OVERLINE

Conducting the inhibition in memory formation

Brainstem nucleus incertus orchestrates the formation of contextual memories by inhibiting hippocampal dendrite-targeting interneurons

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Listening to the harmonic performance of a symphonic orchestra is a peculiar experience indeed. The music is dynamically changing during the performance, sometimes the strenuous voice of the brasses dominate, sometimes only a faltering violin can be heard. The musicians, masters of their instruments, are playing together to form a symphony from the voices of the play. Still, the musicians can form a perfect harmony only if they listen to the conductor. The conductor understands every minute part of the symphony and instructs the musicians to be silent or audible as required by the dynamics of the masterpiece.

The hippocampal network is functioning in a similar manner. The co-firing of a subpopulation of pyramidal cells in the CA1 region encode the episodic memories of our life, like the voices of the instruments form the symphony of the play. To fulfill this role, CA1 pyramidal cells receive multisensory information from the CA3, and direct sensory-related information from the entorhinal cortex (*1*, *2*). These information must be associated on pyramidal cells for appropriate memory formation (*3*, *4*). The number of CA1 pyramidal cells that encode a memory trace must be tightly regulated. The participation of too many pyramidal cells in a cell assembly may engender memory interference, while too few pyramidal cells in the assembly can form only unstable memories (*5*, *6*). This is controlled by GABAergic inhibitory interneurons, just like the number of notes that are played must be controlled by a musician (*7*). Somatostatin (SOM)-positive dendrite-targeting interneurons play an essential role in the selection of the memory-encoding CA1 pyramidal cells. SOM-positive interneurons exclude the direct sensory-related information from the majority of the pyramidal cells, and they are activated by the excitatory glutamatergic and cholinergic cells of the medial septum (MS) (*8*–*10*).

But who is the conductor of this symphony? Which input of the SOM-positive interneurons help fine-tune the number of CA1 pyramidal cells that encode a given memory trace? Together with my mentor, Gábor Nyiri, in the laboratory of Tamás Freund, we hypothesized that the little known nucleus incertus (NI) would be ideal to fulfill this role, as it strongly projects to the septo-hippocampal system (11).

Hippocampal dendrite-targeting interneurons are inhibited by brainstem nucleus incertus both monosynaptically and indirectly via the medial septum

We used cell-type specific anatomical tracing, electron microscopic analysis and in vitro optogenetics to identify the targets of NI in the hippocampus and in the MS. We discovered that NI selectively targets SOM-positive dendrite-targeting interneurons in the CA1 and it establishes GABAergic inhibitory synapses on them. NI also inhibited glutamatergic and cholinergic neurons in the MS and therefore it inhibited the subcortical excitatory inputs of SOM-positive hippocampal interneurons. NI neurons frequently projected into the hippocampus and MS simultaneously, suggesting that they can inhibit hippocampal SOM-positive interneurons through both a direct and an indirect pathway at the same time (*12*).

Nucleus incertus is rapidly activated by salient sensory stimuli

Based on our model, NI neurons should know if something needs to be memorized. We tested whether NI is activated by salient sensory inputs, so they can regulate SOM-positive hippocampal interneurons at the right moment. Therefore, we labeled NI cells with a fluorescent calcium-indicator and performed two-photon imaging of the NI fibers in the CA1 region of head-fixed awake mice. Mice were running on a treadmill, while they received different sensory stimuli with the simultaneous recording of NI fiber activity. We found that NI fibers were activated by the different sensory stimuli at a different rate. Aversive air-puffs and water rewards evoked a larger response in these fibers than light flashes or auditory tones, suggesting that NI is fine-tuned by different sensory inputs based on their relevance and/or modality. Using rabies virus tracing, we also examined, which brain areas modulate NI GABAergic neurons directly. We found that brain areas processing relevant environmental inputs, like the lateral habenula or prefrontal cortex, innervated NI GABAergic neurons monosynaptically for its potentially rapid activation (*12*).

Nucleus incertus bidirectionally controls contextual memory formation

To better understand how NI GABAergic cells orchestrate behavior, we used contextual fear conditioning. We placed virus-injected mice into an unfamiliar environment, where they received mild, aversive foot-shocks. NI GABAergic cells or their fibers in the CA1 region were activated optogenetically precisely during the foot-shocks. On the next day, mice had to

face the same environment. While control mice showed appropriate fear behavior, optogenetically stimulated mice showed no fear in the same environment. This effect was absent, when optogenetic stimulation was not precisely aligned to foot-shock presentation. On the other hand, optogenetic inhibition of NI resulted in excessively enhanced contextual memories, confirming that NI GABAergic cells can regulate episodic memory formation bidirectionally (*12*).

The role of nucleus incertus in normal and pathological memory formation

We showed that NI rapidly processes salient environmental stimuli. Our data suggest that NI tightly controls contextual memory formation by the direct and indirect inhibition of SOM-positive dendrite-targeting interneurons in the CA1, by fine-tuning the selection of memory-encoding pyramidal cells based on the relevance and/or modality of the different environmental inputs. Optogenetic stimulation of NI precisely at the moment of aversive stimulus presentation prevented contextual memory formation. This suggests that NI may play a role in filtering non-relevant everyday experiences, the unnecessary encoding of which could lead to cognitive symptoms (*13*). In contrast, because optogenetic inhibition of NI caused pathologically strong fear memory formation, dysfunction of NI GABAergic neurons may lead to generalized anxiety-like syndromes or to posttraumatic stress disorder, where pathologically strong fear memory formation is present. Our results show that the brainstem NI is an unexpectedly precise conductor of the hippocampal SOM-positive cells, the musicians of the hippocampal network, where the pyramidal cells form the symphony of our episodic memories.

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10.1126/science.