



Supplementary Materials for

Causal evidence for the role of REM sleep theta rhythm in contextual memory consolidation

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This PDF file includes:

Materials and Methods
Figs. S1 to S8
Tables S1 to S9
References (24–27)

Materials and Methods

Animals

VGAT-ires-Cre (VGAT::Cre) transgenic mice (The Jackson Laboratory, stock number 016962) were used in all experiments in order to selectively target Cre recombinase expression to GABAergic neurons. For the collection of *in vivo* data only male mice were used, whereas both females and males were used for the collection of *in vitro* slice electrophysiology data as well as data for FISH experiments. Mice were housed individually in polycarbonate cages at constant temperature (22° C) and humidity (30-50%) and were kept on a regular circadian cycle (12 h:12 h light:dark cycle, lights on at 7:30 a.m.). Mice were provided with food and water ad libitum. Animals were treated according to protocols and guidelines approved by McGill University and the Canadian Council of Animal Care.

Virus-mediated targeting of opsin and eYFP expression

At ~10 weeks age, male VGAT::Cre mice to be used for *in vivo* behavioral testing were anesthetized with isoflurane (5% induction, 1-2% maintenance). 0.6 µL of recombinant AAVdj carrying either Eflα-DIO-ArchT-eYFP or control Eflα-flex-eYFP (plasmids were provided by Dr. K. Deisseroth; viral vectors were packaged at Vollum Vector Core, University of Washington, Washington, USA) were stereotactically injected into the medial septum (MS) (anteroposterior (AP), +0.86; mediolateral (ML), 0.0; dorsoventral (DV), -4.5; all coordinates relative to Bregma) through a 28 G cannula at a rate of 50 nL/min. The injection needle was lowered through a hole drilled lateral to the midline (AP, +0.86; ML, -0.5) at an angle of ~6.4 ° (in the ML axis) relative to the vertical plane in order to avoid the sagittal sinus. For mice that were injected for use in *in vitro* experiments the same procedure was followed, however injections were performed at ~2 weeks of age.

Fluorescent in situ hybridization (FISH) for quantification of construct targeting specificity

Mice that had been injected at least 1 month prior were deeply anesthetized with isoflurane and subsequently decapitated. The brain was quickly removed and rapidly frozen in methyl-butane (-80 ° C). Brains were sectioned at 16 µm thickness using a cryostat and subsequently mounted on RNAase-free glass slides. Slides were first incubated for 20 minutes in filtered 4 % PFA in 1 x PBS made with distilled water containing 0.1% Diethyl pyrocarbonate (DEPC water). This was followed by a wash in PBS and subsequent incubation for 10 minutes in 0.3 % H₂O₂ in PBS, followed by another wash in PBS. Slides were then rinsed in DEPC water before undergoing a 10 minute acetylation in 0.1 M (Tris-acetate EDTA) (TEA) buffer (TEA in DEPC water with 25 % acetic anhydride). Slides were next washed 5 x 5 minutes in 1 x PBS followed by a series of 90 s dehydrations in ethanol (60 %, 80 %, 95 %, 100%) mixed in DEPC water. After allowing the slides to air-dry for 10 minutes the sections on the slides were circumscribed with an immEdge pen. The sections on the slides were then incubated for 2 h at 60 ° C with 300 µL/slide of hybridization buffer (40 % deionized formamide, 10 mM Tris-HCl (pH 8.0), 200 µg/mL yeast tRNA, 10 % dextran sulfate, 1 x Denhardt's solution, 600 mM NaCl, 1 mM EDTA (pH 8.0) dissolved in H₂O). Afterwards, the riboprobe was diluted 1:500 in hybridization buffer and 300 µL of solution was applied

to sections on each slide. The slides were then coverslipped and incubated for 12 h at 60 ° C in a humid chamber. The following day, slides were first washed 5 x 5 minutes in 5x SSC (diluted in sterile H₂O) at 60 ° C followed by a 1 minute wash in 2x SSC at 60 ° C. A wash for 30 minutes at 60 ° C in 0.2x SSC/Formamide (40%) was completed next, followed by two 5 minute washes in 0.2 x SSC and 1x PBS at room temperature. Slides were then blocked in 4 % BSA and 0.5 % blocking reagent in 1x PBS for 1 h. Sections on slides were then covered in rabbit anti-GFP diluted 1:2000 in PBST-BSA 4%, coverslipped, and incubated for 36 h at room temperature. After incubation in the primary antibody, slides were washed 5 x 5 minutes in 1x PBS and subsequently covered in 300 µL sheep anti-DIG-POD diluted 1:500 in 1x PBS, coverslipped, and incubated for 6 h at room temperature. Another 5 x 5 minute wash in 1x PBST was completed after, followed by application of 200 µL TSA-cy3 diluted 1:100 in amplification buffer over sections on each slide which were subsequently coverslipped and incubated for 10 minutes. The slides were then washed 5 x 5 minutes in PBST and sections on each slide were subsequently covered with alexa fluor 488 ex anti-rabbit IgG (H+L) 1:200 in PBST, coverslipped and incubated for 1 h at room temperature. Slides then underwent 3 x 5 minute washes in 1 x PBST and 5 x 5 minutes in 1 x PBS before finally being covered in Fluoromount and permanently coverslipped. Fluorescent images from immunolabelled sections were collected using a fluorescent microscope; quantification of colocalization was completed on sections containing the MS.

In vitro electrophysiology experiment preparation

Three to five weeks following virus injection, mice were deeply sedated using isoflurane, decapitated, and the brain was rapidly removed and cooled in ice-cold high-sucrose cutting solution containing (in mM): 252 sucrose, 26 NaHCO₃, 2.5 KCl, 4 MgCl₂, 1.25 KH₂PO₄, 0.1 CaCl₂ and 10 glucose. All mice were sacrificed during the light phase of the sleep-wake cycle and recordings were performed in late morning and early afternoon. Coronal slices were cut using a vibratome and allowed to recover for ~1.5 h at 22 ± 0.5 ° C in artificial cerebrospinal fluid (ACSF) containing (in mM): 124 NaCl, 5 KCl, 1.25 NaH₂PO₄, 2 MgSO₄, 2 CaCl₂, 26 NaHCO₃, and 10 dextrose saturated with 95 % O₂ and 5 % CO₂ (pH ~7.3, 300-310 mOsm). Individual brain slices were then transferred to the recording chamber and perfused continuously with oxygenated ACSF (1.5-2 mL min⁻¹) maintained at 22 ± 0.5 ° C. Cellular morphology and fluorescence were visualized with an upright fluorescent microscope equipped with a 40x water-immersion objective, differential interference contrast optics, infrared differential interference contrast and a near-infrared fluorescence camera.

In vitro electrophysiology experiment recording and analysis

Micropipettes were prepared from borosilicate glass capillaries (1.0 mm outer diameter, 0.58 mm inner diameter) using a horizontal puller, and had tip resistances of 3-8 MΩ. Tight gigaohm seals (>1 GΩ) were obtained under voltage clamp by applying gentle negative pressure, and whole-cell configuration was achieved using strong negative pressure. Somatic whole-cell current and voltage-clamp recordings from YFP-expressing medial septal neurons were obtained using patch recording pipettes containing (in mM): 120 K-gluconate, 20 KCl, 10 N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES), 7 phosphocreatine di-Tris, 2 MgCl₂, 0.2 ethylene glycol-bis (β-aminoethyl ether)-N,N, N', N'-tetraacetic acid (EGTA), 4 Na²⁺-ATP, and 0.3 GTP-Tris (pH adjusted to 7.20-7.26 using KOH, 275-85 mOsm). Series resistances were

continuously monitored using a brief 5 mV depolarizing voltage step and data were discarded if intrinsic cell properties (resting membrane potential, input resistance) were more than 3 s.d. outside of the group mean and/or access resistance changed by >15 %. An Axopatch 700B amplifier was used for all current-clamp and voltage-clamp recordings and signals were digitized and sampled at 20 kHz for storage on a hard-disk. Current-clamp recordings were filtered at 10 kHz, and voltage-clamp data were filtered at 2 kHz.

For optogenetic inhibition, square pulses of orange (594 nm) light were delivered using lasers connected to a 200 μ m optical fiber and triggered via a built-in transistor-transistor logic (TTL) circuit. Light intensity was tested before each experiment, and was calibrated to emit 20 mW from the tip of the optical fiber.

Electrophysiological characteristics of VGAT neurons were quantified using Clampfit 10.3 software package. Action potential measurements were derived from the first spike in response to a depolarizing intracellular current injection (typically 40-60 pA), and action potential amplitude was calculated from resting membrane potential. Action potential duration and afterhyperpolarizations were calculated from action potential threshold, and input resistance and rectification ratio was calculated in response to a -100 pA and -200 pA hyperpolarizing current pulse, respectively, from a holding voltage of -60 mV.

Electrode construction for *in vivo* experiments

Tetrodes were composed of 17.5 μ m diameter platinum-iridium wire (platinum:iridium 90%:10%) with Teflon and VG bond coating for insulation and heat-induced annealing, respectively. Tetrodes were constructed by twisting together 4 wires followed by briefly heating one end of the tetrode to anneal the VG bond coating on each wire together without damaging the Teflon insulation. Individual wire leads from the non-annealed end were then soldered onto an electrode interface board. For hippocampal area CA1 (CA1) recordings 3 tetrodes were arranged in a linear array (0.4 mm spacing between adjacent tetrodes). Epoxy was used to cement tetrodes in the correct orientation for implantation and also served to insulate any exposed wire at the connection point with the electrode interface board. The annealed tip of each tetrode in the array was cut to equal length using sharp scissors and the cut tetrode ends were cleaned immediately prior to surgery to give an impedance of $\sim$ 1 M Ω .

Electrode and optic fiber implantation for *in vivo* experiments

At $\sim$ 18 weeks mice injected for *in vivo* experiments were anesthetized with isoflurane (5% induction, 0.5-2% maintenance). The skull was completely cleared of all connective tissue and thoroughly dried using alcohol. To facilitate light delivery to the transfection zone within the MS, a hole was drilled in the skull above and lateral to the medial septum (AP, +0.86; ML, -0.5). After gently cutting through the dura an optic fiber implant, constructed by cementing a cleaved piece of optic fiber $\sim$ 12 mm in length into a ceramic ferrule, was lowered through the hole at an angle of $\sim$ 4.5 $^{\circ}$ (in the ML axis) with the optic fiber end location targeted just above the medial septum (AP, +0.86; ML, -0.2; DV, -3.83). For 'optrode' (optic fiber + tetrode) recordings the optic fiber was positioned as described above, however a tetrode was cemented to the optic fiber, with the tetrode tip extending $\sim$ 0.8 mm beyond the tip of the optic fiber. For CA1 tetrode placement, holes were drilled through the skull above the dorsal hippocampus (AP, -2.45; ML, +1.4, 1.8, 2.2). After gently cutting the dura, the tetrode array was slowly lowered

1.3 mm (relative to Bregma) with the end target being deep stratum radiatum of the dorsal CA1 region. For 16-channel (50 μ m site spacing oriented vertically) silicon probe implantation spanning CA1, the same procedure was used with slightly different coordinates (probe tip target: AP, -2.45; ML, +1.50; DV, -1.50). An EEG screw was placed in the skull above the hippocampus in the contralateral hemisphere (AP, -2.3; ML, -1.35). 2 EMG electrodes consisting of stranded tungsten wires inserted into the neck musculature were used to record postural tone. Screws placed in the bone above the frontal cortex and cerebellum served as ground and reference, respectively. Following optic fiber, electrode, ground and reference placement, dental cement was applied to secure the implant permanently to the skull.

Post-surgery habituation to recording setup

Following surgery, mice were allowed to recover undisturbed for at least 1 week. Once mice had recovered from surgery they were briefly anesthetized with isoflurane (5% induction, 2% maintenance) and a custom built headstage pre-amplifier tether was attached to a connector on the top of the implanted electrode interface board. An optic fiber patch cord assembled in-house using a standard kit was connected to the exposed end of the optic fiber implant. Black nail polish was applied to the entire implant as well as the initial portion of the optic fiber patch cord to reduce the amount of light emanating from the implant and therefore prevent excessive disruption of mice from escaping light when the laser was on. Mice were then returned to their home cage and were left undisturbed until they were habituated to being chronically tethered and exhibited a regular sleep-wake cycle; typically 5 to 7 days.

In vivo electrophysiological recording

Recordings began only after mice were habituated to being chronically tethered. All recorded signals from implanted electrodes were amplified by the headstage pre-amplifier tether before being digitized at 16000 Hz using a digital recording system and saved to a hard disk.

Light delivery to the MS in vivo

For optical experiments, orange (594 nm wavelength) light was delivered to the MS from a TTL controlled laser with adjustable output intensity via the optic fiber patch cord-optic fiber implant system. For each AAVdj-ArchT-transduced mouse, the minimum amount of light estimated to be present at the tip of the optic fiber implant required to produce maximal effects on the CA1 theta rhythm during REM sleep (REMS) was determined before behavioral experiments were started in order to minimize unwanted side-effects including light-induced tissue damage as well as excessive disturbance of animals. The estimation was based on pre-surgery testing of transmittance of light through the optic fiber patch cord and optic fiber implant. The estimated amount of light determined using these criteria rarely exceeded 20 mW, and was never allowed to exceed 30 mW. The amount of light that each control AAVdj-eYFP-injected mouse received during all optical experiments was matched with an AAVdj-ArchT-injected mouse.

Baseline recording and characterization of optical inhibition in vivo

Prior to any optical testing or behavioral experiments a 24 h baseline recording was performed to ensure that every mouse demonstrated a regular sleep-wake cycle. Afterwards, baseline optical testing was completed. This consisted of no fewer than 10 relatively brief (5-10 s) isolated periods of light delivery to the MS during REMS as well

as NREMS and active wakefulness to determine both the basic effects of optical inhibition of MS GABAergic (MS^{GABA}) neurons, as well as to calibrate the appropriate amount of light intensity to use for subsequent behavioral experiments as discussed above. Once the baseline recording and baseline optical testing were completed, behavioral experiments commenced. AAVdj-ArchT-injected mice were randomly assigned to either the test (ArchT) or control (ArchT control or ArchT REM control) condition at this time. All AAVdj-eYFP-injected mice were placed in the YFP control group.

Novel object place recognition procedure and behavioral analysis

For all mice the first behavioral experiment to be completed was a novel object place recognition task, a modified version of the novel object recognition task (17). The test area consisted of a 30.5 cm³ open-topped square container. The bottom and walls of the container were painted with white waterproof paint and unique black patterns were present on each of the 4 walls. The test was run on 3 consecutive days within the first two hours of light cycle onset (7:30 a.m. to 9:30 a.m.) in a room separate from the room in which the home cage was located. The precise test time for each mouse was kept consistent for each of the 3 days. On the first day of testing (test area habituation/baseline (Day 0)), mice were placed in the empty test area and allowed to explore freely for 10 minutes before being returned to their home cage. For the second day of testing (Day 1), 2 identical yet individually identified objects (Object 1 and Object 2) were each placed in a randomly assigned quadrant within the test area and mice were again allowed to explore freely for 10 minutes before being returned to their home cage. Upon being placed back in their home cage, EEG, CA1 LFP (CA1LFP) and EMG activity was manually monitored by an experimenter continuously for 4 hours. The real-time behavioral state was determined based on criteria discussed below for vigilance state architecture analysis. Whenever mice in either the ArchT or YFP control groups entered into REMS during the 4 h post-test period, the laser was manually activated and orange laser light was continuously delivered to the MS via the optic fiber patchcord/implant system until the mouse transitioned out of REMS, at which time the laser was turned off until the next REMS episode occurred. An ~10 s delay existed for manual detection of REMS to ensure that mice were in stable REMS. For mice in the ArchT REM control group each REMS episode occurring during the 4 h post-test period was manually documented and the duration noted; however, delivery of laser light to the MS did not occur immediately upon detection of REMS but was instead delayed by ~5 min from the end of the REMS episode. Following the delay laser light was delivered continuously to the MS for a timespan equal to the duration of the preceding REMS episode, unless the rare event of a mouse transitioning to REMS while the laser was on occurred, in which case the laser was turned off. This method was effective in providing a pattern of MS^{GABA} neural photoinhibition that, while statistically the same as that provided selectively during REMS to mice in the ArchT group, almost entirely avoided REMS in the ArchT REM control group of mice. Mice in the ArchT control group did not have light delivered to the MS at any point during behavioral testing. On the third and final day of testing (Day 2) the same 2 identical objects were placed within the test area as done on the day prior with the exception that Object 2 was located in a different quadrant relative to Day 1 position. Mice were given 10 minutes to explore the test area before being returned to their home cage. For each day of testing recordings were obtained from

electrodes during the testing period in addition to the first 5 hours after the mouse had been returned to its home cage immediately following the conclusion of testing. Both the test area and each of the identical objects used in this task were thoroughly washed with Peroxyguard before every test session. Test sessions on each day were recorded with an overhead video camera. For analysis of novel place recognition data, the time spent exploring each object during the test period was measured for each mouse on Day 1 and Day 2 of testing. For statistical analysis, the preference of mice for Object 2 exploration was determined by the following equation:

Object 2 discrimination index (DI)= ((Object 2 exploration (s)-Object 1 exploration (s)))/((Object 2 exploration (s)+Object 1 exploration (s)))

Movement data for mice during each test session was completed through analysis of video recordings from each test session at 1 Hz resolution using custom scripts in Matlab. Fear conditioning procedure and behavioral analysis

For all mice the second and final behavioral task completed involved testing context- and cue-based responses following a standard fear conditioning protocol (24). Testing was completed over two days and always took place within the first 2 hours of the onset of the light cycle (7:30 a.m. to 9:30 a.m.) in a room separate from the room in which the home cage was located. On the first day of testing each mouse underwent the fear conditioning protocol. The floor of the test area consisted of a metal grid through which electrical current could be applied, while the wall panels were composed of opaque white Plexiglas with distinct black markings. The roof panel of the test area was composed of transparent Plexiglas to allow mouse behavior to be recorded with a video camera. A hole in the roof panel allowed the mouse to remain tethered during testing. The conditioning protocol consisted of three 80 dB tones 28 s in duration that were each immediately followed by 2 s of 0.5 mA current delivery through the metal grid floor. Each 30 s tone-shock event was immediately preceded and followed by 120 s blocks of time in which no tone or shock was present. Thus the total test time was 9.5 minutes. Once testing was complete, mice were immediately returned to their home cage. Between each conditioning session the test area was cleaned thoroughly with Peroxyguard. Upon being placed back in their home cage, EEG, CA1LFP and EMG activity was continuously monitored for 4 hours. The laser delivery protocol was the same for each group during the 4 h post-fear conditioning period as for the Day 1 post-test period for novel object place recognition testing discussed in the prior section. The second and final day of testing consisted of contextual and cued memory recall tests that were each completed 1 h apart but both within the first 2 hours of the onset of the light cycle (7:30 a.m. to 9:30 a.m.). For the context test, the exact same test area setup (context A) and test duration (9.5 min) that was used for fear conditioning on the prior day was again used, however no tone-shock events were present. Upon test completion, mice were immediately returned to their home cage for ~1 h until the cue test commenced. The test area was thoroughly cleaned with Peroxyguard between subsequent test sessions. For the cue testing the test area (context B) was substantially different than that used for the conditioning session and context testing. The floor consisted of a black Plexiglas panel covered in woodchip bedding, the walls were black Plexiglas, the shape of the testing area had a triangular configuration as opposed to the square configuration used for fear conditioning and context testing, and 70% ethanol was used to clean the test area between mice as opposed to Peroxyguard. During the cue test

three 80 dB tones 28 s in duration, identical to those produced during fear conditioning in context A the prior day, were evenly spaced over a 9.5 minute test period. EEG, CA1LFP and EMG recordings were made for 5 hours continuously following fear conditioning, and 1 h following completion of cue testing the following day. Test sessions on each day were recorded with a video camera. For the fear conditioning and subsequent contextual and cued recall memory test sessions, fear response was assessed by quantifying observed freezing behavior (25), identified as absence of movement except for breathing, in 30 s bins covering the entire duration of each test session (titled ‘unprocessed’ freezing analysis in figures). For statistical analysis of freezing behavior, data was further binned as described in the text and accompanying figures (titled ‘binned’ freezing analysis in figures).

Vigilance state architecture analysis

For all recordings made during behavioral testing, EEG, CA1LFP and EMG data were plotted and the vigilance state was manually scored in 5 s epochs using a custom written Matlab program. Scoring was based on visual characteristics of the CA1LFP, EEG and EMG data, fast Fourier transform analysis of each epoch scored as well as video monitoring of mouse behavior. Wakefulness was defined by a de-synchronized low-amplitude EEG and CA1LFP and tonic EMG activity with periods of movement-associated bursts of EMG activity. NREMS was defined as synchronized, high amplitude, low-frequency (δ , 1-4 Hz) EEG and CA1LFP activity that was accompanied by reduced EMG activity relative to that observed during wakefulness. REMS was defined as having reduced δ power, a prominent theta rhythm (4-10 Hz) and an absence of tonic muscle activity. In cases where theta power was significantly reduced during REMS due to photoinhibition, reduced δ power, lack of EMG tone and observation of sustained quiescent behavior were found to be highly reliable indicators of ongoing REMS. Hypnogram analysis (cumulative duration and average episode duration for each vigilance state) was completed using custom scripts in Matlab.

Electrophysiological analysis of in vivo MS unit, CA1 unit, EEG, CA1LFP and EMG data

All electrophysiological analysis was completed using custom scripts in Matlab unless otherwise noted.

For recording unit activity in the MS and CA1 pyramidal cell layer, single units were manually isolated from 600 – 6000 Hz filtered tetrode recordings using the clustering method (50 μ V spike detection threshold) with criteria similar to what has been discussed elsewhere (26). Briefly, only units that formed clusters with clear boundaries were used for subsequent analysis. Additional requirements for single units included stability for the duration of recording and the presence of a clear refractory period of at least 3 ms between subsequent spikes determined through inter-spike interval analysis. Once sorting was complete, timestamp and spike waveform data of isolated units was output into Matlab for further analysis. For units isolated at the CA1 pyramidal cell layer, analysis for each treatment group revealed a minority (~3 %) of units possessing a symmetrical as opposed to unsymmetrical waveform which has previously been used as a method to identify putative interneurons and pyramidal cells, respectively (26). However, due to the low n analysis of symmetrical units was severely restricted. Thus, symmetrical units were removed from further analysis and all data shown was obtained from isolated units with unsymmetrical waveforms. Analysis was only completed on unit

data recorded during novel object place recognition testing as there was an insufficient number of stable units contained within the fear conditioning data.

Current source density (CSD) (27) was calculated from 16-channel silicon probe LFP recordings using the following equation:

$$\text{CSD}(x,t) = (\sigma(2(x,t) - (x+\Delta x,t) - (x-\Delta x,t)))/((\Delta x)^2)$$

where (x,t) is the potential at depth x for time point t , with Δx of 50 μm . Conductivity (σ) was assumed constant and units for CSD are reported as $\text{mV}/\mu\text{m}^2$.

Vigilance state-specific spectral analysis of CA1LFP and CSD data was completed using inputs from the Chronux signal processing toolbox (window size = 5 s, step size = 5 s, tapers [3 5]). Analysis for each mouse was completed using the best (optimal combination of stability and size) individual electrode from the most accurately placed tetrode determined histologically (described further below). Analysis of EMG activity was completed by applying a 100 Hz high-pass filter to the data and subsequently integrating the rectified signal over 5 s windows.

Detection of transient EEG spindle and CA1LFP ripple activity during NREMS was completed on 30s windows of continuous NREMS that occurred during the recording period; there was no overlap in subsequent analysis windows. For each window, the signal was first bandpass filtered (EEG/spindle, 9-15 Hz; CA1LFP/ripple, 100-250 Hz) and the absolute amplitude was calculated with set resolution within the analysis window (EEG/spindle, 100 ms; CA1LFP/ripple, 10 ms). The mean and standard deviation (SD) for the absolute amplitude was calculated and the detection threshold was set at 1.5 SD above the mean for spindle analysis and 3 SD above the mean for CA1LFP ripple analysis. Values for detection threshold were selected based on accuracy of automatic detection as compared to manual scoring. EEG spindles and CA1LFP ripples were identified from detected above threshold data time points using additional criteria (EEG/spindle, minimum duration between successive spindles = 0.5 s, maximum duration of spindle = 3 s; CA1LFP/ripple, minimum duration between successive ripples = 30 ms).

Histological confirmation of electrode/optic fiber placement and construct expression

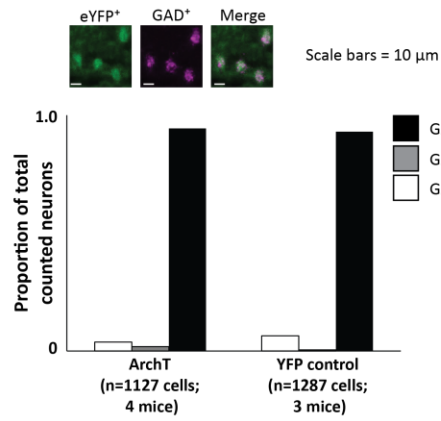
Following completion of all behavioral experiments, mice were deeply anesthetized with ketamine/xylazine/acepromazine (100, 16, 3 mg/kg, respectively, intraperitoneal injection). Electrode sites were marked by passing a current of 10 μA for ~20 s through each of the CA1 electrodes. Mice were then perfused transcardially with 1 x PBS-heparine 0.1%, pH 7.4, followed by 4% paraformaldehyde in PBS (PFA). The brains were then extracted and postfixed overnight in PFA at 4 ° C and subsequently cryoprotected in 30% sucrose dissolved in PBS for an additional 24 h at 4 ° C. Each brain was then frozen and sectioned at 50 μm using a cryostat; odd sections were collected, mounted on glass slides and stained with cresyl violet prior to being coverslipped for confirmation of electrode and optic fiber placement. To confirm construct expression, even sections were first washed in PBS 1x-Triton (0.3 %) (PBST), incubated in blocking solution (4 % bovine serum albumin (BSA) in PBST) for 60 minutes at room temperature and subsequently incubated in rabbit anti-GFP diluted 1:5000 in 4 % BSA overnight at 4 ° C. Sections were then incubated in alexa fluor 488 ex anti-rabbit IgG (H+L) diluted 1:1000 in PBST to detect the primary antibody for 1 h at room temperature and then mounted on glass slides and permanently coverslipped with Fluoromount-G. Images of cresyl violet stained sections were obtained using a light

microscope whereas fluorescent images from immunolabelled sections were collected using a fluorescent microscope. Only mice with histologically confirmed placement of at least 1 tetrode in deep CA1 stratum radiatum and optic fiber placement as well as proper construct expression in the MS were used in the present study. Additionally, only mice with histologically confirmed placement of at least 1 tetrode in the CA1 pyramidal cell layer were further considered for analysis of CA1LFP ripple and unit activity.

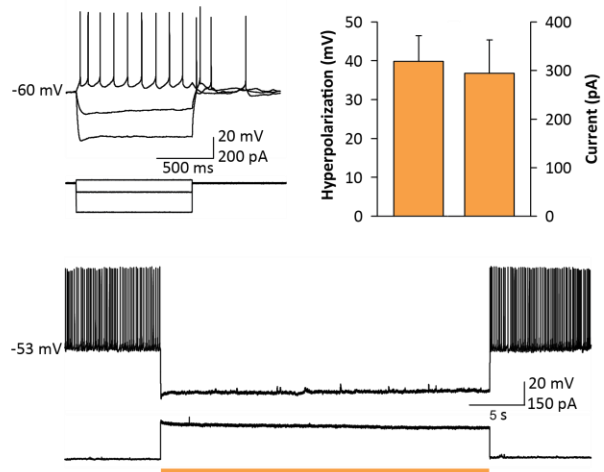
Statistical analysis

Statistical analysis was performed using GB-Stat. All data are presented as mean $\pm$ standard error of the mean (SEM) and statistic test details are described in the text. $P < 0.05$ was considered statistically significant.

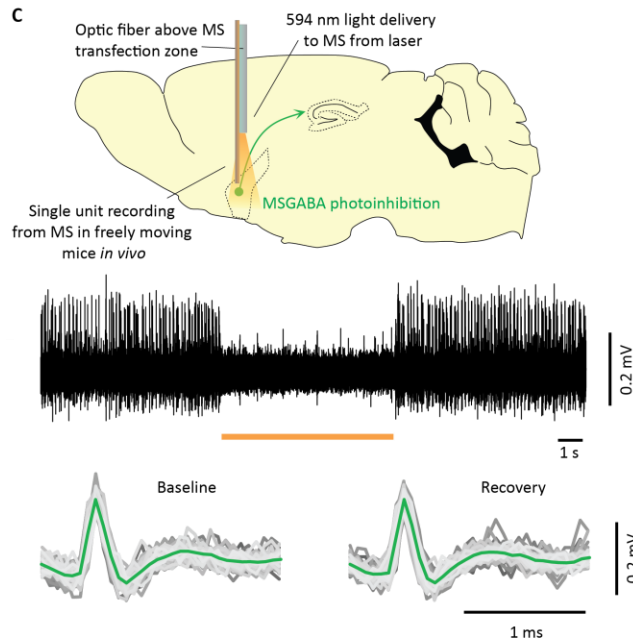
A



B



C



D

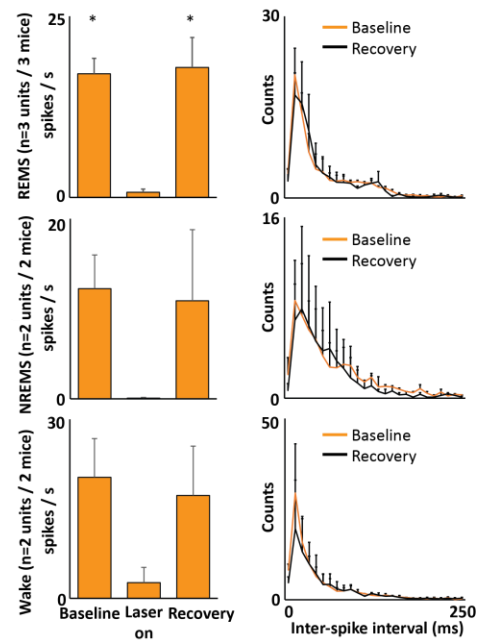


Fig. S1.

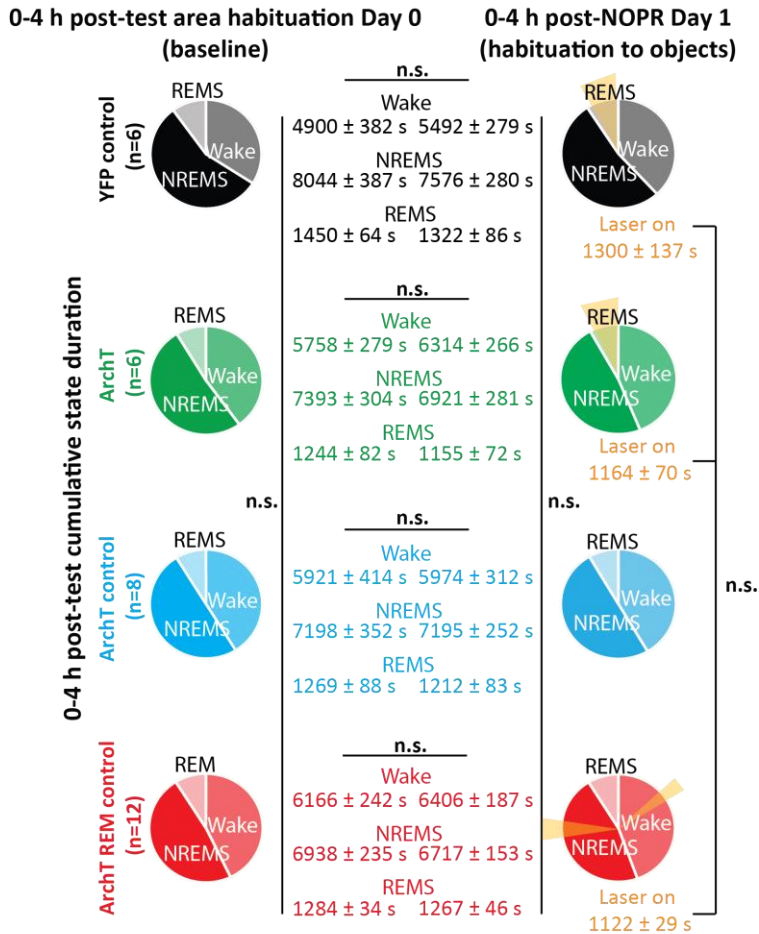
Specific targeting of ArchT-mediated inhibition to MS^{GABA} neurons. (A) Top photos show cell-specific expression of ArchT-eYFP (green) in MS^{GABA} neurons (magenta) in a VGAT::Cre mouse injected with Cre-dependent AAV. Quantified expression shown at bottom. (B) Optical inhibition of MS neurons in brain slices *in vitro*. Representative voltage traces (top, left) from an ArchT-expressing neuron responding to hyperpolarizing and depolarizing current pulses; illumination with orange light (orange bar) resulted in hyperpolarization and an outward current (bottom). Group data shown at top right. (C) Schematic (top) of *in vivo* optrode recording; an optic fiber and tetrode were placed above and within the MS, respectively, for recording transfected MS^{GABA} neurons *in vivo*. A filtered single unit recording during REMS showing precise inhibition of spiking during optical silencing is shown in middle. Spike waveforms for the 5 s pre-inhibition (baseline) period is shown at bottom left with average waveform overlaying in green; 5 s post-inhibition (recovery) waveform data is shown at bottom right. (D) Effect of optical inhibition on spiking rate (left) and inter-spike interval (right) of putative MS^{GABA} neurons during different states in freely behaving mice *in vivo* (n = 3 REMS, n = 2 NREMS/wake; *P < 0.05, Kruskal-Wallis test).

Fig. S2

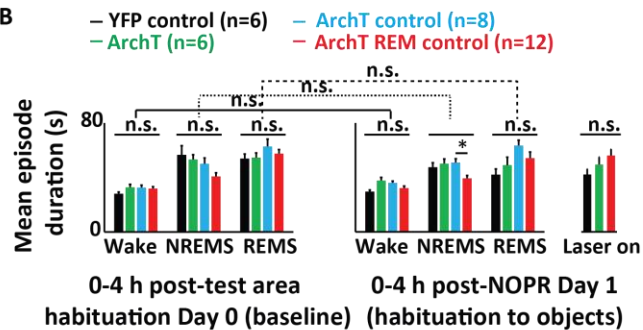
ArchT-mediated inhibition of MS^{GABA} neurons during NREMS and wakefulness. (A) Schematic of *in vivo* recording configuration; an optic fiber placed above the MS (histology sample in first photo at right) delivered orange laser light to the MS transfection zone allowing for optogenetic inhibition of MS^{GABA} neurons while LFP recordings were completed from electrodes implanted in dorsal CA1 (histology sample in second photo at right). An EMG inserted in the neck musculature (not depicted) simultaneously recorded muscle tone. (B) Effect of MS^{GABA} neural inhibition during active wakefulness (data on left) and NREMS (data on right) on CA1LFP and EMG activity. Mice injected with a control virus resulting in expression of only eYFP in MS^{GABA} neurons controlled for use of orange light (YFP control) (n = 9 ArchT, n = 11 YFP control; n.s. = not significant, 2-way ANOVA).

A

Post-test behavioral state analysis



B



C

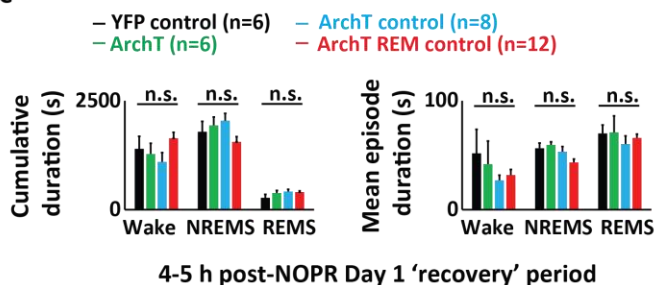


Fig. S3

NOPR post-test vigilance state analysis. (A,B) Comparison of vigilance state cumulative (A) and average (B) duration between groups for 4 h Day 1 (D1) post-test period and within groups vs. baseline condition (4 h period following initial habituation to NOPR test area) (n = 6 ArchT, n = 6 YFP control, n = 8 ArchT control, n = 12 ArchT REM control; *P < 0.05, n.s. = not significant, 2-way ANOVA for between group comparison; 2-way repeated-measures ANOVA for baseline vs. D1 within-group comparison). (C) Comparison of vigilance state cumulative (left) and average (right) duration between groups for 4-5 h D1 post-test 'recovery' period (4-5 h D1 post-test period when MS^{GABA} neural photoinhibition no longer occurs) (n = 6 ArchT, n = 6 YFP control, n = 8 ArchT control, n = 12 ArchT REM control; n.s. = not significant, 2-way ANOVA between group comparison).

Fig. S4

NOPR post-test CA1LFP spectral analysis. (A) Comparison of CA1LFP spectral power for baseline condition and 4 h Day 1 (D1) post-test period ($n = 6$ ArchT, $n = 6$ YFP control, $n = 8$ ArchT control, $n = 12$ ArchT REM control; $^{**}\dagger P < 0.01$ (ArchT vs. all other groups), n.s. = not significant, 2-way ANOVA). (B) Top: Comparison of CA1LFP spectral power between baseline condition, 4 h D1 post-test period, and recovery period (4-5 h D1 post-test period when MS^{GABA} neural photoinhibition no longer occurs) ($n = 6$ ArchT, $n = 6$ YFP control; $^{**}\dagger P < 0.01$ (REM with laser on vs. baseline and recovery REMS), n.s. = not significant, 2-way repeated-measures ANOVA). Bottom: CA1LFP spectral power in ArchT REM control mice on 4 h D1 post-test period when laser is on vs. off ($n = 12$; n.s. = not significant, 2-way ANOVA). (C) Between-group comparison of CA1LFP spectral power during D1 post-test recovery period ($n = 6$ ArchT, $n = 6$ YFP control, $n = 8$ ArchT control, $n = 12$ ArchT REM control; n.s. = not significant, 2-way ANOVA).

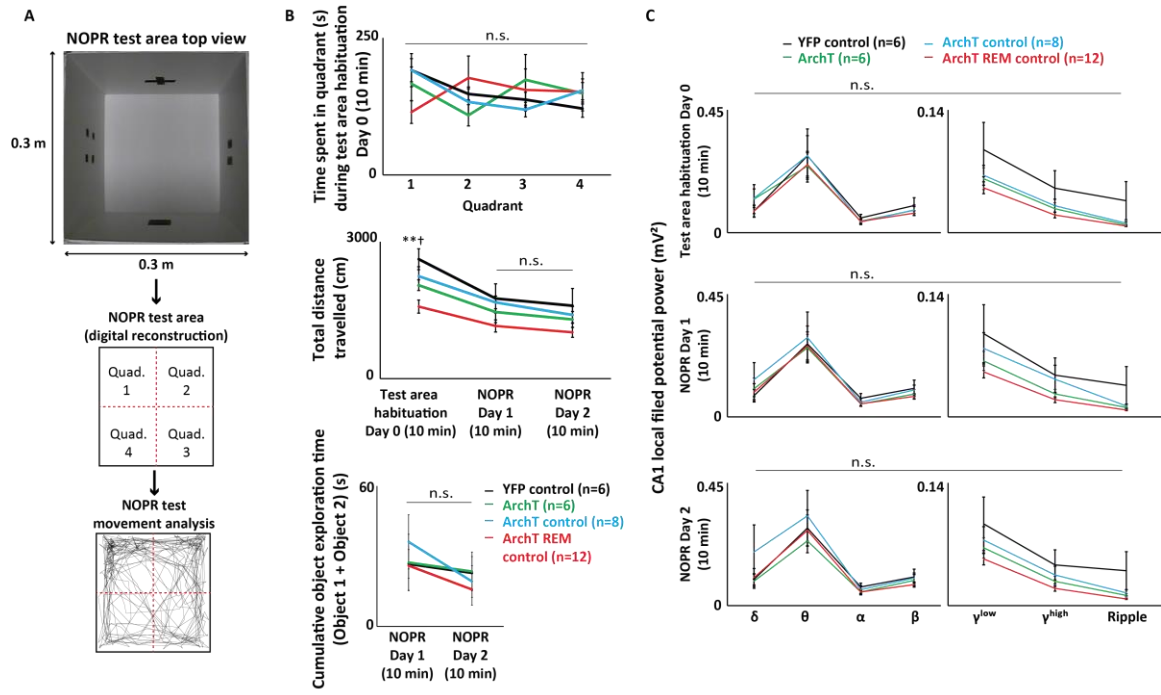


Fig. S5

NOPR test movement and CA1LFP spectral analysis. (A) Schematic of NOPR movement analysis. Movement of mice during each test session (Test area habituation Day 0, Day 1 (D1), Day 2 (D2)) was quantified digitally; for preferred location analysis completed during initial test area habituation, the test area was divided into 4 quadrants digitally. (B) Movement data for NOPR test sessions. Quadrant distribution for first exposure to the NOPR test area when no objects were present (Test area habituation) is shown at top. Distance travelled during each test session is shown in middle. Cumulative object exploration time for D1 and D2 is shown at bottom. (C) Spectral analysis of CA1LFP recordings during each test session. (B-C) (n = 6 ArchT, n = 6 YFP control, n = 8 ArchT control, n = 12 ArchT REM control; **†P < 0.01 (YFP control vs. ArchT REM control), n.s. = not significant, 2-way repeated-measures ANOVA with Tukey post-hoc test 2-way ANOVA).

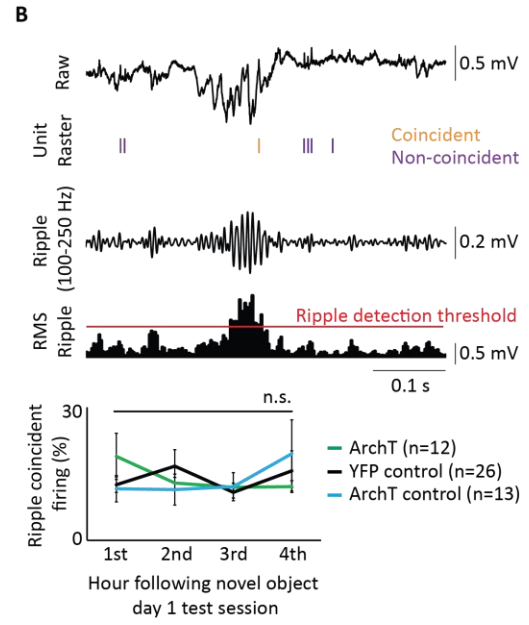
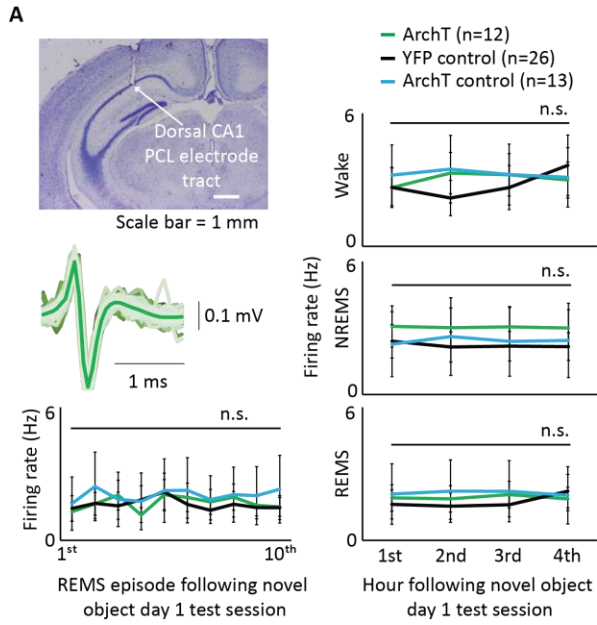
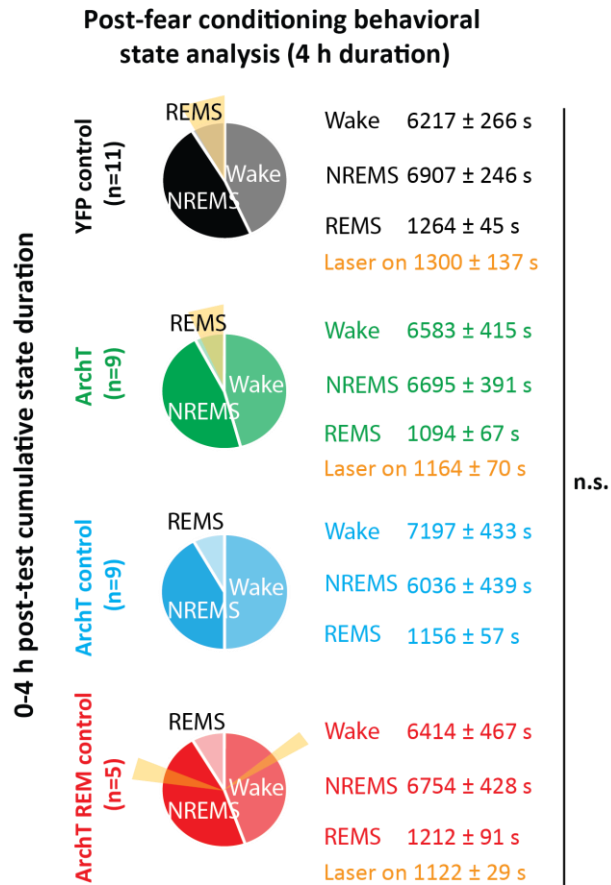


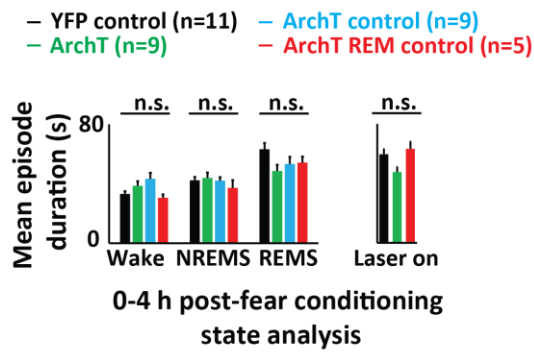
Fig. S6

CA1 physiology during 4 h REMS-selective MS^{GABA} neural silencing protocol. (A) Basic firing properties of neurons isolated from the dorsal CA1 pyramidal cell layer (PCL) (top and middle, left) were analyzed following the NOPR D1 test. Bottom, left: Unit firing frequency during REMS episodes occurring successively following testing. Right: State-specific unit rate analysis 0-4 h following testing (n = 12 units (4 mice) ArchT, n = 26 units (5 mice) YFP control, n = 13 units (5 mice) ArchT control, insufficient n ArchT REM control; n.s. = not significant, Kruskal-Wallis test). (B) Coincident unit firing probability during ripples recorded from the CA1 PCL. Top: Example of ripple detection (bottom bar graph) from ripple-filtered (middle trace) raw data (top trace). The proportion of unit timestamps occurring during detected ripples was calculated 0-4 h following NOPR D1 testing (bottom) (n = 12 units (4 mice) ArchT, n = 26 units (5 mice) YFP control, n = 13 units (5 mice) ArchT control, insufficient n ArchT REM control; n.s. = not significant, Kruskal-Wallis test).

A



B



C

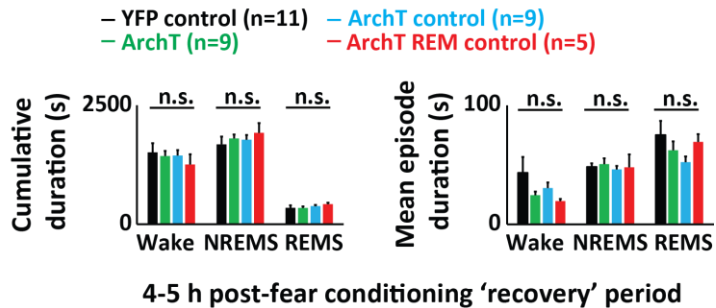
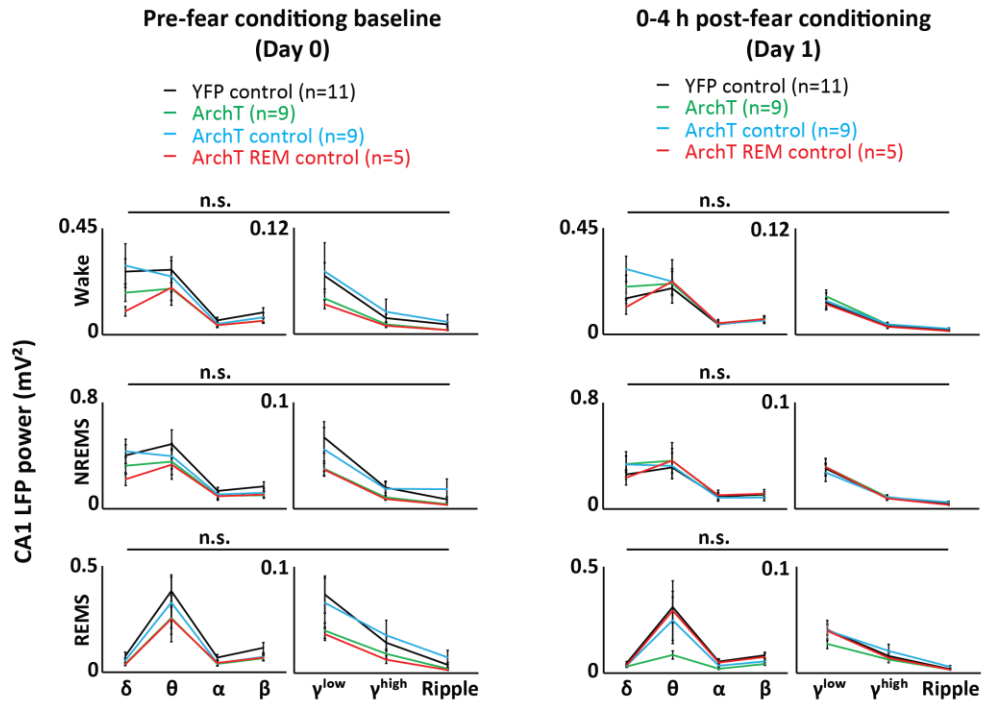


Fig. S7

Comparison of vigilance state cumulative (A) and average (B) duration between groups for 4 h Day 1 (D1) post-fear conditioning period (n = 9 ArchT, n = 11 YFP control, n = 9 ArchT control, n = 5 ArchT REM control; n.s. = not significant, 2-way ANOVA). (C) Comparison of vigilance state cumulative (left) and average (right) duration between groups for 4-5 h D1 post-fear conditioning 'recovery' period (4-5 h period when MS^{GABA} neural photoinhibition no longer occurs) (n = 9 ArchT, n = 11 YFP control, n = 9 ArchT control, n = 5 ArchT REM control; n.s. = not significant, 2-way ANOVA).

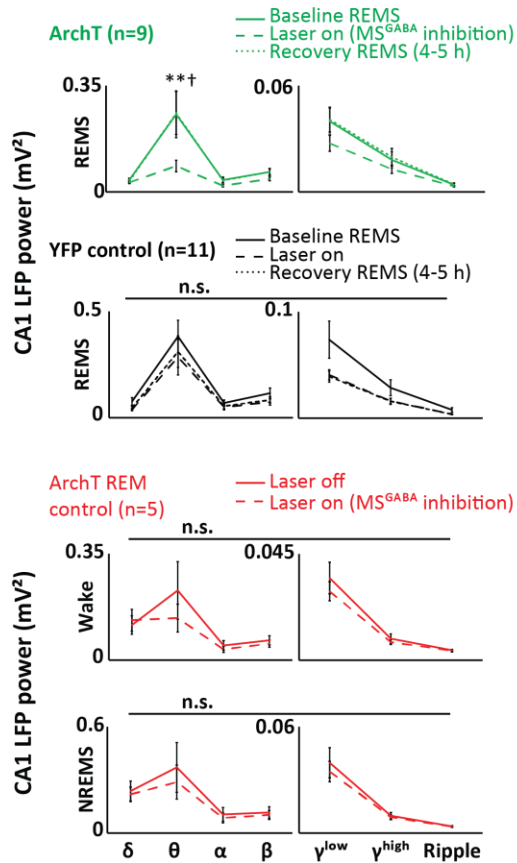
A

CA1 LFP post-test spectral analysis (4 h duration)



B

0-4 h post-fear conditioning (Day 1)



C

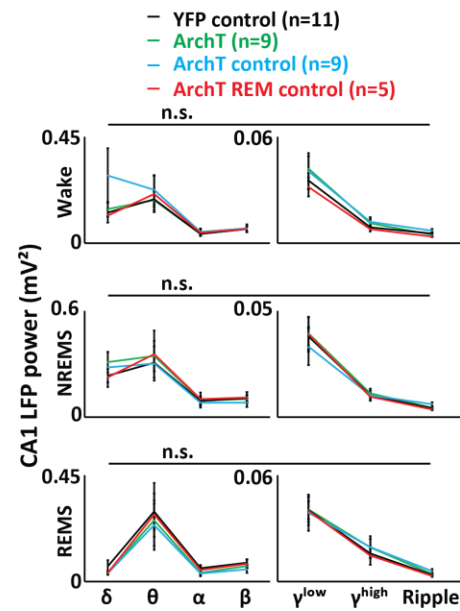
4-5 h post-fear conditioning
'recovery' period

Fig. S8

Post-fear conditioning CA1LFP spectral analysis. (A) Comparison of CA1LFP spectral power for baseline condition (4 h recording starting ~24 h prior to fear conditioning) and 4 h Day 1 (D1) post-fear conditioning period ($n = 9$ ArchT, $n = 11$ YFP control, $n = 9$ ArchT control, $n = 5$ ArchT REM control; n.s. = not significant, 2-way ANOVA). (B) Top: Comparison of CA1LFP spectral power between baseline condition, 4 h D1 post-fear conditioning period, and recovery period (4-5 h D1 post-fear conditioning period when MS^{GABA} neural photoinhibition no longer occurs) ($n = 9$ ArchT, $n = 11$ YFP control; $**\ddagger P < 0.01$ (REM with laser on vs. baseline and recovery REMS), n.s. = not significant, 2-way repeated-measures ANOVA). Bottom: Comparison of CA1LFP spectral power in ArchT REM control mice on 4 h D1 post-test period when laser is on vs. off ($n = 5$; n.s. = not significant, 2-way ANOVA). (C) Between-group comparison of CA1 LP spectral power during D1 post-fear conditioning recovery period ($n = 9$ ArchT, $n = 11$ YFP control, $n = 9$ ArchT control, $n = 5$ ArchT REM control; n.s. = not significant, 2-way ANOVA).

Table S1.

Analysis of EEG spindle characteristics during NREMS occurring within 0-4 h NOPR Day 0 (baseline) post-test period (n = 6 ArchT, n = 6 YFP control, n = 8 ArchT control, n = 12 ArchT REM control; non-significance indicated by absence of symbols, 2-way ANOVA between groups, repeated-measures ANOVA Day 0 vs. Day 1 post-test period (Table S2) within groups).

Spindle Parameter (Window (30 s) mean)	YFP Control (n=6)	ArchT (n=6)	ArchT Control (n=8)	ArchT REM Control (n=12)
Number	2.14 ± 0.02	1.99 ± 0.03	2.06 ± 0.04	2.07 ± 0.04
Density (spindles / s)	0.071 ± 0.001	0.066 ± 0.001	0.069 ± 0.001	0.069 ± 0.001
Spindle duration (ms)	780 ± 6	761 ± 5	774 ± 5	778 ± 6
Max spindle amp (mV)	0.07 ± 0.01	0.06 ± 0.00	0.11 ± 0.04	0.09 ± 0.02
Spindle magnitude (°)	36 ± 3	32 ± 1	58 ± 22	38 ± 10

Table S2.

Analysis of EEG spindle characteristics during NREMS occurring within 0-4 h NOPR Day 1 post-test period (n = 6 ArchT, n = 6 YFP control, n = 8 ArchT control, n = 12 ArchT REM control; non-significance indicated by absence of symbols, 2-way ANOVA between groups, repeated-measures ANOVA Day 0 (baseline (Table S1)) vs. Day 1 post-test period (Day 1) within groups).

Spindle Parameter (Window (30 s) mean)	YFP Control (n=6)	ArchT (n=6)	ArchT Control (n=8)	ArchT REM Control (n=12)
Number	2.14 ± 0.04	2.05 ± 0.04	2.02 ± 0.05	2.08 ± 0.05
Density (spindles / s)	0.072 ± 0.001	0.068 ± 0.001	0.067 ± 0.002	0.069 ± 0.001
Spindle duration (ms)	774 ± 7	783 ± 5	783 ± 12	776 ± 5
Max spindle amp (mV)	0.12 ± 0.05	0.11 ± 0.05	0.07 ± 0.01	0.07 ± 0.00
Spindle magnitude (°)	59 ± 23	54 ± 24	38 ± 3	38 ± 2

Table S3.

Analysis of EEG spindle characteristics during NREMS occurring within 4-5 h NOPR Day 1 post-test ‘recovery’ period (n=6 ArchT, n=6 YFP control, n=8 ArchT control, n=12 ArchT REM control; non-significance indicated by absence of symbols, 2-way ANOVA between groups).

Spindle parameter (Window (30 s) mean)	YFP Control (n=6)	ArchT (n=6)	ArchT Control (n=8)	ArchT REM Control (n=12)
Number	2.08 ± 0.07	2.07 ± 0.06	2.13 ± 0.08	2.07 ± 0.07
Density (spindles / s)	0.069 ± 0.002	0.069 ± 0.002	0.071 ± 0.002	0.069 ± 0.002
Spindle duration (ms)	785 ± 21	786 ± 14	771 ± 13	759 ± 14
Max spindle amp (mV)	0.068 ± 0.006	0.062 ± 0.002	0.073 ± 0.005	0.075 ± 0.003
Spindle magnitude (f)	36 ± 7	32 ± 1	37 ± 3	38 ± 2

Table S4.

Analysis of EEG spindle characteristics during NREMS occurring within 0-4 h fear conditioning post-test period (n = 9 ArchT, n = 11 YFP control, n = 9 ArchT control, n = 5 ArchT REM control; non-significance indicated by absence of symbols, 2-way ANOVA between groups.

Spindle Parameter (Window (30 s) mean)	YFP Control (n=11)	ArchT (n=9)	ArchT Control (n=9)	ArchT REM Control (n=5)
Number	2.12 ± 0.03	2.02 ± 0.03	1.99 ± 0.04	2.08 ± 0.07
Density (spindles / s)	0.071 ± 0.001	0.067 ± 0.001	0.066 ± 0.001	0.069 ± 0.002
Spindle duration (ms)	783 ± 7	775 ± 8	793 ± 15	788 ± 9
Max spindle amp (mV)	0.076 ± 0.006	0.070 ± 0.002	0.067 ± 0.005	0.079 ± 0.008
Spindle magnitude (f)	39 ± 3	36 ± 1	35 ± 3	40 ± 5

Table S5.

Analysis of EEG spindle characteristics during NREMS occurring within 4-5 h fear conditioning post-test ‘recovery’ period (n=9 ArchT, n=11 YFP control, n=9 ArchT control, n=5 ArchT REM control; non-significance indicated by absence of symbols, 2-way ANOVA between groups).

Spindle parameter (Window (30 s) mean)	YFP Control (n=11)	ArchT (n=9)	ArchT Control (n=9)	ArchT REM Control (n=5)
Number	2.17 ± 0.08	2.11 ± 0.06	1.93 ± 0.07	2.01 ± 0.07
Density (spindles / s)	0.072 ± 0.003	0.070 ± 0.002	0.064 ± 0.002	0.067 ± 0.002
Spindle duration (ms)	773 ± 13	783 ± 13	771 ± 15	794 ± 23
Max spindle amp (mV)	0.075 ± 0.007	0.069 ± 0.002	0.069 ± 0.004	0.083 ± 0.016
Spindle magnitude (f)	39 ± 4	35 ± 1	35 ± 3	44 ± 10

Table S6.

Analysis of CA1 cell layer LFP ripple characteristics during NREMS occurring within 0-1 h NOPR Day 1 post-test period. Only data from mice that contributed to CA1 unit analysis is included (n = 4 ArchT, n = 5 YFP control, n = 4 ArchT control; non-significance indicated by absence of symbols, 2-way ANOVA between groups).

Ripple Parameter (Window (30 s) mean)	YFP Control (n=5)	ArchT (n=4)	ArchT Control (n=4)
Number	32.3 ± 1.1	31.5 ± 1.5	32.2 ± 0.4
Density (Ripples / s)	1.08 ± 0.04	1.05 ± 0.05	1.07 ± 0.01
Ripple duration (ms)	23.1 ± 1.0	23.3 ± 1.2	21.0 ± 1.8
Max ripple amp (mV)	0.18 ± 0.03	0.20 ± 0.06	0.14 ± 0.07
Ripple magnitude (j)	3.8 ± 0.6	4.2 ± 1.5	2.9 ± 1.4

Table S7.

Analysis of CA1 cell layer LFP ripple characteristics during NREMS occurring within 1-2 h NOPR Day 1 post-test period. Only data from mice that contributed to CA1 unit analysis is included (n = 4 ArchT, n = 5 YFP control, n = 4 ArchT control; non-significance indicated by absence of symbols, 2-way ANOVA between groups).

Ripple Parameter (Window (30 s) mean)	YFP Control (n=5)	ArchT (n=4)	ArchT Control (n=4)
Number	32.0 ± 0.9	31.4 ± 0.8	33.5 ± 1.4
Density (Ripples / s)	1.07 ± 0.03	1.05 ± 0.03	0.84 ± 0.28
Ripple duration (ms)	23.1 ± 1.0	22.8 ± 1.1	21.2 ± 1.5
Max ripple amp (mV)	0.17 ± 0.03	0.19 ± 0.07	0.16 ± 0.08
Ripple magnitude (j)	3.6 ± 0.5	4.0 ± 1.5	3.2 ± 1.6

Table S8.

Analysis of CA1 cell layer LFP ripple characteristics during NREMS occurring within 2-3 h NOPR Day 1 post-test period. Only data from mice that contributed to CA1 unit analysis is included (n = 4 ArchT, n = 5 YFP control, n = 4 ArchT control; non-significance indicated by absence of symbols, 2-way ANOVA between groups).

Ripple Parameter (Window (30 s) mean)	YFP Control (n=5)	ArchT (n=4)	ArchT Control (n=4)
Number	32.6 ± 1.5	30.7 ± 0.6	32.6 ± 1.0
Density (Ripples / s)	1.09 ± 0.05	1.02 ± 0.02	1.09 ± 0.03
Ripple duration (ms)	22.8 ± 1.0	22.5 ± 0.9	20.4 ± 1.6
Max ripple amp (mV)	0.17 ± 0.03	0.19 ± 0.06	0.14 ± 0.07
Ripple magnitude (j)	3.5 ± 0.5	3.8 ± 1.5	2.7 ± 1.3

Table S9.

Analysis of CA1 cell layer LFP ripple characteristics during NREMS occurring within 3-4 h NOPR Day 1 post-test period. Only data from mice that contributed to CA1 unit analysis is included (n = 4 ArchT, n = 5 YFP control, n = 4 ArchT control; non-significance indicated by absence of symbols, 2-way ANOVA between groups).

Ripple Parameter (Window (30 s) mean)	YFP Control (n=5)	ArchT (n=4)	ArchT Control (n=4)
Number	30.7 ± 1.2	30.3 ± 0.9	32.0 ± 0.6
Density (Ripples / s)	1.02 ± 0.04	1.01 ± 0.03	1.07 ± 0.02
Ripple duration (ms)	23.4 ± 1.0	23.0 ± 1.1	21.1 ± 1.6
Max ripple amp (mV)	0.17 ± 0.03	0.19 ± 0.06	0.14 ± 0.06
Ripple magnitude (j)	3.6 ± 0.6	3.9 ± 1.5	2.7 ± 1.4

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