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Interneuron switching on and off across memory rhythms.

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Main Text

Rhythms in the hippocampus, such as theta and ripple oscillations, are expressed at different stages of memory formation, including encoding (theta-related) and retrieval/ consolidation (ripple-related) of memory contents (Buzsáki, 1989; Girardeau et al., 2009). Both theta and ripple oscillations, are the result of precisely spatiotemporally organized interactions between the excitatory and inhibitory neurons that make up the neural network (Buzsáki, 2015). In addition to several subclasses of pyramidal neurons, the hippocampal CA1 network is composed of more than 20 types of GABAergic interneurons (Klausberger and Somogyi, 2008). This diversity of inhibitory neurons has raised fundamental questions about their contribution to hippocampal oscillations and function: How are these different interneurons involved in oscillogenesis? How is their activity linked to the functional states of the hippocampus? And in what ways do they contribute to computations in the hippocampus? A growing body of literature suggests that some types of interneurons (including cholecystokinin (CCK)-expressing basket cells and vasoactive intestinal polypeptide (VIP)-expressing cells) remain virtually silent during ripples (Dudok et al., 2021; Francavilla et al.,

2018). In contrast, some parvalbumin (PV) expressing subclasses, including the fast-spiking basket cells, are highly active in both rhythms, with spike rates as high as ~130/second during ripples (Varga et al., 2014). In this issue of Neuron (Szabo et al., 2022), shed new light on the inhibitory specific regulation and propagation of CA1 ripples. The authors recorded from CA1 nonpyramidal neurons in behaving mice that were resting or running on a treadmill. Surprisingly, they identified a unique interneuron type that, unlike all others, followed its own activity pattern. This interneuron type remained essentially silent during theta states (**Figure 1A,** left). However, immediately before the onset of the ripple, its spiking activity increased dramatically, while its firing, was maximal before the ripple peak and remained strikingly high during the whole ripple cycle, in both wakefulness and non-REM sleep states (**Figure 1A,** right). The discharge rates of these *theta-OFF, ripple-ON* (TORO) cells, exceeded those of PV-expressing basket cells, reaching spike rates of >200/second in individual TORO cells (average ~174/second). This intriguing finding raises the question of whether TORO cells are necessary and sufficient for the ripple formation in CA1. The authors found that their activity, is positively correlated with the frequency and amplitude, but not with the duration of ripples. So, are TORO cells necessary and sufficient to modulate ripple related activity? Further studies are needed to determine the effects of TORO cell silencing on generation and properties of ripples.

What are the anatomical and molecular features of TORO interneurons? The authors went on to characterize TORO cells anatomically, cytochemically, and electrophysiologically. They found them distributed across the CA1 layers, with a preferential location in the stratum Oriens. Interestingly, all TORO cells were equipped with inhibitory muscarinic type 2 receptors (M2Rs) in their somatodendritic compartments (a subset expressed somatostatin and/or calbindin in addition). This further distinguishes TORO cells from PV cells, where M2Rs are expressed in axonal compartments. M2Rs are also found in the somatodendritic compartments of some VIP-expressing long-range projecting interneurons. However these interneurons remain inactive during ripples (Francavilla et al., 2018), further emphasizing the exclusive identity of TORO cells.

What factors control the activity pattern of TORO cells? The medial septum (MS) is known to be active during theta-related behaviors and to send signals to the hippocampus via cholinergic and GABAergic projections (for review, see Müller and Remy, 2018). Using an *in*

vitro approach, (Szabo et al., 2022) demonstrated hyperpolarization of TORO cells by cholinergic activation, an effect presumably mediated by the abundant expression of M2Rs in TORO cells. Furthermore, applying functional Ca^{2+} imaging *in vivo*, they found that septal GABAergic inputs are a source of inhibition for TORO cells during theta but not during ripples. Taken together, these findings may somehow explain the observed theta-related suppression of TORO cells (**Figure 1B**). However, it is important to mention that other interneurons, e.g., the PV-expressing basket cells, are also inhibited by strong rhythmic GABAergic inputs from the MS (Unal et al., 2018), and yet these cells remain quite active during theta rhythm (e.g., Varga et al., 2014). Thus, whether septal mediated GABAergic input is sufficient to explain the suppression of TORO cells during theta is still unclear. For example, VIP-expressing interneurons that suppress other interneurons have been shown to be very active during locomotion (Turi et al., 2019) and it is conceivable that TORO cells are also controlled by such local disinhibitory interneurons during theta activity. Conversely, are TORO cells completely cut off from excitatory inputs during the theta states? The CA1 network receives, among others theta-related excitatory inputs from CA3 and the entorhinal cortex, (Buzsáki, 2002). It would be interesting to clarify if the TORO cells receive any kind of excitatory drive in this network state, and if so, what its properties are. Lack of or weak excitation, together with septal inhibition and possibly local inhibition, may in combination account for the remarkably low activity of TORO cells during theta oscillations. Future studies might shed more light on the functional connectivity of TORO cells during theta. During ripples, excitatory signals generated in the CA3 area (via the Schaffer collateral pathway), activate the CA1 network and, in particular, the pyramidal and PV interneuron populations, whose interactions are considered crucial for ripple oscillogenesis (Buzsáki, 2015). Using an optogenetic approach, the authors stimulated CA3 excitatory axons, while imaging Ca^{2+} transients in cells of the local network. This allowed them to show that TORO cells are particularly strongly activated by excitatory CA3 input compared to other CA1 interneuron types tested. This finding explains the exceptionally high activity rate of TORO cells relative to other ripple-ON neurons. However, future studies should consider the additional possibility of local and distal sources of excitatory drive of TORO cells.

What are consequences of TORO cell activity in regulating the local and distal neuronal networks? The authors showed that for CA1 and entorhinal cortex that PV- and CCK-

expressing interneurons are in dense contact with M2 receptor-bearing, non-PV-expressing axons, suggesting that these interneuron classes are under the inhibitory control of TORO cells. This astonishing finding, together with the functional result of higher synaptic connectivity of TORO cells to interneurons compared with pyramidal neurons (see above), makes it plausible, that TORO cells, function primarily as disinhibitory circuit elements. Specifically, for the CA1, this implies that the highly active PV-expressing interneurons are under the control of even more active TORO cells during ripples, an observation that may add key aspects to current models of ripple generation (Buzsáki, 2015). Follow-up questions might include: How does ripple-related activity of TORO cells affect the balance of inhibition and excitation in the local network, and what are consequences for the recruitment of pyramidal neurons? Are there rules for plasticity of TORO interneurons, and if so, how would TORO cell plasticity affect ripple generation or function? Future studies will hopefully shed more light on these fundamental issues. Finally, and perhaps most striking of all, the authors found that TORO cells send inhibitory outputs to a number of extrahippocampal areas, including the subiculum, retrosplenial and entorhinal cortex, and others (**Figure 1B**). Indeed, TORO cells could be the major carriers of ripple-related inhibition leaving the hippocampus. Whether their inhibitory output, can causally influence the spread of ripples to other brain regions and/or causally affect the computations associated with ripples in these downstream areas remains to be investigated in future studies. Overall, much remains to be done to elucidate further details of the local synaptic mechanisms and physiological significance of TORO cells. In any case, this groundbreaking research, represents a substantial step forward and provides further evidence for the inhibitory specific regulation and propagation of hippocampal CA1 ripples during memory retrieval and consolidation.

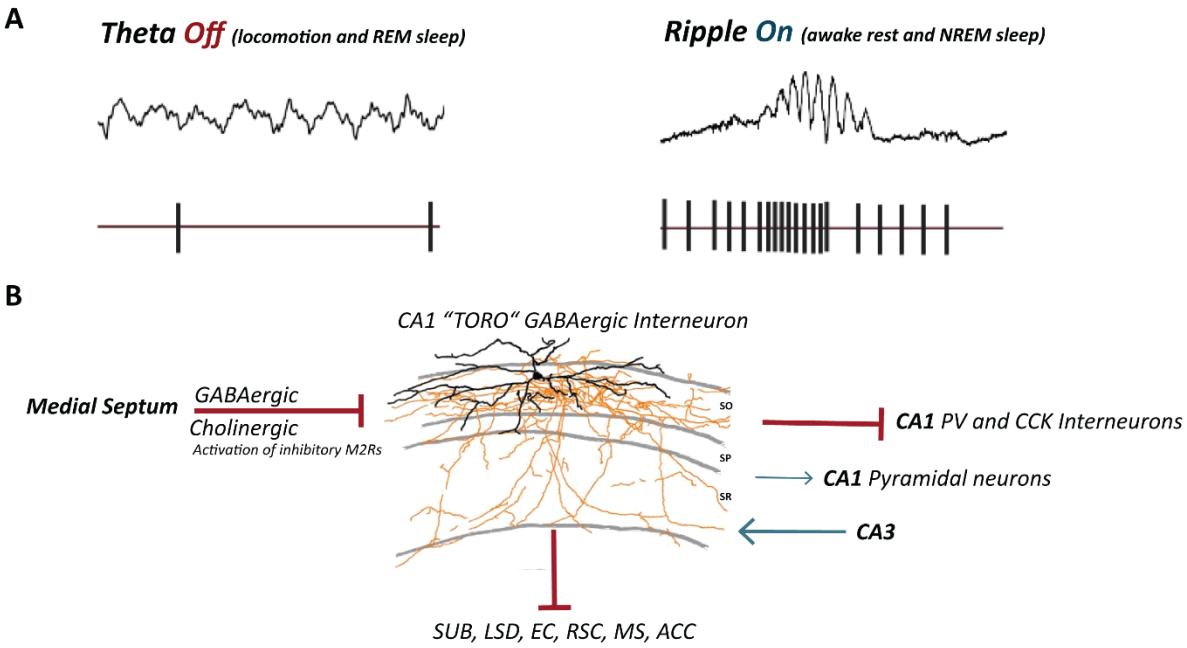

Figure 1. Theta-OFF, ripple-ON (TORO) interneurons activity and input - output patterns during memory related rhythms. A) Theta off – Ripple on (TORO) interneurons remain almost silent during theta oscillations (left panel) while they are actively engaged slightly before and during ripples, maximizing their firing before the ripple peak (right panel). **B)** During theta states, TORO interneurons receive inhibitory GABAergic input from the medial septum. In addition, cholinergic inputs from the medial septum activate inhibitory muscarinic type 2 receptors (M2Rs) expressed in the somatodendritic compartments of TORO cells. During ripples, Schaffer collaterals in CA3 activate TORO interneurons in CA1, which in turn inhibit mainly local PV and CCK interneurons and, to a lesser extent, pyramidal cells. TORO cell axons relay ripple-related inhibitory signals from CA1 to several extrahippocampal areas, including the subiculum (SUB), dorsal lateral septal nucleus (LSD), lateral and medial entorhinal cortex (EC), retrosplenial cortex (RSC), medial septum (MS), and anterior cingulate cortex (ACC). The neuronal reconstruction (modified) is taken from Szabo et al., (2022).

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