in the aging process where its decay correlate with a decrease in the cognitive performance.

Firing-Rate, Inter-Spike Interval, and Burst in the whole hippocampus

Ten hippocampus from 5 GB and 5 BB degus were selected (Fig 10A) for electrophysiology, using a multielectrode array (MEA) method. Hippocampal slices (see methods) from the dorsal hippocampus (right hemisphere) were used for electrophysiology, due to its association with navigation, exploration, and locomotion, (Fanselow & Dong 2010). The left-brain hemisphere was preserved for biochemical assays.

Each experiment consists of 10 min of spontaneous activity (SA) recording, follow by 20 min of recording activity induced by the picrotoxin drug (PTX), an allosteric inhibitor of ionotropic GABA receptors ($GABA_A$). After the experiment, a spike sorting procedure was carried (Yger et al. 2018) to separate single-unit neurons from the MEA recording.

Figure 11A (top) shows a representative raster plot, each line corresponds to a single neuron, sorted from low to high firing rate for BB (n = 162, left) and GB (n = 143, right). The global activity (Fig 11A bottom) was normalized according to the total number of neurons recorded per groups by each bin (1 s). PTX increase neuronal activity in both BB and GB conditions, but GB neurons seem more actives compared to BB. To quantify the effect of the PTX we select the last 10 min of each recording, representing a steady-state level value of the system (Burke & Barnes 2006).

A more quantitative measure was carried comparing the firing rate (FR) (Fig 11B, left), Maximum FR (Fig 11C, left) and the Interspike Interval (ISI) (Fig 11D, left). The BB and GB increased neuronal activity under PTX, both during the steady state FR and during the maximum FR, compared with their

SA (FR BB: SA = 0.81 Hz; PTX = 1.2 Hz; $p<0.0001$. GB: SA = 1.16 Hz; PTX = 2.41 Hz; $p<0.0001$) (Maximum FR BB: SA = 6 Hz; PTX = 8 Hz; $p<0.001$. GB: SA = 6 Hz; PTX = 12 Hz; $p<0.0001$) (Table 2). This was expected because a drug that inhibits the GABAergic system should raise neuronal activity. It should be noted that the values are not presented with an error dispersion value (SD or SEM) since our data are not parametric. For this reason, we represent the data with the median and its distribution (cumulative frequency plot), in addition to applying a statistical test for non-parametric data (Kolmogorov-Smirnov).

When we compare the Maximum FR for GB and BB, they show an acute effect of PTX. This is consistent with the response that PTX elicits over the hippocampal slice, generating epileptic-like responses as seizures due to a disruption of E/I balance (Hablitz 1984; Scharfman 2007; Hashimoto et al, 2017).

An important result here was the observation that BB degus showed a tendency for less SA activity compared to GB degus, and we interpretate it as the presence of a compensatory mechanism, different of the GABAergic system controlling the glutamatergic activity.

To quantify the number of different neurons that were affected by PTX, we separated their responses based on their activity during SA. The separation consisted of identifying those that increased (affected) their activity with PTX from those that decreased (not affected) it (Fig 11B-C, right). The FR during steady state and maximum FR showed a higher number of neurons affected by PTX (FR: BB = 69.75%; GB = 72.72%; $p>0.05$. Max FR BB = 65.0%; GB = 78.9%; $p<0.01$.), where the difference on maximum FR was significant according to Fisher's exact test, supporting the idea that GB tend to preserve more neurons been modulated by the GABAergic system.

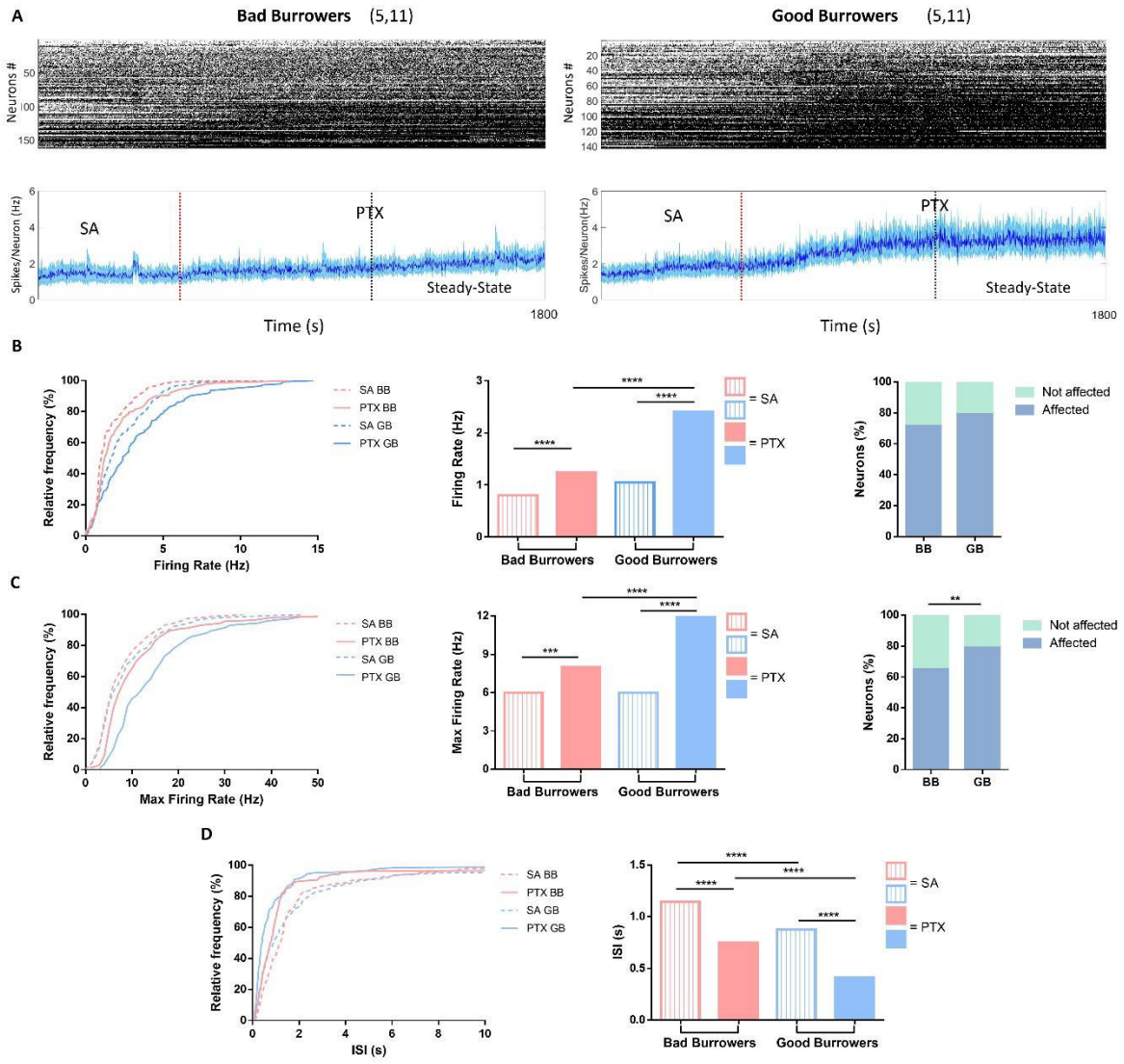

Figure 11. MEA recording for GB and BB degus. A Representative raster plot for whole neurons was recorded, separated according to BT performance into bad (5 degus, 11 slides, n=162 neurons, left) and good (5 degus, 11 slides, n=143, right) burrowers (top). Population activity represented by spikes/neuron (Hz) (blue line) ± 95% of confidence error (blue shaded) for the whole recorded time (1800 s). The redline separates spontaneous activity (SA, 600 s, left) and PTX activity (PTX, 1200 s, right). The black line represents the last 600 s where was compute the steady state firing rate (bottom). Bin selected was 1s. **B** SA (segmented line) and PTX (continue line) steady state firing rate (FR) separated into BB (pink) or GB (blue), represented by cumulative frequency plot (left). Median of FR cumulative frequency distribution for SA (empty bar with lines) and PTX (filled bar), separated into BB (pink) or GB (blue) (center). Number of neurons affected by PTX (calypso blue,) increasing their SA FR > 1 after PTX induction (steady state FR) (right). In Hertz, **C** Maximum FR activity similar to B, using a bin size of 1s. In seconds, **D** Inter-spike interval (ISI) for the same conditions in B (left and center). Data showed median. Statistical nonparametric test Kolmogorov-Smirnov and Fisher's exact test for affected and not affected values. **=p<0.01; ***=p<0.001; ****=p<0.0001. Both rasters were sorted increasingly, according to whole neuronal FR.

	BB (median)			GB (median)		
	SA	PTX	% Change	SA	PTX	% Change
FR (hz)	0.81	1.2	+48%	1.16	2.41	+108%
ISI (s)	1.15	0.75	-35%	0.87	0.41	-53%
Max FR (Hz)	6	8	+33%	6	12	+100%
Burst (Hz)	0.016	0.02	+25%	0.047	0.086	+83%
Burst length (ms)	58.8	55.2	-6%	52.7	54.1	+3%
Spike in Burst (%)	4.1	3.6	-12%	8.1	12.4	+53%

Table 2. Data for single-neuron recording, separated on bad (BB) or good (GB) burrowers and according to SA and PTX activity.

Burst is usually defined as "a group of action potentials generated in rapid succession, followed by a period of relative quiescence" (Zeldenrust et al, 2018). The functional meaning of burst is related to plasticity modulation enhance reliability in information transmission and expand the coding space. We used an algorithm based on ISI distribution, defining a burst detector threshold to each neuron (Chen et al, 2009) and has been used in different works (Bridi et al 2018; Trujillo et al., 2019; Shin et al., 2021). This threshold is a time window whereby at least three spikes to consider a burst event.

Figure 12A shows the burst activity (left and center) and the number of affected neurons by PTX (right). Similar to FR, the burst activity increase under PTX activity on both BB (SA = 0.016 Hz; PTX = 0.020 Hz; $p<0.01$) and GB (SA = 0.047 Hz; PTX = 0.086 Hz; $p<0.0001$). Comparing the burst distribution under PTX activity, we find that the burst activity was higher for GB than BB ($p<0.0001$). The number of neurons affected by PTX also showed a significant sensibility for PTX (BB = 63.8%; GB: = 77.5%; $p<0.01$).

These data strongly suggest that the GABAergic system of GB tend to be more responsible and in a better health condition compared to BB. According to previous descriptions the GABA ionotropic circuits are affected during the aging, losing their functional control of neuronal circuits and therefore their E/I balance (Rozycka & Liguz-Lecznar 2017). The correct E/I balance has been described to be a critical mechanism to preserve cognitive skills during aging (Tran et al. 2018; Tran et al. 2019).

Wherein GB degus performing better in BT suggest that their GABAergic systems are better maintain compared to BB. Moreover, the fact that BB showed a lower SA, both during steady-state FR and Burst, need some explanations. If BBs were to control only their excitatory activity via the GABAergic system, we would expect to see a high SA in line with work that has linked aging with hyperactivity (Haberman et al., 2017).

Figure 12B shows the burst length (left) and spikes contained in bursts (right). In this experiment we did not find a difference among all groups (BB: SA = 58.8 ms; PTX = 55.2 ms; $p=0.202$. GB: SA = 52.7 ms; PTX = 54.1 ms; $p=0.256$). Furthermore, spikes in burst increase under PTX effect but only for GB (BB: SA = 4.09%; PTX = 3.6%; $p=0.964$. GB: SA = 8.13; PTX = 12.4; $p<0.05$). Our results suggest a change in the distribution of spikes in GB, where PTX besides to increases spikes also affect the pattern structure of the burst. BB despite increasing its activity with PTX, its spikes do not follow changes in the burst pattern structure, which is an argument to support the idea that BB does not have a healthy hippocampus (Hablitz 1984). In conclusion, GB responds to PTX more strongly than BB, acutely (maximum FR) and according to PTX effect (bursting activity).

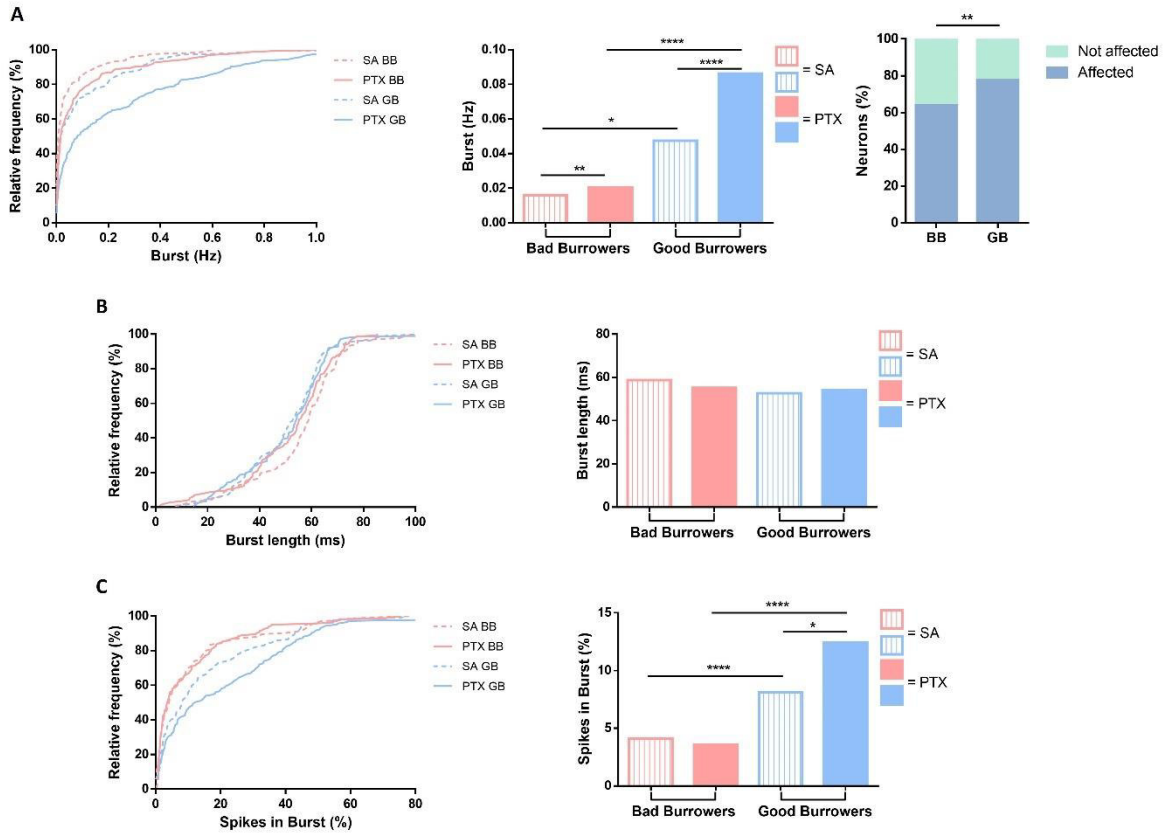

Figure 12. PTX and burst activity. A Burst activity, in Hertz, for SA (segmented line) and PTX (continue line) steady state, separated into bad (BB; pink) or good burrowers (GB; blue), represented by cumulative frequency plot (left). Median of burst cumulative frequency distribution for SA (empty bar with lines) and PTX (filled bar), separated into BB (pink) or GB (blue) (center). Percentage of neurons affected by PTX (calypso blue), increasing more than one times their SA burst on PTX activity (steady state FR) (right). **B** Cumulative frequency plot of burst length (ms, left) and percentage of spikes that belong at a burst (**C**), using the same representation on A. Data shown median. Statistical nonparametric test Kolmogorov-Smirnov and Fisher's exact test for affected and not affected values. *=p<0.05; **=p<0.01; ****=p<0.0001.

Neuronal classification according to hippocampal zones

The hippocampus has been classified into three main zones: CA1, CA3 and DG. These regions possess different features, depending on the wiring, neuronal types, and functionality (Deng et al., 2010). The CA3 region is related to memory processes, encoding spatial representation and episodic memories (Cherubini and Miles 2015). Moreover, CA3 correspond to the core for signal processing and coding, receiving signals by DG granular cells, sending signals to CA1 pyramidal cells, and to CA3 neurons through recurrent excitatory synapses, which are recurrent network (Witter M 2007).

To separate our neurons according to hippocampal zones we used a hippocampal reconstruction map to contrast the recording electrodes and their spike response in each hippocampal slice. Figure 13A shows a picture of the hippocampal slice over our MEA matrix (left) and a map reconstruction (right), where blue circles represent neurons. Some blue circles are located outside of the picture because the camera field does not cover the whole MEA matrix surface.

Once the neurons were localized and classified in a hippocampal zone, their FR of BB and GB in SA and PTX activity were computed (Fig 13B). The results shows that BB increased FR under PTX effect only in the CA1 region, compared with SA ($p<0.01$) (Table 3). In contrast, GB increased FR only in the CA3 region ($p<0.0001$) but in a higher significant difference than BB CA1.

	BB (FR median)			**GB (FR median)**		
	SA	PTX	% Change	SA	PTX	% Change
CA1 (Hz)	0.64	1.56	143%	0.96	1.48	54%
CA3 (Hz)	0.95	1.23	29%	1.73	3.46	100%
DG (Hz)	0.86	0.97	13%	0.73	1.11	52%

Table 3. Data for CA1, CA3 and DG hippocampal zones, separated on bad (BB) or good (GB) burrowers and according to SA and PTX activity.

The number of neurons affected by PTX (> = 1) results similar between BB and GB for CA1 (BB = 76.0%: GB = 76.5%) but not for CA3 (BB = 74.2%: GB = 87.3%) and DG (BB = 61.1%: GB = 46.0%) (Fig 13C, left). The GB degus kept for CA3 a higher inhibition compared to BB degus, could be a key point to preserve a good hippocampal state and good behavioral performance during aging. The

number of neurons recorded from hippocampal zones were similar for BB and GB (BB: CA1 = 32.9%; CA3 = 42.5%; DG = 24.7%. GB: CA1 = 25.4%; CA3 = 42.6%; DG = 31.8.

Besides finding different responses to PTX for BB and GB hippocampus, we also found differences in responses depending on the hippocampal zone. First, DG showed less activity in both groups, and this can be explained since where granular cells have lower firing rates, may be related to their participation in sparse coding for pattern separation (Neunuebel & Knierim 2012; Diamantaki et al., 2016), and being DG the most silent zone of the hippocampal network (Jung & McNaughton 1993). Our findings are in concordance with literature and support our electrophysiology MEA recording.

Our results also show an important inhibition for CA1 in BB degus and in CA3 for GB degus. As we mentioned before the E/I balance is a critical mechanism to preserve cognition during the aging process. Aged rats with similar cognitive skills compared to young rats tend to have similar E/I ratio (small ratio), predominating inhibition (Tran et al., 2018; Tran et al., 2019). However, the E/I balance is complex and delicate subject with some contradictory results. For example, some works have shown cognition impairment with highest CA1 inhibition and LTP decreases (Chapman et al., 1998) (Cunha et al., 2019) and spatial memory deficit (Valbuena et al., 2019). In our hands, we have shown that aged degus showed deficit in LTP and cognition impairment (Ardiles et al., 2012), what can be an orientation for further studies on SP according to degus BT performance.

Place cells are responsible for spatial coding and navigation, and their performance decrease with aging (Cacucci et al., 2008). Since BT has a spatial component (free exploration), it would be interesting to study place cells in aged degus and assess whether the important inhibition suggested for CA1 here, plays a role in these neurons. This could explain the relationship among poor BT performance, high inhibition in CA1 and spatial memory deficit (place cells).

Also is important to mention that CA3, correspond to the most affected zone in our results, and has been related to seizures, neurodegeneration related to memory process during aging, the reason for which has attracted significant attention in recent years (Cherubini & Miles 2015).

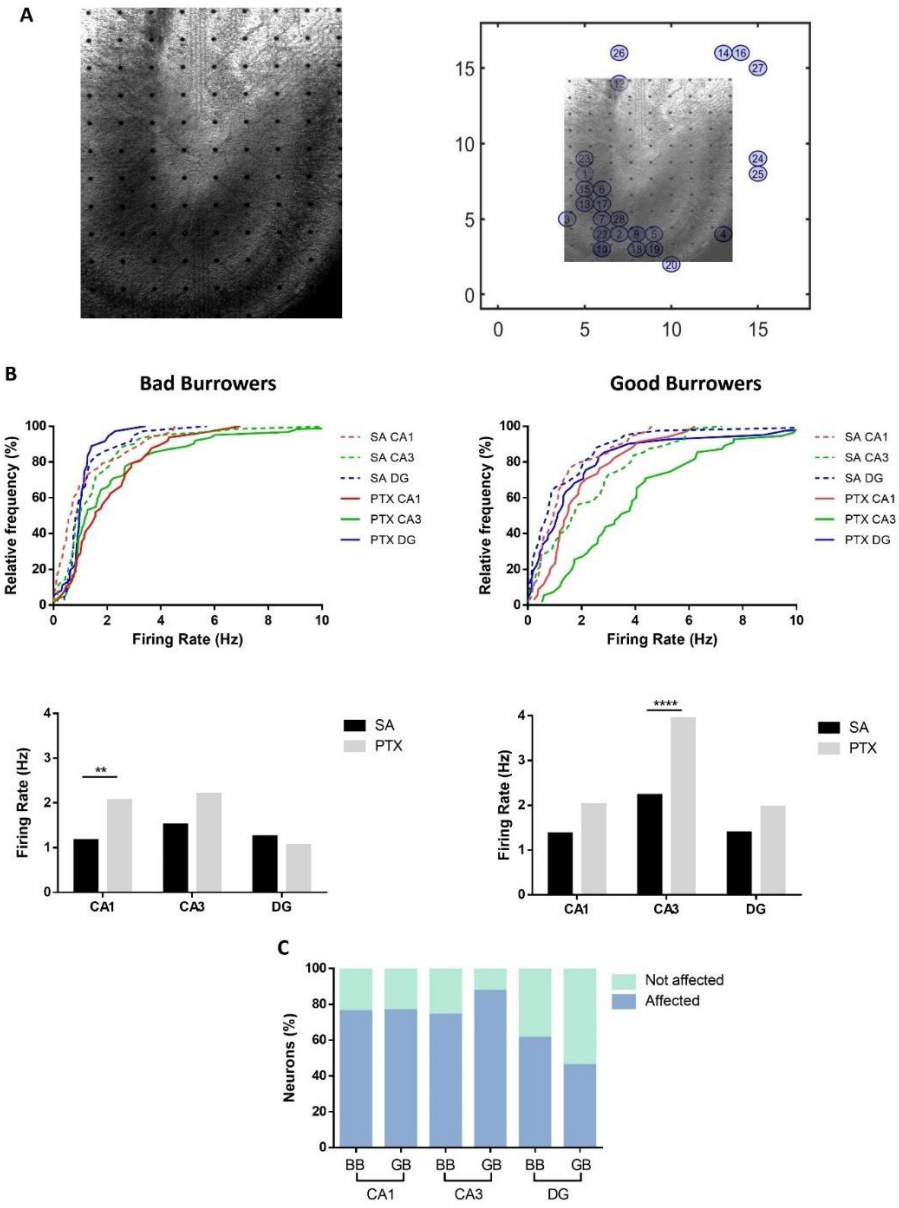

Figure 13. Hippocampal classification according to CA1, CA3 and DG zones. A Hippocampal slice over MEA matrix picture in our recording set-up (left). Hippocampal map reconstruction using neurons recorded and successfully sorted (blue circle) compared with a picture of hippocampal slice on MEA matrix (left) **B** Firing rates (FR) to each hippocampal zone (CA1 red, CA3 green and DG blue) according to their burrowing performance (BB left or GB right) and different activity (SA segmented line, PTX continue line), represented by cumulative frequency plot (top). Median of FR cumulative frequency distribution for SA (black bar) and PTX (gray), separated into BB (left) or GB (right) (bottom). **C** Neurons affected by PTX (calypso blue), increasing more than one times their SA FR on PTX activity (steady state FR) for each hippocampal region. Data shown median. Statistical nonparametric test Kolmogorov-Smirnov and Fisher's exact test for affected and not affected values. **=p<0.01; ****=p<0.0001.

CA3 the most affected zone by PTX drug

The CA3 is the core of neural circuits of the hippocampus and is where we found the highest difference comparing GB and BB (maximum FR, number of neurons affected by PTX, Burts activity) (Fig 14, Table 4). Figure 14A shows a representative raster plot of CA3 neurons using the parameters in Figure 12A (BB left and GB right). We observe that PTX induce an increase of the maximum FR for GB (SA = 5 Hz; PTX = 10 Hz; $p < 0.001$) but not for BB (SA = 6 Hz; PTX = 6 Hz; $p = 0.982$) (Fig 14B). A similar observation for the maximum FR for both groups for SA (BB vs GB), differing to the observation for whole hippocampus steady-state FR.

The number of neurons affected by PTX were higher for GB compared to BB (GB = 83.3%, BB = 54.1%; $p<0.01$). Moreover, the numbers of neurons increasing their maximum FR by > 2 were 40.5% for GB and 8.1% for BB ($p<0.001$). We also observe that the burst activity for the GB increase under PTX effect (SA = 0.014 PTX = 0.042; $p<0.001$) and the neurons affected (BB = 45.2%; GB = 70.4%; $p<0.01$).

Finally, the burst features for the GB were similar to our previous results for the whole hippocampus (Fig 12), without differences on burst length (Table 3. $p=0.995$). However, the number of spikes in burst was higher in GB compared to BB (Table 3. $P<0.001$).

In conclusion, the evidence here shows a significant difference of GABAergic state between BB and GB in the CA3 region, compared with differences observed in the whole hippocampus. Our findings are consistent with previous reports, in terms of the effect of PTX and the induction of epileptic responses for CA3 (Hablitz 1984). Indeed, CA3 pyramidal neurons are a pacemaker of spontaneous burst activity, eliciting this specific activity (Witter M 2007). Inhibition plays a key role in maintaining quiet CA3 neurons through $GABA_A$ receptors (Aradi & Maccaferri, 2004) and, hence, the CA3 network. Our observations, support the significant difference between BB and GB under PTX effect, base a differential inhibition of the GABAergic system, which is more prominent for CA3, and we speculate on the importance of CA3 region to maintain a better performance in our aged GB degus preserving part of some of their cognitive skills.

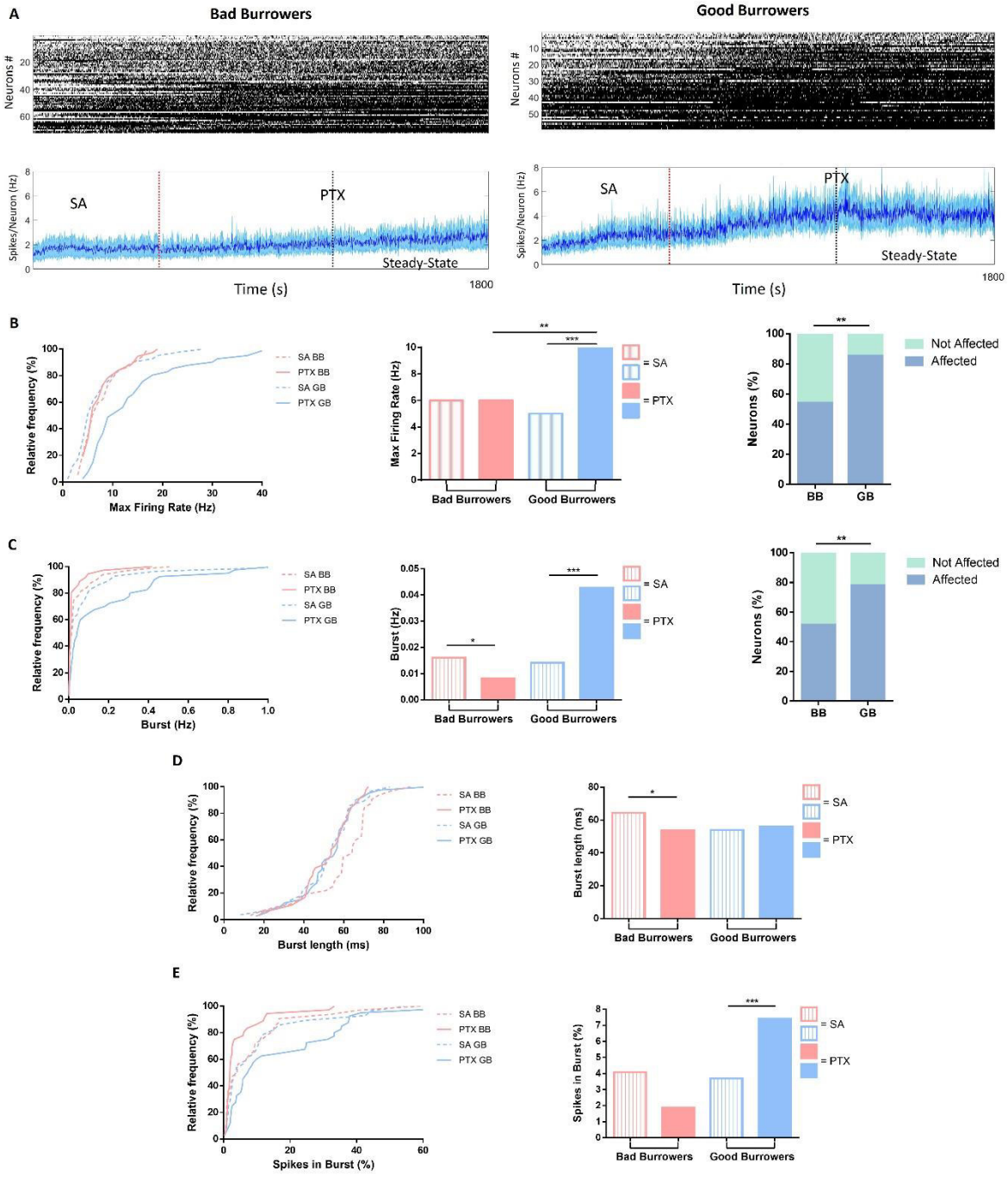
A
Bad Burrowers
Good Burrowers
Neurons #
SA
PTX
Steady-State
Spikes/Neuron (Hz)
Time (s)
1800
B
Relative frequency (%)
Max Firing Rate (Hz)
SA BB
PTX BB
SA GB
PTX GB
Max Firing Rate (Hz)
= SA
= PTX
Bad Burrowers
Good Burrowers
Neurons (%)
Not Affected
Affected
BB
GB
C
Burst (Hz)
Burst (Hz)
D
Burst length (ms)
Burst length (ms)
E
Spikes in Burst (%)
Spikes in Burst (%)

Figure 14. CA3 and PTX. A Representative raster plot for CA3 neurons recorded, separated according to BT performance into bad (n=62 neurons, left) and good (n=55, right) burrowers (top). Population activity represented by spikes/neuron (Hz) (blue line) ± 95% of confidence error (blue shaded) for the whole recorded time (1800 s). Red line separates spontaneous activity (SA, 600 s, left) and PTX activity (PTX, 1200 s, right). Black line represents last 600 s where was compute the steady state firing rate (bottom). Bin selected was 1s. **B** SA (segmented line) and PTX (continue line) maximum FR on steady state, separated into bad (BB; pink) or good burrowers (GB; blue), represented by cumulative frequency plot (left). Median of maximum FR cumulative frequency distribution for SA (empty bar with lines) and PTX (filled bar), separated into BB (pink) or GB (blue) (center). Neurons affected by PTX (calypso blue), increasing more than one times their SA maximum FR on PTX activity (steady state maximum FR) (right). **C** Burst activity, in Hertz, with the same representation in B. **D** Cumulative frequency plot of burst length (ms, left) and percentage of spikes that belong at a burst (right), using the same representation on A. Data shown median. Statistical nonparametric test Kolmogorov-Smirnov and Fisher's exact test for affected and not affected values. *=p<0.05; **=p<0.01; ***=p<0.001.

	BB (median)			GB (median)		
	SA	PTX	% Change	SA	PTX	% Change
FR (hz)	0.95	1.23	29%	1.73	3.46	100%
Maximum FR (Hz)	6	6	0%	5	10	100%
Burst (Hz)	0.016	0.008	-50%	0.014	0.042	200%
Burst length (ms)	64.4	53.7	-17%	54	56	4%
Soike in Burst (%)	4.1	1.9	-54%	3.7	7.4	100%

Table 3. Data parameters for CA3 neurons between BB and GB according to SA and PTX activity.

Putative interneuron and pyramidal cells classification

To understand more precisely the contribution of interneuron and pyramidal neurons to our results, we classified them according to the method describe in (Csicsvari et al, 1998) base in their different waveforms and we represented them by hippocampal zone (Fig 15A, bottom).

The recorded putative interneurons in our experiments did not differ between BB (6.97%) and GB (6.28%) (p = 0.842) (Fig 15B), however we only have a small number of recorded neurons to make this preliminary conclusion a stronger argument. Nevertheless, a limitation of our method is that only fast spiking interneurons representing about 40% of all interneurons in the hippocampus (Tremblay et al. 2016). could be classified using (Csicsvari et al, 1998) method.

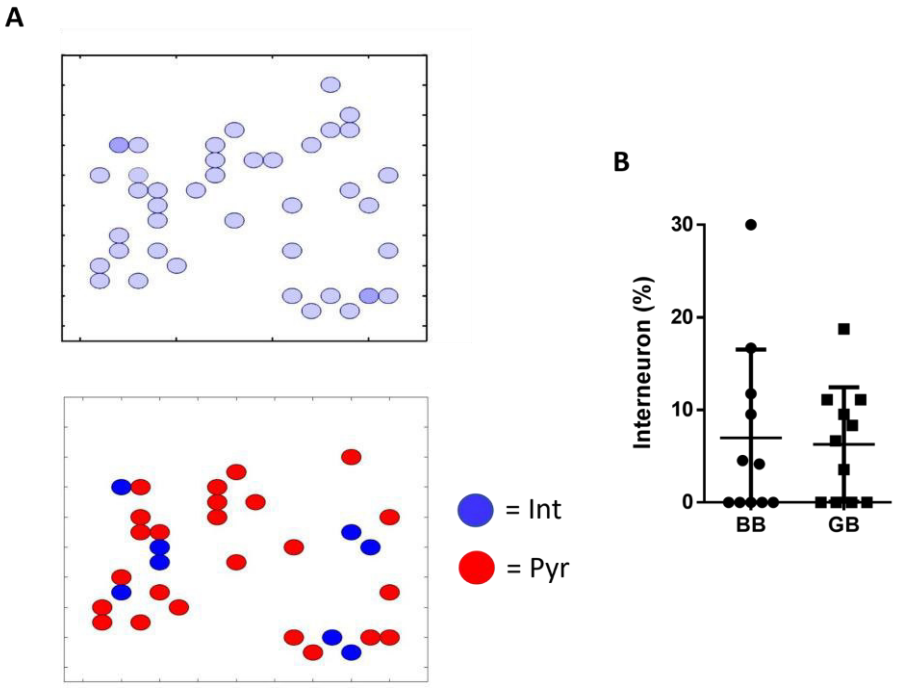

Figure 15. Pyramidal or Interneuron classification. A Hippocampal map by neurons recorded (top) and map according to neuronal type (putative interneuron = blue circle: putative pyramidal cell = red circle; bottom). **B** Quantification of interneuron percentage for BB (circle) and GB (square). Each circle or square represents a hippocampal slice recorded. Data shown as mean ± SD. Statistical t-test was used.

Hippocampal Network: Correlations and coding process

The neural coding population activity is critical to establish learning, memories, and cognition (Doiron et al. 2016). In this context, we assessed a neural population analysis to unveil the hippocampal network state in our GB and BB degus population.

We use a simplest network analysis based on pairwise Pearson correlation. However, since the data are discrete, Pearson values were quite small; hence we introduce a method to find the relevant correlations. Briefly, it consists of taking the spikes of neurons and shuffling, disrupting the time influences and computing Pearson correlation, repeating that process 1000 times. All correlation values higher than "shuffle correlation threshold" (chance threshold) were considered as 1 (relevant connection). Finally, we computed the relevant connections (network connectivity) by each neuron in

the same slice recorded, taking the maximum possible correlation of pairwise neurons dividing the sum of relevant connections per neuron.

Figure 16A shows similar network connectivity for BB in SA (16.7%) and PTX (19.1%) ($p = 0.054$). GB increased network connectivity under PTX effect (SA = 11.1%; PTX = 28.6%. $p<0.0001$) more than twice. The GB degus shows a neural network with less connections compared to BB in SA ($p<0.0001$), even if GB showed higher FR (Fig 11B). Nevertheless, when we compared both groups under the PTX effect, GB increase to higher network connectivity than BB ($p = 0.0001$), reversing previous differences.

Our network connectivity analysis by hippocampal zones showed different results depending on degus group. BB did not show a significant difference between SA and PTX among hippocampal zones (CA1 SA = 22.2%; PTX = 29.9%; $p=0.708$. CA3 SA = 13.0%; PTX = 19.2%; $p=0.614$. DG SA = 14.9%; PTX = 17.4%; $p=0.020$) (Fig 16B, left). CA1 region has few associational connections, being a network focused on smaller cells modules, although it does not mean that CA1 lacks these connections (Yang et al., 2014). This feature of CA1 could explain why it did not increase its network connectivity, despite neural activiry was affected by PTX. Consistent with our finding, a network with few recurrent connections (over them self inputs) is disabled to increase correlation with neurons that constitute the network.

In terms of connectivity, the GB degus showed significant differences in all hippocampal zones (CA1 SA = 12.5%; PTX = 32.6%; $p=0.0013$. CA3 SA = 14.8%; PTX = 31.8%; $p<0.0001$. DG SA = 5.0%; PTX = 22.2%; $p<0.0001$) (Fig 16B, right). The FR, maximum FR and burst activity were increased by PTX only in CA3 (Fig 13B and Fig 14B-C), where network connectivity increases in every zone including DG, where connections from CA3 to DG are almost null. One of the main features that can explain our findings is the recurrent architecture of CA3 network.

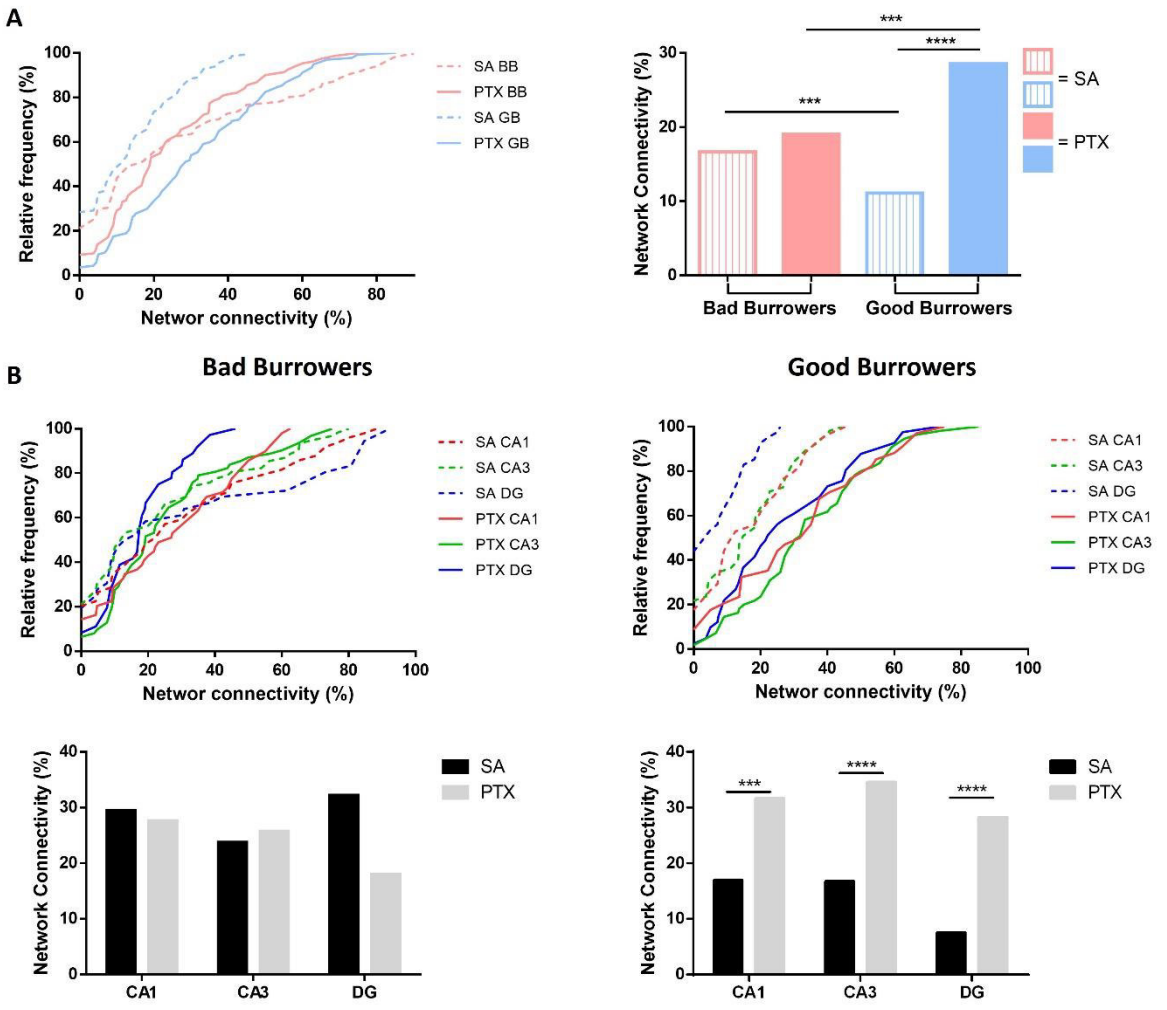

Figure 16. Network connectivity under PTX effect. **A** Network connectivity, computing through Pearson correlation, in spontaneous activity (SA segmented line) and PTX drug (continues line), separated in bad (pink) and good (blue) burrowers, represented by cumulative frequency plot (left). Median of network connectivity cumulative frequency distribution for SA (empty bar with lines) and PTX (filled bar), separated into BB (pink) or GB (blue) (right). **B** Network connectivity to each hippocampal zone (CA1 red, CA3 green and DG blue) according to their burrowing performance (BB left or GB right) and different activity (SA segmented line, PTX continue line), represents by cumulative frequency plot (top). Median of network connectivity cumulative frequency distribution for SA (black bar) and PTX (gray), separated into BB (left) or GB (right) (bottom). Data shown as box plot and median $\pm$ Tukey test distribution. Black circle represents the out-layers. Statistical nonparametric test Kolmogorov-Smirnov. ***=p<0.001; ****=p<0.0001.

III] COMPUTATIONAL ANALYSIS RESULTS

With the evidence presented above, we looked for a quantitative approximation of the inhibition affecting the degu's hippocampus. The study of neural networks needs the recording of many simultaneous neurons. The later was not our case where we only obtained a small number of neurons per slices (around 20). For those reasons, to understand better the implications of our results we introduce a neuronal network model. We have extrapolated some of our empirical data results into a modeled network to better understand the inhibition / excitation neural balance in our experiments. In that context an internship was realize at Bordeaux, during three months in Frederic Alexandre lab (MNEMOSYNE team - INRIA Bordeaux Institut des Maladies Neurodegeneratives CNRS)

The model selected was the "Random balanced network" model (Brunel N, 2000) due to its simplicity, fast implementation, low computational cost, and because it addresses the excitation / inhibition balance problem. Briefly, the model comprises a network of N neurons, represented by the Integrate and Fire (IN) model, for both excitatory and inhibitory neurons. The ratio of excitatory and inhibitory neurons was 4:1, respectively, according to model data and biological proportion (Pelkey et al., 2017). Total neurons of the network were 12500, being 10000 excitatory and 2500 inhibitory. Each neuron receives a random number of connections from other neurons and excitatory neurons outside the network.

Figure 17A shows the network activity (V_0), with neocortex values, in terms of frequency (Hz) as a function of g and at different V_{ext}/V_{thr}. g represents inhibition/excitation ratio, where at higher g values, higher is the inhibition. V_{ext} corresponds to the external stimulation of the neuron given by an independent Poisson process. V_{thr} is the threshold frequency for the neuron to fire an action potential, therefore V_{ext}/V_{thr} represents the excitability of a neuron. The V_0, as a function of V_{ext}/V_{thr}, increases proportionally and does at a higher magnitude with lower values of g (Figure 17A, left). This is because, at lower values of g, the excitation is more significant. Figure 17B shows the change of the

network with CA3 parameters for both graphs, reaching lower values than the neocortex, for g and V_{ext}/V_{thr}.

Figure 17C shows the same graph in 17B left but takes the range of FR values between 0 and 10Hz, similar to our data. At higher V_{ext}/V_{thr}, a higher FR value is observed for the same g value. V_{ext} is higher than V_{thr}, making the neuron more susceptible to fire and more excitable. We selected the value of V_{ext}/V_{thr} =1 since the experiments were done in spontaneous activity, and the activity of this is primarily low, hence a low excitability of neurons.

Figure 17D shows the curve g vs. V_0, with V_{ext}/V_{thr} =1, but comparing three connection probability values, being 0.1 the network's default value. The value of 0.3 was chosen because CA3 is characterized as a network with recurrent connections, a value with which the previous graphs were constructed (Fig 17B and 17C).

After setting CA3 parameters the next step was reproducing the FR from our empirical data and assessing their g value. We took the median for FR from CA3 measure (Table 3), and we interpolated it in the curve of Figure 17C (blue line) (Fig 17E). g values to BB were 4.9 to SA and 4.7 to PTX activity, while GB were 4.6 to SA and 4.2 to PTX activity (Fig 17E). The differences between stimulus and degus group were quite similar, although GB showed a higher contrast with BB, in terms of neuronal activity and network connectivity. This result indicates that the difference between BB and GB could not only be explained by GABAergic, due to the g value for both groups was similar. The model also could not help us quantify the E/I balance properly, because the model was simple and no representative to CA3 network even with parameter proper to this zone. We reproduced the raster plot for four conditions (Supplementary S3), finding a similar pattern in low FR but not large.

Models are always a trade-off between complexity needed and computational requirement, being directly proportional. The choice of model will depend on the question to be answered and the context. Our initial question was to characterize the inhibition/excitation balance of the network using empirical data for the network activity. For this reason, it was decided to use a basic network and that within its parameters, it considered the inhibition/excitation balance and required a low computational cost. To

make progress in this field, our further models should consider including a more sophisticate analysis in terms of a pattern completion model (Guzman et al., 2016), a task related with CA3 (O'Reilly & McClelland 1994).

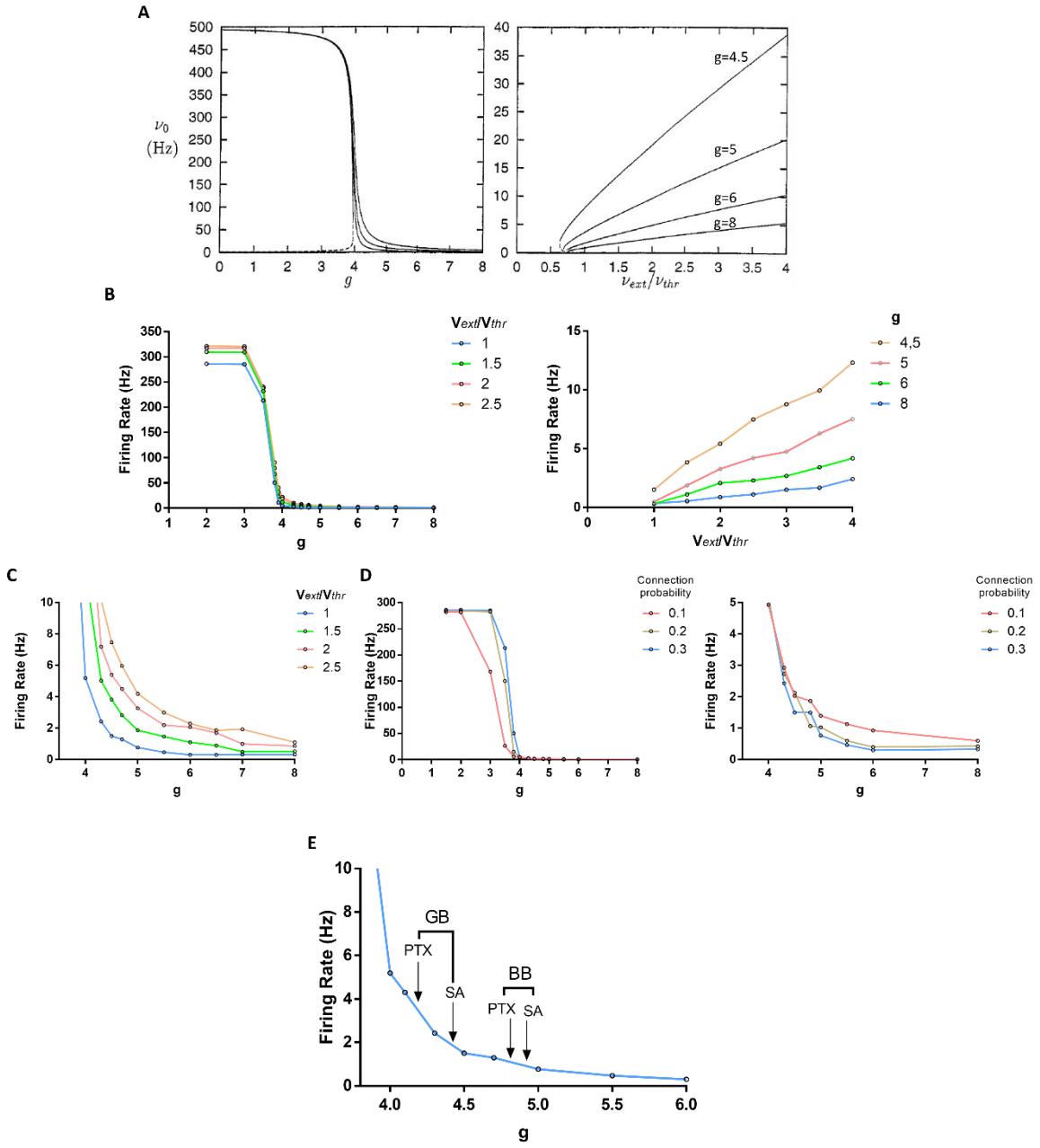

Figure 17. Random balanced network model with CA3 parameters. A Network activity (V_0) in Hz is a function of g, for V_{ext}/V_{thr} = 0, 1, ,2 and 4 (left). V_0 as a function of $V_{ext/Vthr}$, for g = 4.5, 5, 6, and 8. Parameters of the model corresponding to the neocortex. **B** Firing rate (Hz) as a function of g (left) and V_{ext}/V_{thr} (right), for V_{ext}/V_{thr} = 1, 1.5, 2, 2.5 (left) and for g = 4.5, 5, 6, and 8 (right). Parameters of the model corresponding to the CA3 hippocampal zone. **C** Same plot in 1B left but with a different scale, more like our empirical data, choosing V_{ext}/V_{thr} = 1 to build our modelization. **D** Firing rate (Hz) as a function of g for connection probability = 0.1, 0.2 and 0.3 (left). Same plot but with a different scale, choosing connection probability = 0.3 to build our modelization (right). **E** FR Interpolation from empirical data in model curve, separated into BB and GB and SA and PTX activity.

DISCUSSION

Cognitive abilities naturally decrease through the aging process, such as memories and learning (Riepe 2005). Nevertheless, since not all individuals' ages in the same manner, where some keep their cognitive, learning, and memory abilities for very long, we were interested to further explore this subject. The hippocampus, part of MTL and the limbic system (Ulanovsky & Moss 2007), is associated with spatial, episodic and semantic memory (Buzsáki & Moser, 2012), as well as synaptic plasticity and learning (Ball 1977; Landfield et al., 1978; Markowska et al., 1989; Bliss & Collingridge 1993; Foster & Norris 1997) and is susceptible to aging and dementia. Particularly, CA3 region besides being the computational neural core of the hippocampal network (Knierim 2015) is vulnerable to seizures and neurodegeneration processes (Cherubini and Miles 2015). Furthermore, the aging process highly affects the GABAergic system (Rozycka & Liguz-Lecznar, 2017), where preservation means a better cognitive state (Verret et al., 2012; Tran et al., 2018; Tran et al., 2019).

In this work, we used behavioral, electrophysiology, and computational model tools to elucidate the relationship between a healthy state of cognition and the integrity of the GABAergic system in degus during aging. We introduce a burrowing task (BT) which related to a spontaneous behavior assimilate to activities of daily life (ADL), to classify our aged degus into good burrowers (GB) and bad burrowers (BB). Our results suggest a good correlation between GB and the integrity of their GABAergic system, where CA3 presented the best-preserved hippocampal zone for the GABAergic system.

Degus has proven to be a good model for studying aging and neurodegeneration since it can live up to ten years under laboratory conditions (Hurley et al., 2018). Moreover, throughout aging, their cognitive decline is associated with a deficit of synaptic plasticity in the hippocampus (Ardiles et al., 2012). An adequate behavioral test is essential to measure the cognitive status of the animals. The novel object recognition test (NOR) has proven to be a standard test to measure the cognitive status

of degus (Ardiles et al., 2012; Lindsay et al, 2019; Rivera et al, 2021) where in populations older than 24 months most of degus decrease their performance (Ardiles et al. 2012), a similar result obtained here (Fig 5). This is not to say that the animals are no longer functional, cognitively speaking, but rather that the NOR requires a well and strong preserve cognitive abilities (Wooden et al., 2021).

The fact that some old people behave cognitively well during aging call us to find a behavioral test to discriminate between such population. So, we turn to search for a behavioral test relevant to their animal's daily life. The burrowing behavior is spontaneous and is used in the wild by degus to build burrows, protect themselves from weather and predators (Dudek et al. 1983) and has been describe as an ADL-like behavior (Deacon 2009). In addition, it is associated with hippocampal integrity (Deacon et al., 2002) and is associated with AD biomarkers (Deacon et al., 2015).

Using BT, we were able to separate two populations of aged degus older than 24 months (Fig 7). In addition, we ran different controls to ensure that there were no biases in our measurements, such as sex, locomotor activity, and anxiety levels. We did not find differences in such controls between degus with high GB or poor BB (Fig 7; Fig 10). We also measured the effect of aging on BT, showing a clear decrease over time (Fig 10), as well as a decrease in performance, and similarly to NOR (Fig 10). Consistently, BT, as also observed in ADL, deteriorates during aging (Reisberg et al., 2001; Deacon 2009).

It would have been interesting to perform our BT test on young degus but this turns impossible because the young degus we tested presented high anxiety levels, performing freezing and not exploring the maze. This type of behavior could be avoided by increasing the habituation period to the experimenter and the experimental room, what was not possible to carried during the pandemic period.

An irrefutable task would be to measure hippocampal SP, depending on BT performance, to find the significance of burrowing behavior. Synaptic plasticity decreases in aging and is involved in learning and memory (Landfield & Lynch 1977; Deupree et al., 1991; Burke and Barnes, 2006; Boric et al.,

2008). Therefore, it would put burrowing and BT as a cognitive behavior affected by aging, where GB preserves synaptic plasticity.

We found that the FR, maximal FR, and burst increase more in the GB degus compared to BB suggesting they possess a better-preserved GABAergic system. The PTX is characterized by producing an epileptic-like effect, i.e., a sharp and transient increase in neuronal activity, affecting CA3 most strongly (Hablitz 1984; Hashimoto et al, 2017). According, CA3 was the hippocampal area most affected by PTX, both for FR, maximal FR, and burst activity (Fig 13-14), consistent with the drug's effect. Finally, the hippocampal network connectivity, was significant increase with PTX in the GB compared to BB (Fig 15).

Interestingly, the spontaneous activity (SA) of BB was lower than GB, despite PTX affecting them in a lesser manner. One would expect that if BB do not possess a fully functional GABAergic system, SA would be high, as observed in aged animals (Palop et al., 2007; Haberman et al., 2017). We speculate here that the system manages to control SA, and if it does not do it by inhibition, it could be by controlling excitation. Glutamate is an excitatory neurotransmitter, the most abundant on the CNS, and more than 90% of neurons have glutamate receptors (Gasiorowska et al., 2021). Previous works have shown a relationship with beta-amyloid, related with AD, and impairments in synaptic glutamatergic transmission and retraction of excitatory dendritic spines (Palop et al., 2003; Palop et al., 2007). These changes produce a decrease in excitatory transmission in a different manner than a strong GABAergic system. Moreover, in an AD transgenic mouse, LTP deficit was found due to excessive inhibition, where after PTX application, they could not normalize LTP deficit (Palop et al., 2007).

Furthermore, BT has shown a relationship with AD biomarkers, such as beta-amyloid and APP (Deacon et al., 2015) (Inestrosa et al., 2005; Ardiles et al., 2012) and it would be interesting to further analyze the brain tissues preserved for each recorded animal to address this relationship.

In aging, the decrease of $GABA_A$ receptors has been seen in different parts of the brain, including the hippocampus, in a range between 5% to 35% (Shen et al., 2010). It would be interesting to measure

the number of $GABA_A$ receptors in degus brains according to their performance in BT. At the moment, there is no information reported on the GABA receptor expression during degus aging. Indeed, only three papers have been reported in the entire literature on GABA receptor expression in brain degu, all focused on a neonatal stage and from the same group (Ziabreva (a) et al., 2003; Ziabreva (b) et al., 2003; Seidel et al., 2008).

The role of inhibition in preserving cognition in an aging population has been due to increasing synaptic inhibitory strength or restoring E/I balance by a different mechanism than in young animals (Tran et al., 2018; Tran et al., 2019). Another example is the importance of brain oscillations, a phenomenon highly controlled by inhibitory neurons (O'Keefe & Recce 1993; Steriade et al., 1993; Csicsvari et al., 2003). Hippocampal gamma oscillation (30-80 Hz) is fundamental in the synchronization of neuronal assemblies for the correct propagation and storage of information (memory) in neuronal circuits (Harris et al., 2003; Buzsaki & Watson 2012). Moreover, in AD mice with cognitive deficits, associate with alteration in gamma oscillation, and parvalbumin interneuron functionality, a recuperation was possible by recovering interneurons functionality (Verret et al., 2012). Therefore, our results align with a more healthy and operative hippocampal GABAergic system in GB degus which preserve cognitive abilities such the one involved during burrowing.

As computational tools have become powerful, computational neuroscience has taken an important role in neuroscience. Today, it is possible to model different brain areas circuits to test various hypotheses (Blohm et al., 2020). Here, a simple representation model of a randomly connected neural network, considering excitatory and inhibitory connections, and determining the E/I balance of the network (Brunel 2000), was selected. Although our physiological results showed significant differences between BB and GB for SA vs. PTX conditions, calculated values of g (Inhibition/excitation) were similar (Fig 16). When looking at the raster plot of the model compared to the empirical data (Supplementary S3), values of FR greater than 1.5 Hz did not appear similar, the actual parameters used correspond to the neocortex, collected in the literature and according to the

properties of the cell membrane (Kali et al., 2000; Guzman et al., 2016), and in the future should be adapted to CA3 values. Some of the limitation here, was in the context of a short internship (3 months) (Lab Frederic Alexandre, Inria Bordeaux Institut des Maladies Neurodegenerative CNRS) with not enough time to test for more sophisticated models.

Our findings suggest a direct relationship between the cognitive status of aged degus who properly perform BT and the GABAergic status of the hippocampal network, especially with CA3, being consistent with what has been described in previous works.

CONCLUSIONS AND PROYECTIONS

- We described the different cognitive levels of the aged degu population established using behavioral test (Burrowing Task) related to Activity of Daily Living and the physiological characterization of their Hippocampal network using multielectrode array.
- Aged degus Good Burrowers performing well in Burrowing Task maintain a better GABAergic system than degus with poor performance, possible related to a better preserved CA3 hippocampal area.
- The results in aged degus Bad Burrowers suggest the presence of a different mechanism than GABA to maintain a low Spontaneous Activity, perhaps related to the glutamatergic system.
- Although we use a computational model to determine the inhibitory state of the CA3 network, it would be convenient to use a more complex model associated with CA3 functions, such as pattern completion.

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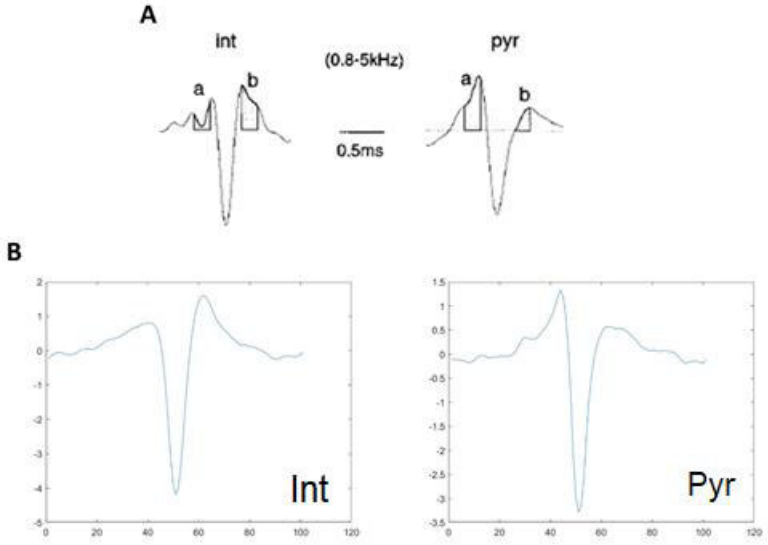
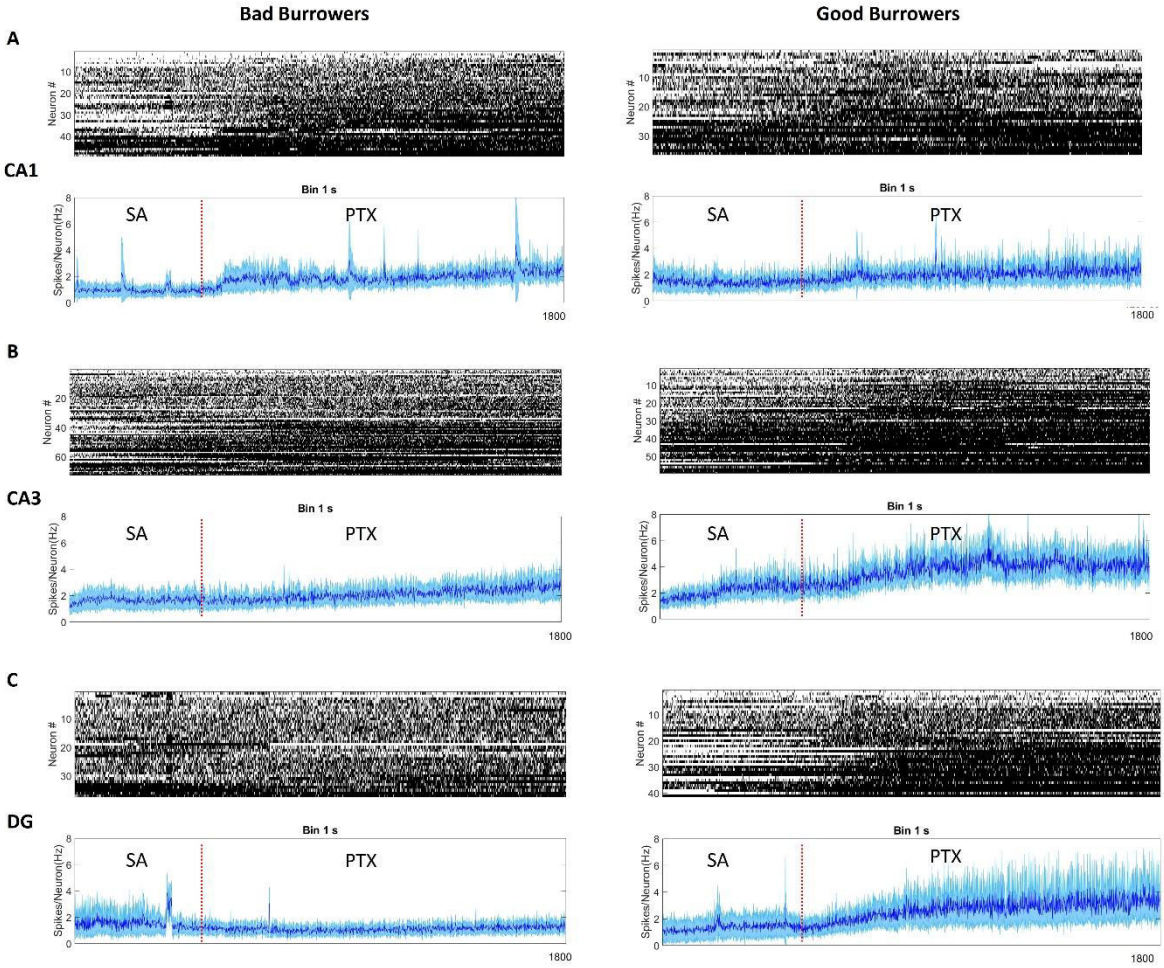
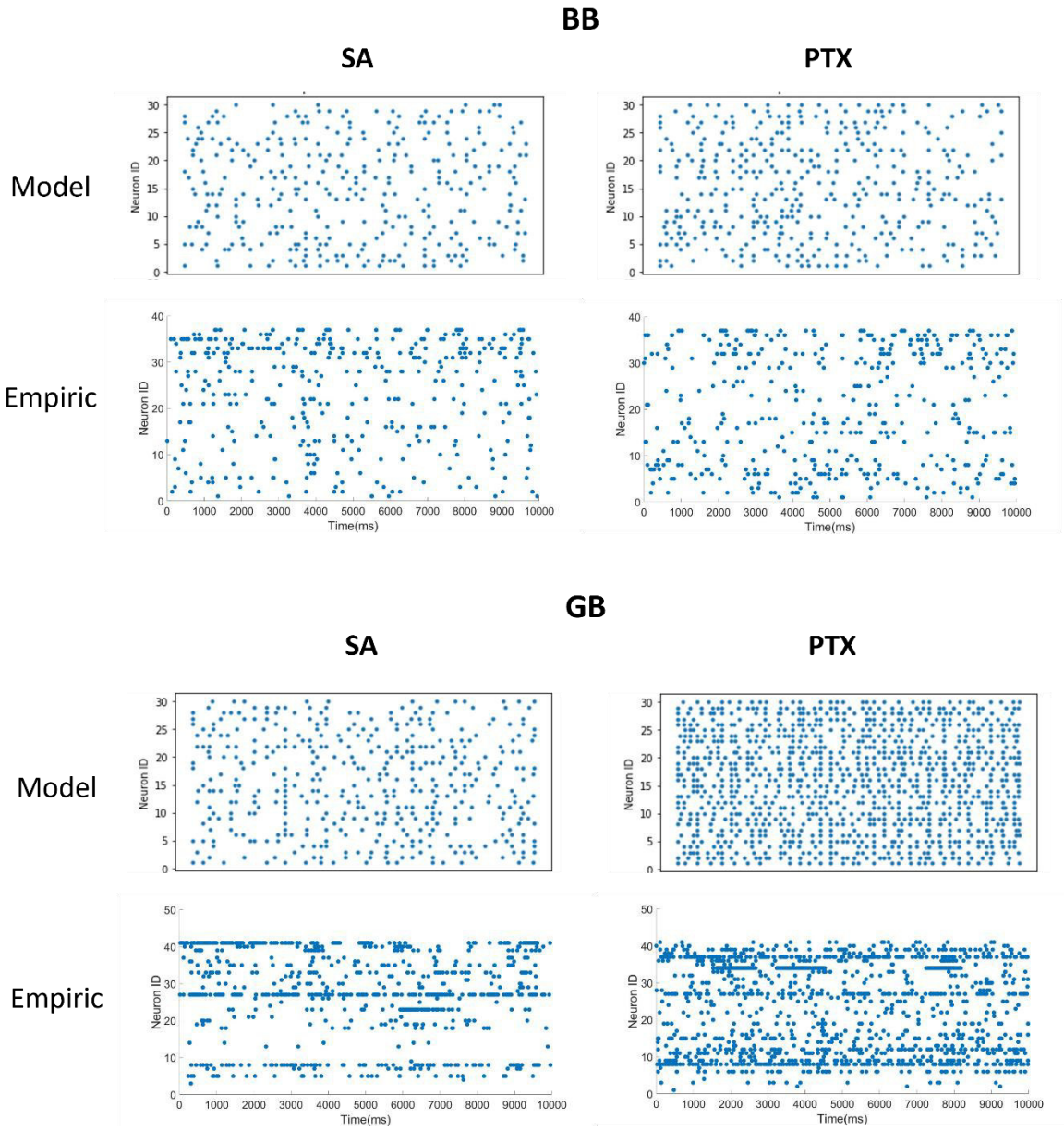
SUPPLEMENTARY



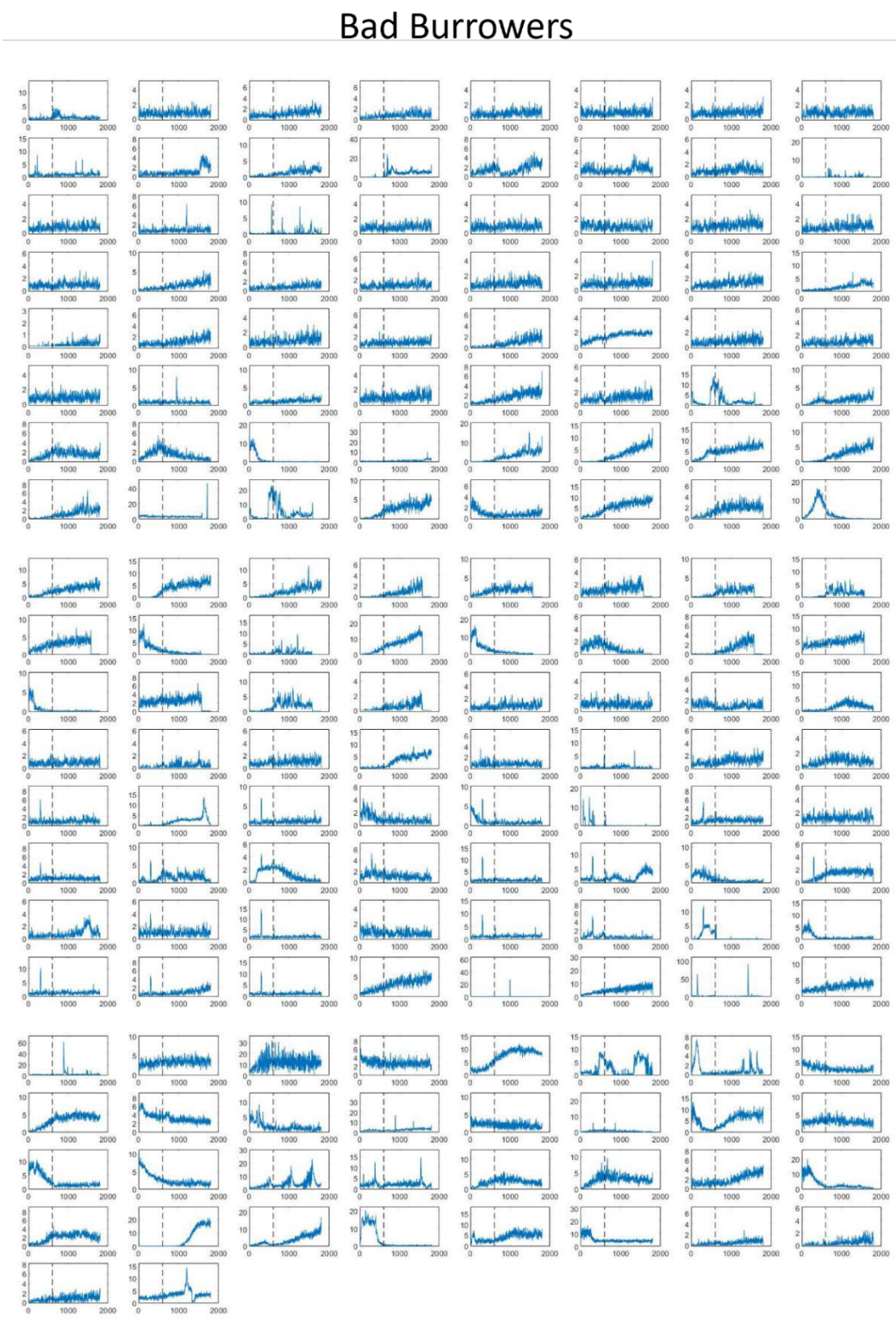
Supplementary S1. The waveform of putative interneuron (Int) and putative pyramidal cell (Pyr). **A** Representative waveform to Int and Pyr (Csicsvari et al., 1998). **B** Representative waveform to Int and Pyr of our neurons.



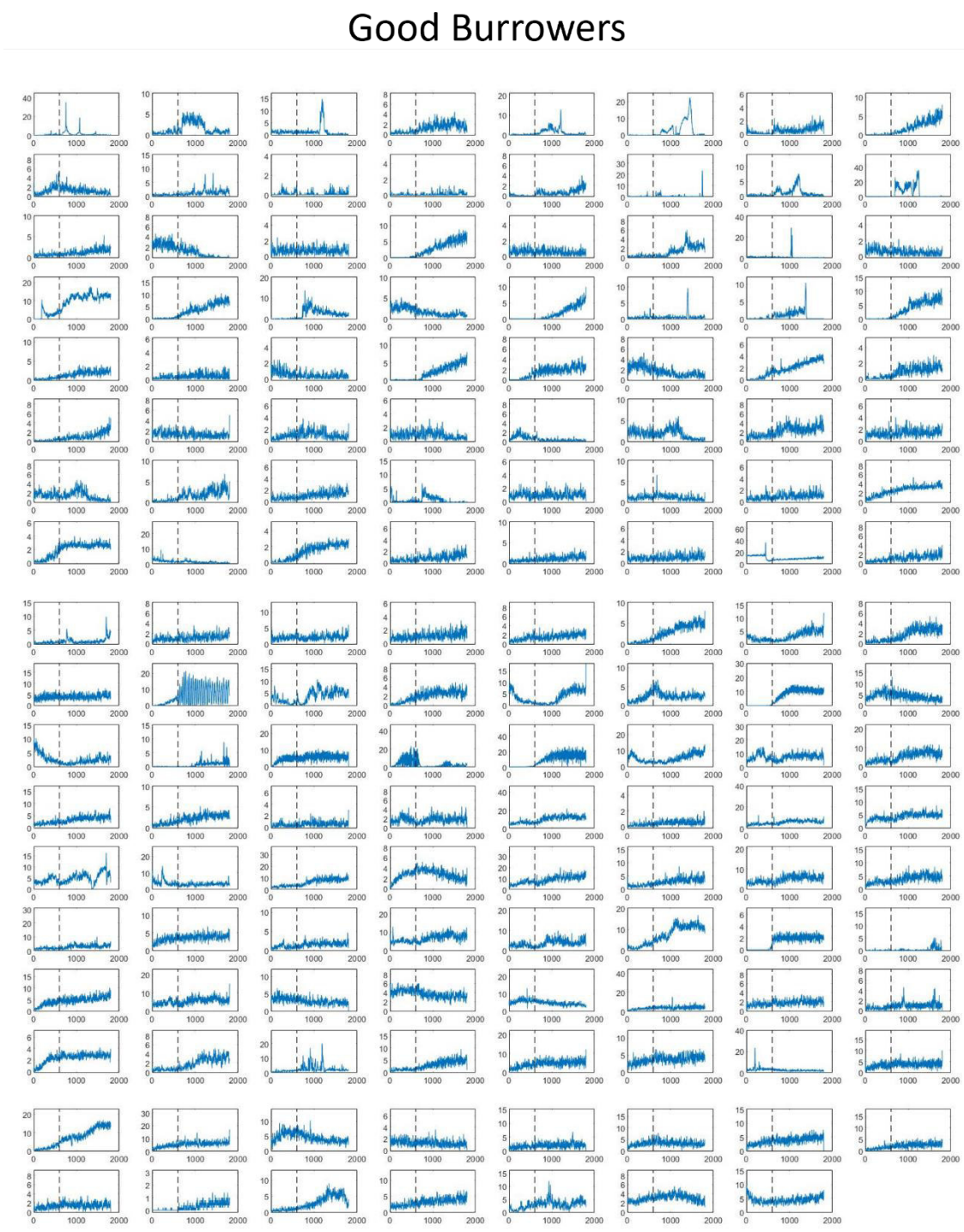
Supplementary S2. Representative raster plot for CA1 (**A**), CA3 (**B**), and DG (**C**) neurons recorded, separated according to BT performance into bad (left) and good (right) burrowers (top). Population activity is represented by spikes/neuron (Hz) for the recorded time (1800 s). The Redline separates spontaneous activity (SA, 600s, left) and PTX activity (PTX, the 1200s, right) (bottom). Bin selected was 1s



Supplementary S3. Raster plot representation for model and empirical data, divided in BB (top) and GB (bottom) and according to SA or PTX activity. Times recording was 10 s. Bin size was 1 ms.



Supplementary S4. Temporal response of each neuron to BB degus. Vertical segment line separate SA activity (left) and PTX (right)



Supplementary S5. Temporal response of each neuron to GB degus. Vertical segment line separate SA activity (left) and PTX (right)

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Review

Alzheimer's Disease, Neural Plasticity, and Functional Recovery

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Abstract. Alzheimer's disease (AD) is the most common and devastating neurodegenerative condition worldwide, characterized by the aggregation of amyloid-β and phosphorylated tau protein, and is accompanied by a progressive loss of learning and memory. A healthy nervous system is endowed with synaptic plasticity, among others neural plasticity mechanisms, allowing structural and physiological adaptations to changes in the environment. This neural plasticity modification sustains learning and memory, and behavioral changes and is severely affected by pathological and aging conditions, leading to cognitive deterioration. This article reviews critical aspects of AD neurodegeneration as well as therapeutic approaches that restore neural plasticity to provide functional recoveries, including environmental enrichment, physical exercise, transcranial stimulation, neurotrophin involvement, and direct electrical stimulation of the amygdala. In addition, we report recent behavioral results in *Octodon degus*, a promising natural model for the study of AD that naturally reproduces the neuropathological alterations observed in AD patients during normal aging, including neuronal toxicity, deterioration of neural plasticity, and the decline of learning and memory.

Keywords: Neural plasticity, neurorestauration, non-transgenic animal models of neurodegeneration

Alzheimer's disease (AD) is one of the most common and devastating neurodegenerative diseases that occur during aging and is characterized by a progressive neurodegeneration process that produces learning and memory loss. Although there is no consensus yet on the origin of AD, we can mention some candidates: the amyloid-β protein (Aβ) cascade, i.e., Aβ accumulation (soluble or in plaques) [1, 2]; the accumulation of phosphorylated tau protein (tangles) [3]; and a neurovascular failure-inducing degeneration [4, 5]. AD is associated with the accumulation and deposition of Aβ, astrogliosis, oxidative injury, the formation of neurofibrillary tangles, cell death, and neurotransmission alterations that impair synaptic plasticity (SP) and cognition. One of the dominant working hypotheses involves the Aβ cascade, which is supported by research that makes use of transgenic mice expressing familial mutations of the human amyloid precursor protein (APP) and presenilin and results in the development of deficits in neural plasticity, learning, and memory. Nevertheless, these transgenic mice rarely develop neurofibrillary tangles and exhibit little synaptic and neuronal loss [6–13].

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Instead of using mice, which are short-lived, another approach involves the use of long-lived animal models that naturally and progressively express hallmarks of AD as they age [13, 14] and constitute more realistic models of AD. These models are vital to test different etiologies, e.g., the amyloid cascade of the disease [15, 16]. In this regard, one promising model is the rodent, *Octodon degus* (degus), which develops brain changes during aging similar to those observed in patients with AD [13, 17–20]. Another critical issue that must be taken into account to advance in the treatments for AD is to include neural plasticity as part of the treatment since it contributes to sustaining memory and learning and the nervous system's self-repair [21–24].

Here we review the advantages and disadvantages of animal models that naturally develop AD neuropathology, as well as some therapeutic procedures, such as environmental enrichment, physical exercise, transcranial stimulation, neurotrophins, and direct electrical stimulation of the amygdala, that improve neural plasticity and achieve a functional recovery of learning and memory [21–24].

NEURAL PLASTICITY AND NEURODEGENERATION

In contrast to what was previously believed, the central nervous system (CNS), unlike thought for many years, can dynamically modify its properties in response to changes in the environment. This view extends to neural plasticity's mechanisms associated with learning and memory and the recovery of function after injury [21–24]. In its broadest sense, neural plasticity means the capacity for functional or morphological changes, of the CNS and its component elements (e.g., nerve cells and synapses), by external agents' actions. This plasticity must be differentiated from genetically programmed modifications. External agents are usually sensory stimuli and traumatic injuries, which make each unique and different experience. Thus, neural plasticity stands out for its adaptive value, allowing the compensatory changes induced by experience to occur in the CNS continuously [21, 25]. The mechanisms of neural plasticity are diverse. They can vary from extensive morphological modifications, such as those observed in the regeneration of axons and new synapse formation, to subtle molecular changes that alter the cellular response to neurotransmitters [26, 27]. In this sense, two neural plasticity forms can differentiate morphological or growth plasticity and functional plasticity [21, 28], where morphological plasticity includes neurogenesis, regeneration, axonal collateral formation, and reactive synaptogenesis. Santiago Ramón y Cajal was the first to propose plasticity in the number and strength of neural connections as the physical basis of learning and memory.

Years later, the psychologist Donald Hebb [29] proposed plasticity as the mechanism by which the coincidence of pre- and postsynaptic activity could modify the neural connections in specific structures of the brain. In 1973, the first experimental evidence was found that supported the hypothesis of Cajal and Hebb, that is, that synapses can change as a result of their activity [30–33]. This phenomenon has been known as long-term potentiation (LTP) and consists of a sustained increase in synaptic transmission efficiency after stimulating an afferent pathway with high-frequency stimuli. The entire transmission process occurs faster and to a greater extent [34, 35]. Such changes happen immediately and have a variable duration, depending on the protocol used for their induction, ranging from a few hours and days to weeks. Since its discovery, LTP is proposed to be the cellular basis of the processes that underlie learning and memory [34–36]. In particular, when LTP's efficacy decreases during, e.g., neurodegeneration and or aging, there is also a decline in subjects' cognitive capacity. The presence of amyloid plaques, neurofibrillary tangles, Lewy bodies, synaptic dystrophy, synaptic loss, loss of dendritic extent, and neurons loss in the brain [37, 38] has been described as a normal process observed during human aging. Although these changes are more subtle and selective than in AD patients, a critical consequence is a decay in neural plasticity (e.g., LTP) during aging [39]. Hence, failing neural plasticity mechanisms could accelerate transit from normal aging to neurodegeneration [40]. Numerous studies have shown that AD patients' memory failures do not correlate well with the amyloid plaque burden in recent years. Instead, the loss of synaptic markers in the human cortex and hippocampus is a better predictor of clinical symptoms and disease progression [41–44]; however, there is no biomarker to measure the synaptic integrity directly over time in AD patients. The methods used most frequently to assess synaptic integrity are electrophysiological and neuroimaging [44]. More direct methods include neuroanatomical studies [45, 46] and measuring mRNA or synaptic proteins [47, 48]. Of particular interest are soluble oligomeric species that may play an essential role in synaptic dysfunction

and neuronal loss in AD since current evidence indicates that neuronal and a rise of Dickkopf-1 may cause synapse loss, an antagonist of the endogenous intracellular wnt pathway [49, 50], rather than by Aβ plaque deposition per se [51, 52].

Tau hyperphosphorylation seems to play a more critical role in synaptic dysfunction and cognitive decline, affecting organelles' axonal transport, including the mitochondria [53, 54] and impair AMPA receptor clustering [55]. The colocalization of Aβ and tau [56], observed in AD patients, suggests potentiation of these adverse effects because tau could become hyperphosphorylated in the amyloid presence [57].

We hypothesize neurodegeneration and cognitive decay during aging could, at least in part, be related to a failure of neural mechanisms to process information and to accommodate new learning and memory, practically a loss of neural plasticity. Therefore, the modulation of neural plasticity mechanisms could potentiate the recovery of lost functions in AD patients. Following this idea, we will expose some evidence that strongly supports our hypothesis.

ENVIRONMENTAL ENRICHMENT AND NEURAL PLASTICITY

Early evidence showed that environmental enrichment produces changes in cortical weight and thickness [58], and an increase of dendritic branching and length, the number of dendritic spines, and the size of synapses of some neuronal populations [59, 60] and dental gyrus neurogenesis [61]. Additionally, at the molecular level, environmental enrichment induces the expression of brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF) [62, 63], synaptic proteins [64, 65], and NMDA (N-methyl-d-aspartate) and AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) receptor subunits [66]. As a result, environmental enrichment increases SP, such as LTP [67]. More importantly, environmental enrichment improves learning and memory at the behavioral level in young and old animals [68, 69]. Exciting results have shown that environmental enrichment enhances learning and memory in rodent models of neurodegenerative diseases such as AD. At the molecular level, there is an increase in synaptophysin, NGF, and neprilysin expression. Also, Adlard et al. [70, 71] using mice expressing a double mutant form of APP, showed an increased learning rate in the water maze test. They decreased the expression of Aβ plaques combining environmental enrichment with running wheels for five months.

OCTODON DEGUS, A NATURAL MODEL OF AGING AND NEURODEGENERATION

Although transgenic models have been instrumental in understanding familial forms of AD (5% of cases), these models, by not reproducing the full spectrum of neurodegeneration, are ineffective for clinical trials for sporadic AD (95% of cases) [12]. Aging is a critical factor that allows the gradual manifestation of the pathological mechanisms that accompany neurodegeneration and dementia in patients with AD. In general, the use of transgenic animals, although useful, is limited to detailed comparisons and related to the overexpression of specific proteins, such as the amyloid-β protein precursor. Another drawback in mice is that their lifespan is relatively short, 18–24 months, which is not sufficient to study the slow process that accompanies aging. In this respect, a limitation of currently validated animal models is that few allow for studying the real impact of natural aging on neurodegeneration development.

In the last few years, we have introduced the rodent, *Octodon degus* (degus), a natural model candidate to study aging and neurodegeneration since their brain reproduces changes observed in AD patients [13, 17–20]. Degus are mainly diurnal, medium-sized rodents and live in groups with high social interaction in the wild and under laboratory conditions [72]. Degus Aβ peptide shows a high 97.5% amino acid homology with humans, differing in only one amino acid, unlike rat and mouse, which differ from the human sequence in 3 amino acids [16]. Perhaps because of this, some aged degus naturally develop AD-like pathologies, including the brain expression of the neuronal AβPP (β-APP695), display both intracellular and extracellular deposits of Aβ, intracellular accumulations of tau-protein and ubiquitin, a strong astrocytic response, and acetylcholinesterase-rich pyramidal neurons. Moreover, during aging, degus present symptoms associated with neurodegeneration and develop cognitive impairments including object recognition, spatial memory, and SP associated with the NMDAR-dependent process, which declines in an age-dependent manner (LTP and LTD). In degus, these impairments correspond to a form of sporadic AD with an increase of Aβ and soluble Aβ*56 (12-mer) oligomer, suggesting a critical factor for neural toxicity [51, 73], and tau deposition [74].

Consequently, SP in degus is affected during aging, especially at the postsynaptic level, with a decrease in LTP, protein expression (PSD-95, GluR2, NR2B), and cognitive performance (object recognition, T-maze), as we have described earlier [17, 51]. Here we present behavioral results based on a natural burrowing test for degus [75], that promise is an excellent biomarker for AD neurodegenerative disorder.

ACTIVITIES OF DAILY LIVING

A wide variety of behavioral tests are designed to assess animals' cognitive states and defined brain structures. We have used a behavioral test based on rodents' natural and spontaneous affinity to burrow, observed in rugged environments [76]. Importantly, designing behavioral tests based on natural or spontaneous behaviors provides a clear advantage in motivating animals for testing and reducing stress levels [77]. For example, natural or spontaneous behaviors have shown a good association with Activities of Daily Living (ADLs). Thus, in humans, ADLs are among the first activities affected in neurodegenerative diseases and are defined as necessary personal care activities (dressing, grooming, bathing, toileting, eating, and ambulation) or complex activities (meal preparation, shopping, telephone use, among others) [78]. The latter was one reason why we wanted to study the task of burrowing or digging to characterize the natural cognitive state of our subjects [79]. Burrowing is a natural behavior expressed by many rodent species, as they take advantage of their natural environment to protect themselves from predators, adverse weather conditions or store food and build a shelter for their offspring [80].

A burrowing task (BT) corresponds to the ADL type's spontaneous activity and requires hippocampus function, as the induction of a cytotoxic injury decreased the burrowing performance [81]. Importantly, this test is fast, economical, and easy to implement in the laboratories in its practical part. In a preliminary study (see methods in the Supplementary Material), we tested twenty-five degus aged between 40–75 months. The results show that 44% ($n = 11$) of degus exceeded the 10% threshold (grams of burrowed pellets) and were classified as Good Burrowers (GB), while 56% (14 degus) were below the threshold and were classified as Bad Burrowers (BB) ($p < 0.05$) (Fig. 1B). No statistical differences were found either by sex ($p > 0.05$) (Fig. 1C) or age (40–55 versus 56–75 months old, $p > 0.05$) (Fig. 1D). Figure 1E shows the GB number that changed from 58.3% to 50%, from 40–55 versus 56–75 months old, respectively. ADL-type behaviors are one of the first tasks that humans lose with aging and neurodegeneration [79]. To check if degus BT classification is related to their motor performance, we carried an open field (OF) test in which the degu is free to explore for 5 min. The results shown in Fig. 1F show that BB traveled a distance of $28.5 + 5$ m, while GB traveled 32.4 ± 3 m, with no significant differences ($p > 0.05$). The differential exploration of the center versus periphery of an OF is used to determine the animal's anxiety level [82]. Figure 1G indicates individual results, where -1 indicates more time spent exploring the center, and +1 indicates a preference for the periphery. We did not see a particular difference between GB and BB (Fig. 1G) (BB: -0.39 ± 0.13, GB: -0.27 ± 0.09 m, $p = 0.49$), indicating that degus are not influenced by anxiety. Previous related results, according to burrowing test performance in degus, have established a good correlation with AD biomarkers of neuroinflammation [75].

PHYSICAL EXERCISE AND NEURAL PLASTICITY

Previous studies have demonstrated that physical exercise can improve neural plasticity mechanisms, increase BDNF, vascular endothelial growth factor (VEGF) and insulin-like growth factor 1 (IGF-1) [83–88]; enhance LTP and LTD in the dentate gyrus, increase spine density, dendritic branching, and neurogenesis [89–91], suggesting that all these factors contribute to the cognitive and neural plasticity imp rovement observed in physically trained model animals. On the other hand, it has been observed that exercise increases capillarization [92], decreases oxidative stress [93], and reduces Aβ load and the levels of hyperphosphorylated tau proteins [70, 94–97]. More importantly, physical exercise improves physical and executive function and spatial memory of patients with mild, moderate, or severe AD [88, 93, 95, 98]. Several studies have suggested that the activity carried out by free access to a wheel can prevent or delay cognitive deterioration occurring during a neurodegenerative process.

In a preliminary experiment in our laboratory, we have studied in degus of different ages the effect of voluntary long-term physical exercise on their cognitive capacities. Specifically, we have tested the locomotor activity (open field), object recognition, and spatial (8-arm-maze) memory in young and old

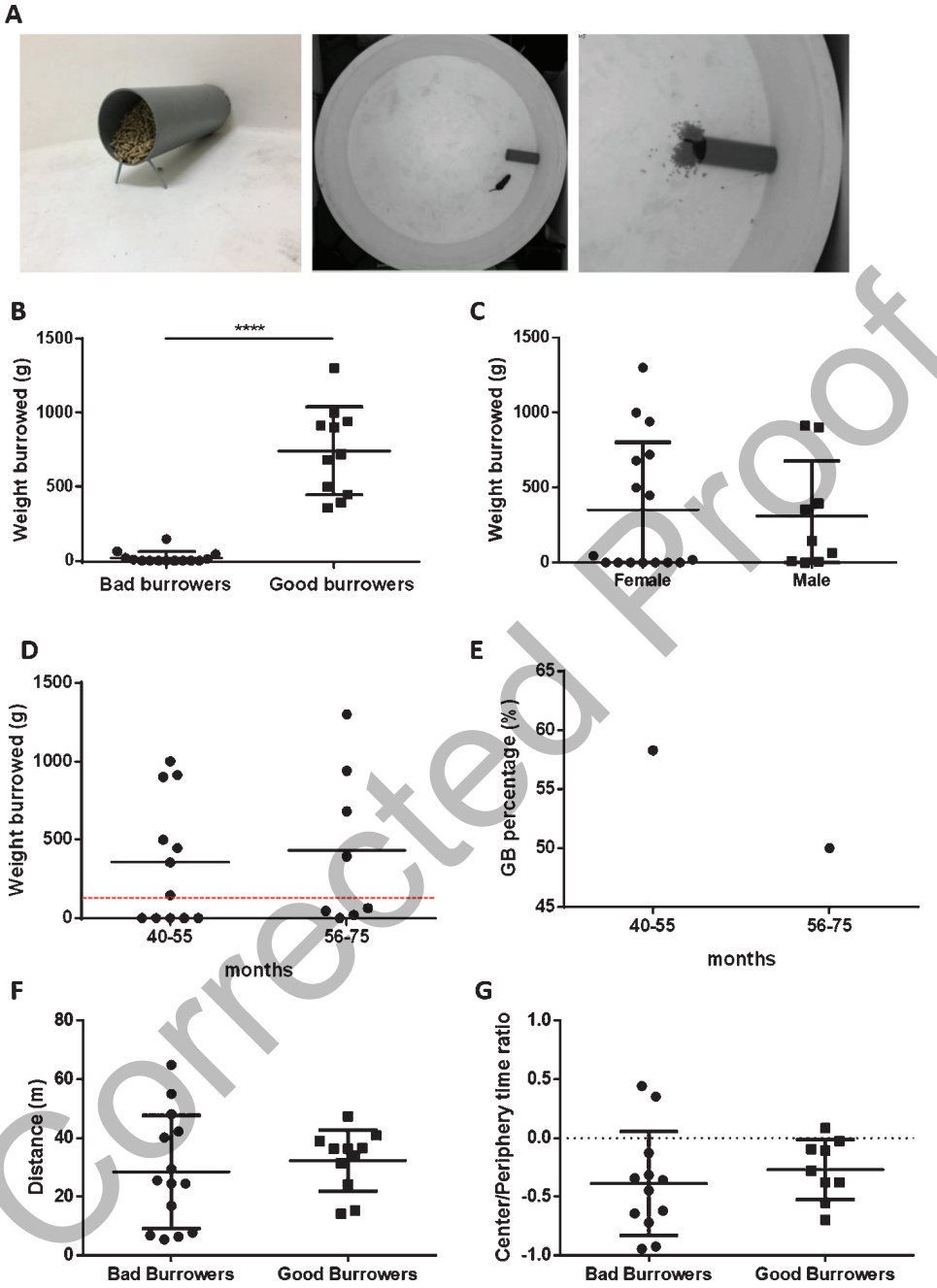

Fig. 1. The burrowing task in aged degus. A) Burrowing setup. Left: Burrowing apparatus (gray plastic tube with two screws at the entrance for support) filled with rabbit food pellets (1,300 g). Center: localization of the setup against the wall of a circular OF (diameter 180 cm). Right: degus put in the maze for free exploration; the burrowing content is measured as grams of pellets displaced out of the tube, corresponding to the BT performance. B) Burrowing classification according to degus performance in terms of the weight of pellet burrowed ($n = 25$). A threshold value of 10% of the total pellet burrow was determined (130 g) to separate Good Burrowers (GB) from Bad Burrowers (BB). C) Burrowing performance, according to sex. D) Burrowing performance in animals aged 40–55 months and 56–75 months. The red line represents the threshold value. E) Percentage of GB as a function of age. F) Distance traveled in an OF. G) Time in center versus periphery in the OF to measure anxiety level. The black line corresponds to the ratio of exploration time in center versus periphery, which were the same. Data are mean ± SD. Statistical analysis using the *T*-test. ****$p < 0.0001$.

degus. Our results (unpublished) suggest that both young and old exercised degus reach a better cognitive performance than degus without (wheel) activity. Moreover, after four months without access to the freewheel, both degu groups show an increased cognitive deterioration [99]. Therefore, voluntary exercise may be an effective therapeutic strategy to reduce AD's cognitive impairment. In another study (unpublished), we conducted a pilot study to determine the hippocampus's neurogenesis level during aging, which usually decreases in rodents and primates, including humans. For this, we studied the morphology of the hippocampus (gyrus dentate) during aging, finding a dramatic decrease in neurogenesis between 7 to 96 months in degus, which contrasts with the number of cells in CA1, which do not change with age [100].

TRANSCRANIAL STIMULATION AND NEURAL PLASTICITY

Over the last few years, transcranial stimulation has been shown to promote neural plasticity mechanisms and cognitive improvement in neurodegenerative disorders. Early research in humans showed that repetitive transcranial magnetic stimulation (rTMS) produces a neural potentiation measured at EEG electrodes located bilaterally over the premotor cortex [101]. Interestingly, high-frequency rTMS induces LTP-like cortical plasticity within the precuneus in AD patients [102]. However, some results are contradictory, perhaps due to different protocols utilized in each study. For example, a study accomplished by Chen et al. [103] in an animal model of AD using rTMS showed an enhancement of cognitive function, a reduction of neuronal apoptosis, and an increase in the levels of BDNF, NGF, and doublecortin.

On the other hand, repetitive transcranial direct current stimulation (tDCS) produced spatial memory recovery in an AD rat model [104]. These authors suggest that the improvement is due to tDCS modulating synaptic plasticity through calcium or sodium channel regulation and increasing cell proliferation in the subventricular zone. However, Gondard et al. [105], using the same technique, did not find positive effects on learning and memory. Moreover, few randomized controlled trials using rTMS or tDCS in patients with mild to moderate cognitive impairment and AD demonstrated an improvement in cognitive functions in a different cognitive test such as the Mini-Mental State Examination (MMSE) or the AD Assessment Scale-Cognitive Subsection (ADAS-cog) [106–115]. In addition, it has been demonstrated by Zhang et al. [116] that rTMS combined with cognitive training improves cognitive function, which suggests that combined therapies could lead to better results in AD patients.

NEUROTROPHINS AND NEURAL PLASTICITY

Neurotrophins are growth factors that are essential in neuronal development, function, survival, and plasticity in the developing and adult CNS. They consist of four structure-related proteins: nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), and neurotrophin-4 (NT-4/5) [117]. Neurotrophins exert their effects through membrane receptors that connect with different intracellular molecular cascades, such as MAP-kinase, PKC, and phosphatidylinositol 3-kinase (PI3-K), modifying gene expression and causing the synthesis of proteins [118]. The latter enables them to induce and modulate growth and functional neuroplasticity [119, 120]. On the other hand, neurotrophins can also indirectly support SP processes and reinforce the influence of non-glutamatergic afferents modulating LTP [121, 122]. BDNF in the adult brain increases synaptic transmission, facilitates SP, and promotes synaptogenesis [123]. Previous studies in healthy animals have shown that BDNF is crucial for forming and retaining hippocampal-dependent memory, fear memory extinction, and motor learning [124]. Moreover, previous AD animal models have demonstrated that BDNF administration decreases cognitive impairment and synapse loss, and neuronal abnormalities without causing Aβ and tau pathology [125–131]. Other authors have found that BDNF in neuronal cultures decreases production, and its removal contributes to an increase of Aβ [132, 133]. Likewise, Murer et al. [134] showed that neurons expressing BDNF did not present NFT, and by contrast, neurons with NFT did not express BDNF. In addition, Wang et al. [135], report that TrkB, an agonist antibody AS86 induces neurite outgrowth and enhanced spine growth with decreased cell death in cultured neurons. Furthermore, in this study, the use of AS86 rescued the cognitive impairments in APP/PS1 mice. On the other hand, the expression of BDNF in the hippocampus, temporal cortex, and parietal cortex is reduced in AD patients [136–138]. Also, in patients with sporadic and autosomal dominant

AD, the BDNF Val66Met polymorphism impairs episodic memory and hippocampal activity when measured by Fluorodeoxyglucose-positron emission tomography (FDG-PET) [139, 140].

AMYGDALA STIMULATION AND NEURAL PLASTICITY

The amygdala is a subcortical structure critical for emotional and motivational reactions [141, 142]. It also contributes to memory consolidation occurring in other brain areas [143]. Moreover, electrical stimulation of the basolateral amygdala (BLA) can reinforce memory-related synaptic mechanisms like LTP [144] via cholinergic afferents to the locus coeruleus and noradrenergic afferents to the medial septum [145]. Interestingly, natural emotional and motivational stimuli, like drinking water after two hours of deprivation, prolong LTP if temporally related to LTP induction [146]. This phenomenon, recognized as LTP-behavioral reinforcement, is mediated by noradrenergic receptors [147], dependent on the synthesis of new plasticity-related proteins [148], and the amygdala is an essential part of the neural circuit involved [149]. Following this idea, we have shown that post-training BLA electrical stimulation in healthy animals accelerates the acquisition of a motor skill in the staircase task [150] and improves spatial memory in fimbria-fornix (FF) lesioned animals [151]. It also increased BDNF protein expression and arc gene expression in the hippocampus [152, 153], an increase of the synaptogenesis related proteins MAP-2 and GAP-43 in the hippocampus and prefrontal cortex [154]. Furthermore, the amygdala's stimulation produces an increase of c-Fos protein, an early expression transcription factor related to neural plasticity and memory, in brain regions like the hippocampus and prefrontal cortex [155]. Interestingly, Inman et al. [156] demonstrated that the amygdala's direct electrical stimulation enhances humans' declarative memory. BLA electrical stimulation shortly after the performance of the behavioral task produces a functional recovery by directly promoting plastic changes in the brain structure involved in the task or by activating other modulatory regions like the locus coeruleus or the septal area, which, in turn, modulate the neural plasticity mechanisms involved in memory in relevant areas, especially in the hippocampus and the prefrontal cortex. Interpreting these and previous results [152–155], we propose that BLA stimulation promotes norepinephrine and dopamine release in the prefrontal cortex. In contrast, norepinephrine and acetylcholine are released in the hippocampus, which via G protein activates CREB and c-fos, BDNF, and arc gene expression.

In turn, c-Fos and BDNF could induce synaptogenesis-related proteins like MAP-2 (post-synaptically) and GAP-43 (pre-synaptically), contributing to the observed behavioral recovery. In the lesioned hippocampus, since the fimbria fornix lesion eliminates most subcortical afferents, similar plastic mechanisms could be initiated via entorhinal cortex afferents and be triggered by glutamate activation of NMDA and metabotropic receptors. The latter could improve the spatial memory storage but might also explain why the recovery is only partial. In a pharmacological study, we have demonstrated that noradrenergic agonists applied 10 min after the induction of an early-LTP could mimic BLA stimulation's reinforcing effect. In contrast, cholinergic agonists were not able to do so [145]. Catecholaminergic afferents appear to be relevant to LTP's early maintenance, while cholinergic afferents are required later. According to the model proposed in Fig. 2, the stimulation of the BLA in FF-lesioned and trained rats can partially activate the molecular mechanisms leading to neural plasticity and trace formation, producing a recovery of spatial memory. Altogether, BLA stimulation can improve or modulate the neuroplastic process implicated in recovering lost functions due to CNS injury. We expect that modulating neural plasticity mechanisms through BLA stimulation can also rescue lost functions, such as memory, in AD models.

CONCLUSIONS

Neural plasticity is a fundamental property of a healthy CNS which supports functions like learning and memory and functional recovery based on synaptic efficacy modification, synaptogenesis, sprouting, axonal regeneration, and neurogenesis. In contrast, the cognitive decline that occurs during aging is accompanied by an increase and accumulation of Aβ protein in the brain, neurofibrillary tangles, synaptic dystrophy, loss of neurons, and reduction of brain volume. These changes could overcome a physiological threshold beyond which neural plasticity mechanisms fail, and thus neurodegeneration is triggered. Exciting results have shown that optogenetic induction of LTP in the perforant path synapses of dentate-gyrus cells or optogenetic reactivation of the dentate gyrus cells in double transgenic mouse models of AD restore

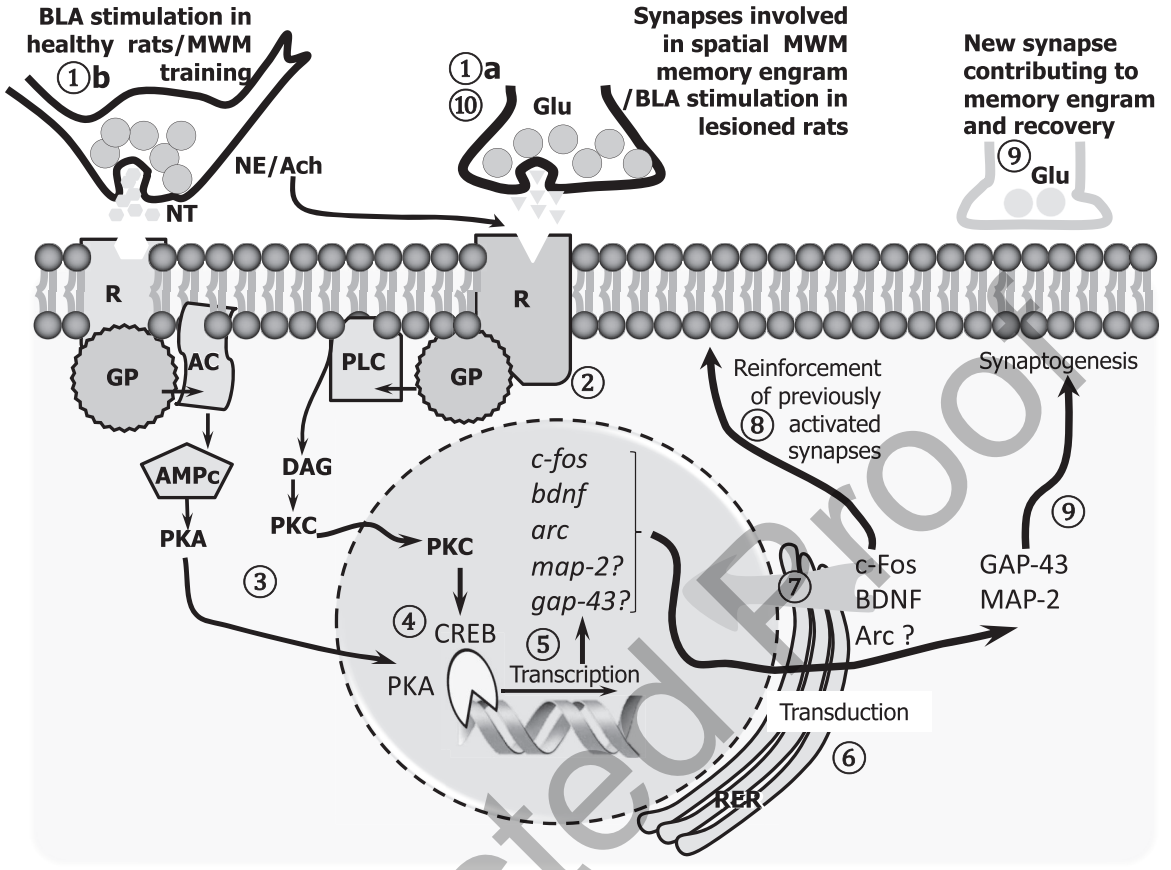

Fig. 2. A cell model interpreting the synaptic plasticity mechanisms triggered by BLA stimulation on engram cells, promoting spatial memory in healthy and lesioned animals. In normal animals, the glutamatergic afferents (1a) to the dentate gyrus (DG) carry the information to be stored within the hippocampal memory system, probably as a long-lasting increase in the efficacy of those activated synapses (LTP). The activation of the amygdala contributes to reinforcing the LTP in the DG via the activation of norepinephrine (NE) afferents from the locus coeruleus, which also activates the septal cholinergic input required mainly by late phases of LTP (1b). Both transmitters activate intracellular second messenger cascades (2,3) that modify pre-existing proteins and regulate the expression of plasticity-related genes (4,5) like BDNF, Arc (functional plasticity), MAP-2, and GAP-43 (structural plasticity) (6,7). Altogether, the potentiation of existing synapses (8) and the formation of new ones (9) are cellular mechanisms by which memory is stored. In FF lesioned animals, this sequence is affected by the interruption of both NE and ACh afferents; however, the stimulation of the BLA can still (at least in part) contribute to consolidation via the glutamatergic afferents from the entorhinal cortex (10), probably by the activation of metabotropic glutamate receptors, which share common postsynaptic molecular cascades with other transmitters (2). Such a partially restored function could explain the memory recovery achieved by BLA post-training stimulation, resulting in an amelioration, but not in a full recovery.

long-term memory and spine density [157, 158]. Moreover, environmental enrichment, natural behavioral tests, physical exercise, transcranial stimulation, neurotrophins such as BDNF, and direct amygdala electrical stimulation all induce plastic changes, rescue damaged synapses, and improve memory. As discussed here, the use of natural animal models, which recapitulate the main findings associated with the neurodegenerative diseases that occur during the slow, progressive, aging process seen in humans, is critical when evaluating neurorestauration alternatives. As illustrated in this work, the combined use of natural models with a relatively long lifespan combined with interventions that promote neural plasticity represents an effective way to screen for AD preclinical treatments.

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SUPPLEMENTARY MATERIAL

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