Uncovering optogenetic and chemogenetic induction of cognitive deficits: Efficient techniques for manipulating and observing specific neural activities

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The hippocampus is part of the brain limbic system and plays an important role in learning and memory. Moreover, its ability to form, consolidate, and retrieve different types of memories makes it a central component in the cognitive functions necessary for everyday life. Understanding the role of the hippocampus helps comprehend how memories are created, stored, and recalled and sheds light on the impact of hippocampal damage in conditions such as Alzheimer's disease and other forms of dementia. Optogenetics and chemogenetics are powerful tools that have been used to investigate the role of the hippocampus in learning and memory by allowing precise control and manipulation of specific neural circuits within the brain. While optogenetics utilizes light pulses to control light-sensitive ion channels (opsins), chemogenetics employs a designer drug to modulate designer receptors exclusively activated by the designer drug. These tools have significantly advanced our understanding of the role of the hippocampus in learning and memory by enabling researchers to unravel the complex interactions within the hippocampus that underlie cognitive functions. Further, these techniques allow researchers to manipulate and observe the effects of specific neural activities, thereby providing a deeper understanding of the mechanisms through which the hippocampus supports learning, memory formation, and recall.

Traditionally, brain information processing has been viewed as primarily a neuronal function. Astrocytes in the central nervous system have been considered mainly supportive of neurons. However, growing evidence indicates that astrocytes play active roles, including signal transmission and regulation of synaptic plasticity. Recent research has linked astrocytes to various behavioral states and brain pathologies in animal models, showing that cognitive processes such as learning and memory require a coordinated interaction between astrocytes and synaptic networks in the hippocampus (Bohmbach et al., 2023). Thus, the coordinated actions of glial–neuron networks are likely fundamental to many brain functions, including cognition. Nonetheless, the physiological contribution of hippocampal astrocytes to cognitive function and the underlying mechanisms remain largely unexplored. Several studies have highlighted the importance of astrocytes in memory, showing that disrupting astrocyte function leads to memory impairments, and correcting astrocyte genotypes in genetic models of cognitive deficit can alleviate memory impairments (Escalada et al., 2024). For example, optogenetic stimulation of hippocampal CA1 astrocytes in a transgenic animal has been shown to attenuate contextual fear memory (Li et al., 2020). Conversely, activating a particular G protein-coupled pathway in astrocytes can enhance memory in mice (Adamsky et al., 2018). Another study suggests that certain astrocytic intracellular pathways may detrimentally affect memory function (Escalada et al., 2024). Thus, these results indicate a bell-shaped curve for astrocytic activity in memory, where an optimal level supports intact memory, but deviations from this optimal level can be harmful.

Astrocytes and microglia have been implicated in the pathogenesis of Alzheimer's disease and other neurodegenerative conditions (Liddelow et al., 2024). These brain-resident glial cells are the primary drivers of neuroinflammation, common in various nervous system pathologies. Microglia respond to brain insults by translating signals into diverse molecules that regulate the reactivity of astrocytes. Although astrocytes are an integral component of synapses regulating various brain physiologies, reactive astrocytes can influence microglia phenotypes, with their interaction playing a significant role in neuroinflammation and disease progression. Proinflammatory cytokines, under normal conditions, are involved in memory formation, but excessive cytokine release under pathological neuroinflammatory conditions can lead to overactivation of astrocytes, resulting in neuronal injury and cognitive decline. Lipocalin-2 (LCN2) has previously been identified as a mediator of reactive astrocytosis (Lee et al., 2009). Studies have also linked LCN2 to neurodegenerative and cognitive disorders characterized by neuronal loss, astrocyte alterations, neuroinflammatory responses, and synaptic dysfunction (Afridi et al., 2024). However, the connection among LCN2, chronic neuroinflammation in the hippocampus, and cognitive impairment still needs to be fully established. It remains unclear whether hippocampal inflammation persists with chronic reactive astrocytosis. LCN2 has been reported to have both proinflammatory and anti-inflammatory effects, and its role in being neurotoxic or neuroprotective is still debated.

To understand the mechanisms of astrocyte reactivity in disease, it is crucial to investigate the relationship between prolonged astrocytic signaling and neuronal function. A recent study used optogenetic and chemogenetic strategies combined with electrophysiology and behavioral assessments to explore the role of CA1 astrocytes and the effects of their aberrant activation on hippocampal synaptic activity and cognitive decline (Kim et al., 2024). This study has demonstrated that sustained stimulation of hippocampal CA1 astrocytes using optogenetic and chemogenetic tools induces neuroinflammation and a reactive astrocyte phenotype, resulting in cognitive impairment (**Figure 1**). LCN2 released from reactive astrocytes decreased NMDA receptor-mediated synaptic activity, affecting the long-term synaptic plasticity of hippocampal CA1 neurons and leading to cognitive decline. A series of experiments confirmed that hippocampal astrocyte activation is both necessary and sufficient for cognitive decline. The detection of astrocyte activation under neuroinflammatory conditions *in vivo* using Ca^{2+} fiber photometry further supported these findings. Prolonged activation of hippocampal CA1 astrocytes impairs cognitive function, mimicking pathological conditions where astrocytes are aberrantly activated to disrupt cognitive function. Unlike neurons, astrocytes require longer stimulation periods to exhibit responses. Thus, previous studies have used prolonged optogenetic stimulation of astrocytes in various contexts, including anxiety and spinocerebellar ataxia. Indeed, Kim et al. (2024) applied daily optogenetic stimulation over three days to induce hippocampal inflammation, consistent with the time required for neuroinflammation to peak in mouse models.

Although the photostimulation of channelrhodopsin-expressing astrocytes may differ from physiological stimuli, optogenetic manipulation has been successfully used to study various behavioral responses. Previous works by the same group showed that photostimulation increases intracellular Ca^{2+} concentration and sustained oscillation in channelrhodopsin-expressing astrocytes, triggering gliotransmitter release (Kim et al., 2020, 2025). While astrocyte Ca^{2+} signaling is crucial for astrocyte–neuron communication, its functional relevance remains under investigation. Astrocytes undergo remodeling in response to central nervous system injury, disease, or infection, varying dramatically across different conditions. These reactive states must be carefully modeled to replicate disease-specific conditions accurately.

Neuroinflammation is regulated by complex crosstalk between microglia and astrocytes (Bhusal et al., 2023). Following brain insults, microglia release cytokines and inflammatory mediators, which facilitate microglia–astrocyte communication. Subsequently, astrocytes secrete cytokines that regulate microglia function. Kim et al. (2024) found increased proinflammatory cytokines and LCN2 expression in optogenetically stimulated astrocytes, suggesting that reactive astrocytes may promote microglia activation through cytokines or LCN2—a key mediator of neuroinflammation that influences activation phenotypes of astrocytes and microglia. Thus, it is suggested that LCN2 released from optogenetically stimulated hippocampal CA1 astrocytes may either facilitate microglial activation or modulate

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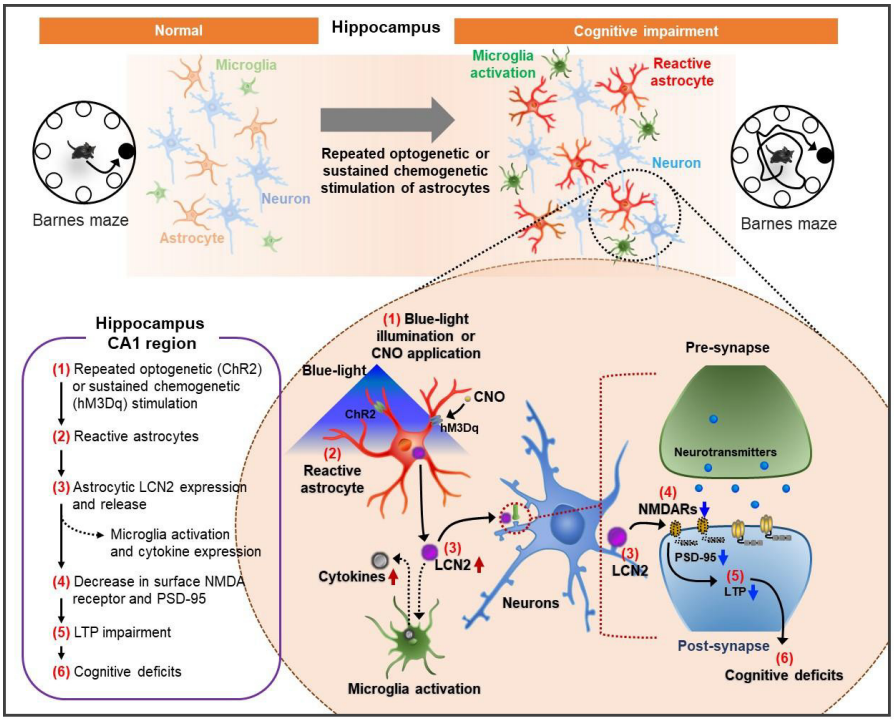

Figure 1 | **LCN2-dependent mechanism of cognitive dysfunction after optogenetic or chemogenetic stimulation of hippocampal astrocytes.**
Prolonged optogenetic or chemogenetic stimulation of astrocytes induces their reactivity. LCN2 released from reactive astrocytes in the hippocampus causes cognitive impairment in mice, as measured by Barnes maze task, through a series of events: (1) prolonged optogenetic or chemogenetic stimulation of hippocampal astrocytes; (2) induction of reactivity of astrocytes; (3) LCN2 release from reactive astrocytes and subsequent activation of microglia in the vicinity; (4) reduction of NMDA receptor and PSD-95 on neuronal membrane; (5) decrease in the extent of hippocampal LTP; (6) induction of cognitive deficits. Reprinted from Kim et al. (2024) based on the Creative Commons Attribution 4.0 International (CC BY) license. CA1: Cornu Ammonis 1; ChR2: channelrhodopsin 2; CNO: clozapine N-oxide; hM3Dq: human M3 muscarinic receptor; LCN2: lipocalin-2; LTP: long-term potentiation; NMDAR: N-methyl-D-aspartate receptor; PSD-95: post-synaptic density 95.

hippocampal synaptic activity to influence cognitive function. Further, Kim et al. (2024) showed that LCN2 reduces NMDA receptor surface expression, inhibits synaptic long-term potentiation, and affects CA1–CA3 synapse excitability. Although the precise molecular mechanisms are unclear, these findings emphasize the role of LCN2 in astrocyte-mediated cognitive decline.

In summary, aberrant activation of hippocampal astrocytes appears to impair excitatory synaptic transmission and cognitive function. LCN2 released from reactive astrocytes in the inflamed hippocampus may mediate these synaptic alterations. Targeting astrocyte pathways and/or LCN2 could offer new therapeutic approaches to treat cognitive decline in diverse central nervous system conditions.

Repeated and prolonged stimulation of astrocytes leads to a reactive astrocyte phenotype through an LCN2-dependent mechanism, resulting in reduced synaptic plasticity and cognitive impairment in mice (**Figure 1**). The study by Kim et al. (2024) has highlighted numerous avenues for further research. One area of interest is the disease-specific nature of astrocyte reactivity (as commented in Bohmbach and Henneberger, 2024). While some characteristics are common across different diseases, many aspects are unique to a particular condition or trigger. This raises the question of which diseases exhibit astrocyte reactivity similar to that induced by Kim et al. (2024), ultimately sharing the same pathogenic mechanism. Conversely, identifying which brain diseases or conditions with astrocyte reactivity might benefit most through manipulating LCN2 signaling could have significant therapeutic implications. Additionally, the specialization of astrocytes across different brain regions and within specific areas presents another intriguing research avenue. It remains to be determined how astrocyte reactivity and its impact on synapse function vary between brain regions and synaptic circuits. Finally, future studies need to offer an improved definition of the astrocyte–microglia crosstalk in molecular terms. Under neuroinflammatory conditions, reactive astrocytes inevitably ensue microglial activation and vice versa. Communication between these glial cells via various regulatory mediators likely governs the overall extent of neuroinflammation and shapes consequent behavioral responses, including learning and memory.

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