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Research report

Septohippocampal acetylcholine and theta oscillations can modulate memory encoding and retrieval: Insights from a neural masses network

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ABSTRACT

The hippocampus' ability to encode new information while simultaneously avoiding disruptive interference poses a fundamental challenge to cognitive neuroscience. It has been supposed that dynamical changes in acetylcholine (ACh), a neurotransmitter involved in learning and memory, can facilitate a shifting between encoding and retrieval: high ACh levels promote encoding by enhancing synaptic plasticity while concurrently suppressing retrieval-related networks; low ACh levels favor retrieval, suppressing external inputs and synaptic potentiation. The primary source of ACh in the hippocampus, the medial septum/diagonal band of Broca, is also a key determinant of hippocampal theta: these two aspects could therefore be integrated, with ACh and theta fluctuations modulating encoding and retrieval phases. Here, we present a computational model based on neural masses, simulating the possible role of ACh on hippocampal function. A first set of simulations was performed assuming that ACh's dynamics are comparable to those of theta. Simulations support the hypothesis that ACh can orchestrate encoding and retrieval at different phases of the theta cycle, but they require an ACh time constant of the order of a few milliseconds, which is much faster than that currently measured. A second set of simulations considers the effect of a slower ACh time scale. Moreover, the network isolated from the environment with constant low ACh levels, spontaneously retrieves stored information, offering early insights into the hippocampal role during states such as imagination, rumination, and slow-wave sleep. Finally, sensitive analysis of model parameters may elucidate the pathophysiology of mnemonic disorders characterized by cholinergic dysfunction, like dementia and amnesia.

1. Introduction

To properly guide our daily behavior, the brain must continuously encode new information while simultaneously avoiding disruptive interference from the past (Epp et al., 2016). The hippocampus plays a pivotal role in the formation of declarative episodic memories, i.e., the recollection of past experiences related to specific times and places (Eichenbaum et al., 1999).

Brain rhythms, the result of the synchronized activity of large neuronal populations, have emerged as a crucial component of many cognitive functions (Buzsáki and Draguhn, 2004). Among the various oscillatory phenomena in the hippocampus, the coupling between theta (4–8 Hz) and gamma (>30 Hz) bands plays a key role (Belluscio et al., 2012; Colgin, 2015; Mysin and Shubina, 2022). First predicted in the 1950s (Miller, 1956), this mechanism gradually gained notoriety in the scientific world following the seminal work of O'Keefe and Dostrovsky, which led to the discovery of place cells in free-moving rats (O'Keefe and Dostrovsky, 1971). Today, the theta-gamma code is widely considered a crucial mechanism the brain uses to organize multiple items in memory (Ursino and Pirazzini, 2024). Theta-gamma coupling is particularly evident during waking under active exploration conditions, such as maze navigation in rodents or when a subject is paying attention to events in the surrounding environment (Tort et al., 2009). Interestingly, the degree of coupling has also been positively correlated with improved memory performance, movement speed, and respiratory rate (Colgin, 2015; Tamura et al., 2017; Hammer et al., 2021). However, the theta rhythm disappears or reduces its frequency in other waking conditions, such as mind-wandering or rumination, when the subject is not focused on the external environment, and also during slow-wave sleep (SWS) stages (Mysin and Shubina, 2022). Still, some data suggest that theta-gamma entrainment also plays a role during rapid eye movement (REM) sleep, although more controversial opinions can be found on this

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point (Ursino and Pirazzini, 2024; Scarpelli et al., 2019).

Although isolated hippocampal circuits can autonomously generate a theta rhythm (Hammer et al., 2021), hippocampal theta results from a combination of multiple independent oscillators (Buzsáki, 2002). The medial septum and the vertical limb of the diagonal band of Broca (MSDB) play a key role in this rhythmogenesis through glutamatergic, GABAergic, and cholinergic projections (Robinson et al., 2016). The hippocampus and the MSDB are reciprocally interconnected via the fimbria and dorsal fornix, forming a single continuous anatomical structure (Teles-Grilo Ruivo and Mellor, 2013). The critical role of MSDB in modulating hippocampal oscillatory properties is supported by studies performing targeted lesions or pharmacological inactivation (Mitchell et al., 1982; Mizumori et al., 1990), which led to the disruption of hippocampal theta.

An influential hypothesis is that the septohippocampal interaction and the consequent theta-gamma code play a role in the memorization of episodes in our autobiographical memory (Nyhus and Curran, 2010). According to this idea, cell assemblies that represent individual items fire in synchronism in the gamma band, while their phase within the theta period defines the sequential order of the items (Lisman and Jensen, 2013). Of course, the realization of such a sequential memory requires two distinct mechanisms: an encoding one through which items are memorized within the hippocampus synapses, and a retrieval one which exploits already formed synapses to recover previously stored items starting from an external cue. The hippocampus supports these processes via a trisynaptic circuit, which involves three main synaptic connections between four major regions. This circuit comprises projections from the entorhinal cortex (EC) to the dentate gyrus (DG) via the perforant path, from the DG to the CA3 nucleus via mossy fibers, and from the CA3 to the CA1 nucleus via Schaffer collaterals. Within this circuit, granule cells in the DG are thought to be involved in pattern orthogonalization, preventing interference, while the CA3 subfield exhibits a dense network of recurrent collateral connections, which has inspired a series of influential theories regarding CA3's role in supporting multiple mnemonic processes. In particular, the retrieval of stored patterns in the hippocampus is based on pattern completion in the CA3 nucleus, following attractor dynamics driven by Hebbian auto-associative mechanisms (Rennó-Costa et al., 2014).

Within this scenario, a critical aspect is that the encoding of novel information necessitates inhibition of the pattern completion mechanism to prevent past associations from interfering with new ones. Some authors suggested that acetylcholine (ACh), an essential neurotransmitter in the mammalian central nervous system, can be implicated in learning and memory (Levin and Simon, 1998; Gold, 2003) by facilitating synaptic plasticity in response to novel information (Hasselmo, 2006; Drever et al., 2011). In particular, early models (Hasselmo, 1999; Hasselmo and Fehlau, 2001) propose a crucial role for ACh in managing the encoding and retrieval phases in the hippocampus: ACh may suppress the CA3-related activity in response to novel information while still allowing input from the EC to the hippocampus and long-term potentiation (LTP) mechanisms (essential for encoding).

Notably, besides being involved in theta rhythm generation, the septohippocampal pathway also constitutes the primary source of ACh in the hippocampus (Dutar et al., 1995). It is thus possible that these two mechanisms - theta rhythm generation and ACh release by the MSDB, with consequent modulatory effects on synaptic dynamics - are strictly related to supporting memory processes. Consequently, recent models (Hasselmo, 2006; Hasselmo et al., 2002; Kunec et al., 2005; Cutsuridis et al., 2010; Decker and Duncan, 2020) suggested that the hippocampal theta phase could not be only useful to encode different items but also dynamically separate encoding and retrieval by exploiting ACh release: encoding would preferentially occur during the troughs of CA3 pyramidal theta, while retrieval during its peaks.

Recent findings (Rizzuto et al., 2006; Lever et al., 2010; Jezek et al., 2011; Siegle and Wilson, 2014; Kay et al., 2020; Rayan et al., 2022; Kerrén et al., 2022; Saint Amour di Chanaz et al., 2023) support these latter models, showing that during storage or recovery of information, as well as during exposure to novel versus familiar environments, hippocampal pyramidal cells fire at distinct phases of the theta rhythm.

This interpretation, however, implies that ACh operates on a timescale comparable to theta oscillations, an argument that remains controversial since, according to present data, the minimum timescale for ACh dynamics seems appreciably slower than the theta one (Teles-Grilo Ruivo and Mellor, 2013). However, the study of cholinergic dynamics is challenging. The major obstacle to understanding the dynamics of the cholinergic system has been the lack of adequate methodological approaches to map the spatiotemporal pattern of ACh in real-time with optimal sensitivity (Xia et al., 2021). Only the recent development of new optogenetic techniques (Jing et al., 2020) has enabled the first measurements of sub-second ACh fluctuations in vivo in mice (Lohani et al., 2022; Krok et al., 2023). Nevertheless, despite these latest advances, reports on the measurement of real-time ACh dynamics are still scarce (Mineur and Picciotto, 2023).

This body of evidence underscores that our understanding of the role of cholinergic modulation and theta rhythm in episodic memory is still limited. Several questions persist, such as (Teles-Grilo Ruivo and Mellor, 2013): *i)* which firing patterns of MSDB cholinergic neurons could optimally coordinate encoding and retrieval? *ii)* what are the hippocampal ACh concentrations, and which spatiotemporal dynamics could facilitate these mechanisms' implementation? *iii)* how could the dependence of synaptic strength and plasticity on ACh prevent interference during encoding?

In this scenario, computational modeling emerges as a powerful tool, providing theoretical frameworks that can complement experimental data, formulate hypotheses in rigorous quantitative terms, guide future investigation, and provide deeper insights into how neural mechanisms can support cognitive phenomena.

Recently, we developed a neurocomputational model of episodic memory based on neural masses to simulate the encoding and retrieval of temporal information in the hippocampus by exploiting theta-gamma coupling (Pirazzini and Ursino, 2024). Although it considers important physiological aspects, such as external modulation of hippocampal theta rhythm, this model lacks a mechanism to dynamically manage encoding and retrieval.

To address this limitation, a new version of the model is presented here. We implement the synthesis and release of ACh by MSDB and its subsequent binding to hippocampal receptors. Following the previous insights, we simulate ACh's ability to modulate hippocampal connections and LTP mechanisms selectively. The model integrates and synthesizes experimental results (Kay et al., 2020; Saint Amour di Chanaz et al., 2023; Douchamps et al., 2013; Kerrén et al., 2018; Robinson et al., 2023) while predicting unexplored scenarios. In the first set of simulations, we introduce the provocative hypothesis that ACh operates at finer timescales than those previously considered in the literature (Hasselmo and Fehlau, 2001), i.e., a time scale comparable with the theta one. We test the possibility that under this hypothesis, encoding and retrieval robustly occur at different theta phases. In the last part, we test the less challenging hypothesis that ACh dynamics occur at a timescale lower than the theta one and discuss the consequent implications. A subsequent sensitivity analysis provides testable predictions that can be experimentally validated. Preliminary results indicate potential relevance for understanding memory disorders such as retrograde and anterograde amnesia, which are associated with deficits in cholinergic synthesis and transmission (Mineur and Picciotto, 2023; Cummings et al., 2019).

Finally, it is well-established that, during offline or resting states, the hippocampus spontaneously reproduces previously stored information (Ólafsdóttir et al., 2018). This phenomenon, known as replay, occurs prominently during the slow-wave phases of sleep, playing a crucial role in long-term memory consolidation (Rasch and Born, 2013). During SWS, cholinergic activity reaches minimum levels, facilitating the retrieval of previously stored memories and the continuous transfer of

information to the cortex (Platt and Riedel, 2011). We show that the model can simulate this process by suppressing ACh synthesis in the MSDB.

In the present work, we focus solely on the role of the neurotransmitter acetylcholine. The contribution of other neurotransmitters (such as dopamine or norepinephrine) and neurotrophins (like the brain-derived neurotrophic factor (BDNF)) to hippocampal functioning (Hörtnagl et al., 1991) may be the subject of upcoming investigation. Additionally, in the future, the model can be integrated with both subcortical and cortical modules to realize a more comprehensive network of long-term memory consolidation and episodic-semantic integration.

2. Methods

The network is a modification of a previous model developed by the authors (Pirazzini and Ursino, 2024). The novelties introduced will be described below; for all other details, please refer to the previous article (and "Supplementary Materials").

2.1. The neural mass Units

Each computational Unit in the network is described through a neural mass model, consisting of the feedback arrangement of excitatory and inhibitory populations, able to produce a brain rhythm when stimulated by an external input. Depending on the particular working conditions (see below), each Unit can receive this input either from the "outside" (i.e. from layers not explicitly present in the model, simulating the environment or other brain regions) and/or from excitatory and inhibitory synapses from other Units. Moreover, each Unit receives a random white noise of assigned standard deviation. Two different types of Units are used to simulate a gamma rhythm (*Gamma Unit*, ~40 Hz, located in the medial prefrontal cortex (mPFC), the CA3 and CA1 nuclei) or a theta rhythm (*Theta Unit*, ~4 Hz, represented by a single Unit in the MSDB). Parameters in the two Units are accordingly different ("Supplementary Materials").

The *Gamma Unit* includes four populations (pyramidal neurons, excitatory interneurons, and inhibitory interneurons with slow and fast dynamics), as in previous works by the authors (Fig. 1, *left panel)*) (Ursino et al., 2010 Sep 1).

The *Theta Unit* includes a fifth population, representing the activity of a large ensemble of cholinergic neurons (Fig. 1, *right panel)*). In agreement with recent studies, cholinergic activity oscillates in the theta range (Brazhnik and Fox, 1999; Lee et al., 2005; Zhang et al., 2010; Mamad et al., 2015) and in antiphase with respect to other populations in the MSDB (Ma et al., 2020; Dragoi et al., 1999). MSDB pyramidal neurons provide significant excitatory inputs to most local fast inhibitory interneurons, synchronizing them in theta rhythm (Robinson et al., 2016; Robinson et al., 2023; Manseau et al., 2005; Müller and Remy, 2018; Colom et al., 2005; Huh et al., 2010; Leão et al., 2015). The latter, in turn, rapidly inhibits cholinergic neurons through strong inhibitory inputs (Rasch and Born, 2013).

Connections among different Units - Units in the model can be connected through three types of synapses, each emerging from the pyramidal populations of the pre-synaptic Unit: *i)* glutamatergic synapses directed to pyramidal neurons in the postsynaptic Unit (excitatory connection); *ii)* glutamatergic synapses targeting the fast inhibitory interneurons of the postsynaptic Unit (bisynaptic inhibitory connection); *iii)* inhibitory synapses similar to type *ii),* but with much faster dynamics. These connections play distinct roles in the model: type *i)* connections are used to retrieve features of an episode, to recall the following episode within a sequence, or to propagate theta rhythm from the MSDB to the hippocampus, type *ii)* connections help synchronize features within the same episode, and type *iii)* connections allow desynchronization of features belonging to different episodes (Pirazzini and Ursino, 2024) ("Supplementary Materials").

2.2. Architecture of the model

The complete network comprises three different layers (Fig. 2) that simulate the activity of the mPFC and the hippocampal CA3 and CA1 nuclei. Each layer comprises 75 identical Units oscillating in the gamma band; each Unit codes for a different feature, replicated identically in the mPFC, CA3, and CA1. A further external Unit simulates the theta activity of the MSDB.

Each Unit in a layer receives an excitatory glutamatergic synapse from the corresponding Unit in the previous layer; hence, all Units encoding the same feature are connected according to a feedforward schema. This feedforward pathway reproduces the well-known traditional unidirectional organization of the hippocampus, from the Dentate Gyrus to CA3 to CA1 and the subiculum (Swanson et al., 1978). In addition, all Units in the CA3 nucleus receive an excitatory synapse from the Unit simulating the MSDB activity (Goutagny et al., 2009; Brazhnik and Fox, 1999; Lee et al., 2005; Manseau et al., 2005; Disney and Higley,

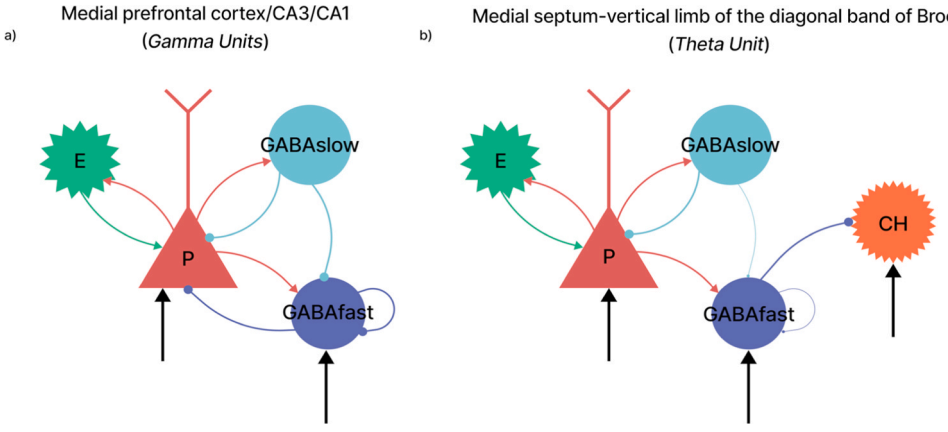

Fig. 1. *The neural mass Units.* Diagrams of the models used to simulate the dynamics of each Unit in the different areas of the network. In all the panels, red and green arrowed lines indicate glutamatergic excitatory synapses (from pyramidal and excitatory populations, respectively), blue circled lines indicate GABAergic fast inhibitory synapses and light blue circled lines indicate GABAergic slow inhibitory synapses. Thick black arrows are used to indicate different inputs, which can represent either glutamatergic synapses from other Units or the external environment, or spontaneous activation. *Left panel)* A well-established neural mass model, consisting of four different neuronal populations (Ursino et al., 2010), is used to simulate the activity of each Unit in the CA3 and CA1 nuclei and the mPFC, oscillating with a gamma rhythm ("*Gamma Units*"). *Right panel)* To simulate the MSDB, the previous model is enriched by introducing a fifth population, simulating the activity of cholinergic neurons. The parameters are different for realizing theta rhythm ("*Theta Unit*").

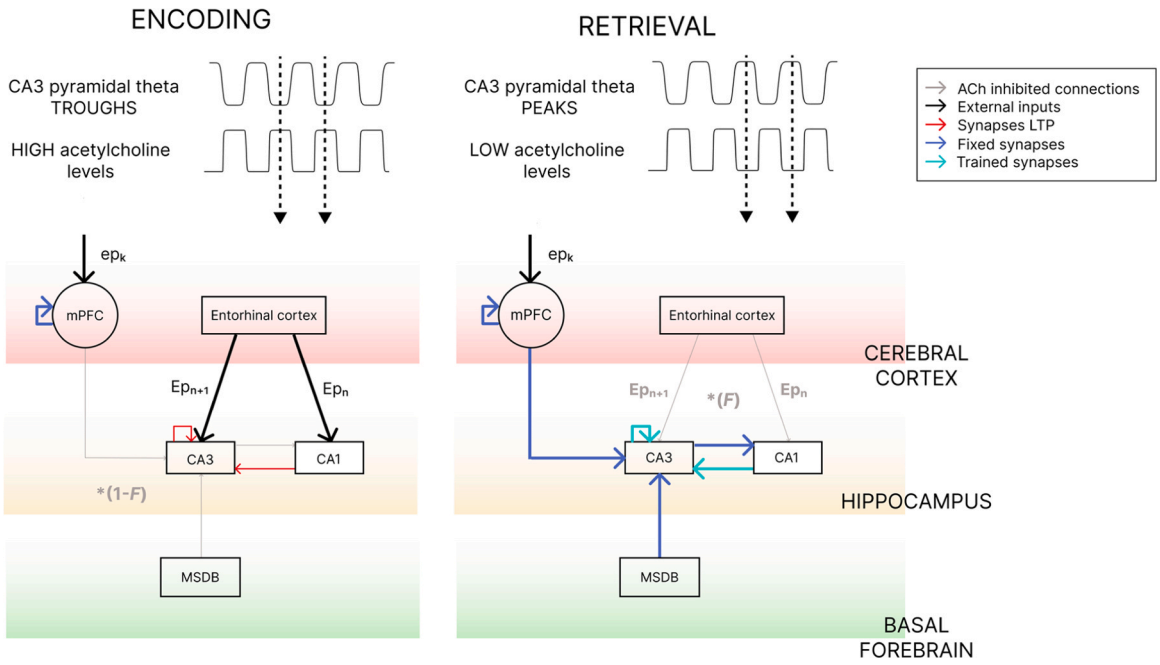

Fig. 2. *Network architecture and operation.* In all the panels, black boxes are used to indicate the four layers implemented in the model (medial prefrontal cortex, medial septum-vertical limb of the diagonal band of Broca, and the CA3 and CA1 nuclei, plus the entorhinal cortex (included in the figure for a better understanding). Red arrows indicate synapses subject to LTP, light blue arrows enhanced synapses after LTP, blue arrows fixed synapses, grey arrows synapses inhibited by high ACh levels (*left panel*) or by low ACh levels (*right panel*), and black arrows the input synapses coming from the external world (areas not implemented in the model, like the entorhinal cortex or sensory areas). Ep_n and Ep_{n+1} represent two consecutive episodes of the same sequence; ep_k is a partial sub-set of a generic episode. Please note that this figure illustrates the scenario of very fast acetylcholine dynamics. *Left panel)* During the troughs of the CA3 pyramidal theta, when the fraction of ACh bound to the receptor sites (F) is high, the network is able to encode new sequences. This is accomplished by inputs directly coming from the EC and by LTP mechanisms involving synapses within CA3 and from CA1 to CA3. *Right panel)* During CA3 pyramidal theta peaks, when the fraction of ACh bound to the receptor sites (F) is minimal, the network retrieves previously learned sequences. This occurs due to incoming synapses from the mPFC (which plays the role of working memory) and the high level of synapse transmission within CA3 and between CA1 and CA3.

2020), thus inheriting the theta rhythm. These synapses are fixed and, therefore, not subject to LTP.

Moreover, Units in CA3 receive synapses of type *i)*, *ii)*, and *iii)* from other units in CA3, implementing an auto-associative network. Auto-associative plastic connections are well-documented in CA3 and are at the basis of most auto-associative models (Guzman et al., 2016). An original aspect of our schema is that plastic synapses of type *i)* also originate from CA1 to CA3, implementing a hetero-associative network. The presence of feedback non-canonical connections, directed in the opposite direction compared with the traditional ones, has been documented in recent studies (Lin et al., 2021; Sun et al., 2018; Xu et al., 2016). All these synapses are plastic and subject to LTP. It is important to note that, unlike the previous model, the present model's synaptic LTP is regulated by the cholinergic pattern. Although we have no data on the plasticity of CA1 to CA3 connections, recent studies document that they are involved in object memory, a task that certainly requires learning (Lin et al., 2021; Treves, 2004).

Medial prefrontal cortex - The purpose of the mPFC layer is to keep information in memory even when external input ceases, protecting it from interference and updating it as soon as new input arrives. Our model realizes this behavior, which is typical of working memories, through an excitatory auto-loop in each pyramidal population, which mimics feedback from the basal ganglia or thalamus (Bolkan et al., 2017; Nir-Cohen et al., 2020; Ursino et al., 2023). Moreover, this self-excitation is reset when a new input is detected. The activity of the mPFC is then transmitted to the "CA3" layer.

CA3 nucleus – All Units of this layer are typically inhibited but receive rhythmic disinhibition from the pyramidal neurons of the "MSDB" layer (implementing the theta-gamma coupling), or are excited up to saturation by external inputs from the EC. Moreover, when a particular sequence is provided as input (see "*Simulations of the network*" below), plastic synapses (excitatory, inhibitory, and desynchronizing ones) between its different Units are modified according to Hebbian and anti-Hebbian mechanisms. Specifically, the encoding of new information occurs when ACh levels are high (see below). As a result, the layer behaves as an auto-associative memory, allowing all features of an episode to be encoded in the synapses and subsequently retrieved (during theta peaks) in a highly synchronized manner. The activity of the CA3 nucleus is then transmitted to the "CA1" layer. The presence of inputs reaching both the CA1 and the CA3 from the EC is well documented (Sun et al., 2018).

CA1 nucleus – Feedback synapses from the "CA1" layer to the previous "CA3" layer implement a hetero-associative memory able to reconstruct ordered sequences of episodes. During encoding, the features of one episode are given to CA1, while the features of the subsequent episode of the same sequence are stimulated in CA3 (both through the EC). Feedback excitatory connections are then potentiated from Units in CA1 to future Units in CA3, allowing restoration of the overall sequence from the reconstruction of the first episode.

Medial septum–vertical limb of the diagonal band of Broca - The "MSDB" layer implements both the generation of hippocampal theta rhythm and the synthesis of ACh by cholinergic neurons (see "*Acetylcholine modeling*" below).

2.3. Synaptic learning

Excitatory and inhibitory synapses of type *i)* and *ii)* within the CA3 and excitatory synapses of type *i)* between CA1 and CA3 are trained with a Hebbian rule. Instead, the desynchronizing synapses of type *iii)* within CA3 are trained with an anti-Hebbian rule. Normalization mechanisms are also implemented to simulate the physiological limitation of neurotransmitters (i.e., the sum of synapses entering a Unit must not

overcome a maximum value). For parsimony's sake, in the present model version, neither a fatigue mechanism for the synapses (which has been observed in the hippocampus (Abrahamsson et al., 2005)) nor any form of long-term depression has been included. More details can be found in previous papers (Ursino et al., 2023; Pirazzini and Ursino, 2024) or in the "Supplementary Materials".

2.4. Acetylcholine modeling

Acetylcholine synthesis - The synthesis of ACh by cholinergic neurons is simulated through first-order dynamics with unit gain:

$$\tau_c \cdot \frac{dACh(t)}{dt} = -ACh(t) + z_c(t)$$

where $ACh(t)$ is the concentration of acetylcholine, τ_c the time constant of Ach release by cholinergic neurons, and $z_c(t)$ the average density of spikes of cholinergic neurons. τ_c was first set to 4 ms and then subject to a sensitivity analysis. This very rapid ACh dynamics was assumed to test the hypothesis that ACh works with a timescale compatible with the peaks and troughs of the theta wave. This is a strong model assumption, still not verified experimentally (see "Discussion" for a critical analysis) but which may be at least partly justified by the absence of sufficiently rapid experimental techniques for detecting spatiotemporal variations in ACh. To strengthen model applicability, however, we also tested its behavior assuming a higher value of the time constant (τ_c=250 ms) more coherent with the values measured through current techniques (Zhang et al., 2010).

A*cetylcholine binding - ACh(t)* determines the fraction of receptor sites for acetylcholine, *F*, occupied at the hippocampal level. This is described through the Hill's equation (Hill, 1910; Goutelle et al., 2008):

$$F(t) = \frac{F_{\max} \cdot ACh(t)^{n_c}}{k_c^{n_c} + ACh(t)^{n_c}}$$

where F_{max} is the maximum achievable effect, n_c is the degree of acetylcholine cooperativity (i.e., the ability of the ligand to interact with receptor sites), and k_c is the dissociation constant (i.e., the ACh concentration for which 50 % of maximum effect is obtained). In the present work, the maximum F_{max} was set to 1. Hence, $F(t)$ corresponds to the fraction of receptor sites occupied at a given instant *t*. The cooperativity coefficient n_c was set equal to 2 while the dissociation constant k_c to 0.7 (Wagner, 1968; Weiss, 1997).

2.5. The role of acetylcholine

We assumed that ACh acts on different portions of the model, allowing dynamic scheduling of encoding and retrieval (Hasselmo, 2006; Hasselmo, 1999; Hasselmo et al., 2002). In particular, the inputs from the EC, the Hebbian learning rates, and all internal synapses in the model are modulated by the fraction of receptors bound by ACh, *F*. Specifically:

i) high ACh levels inhibit internal model synapses, both feedback synapses within CA3, feedback synapses from CA1 to CA3, and feed-forward synapses from mPFC to CA3 and from CA3 to CA1. At the same time, input synapses from the EC and the Hebbian learning rates maintain their normal values. This implements an *encoding phase* during which the inputs are transmitted to CA1 and CA3, and feedback synapses between CA3 and from CA1 to CA3 are potentiated.

ii) low ACh levels inhibit the inputs from the EC and reduce the learning rates for Hebbian LTP. Conversely, all synapses within the model are active, allowing the completion of episodes in CA3 from partial cues via the mPFC and the retrieval of entire sequences via CA1–CA3 hetero association.

To realize this behavior, EC inputs and the learning rate in the model are multiplied by the fraction *F*. In contrast, synapses within the model are multiplied by the quantity (1 – *F*), representing the fraction of receptors unbound by ACh.

2.6. Simulations of the model

All scripts were developed by the authors in the MATLAB environment and are available on GitHub platform. The simulations presented in this manuscript are fully reproducible, and the data to realize each individual Figure requires, on average, less than 10 s to generate on a mid-to-high-end laptop computer.

Encoding - Since we did not implement entorhinal cortex activity, for simplicity's sake, inputs from EC were modelled as sinusoidal functions oscillating at the same frequency as our *Gamma Units*. Each episode of a new sequence is given directly from the EC to the "CA3" and "CA1" layers by stimulating the pyramidal neurons and fast inhibitory interneurons of the corresponding features per 250 ms. This represents the shortest period that allows new episodes always to be learned correctly at whatever time they are provided (considering fast ACh dynamics). To avoid interference, information on the two subsequent episodes was delivered interspersed with a 30 ms interval. Specifically, naming the episodes of a sequence as Ep_1, Ep_2, …, Ep_N, the "CA3" neurons are stimulated by Ep_j and the "CA1" neurons by the preceding episode $Ep_{(j-1)}$ (with j = 1, 2, …, N), where Ep_0 represent a "null" episode, i.e., no features are stimulated. Encoding occurs automatically only when ACh levels are high.

The simulations in Figs. 3, 4, 6, 7, and 8 involved sequences of five orthogonal episodes each (i.e., episodes without any common features). The simulation in Fig. 5 involved two non-orthogonal sequences (sharing the first two episodes) still of five episodes. In all cases, the first and last episodes of each sequence consist of 4 features, the second and fourth of 6 features, and the third of 5 features. Of course, sequences longer than five episodes can be encoded equally well by the network but cannot be evoked within a single theta cycle. More complex episodes can also be correctly encoded and retrieved by the network. We tested the model's ability to accurately handle up to fifty features per episode (unpublished results). The presence of none or just a moderate number of common features between episodes is justifiable by the well-known sparse-coding performed by the DG at the entrance to the hippocampus, making the input patterns quasi-orthogonal (Kesner, 2013).

Retrieval – To implement recovery, one feature of the first episode in a sequence is given as input to the mPFC for a brief period (50 ms). The mPFC maintains this feature in memory until a new input is presented. The auto-associative network in CA3 is able to reconstruct the overall first episode and transmit it to CA1. The hetero-associative synapses from CA1 to CA3 can reconstruct the overall sequence, oscillating with the gamma rhythm during the peaks of the theta cycle (when ACh levels are low).

3. Results

As explained in the "Methods" section above, ACh modulation was introduced to handle the encoding and retrieval phases in the hippocampus within the same simulation. Specifically, during high ACh levels, the CA3 and CA1 nuclei directly receive inputs from the EC and store information from the outside world through LTP mechanisms. In contrast, during low ACh levels, the hippocampal nuclei receive inputs from the medial prefrontal cortex (mPFC), and recovery of stored information occurs via synaptic transmission within the CA3 nucleus and between the CA1 and CA3 nuclei.

In the following, modulation by the ACh is performed under two alternative hypotheses: *i)* ACh dynamics exhibits a few milliseconds time constant; hence, ACh changes follow the variations of the theta rhythm imposed by the MSDB; *ii)* ACh dynamics are appreciably slower than the theta time scale; hence, ACh changes are low-pass filtered compared with the theta rhythm.

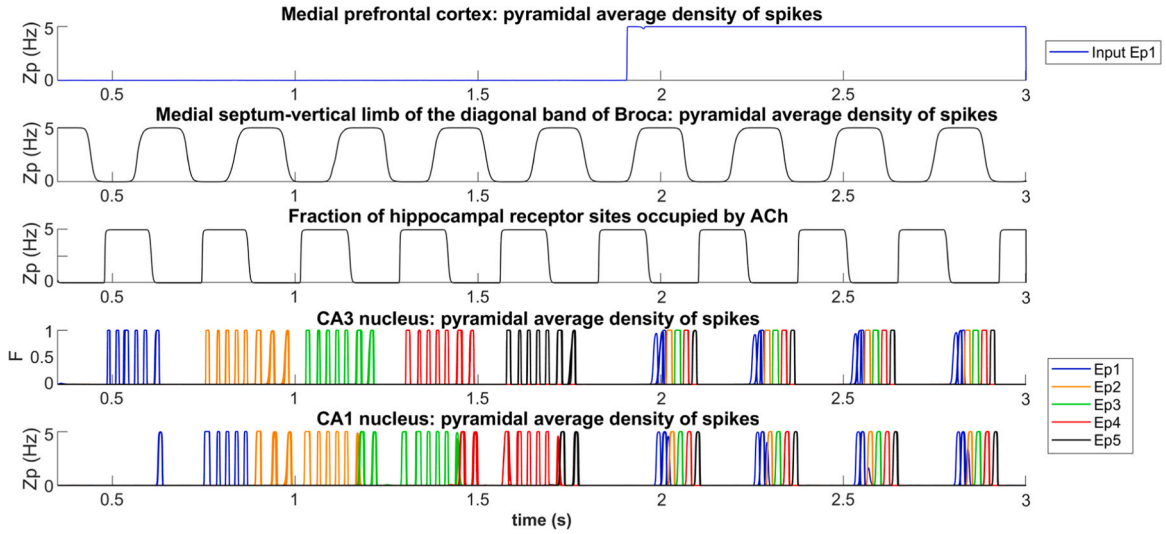

Fig. 3. *Encoding and retrieval of a single sequence with fast ACh dynamics.* Average spike density of the pyramidal neurons of the different layers of the network: "mPFC", "MSDB", "CA3", and "CA1", along with the pattern of ACh bound to the receptor sites in the hippocampus, during the encoding and retrieval of a single five-episode sequence with fast cholinergic dynamics. The features belonging to the episodes are represented through a list of colors (see the legend). In the initial part of the simulation (between 0 and 1.8 s), all five episodes of the sequence are provided in ordered pairs as input from the EC. Only during the peaks of ACh are these inputs transferred to CA1 and CA3, causing LTP of auto-associative synapses in CA3 and hetero-associative synapses from CA1 to CA3. After encoding, a feature of the first episode is provided as input to the mPFC for a short time (50 ms) and maintained in memory (between 1.9 and 3 s), thus testing the retrieval of the learned sequence. During the peaks of pyramidal theta (low levels of ACh), the network can reconstruct the entire sequence starting from a single feature, exploiting the mechanism of theta-gamma coupling.

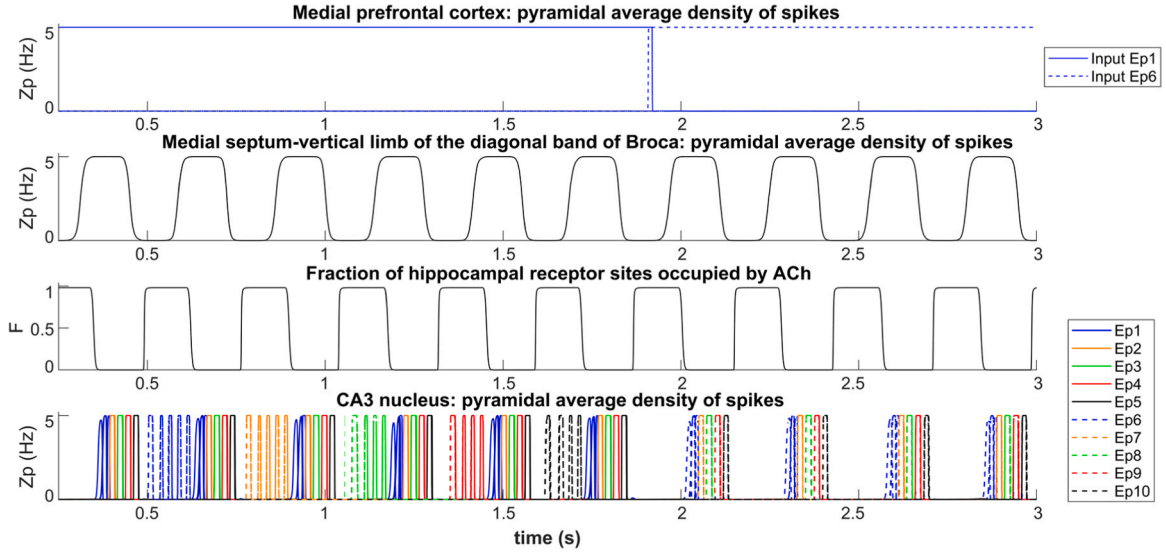

Fig. 4. *Encoding of a new sequence while simultaneously retrieving a previously learned one.* Average spike density of the pyramidal neurons of different layers of the network: "mPFC", "MSDB", "CA3", and of ACh bound to the receptor sites in the hippocampus, during the encoding of a new sequence and the simultaneous retrieval of a previously learned one. During the first part of the simulation, a feature belonging to the first episode of the previously learned sequence (Fig. 3) is provided as input to the mPFC for a short time and maintained in memory (interval 0–1.9 s), allowing the first sequence to be recovered during pyramidal theta peaks (low ACh level). In the meantime, the episodes of a second sequence are provided in pairs as input to the EC. Thanks to fast modulation by ACh, the network is able to encode the second sequence during high ACh levels (theta rhythm troughs), avoiding any interference between the sequences. To test the reliability of the new encoding, a feature belonging to the first episode of the second sequence is provided as input to the mPFC and maintained in memory between the instants 1.9 and 3 s while setting the EC inputs to zero. For simplicity, only the activity of the pyramidal neurons of the CA3 nucleus is shown. The features belonging to the episodes constituting the sequences are represented through a list of colors, while the two sequences are demarcated using different hatching styles (seq1: continuous, seq2: dashed).

3.1. Fast ACh dynamics

3.1.1. Encoding and retrieval of a single sequence

We first tested the encoding and then the retrieval of a single sequence under the hypothesis of fast ACh dynamics (Fig. 3). Both stages (encoding and retrieval) are accomplished within a single simulation. Encoding occurs between the instants 0 and 1.8 s. During this phase, the mPFC receives no input, while the features of each episode are sequentially given as inputs to the CA3 and CA1 nuclei through the EC ("*Methods*"). The retrieval is simulated between the instants 1.9 and 3 s. In this phase, the external inputs to CA3 and CA1 are set to zero, and only one feature belonging to the first episode is given to the mPFC. The

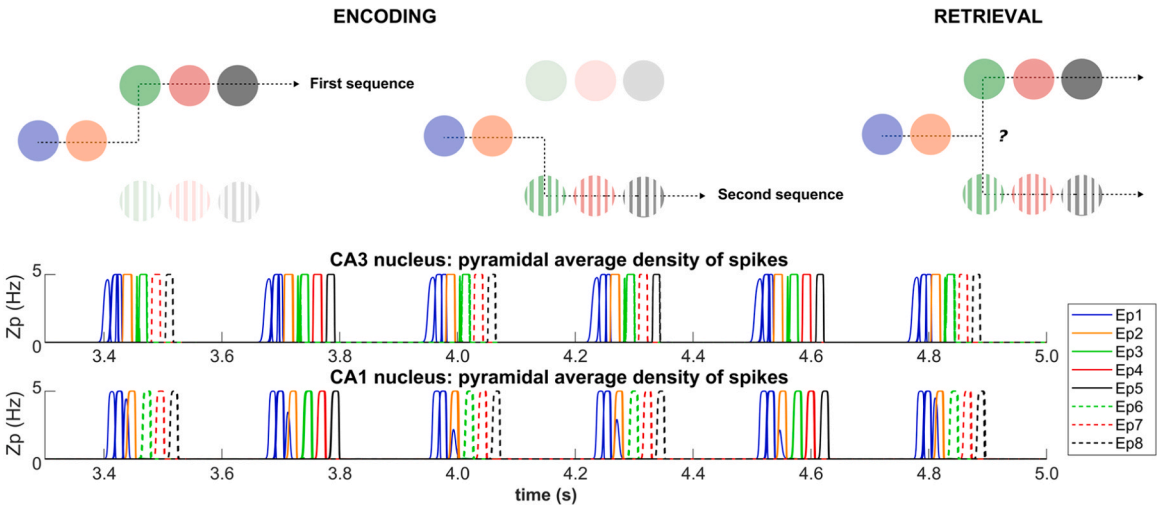

Fig. 5. *Encoding and retrieval of two partially overlapped sequences. Upper panel)* Conceptual representation of the simulations: after encoding a first sequence, a second sequence, which shares the first two episodes with the previous one, is provided as input to the network. Due to the ACh modulation, the two sequences are stored correctly, but a mechanism to discriminate which one to retrieve is lacking. *Lower panel)* Average spike density of the pyramidal neurons of network "CA3" and "CA1" layers, during the retrieval of the overlapped sequences. After learning, a feature of the first episode (common to both sequences) is provided as input to the mPFC and maintained in memory between 3.3 and 5 s. Consequently, the network retrieves the first or the second sequence randomly, depending on the noise. The features belonging to the episodes are represented through a list of colors, while the two sequences are demarcated using different hatching styles (seq1: continuous, seq2 (non-overlapping episodes): dashed).

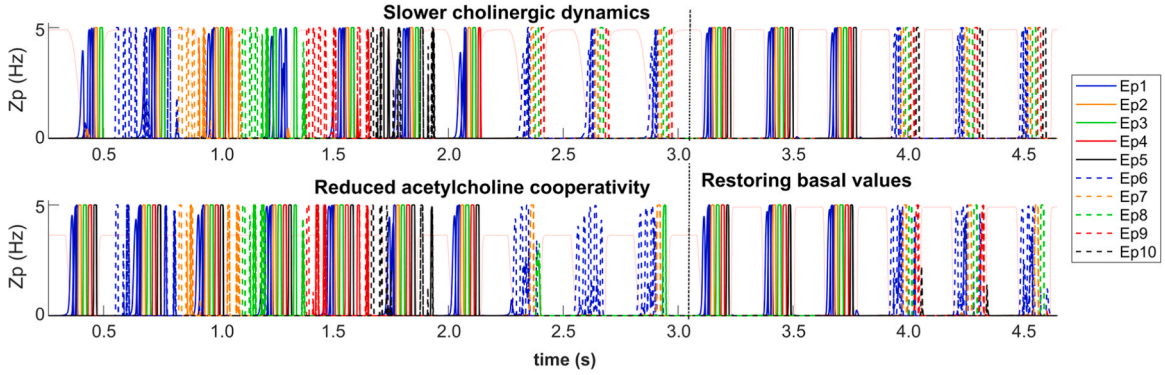

Fig. 6. *Cholinergic dysfunction and mnemonic impairments.* Average spike density of the pyramidal neurons in the "CA3" layer of the network, along with the pattern of ACh (superimposed in transparency), during the encoding of a new sequence while the network retrieves a previously learned one (same simulation as in Fig. 4). Please note that the *F* values are renormalized to fit pyramidal activity (so a value of 5 now corresponds to 100 % occupied sites). In the first part of the simulation, a different parameter inherent in ACh modeling is set to a value that makes the network's behavior fail. Specifically, *in the top row,* an increase in the time constant of cholinergic neurons causes ACh synthesis to be more widely distributed over time. Thus, the period devoted to retrieval decreases, and the network can no longer recall previously learned sequences while maintaining optimal functioning during encoding phases; *bottom row)* by decreasing the degree of ACh cooperativity, the binding sites in the hippocampus are no longer fully occupied during the troughs of theta rhythm. This time, the behavior of the network fails in the opposite manner. Previously learned sequences can still be retrieved, while the encoding of new information is no longer performed correctly (as can be seen by the subsequent failed retrieval). Depending on the parameter alteration involved, the network, therefore, fails in the individual encoding or retrieval phases. In the second part of the simulation, the parameters are restored to the basal value, and the retrieval of the two sequences is tested by providing a feature of their first episode as input to the mPFC (seq1: between 3 and 3.8 s; seq2: between 3.8 and 4.6 s).

simulation suggests that hippocampal CA3 and CA1 nuclei are able first to store and then recover a sequence made up of five episodes of different sizes. Cholinergic modulation, under the assumption of a few milliseconds time constant, allows encoding when ACh levels are high and retrieval when ACh levels are low. During encoding, two consecutive episodes are activated in CA3 and CA1 cells, allowing potentiation of auto-associative synapses within CA3 and of feedback hetero-associative synapses from CA1 to CA3. During retrieval, all features belonging to the same episode fire in a highly synchronized manner in a single gamma period, and all episodes of a sequence are nested within the ON phase of the theta period. We consider an episode recovered if all its features are simultaneously above a threshold (90 % of the maximum activity of pyramidal neurons) for at least 1 ms. In addition, a sequence is considered recognized within a theta cycle if all its episodes are recovered in the correct order.

Our simulations put in evidence that a minimum time is required to encode or retrieve a sequence. Regarding encoding, if this occurs in the presence of an oscillatory theta rhythm (as in Fig. 3 and the following Fig. 4), memorization of a five-episode sequence requires five theta rhythms (since encoding of each episode occurs during the OFF phase of the theta, when ACh levels are high). Hence, the total phase must take at least approximately 1.5 s. This time can be reduced if, as will be discussed in the subsequent *"Slow ACh dynamics"* section, encoding occurs without a theta rhythm but with a constant high level of ACh. In that case, the five episodes could be compressed within approximately 0.8 s. Regarding retrieval, a five-episode sequence requires five gamma

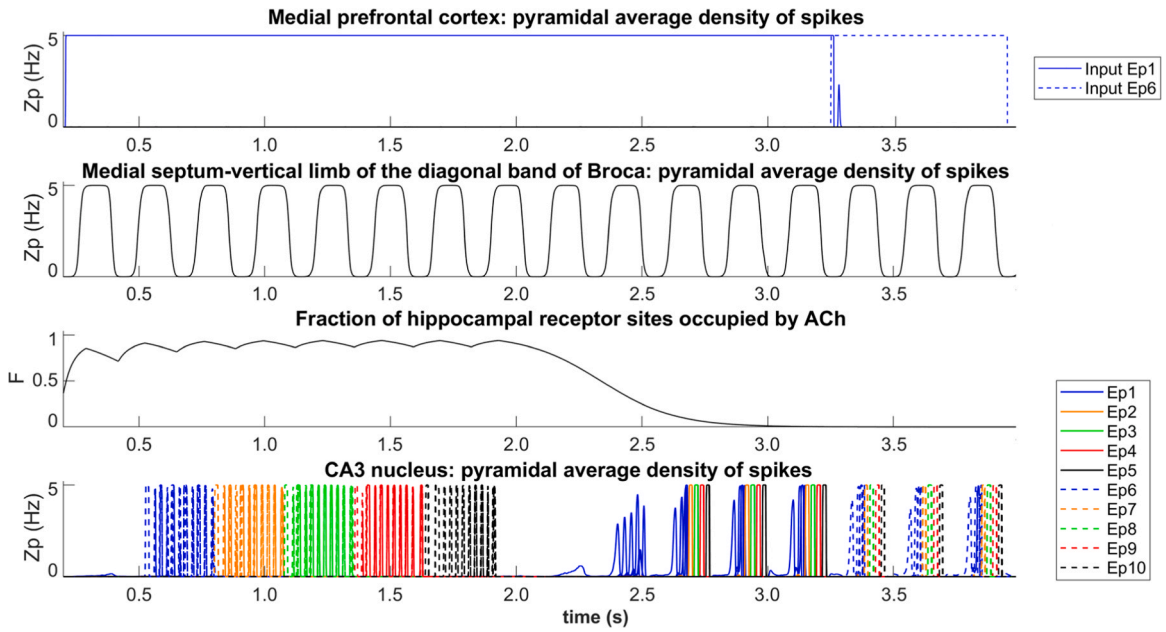

Fig. 7. *Encoding and retrieval of sequences with slow cholinergic dynamics.* The ability of the network to encode a new sequence while a previously learned one is retained in memory, this time with slower cholinergic dynamics. During the first part of the simulation, a feature belonging to the first episode of a previously learned sequence is provided as input to the mPFC for a short time and maintained in memory (interval 0–3.25 s). Due to the new, slower ACh dynamics, the network does not recover the sequence (ACh levels are persistently high). Simultaneously, the episodes of a second sequence are provided in pairs as input to the EC. Thanks to ACh modulation, the network is able to encode the new sequence during the theta valleys, avoiding interferences with the one in memory. When ACh levels return low, the network starts to retrieve the first learned sequence (still present as input to the mPFC) during the theta peaks. Then, to test the reliability of the new encoding, a feature belonging to the first episode of the second sequence is provided as input to the mPFC and maintained in memory (3.25–4 s). The network is also able to retrieve the second sequence correctly.

periods during the ON phase of the theta, hence approximately 0.1–0.12 s, and can be repeated indefinitely until the input stimulus is reset.

Please note that the times of our simulations are consistent with those verified experimentally (Hong et al., 2023; Yaffe et al., 2014; Hasselmo, 2025).

3.1.2. *Encoding of a new sequence while simultaneously retrieving the previously learned one*

Thanks to the fast cholinergic modulation, the network can

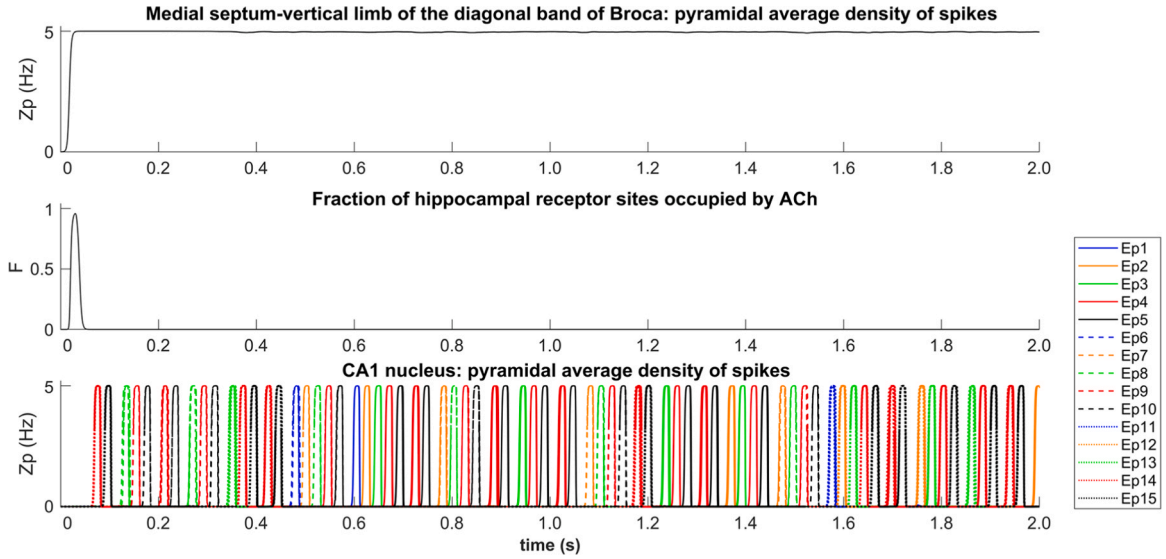

Fig. 8. *Offline memory replay.* Average spike density of pyramidal neurons from the "MSDB" and "CA1" layers of the network, together with the pattern of ACh bound to the hippocampal receptor sites, during a limit case simulation where the MSDB pyramidal neurons saturate their activity. As a result, the cholinergic neurons are constantly inhibited and no longer synthesize ACh. Furthermore, we assumed that the CA3 nucleus is disconnected from the mPFC and receives only high and uniform noise as input. For simplicity, only the activity of the pyramidal neurons of CA1 is shown. The network can spontaneously and randomly recall the three previously learned sequences through the mere presence of high input noise, with the correct order of episodes in each sequence. In addition, the absence of theta rhythm (and consequently of ACh bound to hippocampal receptor sites) allows the network to continuously recall sequences in an uninterrupted retrieval phase without encoding new information. Episodes consisting of a larger number of features (i.e., the second and fourth episodes of each sequence) are more easily recalled due to random noise along the simulation. The three sequences are distinguished through different hatching styles (seq1: continuous, seq2: dashed, seq3: dotted).

simultaneously encode new information and retrieve previously learned associations. New encoding can take place during high levels of ACh, while old information can be retrieved when ACh levels are low (Fig. 4). During this simulation, one feature of the first episode of the previously memorized sequence is maintained in mPFC between the instants 0 and 1.9 s, while ordered episodes of a new sequence are sequentially given as input to the CA3 and CA1 nuclei through the EC. Hence, the network is required to simultaneously encode and retrieve information without generating interference between the two sequences. The behavior is satisfactory. As expected, when F is high, the external inputs allow synapses LTP. When F is low, the previously memorized sequence is entirely recovered. To test the correct encoding, during a subsequent temporal period (between 1.9 and 3 s), one feature of the first episode of the new sequence is given to the mPFC, while the EC inputs to CA3 and CA1 are set at zero. The entire new sequence is retrieved correctly by exploiting the theta-gamma code.

Interestingly, our Units do not have fixed roles as "encoding" or "retrieval" neurons. Rather, they dynamically transit from one functional state to another in response to ongoing inputs and ACh levels.

To better quantify the model's behavior, the simulation in Fig. 4 was repeated twenty times by varying the noise seed and the input times randomly (from both the EC and mPFC). In all cases, the network is able to optimally encode a new sequence and simultaneously retrieve a previously learned one. Quantitative details of this analysis can be found in the Table S1 of the "Supplementary Materials".

3.1.3. Sensitivity analysis

To further stress the robustness of the model, the same test was repeated by progressively increasing the noise standard deviation to the different neuronal populations and the value of some critical parameters related to ACh modeling. These are *i)* the time constant of cholinergic neurons, *ii)* the cooperativity coefficient of ACh, *iii)* the dissociation constant of ACh, *iv)* the connectivity constant between pyramidal neurons and GABAfast (in the MSDB), and *iv)* the connectivity constant between GABAfast interneurons and cholinergic neurons (in the MSDB). The performance of the model was considered satisfactory when, within all the different simulations, the network was still able to retrieve all episodes of the sequences at least 90 % of the time. The results of this analysis are shown in Table 1, reporting for each parameter the minimum and maximum values maintaining satisfactory network operation (along with the basal values used in this work). Results demonstrate that the model is quite robust, and the correct behavior is not crucially dependent on a specific parameter value. However, the connectivity constants in the MSDB must be high enough, and the cholinergic time constant must be small enough to allow the rapid changes required for ACh dynamics. This aspect will be critically discussed in the next section.

Table 1
Sensitivity analysis.

	Minimum value	Basal value	Max value
σ *(input noise standard deviation)*	[0]	5	20
τ_c *(cholinergic time constant)*	[0]	4 ms	9 ms
N_c *(ACh cooperativity coefficient)*	1.3	2	[∞]
K_c *(ACh dissociation constant)*	0.3	0.7	1.4
C_{fp} *(pyramidal→GABAfast connectivity constant)*	70	81	160
C_{cf} *(GABAfast→cholinergic connectivity constant)*	45	61	320

Results of the network robustness and sensitivity analysis performed during a behavior similar to Fig. 4 (encoding of a new sequence while retrieving a previously learned one). The simulations were repeated twenty times after altering the value of some critical parameters related to ACh modeling to estimate the range for which the model continues to perform satisfactorily. Square brackets were used to represent physiological value limits.

3.1.4. Encoding and retrieval of two partially overlapping sequences

Then, we tested the model's behavior with two partially overlapping sequences sharing the first two episodes (Fig. 5, upper panel). Results suggest that thanks to the ACh modulation, the two sequences can be correctly memorized despite the presence of superimposed episodes. After encoding, if one feature of the first episode is given as input to the mPFC (Fig. 5, bottom panel), the network is able to recall both sequences, but they appear randomly depending on noise. Hence, as discussed in the next section, the choice of one or another sequence requires some additional information.

3.1.5. Cholinergic dysfunction and mnemonic impairments

As deficits in ACh dynamics are associated with different memory disorders, the previous simulation (Fig. 4) was repeated by altering two crucial parameters related to ACh modeling. Specifically, we tested the effect of increasing the time constant of cholinergic neurons (to 16 ms) or the effect of a reduction in ACh binding to the receptor sites (decreasing the degree of cooperativity to 0.5). In both cases, the network can no longer recover the second sequence correctly (see Fig. 6). However, the two behaviours are different. In the first case (increased time constant), the period devoted to retrieval decreases since ACh takes a longer time to fall to zero during theta peaks, and the network can no longer recall previously learned sequences while maintaining optimal functioning during the encoding phase. Conversely, in the second case (reduced cooperativity), the binding sites in the CA3 nucleus are no longer fully occupied during the troughs of theta rhythm, and the network loses its encoding capacity. These differences point out two alternative pathological conditions, one related to encoding and the other to the retrieval of memories. Accordingly, if parameters are restored to basal values, older information is always recovered. In contrast, more recent memories are retrieved only if the pathology has not affected the encoding phase (i.e. in the first case, but not in the second). Similar cases obtained by modifying the remaining parameters related to ACh modeling (Table 1) are shown in the "Supplementary Materials" (Figure S1)

Finally, we repeated the simulation in Fig. 5 (partially overlapping sequences) without the ACh mechanism. In this condition, the network cannot encode the two sequences correctly, demonstrating that the presence of cholinergic modulation is essential to avoid proactive interference phenomena ("Supplementary Materials", Figure S2).

3.2. Slow ACh dynamics

The previous simulations were performed assuming very fast ACh dynamics (transients as low as 15–20 ms), a value that has still not been experimentally demonstrated. To strengthen the value of the present model, we also simulated the case of slower ACh dynamics using a time constant as high as 250 ms. In this way, using the same MSDB model as before, fluctuations in ACh concentration now become rather negligible, and the fraction of binding sites occupied by ACh settles at a rather constant level (approximately 0.9, see Fig. 7). This means that only the encoding phase can be performed since the high occupancy level almost completely inhibits internal synapses in the model, which are necessary for retrieval. To allow both encoding and retrieval in separate periods, we need to include a further assumption that was not necessary with the previous simulations: i.e., during retrieval the external input to cholinergic neurons in the MSDB (see Fig. 1) is set to 0 to suppress cholinergic activity and reduce the concentration of ACh and the fraction of occupied receptors to minimal levels.

Fig. 7 shows a simulation in which the external input to cholinergic neurons in the MSDB is maintained at the same values as in the previous simulations during the first 2 s; hence, ACh receptor occupancy is high. During this period, one feature belonging to the first episode of a previously memorized sequence is given as input to the mPFC, while a new sequence is given as input to the CA3 and CA1 nuclei through the EC. The new sequence is encoded correctly without any interference with

the previous one, despite the presence of the mPFC feature input. In fact, no retrieval of the previous sequence occurs. Subsequently, the input to the cholinergic neurons is set to zero (interval 2–3.9 s). Retrieval now occurs without encoding, and the previously memorized sequence is correctly recovered with the theta-gamma code. Interestingly, suppose that the input to the mPFC is replaced from one feature of the old sequence to one feature belonging to the first episode of the new sequence (instant 3.5 s). In that case, the second sequence is correctly recovered instead of the older one, demonstrating that the recent encoding was actually successful.

3.3. Offline memory replay

Finally, we simulated a condition of spontaneous replay, such as mind-wandering or SWS (Fig. 8). We first memorized three orthogonal sequences with a procedure similar to that in Fig. 3. Then, to mimic an offline state, the CA3 nucleus was isolated from the mPFC, thus receiving only high random noise with uniform distribution as input. Furthermore, the MSDB pyramidal neurons were excited to the upper saturation level (Rasch and Born, 2013 Manseau et al., 2005; Müller and Remy, 2018). In this way, the theta rhythm is abolished, and the ACh synthesis is suppressed (as a consequence of the constant inhibition of cholinergic neurons, which become silent) (Robinson et al., 2023; Ma et al., 2020; Vandecasteele et al., 2014). Simulations show that thanks to the presence of high noise input, the network can spontaneously retrieve the previously learned sequences, preserving the correct order of episodes, without encoding new information. Sequences can start from any episode and alternate randomly. A discussion of possible future aspects concerning the role of ACh in rumination states or long-term memory consolidation is given in the next section.

4. Discussion

Competition between overlapping memories represents a significant challenge in mnemonic tasks, often leading to forgetting or distorted memorization. This problem is particularly evident when overlapping information is stored before the appearance of a new learning target. More generally, associative memories based on Hebbian learning require that the encoding timing is separate from retrieval to avoid interference between recovered and stored items. The previous questions gain particular attention in the study of the hippocampus. This crucial brain region plays a pivotal role in the memorization and recovery of recent spatiotemporal autobiographic memories.

An influential model developed in the past decades (Hasselmo, 2006; Hasselmo, 1999) ascribes a critical role to ACh in separating encoding from retrieval, thus avoiding dangerous interference. Several results, in fact, show that high ACh levels enhance afferent inputs from the EC to the hippocampus while concurrently suppressing CA3 recurrent circuits (Hasselmo and Schnell, 1994; Hasselmo and Wyble, 1997). Other studies point out that cholinergic neuromodulation is essential to induce hippocampal LTP (Hangya et al., 2015; Gu et al., 2012). Conversely, low ACh levels reduce LTP and suppress external inputs while preserving a role for feedback synaptic connections essential for the associative retrieval of previously memorized items (Gold, 2003; Hasselmo and Wyble, 1997). Hence, ACh modulation appears as a natural way for biasing hippocampal circuits towards encoding (high ACh levels) or retrieval (low ACh levels), separating their respective timing. Supporting this hypothesis, Douchamps et al. (Douchamps et al., 2013) demonstrated that elevated ACh tones in mice, during exploration of novel environments, shift the encoding-retrieval dynamics toward encoding, and that administration of scopolamine, a cholinergic antagonist, promptly disrupts this shift.

These findings have significant implications, as they could lead to a deeper understanding of learning and memory processes and potential therapeutic interventions.

Within this context, several questions remain unanswered. The primary source of ACh in the hippocampus is cholinergic innervations from the medial septum and the vertical limb of the diagonal band of Broca, with the MSDB also being the primary determinant of hippocampal theta oscillations. A compelling hypothesis integrates these two phenomena, suggesting that encoding and retrieval can be separated by the phase of the ongoing theta wave, under the influence of ACh tones fluctuating within the same period. This assumption, which has been hypothetical for many years, has recently been significantly supported. Various findings in rodents and humans show separate phases for encoding and retrieval within individual theta cycles (Rizzuto et al., 2006; Lever et al., 2010; Jezek et al., 2011; Siegle and Wilson, 2014; Kay et al., 2020; Rayan et al., 2022; Saint Amour di Chanaz et al., 2023). Notably, a recent study by Kerren et al. (Kerrén et al., 2022) confirms that reactivations of targets and competitors occur at different phases of the hippocampal theta rhythm. Building on a previous model by Norman et al. (Norman et al., 2007), the authors suggest that target memories would be strengthened during a high inhibition phase of the theta rhythm. In contrast, competitors would be reactivated during a later low-inhibition phase. Although the authors do not explicitly mention ACh, the simultaneous dependence of the theta rhythm and ACh release on the activity of the MSDB suggests a close relationship between these phenomena.

A fundamental problem, however, concerns the temporal pattern of ACh extracellular levels in the hippocampus. A traditional viewpoint is that extracellular ACh levels exhibit volume transmission with slowly changing dynamics (in the scale of seconds), which seems incompatible with the hypothesis that ACh is modulated with the theta rhythm. Still, all previous results are biased by the absence of tools in past years that were able to measure extracellular ACh with sufficient spatial and temporal resolution due to the low concentrations and rapid turnover of this neurotransmitter (Mineur and Picciotto, 2023). More recent work, based on advanced recording techniques like genetically encoded fluorescent sensors, emphasizes a function for rapid phasic changes in cholinergic signaling (Jing et al., 2020; Lohani et al., 2022; Krok et al., 2023), indicating that the latter can be spatially and temporally more specific (down to milliseconds and micrometers, respectively). However, these findings represent only a preliminary step, and further studies are necessary.

Due to the previous limitations, the precise spatiotemporal profile of ACh release remains uncertain, making it challenging to ascribe a definite role to cholinergic modulation in the hippocampus (see (Disney and Higley, 2020; Sarter and Lustig, 2020) for a discussion on this subject).

In this regard, neurocomputational models can play a crucial role in evaluating whether the different hypotheses are more or less plausible, reaching a deeper insight into the different mechanisms involved, and assessing which aspects of ACh dynamics are compatible with a role in encoding and retrieval.

Here, we developed a neurocomputational model based on neural masses to simulate the encoding and retrieval of temporal information by exploiting theta-gamma coupling and ACh modulation on hippocampal connections and LTP mechanisms. We assumed two different time scales: one comparable to that of theta oscillations (very fast), and another slower than theta (in the order of seconds). In the following, the main structure of the model is first critically analyzed against data in the literature, and then aspects of ACh dynamics will be discussed.

4.1. Model structure

The present model exhibits a simple structure, which, however, reflects our basic knowledge about the mPFC, hippocampus, and MSDB arrangement. First, the main structure of the model reproduces the traditional feedforward pathway in the hippocampus, from the DG to CA3, to CA1, and then to the subiculum. In our model, this pathway transmits information from the mPFC to the first layer, then to the second layer, and finally outside through strong fixed connections.

In addition, the model includes the well-known pathway from the

MSDB to CA3, transmitting the theta rhythm. It is worth noting that, for the sake of simplicity, we neglected possible feedback from CA3 to the MSDB. A question for our model is that the theta rhythm oscillates at 4 Hz, while several papers in the literature refer to a 6–8 Hz rhythm in the hippocampus. However, a 4 Hz oscillation in the hippocampus has also been frequently observed and related to memory encoding and recall (Fujisawa and Buzsáki, 2011; Domanski et al., 2023).

All computational Units in the model include the feedback arrangement of four populations, which reflect previous hippocampal modeling (see for example (Wendling et al., 2002)). Moreover, the MSDB also includes a cholinergic population, according to the literature (see *"Methods"* section for a detailed explanation).

As is the case of most traditional hippocampal models, and experimentally well documented, our structure also includes recurrent connections among CA3 neurons. They involve both excitatory and inhibitory connections and exhibit Hebbian (or anti-Hebbian) plasticity.

Furthermore, the model assumes that the CA3 and CA1 units also receive further external inputs, which do not follow the traditional feedforward pathway. These inputs probably come from the EC via the perforant path and are well documented in the literature (Kesner, 2013; Sun et al., 2018). In particular, it is known that the temporoammonic branch (TA-CA1) of the perforant path mediates memory consolidation (Remondes and Schuman, 2004).

An essential model aspect, partly diverging from the traditional schemas and deserving discussion, concerns the presence of feedback plastic connections from CA1 to CA3, realizing a hetero-associative network able to recover future episodes from previous ones. Although this pathway is not included in traditional hippocampal connectivity studies, recent data obtained with genetically virus-based retrograde tracing show the existence of a significant non-canonical retrograde pathway (Lin et al., 2021; Xu et al., 2016; Sun et al., 2018). Of particular relevance for our model is a recent study by Lin et al. (2021) supporting the presence of non-traditional synapses from the ventral hippocampus CA1 to the dorsal CA3. Moreover, using functional connectivity estimation techniques, Sandler et al. (2015) found a significant causal connection in the sense of "Granger" from CA1 to CA3. It is worth noting that, in our model, these back-projections are not initially present (whereas the feedforward ones are present from the beginning and fixed) but develop only after training to recover specific sequences. This can explain why they are experimentally more elusive than the feedforward ones. Our model assumes that these back-projections are also subject to Hebbian learning. We are not aware of experimental data supporting this point, which thus represents a testable aspect. However, Lin et al. (2021), using genetic inactivation techniques, found that inactivation of backward ventral CA1 to dorsal CA3 projections impairs object learning and memory. We claim this result constitutes indirect support for the idea that these synapses are plastic and involved in learning.

4.2. ACh dynamics

The primary objective of the implemented model was to investigate the possible relationships between MSDB activity, theta oscillations in the hippocampus, and ACh dynamics. The first fundamental testable hypothesis was that ACh levels in the hippocampus fluctuate with a theta frequency and allow the occurrence of encoding and retrieval at different phases of the theta cycle. Subsequently, we relaxed this strict hypothesis assuming that ACh dynamics is appreciably slower than the theta one, and tested encoding and retrieval in this condition.

Hypothesis 1. (fast Ach dynamics) - Results of the present simulations and the sensitivity analysis suggest that the first hypothesis works, provided a few fundamental conditions are verified:

i) high levels of extracellular ACh concentration in the hippocampus dampen internal synapse strength while preserving entorhinal inputs and enhancing LTP, thus favoring encoding. Conversely, low levels of extracellular ACh concentration reduce external inputs and LTP but spares CA3 recurrent activity, thus favoring retrieval. Hence, ACh must modulate network activity and plasticity efficiently and with high temporal precision. Various experimental data support these hypotheses (Hasselmo, 1999; Norman et al., 2007; Sarter and Lustig, 2020).

ii) cholinergic neurons in the MSDB oscillate in antiphase compared with pyramidal neurons in the hippocampus. Their activity is minimal when pyramidal neurons are recovering internal representations and maximal when pyramidal neurons are silent but excitable from the external. Experimental data by Dragoi et al. (1999) and Ma et al. (2020) support this assumption. To obtain this result, however, model synapses within the MSDB must be strong enough to induce fast phasic inhibition and excitation of cholinergic neurons within each theta cycle (see (Robinson et al., 2016; Manseau et al., 2005; Colom et al., 2005; Huh et al., 2010; Leão et al., 2015) for some experimental confirmation);

iii) the temporal dynamics of extracellular ACh release and clearance must be high-speed. Using a value for the ACh cooperative coefficient $N_c = 2$ (Wagner, 1968; Weiss, 1997), the present model requires a time constant of ACh release lower than 8 ms to work properly. This value, however, may be affected by other parameters. For instance, in the present model, we assumed that the entorhinal inputs and the learning rate of LTP are proportional to the fraction of receptors bound by ACh, F, while the strength of hippocampal synapses is proportional to the unbounded fraction $1 - F$. This signifies that F must be either close to 1 or close to 0 to have maximal efficacy in the model. A different dependence on F (for instance, a sharper one, like quadratic or cubic) might be compatible with slower dynamics since even a moderate deviation from the mean value $F = 0.5$ could exert significant effects on synapses.

All previous points can be the target of testing experiments.

In the present work, we primarily evaluated encoding and retrieval considering orthogonal sequences. It is a widely accepted notion that the dentate gyrus performs pattern separation on sensory inputs prior to their storage in CA3 cells (Kesner, 2018). Hence, the hypothesis of orthogonality is not unphysiological. Nevertheless, we deemed it essential to assess network performance also using non-orthogonal sequences sharing an initial part; i.e., starting from the same initial episodes, sequences diverge into two alternative paths (this can be a common scenario in spatial navigation but can also occur in more general life experiences). Results suggest that, in this condition, ACh modulation is essential to allow correct storage of both sequences, avoiding proactive interference. In fact, if the ACh dynamics are impaired, the two sequences cannot be stored autonomously, even if provided in temporally segregated intervals, resulting in confusion between the respective episodes (Robinson et al., 2023). Interestingly, even in the case of correct memorization of the non-orthogonal sequences, the network recovers the first or second sequence casually in response to a cue of the first episode, depending on the superimposed noise. This signifies that additional mechanisms - not implemented in our simulations - must be recruited by the network to determine a single choice, avoiding random fluctuations among alternative possible behaviors.

Hypothesis 2. (slower Ach dynamics) – In the second assumption, by providing the same input as before to the cholinergic neurons, ACh receptor occupancy remains high despite theta fluctuations of cholinergic neuronal activity, due to low-pass filtering, allowing only encoding without retrieval of previously learned information. Hence, this scenario requires the presence of a variable external input to the MSDB cholinergic neurons, which is high during encoding but falls to zero when retrieval starts. Then, ACh remains low for an extended period, permitting the restoration of previous experiences without storing new information. While plausible, this scenario precludes the simultaneous occurrence of encoding and retrieval within the same theta cycle. With the chosen parameter values, we showed that a valid separation between encoding and retrieval requires about 1.5–2 s. In contrast, the previous assumption ("Hypothesis 1" above) allowed the possibility of encoding

new episodes while restoring others already stored, i.e., storage and recovery could occur simultaneously without generating interference.

4.3. Cholinergic dysfunction and mnemonic impairments

Another significant aspect of our work is the possibility of simulating different pathological conditions. Given the implicated role of cholinergic dysfunctions in the etiology of several neurological disorders such as AD, dementia, amnesia, and attentional deficits (Cummings et al., 2019; Schliebs and Arendt, 2011; Mineur and Picciotto, 2021), a deeper understanding of these mechanisms can have relevant future clinical implications. It is worth noting that we tested pathological deficits in ACh only under the first hypothesis, i.e., fast Ach dynamics.

Specifically, assuming impairments in some cholinergic features, we found two different failures for the network, which can be correlated with different pathological conditions. In the first scenario, characterized by a slow ACh temporal descent (obtained by increasing the time constant of cholinergic neurons), the network cannot correctly recall all the previously learned sequences (like in retrograde amnesia) while still maintaining optimal functioning during the encoding phase. Notably, suppose that the correct cholinergic dynamics are subsequently restored (i.e., the correct parameters are restored, for instance, through pharmacological intervention). In that case, the network starts working appropriately again and recovers all previously memorized sequences (both older and newer). Conversely, in the second condition (characterized by impaired ACh binding to hippocampal receptors), previously learned sequences can still be retrieved. In contrast, the encoding of new information is no longer performed correctly (anterograde amnesia). In this case, restoration of normal ACh dynamics enables the encoding of new memories again, but the ability to retrieve memories encoded during the period of dysfunction is still not recovered.

The condition of impaired ACh binding resembles that observed after scopolamine administration, a cholinergic antagonist that selectively impairs encoding rather than retrieval (Douchamps et al., 2013; Rogers and Kesner, 2003; Atri et al., 2004), thus causing anterograde amnesia. Other differences in cholinergic impairments can also be helpful in discriminating between anterograde and retrograde amnesia. A number of studies have shown that cholinergic blockade of the striatum produces anterograde amnesia (Kopelman and Corn, 1988; Kopelman et al., 1999). On the other hand, cholinergic deafferentation of the hippocampus induces retrograde amnesia (Köppen et al., 2016).

4.4. Offline memory replay

A further intriguing result of our work regards the simulation of conditions in which the network is isolated from the environment. Here, we assumed that the MSDB pyramidal neurons are maximally engaged; consequently, the activity of cholinergic neurons is disrupted, ACh levels are minimal, and hippocampal circuits are continuously disinhibited. While this condition shares similarities with the retrieval phase, it is distinguished by the absence of input from the WM layer and the lack of coherent theta oscillations. Due to the absence of any external cue, the network can be activated only by assuming a higher level of noise compared with the one used during the previous encoding and retrieval periods. Now, retrieval occurs randomly, and the recovered sequences prolong their activity for the overall simulation period, causing the appearance of an accidental alternation of the stored sequences, which follow each other casually. These simulations can shed light and provide interesting insights into offline mental processes such as imagination or SWS. Notably, although our model does not consider complex rhythmic phenomena (such as sharp waves and ripples), its prediction captures a key principle: spontaneous replay of previously stored information can occur only if cholinergic activity is interrupted. This is conceptually consistent with recent experimental observations that optogenetic activation of MSDB cholinergic neurons during SWS suppresses CA1 ripple oscillations, a hallmark of offline memory consolidation (Ma et al., 2020; Vandecasteele et al., 2014; Jarzebowski et al., 2021).

Moreover, it is known that excessive retrieval of autobiographical memories, especially negative ones, may contribute to rumination, a cognitive pattern associated with anxiety and depression. Several studies revealed altered activity in the hippocampus and medial prefrontal cortex in these conditions (Belleau et al., 2019). If such a pattern of excessive memory retrieval becomes persistent, it can compromise cognitive flexibility and strengthen negative memory networks. This can potentially maintain or exacerbate depressive symptoms. As discussed above, our model is able to simulate a condition of sustained retrieval by imposing low ACh levels. However, the current version does not implement any plasticity mechanism during recovery, since low ACh levels jeopardize synapse long-term potentiation in the hippocampus.

In order to account for these effects on memory, a future improved model version should simulate the consolidation of long-term memory in the neocortex during retrieval phases, i.e., the transmission of memorized patterns from the hippocampus to the cortex and their subsequent generalization (see *"Model limitations and future research"* below). The inclusion of these consolidation mechanisms may allow the study of cognitive processes such as slow-wave sleep, as well as the link between rumination, memory and mood disorders.

4.5. Testable predictions

From a modeling perspective, it is valuable to propose testable predictions and future experimental directions, which can help verify the different hypotheses. A first prediction is that the activity of MSDB cholinergic neurons oscillates with a theta rhythm during conditions requiring encoding of external information while remaining quiescent during states like mind-wandering or SWS, when the network is functionally isolated. Another fundamental testable prediction concerns the temporal dynamics of extracellular ACh concentration and its effect on synaptic behavior. The total effect (including ACh synthesis, release, receptor binding, and effect on synapses) must occur with a time constant equal to or less than approximately 8–10 ms. Given that the peaks and troughs of the theta wave last approximately 125 ms, the ACh overall transient periods (rising or falling times) must be below 15–20 ms to allow sufficient time to remain for the encoding and retrieval of 4–5 different gamma waves within half a theta period. As described above, to date, this hypothesis has not been subjected to direct experimental validation due to a lack of tools able to detect ACh concentration fluctuations with sufficient spatiotemporal resolution. However, the first indirect support for this model's predictions could be obtained by experimentally testing the capacity to encode new information while simultaneously recovering a previously stored sequence.

Importantly, if the prediction of very fast ACh dynamics were not verified, the model could still work. However, an external input is required to shift between encoding and retrieval; in this case, the two phases should be separated by a few seconds.

Additional testable predictions may concern alterations in memory storage and recall following dysfunctions of the cholinergic system and of the MSDB function, compared with model simulations. Such comparisons can concern behavioral alterations (e.g., difficulty either in storing novel information or in recovering old information), as well as perturbations in theta-gamma cross-frequency coupling.

4.6. Model limitations and future research

Finally, we wish to discuss the main model limitations and lines for future research.

In the present model, we forced inputs from the EC during the encoding phase. While several data (Colgin, 2015; Bieri et al., 2014) suggest that hippocampal circuits oscillate with a slower gamma (40 Hz, similar to that used in the present work) during retrieval, it remains debated whether the same frequency or even a much faster gamma (>60 Hz), is engaged during encoding (Yamamoto et al., 2014). Future

simulations could investigate these dynamics, implementing an information flow between the EC and the hippocampus synchronized with a higher gamma frequency (Colgin et al., 2009).

Some models (Hasselmo et al., 2002) assume that long-term depression of previously stored representations occurs concomitantly with the encoding of novel information. Additionally, synapses in the hippocampus are known to be subject to a fatigue phenomenon when they are stimulated repeatedly (Zhang and Huang, 2024). We have not included these mechanisms in the present model version to reduce the number of assumptions on synapse plasticity. Still, we show that the model can work correctly. Future simulations could incorporate a synaptic depletion component to mimic synaptic fatigue (see, for instance, Singh et al., 2022). We claim this addition would improve the network capacity to retrieve sequences consisting of non-orthogonal episodes, without generating disruptive interference. In particular, if synapses in a previous episode exhibit a fatigue phenomenon, it will be easier to recover future episodes, avoiding the re-occurrence of the previous interfering features.

Furthermore, several authors pose CA1 as a match/mismatch detector, which evaluates the predictions provided by CA3 through a dynamic interaction between encoding and retrieval (McClelland and Goddard, 1996; Lisman, 1999). Future work may incorporate these mechanisms, in addition to the current one, and explore the role of theta-gamma coupling and neuromodulators in predictive processing and mnemonic updating (Köster, 2024).

Moreover, the ACh shifting between the retrieval and encoding phases is not the only mechanism involved in the spontaneous recovery of stored memories. Changes in other neurotransmitters' levels, such as dopamine or norepinephrine, also play an important role. For example, dopamine is involved in the retrieval of reward-related memories (Kesner et al., 2022), while norepinephrine regulates levels of attention, thereby facilitating memory access processes (Bacon et al., 2020). Additionally, brain-derived neurotrophic factor (BDNF) is a key modulator of hippocampal synaptic plasticity. It facilitates LTP via several mechanisms, such as enhancing NMDA receptor function or promoting dendritic growth (Cunha et al., 2010). Furthermore, several studies have found a correlation between abnormal levels of BDNF and memory-related disorders (such as Alzheimer's) (Tapia-Arancibia et al., 2008). Due to its role in synaptic plasticity, it is possible to assume that the learning factor in our model is affected by BDNF levels, resulting in impaired plasticity, insufficient learning during the encoding phases, and depressed memory responses when BDNF is low. Future versions of the model could consider the role of these additional biological mechanisms in hippocampal functioning.

Finally, although the existence of hippocampal feedback projections to the MSDB is well-established (Manseau et al., 2008), the functional properties of these connections remain poorly characterized and lie beyond the scope of our current model, and therefore have not been implemented.

Future extensions concern how the present model can be linked with other brain regions, for instance, the parieto-temporo-occipital cortex, the thalamus, or the amygdala. A first extended network (hippocampus+neocortex) can be implemented to study how patterns stored in the hippocampus are transferred to the cortex and how these patterns can be consolidated or generalized during sleep or rumination periods. Such an extended model may provide a computational framework for studying the interactions between episodic and semantic memory. In particular, recently we proposed a semantic memory model that oscillates with a gamma rhythm (Ursino and Pirazzini, 2024; Ursino et al., 2009). This model could be integrated with the present one to study a generalization of autobiographical memory into more abstract knowledge. The relationship between the hippocampus and the thalamus can be of value in reaching a better description of sleep-associated phenomena (such as sharp wave ripples and spindles (Cona et al., 2014)). Finally, the relationship between the hippocampus and the amygdala may elucidate the influence of emotions in episodic memory formation. Converging evidence suggests that theta oscillations are enhanced in the amygdala and cingulate cortex during emotional learning (especially aversive (Pirazzini et al., 2023)), with this activity subsequently transmitted to hippocampal circuits (Chaaya et al., 2018)).

Data and code availability

The datasets generated during the current study will be available after acceptance in the GitHub repository: https://github.com/gpirazzini.

Declarations and statements

None.

CRediT authorship contribution statement

Gabriele Pirazzini: Writing – review & editing, Writing – original draft, Visualization, Software, Methodology, Formal analysis, Data curation. **Mauro Ursino:** Writing – review & editing, Writing – original draft, Supervision, Software, Methodology, Funding acquisition, Formal analysis, Conceptualization.

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Declaration of Competing Interest

The authors have no competing interests to declare.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.brainresbull.2025.111465.

Data availability

After acceptance, data and codes will be available on the GitHub platform

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