Disrupted neural correlates of anesthesia and sleep reveal early circuit dysfunctions in Alzheimer models

Highlights
- CA1 firing rates are similar between WT and fAD model mice in active wakefulness
- Down-regulation of CA1 firing rates by NREM sleep and anesthesia fails in fAD mice
- fAD mutations impair stabilization of lower firing rate set points by anesthetics
- DHODH inhibition suppresses CA1 hyperexcitability under anesthesia in fAD mice

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In brief
Zarhin et al. show that firing rate dyshomeostasis is masked during active wakefulness but surfaces during anesthesia and NREM sleep in hippocampal circuits, preceding global sleep and memory disturbances in a mouse model of familial Alzheimer’s disease.
Disrupted neural correlates of anesthesia and sleep reveal early circuit dysfunctions in Alzheimer models

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SUMMARY

Dysregulated homeostasis of neural activity has been hypothesized to drive Alzheimer’s disease (AD) pathogenesis. AD begins with a decades-long presymptomatic phase, but whether homeostatic mechanisms already begin failing during this silent phase is unknown. We show that before the onset of memory decline and sleep disturbances, familial AD (fAD) model mice display no deficits in CA1 mean firing rate (MFR) during active wakefulness. However, homeostatic down-regulation of CA1 MFR is disrupted during non-rapid eye movement (NREM) sleep and general anesthesia in fAD mouse models. The resultant hyperexcitability is attenuated by the mitochondrial dihydroorotate dehydrogenase (DHODH) enzyme inhibitor, which tunes MFR toward lower set-point values. 

Ex vivo fAD mutations impair downward MFR homeostasis, resulting in pathological MFR set points in response to anesthetic drug and inhibition blockade. Thus, firing rate dys-toneostasis of hippocampal circuits is masked during active wakefulness but surfaces during low-arousal brain states, representing an early failure of the silent disease stage.

INTRODUCTION

Alzheimer’s disease (AD) is a progressive neurodegenerative disorder accounting for the vast majority of dementias. Hippocampal and cortical circuit dysfunctions are hypothesized to cause cognitive deficits in AD, such as episodic and spatial memory impairments. However, the onset of amyloid-β (Aβ) depositions precedes cognitive impairments by at least 10–20 years, marking a significant presymptomatic disease stage (Bateman et al., 2012; Long and Holtzman, 2019; Vermunt et al., 2019). By the time the earliest AD clinical symptoms are detectable, Aβ accumulation is close to reaching its peak, followed by intracellular aggregation of tau (Morris and Price, 2001). This suggests that homeostatic mechanisms successfully maintain critical aspects of neural circuits during early impairments of Aβ and tau proteostasis, but fail at some point, driving the emergence of the first symptoms (Frere and Slutsky, 2018; Styr and Slutsky, 2018). Identifying how circuit-level signatures are altered during the presymptomatic AD stage is crucial for understanding the transition from “silent” pathophysiology to clinically evident impairments.

Extensive experimental efforts over the past decades have identified the role of familial AD (fAD) mutations in early impairments of synaptic transmission and plasticity in hippocampal circuits (Fogel et al., 2014; Hsia et al., 1999; Li et al., 2009; Rice et al., 2019; Shankar et al., 2008; Wang et al., 2017; Willem et al., 2015). In addition to synaptic plasticity deficits, emerging evidence points to hyperexcitability of hippocampal and cortical neural networks in patients with amnestic mild cognitive impairment (Bakker et al., 2012), with AD (Horvath et al., 2021; Lam et al., 2017; Quiroz et al., 2010; Vossel et al., 2013, 2016), and in distinct AD mouse models (Busche et al., 2008, 2012; Hall et al., 2015; Minkeviciene et al., 2009; Palop...
Why is the activity of cortico-hippocampal circuits destabilized in early AD stages? It is hypothesized that homeostatic plasticity bidirectionally regulates neuronal activity around a stable set point to compensate for learning-related plasticity (Davis, 2006; Marder and Goaillard, 2006; Turrigiano and Nelson, 2004). Emerging evidence suggests that the distribution of firing rates among neurons in a neuronal circuit and their mean firing rate (MFR) are the key variables that are maintained around a set-point value in a process called firing rate homeostasis (Barnes et al., 2015; Hengen et al., 2013, 2016; Keck et al., 2013; Slomowitz et al., 2015; Styr et al., 2019). Our recent study suggests that homeostatic MFR set points are not predetermined but can be tuned by readjusting the compensatory feedback mechanisms to maintain a distinct MFR set-point value (Styr et al., 2019). Indeed, firing rate distributions and their means are physiologically regulated by sleep-wake states in distinct neural circuits, and this state-dependent regulation has been proposed to be homeostatic (Levenstein et al., 2017; Watson et al., 2016). MFRs decrease during sleep but return to higher MFR set points following transitions to active wakefulness in the rodent hippocampus and some areas of the cortex (Ju et al., 2014; Miyawaki and Diba, 2016; Niethard et al., 2021; Senzai et al., 2019; Vyazovskiy et al., 2009; Zhou et al., 2019). Other parameters of firing rate statistics are also regulated by sleep. For example, non-rapid eye movement (NREM) sleep, which makes up 80% of all sleep, is associated with homogenization of firing rate distributions, differentially regulating MFR of high-firing- and low-firing-rate neurons (Watson et al., 2016). This homeostatic regulation can operate at a local, layer-specificity scale in the cortex (Senzai et al., 2019). Thus, malfunction of state-dependent firing rate homeostasis in local neural circuits may be at the core of the early AD progression.

State-dependent changes in firing rates may be important for early progression of AD pathology. Aβ and tau soluble protein levels and aggregates are influenced by the sleep-wake cycle (Roh et al., 2012; Wang and Holtzman, 2020): their levels in the interstitial fluid are decreased by sleep increase and by sleep deprivation (Holth et al., 2019; Ju et al., 2017; Kang et al., 2009; Lucey et al., 2018; Xie et al., 2013). Furthermore, sleep is progressively deteriorated in patients with AD and in fAD mouse models, resulting in disrupted slow-wave activity (SWA) during NREM sleep, sleep fragmentation, and reduction in sleep time (Ju et al., 2017; Mander et al., 2015; Roh et al., 2012; Wang and Holtzman, 2020). We, therefore, hypothesized that local homeostatic dysregulation of MFRs in hippocampal circuits may take place before global changes in sleep become evident.

In addition to natural sleep, general anesthesia may constitute a distinct low-arousal brain state that could also reveal early firing rate dyshomeostasis.

We combined large-scale in vivo Ca2+ imaging and electrophysiology to study the functional changes in the hippocampal circuits of fAD mouse models before the onset of cognitive decline. Our results demonstrate that CA1 neural dynamics and MFRs were preserved during active wakefulness but disrupted during NREM sleep and anesthesia, resulting in pathological CA1 hyperexcitability. In line with our hypothesis, this local dysregulation of CA1 MFRs precedes global disturbances in SWA during NREM sleep. Studying network-level firing rate homeostasis ex vivo suggests that fAD mutations disrupt the basic regulation of MFR set points by general anesthetic and homeostatic response to inhibition blockade, resulting in pathological MFR set points. Teriflunomide (TERI), an inhibitor of the mitochondrial enzyme dihydroorotate dehydrogenase (DHODH), a recently identified signaling pathway of MFR set-point downregulation (Styr et al., 2019), suppressed CA1 hyperexcitability in anesthetized fAD mice. Overall, our study identifies a central role of low-arousal brain states in early vulnerability of hippocampal circuits in fAD models. Furthermore, it proposes that lowering firing rate set points during such states may present a new conceptual strategy for treating or preventing pathological hippocampal activity during the presymptomatic AD phase.

RESULTS

CA1 population activity is normal during active wakefulness in APP/PS1 mice

First, we characterized amyloid homeostasis and hippocampus-dependent memory in 4- to 5-month-old APP/PS1 (APP Swe/PS1ΔE9) mice. These mice display pathologically increased Aβ40, Aβ42, and Aβ42/Aβ40 ratio for both soluble and insoluble Aβ fractions (Figures S1A–S1C). Hippocampus-dependent memory functions, such as spatial working memory and context-dependent fear memory, were unimpaired at this age (Figures S1D–S1F). Notably, memory decline was evident at a more advanced disease stage, in 9-month-old APP/PS1 mice (Figures S1G and S1H).

To analyze neuronal activity in large neuronal populations in freely behaving mice, we employed wide-field, head-mounted miniaturized microscopes (Cai et al., 2016; Ghosh et al., 2011; Ziv et al., 2013) (Figures 1A and S2A–S2C). This technique enables tracking of Ca2+ dynamics with single-neuron resolution (Barretto et al., 2011; Ziv and Ghosh, 2015) as a proxy for neuronal activity (Chen et al., 2013). The integrated miniaturized microscope allows for high-speed and large-scale longitudinal recordings of Ca2+ dynamics from genetically defined neuronal populations in various deep brain structures, including the hippocampus, in freely behaving mice. Utilizing this method, we monitored activity patterns of thousands of CA1 pyramidal neurons and analyzed active neurons (NAs) at each session during active wakefulness. We imaged fluorescence generated by a genetically encoded Ca2+ sensor GCaMP6f (Chen et al., 2013) expressed in excitatory CA1 pyramidal neurons under the Ca2+/calmodulin-dependent protein kinase Ilz (CaMKIlz) promoter. Experiments were performed in six APP/PS1 mice (3,973 neurons total) and six wild-type (WT) littermates (3,846 neurons total), while they explored a
Figure 1. No deficits in the CA1 network activity and CA3-CA1 synaptic transmission during active wakefulness in APP/PS1 mice
(A) Large-scale Ca²⁺ imaging of excitatory cells expressing GCaMP6f using wide-field, head-mounted miniaturized fluorescence microscope in freely behaving mice during exploration of familiar environment.
(B) Relative fluorescence change traces of 10 cells randomly selected from the spatial locations depicted in Figure S3A, representing Ca²⁺ transients of cells detected in WT (left, blue traces) and APP/PS1 (right, red traces). Scale bars: 5 min; 10 Z scores.
familiar open field. Imaging was performed daily at regular hours during light phase to avoid circadian effects. Firing rate of single cells was approximated from the Ca\(^{2+}\) event rates using the CMNF-E method (Zhou et al., 2018) optimized for one-photon Ca\(^{2+}\) imaging (Figures 1B, 1C, and S2D–S2Q). No detectable changes in Ca\(^{2+}\) event rate distributions of CA1 neuronal populations were found between WT and APP/PS1 mice in active wakefulness (Figures 1B and 1C; Videos S1 and S2). Mean Ca\(^{2+}\) event rate (mCaR) per cell was very heterogeneous in both WT and APP/PS1 mice (Figure 1C). No differences were observed in physical activity of WT versus APP/PS1 mice during exploration of a familiar environment (Figures S3A–S3E). Detailed analysis of all recorded neurons revealed no difference in the median mCaR (Figure 1D) or in the number of N\(_S\) (Figure 1E), resulting in similar total level (N\(_S\), mCaR) of CA1 activity between WT and APP/PS1 mice (Figure 1F). Also, no differences were detected in the statistical attributes of CA1 network activity between behaving WT and APP/PS1 mice (Figures S3F–S3H).

Although somatic Ca\(^{2+}\) signals in neurons are used as a proxy of spiking activity, whether spike-to-Ca\(^{2+}\) transfer function is preserved under variable experimental conditions is unknown, and the sensitivity of microendoscopy for Ca\(^{2+}\) transients evoked by a single action potential remains to be improved. Therefore, we have chronically implanted tetrodes to directly record single-unit spiking activity in behaving WT and APP/PS1 mice. The recordings of CA1 firing rates were performed during the same hours of light phase as Ca\(^{2+}\) recordings, in a familiar environment (home cage). Vigilance state analysis was performed by analyzing local field potential (LFP) and electromyogram (EMG) in the same mice with single-unit recordings (Figures S6A and S6B) and in a separate batch of mice by analyzing electroencephalogram (EEG)/EMG and video recordings (Figure S4). Criteria for clustering of single units (Figures S5A–S5C) and separation of regular spiking (RS), putative pyramidal neurons from fast-spiking (FS), putative interneurons (Figures S5D–S5F) were based on a previous analysis (Petersen et al., 2021) and optimized for our recording conditions. Our results show no difference (p = 0.82) in MFR of the CA1 population of RS neurons between WT (2.01 ± 0.195 Hz) and APP/PS1 mice (1.88 ± 0.15 Hz) in active wakefulness (Figures 1G–1I). Furthermore, no difference was observed in MFRs of CA1 FS neurons (Figure S5G, p = 0.22). The duration of active wake state (Figure S6C) and physical activity of mice (Figures S6D–S6E) were similar between groups. Thus, two independent recording methods with single-neuron resolution, Ca\(^{2+}\) microendoscopy and single-unit electrophysiology, demonstrate that CA1 MFRs are unaltered during active wakefulness in early-stage APP/PS1 mice.

Finally, we recorded the extracellular field excitatory postsynaptic potentials (fEPSPs) in the CA3-CA1 pathway in awake WT and APP/PS1 mice. No changes were observed in CA3-CA1 synaptic transmission and short-term synaptic plasticity between awake WT and APP/PS1 mice (Figures 1J and 1K). Overall, these results demonstrate that CA1 firing rates, CA3-CA1 synaptic transmission, and short-term plasticity are not impaired during active wakefulness in APP/PS1 mice before the onset of memory decline.

**Dysregulation of CA1 MFRs during NREM sleep in APP/PS1 mice**

Next, we asked whether local homeostatic mechanisms underlying down-regulation of CA1 neuronal activity by NREM sleep (Miyawaki and Diba, 2016; Zhou et al., 2019) are maintained in the early-stage fAD mice. To analyze regulation of CA1 neuronal activity by sleep, we imaged CA1 dynamics, in parallel with LFP/EMG recordings, during the sleep-wake cycle of mice. The recordings were performed in five WT and four APP/PS1 mice during the same hours of the light cycle. Ca\(^{2+}\) imaging was analyzed during periods of wake-dense episodes dominated by active wakefulness (Figures 2A and 2E) and sleep-dense episodes dominated by NREM sleep periods (Figures 2B and 2F). As expected, WT mice showed ~60% reduction in total CA1 activity during NREM sleep because of a reduction in the number of N\(_S\) and the mCaR, in comparison to active wakefulness (Figures 2A–2D and 2). In contrast to WT mice, neither the number of N\(_S\) nor the mCaR was significantly changed during NREM sleep of APP/PS1 mice, resulting in similar total activity levels during active wake and NREM sleep periods (Figures 2G–2H and 2J). Thus, the typical negative regulation of CA1 population activity by NREM sleep was significantly diminished in APP/PS1 in comparison to WT mice (Figure 2K). These results indicate that homeostatic regulation of CA1 MFRs is impaired in a state-dependent manner in APP/PS1 mice.

**Local dysregulation of CA1 firing rates during NREM sleep precedes global deterioration of SWA in APP/PS1 mice**

Next, we asked whether local homeostatic regulation of firing rates by NREM sleep is impaired because of the deterioration of...
slow-wave oscillations, as reported in patients with AD (Lucey et al., 2019; Mander et al., 2015) and in fAD mouse models after the onset of cognitive decline (Kent et al., 2018). To compare CA1 firing rates between WT and APP/PS1 mice, we used single-unit recordings (Figures 3A and 3B). In WT mice, MFRs of CA1 RS neurons were decreased on average from 2.01 ± 0.195 to 1.51 ± 0.12 Hz during NREM sleep in comparison to active wakefulness (Figure 3C). We proceeded to quantitatively compare MFR of each unit in active wakefulness and NREM sleep across the entire dataset. A sub-population of CA1 high-rate excitatory neurons (defined as neurons whose MFRs during active wakefulness were above the median of 1.4 Hz) showed attenuation with median gain factor of −21% in NREM sleep (45% of all the units, Figure 3D). However, a sub-population of low-firing neurons (defined as neurons whose MFRs were below the median) displayed heterogeneous gain factors with a tendency toward a small increase (p = 0.055; Figure 3D), confirming the results of a previous study conducted in the cortex (Watson et al., 2016). This physiological regulation of firing rates in CA1 pyramidal neurons by NREM sleep was disrupted in APP/PS1 mice. On average, MFRs of the CA1 network were not different (1.88 ± 0.15 Hz in AW versus 1.91 ± 0.13 Hz in NREM; p = 0.53) between active wakefulness and NREM sleep (Figure 3E). The loss of negative regulation of the network MFRs by NREM sleep was due to the reconfiguration of MFRs within the local CA1 network. Namely, low-firing-rate units showed an increase in NREM sleep with median gain factor of +12% (p < 0.0001, 50% of all the units; Figure 3F), while the gain factor of high-firing-rate units was not affected by NREM sleep (p = 0.28; Figure 3F). Thus, fAD mutations augmented a positive effect of NREM sleep on low-firing-rate neurons, but diminished its negative effect on high-firing-rate neurons. FS neurons in the CA1 showed a similar regulation by NREM sleep: MFR reduction was prominent in WT but lost in APP/PS1 mice (Figures S5H and S5I). The duration of active wake and NREM sleep states was not different between the genotypes (Figure S6C).
Because global SWA is known to be disrupted in patients with AD (Lucey et al., 2019; Mander et al., 2015) and in fAD mouse models after the onset of cognitive decline (Kent et al., 2018), we used EEG/EMG recordings (Figures S4A–S4C) to test how global SWA is affected in APP/PS1 mice before robust cognitive impairments are evident. No differences in EEG SWA (spectral power of 0.5–4 Hz) during NREM sleep were found between WT and APP/PS1 mice (Figure 3G). Furthermore, EEG power spectra were not different for other vigilance states, as well as between WT and APP/PS1 mice (Figures S4D–S4F). In contrast to the similar global SWA in 4- to 5-month-old mice, EEG SWA significantly decreased in older 9-month-old APP/PS1 mice showing memory decline (p = 0.002; Figure S4G), as expected (Kent et al., 2018). Notably, the LFP SWA measured by tetrodes in the local CA1 circuitry showed a tendency toward lower levels during NREM sleep in 4- to 5-month-old APP/PS1 mice but did not reach statistical significance (p = 0.14; Figure 3H). This non-significant trend for lower SWA in CA1 LFPs represents an intermediate scale between the observed differences at the local single-neuron level and the lack of differences at the global EEG scale.

Taken together, Ca²⁺ imaging and electrophysiological data with single-cell resolution suggest that local down-regulation of CA1 MFRs by NREM sleep is disrupted in early-stage APP/PS1 mice, and this homeostatic dysregulation of firing rates precedes global deterioration of slow-wave oscillations.

**Loss of neuronal suppression during general anesthesia in APP/PS1 mice**

We next asked whether APP/PS1 mice also display dysregulated CA1 activity under a distinct low-arousal state, such as general anesthesia. We first used the volatile gas anesthetic isoflurane because of its fast kinetics. We assessed three consecutive conditions associated with distinct LFP patterns (Figures 4A and 4B; Figures S7A–S7C): exploration in familiar environment in active wakefulness (low-amplitude high-frequency activity), moderate anesthesia (1% isoflurane, high delta 1–4 Hz power, sporadic...
responses to tail pinching), and deep anesthesia (1.5% isoflurane, burst suppression, unresponsiveness to tail pinching). Importantly, electrophysiological markers of anesthetic depth (Figure S7D) and respiratory rate (Figures S7E and S7F) were closely monitored and showed no differences between anesthetized WT and APP/PS1 mice, ruling out altered respiration or pharmacokinetics as potential factors.

In WT mice, isoflurane caused a pronounced inhibition of CA1 population activity (Figures 4A and 4C; Video S3). The distribution of mCaRs across cells revealed suppression of total CA1 activity by ~57% and ~87% during moderate and deep anesthesia, respectively (Figures 4C and 4E), mainly because of reduction in the number of N_{As} in WT mice (Figure 4C). General anesthesia had a different effect on CA1 population activity in APP/PS1 mice when compared to WT mice. Both moderate and deep anesthesia states expressed a reduced number of N_{As} that was accompanied by an increase in mCaR (p < 0.05, right shift in the distribution; Figures 4D and 4E). As a result, total activity level was maintained across wakefulness and anesthesia (Figures 4D and 4E; Video S4). The loss of a typical response to general anesthetic resulted in CA1 hyperactivity in anesthetized APP/PS1 relative to WT mice (Figure 4E). Moreover, the number of discriminable micro-patterns of activity (microstates) was also reduced under anesthesia in WT, but not in APP/PS1, mice (Figure S8). CA1 population activity became hyper-synchronized in anesthetized APP/PS1 mice, in comparison to WTs, as reflected by more neurons participating in network bursts (Figure 4F) and more spikes evoked per network bursts (Figure 4G).

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transmission and spontaneous CA1 spiking activity were selectively impaired in anesthesia, but not in active wakefulness, in APP/PS1 mice.

To test whether the aberrant activity of CA1 neurons in APP/PS1 mice is specific to isoflurane or a general feature of anesthesia, we recorded CA1 activity under ketamine-xylazine (KX) anesthesia. Ketamine is a dissociative anesthetic, inducing anesthesia mainly through blockade of N-methyl-D-aspartate (NMDA)-type glutamate receptors (Franks, 2008), and is often supplemented with xylazine, an α2-adrenergic receptor agonist. In WT mice, KX anesthesia resulted in a profound reduction in CA1 activity but was much less efficient in APP/PS1 mice (Figures S9C–S9E). Finally, medetomidine (MED), another general anesthetic that selectively activates α2-adrenergic receptors, caused pronounced inhibition of CA1 activity in WT mice, while a much lower level of suppression was observed in APP/PS1 mice (Figures S9F–S9H). Collectively, our results indicate that APP/PS1 mice express abnormal profiles of neural activity induced by several distinct classes of anesthetics, and that such changes are not due to a specific anesthetic drug.

**Distinct fAD models express CA1 hyperexcitability during anesthesia**

Augmented and hyper-synchronous Ca2+ dynamics in the soma of excitatory CA1 neurons of anesthetized fAD model mice prompted us to investigate whether these mice express silent epileptiform spikes, similarly to those detected in sleeping patients with AD (Lam et al., 2017). To address this question, we tested how general anesthesia affects CA1 network excitability using *in vivo* electrophysiological recordings in the CA1 stratum radiatum across different mouse fAD models. In addition to the APP/PS1 model, we made measurements in 5XFAD (Oakley et al., 2006) and AppNl-ΔG-ΔF knockin model (Saito et al., 2014) (APP-KI). APP-KI mice express physiological APP levels (Saito et al., 2014) but demonstrate an increased Aβ42/Aβ40 ratio (Figures S1A–S1C). In brief, epileptiform high-voltage spikes were detected across all fAD models but rarely in WT mice during isoflurane anesthesia (Figures 5A and 5B). To further understand the relationship between anesthesia states and CA1 hyperexcitability, we quantified the frequency of abnormal spiking activity across anesthetics depth. State-specific analysis revealed higher epileptiform spike frequency for all three fAD models compared to WT, for both moderate and deep anesthesia (Figure 5C; Videos S5 and S6). Importantly, respiration rate showed no differences between all three fAD models and WT anesthetized mice at both moderate and deep anesthesia (Figures S7E and S7F). These results indicate that pathological CA1 hyperexcitability is a common neuronal network dysfunction that emerges during low-arousal states. Moreover, the robustness of anesthesia-induced CA1 hyperexcitability in APP-KI mice indicates that fAD mutations on their own, even without APP overexpression, are sufficient to cause CA1 hyperexcitability under anesthetic-induced alerted states of arousal.

**Downward firing rate homeostasis is impaired by fAD mutations**

The exposure of CA1 hyperexcitability under general anesthesia may be caused by impaired homeostatic regulation of MFRs. Hippocampal networks grown *ex vivo* on multi-electrode arrays (MEAs) have been previously established as an excellent model for dissecting the mechanisms of MFR homeostasis (Slomowitz et al., 2015; Styr et al., 2019). Therefore, we used this *ex vivo* platform to address the role of fAD mutations in firing rate homeostasis in response to the general anesthetic isoflurane. Spontaneous spiking activity of cultured hippocampal neurons grown on 120-channel MEAs was continuously monitored in an incubator chamber during a baseline recording period and for 24 h following application of isoflurane. Infusion of isoflurane (1%, 40 mL/min) stably reduced MFR in WT neurons without inducing a compensatory response (Figures 6A and 6C). This is in striking contrast to a typical MFR renormalization to the baseline level during 1 day in response to inactivity, induced by GABAA receptor agonist baclofen as an example (Figure 6F) (Slomowitz et al., 2015). The observed compensatory response...
to decreased spiking activity confirms the idea that homeostatic mechanisms maintain stable circuit function by keeping network MFR around a set point. Moreover, our data demonstrate that this effect is not specific to isoflurane but is characteristic of structurally distinct anesthetics (Figures S10A and S10B). These results indicate that anesthetic drugs disable compensatory homeostatic mechanisms; thus, they constitute negative regulators of MFR set points. In contrast to the persistent suppression of MFRs in WT networks, isoflurane induced smaller and transient MFR decrease in APP/PS1 networks, accompanied by a fast MFR compensation to the original set point (Figures 6B and 6D). As a result, significant hyperexcitability was observed following isoflurane application in APP/PS1 in comparison to WT networks (Figure 6E). Notably, fAD mutations did not significantly impair homeostatic MFR response to inactivity (Figures 6G and 6H). These results suggest that IAD mutations disrupt basic homeostatic regulation of MFR set points by isoflurane.

Because isoflurane and other volatile anesthetics are known to augment inhibition by prolonging inhibitory currents mediated by GABA<sub>A</sub> receptors (GABA<sub>A</sub>Rs) in hippocampal neurons (Jones and Harrison, 1993), among other targets, we decided to test how fAD mutations affect the homeostatic response to hyperactivity imposed by GABA<sub>A</sub>R blockade. As expected (Vertkin et al., 2015), application of a GABA<sub>A</sub>R antagonist gabazine (30 μM) caused a fast and pronounced increase in the population firing rate that gradually declined within 2 days to the set-point level (Figure 6I), despite the constant presence of gabazine. However, fAD mutations impaired MFR compensation in response to GABA<sub>A</sub>R blockade (Figure 6J), resulting in pathologically high MFR set points in APP/PS1, in comparison to WT networks (Figure 6K). Taken together, these results demonstrate that IAD...
mutations result in severe impairments of homeostatic MFR regulation, and these deficits can be uncovered even in 3-week-old cultured hippocampal neurons in the dish, disconnected from other brain structures. Specifically, fAD mutations dysregulate the downward homeostatic mechanisms that normally keep lower MFR set points by general anesthetics or return MFRs from hyperactivity.

DHODH inhibition suppresses CA1 hyperexcitability under anesthesia

Finally, we asked whether lowering MFR set points can present an effective way to suppress CA1 hyperexcitability under anesthesia in fAD mice. Our recent work has uncovered the mitochondrial DHODH enzyme as a regulator of MFR set points and Ca²⁺ buffering by mitochondria during spiking activity (Styr et al., 2019). Namely, we showed that DHODH inhibition by TERI negatively regulates CA1 MFR set points and suppresses CA1 hyperexcitability in a genetic model of Dravet syndrome (Styr et al., 2019), one of the most intractable and severe forms of childhood epilepsy. To test whether DHODH inhibition suppresses CA1 hyperexcitability associated with fAD mutations, we first compared the dose response of TERI on CA3-CA1 synaptic transmission in hippocampal slices prepared from WT and APP/PS1 mice. The IC₅₀ of TERI on fEPSP amplitude was not different between genotypes (Figure 7A; 17.6 µM in WT and 19.1 µM in APP/PS1), indicating that DHODH activity is not altered by fAD mutations. Moreover, a negative regulation of MFR set points by TERI was preserved in APP/PS1 networks (Figures S10C and S10D), suggesting that fAD mutations do not impair homeostatic regulation of MFR set point by DHODH. In addition, the concentration of orotate, the direct product of DHO oxidation by DHODH, was similar in the hippocampi of anesthetized WT and APP/PS1 mice (Figure 7B), confirming that DHODH activity is not affected by fAD mutations. Based on these results, we decided to inhibit cerebral DHODH in vivo and test how it affects pathological CA1 activity under anesthesia in APP/PS1 mice. Because TERI does not cross the blood-brain barrier efficiently (Vidal-Jordana et al., 2015), we used intracerebroventricular (i.c.v.) infusion of TERI (27 µg in 1 µL) versus vehicle (VEH; 1 µL) (Styr et al., 2019) to test the effect of DHODH inhibition on aberrant CA1 activity under anesthesia in APP/PS1 mice. On average, i.c.v. infusion of TERI caused a ~50% decrease in the rate of epileptiform high-voltage CA1 spikes in comparison to baseline recordings (Figures 7C, 7D, and 7F). In contrast, a similar amount of VEH did not affect...
aberrant CA1 activity (Figures 7E and 7F). These results indicate that cerebral DHODH inhibition dampens CA1 hyperexcitability under anesthesia in APP/PS1 mice.

**DISCUSSION**

Understanding how neural circuits are dysregulated at the early presymptomatic stages of AD is of utmost importance for detection, treatment, and perhaps even prevention of the disease. Electrophysiological and imaging studies provide compelling evidence of network-wide dysfunctions of neural circuits in patients with AD and animal models (Frere and Slutsky, 2018; Palop and Mucke, 2016; Vossel et al., 2017; Zott et al., 2018). Moreover, impairments of homeostatic control have been associated with a wide range of brain disorders, including neurodevelopmental (Mullins et al., 2016; Ramocki and Zoghbi, 2008) and psychiatric (Kavali and Monteggia, 2020) disorders. However, whether failures in firing rate homeostasis are gated by specific brain states and contribute to the disease progression remain unknown. Our results provide converging evidence on disrupted neural signatures of two low-arousal brain states, anesthesia and sleep, by fAD mutations at early stages in the disease progression.

Our study demonstrates that during active wakefulness, APP/PS1 mice are no different than WT mice in terms of CA1 firing rate distributions, CA3-CA1 synaptic transmission, and short-term synaptic plasticity, before the onset of hippocampus-dependent memory decline. The similarity of CA1 MFRs between WT and APP/PS1 mice in active wakefulness was confirmed by two independent recording methods with single-cell resolution: Ca²⁺ microendoscopy and single-unit electrophysiology. However, pronounced dysregulations of CA1 circuits were exposed by NREM sleep in fAD mice. Specifically, the down-regulation of CA1 MFR by NREM sleep was disrupted in fAD mice. If MFR fluctuates between stable homeostatic set-points across sleep-wake cycle, the observed MFR dysregulation during NREM sleep may be caused by fAD-related impairment of downstream MFR homeostasis (Figure 6) that is typically induced during extended sleep periods (Torrado Pacheco et al., 2021). Alternatively, dysregulation of MFRs during NREM sleep may result from the impairment of set-point establishment mechanisms. In this case, all the compensatory mechanisms may act in reference to this pathological set-point value, being detrimental for hippocampal functioning. The mechanisms leading to a pathological set point may be associated with abnormal changes in the neuromodulatory tone, which is typically diminished during wake-NREM transitions for the major neurotransmitters and neuromodulators, including acetylcholine, noradrenaline, histamine, orexins, dopamine, serotonin (Brown et al., 2012), and adenosine (Peng et al., 2020). Notably, cholinergic (Mesulam, 2013) and noradrenergic (Gruzdien et al., 2007) dysfunctions are evident in mild cognitive impairment and patients with early-stage AD. Further studies may shed some light on malfunctions in neuromodulatory signaling pathways that underlie dyshomeostasis of CA1 firing rates by NREM sleep in the presymptomatic AD stages.

Importantly, our data show that the observed local dysregulation of CA1 MFR set points during NREM sleep precedes global deterioration of SWA, an indicator of AD pathology at early stages of the disease (Lucey et al., 2019). Thus, the loss of natural brakes on spontaneous spiking activity during NREM sleep in local hippocampal networks may represent the earliest state-dependent homeostatic failure induced by fAD mutations. The homeostatic dysregulation of MFR set points during NREM sleep may underlie subclinical epileptiform activity, detected during sleep in a fraction of human patients with AD (Brown et al., 2018; Lam et al., 2017; Vossel et al., 2016) and in fAD models (Brown et al., 2018; Kam et al., 2016). How AD-related changes in synaptic and intrinsic neuronal properties (Frere and Slutsky, 2018; Palop and Mucke, 2016; Selkoe and Hardy, 2016; Sty and Slutsky, 2018; Zott et al., 2018) are altered by brain states and cause state-dependent dysregulation of activity set points remains to be discovered.

The results of this work, taken together with previous studies on the reciprocal relationship between neuronal activity and AD-related pathology (Ju et al., 2014; Wang and Holtzman, 2020), point to a complex role of NREM sleep in AD progression. On the one hand, accumulated evidence suggests that sleep mitigates AD-related pathology. Extracellular Aβ levels are regulated by spiking and synaptic activity, being decreased by activity suppression (Bero et al., 2012; Cirrito et al., 2005; Dolev et al., 2013; Kamenetz et al., 2003). Interstitial Aβ and tau levels are decreased by sleep and increased by sleep deprivation (Hoeh et al., 2019; Kang et al., 2009; Lucey et al., 2018; Xie et al., 2013). Because sleep is associated with a homeostatic decrease of MFRs (Mirowski and Diba, 2016; Senza et al., 2019; Vyazovskiy et al., 2009) and homeostatic down-scaling of excitatory synapses (de Vivo et al., 2017; Diering et al., 2017), this may be the cause of physiological fluctuations in the interstitial Aβ levels during the sleep-wake cycle (Roh et al., 2012). On the other hand, our results point to homeostatic dysregulation of firing rates by NREM sleep in local CA1 hippocampal circuitry of APP/PS1 mice. This may be a potential reason for disrupted Aβ fluctuations during the sleep-wake cycle in APP/PS1 mice (Roh et al., 2012). Because homeostatic dysregulation of CA1 MFRs precedes global sleep disturbances, rescue of these homeostatic mechanisms during sleep may provide an early opportunity to prevent or slow down deterioration of sleep patterns and subsequent accumulation of pathological AD hallmarks.

In addition to sleep, physiological regulation of firing rates was impaired during general anesthesia in fAD mice. General anesthesia is defined as a drug-induced reversible behavioral state that is associated with unconsciousness, amnesia, analgesia, and akinnesia, with concomitant homeostasis of vital physiological functions (Brown et al., 2011). Despite decades of research, experimental evidence is lacking on how anesthetic drugs affect homeostatic regulation of central neural networks. Utilizing an ex vivo system that enables long-term spike recordings from the same neurons in neural networks, we were able to show that distinct anesthetic drugs lower MFR set point in WT neural networks. However, the homeostatic response of APP/PS1 neurons to isoflurane was completely disrupted. Instead of a stable suppression, MFR was rapidly compensated and returned within 1–2 h to the original set point despite the presence of isoflurane. This disruption of homeostatic set-point regulation by anesthetic drug in hippocampal networks ex vivo, disconnected from other
brain structures, is translated to profound dysregulation of hippocampal activity under general anesthesia in fAD mice in vivo. At the level of the CA1 population, the hallmarks of anesthesia-induced changes in neuronal activity—suppression of firing rates and reduction in the number of discriminable network microstates—were impaired across anesthetic depth. As a result, hyperactivity and hypersynchrony of CA1 networks were exposed by anesthesia in several fAD models, including APP/PS1, 5XFAD, and APP-KI. In light of these results, anesthesia may account for some of the causes of neuronal hyperexcitability described in earlier two-photon Ca²⁺ imaging studies performed under isoflurane in the APP/PS1 model (Busche et al., 2008, 2012, 2019; Grienberger et al., 2012).

It is worth mentioning that only a few studies performed state-dependent analysis of aberrant brain activity in fAD mouse models. Among those, epileptiform activity was completely absent from wakeful behavior in Tg2576 mice (Kam et al., 2016). Interestingly, J20 mice showed fewer aberrant spikes during exploratory behavior associated with increased gamma activity (Hanson et al., 2020; Verret et al., 2012). At the single-unit level, APP-KI (AppNL-G F) mice displayed normal CA1 MFRs during active wakefulness, while exploring a familiar environment (Jun et al., 2020). In addition, normal MFRs were detected in the CA3 and dentate gyrus of APP/PS1 mice during exploratory behavior (Rechnitz et al., 2021). Even if some abnormal brain activity can be revealed under specific conditions in active wakefulness, our results strongly suggest a robust increase in excitability by general anesthetics as a common feature of different fAD models.

The anesthetic state and natural sleep share many neurobiological features, yet they are two distinct states (Akeju and Brown, 2017). Therefore, different molecular mechanisms may underlie dysregulation of neural correlates of NREM sleep and general anesthesia by fAD mutations. In this study, we found that fAD mutations do not impair DHODH enzymatic activity under anesthesia; thus, inhibition of cerebral DHODH by TERI effectively suppressed MFR set point ex vivo and pathological CA1 hyperexcitability in anesthetized APP/PS1 mice in vivo. These results suggest that identification of signaling pathways that (1) negatively regulate MFR set points and (2) are resilient to fAD mutations may present a therapeutic opportunity to prevent hippocampal hyperexcitability during low-arousal brain states. Notably, DHODH is primarily expressed in neurons in the brain (Schaefer et al., 2010) and regulates MFR via mitochondrial Ca²⁺ (Styr et al., 2019). Because dysregulation of mitochondrial Ca²⁺ has been recently implicated at the more advanced disease stages in the APP/PS1 model and in patients with AD (Calvo-Rodriguez et al., 2020), DHODH inhibitors may be powerful blockers of activity-dependent mitochondrial Ca²⁺ overload (Styr et al., 2019). Interestingly, the concentration of orotate, the direct product of DHODH enzymatic reaction, is decreased during NREM sleep in the cortex of WT mice (Bourdon et al., 2018). How fAD mutations affect cellular-molecular mechanisms underlying MFR down-regulation by anesthesia versus NREM sleep remains an important challenge for future investigations.

In summary, our data demonstrate that anesthesia and sleep, two distinct altered states of low arousal, expose CA1 network dysfunctions that are hidden during active wakefulness. The observation that early hippocampal pathophysiology emerges only during specific brain states provides perspectives on the circuit-level basis of AD-related vulnerability. Based on our data, we propose that sleep fragmentation and decrease in sleep time, detected at later AD stages, may present a compensatory response to CA1 neuronal hyperexcitability during NREM sleep in an effort to maintain daily MFR homeostasis. Whether impaired neural correlates of anesthesia and sleep constitute an early pathophysiological hallmark of presymptomatic AD remains to be determined by future human studies.

Limitations of the study
Some limitations should be considered when interpreting our results. First, the homeostatic nature of MFR downregulation during NREM sleep is still awaiting further experimental support. Second, although we demonstrate that fAD mutations impair upward MFR homeostasis in response to a chronic perturbation ex vivo, these results should be validated in vivo. Finally, although the DHODH inhibitor presents an opportunity to suppress AD-related epileptiform spikes under anesthesia, its role in sleep disturbances remains to be established.

STAR★METHODS
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Supplemental information can be found online at https://doi.org/10.1016/j.celrep.2021.110268.

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Author Contributions

D.Z. and R.A. equally contributed to this work. D.Z., R.A., and I. Slutsky designed the project. D.Z. designed, performed, and analyzed Ca2+ imaging and fEPSP/LFP experiments in behaving and anesthetized mice, with the help of H.B., S.S., O. Shinkamin, and A.S. R.A. designed, performed, and analyzed single-unit experiments, with the help of L.R.H. H.B. designed, performed, and analyzed EEG/EMG experiments. A.R. designed, performed, and analyzed MEA experiments, with the help of B.S. O. Scharf performed, and analyzed EEG experiments and analysis of samples prepared by M.H. and T.G. performed liquid chromatography-tandem mass spectrometry (LC-MS/MS) experiments and analysis of mouse brain. This work was performed in partial fulfillment of the requirements for a PhD degree by D.Z. and R.A. at the Sackler Faculty of Medicine, Tel Aviv University, Israel.

Declaration of Interests

The authors declare no competing interests.

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# STAR METHODS

## KEY RESOURCES TABLE

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| **Bacterial and virus strains** | | |
| AAV5-CaMKIIz-GCaMP6f | University of North Carolina Vector Core | Custom preparation |
| **Chemicals, peptides, and recombinant proteins** | | |
| Isoflurane | Sigma Aldrich | CAS: 26675-46-7 |
| Ketamine hydrochloride | Sigma Aldrich | CAS: 1867-66-9 |
| Xylazine hydrochloride | Sigma Aldrich | CAS: 23076-35-9 |
| Teriflunomide | Tocris Bioscience | Cat# 5069;CAS: 108605-62-5 |
| R-Baclofen | Tocris Bioscience | Cat# 0796;CAS: 69308-37-8 |
| Gabazine | Abcam | Cat# ab120042;CAS: 104104-50-9 |
| Buprenorphine | Sigma Aldrich | CAS: 52485-79-7 |
| **Experimental models: Organisms/strains** | | |
| Mouse: C57BL/6J | The Jackson Laboratory | JAX: 000664 |
| Mouse: APP/PS1 hemizygotes on a C57BL/6J-congenic background | The Jackson Laboratory | JAX: 005864 |
| Mouse: homozygous App<sup>K/L-G-F</sup> knock-in mice | Saito et al. (2014) | N/A |
| Mouse: heterozygous 5xFAD mice | Oakley et al. (2006) | N/A |
| **Software and algorithms** | | |
| Inscopix Data Processing Software 1.2.1 | Inscopix | https://www.inscopix.com |
| MATLAB | MathWorks | MATLAB 2019 |
| GraphPad Prism 8 | GraphPad | https://www.graphpad.com/scientific-software/prism/ |
| CNMF-E | Zhou et al., 2018 | https://github.com/zhoupc/CNMF_E |
| AccuSleep – sleep scoring software | (Barger et al., 2019) | https://github.com/zekebarger/AccuSleep |
| KlustaKwik – spike sorting software | Kadir et al. (2014) | http://klustakwik.sourceforge.net/ |
| Cell Explorer – cell type characterization | (Petersen et al., 2021) | https://cellexplorer.org/ |
| Custom MATLAB code used to produce the analysis results | This paper | https://doi.org/10.5281/zenodo.5779904 |
| **Other** | | |
| nVistaHD 2.0 or nVista 3.0 miniscopes + DAQs | Inscopix | https://www.inscopix.com |
| GRIN Lens Probe | Inscopix | 1050-002176 |
| Glass tube | vitroCom | CV1518-B-003 |
| Custom coverslips | Thermo scientific | 25 mm #0 (round) |
| Baseplate | Inscopix | 1050-004201 |
| Baseplate cover | Inscopix | 1050-002193 |
| Coated stainless steel wire | A-M system | 791400 |
| Male connectors | Harwin Inc. | M20-9990545 |
| Amplifier | custom-made amplifier | N/A |
| Digitizer | Digidata 1440A by Molecular Devices | https://www.moleculardevices.com/ |
| Monochrome camera | GigE Vision, Basler AG | https://www.baslerweb.com/en/ |
| Microdrive | Custom-built by Rogat Enterprises | N/A |
| Electronic interface board (EIB) | Custom-built by Rogat Enterprises | N/A |
| Recording wires (tetrodes) | California Fine Wire | Model:M283720 size: 17mm, Platinum 10% Indium |

(Continued on next page)
**RESOURCE AVAILABILITY**

**Lead contact**
Further information and requests for resources should be directed to and will be fulfilled by the lead contact, Inna Slutsky (islutsky@tauex.tau.ac.il).

**Materials availability**
This study did not generate new unique reagents.

**Data and code availability**
Datasets generated during the study will be shared by the lead contact upon request.

All original code has been deposited at Zenodo and is publicly available as of the date of publication. DOIs are listed in the key resources table.

Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

**EXPERIMENTAL MODEL AND SUBJECT DETAILS**

All animal experiments were approved by the Tel Aviv University Committee on Animal Care. In vivo experiments were performed on 4-5 month old male and female APP/PS1 (APP<sub>695/717</sub>/PS1<sub>ΔE9</sub>) hemizygotes (Stock No. https://www.jax.org/strain/005864, The Jackson Laboratory) and their wild-type littermates. In vivo electrophysiological experiments were performed on the following 4-5 month old transgenic mice and their littermates: (1) APP/PS1 mice, (2) homozygous App<sup>NL,G-F</sup> knock-in mice (Saito et al., 2014) (APP-KI), co-expressing Swedish (KM670/671NL), Iberian (I716F) and Arctic (E693G) mutations, and (3) heterozygous 5xFAD mice https://www.nature.com/articles/s41593-020-0624-8 - ref-CR12 (Oakley et al., 2006) (provided by Dr. Dan Frenkel lab), co-overexpressing mutant forms of human APP associated with the Swedish, the Florida (I716V) and the London (V717I) mutations. Only male mice were used for memory and sleep experiments, while both male and female mice were used for electrophysiological and Ca<sup>2+</sup> imaging recordings under anesthesia. All mice were on a C57BL/6J-congenic background. All animals were kept in a normal light/dark cycle (12h/12h, lights on at 7AM) with free access to food and water. Mutant and wild-type mice were housed together for behavioral, biochemical and EEG/EMG experiments. Mice for Ca<sup>2+</sup> imaging / single-unit recordings were singly housed after the microendoscopy / microdrive implantation.

**METHOD DETAILS**

**General surgical procedures**
In all the surgical procedures, the mice were anaesthetized with 5% isoflurane by volume for induction, injected i.p. with ketamine/xylazine (100 mg/kg ketamine and 8 mg/kg xylazine), head fixed to a stereotaxic apparatus (David Kopf instruments) and then maintained anesthetized by continuous isoflurane (1.5%) inhalation throughout the surgical procedure. Eye ointment was used to protect the mice eyes (Duratears, Vetmarket) and body temperature was recorded and maintained by a heating pad (FHC, DC temperature controller) at 34°C throughout the surgery. At the beginning of each surgical procedure the mice were injected sub cutaneous with Carprofen (5mg/kg) to reduce inflammation and pain. The mice were then allowed to recover in their home cage for at least 1-2 week before the subsequent surgical procedure or experiment began. When studying the effects of distinct anesthetics on physiological CA1 properties, individual anesthetics were used as specified in the figure legends.
For ICV injections, a small hole was drilled in the skull above the left lateral ventricle (0.7 mm posterior, 1.2 mm lateral to bregma), and a 5mm guide cannula was slowly inserted into the ventricle and fixed to the skull by dental cement (C&B Metabond, Parkell). The guide cannula was sealed with a 5 mm sterile metal bar to prevent CSF leakage and possible infections. 1-2 weeks after the surgery, the mice received i.c.v. injections using a 10 μl syringe (Hamilton). The mice were injected with 1 μl containing 27 μg of Teriflunomide (TERI), dissolved in DMSO or vehicle (VEH, 1 μl of DMSO) with speed of 0.15 μl/min (Nano Jet stereotactic syringe pump).

**LFP/fEPSP recordings**
Small diameter holes were drilled in the skull at the position of the recording and stimulating electrodes. The recording electrode (bipolar, PFA-coated stainless steel; 0.127 mm diameter) was slowly lowered through the cortex into the CA1 *stratum radiatum* (2.06 mm posterior to bregma; 1.5 mm mediolateral, ML; 1.5 mm dorsoventral from bregma, DV), and the stimulating electrode (bipolar stainless steel; 0.127 mm diameter) was slowly lowered through the cortex into the Schaffer Collateral (SC, 2.54 mm posterior to bregma; 2.75 mm ML; 2.2 mm DV). Ground electrode was screwed to the skull above the cerebellum. Test stimuli of 50-100 μA were delivered to the SC at 0.06 Hz to verify the proper location of the electrodes and to estimate the stability of the signal. The electrodes were firmly fixed to the skull by dental cement (C&B Metabond, Parkell) and dental acrylic. Operated mice recovered in their home cage for at least a week following electrodes implantation. For evoked fEPSP recordings in CA3-CA1 synapses in awake mice (Figures 1J and 1K), mice were habituated for a few days to the experimental device that was composed from a running wheel and metal bar in which the mice were head fixed into throughout the experiment with a screw. Following habituation, the mice were head fixed to the experimental device, and CA3-CA1 fEPSP measurements were taken 30 minutes after.

Extracellular field potentials and electromyograms were amplified x100 using a custom-made amplifier, band-pass filtered between 0.1 Hz and 4 KHz, and digitized by Digidata 1440A at 56 kHz sampling rate (Molecular Devices). Data were analysed using Clampfit 10.7 (Molecular devices) for fEPSP’s or custom MATLAB functions (MathWorks).

**EEG/EMG recordings**
EEG screws were placed over the frontal and parietal cortices. Ground and reference screw electrodes were placed above the cerebellum. Neck muscle electrodes were implanted bilaterally, and bipolar referencing was used for EMG. A custom-made EEG/EMG connector was fixed on the skull with dental cement for sleep recordings.

**Surgical procedure for Ca²⁺ imaging**
The surgical procedures were previously described (Ziv et al., 2013). First, 500 nL of the viral vector AAV5-CaMKII-GCaMP6f (prepared by University of North Carolina Vector Core) was injected into the CA1 pyramidal layer at the following coordinates: −2.1 mm AP, −1.5 mm ML, and −1.3 mm DV to bregma. The skin was sutured and disinfected using Betadine solution. Two weeks after virus injection, a glass guide tube was implanted directly above CA1. For this, a trephine drill was used to remove a circular part of the skull located postero-lateral to the viral injection site, and the dura, cortex and the hippocampal commissures above the CA1 were removed by suction with a 29 gauge blunt needle while constantly washing the exposed tissue with sterile PBSx1. A glass guide tube was then implanted above the CA1 *stratum pyramidale*. A recording electrode (bipolar stainless steel; 0.127 mm diameter) was slowly lowered through a hole that was drilled adjacent to the guide tube into CA1 *stratum radiatum* (1 mm DV). The space between the skull and the optical guide tube was sealed using a low toxicity silicone adhesive (Kwik-Sil, WPI surgical instruments) and the remaining exposed area of the skull was covered with dental cement and dental acrylic. A metal bar was added to the posterior aspect of the construct in order to head fix the animal when needed.

**Anesthetic depth analysis**
Anesthetic depth was modulated by isoflurane level: 1% for moderate anesthesia and 1.5% for deep anesthesia. For vital physiological measurements, mice were placed in an induction chamber connected to isoflurane vaporizer (Isotec 5), gas flow rate was turned on to 0.8 LPM and the vaporizer was set at 5% isoflurane. When mouse’ breathing slowed down and became rhythmic, it was moved to physiological monitoring system (75-1500 Harvard Apparatus) and isoflurane level was reduced gradually and slowly to either 1.0% or 1.5%. Respiratory rate was monitored and analyzed by the physiological monitoring system. Temperature was also maintained by the device at 37°C throughout the procedure. No changes in respiratory rate was found between all the genotypes used in the paper at 1.0% and 1.5% isoflurane (Figure S9). Analysis of burst-suppression ratio (BSR) is described in ‘quantification and statistical analysis’ section.

**Ca²⁺ imaging in behaving and anesthetized mice**
For time-lapse imaging in behaving mice we used an integrated miniature fluorescence microscope (nVistaHD 2.0 or nVista 3.0, Inscopix) as previously described (Ziv et al., 2013). At least two weeks after the glass guide tube implantation, we inserted a microendoscope consisting of a metal guide cannula (~3.1mm length, 1.8mm outer diameter) and a single gradient refractive index lens (4.0mm length, 1.0mm diameter) into the implanted glass tube and examined Ca²⁺ indicator expression in the operated mice (Inscopix data acquisition software, Inscopix). We selected for further imaging only those mice that exhibited homogenous GCaMP6f expression throughout the field of view, without signs of injury or inflammation (~85% mice passed the selection criteria). For the selected mice, we then affixed the microendoscope within the glass guide tube using ultraviolet-curing adhesive (Flow-It A3,
Pentron). Next, we attached the miniature microscope’s magnetic base plate to the dental acrylic surface with the ultraviolet-curing adhesive. A day later, the mice were habituated for 4-5 days to freely explore a 50 x 50cm open-field, with the miniature microscope. Before the beginning of each session, the open-field was thoroughly cleaned with a 70% Ethanol solution. To record mouse behavior, we used an overhead monochrome camera (GigE Vision, Basler AG), which we synchronized with the miniature microscope. Behavioral analysis was performed with ToxTrac software (Rodriguez et al., 2018). Ca^{2+} imaging was performed at 10 Hz. Imaging sessions consisted of 5-15-min-long trials, while the inter-trial interval was ≥ 15 min.

For Ca^{2+} imaging sessions during different types of anesthesia, the mice were head-fixed to a stereotactic device before the application of anesthesia and their body temperature was maintained by a temperature controller (FHC, 40-90-8D). LFP activity in the CA1 stratum radiatum was recorded throughout the duration of anesthesia and Ca^{2+} measurements were taken once a stable LFP pattern was observed, 60 minutes following anesthesia induction. For the inhalatory anesthetic isoflurane, experiments were performed under 1.0% and 1.5% isoflurane, combined with oxygen (100%). Ketamine (100 mg/kg), supplemented with xylazine (8 mg/kg), were injected i.p. Medetomidine (0.3 mg/kg) was injected i.p and stopped using the synthetic α2 adrenergic receptor antagonist Antipamezole (1mg/kg, i.p). The drugs were diluted with sterile PBS before injection.

For Ca^{2+} imaging during sleep-wake cycle, two stainless-steel wires were inserted to either side of neck muscles, and referenced bipolarly, to measure EMG activity. Imaging analysis was performed during extended (≥ 5 min with less than 30 sec interruption by the other state) bouts of active wakefulness or NREM sleep based on LFP/EMG recordings during 6 hours of the light cycle (10 AM – 4 PM). 5 kHz noise in the LFP/EMG recordings generated by electronic focus of the nVista3 microscope was filtered out by a band-pass filter (1-300 Hz). Mice were habituated to the recording chamber for several days before the experiment.

**Single-unit surgery and data acquisition**

Mice were implanted with a costume-made microdrive (custom printed circuit board and drive by Rogat, Carmiel, Israel) along with neck muscle electrodes implanted for electromyography (EMG). The microdrive contained a moveable assembly of 4 tetrodes (17-μm, Platinum 10% Iridium, California Fine Wire) and was connected to the recording setup via an Omnetics headstage connector (Connector Corporation, Minneapolis MN, USA). Two holes were drilled in the skull: one in the frontal bone plate for a screw serving as ground; the second hole for the electrodes implanted in the parietal cortex (1.94 mm posterior of bregma; 1-1.2 mm medial lateral axis; 1-1.2 mm dorsal ventral axis). After 7 days of monitored recovery, subsequent downward movements of the microdrive were made in 25- to 50-μm increments over 24-hr intervals until approaching the CA1 pyramidal cell layer, recognized by the appearance of multiple high-amplitude units and spontaneous ripple events. At the end of the experiment, a small electrolytic lesion was made (30 μA for 20 sec) under anesthesia. Two days after, histology procedure was performed to verify electrodes location as described (Weiss et al., 2017).

Animals were recorded during the first half of the light cycle in a familiar environment (home cage). Raw data were sampled at 24 KHz using a Neurophysiology Workstation (RZ5D base processor and PZ5 NeuroDigitizer amplifier, Tucker-Davis Technologies Inc).

**Detection of pathological spikes in FAD models**

High-voltage pathological spikes were detected by setting a threshold 10 z-scores above and below the mean voltage during the entire recording and accepted only if their peak-to-peak value within 30 ms was greater than 10 z-scores. A dead-time of 50 ms was set to assure the same spike was not counted twice. Subsequently, all spikes were manually inspected and approved.

**Scoring of vigilance stages**

Vigilance states were manually scored in 5-s epochs based on visual inspection of frontal EEG or CA1 LFP and EMG as previously described (Barger et al., 2019). Wakefulness was defined by low-amplitude, high-frequency EEG/LFP activity, recorded in the frontal lobe, and high EMG activity. Wakefulness was further divided to states of active wake (exploration, grooming, eating) and quiet wake based on video and EMG recording. NREM sleep was defined by high-amplitude, low-frequency EEG/LFP, and reduced EMG tone. REM sleep was defined by low-amplitude, high-frequency EEG/LFP, dominated by theta activity, and with flat EMG. States transitions and artifacts (<3% of recording time) were excluded from further analysis. Spectrograms were constructed by Fourier frequency transformation (1 second bins) of the EEG signal.

**MEA**

Postnatal hippocampal cultures were plated on MEA plates containing 120 titanium nitride (TiN) electrodes, in addition to 4 internal reference and 4 ground electrodes (Styr et al., 2019). Each electrode has a diameter of 30 μm and electrodes are arranged in a 12X12 grid (sparing 6 electrodes in each corner), spaced 100-200 μm apart on average [Multi Channel Systems (MCS), 120MEA200/30IR-Ti]. Data acquisition was done in 3-weeks-old cultures using a standard MEA2100-Systems and MEA2100-mini-Systems (MCS) with a hardware filter cut-off of 3.3 kHz and sampling rate of 10 kHz per electrode. Recordings were carried out under constant 37°C and 5% CO2 levels.

**Electrophysiology in slices**

Acute hippocampal slices (coronal, 400 μm) were prepared from WT and APP/PS1 mice. Slices were transferred to a submerged recovery chamber at 32°C containing oxygenated (95% O2 and 5% CO2) artificial cerebrospinal fluid (ACSF) for 1h before the
experiment. The ACSF contained, in mM: NaCl, 125; KCl, 2.5; CaCl₂, 1.2; MgCl₂, 1.2; NaHCO₃, 25; NaH₂PO₄, 1.25; glucose, 25. fEPSPs were recorded in acute hippocampal slices with a glass pipette containing Tyrode solution (1 – 2 MΩ) from synapses in the CA1 stratum radiatum using a MultiClamp700B amplifier (Molecular Devices). Stimulation of the Shaffer Collateral (SC) pathway was delivered through a glass suction electrode (10 – 20 μm tip) filled with Tyrode. Data were analyzed using pClamp10 (Molecular Devices).

**Protein extracts and ELISA for Aβ**

After transcardial perfusion with cold PBS hippocampus were dissected, snap-frozen in liquid nitrogen, and stored at –80°C until use. Proteins from both hippocampus of 5 month old APP/PS1 and APP-KI mice were sequentially extracted in a 2-step procedure. Tissue (~0.2 mg/mL wet weight) was homogenized using a mechanical homogenizer in 50 mM Tris-HCl, pH 8.0, 150 mM NaCl, complete proteinase inhibitor cocktail (Roche) and 10 mg/mL Pepstatin A, centrifuged at 100,000 g for 1 hour at 4°C, and supernatants containing the soluble fraction were collected, stored at –80°C, and used for quantification of soluble Aβ. Pellets were resuspended, incubated on ice for 1h and sonicated in 6 M guanidine-HCl, 50 mM Tris-HCl, pH 7.4, centrifuged at 100,000 g for 1 h at 4°C, and supernatants containing membranous fraction and insoluble aggregates were collected, stored at –80°C, and used for quantification of insoluble Aβ. Protein quantification was performed using Bradford method. The levels of soluble Aβ40 and Aβ42 in mice hippocampus extracts were detected using sandwich ELISA kits (Wako, Japan), according to the manufacturer’s instructions. Concentrations of soluble and insoluble Aβ are expressed in pM per μg of total soluble or insoluble protein, respectively.

**Spatial working memory test**

Spatial working memory was examined using a continuous variation of the T-maze (Wood et al., Neuron, 2000). In sum, mice were required to traverse the central arm of a delta-shaped maze and alternate between left and right turns during subsequent trials. Correct trials were reinforced with a drop of condensed milk at the edge of the selected side arm. After entering one of the side arms, retracing was prevented by hinged doors such that mice could initialize a new trial only by returning to the base of the central arm via a connecting arm. At the base, mice were confined for a specified delay before the door to the central arm was opened and a new trial commenced. The first trial of each session contained a reward in both side arms of the maze. Prior to the onset of training, mice were habituated to the apparatus for 15 min a day for 5 consecutive days. During training, mice performed 10 trials of the task with a 10 s delay. During testing, the delay varied between days from 10 to 180 s. To facilitate learning and assure consistent motivation when performing the task, food intake of mice was restricted such that their weight was kept at 85 – 90% relative to ad libitum feeding. Success rate was defined as the percentage of correct alternations relative to the number of trials.

**Contextual fear conditioning test**

Contextual fear conditioning (CFC) was performed in a 25.5 x 25.5 x 36 cm chamber with electric grid floor. For the conditioning session, the mice were placed in the CFC apparatus for 2.5 min, and then a pure tone (2.9 kHz) was introduced for 20 sec, followed by a 0.6 mA foot shock for 2 sec. Another tone and shock were introduced again after 1 min, and then, 30 sec after the second shock, the mice were returned to their home cages. The context in which the mice received foot shock consisted of green lighting, white noise, vanilla scent and square perspex walls. To test contextual fear memory, mice were placed back in the familiar context for 5 min. The mice were later placed in a novel context for 2.5 min, consisted of white lighting, rum scent and round walls. The apparatus was cleaned between every session using 70% Ethanol and Virusolve. Automatic freezing detection of the recorded videos was conducted using the EthoVision software.

**Histological verifications**

To check the expression of AAV5-CaMKII-GCaMP6f in the CA1 and the precision of the injection location and micro endoscope implantation site, we used 2-photon microscope (LSM 7 MP, Zeiss) to image the pyramidal layer in hippocampal slices. Chameleon Ti:Sapphire laser system with a 80 MHz repetition rate was used to excite the sample. The excitation wavelength was 920 nm. Emission light was filtered by 500 – 550 nm band-pass filter. Three-four weeks after injection of the virus or at the end of the behavioral experiments, we perfused mice with phosphate-buffered saline (PBS) followed by cold 4% paraformaldehyde (PFA). We then removed the perfused brains and kept them in PFA solution for 24h. 70-μm coronal slices were obtained from the perfused brains using a Leica VT1200 vibrating microtome and stored in PBS for further imaging. Validation of the injection site, implanted micro endoscope location and viral expression was obtained by imaging in 2-photon microscope.

**Metabolic profiling**

For determining orotate concentration in the hippocampus of WT and APP/PS1 mice, mice were anesthetized by 1.5% isoflurane and hippocampi were dissected and then homogenized by Bullet Blender homogenizer (Next Advance) at 4°C with methanol: acetonitrile: water (5:3:2 ratio) solution at 40 mg/ml concentration. Homogenized samples were centrifuged for 15 min at 4°C at 16,000 x g. The supernatants were transferred to glass HPLC vials and stored at –80°C. Metabolic profiling was done using an Ultimate3000 UHPLC system (Dionex, Thermo Scientific) coupled to a Q-Exactive Plus mass spectrometer (Thermo Scientific). Metabolite separation was done using a 49 min gradient of buffer A (95% acetonitrile) and buffer B (50 mM ammonium carbonate, pH10, 5% acetonitrile) using SeQuant ZIC-pHILIC column (Merck; 150 3 2.1 mm, 5 mm) coupled to a SeQuant ZIC-pHILIC guard column (Merck; 20 3 2.1 mm,
CellExplorer open source software (Petersen et al., 2021), clusters were separated to regularly-spiking and fast-spiking units semi-automatically by inspecting their waveform, autocorrelations and burst index (Royer et al., 2012).

Next, we measured the relative synchronized activity per time bin of 100 ms, defined as:

\[
(\text{Relative Synchronization})_n = \frac{\sum (\text{Active cells})_n}{(\text{Total cells detected})}
\]

In which "n" equals to the time bine analyzed. Synchronization vector peaks that were higher than the 90th percentile in exploration sessions, or higher than 0.05 in anesthesia sessions were defined as network bursts. Threshold of 0.05 was chosen for anesthesia sessions since it gave the most reliable network bursts detection based on visual inspection and verification of the data by the experimenter. Similar results were obtained when testing different thresholds (e.g. 0.1 and 0.15, data not shown). We than analyzed
the inter-burst interval, number of cells that were active in each network burst, and number of spikes that comprised each network burst. 20 random samples were taken from each mouse for pooled analysis.

**Burst-suppression ratio analysis**

For detecting burst-suppression (deep anesthesia), raw LFP recordings were divided into 500 ms bins and for each bin three parameters were calculated: (1) maximum absolute value, (2) standard deviation, and (3) the first principle component of the spectrogram calculated between 1-100 Hz. Each of these parameters showed a bimodal lognormal distribution and thus were used to classify the bins as periods of bursts or suppression using a gaussian mixture model. Epochs of bursts were merged if the inter-burst interval was less than 2 seconds and were accepted as bursts only if their duration was greater than 2 seconds. These criteria were empirically selected as they increased the algorithm’s robustness. Further, manual scoring revealed that indeed >95% of bursts were longer than 2 seconds. Burst-suppression ratio (BSR) was calculated in ~1 min bins (52.428 s) as the fraction of time in suppression. Overall, this algorithm demonstrated >92% accuracy when compared to manual scoring of two data sets from different genotypes. Relative delta power was calculated in ~1 min bins as the power spectral density (PSD) between 1-4 Hz divided by the broadband PSD in 1-100 Hz. This ratio was z-scored and normalized between 0 and 1. Epochs of deep anesthesia were defined as 0.3 < BSR < 0.8 and epochs of moderate anesthesia were defined as BSR < 0.3 and relative delta power > 0.5. These epochs were merged if the inter-epoch interval was less than 1 min and were accepted only if their duration was greater than 1 minute.

**Microstate clustering**

**t-SNE/WS algorithm**

We identified microstates of CA1 hippocampal neuronal population using the unsupervised nonlinear embedding method, t-Distributed Stochastic Neighbor Embedding (Laurens van der Maaten, 2008) (t-SNE) and an image processing algorithm – watershed (WS) combination into t-SNE/WS clustering algorithm. The input for the clustering algorithm was the inferred, de-convoluted spiking patterns based on Ca²⁺ signals provided by the CMNF-E algorithm. The analysis was performed on active frames that included at least 2 co-active neurons as described in an earlier study (Wenzel et al., 2019), with some modifications related to initial dimensionality reduction and perplexity value calculation. For noise reduction, we used an initial dimensionality reduction based on principle component analysis (PCA). The principal components >30th percentile of the derivative of the cumulative explained variance were selected and the perplexity parameter for the t-SNE was calculated as \[ \sqrt{\text{number of active frames}} \] in each experiment. Using the calculated perplexity value and initial dimensionality reduction, t-SNE was applied with 1000 iterations to produce a robust 2D embedding space that could be analyzed and visualized. To create density maps, the embedded points on the 2D map, representing patterns of coactive cells per frame, were smoothed by a Gaussian kernel with a standard deviation equal to 1/60 of the maximum coordinate in the embedded space. To turn patterns of coactive neurons into separated microstates, we used a watershed algorithm (Wenzel et al., 2019) on the density map. A microstate was defined as a region created by the WS algorithm which contains a cluster of similar co-activity patterns based on Ca²⁺ imaging data analyzed in Figure 2. The size of each microstate depends on the frequency of a specific pattern during the recording session. For clustering validation, we used an inter- and intra-cluster correlation score, calculated based on PCA analysis. In order to calculate the number of microstates in a randomized data, we permuted the activity in each frame, so the number of co-active cells in each frame was maintained, while the cells’ identity was different.

**Affinity propagation clustering**

We used the affinity propagation cluster (APC) algorithm (Frey and Dueck, 2007) as an additional microstates clustering method. It is an efficient clustering algorithm that takes as inputs the similarities between pairs of observations in the dataset (frames, in our case), and finds exemplars and the observations they represent by exchanging real-valued messages between data points. We used the MATLAB (Mathworks) ‘apcluster’ function made available by the authors. We used the post-PCA correlation between frames as the measure of similarity used by the algorithm. We also used convicts = 10 and a preference equal to the median similarity of the dataset.

**Hierarchical clustering**

We used MATLAB’s ‘linkage’ function to build a hierarchical bottom-up tree with weighted correlation metric based on the PCA data. ‘Cluster’ function was used to cluster the tree with distance cutoff of 0.75.

**MEA data analysis**

Raw data was filtered, offline, at 200 Hz using a Butterworth high-pass filter. Spikes were then detected, offline, using MC Rack software (MCS) based on a fixed threshold set to between 5-6 standard deviations from mean. Twenty minutes of each hour (that were previously shown to reliably represent the MFR of the entire hour, were used for analysis to reduce processing time and analyzed using custom-written scripts in MATLAB (Mathworks) as previously described (Slomowitz et al., 2015)). Channels with unstable (>30% change of MFR) baseline recordings during 3-4 hr prior to a perturbation were excluded from the analysis.

**Statistical analysis**

Error bars shown in the figures represent SEM. All the experiments were repeated at least in three different animals and repeated within the animal at least twice. Statistical significance was assessed by unpaired or paired Student’s t-tests, Mann-Whitney U-tests,
one-way analysis of variance (ANOVA), or two-way ANOVA, where appropriate (multiple comparison tests are specified in figure legends). Normality was assessed using the Shapiro-Wilk test. For non-normal distributions, differences between groups were tested with Wilcoxon signed-rank test for paired data and Mann-Whitney test for unpaired data. Comparison of distributions was performed by Kolmogorov-Smirnov test. Statistical analysis was performed using Prism 8.0 GraphPad. The statistical test, p value and the number of cells / mice that went into the calculation are reported in figure legends. Significance was declared at p < 0.05 and all tests were two-sided.
Supplemental information

Disrupted neural correlates of anesthesia and sleep reveal early circuit dysfunctions in Alzheimer models

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SUPPLEMENTARY INFORMATION

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This file includes:

Figures S1 to S10
Movies S1 to S6
Figure S1. Soluble and insoluble Aβ40 or Aβ42 levels in the hippocampus of WT and cognitively-unimpaired APP/PS1 mice

(A) Soluble Aβ40 or Aβ42 levels in WT (n = 5), APP/PS1 (n = 5) and APP-KI (n = 9) 5-m.o. mice.

(B) Insoluble Aβ40 or Aβ42 levels in WT (n = 5), APP/PS1 (n = 5) and APP-KI (n = 9) 5-m.o. mice.

(C) Aβ42/Aβ40 ratio in soluble and insoluble fractions (the same data as in A,B).
(D) Success rate in a continuous variation of the T maze revealed no difference in spatial working memory between WT (n = 12) and APP/PS1 (n = 11) mice (p = 0.69, Two-way ANOVA with Sidak’s multiple comparison tests: 10 sec p = 0.66, 1 min p = 0.62, 3 min p = 0.49).

(E) No difference in contextual fear memory, tested 1 day after acquisition, between 4-5 m.o. WT and APP/PS1 mice (p = 0.29, WT n = 25, APP/PS1 n=19).

(F) Contextual fear memory was specific, reflected by reduction in freezing in the novel context in both, WT and APP/PS1 mice (the same mice as in E).

(G) Contextual fear memory was impaired in 9-m.o. APP/PS1 mice in comparison to WT littermates (p = 0.029, WT n = 14, APP/PS1 n = 14).

(H) Contextual fear memory was specific, reflected by reduction in freezing in the novel context in both, WT and APP/PS1 mice (paired t-test, the same mice as in G).

Two-way-ANOVA with Dunnett’s (A-C) and Sidak (D) multiple comparisons test, un-paired t-test (E,G), paired t-test (F,H) were used for the analysis. *p < 0.05, **p < 0.01, ***p<0.001, ****p<0.0001, ns – non-significant. Error bars represent SEM.
Figure S2. Large-scale Ca$^{2+}$ imaging using wide-field, head-mounted miniaturized fluorescence microscope

(A) Coronal section of the cortex and hippocampus showing the area of cortex aspirated (red dotted line) and labeled excitatory CA1 pyramidal neurons expressing the genetically encoded Ca$^{2+}$ sensor GCaMP6f (white dotted square).
(B) Enlargement of a, showing individual excitatory CA1 pyramidal neurons expressing the genetically encoded Ca\textsuperscript{2+} sensor GCaMP6f and the approximate location of the GRIN lens used for micro-endoscopic imaging (red dotted line).

(C) A mouse carrying a miniaturize fluorescence microscope.

(D) Representative traces obtained from the imaging data using the CNMF-E (Zhou et al., 2018) algorithm. Relative scaled fluorescence changes (blue traces) and the denoised versions (black line) of three signals with different peak-to-noise ratios are presented. Scale bars: 1 minute, 20 z-scores (two upper traces) and 5 z-scores (bottom trace).

(E-G) ROIs were limited based on different exclusion criteria: minimum peak-to-noise ratio of 8 (G), ROI size below 30 and above 300 pixels (E,G) and minimum circularity estimate of 0.5 (F,G).
Figure S3. No difference in CA1 neuronal activity pattern and behavioral activity in the open field between WT and APP/PS1 mice

(A) Representative traces of WT and APP/PS1 mice locomotor activity while freely exploring a familiar open field for 15 minutes.

(B-E) The two experimental groups (6 mice in each group) showed no behavioral differences in the mobility rate (D, p = 0.59), average speed across the imaging session (E, p = 0.60), average acceleration across the imaging session (F, p = 0.94) and total traveled distance (G, p = 0.99). The analysis relates to the mice analyzed in Figure 1C-F. Mann-Whitney U test (D-G) was used for the analysis. ns, non-significant. Error bars represent SEM.

(F-H) Patterns of Ca^{2+} transients during free exploration of a familiar environment were similar between WT (6 mice) and APP/PS1 (6 mice). Specifically, no difference was found in the inter-network burst interval (A, p = 0.60), number of cells that participate in each network
burst (B, \( p = 0.55 \)), and the number of spikes that constitute each network burst (C, \( p = 0.59 \)).
Relates to the data in Figure 1C-F. Kolmogorov-Smirnov test.

ns, non-significant. Error bars represent SEM.
Figure S4. Vigilance states scoring and state-dependent EEG spectral power in WT and APP/PS1 mice

(A-C) Representative data from a WT mouse.

(D) Active Wake

(E) Quiet Wake

(F) REM

(G) NREM (9-month-old mice)
(A) Representative EEG (top panel) and EMG (bottom panel) traces in different vigilance states: Active WAKE, Quiet WAKE, NREM and REM sleep stages.

(B) Representative hypnogram over 12 hours of EEG/EMG recordings during light phase. Brain states are color-coded: yellow - active wake; grey - quite wake, blue – NREM sleep, purple - REM sleep. Bottom: EMG trace for the entire 12 hours of recording.

(C) Representative scatter plot distribution showing normalized EMG root mean square (y axis) versus EEG delta/theta power ratio in 6 sec epochs. Note that NREM and REM sleep are characterized by low EMG levels, and REM sleep and wakefulness are characterized by low delta/theta ratio in the EEG.

(D-F) Frontal EEG spectra during active wake (D), quiet wake (E) and REM (F) states in 4-5-month-old WT (n = 5, blue) and APP/PS1 (n = 5, red) mice.

(G) Frontal EEG spectra during NREM sleep in 9-month-old WT (n = 5, blue) and APP/PS1 (n = 5, red) mice. Post-hoc comparisons revealed significant decrease in the SWA frequency bins in NREM state (spectral power of 0.5-4 Hz, p = 0.002, two-way ANOVA).

Two-way ANOVA with Sidak multiple comparison test (D, F). **p<0.01, ns – non significant. Error bars represent SEM.
Figure S5. Single-unit recordings in the CA1 of behaving mice (related to Figure 3)

(A) Example of three clusters (color coded) recorded from the same tetrode and projected onto the first principle component of channels 1 and 3. Only well isolated clusters (isolation distance > 10) were included in the analysis.
(B) Mean ± STD waveform traces of the clusters in (A). Scale bars: 0.05 mV, 1 ms.

(C) Inter-spike interval (ISI) histogram of the clusters in (A), vertical line at 2 ms. Only clusters with a well-defined refractory period (less than 0.5% of ISI < 2 ms) were included in the analysis.

(D) Separation of regularly-spiking (RS) putative pyramidal neurons (red) and fast-spiking (FS) interneurons (blue) based on trough to peak time and burst index values.

(E) Mean ± STD of z-scored waveforms of RS (red) and FS (blue) units.

(F) Representative autocorrelogram of an RS unit (left, red) and an FS unit (right, blue).

(G) MFR of CA1 FS neurons was not different (p = 0.22) between WT (7.07 ± 1.25 Hz, 42 single units) and APP/PS1 (5.92 ± 1.84 Hz, 23 single units) during active wakefulness (AW).

(H) NREM sleep caused a reduction (p < 0.001) in CA1 MFR of FS interneurons from 7.06 ± 1.25 Hz in AW to 5.9 ± 1.12 Hz in WT mice (42 single units).

(I) MFR of CA1 FS interneurons was not different (p=0.33) between AW (5.91 ± 1.84 Hz) and NREM sleep (6.10 ± 1.66 Hz) in APP/PS1 mice (23 single units).

Wilcoxon test (H,I), Mann-Whitney U test (G). ***p<0.001, ns, non-significant. Error bars represent SEM.
Figure S6. Sleep-wake states analysis in WT and APP/PS1 mice (related to Figure 3)

(A-B) An example of wake-dense (A) and sleep-dense (B) recordings from a WT (top) and APP/PS1 (bottom) mouse. Top: Hypnograms, generated by manual brain state segregation. Brain states are color-coded: yellow - active wake; grey - quiet wake, blue – NREM sleep, purple - REM sleep. Middle: EMG traces (scale bar: 1 mV). Bottom: Fourier transform-based LFP power spectrograms.

(C) Percent of time spent in AW (27.0 ± 2.86 for WT, 25.5 ± 3.07 for APP/PS1) and NREM (44.25 ± 1.93 for WT, 41.0 ± 1.16 for APP/PS1) is not different (p = 0.89 for AW and p = 0.58 for NREM) between WT (n=4) and APP/PS1 (n=4) mice across 6 hours of recording in home cage during light phase.
(D-E) No difference in average speed (D, $p = 0.40$, $n = 4$) and total distance (E, $p > 0.9$, $n = 4$) between WT (blue) and APP/PS1 (red) mice across 6 hours of recording in home cage during light phase.

Two-way ANOVA with Sidak's multiple comparisons test (C), Mann-Whitney U test (D-E), ns, non-significant. Error bars represent SEM.
Figure S7. Burst-suppression and abnormal spike detection during different states of anesthesia (related to Figures 4 and 5)

(A) From top to bottom: 5 minutes of raw LFP recordings from a representative WT mouse during moderate (left) and deep (right) anesthesia (top), the corresponding spectrogram
based on short-time Fourier transformation (middle), and an expanded view of the LFP trace marked by a dashed box (bottom). Epochs of suppression are shaded gray.

(B) Same as (A) for an APP/PS1 representative mouse.

(C) Raw LFP recordings were divided to 500 ms bins and separated to epochs of bursts (red dots) and suppression (black dots) by a gaussian mixture model comprised of three dimensions: standard deviation, first principle component of the spectrogram, and maximum absolute value (not shown). Each of these parameters showed a bimodal lognormal distribution (inset to the right).

(D) Comparison of burst-suppression ratio (BSR) in deep anesthesia between WT (27 mice) and APP/PS1 (30 mice). No significant difference was observed (p = 0.98). Mann-Whitney nonparametric test was used for the analysis. ns, non-significant.

(E) Respiration rate per minute (RPM) is not significantly different between WT (n = 11) and APP/PS1 (n = 12, P = 0.86), FADx5 (n = 9, P = 0.90) and APP-KI (n = 6, P = 0.98) mice under deep anesthesia (1.5% isoflurane).

(F) RPM is not significantly different between WT (n = 8) and APP/PS1 (n = 8, P = 0.96), FADx5 (n = 9, P = 0.99) and APP-KI (n = 6, P = 0.93) mice under moderate anesthesia (1.0% isoflurane). RPM was significantly lower at 1.5% isoflurane in comparison to 1.0% isoflurane for all the groups (p = 0.005 for WT, p = 0.0001 for APP/PS1, p = 0.013 for FADx5, p = 0.020 for APP-KI, two-way-ANOVA with Sidak's multiple comparisons tests).

One-way-ANOVA with Dunnett’s multiple comparisons tests was used for the analysis (E,F). Error bars represent SEM.
Figure S8. Anesthesia-induced reduction in the number of CA1 microstates is impaired in APP/PS1 mice (Related to Figure 4)

(A-B) Representative density maps of microstates, visualized by 2D t-distributed stochastic neighbor embedding (t-SNE) in CA1 circuits of WT and APP/PS1 mice across arousal states: Active Wake (exploration, left panel), moderate anesthesia (central panel), deep anesthesia (right panel) in WT (A) and APP/PS1 (B).
(C) t-SNE/WS (watershed segmentation) clustering analysis in WT (n = 6) and APP/PS1 (n = 6) mice shows that reduction in the mean number of microstates at anesthetic states is impaired in APP/PS1 mice. Note that no difference was observed in wakefulness between WT and APP/PS1 mice (p = 0.52).

(D) Bar plot of inter- and intra-cluster correlation in the post-PCA space (after data was projected on chosen PCs) for WT and APP/PS1 mice during 3 arousal states; awake, moderate and deep anesthesia.

(E) Number of microstates normalized to the number of microstates in day 1 for WT (n=4) and APP/PS1 (n=4) mice during exploration at 2 days interval.

(F-G) Number of microstates determined by affinity propagation clustering (APC, F) and hierarchical clustering (G) in WT (n = 6) and APP/PS1 (n = 7) mice across arousal states show that reduction in the mean number of microstates at anesthetic states is impaired in APP/PS1 mice.

(H) Number of microstates determined by t-SNE/WS on 100 randomized datasets derived from within-frame shuffling of recorded data. In 5 out of 6 experimental groups, the number of generated microstates was significantly higher in the randomized compared to recorded data (normalized to recorded data).

Two-way ANOVA with Sidak's multiple comparison test were used for the inter-group analysis (C,E-H) and two-way ANOVA with Tukey's multiple comparisons test (D) were used for analysis. *p<0.05, **p<0.01, ***p<0.001, ****p < 0.0001. ns – non-significant. Error bars represent SEM.
Figure S9. Impairment of neuronal inhibition in APP/PS1 mice by distinct anesthetic drugs (Related to Figure 4)

(A) *Left*: Response of fEPSP amplitude recorded in CA1 stratum radiatum to increased current injection in the ipsilateral Schaffer Collaterals shows increased input-output slope in isoflurane-anesthetized (1.5% isoflurane) APP/PS1 (9 mice) versus WT (8 mice). *Right*: representative traces of fEPSP of WT and APP/PS1 evoked by 60, 90,120 µA stimulation. Scale bars: 10 ms, 1 mV.

(B) *Left*: fEPSP amplitude normalized to the first response during a burst stimulation (five stimuli, 50 Hz) shows similar level of synaptic facilitation between isoflurane-anesthetized (1.5% isoflurane) WT and APP/PS1 (p = 0.42). *Right*: representative traces of fEPSP evoked by five stimuli at 50 Hz. Scale bars: 20 ms, 1 mV.
(C-D) Effect of Ketamine (100 mg/Kg, i.p.) combined with Xylazine (8 mg/Kg, i.p.) (KX) anesthesia on average mCaR distribution in CA1 of WT (5 mice, C) and APP/PS1 (4 mice, D) groups.

(E) Total activity was 6.8-fold higher under KX in APP/PS1 mice (the same data as C-D).

(F-G) Effect of Medetomidine (MED, 0.3 mg/Kg, i.p.) anesthetic on average mCaR distribution in CA1 of WT (4 mice, F) and APP/PS1 (4 mice, G) groups of mice.

(H) Total activity was 4.3-fold higher under MED in APP/PS1 mice (the same data as F-G).

Two-way-ANOVA with Sidak’s multiple comparisons test (A,B) and Mann-Whitney U test (E,H) was used for the analysis. *p<0.05, ****p < 0.0001. Error bars represent SEM.
Figure S10. Modulation of MFR set points by anesthetics and DHODH inhibitor (Related to Figure 6)

(A) Diazepam (5 µM) stably reduces MFR by ~56% in WT neural networks (5 experiments, 333 channels).

(B) Propofol (5 µM) stably reduces MFR by ~40% in WT neural networks (3 experiments, 195 channels).

(C-D) Teriflunomide (TERI, 100 µM) stably inhibits MFR by ~45% in WT (A, 7 experiments, 423 channels) and by ~51% in APP/PS1 (B, 3 experiments, 230 channels) neural networks.

Paired t-test for the last 4 hours of a perturbation versus baseline (A-D), **p<0.01, ***p<0.001. Error bars represent SEM.