Early remodeling of the neocortex upon episodic memory encoding

The MIT Faculty has made this article openly available. Please share how this access benefits you. Your story matters.

| As Published | http://dx.doi.org/10.1073/pnas.1408378111 |
| Publisher | National Academy of Sciences (U.S.) |
| Version | Final published version |
| Accessed | Wed Jan 09 00:46:48 EST 2019 |
| Citable Link | http://hdl.handle.net/1721.1/95756 |
| Terms of Use | Article is made available in accordance with the publisher's policy and may be subject to US copyright law. Please refer to the publisher's site for terms of use. |
| Detailed Terms | |
Early remodeling of the neocortex upon episodic memory encoding

Adam W. Bero¹, Jia Meng¹, Sukhee Cho¹, Abra H. Shen¹, Rebecca G. Canter¹, Maria Ericsson², and Li-Hsin Tsai¹,²,¹

¹Picower Institute for Learning and Memory, Department of Brain and Cognitive Sciences, Massachusetts Institute of Technology, Cambridge, MA 02139; ²Broad Institute of Harvard University and Massachusetts Institute of Technology, Cambridge, MA 02142; and ³Department of Cell Biology, Harvard Medical School, Boston, MA 02115

Understanding the mechanisms by which long-term memories are formed and stored in the brain represents a central aim of neuroscience. Prevailing theory suggests that long-term memory encoding involves early plasticity within hippocampal circuits, whereas reorganization of the neocortex is thought to occur weeks to months later to subserve remote memory storage. Here we report that long-term memory encoding can elicit early transcriptional, structural, and functional remodeling of the neocortex. Parallel studies using genome-wide RNA sequencing, ultrastructural imaging, and whole-cell recording in wild-type mice suggest that contextual fear conditioning initiates a transcriptional program in the medial prefrontal cortex (mPFC) that is accompanied by rapid expansion of the synaptic active zone and postsynaptic density, enhanced dendritic spine plasticity, and increased synaptic efficacy. To address the real-time contribution of the mPFC to long-term memory encoding, we performed temporally precise optogenetic inhibition of excitatory mPFC neurons during contextual fear conditioning. Using this approach, we found that real-time inhibition of the mPFC inhibited activation of the entorhinal–hippocampal circuit and impaired the formation of long-term associative memory. These findings suggest an early role for the prefrontal cortex as a critical regulator of encoding-induced hippocampal activation and long-term memory formation, and have important implications for understanding memory processing in healthy and diseased brain states.

Learning | transcriptome | neuroplasticity | optogenetics | hippocampus

Long-term memory is essential for cognition and its disruption is central to myriad neurological disorders. Therefore, a deeper understanding of the mechanisms by which long-term memories are formed in the brain and lost in neurological disease states represents a critical objective in neuroscience. To this end, a wealth of studies has demonstrated that encoding of long-term memory involves early and rapid enhancements in neuronal activation, synaptic plasticity-related gene expression, structural synaptic plasticity, and synaptic efficacy within hippocampal circuits (1–5). Accordingly, lesions of the hippocampus or surrounding medial temporal lobe structures elicit severe memory impairment (6, 7). Over the weeks to months that follow the initial memory encoding event, repeated hippocampal–neocortical interactions slowly reorganize the synaptic architecture in the neocortex and gradually instantiate the memory trace within neocortical circuits, particularly the medial prefrontal cortex (mPFC) (5, 8–10). Collectively, these observations have given rise to the prevailing view that long-term memory encoding requires early and rapid plasticity within the hippocampus, whereas reorganization of the neocortex is limited to the late stages of memory processing to subserve remote memory storage and recall (11, 12).

Intriguingly, however, neuroimaging studies from mouse to man demonstrate that the hippocampus and prefrontal cortex are coactivated during memory encoding (13–20). Indeed, the magnitude of prefrontal activation during memory encoding has been shown to be predictive of the ability to later recall the experience (13), and prefrontal lesions are associated with impaired memory formation (15–17, 21–25). Nonetheless, the mechanisms by which the prefrontal cortex contributes to long-term memory encoding remain poorly understood. Herein, we provide evidence that long-term associative memory encoding activates a synaptic plasticity-related transcriptional program in the mPFC that is accompanied by rapid structural and functional plasticity of mPFC synaptic circuits. Using an in vivo optogenetic approach to examine the real-time contribution of mPFC activation to long-term memory encoding, we further show that excitatory mPFC neurons drive activation of the entorhinal–hippocampal circuit and regulate the formation of long-term memory. These results suggest a critical role for the prefrontal cortex in long-term memory encoding and have implications for understanding memory function in healthy and diseased brain states.

Results

To investigate the mechanisms by which the prefrontal cortex contributes to long-term episodic memory encoding, we used contextual fear conditioning, in which mice learn an association between a novel context and an event (foot shock) that occurs in that context. Single-trial contextual fear conditioning generates a temporally defined, long-lasting associative memory trace and is thus well suited for investigating the mechanisms that mediate long-term memory encoding in the brain (26). Moreover, a critical regulator of encoding-induced hippocampal activation and long-term memory formation, and have important implications for understanding memory processing in healthy and diseased brain states.

Author contributions: A.W.B. and L.-H.T. designed research; A.W.B., J.M., S.C., A.H.S., R.G.C., and M.E. performed research; A.W.B., J.M., S.C., and A.H.S. analyzed data; and A.W.B. wrote the paper.

The authors declare no conflict of interest.

*This Direct Submission article had a prearranged editor.

Data deposition: The data reported in this paper have been deposited in the Gene Expression Omnibus ( GEO) database, www.ncbi.nlm.nih.gov/geo (accession no. GSE58510).

1To whom correspondence should be addressed. Email: lhtsai@mit.edu.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1408378111/-/DCSupplemental.
previous studies suggest that associative fear learning increases neuronal activity in the mPFC (15) and that the mPFC is critical for long-term storage and recall of associative fear memory (9). To verify that this paradigm increases neuronal activity in the mPFC, we examined expression of the neuronal activity-dependent immediate early gene, early growth response 1 (ezf268/egr-1), in the mPFC 1 h following contextual fear conditioning (27). In accord with previous data (15), we found that fear conditioning significantly increased immediate early gene expression in the mPFC compared with control animals exposed to either the context or foot shock alone, suggesting that the mPFC exhibits associative learning-specific neural activation (Fig. S1). Additionally, as immediate early gene expression in the mPFC was significantly greater in context-exposed control animals relative to control animals exposed only to foot shock, context-exposed mice served as controls in subsequent experiments (Fig. S1). Together, these data suggest that associative memory encoding increases neuronal activity in the mPFC and further validate contextual fear conditioning as a useful paradigm by which to examine the role of the prefrontal cortex in long-term associative memory encoding.

Next, we sought to determine whether the memory encoding-induced increase in mPFC neuronal activity was associated with a shift in the mPFC transcriptome. To this end, we performed genome-wide RNA sequencing (RNA-seq) of mouse mPFC 1 h following contextual fear conditioning. Strikingly, we found that 342 genes (121 up-regulated, 221 down-regulated) were differentially expressed in the mPFC of mice that underwent fear conditioning compared with control animals (Fig. 1A and Table S1). Gene ontology and gene network analyses of differentially expressed genes revealed that genes up-regulated in the mPFC during associative memory encoding form a highly interconnected network involved in biological processes that promote synaptic plasticity and memory formation, including synthesis of cAMP, neurotransgenesis, long-term potentiation, and axon guidance (28) (Fig. 1 B and C and Table S2). Conversely, genes down-regulated in the mPFC during associative memory encoding are implicated in biological processes that have been shown to suppress synaptic plasticity and memory function, such as proliferation of macroglia, activation of phagocytes, and apoptosis (29, 30) (Fig. 1 B and C and Table S2). Quantitative RT-PCR analysis of independent samples confirmed these expression changes (Fig. 1D and Table S3). These results suggest that the mPFC transcriptome is reprogrammed rapidly during associative memory encoding and that memory encoding primes the mPFC transcriptome for neuroplasticity.

Given that changes in gene expression are required to drive long-lasting forms of synaptic plasticity (28), we reasoned that the memory encoding-induced gene expression changes we observed in the mPFC might be associated with early remodeling of the local synaptic architecture. To test this hypothesis, we performed transmission electron microscopy (TEM) to analyze the ultrastructure of individual mPFC synapses 1 h following contextual fear conditioning. Specifically, we assessed the length of the active zone and postsynaptic density as well as the number of docked synaptic vesicles per synapse, as these metrics represent structural indices of synaptic strengthening and are increased under conditions associated with memory formation (31, 32). We found that associative memory encoding led to a rapid induction of ultrastructural synaptic plasticity in the mPFC, as contextual fear conditioning significantly expanded the size of the active zone and postsynaptic density (Fig. 2 A and B and Fig. S2 A and B) and increased the number of docked vesicles within individual mPFC synapses relative to controls (Fig. 2 C and Fig. S2 C). In accord with previous studies (33, 34), we found that active zone length, postsynaptic density length, and docked synaptic vesicle number were highly correlated at the level of individual synapses (Fig. 2 D and Fig. S2 D and E). Consistent with these observations, we found that expression of the presynaptic marker, synaptophysin, was significantly increased in the mPFC 1 h following contextual fear conditioning compared with controls (Fig. 2 E and Fig. S3).

To further examine whether memory encoding elicits early plasticity within postsynaptic mPFC circuit elements, we used Golgi-Cox impregnation to investigate the morphology of individual dendritic spines on mPFC pyramidal neurons. Dendritic spines represent the postsynaptic component of excitatory synapses and exhibit morphological and functional continua, ranging from thin spines with a small head (i.e., thin spines) that are highly plastic and are hypothesized to underlie experience-dependent rewiring of neural circuits, to spines with a large, mushroom-like head (i.e., mushroom spines) that are more stable and are hypothesized to represent the physical substrates of long-term memories (35). We found that the ratio of thin spines to mushroom spines in the mPFC was significantly increased 1 h following contextual fear conditioning compared with controls (Fig. 3 A and B), suggesting that associative memory encoding increases dendritic spine plasticity in mPFC circuits. Next, to determine whether memory encoding-induced structural plasticity in mPFC circuits is associated with functional alterations in excitatory synaptic strength, we performed ex vivo whole-cell patch-clamp recording of mPFC pyramidal neurons to examine miniature excitatory postsynaptic currents (mEPSCs) (36). We found that mEPSC frequency, but not

![Fig. 1. Associative memory encoding primes the mPFC transcriptome for neuroplasticity.](image)
Associative memory encoding enhances dendritic spine plasticity and hippocamal circuit activity during long-term associative memory encoding. Interestingly, optical inhibition also reduced the expression of synaptophysin in the mPFC of mice expressing eNpHR3.0-EYFP, but not EYFP alone (Fig. 4 E and F), suggesting that early plasticity of mPFC circuits during associative memory encoding requires excitatory neuronal activity. To determine whether the mPFC regulates hippocampal circuit activity during memory encoding, we next examined the effect of real-time optogenetic mPFC inhibition on hippocampal area CA1, an area critical for long-term memory formation (7). Remarkably, we found that optogenetic inactivation of the mPFC during memory encoding significantly reduced the expression of Zif268 and synaptophysin in hippocampal area CA1 of mice expressing eNpHR3.0-EYFP compared with mice expressing EYFP alone (Fig. 4 G and H), suggesting that the mPFC is neuronal activity (Fig. S6).

Stereotaxic injection of the CaMKIIα-eNpHR3.0-EYFP vector into mPFC resulted in mPFC-specific expression (Fig. 4A), and within mPFC, eNpHR3.0-EYFP was expressed in CaMKIIα-positive excitatory neurons (Fig. 4B). Single-cell recordings from acute mPFC slices confirmed that optical (593 nm) inhibition dampened action potential spiking in excitatory neurons expressing eNpHR3.0-EYFP (Fig. 4C). Next, we found that real-time optogenetic inhibition of the mPFC during contextual fear conditioning (Fig. 4D) significantly reduced Zif268 expression in the mPFC of mice expressing eNpHR3.0-EYFP compared with mice expressing EYFP alone (Fig. 4 E and F), confirming that optogenetic inhibition prevented mPFC activation during long-term associative memory encoding. Interestingly, optical inhibition also reduced the expression of synaptophysin in the mPFC of mice expressing eNpHR3.0-EYFP, but not EYFP alone (Fig. 4 E and F), suggesting that early plasticity of mPFC circuits during associative memory encoding requires excitatory neuronal activity. To determine whether the mPFC regulates hippocampal circuit activity during memory encoding, we next examined the effect of real-time optogenetic mPFC inhibition on hippocampal area CA1, an area critical for long-term memory formation (7). Remarkably, we found that optogenetic inactivation of the mPFC during memory encoding significantly reduced the expression of Zif268 and synaptophysin in hippocampal area CA1 of mice expressing eNpHR3.0-EYFP compared with mice expressing EYFP alone (Fig. 4 G and H), suggesting that the mPFC is neuronal activity (Fig. S6).

Stereotaxic injection of the CaMKIIα-eNpHR3.0-EYFP vector into mPFC resulted in mPFC-specific expression (Fig. 4A), and within mPFC, eNpHR3.0-EYFP was expressed in CaMKIIα-positive excitatory neurons (Fig. 4B). Single-cell recordings from acute mPFC slices confirmed that optical (593 nm) inhibition dampened action potential spiking in excitatory neurons expressing eNpHR3.0-EYFP (Fig. 4C). Next, we found that real-time optogenetic inhibition of the mPFC during contextual fear conditioning (Fig. 4D) significantly reduced Zif268 expression in the mPFC of mice expressing eNpHR3.0-EYFP compared with mice expressing EYFP alone (Fig. 4 E and F), confirming that optogenetic inhibition prevented mPFC activation during long-term associative memory encoding. Interestingly, optical inhibition also reduced the expression of synaptophysin in the mPFC of mice expressing eNpHR3.0-EYFP, but not EYFP alone (Fig. 4 E and F), suggesting that early plasticity of mPFC circuits during associative memory encoding requires excitatory neuronal activity. To determine whether the mPFC regulates hippocampal circuit activity during memory encoding, we next examined the effect of real-time optogenetic mPFC inhibition on hippocampal area CA1, an area critical for long-term memory formation (7). Remarkably, we found that optogenetic inactivation of the mPFC during memory encoding significantly reduced the expression of Zif268 and synaptophysin in hippocampal area CA1 of mice expressing eNpHR3.0-EYFP compared with mice expressing EYFP alone (Fig. 4 G and H), suggesting that the mPFC is neuronal activity (Fig. S6).

Stereotaxic injection of the CaMKIIα-eNpHR3.0-EYFP vector into mPFC resulted in mPFC-specific expression (Fig. 4A), and within mPFC, eNpHR3.0-EYFP was expressed in CaMKIIα-positive excitatory neurons (Fig. 4B). Single-cell recordings from acute mPFC slices confirmed that optical (593 nm) inhibition dampened action potential spiking in excitatory neurons expressing eNpHR3.0-EYFP (Fig. 4C). Next, we found that real-time optogenetic inhibition of the mPFC during contextual fear conditioning (Fig. 4D) significantly reduced Zif268 expression in the mPFC of mice expressing eNpHR3.0-EYFP compared with mice expressing EYFP alone (Fig. 4 E and F), confirming that optogenetic inhibition prevented mPFC activation during long-term associative memory encoding. Interestingly, optical inhibition also reduced the expression of synaptophysin in the mPFC of mice expressing eNpHR3.0-EYFP, but not EYFP alone (Fig. 4 E and F), suggesting that early plasticity of mPFC circuits during associative memory encoding requires excitatory neuronal activity. To determine whether the mPFC regulates hippocampal circuit activity during memory encoding, we next examined the effect of real-time optogenetic mPFC inhibition on hippocampal area CA1, an area critical for long-term memory formation (7). Remarkably, we found that optogenetic inactivation of the mPFC during memory encoding significantly reduced the expression of Zif268 and synaptophysin in hippocampal area CA1 of mice expressing eNpHR3.0-EYFP compared with mice expressing EYFP alone (Fig. 4 G and H), suggesting that the mPFC is neuronal activity (Fig. S6).

Stereotaxic injection of the CaMKIIα-eNpHR3.0-EYFP vector into mPFC resulted in mPFC-specific expression (Fig. 4A), and within mPFC, eNpHR3.0-EYFP was expressed in CaMKIIα-positive excitatory neurons (Fig. 4B). Single-cell recordings from acute mPFC slices confirmed that optical (593 nm) inhibition dampened action potential spiking in excitatory neurons expressing eNpHR3.0-EYFP (Fig. 4C). Next, we found that real-time optogenetic inhibition of the mPFC during contextual fear conditioning (Fig. 4D) significantly reduced Zif268 expression in the mPFC of mice expressing eNpHR3.0-EYFP compared with mice expressing EYFP alone (Fig. 4 E and F), confirming that optogenetic inhibition prevented mPFC activation during long-term associative memory encoding. Interestingly, optical inhibition also reduced the expression of synaptophysin in the mPFC of mice expressing eNpHR3.0-EYFP, but not EYFP alone (Fig. 4 E and F), suggesting that early plasticity of mPFC circuits during associative memory encoding requires excitatory neuronal activity. To determine whether the mPFC regulates hippocampal circuit activity during memory encoding, we next examined the effect of real-time optogenetic mPFC inhibition on hippocampal area CA1, an area critical for long-term memory formation (7). Remarkably, we found that optogenetic inactivation of the mPFC during memory encoding significantly reduced the expression of Zif268 and synaptophysin in hippocampal area CA1 of mice expressing eNpHR3.0-EYFP compared with mice expressing EYFP alone (Fig. 4 G and H), suggesting that the mPFC is neuronal activity (Fig. S6).
Fig. 4. Optogenetic silencing of excitatory mPFC neurons impairs entorhinal–hippocampal circuit activation during associative memory encoding. (A) AAV-CaMKII-eNpHR3.0-EYFP was injected into bilateral mPFC to selectively transduce mPFC excitatory neurons and permit optogenetic inhibition thereof. (Scale bar, 500 μm.) (B) Representative immunohistochemical images depicting CaMKIIa expression in mPFC neurons transduced with CaMKIIa-eNpHR3.0-EYFP. (Scale bar, 10 μm.) (C) Ex vivo optical (593 nm) inhibition of action potential spiking (50 pA current injection) in a representative mPFC pyramidal neuron expressing eNpHR3.0-EYFP. (Scale bar, 20 μm, 100 ms.) (D) Schematic of the experimental paradigm used for in vivo mPFC optogenetic inhibition experiments shown in E–J. Animals injected with AAV-CaMKII-eNpHR3.0-EYFP or AAV-CaMKIIa-eNpHR3.0-EYFP control virus received continuous optogenetic inhibition of excitatory mPFC neurons during FC. One hour later, tissue was harvested for analysis. (E–J) Representative immunohistochemical images depicting Zif268 and synaptophysin immunoreactivity in the mPFC (E and F), hippocampal area CA1 (G and H), and entorhinal cortex (EC, I and J) of mice expressing eNpHR3.0-EYFP or EYFP alone following continuous optogenetic inhibition of mPFC during FC. (Scale bars, 50 μm.) (F) Quantification of Zif268 expression (E n = 3–4 mice per group). (G) Quantification of G (n = 3–4 mice per group). (H) Quantification of I (n = 3–4 mice per group). *P ≤ 0.05; **P ≤ 0.01. Values represent mean ± SEM.

Finally, to directly examine the effect of real-time optogenetic mPFC silencing on long-term memory formation, separate cohorts of mice were injected bilaterally with either the eNpHR3.0-EYFP or EYFP vector, implanted with a fiber optic in the mPFC, and received continuous optogenetic inhibition of mPFC excitatory activity during contextual fear conditioning as described above. One day and 30 d after training, mice were returned to the experimental context to assess recent and remote long-term memory, respectively (Fig. 5A). Interestingly, we found that mice expressing eNpHR3.0-EYFP spent significantly less time freezing during both the recent (Fig. 5B) and remote (Fig. 5C) memory tests compared with EYFP controls, suggesting that optogenetic inhibition of excitatory mPFC neurons during memory encoding impaired long-term memory formation. Importantly, neither basal locomotor activity (Fig. 5D) nor sensitivity to foot shock (Fig. 5E) differed significantly between the groups. These results suggest that real-time silencing of mPFC excitatory neurons during associative memory encoding is sufficient to impair the formation of long-term associative memory.

Discussion

Pioneering work on the mechanisms of memory suggests that episodic memory encoding requires early and rapid alterations in gene expression and synaptic efficacy within the hippocampus, whereas reorganization of neocortical circuits occurs weeks to months later to subserve long-term memory storage and recall (12). Intriguingly however, neuroimaging studies from mouse to man consistently report coactivation of the hippocampus and prefrontal cortex during memory encoding (13–17), thus raising the possibility that the neocortex may play a heretofore undefined role in the early stages of memory processing in the brain. In the present work, genome-wide RNA-seq analysis revealed that long-term associative memory encoding triggers a synaptic plasticity-related transcriptional program in the mPFC. Consistent with these gene expression changes, analysis of synapse ultrastructure, dendritic spine morphology, and synaptic transmission showed that memory encoding induced early remodeling of the mPFC synaptic architecture and enhanced local synaptic efficacy. Finally, using in vivo optogenetic silencing, we found that real-time inactivation of mPFC excitatory neurons during associative memory encoding impaired entorhinal–hippocampal circuit activation and the formation of long-term associative memory. Collectively, our results provide, to our knowledge, the first evidence that episodic memory encoding can induce early transcriptional, structural, and functional remodeling of the neocortex and identify the mPFC as a critical regulator of entorhinal–hippocampal circuit activation and the formation of long-term memory.

Whereas decades of investigation in humans and animal models have established a central role for the hippocampus in episodic memory encoding (1), a large body of literature suggests that the prefrontal cortex may also represent an integral component of the neural memory system. For instance, positron emission tomography and functional magnetic resonance imaging studies in humans and nonhuman primates demonstrate that the prefrontal cortex is activated during working memory and associative memory-encoding paradigms and that the magnitude of prefrontal cortex activation at the time of encoding is predictive of the ability to subsequently remember an event (13–20). In accord with these findings, prefrontal lesions have been shown to impair episodic memory formation across species (15–17, 21–25). Interestingly however, mechanistic investigation of the role of the prefrontal cortex in memory processing has largely focused on its role in memory consolidation and retrieval. In this regard, data in animal models suggest that glucose utilization (8), activity-dependent immediate early gene expression (9, 10), and dendritic spine density (5) are increased in the prefrontal cortex following consolidation of remote, but not recent, long-term memory and
that blockade of mPFC neuronal activity during memory recall impairs recall of remote, but not recent, long-term memory (7, 9, 10). Thus, whereas previous reports have established the importance of the mPFC in late-stage memory consolidation and storage, the mechanisms by which the mPFC regulates memory encoding remain unclear. To this end, our present results suggest that the mPFC exhibits early transcriptional, structural, and functional plasticity upon long-term associative memory encoding and that activation of excitatory neurons in the mPFC drives hippocampal activation and long-term memory formation. Importantly, our findings are consistent with recent reports suggesting that neurons in the prefrontal cortex are “tagged” during long-term memory encoding (16) and required for the incorporation of newly learned information into existing neural schemas (17) and thus suggest that early remodeling of the neocortical architecture may represent a fundamental feature of episodic memory encoding in the brain.

According to the theoretical modeling work of Marr (43) and the standard model of systems-level memory consolidation (11), the hippocampus is believed to integrate input conveyed from distributed neocortical circuits with self-generated spatial information at the time of memory encoding to generate a unified, spatially contextualized memory trace. However, the neocortical inputs that drive hippocampal activation during memory encoding, and thus regulate hippocampal-dependent memory formation, remain unclear. In this regard, our present data suggest that the activity of excitatory mPFC neurons is required for hippocampal activation and long-term memory formation and thus suggest that excitatory mPFC neurons may represent a critical source of hippocampal input during episodic memory encoding. As previous data indicate that late-stage memory consolidation requires hippocampus-driven reorganization of neocortical circuits (5, 16), our data support a stage-dependent directionality model of memory-relevant information flow, in which neocortex-driven hippocampal activity is critical for memory encoding and formation, whereas hippocampus-driven neocortical reorganization mediates subsequent memory consolidation and storage.

Neuroimaging data in humans and animal models indicate that the mPFC serves as a hub of information processing in the brain and exhibits functional connectivity with the hippocampal formation (37, 40, 41). Moreover, a recent study suggests that the mPFC exhibits significantly elevated resting-state aerobic glycolysis (44), suggesting that the mPFC may be uniquely suited to respond to learning-induced biosynthetic demands. Indeed, elevated aerobic glycolysis in the mPFC is closely associated with enhanced expression of genes related to synaptic remodeling and memory formation (45). Taken together with our present data suggesting that the mPFC exhibits rapid encoding-induced plasticity and represents an integral source of neocortical input to the hippocampus during memory encoding, the mPFC may indeed represent a critical node of memory processing in the brain. However, as memory encoding elicits the coordinated activity of widely distributed neural circuits (14), future studies delineating the relative contributions of various nodes of the neocortical network to hippocampal-dependent memory formation will likely provide critical insights into systems-level memory processing in the brain.

Converging evidence from human and animal studies suggests that metabolic demands render the prefrontal cortex preferentially vulnerable to Alzheimer’s disease (AD)-related neuropathology (40, 46–49). For instance, the mPFC is among the first brain regions to develop amyloid-β (Aβ) plaque deposition, an early pathological hallmark of AD (50). However, the mechanisms by which pathological alterations in the prefrontal cortex may contribute to memory impairment in AD remain unclear. Based on the present results, one possibility is that Aβ pathology in the prefrontal cortex may disrupt prefrontal-dependent activation of the entorhinal–hippocampal circuitry and thereby impair encoding of new long-term memories. In support of this view, human neuroimaging data suggest that functional connectivity between the prefrontal cortex, entorhinal cortex, and hippocampus is substantially decreased in the AD-affected brain (51). Finally, it is noteworthy that the pattern of gene expression we observed in the mPFC following memory encoding (i.e., up-regulation of synaptic plasticity-related genes, down-regulation of immune-related genes) is the inverse of the gene expression profile characteristic of AD (52) (i.e., up-regulation of immune-related genes, down-regulation of synaptic plasticity-related genes). As emerging evidence suggests that microglia, the resident CNS immune cells, induce synaptic pruning under conditions of dampened neural activity (53), dissection of the interplay between neuronal activity, synaptic plasticity, and immune cell activation is
Materials and Methods

Wild-type (B6SJL; Taconic Farms) male mice were used at 2.5 ± 0.5 mo of age for experimentation. All animal work was approved by the Committee for Animal Care of the Division of Comparative Medicine at the Massachusetts Institute of Technology. Materials and methods regarding behavioral analysis, immunohistochemistry, genome-wide RNA-seq, quantitative RT-PCR, electron microscopy, Golgi–Cox impregnation, electrophysiology, optogenetics, and statistical analysis are described in detail in SI Materials and Methods.

ACKNOWLEDGMENTS. We thank J. Gräff, R. Madabhushi, and T. X. Phan for reading and commenting on the manuscript; M. Taylor for mouse colony maintenance; and the Massachusetts Institute of Technology (MIT) BioMicro Center for technical assistance. This work was supported by BrightFocus Foundation Research Fellowship A2010D26F (to A.W.B.), the MIT Department of Brain and Cognitive Sciences (R.G.C.), and the MIT Picower Innovation Fund (L-H.T.).