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Midazolam and Atropine Alter Theta Oscillations in the Hippocampal CA1 Region by Modulating Both the Somatic and Distal Dendritic Dipoles

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Abstract

Theta (4-12 Hz) oscillations in the hippocampus play an important role in learning and memory. They are altered by a wide variety of drugs that impair memory, and these effects may underlie or contribute to drug-induced amnesia. However, the network mechanisms linking drug actions with changes in memory formation remain poorly defined. Here, we used a multisite linear electrode array to measure local field potentials simultaneously across the CA1 layers of the hippocampus during active exploration, and employed current source density analysis and computational modeling to investigate how midazolam and atropine – two amnesic drugs that are used clinically and experimentally – change the relative timing and strength of the drivers of θ -oscillations. We found that two dipoles are present, with active inputs that are centered at the soma and the distal apical dendrite and passive return pathways that overlap in the mid-apical dendrite. Both drugs shifted the position of the phase reversal in the local field potential that occurred in the mid-apical dendritic region, but in opposite directions, by changing the strength of the dendritic pole, without altering the somatic pole or relative timing. Computational modeling showed that this constellation of changes, as well as an additional effect on a variably present mid-apical pole, could be produced by simultaneous changes in the active somatic and distal dendritic inputs. These network-level changes, produced by two amnesic drugs that target different types of receptors, may thus serve as a common basis for impaired memory encoding.

Keywords

In vivo recording; electrophysiology; local field potential; current source density analysis; compartmental modeling

Introduction

Throughout the brain, neuronal networks generate rhythmic activity patterns that encompass a wide range of frequencies and play a variety of roles in brain function (Buzsáki, 2006). In the hippocampus, prominent 4-12 Hz “ θ ” oscillations are thought to contribute to decision-making and mnemonic function, including both memory encoding and retrieval, in animals and in humans (Battaglia et al., 2011; Düzel et al., 2010; Guderian et al., 2009; Jacobs et al., 2006; Montgomery et al., 2009). Lesions that disrupt hippocampal θ -oscillations impair memory (Lipponen et al., 2012; McNaughton et al., 2006; Winson, 1978) and successful completion of memory tasks is correlated with the strength of hippocampal θ -oscillations in humans and in rodents (Lega et al., 2012; Montgomery et al., 2009). Moreover, the phase of underlying θ oscillations has been shown to be crucial in determining the direction of plasticity induced by brief bursts of stimuli *in vitro* (Huerta and Lisman, 1995) and *in vivo* (Hölscher et al., 1997; Hyman et al., 2003). Taken together, these findings provide strong evidence that θ oscillations play an important role in hippocampus-dependent memory.

θ -oscillations are generated in the hippocampal CA1 region through a combination of intrinsic θ -frequency resonance of pyramidal neurons (CA1-PC) and interneurons (Leung and Yim, 1991; Leung and Yu, 1998; Pike et al., 2000), local circuit connectivity (Freund and Buzsáki, 1996; Rotstein et al., 2005), and θ -frequency drive from extra-hippocampal sources (medial septum – diagonal band (MS-DB)), entorhinal cortex, thalamus, amygdala) (Buzsáki, 2002; Chamberland and Topolnik, 2012; Sainsbury and Bland, 1981). The aligned dendrites of CA1-PCs receive organized, domain-specific inputs (Amaral and Lavenex, 2007), resulting in a laminar pattern of synchronized oscillations with a gradual $\sim 180^\circ$ phase reversal in the local field potential (LFP) across hippocampal layers. A two-dipole volume conduction model was proposed by Winson (Winson, 1974) and Bland et al., (Bland et al., 1975) to explain the θ phase reversal, with dipole I produced by rhythmic inhibition at the soma and dipole II by rhythmic phase-shifted excitation from the entorhinal cortex (ECtx) impinging on the distal apical dendrites. Subsequently, using compartmental modeling, Leung (Leung, 1984a) showed that the gradual phase reversal can be accounted for by the presence of phase-offset inputs at the soma and the distal apical dendrites.

A wide variety of drugs that impair (or enhance) memory alter theta oscillations (Hajós et al., 2008; Leung, 1984b; Robbe et al., 2006), suggesting that modulation of the theta rhythm may underlie or contribute to drug-induced amnesia. However, the network mechanisms linking drug actions with changes in memory formation remain poorly defined. In the present study we used multielectrode arrays to record LFPs simultaneously across all CA1 layers during active exploration, and current source density (CSD) analysis and computational modeling to investigate changes in the relative timing and strength of the drivers of θ -oscillations caused by two amnesic agents that are used clinically and experimentally: midazolam, a positive modulator of the GABA_A receptor; and atropine, a muscarinic acetylcholine receptor antagonist. We found that both drugs shifted the position of the LFP phase reversal, but in opposite directions, by altering the amplitude of the dendritic pole in the CSD profile, without altering the somatic pole or relative timing. Computational modeling showed that this constellation of changes, as well as an additional effect on a variably present mid-apical dendritic peak in the CSD signal, could be produced

by simultaneous changes in the active somatic and distal dendritic inputs. These network-level changes, produced by two amnesic drugs that target different types of receptors, may thus serve as a common basis for impaired memory encoding.

Materials & Methods

In vivo methods

We studied the male homozygous offspring of heterozygous GABA_AR $\alpha 5$ -H105R “knock-in” mice (Crestani et al., 2002; Zarnowska et al., 2009). We report here the characteristics of θ -oscillations and their modulation by midazolam and atropine in wild type mice. Studies comparing drug effects on θ -oscillations, γ -oscillations, and θ - γ cross frequency coupling across genotypes, as an indication of the contribution of $\alpha 5$ subunits, will be reported in a separate manuscript. Breeding pairs of mice were generously provided by Uwe Rudolph (Zurich, Switzerland), and backcrossed every five generations with 129SvJ mice (Jackson Laboratories, Maine). The experimental protocol was approved by the University of Wisconsin Institutional Animal Care and Use and complied with National Institutes of Health guidelines.

Chronic electrode implantation

Seven wild-type adult mice between the ages of 70-113 days (average 91 days) and average weight of 26.8 gm were chronically implanted with 15 μ m thick, 3 mm long, single shank silicon micro fabricated probes from NeuroNexus Technologies (‘A’ type probe in CM-series package; Fig. 1A). The electrodes contained 16 recording sites spaced 50 μ m apart between recording sites.

Surgery—Mice were anesthetized using 2.8% isoflurane in an acrylic induction chamber. After they lost their righting reflex they were transferred to a stereotaxic apparatus (KOPF® Instruments, Model 900) with a heated platform (STOELTING Co) maintained at 37°C using a circulating water bath. Anesthesia was maintained by an inhaled concentration of 2.5% isoflurane, administered using a custom-made gas delivery mask that enclosed the clamped snout. Respiratory rate, temperature, and movement were monitored throughout surgery. The concentration of isoflurane was adjusted as needed to maintain regular respiratory rhythm and prevent movement. To prevent corneal drying, sterile lubricant eye ointment (Walgreens®) was applied. The skull was secured in position with ear bars (STOELTING Co, Delrin® ear bars), such that the top surface was parallel to the floor of the apparatus. Commercially available depilatory cream (Nair) was used to remove the fur over the area of the scalp to be incised. The scalp was cleaned with sterile normal saline, a midline ~1 cm-long rostro-caudal incision was made, and the skin was retracted using sterile metal hooks to expose the dorsal surface of the skull. For the ground/reference electrode, a hole was drilled using a 0.7 mm burr (Precision Twist Drill) over the cerebellum on the left occipital bone, and a sterile self-tapping bone screw 0.85 mm diameter (FST®) was inserted and advanced until contact was made with dura matter. A second hole was drilled on the left side for insertion of the recording electrode, 1.9 mm caudal to bregma and 1.5 mm lateral to the midline suture. The skull surface was dried and dental bonding agent (Clearfil DC Bond from Kuraray Medical Inc.) was applied, taking care to prevent it from

coming into contact with skin or drilled holes. A single wire that served as both reference and ground was wrapped around the cerebellar screw and silver paint was applied to ensure good electrical contact. The recording electrode was then lowered using a manual hydraulic-driven micromanipulator (KOPF® Instruments) until it pierced the dura, and advanced to the target coordinate 1.5 mm, to span from alveus to the hippocampal fissure in the CA1 region. The final position was adjusted by monitoring the position of the phase reversal of θ -oscillations induced by tail pinch. The electrode was secured using UV-curing dental composite (FLOW-IT ALC, Pentron Clinical Technologies), the wound closed with sutures, and Triple Antibiotic Ointment (Walgreens®) applied. The mouse was administered Buprenorphine (0.1 mg/kg s.c.) immediately post-surgery, observed during recovery until it was awake and mobile, and returned to its home cage. The first recording session was performed 7-10 days after surgery.

Post-recording histology—Histology was performed on all animals following completion of electrophysiological experiments to verify the locations of the implanted electrodes. The mouse was anesthetized using 2.8% isoflurane in the acrylic induction chamber then maintained with 2.0% isoflurane administered through the nose cone. An electrical lesion was created at the recording site closest to the electrode tip by passing a DC current of 50 μ A for 5 s. After recovery from anesthesia the mouse was returned to its home cage. The following day the mouse was anesthetized using a high dose of isoflurane (5%) until respiration was suppressed, and the brain was fixed by transcardiac perfusion using 0.1 M PBS followed by 4% paraformaldehyde. The brain was removed from the skull and placed in 4% paraformaldehyde for a minimum of 24 hours before slicing using a Leica vibrating microtome (VT 1000S), at a thickness of 50 μ m. The slices were wet-mounted in saline and imaged using bright field microscopy. The laminar positions of electrode locations were estimated based on electrode spacing and tract and lesion locations (Fig. 1B).

Electrophysiological recordings/Experiments

Data acquisition—*In vivo* local field potentials were acquired with Tucker Davis Technology (TDT®) 16-channel recording system (System 3). The mouse was placed in an open top glass aquarium (15 $\times$ 30 cm) cleaned with 70% ethanol and air-dried. The implanted electrode was connected to a 16-channel TDT® headstage (model LP16CH) connected by flexible cable and motorized commutator (AC32) to a 16-channel preamplifier (RA16PA Medusa), all enclosed within a custom-made Faraday cage. The preamplifier was connected via fiber optic cable to a recording base station (RX5 Pentusa). OpenEx software (TDT®) was used to collect data, with 2 Hz high pass filter, 6000 Hz low pass filter, and 12 KHz sampling frequency. At the conclusion of the recording session, the dataset was converted to a MATLAB® (v 2008-2012a) format, digitally low pass filtered at 500 Hz (Butterworth filter, using filtfilt for zero-phase distortion) and down-sampled to 1 KHz for subsequent analysis. (Low pass filtering before down-sampling prevents aliasing.) During recording sessions, animals were allowed to explore their environment freely. Behavior was scored online by the experimenter and classified as immobile, exploring, or grooming, using time-stamped data acquisition through a TDT® scoring box (BBOX) connected to the TDT® base station. Only electrophysiological signals collected during periods of exploration (i.e. walking, running, rearing, sniffing, head movements and changes in body

position) are considered here. As first described by Vanderwolf (Vanderwolf, 1969), these behaviors have been associated with so-called “Type1 θ ”. Figure 1C shows an example of one second of raw data recorded during exploration (left panel) and after filtering at θ -frequency (4-12 Hz, right panel), from all 16 recording sites.

Drug administration protocols—Midazolam was administered to all mice ($n=7$) at a dose of 1.25 mg/kg subcutaneous (s.c.). In a separate study, this dose was found to cause a $\sim 50\%$ decrease in freezing to context when conditioned 40 minutes after drug administration (V. Rau and E.I. Eger II, unpublished data). Mice were mildly sedated at this dose; they continued to explore their environment even without any external stimuli, but to increase the fraction of time they engaged in exploratory behavior, the white light in the experimental room was switched on and off intermittently. Atropine sulfate (Sigma®), a muscarinic antagonist that produces amnesia but not sedation (Pan et al., 1998; Solana-Figueroa and Prado-Alcalá, 1990), was administered at a dose of 50 mg/kg intra-peritoneal (i.p.) to a subset of the animals ($n=5$). This dose blocks “type 2” θ -oscillations in urethane-anesthetized rats (Kramis et al., 1975), and is saturating with respect to modulation of the θ -rhythm (Buzsáki et al., 1983; Hentschke et al., 2007). Sterile 0.9% saline was used as a diluent/vehicle for both drugs.

A subcutaneous injection of sterile saline served as a control for drug administration (control for midazolam $n=6$; control for atropine $n=4$). Although animals did explore their environment without any external stimuli after saline injection, to maintain consistency with drug treatment the white light in the room was switched on and off intermittently after saline injection as well. A mouse was randomly administered either drug or saline, restricted to one experimental session each day; if a drug was administered, no experiment was conducted the following day. Not all animals underwent the entire set of drug protocols due to various technical limitations (e.g. electrode failure, recording equipment breakdown).

Each recording session was comprised of 3 segments (Figs. 1E, F). First, a pre-injection baseline was recorded for 10 minutes. The animal was then injected with either drug or saline (treatment) and returned to the recording chamber. After a waiting period of 20 minutes for atropine or 40 minutes for midazolam, the mouse was transferred to a separate circular plastic enclosure of diameter 25.5 cm, and recording was continued for an additional 10 minutes. For experiments with midazolam/saline-control we analyzed the first 3-minute block of pre-injection baseline and 3-minute block after introduction into the circular enclosure (Fig. 1E). This analysis period corresponded to the time period during which the animal was exposed to a novel environment in corresponding memory tests (V. Rau and E.I. Eger II, unpublished data). The average amount of time spent exploring within the 3-minute analysis period for midazolam was 82 ± 37 sec and for the respective saline-control 129 ± 27 sec. For atropine, we analyzed the full 10 minutes of data (Fig. 1F), as this period of time corresponded to the peak effect on θ -oscillations (Hentschke et al., 2007). With atropine the average time spent exploring was 534 ± 51 sec, almost two times that seen with its respective saline-control (270 ± 61 sec) within the 10-minute analysis period.

Data analysis: The θ -band frequency and phase analysis was performed using a MATLAB® v2008 routine written by Harald Hentschke, as described previously

(Hentschke et al., 2007). The raw signal was bandpass filtered between frequencies of 4-12 Hz and divided into ~4 second segments of 4096 points with one-third overlap. The spectral and cross-correlation values were computed for each such segment and averaged for a single experiment. Power spectral density (PSD) was estimated with a fast Fourier transform (FFT) using a Hamming window of the same length. The oscillation phase at each site with respect to the reference channel at the fissure, identified as the recording site with maximum amplitude of θ and confirmed using post-hoc histological analysis, was estimated by extracting time lags from segment-wise cross-correlations and converted to radians using the θ peak frequency of the reference channel.

Further analyses of the local field potentials and the derived current source densities were implemented as newly created routines using MATLAB® v2012a. For LFP analysis, in order to identify the cell body layer, recordings obtained during immobility (the only use we make here of data obtained during immobility) were bandpass filtered at “ripple frequency” (170-250 Hz). *Stratum pyramidale* was taken as the channel with maximal ripple power (Buzsáki et al., 2003) and confirmed by post-hoc histological analysis.

For CSD analysis, we used the spline iCSD method (MATLAB® Toolbox CSDplotter). This approach to CSD estimation is based on inversion of the electrostatic forward solution, as described by Pettersen et al. (Pettersen et al., 2006). The use of cubic splines presents a more smoothly varying CSD along the electrode (z) axis and reduces the prediction of spurious sources or sinks (Pettersen et al., 2006). Raw EEG signals were θ -bandpass filtered (4-12 Hz) and the spline iCSD method was applied to contiguous 1-second segments of exploring data. The strength of the oscillation in the CSD signal at each interpolated position was quantified by computing the root mean square value (rmsCSD) for each 1-second data segment. This analysis revealed prominent CSD peaks at locations corresponding to *stratum pyramidale* and the fissure, referred to here as the soma pole (S_{pole}) and the distal apical dendritic pole (D-AD_{pole}). In approximately one-half of the recordings an additional peak was present in the mid-apical dendritic region within *stratum radiatum*, referred here as M-AD_{pole}. The rmsCSD measure lacks information about the relative timing of oscillations, so to characterize the phase relationships between the individual poles we used the Hilbert transform of the CSD signal to derive the instantaneous phase angles for all sites.

Data normalization and statistics: To account for differences in electrode implantation angle and local anatomical characteristics (i.e. distance between *stratum pyramidale* and the fissure), positions of LFP signals were normalized using histological measurements. The recording site at *stratum pyramidale* was assigned the value ‘0’, and the site at the fissure was assigned a value of ‘1’. For each experimental session, we identified the midpoint of the θ phase reversal by two methods. First, we performed a linear interpolation between electrode sites with values straddling $\pi/2$ (i.e. one-half of a full phase reversal). Secondly, we fit the LFP phase values to a sigmoidal function using the ‘dose response curve’ in Origin® v 8.6. The midpoints for pre-injection baseline and treatment blocks were grouped by treatment conditions. Differences were tested by one-way ANOVA with Bonferroni post hoc test, using GraphPad Prism® v5.

To control for changes in signal amplitudes between recording sessions, and to combine results from different animals, the rmsCSD of S/M-AD/D-AD_{pole} for each treatment block was normalized to its median value during the pre-injection baseline. Treatment effects were visualized by plotting values as cumulative frequency distributions (cumulative probability), and differences were tested using one-way ANOVA with Bonferroni post hoc test (GraphPad Prism® v5). In several recordings, noise/artifacts were evident, particularly in the center channels corresponding to the location of the M-AD_{pole}. These data were excluded from analysis of CSD amplitude and phase.

Circular statistics (MATLAB® Toolbox CircStat), as described by Philip Berens (Berens, 2009), were used to estimate the mean and variances of the difference between the instantaneous phase angles of the S_{pole}/M-AD_{pole} with reference to D-AD_{pole}.

All reports of mean values are accompanied by their respective standard deviations (mean $\pm$ SD).

In Silico Methods

The NEURON model: The laminar profile of LFPs in the CA1 pyramidal layer was simulated using NEURON v7.1 (Carnevale and Hines, 2009). The model of a CA1 pyramidal neuron, consisting of 335 segments/compartments, was downloaded from ModelDB; accession number 20212 (Poirazi et al., 2003). Extracellular recording sites were simulated using the 'Extracellular stimulation and recording' module (Carnevale, 2005). This method is based on the assumption that the extracellular medium is homogeneous, so that the interaction between the recording electrodes and the model CA1 cell can be estimated as transfer resistances. To simulate activity during θ -oscillations, 8 Hz sinusoidal conductance changes were modeled as point processes, with reversal potential (e_{rev}) set at 0 mV for excitatory and -75 mV for inhibitory inputs. The default resting membrane potential for the model cell was -70 mV. Similar to the approach taken by other investigators (Kopell et al., 2010), the strength of the inputs was chosen such that action potential spikes did not occur. The 16 extracellular recording sites were modeled 50 μ m apart to simulate the arrangement of recording sites *in vivo*. The resistivity of the media was modeled at 180 Ω -cm (Andersen et al., 2007; Golding et al., 2005). The model run time was set to 1000 ms and the integration interval (dt) was set at 1 ms; longer run times and a larger value for dt (40 ms) in our preliminary simulations produced similar results.

Three inputs were simulated in the model for θ LFPs. The somatic input consisted of an 8 Hz inhibitory sinusoidal current injected into a soma compartment (soma[1]). A single compartment in the distal apical dendrite (apical_dendrite[92]) received 8 Hz excitatory sinusoidal input, in phase with the somatic inhibition based on spike timing of basket cells and neurogliaform cells, the firing of which is thought to be coincident with the synaptic volley from entorhinal cortex (Buzsáki, 2002; Fuentealba et al., 2010). This same compartment (apical_dendrite[92]) received 8 Hz inhibitory sinusoidal input, delayed by 108° based on the difference between spike timing of O-LM cells *versus* basket cells recorded *in vivo* (19° versus 271° respectively) (Klausberger et al., 2003). The strength of

the inputs at soma and D-AD were chosen to match the ratio of the S_{pole} to $D-AD_{pole}$ amplitudes seen *in vivo*, using equal strengths of excitatory and inhibitory D-AD inputs.

Data analysis: The 16 channel LFP generated by the model was analyzed by custom routine developed in MATLAB® v2012a. Simulated LFPs were bandpass filtered at 6-10 Hz. The first 250 ms of data were discarded to account for the model settling time. The frequency and phase of the LFP in each channel were estimated using FFT. The CSD was computed using the MATLAB® Toolbox CSDplotter – Spline iCSD method (Pettersen et al., 2006). The strength of the dipoles was quantified as rmsCSD over the 750 ms period.

Results

We first present physiological data obtained from multichannel recordings *in vivo*, examining the effects of midazolam, atropine, or saline (control). We then compare these physiological results with simulated θ -band local field potentials generated using an *in silico* model of CA1 pyramidal cells in the NEURON simulation platform.

1. In vivo

Effects of midazolam and atropine on θ -oscillation frequency and phase

profile—During periods of open field exploration, local field potentials recorded from the dorsal hippocampus showed prominent θ -frequency oscillations (Fig. 1C). The amplitude of the oscillation was maximal at the hippocampal fissure, and there was a gradual shift in the phase of the oscillation recorded from different channels, with a complete reversal in phase occurring between *str. pyramidale* and *str. lacunosum-moleculare*. These results are similar to those reported previously in mice (Buzsáki et al., 2003) and rats (Buzsáki et al., 1983; Winson, 1974).

To test the effects of midazolam and atropine on the frequency of θ -oscillations, we performed an FFT-based spectral analysis. Figure 1D shows power as a function of frequency for unfiltered data. A prominent peak is present within the θ frequency range (4 -12 Hz). Subsequent analysis was restricted to θ -band pass filtered data within this range. Midazolam decreased peak frequency by approximately 1 Hz (Table 1; $p < 0.001$ one-way ANOVA), whereas atropine had no effect on peak frequency of the θ -oscillation (Table 1).

To test whether midazolam or atropine alter the θ -oscillation phase profile, we compared the positions of the phase reversals under different treatment conditions. Figure 2 shows the effect of treatment on the phase profile in a single animal; figure 3 shows combined data for pre-injection baseline and treatment conditions in all animals. Saline (black symbols/lines) did not alter the phase profile, whether measured 40 minutes (Fig. 2A) or 20 minutes (Fig. 2C) after the injection (the different timing matches the protocols for midazolam and atropine), in any of the animals that were tested (Fig. 3A; $n=6$; saline-control for midazolam and fig. 3C; $n=4$ saline-control for atropine). However, midazolam shifted the midpoint of the phase reversal towards the soma (Fig. 2B - blue symbols/line). On average, the shift induced by midazolam was $-57 \pm 23 \mu\text{m}$, as assessed by fitting the phase curve using a sigmoidal function (Fig. 3B; $n=7$, $p < 0.01$ one-way ANOVA). Atropine produced the opposite effect: the midpoint of the phase reversal was shifted away from the soma, towards

the distal apical dendrite (D-AD) (Fig. 2D – orange traces). The average shift was $29 \pm 11 \mu\text{m}$ (Fig. 3D; $n=5$, $p<0.001$ one-way ANOVA). Similar values were obtained by linear interpolation (midazolam $-49 \pm 22 \mu\text{m}$, atropine $29 \pm 12 \mu\text{m}$). We found no significant differences in the Hill slope values in any of the treatment blocks with respect to their pre-injection baselines. These results demonstrate that both midazolam and atropine alter the characteristics of the θ -oscillation in awake, exploring mice – in the case of atropine without altering the frequency of the oscillation.

CSD analysis—The θ -oscillation in the CA1 region is thought to be generated by phase-offset inputs that impinge on the soma and the distal apical dendrites, driving or entraining oscillations in the CA1 pyramidal neurons (Bland et al., 1975; Winson, 1974). The change in the extracellular phase profile with midazolam and atropine might therefore result from changes in the strength or the timing of either of these inputs.

To quantify the location, strength, and timing of the driving inputs in the absence and the presence of midazolam and atropine, we performed current source density analysis of the θ -band LFP. Figure 4 shows an example of a CSD signal derived from the θ -band LFP under control conditions, plotted both in 3-D (Fig. 4A) and 2-D (Fig. 4B) false color profiles. Consistent with the proposed model, there were two peaks in the CSD plots, centered on the soma and on the distal dendrites. We quantified the strength of the two poles by calculating the root mean square (rms) amplitude of the CSD signal for each 1 sec contiguous data segment during exploration, and plotted these rmsCSD amplitudes as a function of position (Fig. 4C). The locations of the peak values (Fig. 4C, red boxes) corresponded to soma and D-AD. We quantified the timing of the two poles by computing the instantaneous phase difference between the channel locations corresponding to the somatic and D-AD poles, and visualized the results by plotting the phase difference as a circular histogram (rose plot). This analysis showed that under control conditions, somatic sinks coincided with dendritic sources, and vice versa, as evident in the 2-D CSD plot (Fig. 4B) and revealed by the phase difference of approximately 180° (Fig. 4D).

To examine the effects of midazolam and atropine on the strength of the somatic and dendritic inputs, we compared their effects on normalized rmsCSD peak values derived from 1 sec data segments (as in Fig. 4C) with saline-controls, plotted for all animals as cumulative frequency (cumulative probability) distributions (Fig. 5). Neither midazolam nor atropine altered the strength of the somatic pole compared to saline-controls (Fig. 5A, C, $p>0.05$, one-way ANOVA). However, both drugs did alter the D-AD pole: midazolam significantly reduced its amplitude (Fig. 5B: midazolam/saline-control $n=7/6$, $p<0.001$ one-way ANOVA), and atropine increased its amplitude (Fig. 5D: atropine/saline-control $n=5/4$, $p<0.001$ one-way ANOVA). Neither drug changed the relative timing of the two poles (Table 2), though atropine did increase the variability of the instantaneous phase difference (Fig. 5E – orange: atropine/saline-control $n=5/4$, $p<0.05$, one-way ANOVA), consistent with a previous report that atropine causes the θ rhythm to be less regular (Hentschke et al., 2007). Midazolam produced no significant changes in phase variability (Fig. 5E – blue: midazolam/saline-control, $n=6/4$, $p>0.05$, one-way ANOVA).

A variably present mid-apical dendritic dipole (M-AD_{pole})—In the CSD analysis presented above we found that the two inputs driving θ -oscillations were centered at the soma and the distal apical dendrite. The somatic component did, however, sometimes display an inflection on its shoulder extending into *stratum radiatum* (e.g. Fig. 4C, recording sites 4-5), and in approximately one-half of the animals (4 of 7) this component appeared as a third distinct peak in the mid-apical dendritic regions in the spatial distribution of CSD. Figure 6 shows an example from one mouse that displayed a particularly prominent mid-apical peak. Indeed, this “M-AD_{pole}” was even larger than the S_{pole} over the 1 s data segment plotted here as a 3-D (Fig. 6A) and 2-D (Fig. 6B) CSD signal. Figure 6C shows the rmsCSD values for all 1 s data segments from this animal, demonstrating the clear presence of three distinct peaks in the CSD signal in this animal. The phase of the difference between D-AD_{pole} and M-AD_{pole}, $154^\circ \pm 0.7^\circ$ (Fig. 6E), was smaller than the difference between D-AD_{pole} and S_{pole} (Fig. 6D; $186^\circ \pm 0.7^\circ$).

The M-AD_{pole} may be caused by a third, independent, variably present, driver of θ -oscillations. Alternatively, it may represent a return pathway associated with the somatic or distal apical dendritic inputs, i.e. the passive sink/source associated with the active source/sink at one (or both) of these inputs. If this latter explanation is correct, its timing suggests that the M-AD_{pole} is driven primarily by the D-AD_{pole}, since the M-AD_{pole} and D-AD_{pole} are nearly antiphasic (Fig. 6E). If the M-AD_{pole} is indeed the passive component of the dipole centered on the D-AD, its amplitude would be expected to covary more strongly with the D-AD_{pole} than the S_{pole}. To test this prediction, we examined the relationship between the amplitude of the M-AD_{pole} and the S_{pole} or D-AD_{pole} during the pre-injection baseline period, and after administering saline, midazolam, or atropine. When examined on a cycle-by-cycle basis, the amplitude of the M-AD_{pole} did vary more strongly with the D-AD_{pole} than the S_{pole}, under a variety of conditions (Figs. 7A-C). Administration of midazolam reduced the amplitudes of both the D-AD_{pole} and M-AD_{pole} (Fig. 7B). By contrast, atropine increased the amplitudes of both the M-AD_{pole} and D-AD_{pole} (Fig. 7C), and preserved their strong cycle-by-cycle correlation. On average, changes in the amplitude of the M-AD_{pole} caused by midazolam (Fig. 7D) and atropine (Fig. 7E) mirrored the changes in the D-AD_{pole} presented previously (Fig. 5B, D). Taken together, these results are consistent with a passive origin for the variably present M-AD_{pole}.

2. In Silico

The physiological data presented above show that the direction of the drug-induced shift in the LFP θ phase profile is predicted by the polarity of the change in the amplitude of the CSD-derived distal apical dendritic dipole, and that the characteristics of the M-AD_{pole} are consistent with it being the passive source associated with an active dendritic sink. These findings suggest that the changes in the LFP produced by midazolam and atropine are caused by changes in the strength of the synaptic inputs that drive or entrain θ -oscillations. To further our understanding of their relationship, we performed compartmental modeling to simulate θ -oscillations in the CA1 region, using the NEURON modeling platform. We examined the impact of changing the strength of somatic and D-AD inhibitory and excitatory synaptic inputs on the θ -oscillation phase profile and on the strength and timing of active and passive components of the CSD signal.

Synaptic inputs were modeled as sinusoidally varying conductances (Fig. 8A), with the timing of the oscillations based on established firing properties of the cellular elements that are thought to be the primary drivers of θ -oscillations. Thus, the somatic input was modeled as an inhibitory conductance centered at 0 degrees based on the firing properties of parvalbumin-positive basket cells (PV-BC's), the main source of inhibitory input to CA1 pyramidal cell somata (Freund and Buzsáki, 1996; Fuentealba et al., 2010; Lapray et al., 2012). The input to the distal apical dendrite was modeled as a combination of synaptic excitation arising from layer III of the entorhinal cortex (Desmond et al., 1994) and inhibition from O-LM inhibitory interneurons. The timing of the excitatory input was inferred to be in-phase with somatic inhibition (i.e. 0 degrees) based on the firing properties of NG cells that are also driven by excitatory input from layer III entorhinal cortex neurons (Fuentealba et al., 2010; Price et al., 2005). The dendritic inhibitory input timing was set at 108 degrees, based on direct measurements of OLM cell firing *in vivo* (Klausberger et al., 2003; Royer et al., 2012). A similar approach based on phase-offset dendritic inhibition and excitation was used in an encoding and retrieval model by Cutsuridis and Hasselmo (Cutsuridis and Hasselmo, 2012).

Using these timing parameters, and adjusting the relative amplitudes of the inputs to match the amplitudes of the S_{pole} and $D\text{-}AD_{\text{pole}}$ observed in our physiological experiments, the model CA1 neuron generated oscillations in the local field potential that reversed phase over approximately 200 μm through *stratum radiatum* (Fig. 8B). The 3-D CSD plot generated from the LFP under these “control conditions” exhibited some cycle-by-cycle variability in dipole amplitudes, despite the unvarying nature of the driving signals (Fig. 9A). This feature was not explored in detail, but it likely reflects the complex kinetic properties of multiple channels present in the model cell, as the oscillation was initiated from a ‘resting state’. Figure 9B shows these same data plotted as a 2-D false color map, demonstrating that the two dipoles generated by the simulation are in opposing phases at each instant in time. The strength of the dipoles was quantified as the RMS amplitude of the CSD signal, averaged over the 750ms segment of simulated data (Fig. 9C). Several characteristics of the simulated oscillations matched the spatiotemporal properties of the physiologically derived CSD signals, including the relative amplitudes and timing of the S_{pole} and $D\text{-}AD_{\text{pole}}$ and the shoulder on the *stratum radiatum* side of the S_{pole} (cf. Fig. 4). One characteristic that did not match as well was the steepness of the phase reversal, which occurred over approximately 200 μm in the model, compared with 300 - 400 μm *in vivo* (Figs 2, 3).

Overlap of return pathways leads to generation of $M\text{-}AD_{\text{pole}}$ —We used this computational model to explore the origin of the shoulder on the S_{pole} , which in some cases appeared as a separate $M\text{-}AD_{\text{pole}}$. Figures 10A and 10B show respectively the θ phase LFP profile and the quantification of the CSD derived from the LFP, i) under “control conditions” (all three inputs present, black traces); ii) with two of the three inputs disabled, so that only a single input drove the oscillation (red, blue, grey); and iii) in the presence of somatic inhibitory and dendritic excitatory input alone (green). As demonstrated previously by Leung (Leung, 1984a), single inputs produced abrupt phase reversals in the LFP (Fig. 10A), with the midpoint shifted toward the source of the input for somatic inhibition or dendritic excitation, compared to the more gradual phase reversal with all inputs present

(Fig. 10A). Notably, the return pathways in the rmsCSD profile for single inputs either at the soma (red trace) or at the distal apical dendrite (blue, grey traces) were largest in *stratum radiatum*, at approximately the location of the S_{pole} shoulder (or the $M\text{-AD}_{\text{pole}}$ when it was present) (Fig. 10B). This finding shows that the location of the $M\text{-AD}_{\text{pole}}$ is consistent with its generation as a passive return pathway. In addition, the timing of the simulated $M\text{-AD}_{\text{pole}}$ was similar to our *in vivo* recordings: the phase difference between the D-AD dipole and the $M\text{-AD}_{\text{pole}}$ (i.e. the location that corresponded to the maximal amplitude of the rmsCSD at *stratum radiatum*) was 168° , whereas the D-AD-to-somatic dipole offset was greater, 203° - again closely matching our *in vivo* observations (Figs. 6D, E), and further supporting the interpretation that the $M\text{-AD}_{\text{pole}}$ represents an overlapping passive return pathway.

To examine the contribution of dendritic inhibition specifically to the CSD and LFP patterns, we also simulated its selective removal (Fig. 10, green traces). This change led to a marked decrease in the amplitude of the $D\text{-AD}_{\text{pole}}$, indicating that combined phase offset excitation and inhibition serve to strengthen this active component of the dipole. This change also eliminated the mid-apical shoulder, but it had little impact on the amplitude of the S_{pole} amplitude, showing that the location of the passive CSD component is influenced by both of the phase-offset dendritic inputs.

Changing input strength predicts direction of phase shift—We next explored how changing the strength of individual inputs, while leaving the other inputs unchanged, would affect the LFP phase profiles and the derived CSD signals. Here we restrict ourselves to reporting only the effects of increasing input amplitudes, as decreasing the amplitudes produced effects of similar magnitude but in the opposite direction, for both the LFP and rmsCSD profiles.

Increasing somatic inhibition shifted the phase reversal towards the soma (Fig. 11A) and increased the strength of the S_{pole} in the rmsCSD (Fig. 11B). Similarly, increasing D-AD excitatory input shifted the position of the phase reversal towards the D-AD (Fig. 11C) and increased the strength of $D\text{-AD}_{\text{pole}}$ (Fig. 11D). Surprisingly, increasing the strength of the inhibitory input at the D-AD shifted the phase reversal away from the D-AD, towards the soma (Fig. 11E) and decreased the amplitude of the $D\text{-AD}_{\text{pole}}$ (Fig. 11F). Modulating only the D-AD input (excitatory or inhibitory) changed the amplitude of the rmsCSD peak (increased or decreased respectively) in the mid-apical dendritic region corresponding to the overlap of the return current pathways, similar to the correlation between the cycle-by-cycle variability in $D\text{-AD}_{\text{pole}}$ and $M\text{-AD}_{\text{pole}}$ amplitudes observed *in vivo* (Figs. 7A-C), as well as corresponding changes in $D\text{-AD}_{\text{pole}}$ and $M\text{-AD}_{\text{pole}}$ induced by administration of midazolam or atropine (Figs. 5B, D, 7D, E). Changing the strength of the D-AD input did also influence the somatic dipole (Fig. 11D), but changing the strength of the somatic input resulted in little or no change in the $D\text{-AD}_{\text{pole}}$ (Fig. 11F), perhaps reflecting the same electrotonic characteristics that produce a direction-dependent voltage attenuation in CA1 pyramidal cells (Carnevale et al., 1995).

Simultaneous offsetting changes at somatic and dendritic sites—Although changes in either excitation or inhibition alone at the distal apical dendrite input did alter the strength of the active D-AD pole and shifted the phase reversal in the LFP in the appropriate

direction to simulate effects of atropine and midazolam, these changes also altered the amplitude of the passive return pole at the soma (Figs. 11D,F). This pattern differs from our physiological results, wherein the shift in phase reversal was accompanied by a change only in the D-AD pole but not somatic pole (Figs. 2, 5). This discrepancy suggests that any change in the strength of the D-AD input may have been accompanied by an offsetting change in the somatic input. For example, an increase in D-AD excitation that would have led to an increase in the amplitude of both the D-AD_{pole} the S_{pole} (Fig. 11D) might have been accompanied by a decrease in the somatic inhibitory input – which as our previous simulation showed would decrease the amplitude of the S_{pole} but have little influence on the D-AD_{pole} (Fig. 11B).

To test whether simultaneous, offsetting changes at both locations would reproduce our physiological results, we simulated changes in the strength of both the dendritic and somatic inputs. We found that when the strength of the D-AD input was increased, reducing the strength of somatic inhibition did indeed bring the amplitude of the S_{pole} back to its baseline value, and that the shift in the phase reversal in LFP away from the soma remained (Figs. 11 G, H, blue traces). This combination thus simulated the pattern of changes induced by atropine. Similarly, increasing the amplitude of somatic inhibition compensated for the increase in the S_{pole} produced by an increase in dendritic inhibition, resulting in a selective reduction in the D-AD_{pole} and a shift of the LFP toward the soma (Figs. 11G, H, grey traces), thereby simulating the pattern produced by midazolam. The similarities in the LFP and CSD patterns of change therefore support a model in which these drugs modulate simultaneously both somatic and dendritic inputs to produce the observed pattern of altered θ -oscillations in the hippocampal CA1 region.

Influence of oscillation frequency—Since the distribution and dynamic response of voltage-gated channels will influence the currents produced by oscillations of different frequency, we considered whether the decrease in frequency produced by midazolam might contribute to the pattern of changes that we observed. Thus, we modeled the driving inputs as 7 Hz sinusoidal conductance waveforms to match the effect of midazolam *in vivo*. This change did not substantially alter either the LFP or CSD phase profiles. However, imposing oscillations at the extremes of the θ range (4 Hz and 12 Hz) did lead to a strong attenuation of CSD amplitudes, and shifted the phase reversal towards the D-AD at 4 Hz and towards soma at 12 Hz (data not shown). These findings demonstrate that membrane characteristics are tuned to reinforce oscillations in the center of the θ -frequency range, and that although extreme changes in oscillation frequency can influence the LFP and CSD, that the modest decrease in frequency produced by midazolam cannot account for the drug-induced shift in phase reversal or change in CSD amplitude at the D-AD that we observed.

Influence of leak conductance—Midazolam has been shown to increase the tonic current amplitude mediated by GABA_A receptors (Bai et al., 2001). To explore the possibility that changes in the θ -oscillation profile *in vivo* caused by midazolam arise from an increase in tonic current, we performed simulations using an increased chloride leak conductance. We based the scale of the impedance change on findings from intracellular recordings in urethane anesthetized rats in which the input resistance decreased by 39%

during theta occurring spontaneously or elicited by tail pinch (Kamondi et al., 1998), and on experiments in mouse hippocampal slices in which bicuculline increased the input resistance by ~13% (Glykys et al., 2008). Thus, the leak conductance was changed step wise to achieve a net decrease in the impedance measured at the distal apical dendrite by 13% and 44%. Contrary to the physiological effect of midazolam, increasing the leak conductance in the model shifted the phase reversal towards the D-AD (Fig. 12A), i.e. in a direction opposite to that seen *in vivo* (Fig. 2B). Also, the amplitudes of both the S_{pole} and $D-AD_{pole}$ were decreased; again contrary to the observation that midazolam alters only the $D-AD_{pole}$ (Fig. 5A, B). Hence, the effects of midazolam that we observed *in vivo* are not accounted for by any changes in tonic inhibition that this drug may produce.

Discussion

The combined electrophysiological and computational modeling results presented above demonstrate that θ -oscillations recorded extracellularly in the CA1 region can be accounted for by driving inputs centered at the somatic and distal apical dendritic regions of pyramidal neurons, confirming the findings and conclusions of previous investigators (Bland et al., 1975; Leung, 1984a; Winson, 1974). In addition we identified a variably present mid-apical dendritic pole in the CSD signal and showed that it likely represents the overlapping return pathways of the somatic and distal dendritic inputs. Finally, we found that midazolam and atropine shift the position of the phase reversal in the LFP and alter the strength but not the timing of the distal apical dendritic pole. This pattern of changes was reproduced in our model by changing in the strength of the driving inputs at both the soma and dendrite. These network-level changes, produced by two drugs that target different receptors, may thus serve as a common basis for impaired memory encoding.

Because CA1 pyramidal cells are arranged parallel to each other, with their inputs aligned (Andersen et al., 2007), the dipoles generated in the CA1 region are reinforcing. This leads to LFPs with well-defined phase relationships that can be modeled using a relatively simple one-cell compartmental model. Thus, based upon the assumption that the extracellular space can be represented as a homogenous and isotropic medium (Buzsáki and Wang, 2012; Pettersen et al., 2006), a model that included only somatic and distal dendritic inputs was sufficient to reproduce the patterns in the CSDs and the LFPs that we observed *in vivo*. This approach, and our findings, are similar to those of Leung (Leung, 1984a), but extended here to incorporate i) inhibitory as well as excitatory dendritic inputs, ii) timing of afferent activity based upon firing properties measured *in vivo*, and iii) a more detailed compartmental model.

Although we based the model structure and parameters on anatomical and physiological data, it does have several limitations: 1) We modeled the excitatory and inhibitory inputs as continually varying point processes, but synaptic inputs consist of multiple, discrete anatomically distributed events. 2) Several different classes of interneurons target the apical dendrites, but we included only O-LM cells in the model. 3) To model oscillatory synaptic drive we assigned conductance values that reproduced observed LFP and CSD patterns. It would have been better to use information from independent physiological measurements; unfortunately such information is not available at present. 4) The compartmental model that

we used was based upon the reconstruction of a rat CA1 pyramidal neuron; it is possible that there are important morphological differences between pyramidal neurons of rats and mice. These differences, and the absence of any heterogeneity in the spatial and temporal properties of the multiple cells that generate the theta signal *in vivo*, may account for the relatively steep phase shift in our model compared to our physiological recordings. Despite these limitations, the correspondence between our modeling and physiological results supports the conclusion that the observed shifts in the LFP phase profile resulted from changes in the strength, but not the location or timing, of the synaptic inputs targeting both the soma and the distal apical dendrite (*cf* Figs. 2, 5, 11G, 11H).

A second important contribution from our modeling studies is the conclusion that the mid-peak in the CSD *in vivo* (M-AD_{pole}) reflects the overlap of the passive current return pathways of the dipoles at the soma and the D-AD. Again, the correspondence between the modeling result – showing that the return pathways of somatic and dendritic inputs overlap in this region (Fig. 10B) – and the *in vivo* experimental result – that the amplitudes of the M-AD_{pole} and D-AD_{pole} co-vary (Figs. 7A-C) – supports this conclusion. The similarity between the timing of the M-AD_{pole} *in silico* and *in vivo* lends further support. Although phase-locked inputs from the Schaffer collateral pathway might also contribute to at least some degree to generating the M-AD_{pole}, their sparse firing might also be ‘drowned out’ by stronger signals originating elsewhere in the cell. Further studies will be required to address this issue. An interesting possibility is that the position and timing of the overlapping influences of the somatic and distal dendritic inputs within the termination zone of the CA3 projection may combine to influence the membrane potential in the diagonal branches targeted by the Schaffer collateral pathway, and thereby support the θ -phase-dependent plasticity that occurs at these synapses (Huerta and Lisman, 1995; Hyman et al., 2003).

As in other brain areas, the local field potential in the CA1 region reflects the summed activity of multiple neurons embedded in a complex network. What circuit elements might be targeted by midazolam and atropine to produce simultaneous changes in both somatic and dendritic inputs? One likely possibility is O-LM interneurons. Indeed, these cells appear to be key players in the network that generates θ -oscillations in the CA1 region. O-LM interneurons resonate at θ -frequency (Pike et al., 2000), and modeling studies indicate that this biophysical property supports their ability to participate in θ -oscillations (Rotstein et al., 2005). However, rather than serving as intrinsic oscillators, they help generate the θ -rhythm through their interactions with fast-spiking cells, such as basket cells, which inhibit O-LM neurons and so lead to rebound spiking in the O-LM cells (Rotstein et al., 2005). Since O-LM cells also provide inhibitory input to the apical dendrites of pyramidal neurons, they are in a position to coordinate the somatic and dendritic inhibitory inputs.

Since θ -oscillations at the soma are driven by inhibitory inputs (Freund and Buzsáki, 1996) and midazolam targets inhibitory synapses, we were surprised to find that the strength of the S_{pole} was unaffected by midazolam. Similarly, since cholinergic inputs from MS-DB target the perisomatic region and modulate the excitability of CA1-PCs and soma-targeting interneurons (Buzsáki, 2002), we were again surprised that the S_{pole} was unaffected by atropine. This was particularly unexpected as it has been reported previously that in animals under urethane anesthesia, in which the θ phase profile is similar to that following surgical

removal of ECtx input to CA1 (Buzsáki et al., 1983; Ylinen et al., 1995), atropine abolished the residual θ oscillations (Buzsáki et al., 1983; Kramis et al., 1975) – presumably by interrupting the somatic input (Buzsáki, 2002). Our simulations showing that offsetting or complementary changes in driving inputs at both the soma and dendrite reproduce our physiological results resolves this paradox and provides a good explanation for the unchanged amplitude of the S_{pole} .

In contrast to the S_{pole} , both drugs modulated the $D\text{-AD}_{\text{pole}}$. Midazolam's effect is relatively straightforward to explain, given the results of our modeling studies: increased inhibition produced by direct modulation of the postsynaptic inhibitory receptors that are activated phasically by O-LM interneurons, neurogliaform cells, and other dendrite-targeting interneurons (Freund and Buzsáki, 1996; Maccaferri and McBain, 1996) led to a decrease in amplitude of the active $D\text{-AD}_{\text{pole}}$ (Figs. 5B, 11F) and of the associated passive $M\text{-AD}_{\text{pole}}$ (Figs. 7D, 11F). These changes resulted in a shift in the position of the LFP phase reversal toward the soma (Figs. 2B, 11E). Since midazolam was administered systemically, it is also possible that it acted elsewhere, outside of the hippocampus; for example, a reduction in excitatory drive from the ECtx could also have contributed to the observed effects. Although midazolam has been found to increase tonic currents *in vitro* (Bai et al., 2001), our modeling studies indicated that this would cause a shift in the phase reversal in the opposite direction to what we observed, making it an unlikely contributor to the changes we observed *in vivo*.

It is less straightforward to account for atropine's effect on the $D\text{-AD}_{\text{pole}}$, particularly since immunoreactivity to the m2 subtype of the mAChR (along with m1 and m3) is concentrated within *stratum oriens* (Levey et al., 1995), and the somata of neurons in *stratum oriens* are prominently labeled by m2 immunoreactive agents (Bernard et al., 2003). A possible link again involves dendrite-targeting O-LM interneurons. Lawrence et al. (Lawrence et al., 2006) demonstrated that mAChR signaling increases O-LM interneuron excitability; the mAChR antagonist atropine would produce the opposite effect – i.e. reduced firing, decreased dendritic inhibition resulting in increased amplitude of the $D\text{-AD}_{\text{pole}}$, and a shift in the position of the phase reversal away from the soma. This mechanism would also account for the increased amplitude of the $M\text{-AD}_{\text{pole}}$. An alternative explanation for the change in the $D\text{-AD}_{\text{pole}}$ is that excitatory input from the ECtx may have increased. However, since blocking muscarinic receptors with scopolamine does not alter the amplitude of θ -oscillations expressed by the medial ECtx (Newman et al., 2013), and the cholinergic MS-DB input to the ECtx is thought to be mediated by nicotinic ACh receptors (Buzsáki, 2002), this mechanism seems less likely.

The drugs that we administered clearly altered the animals' motor behavior, as evidenced by changes in the amount of time they spent exploring. These changes were superimposed on time-dependent changes in mobility even under control conditions. Thus, the smaller fraction of time the mice spent exploring during the 10-minute block following saline injection as a control for atropine (270 seconds / 10 minutes = 27 s/min) compared to the time they spent exploring during the 3-minute block as a control for midazolam (129 seconds / 3 minutes = 43 s/min) likely reflects the gradual decrease in exploratory behavior that occurs over time as animals become accustomed to their surroundings (Benkowitz et al., 2007).

The greater amount of time spent exploring after administration of atropine compared to saline is similar to findings of other investigators following systemic administration of atropine (Buzsáki et al., 1983) or direct administration of the drug into the septum or hippocampus (Leaton and Rech, 1972). It is tempting to infer that the animals continued to explore their surroundings because atropine impaired their learning. However, given the widespread projection of cholinergic fibers to brain regions outside the hippocampus, it is also possible that other mental processes that influence motor behavior such as arousal, anxiety, etc., also contributed to this effect. Similar considerations apply to midazolam, which produces sedation as well as amnesia, though the two actions can be separated and may be brought about through different circuit- and network-level mechanisms (Veselis et al., 2009; Veselis et al., 2001).

Could changes in the animals' motor activity patterns driven by other external influences have contributed to the electrophysiological effects of atropine and midazolam that we observed within the hippocampus? Several groups have shown that the frequency of hippocampal θ -oscillations and running speed in a linear track/ treadmill are correlated (Li et al., 2014; McFarland et al., 1975; Whishaw, 1972), but variations in θ power were attributed primarily to mnemonic processes rather than running velocity or acceleration (Montgomery et al., 2009). Also, the frequency of hippocampal θ can remain unchanged even if the firing rates of neurons increases with running speed (Czurkó et al., 1999). Thus, the relationship between motor activity and hippocampal θ -oscillations is complex. Nonetheless, since θ -oscillations and motor behavior are clearly linked, it is certainly possible that these “indirect actions” of atropine and midazolam contributed to their electrophysiological effects. Disentangling changes in hippocampal oscillations produced by these drugs acting within the hippocampus versus elsewhere throughout the brain will require the use of methods that permit selective modulation of receptors in different types of cells or brain structures.

Our finding that atropine alters θ -oscillations during exploration supports the sensorimotor model of θ -rhythm generation (Bland, 1986; Bland et al., 2006; Bland and Oddie, 2001). In this model, atropine-sensitive θ -oscillations occur not only during alert immobility but also during type I (exploratory) behaviors, in conjunction with atropine-insensitive oscillations imposed by the ECtx. Although atropine did not alter oscillation frequency, nor did it abolish θ -oscillations (as it does under anesthesia or during immobility), it did clearly alter the θ -rhythm. Thus, an atropine-sensitive component of the θ -oscillation is present during active exploration. Evidently a simple model in which an atropine-sensitive oscillation is imposed on the soma while an atropine-insensitive oscillation is imposed on the distal dendrite (Buzsáki, 2002) is not sufficient to account for our findings. The fact that atropine sensitivity is present during exploration is an important finding that must be incorporated into a full account of the generation of the θ -rhythm, and that may underlie its ability to interfere with memory.

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Figure 1.

Measurement of local field potentials (LFPs) in the CA1 region of the dorsal hippocampus. **A**, Neuroxexus 'A' type probes in CM-series package (length 3 mm) were used to record LFPs from 16 recording sites that spanned 800 μm (adapted from www.neuroxexus.com). **B**, Light microscopy image of the left dorsal hippocampus showing electrode track in the CA1 region. **C**, Example of a 1 s data block recorded during active exploration. Left panel shows raw traces at the 1 kHz sampling frequency; right panel is θ -filtered signal (4-12 Hz) of the same raw traces. The filled circles on the dotted line show the 180° phase reversal between recording site 3 (at *stratum pyramidale*) to recording site 9 (at the hippocampal fissure). **D**, Power spectral density (PSD) of the raw signal from all 16 channels. The reference channel at the fissure (grey) has the maximum power within the θ -band. **E**, **F**, Experimental protocols used to assess effects of midazolam and atropine. Grey bars show periods used for data analysis.

Figure 2.

Representative phase plots derived from θ -filtered LFP recordings from one animal. Local phase was derived from cross correlation analysis referenced to the recording site at the hippocampal fissure, and plotted for each recording site. **A, C**, Saline-control for midazolam and atropine. There was no significant difference between the pre-injection baseline (black trace) and treatment block (grey trace). **B**, Administration of midazolam (blue) shifted the midpoint of the phase reversal 38 μm towards the soma, compared to pre-injection baseline (black). **D**, Administration of atropine (orange) shifted the midpoint of the phase reversal toward the distal apical dendrite (D-AD) by 37 μm with respect to pre-injection baseline (black). The somatic recording site was at position '3' and the recording site close to the hippocampal fissure at position '9' on the x-axis for this mouse.

Figure 3.

Phase plots – grouped data for all animals. **A**, Saline (vehicle control for midazolam) administration had no significant effect on the θ -phase profile for all animals compared to midazolam (**B**) which shifted the mid-point of the phase reversal towards the soma. **C**, **D**, Shows effect of injection of saline (vehicle control for atropine) and atropine on the phase reversal. Administration of atropine shifted the midpoint of the phase reversal toward the distal apical dendrite. These treatment effects are consistent across individual mice represented by symbols of the same shape connected by dashed lines. The solid lines derived from sigmoidal fitted curve for all animals for pre-injection baseline and post treatment effects, illustrate the general direction of phase shift after treatment (Black – pre-injection baseline; Grey – saline; Blue – midazolam; Orange -atropine).

Figure 4.

Current Source Density (CSD) analysis of θ -oscillations. **A**, CSD derived from a 1 s data block, analyzed using the spline iCSD method, and plotted as a 3-D view. **B**, 2-D view of data plotted in part **A** illustrating the phase reversal between the two dipoles. **C**, Root mean square amplitude of the CSD signal (rmsCSD) plotted as a function of position. Each trace is derived from a 1 s block of data. This example is from an exploring animal. Peaks in the CSD signal were present at the soma (S_{pole} , recording site 2), and the distal apical dendrite ($D\text{-AD}_{\text{pole}}$, recording site 10). The peak values for each 1 s segment average have been marked by red squares. **D**, Histogram of phase difference between S_{pole} and $D\text{-AD}_{\text{pole}}$ determined by Hilbert transform, analyzed by circular statistics (Philip Berens, 2009), and plotted as a rose plot. There was $\sim 180^\circ$ phase offset between the two dipoles (Circular mean $\pm$ SD: $183^\circ \pm 0.3^\circ$).

Figure 5.

Effects of midazolam and atropine on amplitude of somatic and dendritic dipoles. Peak amplitudes were expressed as rmsCSD values, normalized to the median of pre-injection baseline from all animals, and plotted as cumulative distribution functions. **A, C**, Neither midazolam (blue) nor atropine (orange) altered the amplitude of the somatic pole (S_{pole}) compared to saline-control. **B, D**, Midazolam (blue) significantly reduced, and atropine significantly increased, the amplitude of distal apical dendritic pole ($D-AD_{pole}$) compared to saline-control (one-way ANOVA, $p < 0.001$ for both). **E**, Variance of the phase difference between $D-AD_{pole}$ and S_{pole} before (pre-injection baseline open symbols) and after treatment (filled symbols). Atropine (orange) significantly increased the variance with reference to the pre-injection baseline (one-way ANOVA $p = 0.01-0.05$) whereas saline-control (grey) and midazolam (blue) did not.

Figure 6.

Properties of a variably present mid-apical dendritic pole in the CSD signal. **A**, CSD derived from a 1 s data block, analyzed using the spline iCSD method, and plotted as a 3-D view. **B**, shows the same data as in **A** in 2-D. A prominent peak in the mid-apical dendritic region (recording site 8) is evident in this data segment, larger than the peak at the soma (recording site 3). **C**, Root mean square amplitude of the CSD signal (rmsCSD) plotted as a function of position. Three peaks are clearly seen, corresponding to a somatic (S_{pole}), a mid-apical ($M\text{-AD}_{\text{pole}}$), and a distal apical dendritic pole ($D\text{-AD}_{\text{pole}}$). The peak values for each 1 s segment average have been marked by red squares. **D**, Polar histogram (rose plot) of the phase difference between $D\text{-AD}_{\text{pole}}$ and S_{pole} , with the mean vector centered at $186^\circ \pm 0.7^\circ$. **E**, Polar histogram (rose plot) of the phase difference between $D\text{-AD}_{\text{pole}}$ and $M\text{-AD}_{\text{pole}}$, with the mean vector centered at $154^\circ \pm 0.7^\circ$.

Figure 7.

Effects of midazolam and atropine on the relationship between the M-AD_{pole} and the S_{pole} or D-AD_{pole}. **A-C**, Amplitudes of the D-AD_{pole} or S_{pole}, as analyzed on a cycle-by-cycle basis, are plotted as a function of M-AD_{pole} amplitude during the pre-injection baseline (open symbols) and after injection (solid symbols) of saline (gray), midazolam (blue), or atropine (orange) for a single mouse. Solid lines show linear fits to the data. The amplitude of the D-AD_{pole} but not S_{pole} varied with the amplitude of the M-AD_{pole}. **D, E**, Midazolam (blue) significantly decreased, and atropine (orange) increased M-AD_{pole} amplitude, compared to saline-controls (one-way ANOVA $p < 0.001$ for both). Peak amplitudes were expressed as rmsCSD values, normalized to the median of pre-injection baseline from all animals, and plotted as cumulative distribution functions, for all mice in which an M-AD_{pole} was present (four of seven).

Figure 8.

Simulation of extracellular field θ -oscillations by compartmental modeling. **A**, Morphology of the CA1 pyramidal cell used for compartmental modeling. Positions of the 16 simulated extracellular recording sites are indicated by the small filled circles; locations of the 8 Hz somatic and distal apical dendrite (D-AD) inputs are indicated by the large filled circles. **B**, Simulated LFP from the 16 extracellular recording sites, with oscillations driven by somatic inhibitory and dendritic inhibitory plus excitatory inputs. The superimposed black lines illustrate the phase reversal of theta between the somatic and D-AD recording sites.

Figure 9.

CSD analysis of simulated θ -oscillations. The locations of the recording sites are normalized, with '0' corresponding to the somatic input and '1' corresponding to the distal apical dendritic input. **A**, CSD derived from a 1 s data block, analyzed using the spline iCSD method, and plotted as a 3-D view. **B**, 2-D view of data plotted in part **A** illustrating the phase reversal between the two dipoles. **C**, Root mean square amplitude of the CSD signal illustrated in **A**, plotted as a function of position.

Figure 10.

Comparison of simulated oscillations driven by multiple versus individual inputs. Black traces represent the “control condition” with all 3 inputs present: somatic inhibition (0°) plus distal apical dendritic (D-AD) inhibition (108°) and excitation (0°). The red traces represent somatic inhibition alone, blue traces D-AD excitation alone, and the grey traces D-AD inhibition alone. The green traces show the effect of removing dendritic inhibition, leaving only somatic inhibition and dendritic excitation. **A**, Phase profile of simulated θ -filtered LFP. Single inputs resulted in a steep phase reversal. With all inputs present the phase reversal was more gradual, occurring over $\sim 200 \mu\text{m}$. Somatic inhibition plus dendritic excitation produced an intermediate steepness. **B**, Amplitude of CSD signal (rmsCSD) showing that the spread of currents depended on the location and type of inputs. The arrows point to the peak of passive return current when a single input was present. The return pathways of all inputs overlap at the mid-apical dendritic region. In the absence of dendritic inhibition, the amplitude of the dendritic pole and mid-apical shoulder are reduced.

Figure 11.

Simulation of drug effects by changing the strength of individual inputs. The black traces represent “control conditions” with all 3 inputs present. For each input in the first 3 rows, two step-wise increases were tested, with the dashed lines indicating the smaller increase and the solid lines the higher increase in input strength. **A, C, E, G** Phase profiles of θ -filtered simulated LFPs. **B, D, F, H** Corresponding amplitudes of CSD signals as a function of position. **A, B**, Increasing inhibition at the soma (red) shifted the phase profile towards the soma and increased the rmsCSD amplitude at the soma. **C, D**, Increasing excitation at the D-AD (blue) shifted the phase profile towards the D-AD and increased the rmsCSD amplitude at the soma and mid-apical dendritic sites as well as at the D-AD. **E, F**, Increasing inhibition at D-AD (grey) shifted the phase profile towards the soma and decreased the rmsCSD amplitude at the soma and mid-apical dendritic sites as well as distal dendrites. **G, H**, Decreasing inhibition at the soma and increasing excitation at the D-AD increased the D-AD_{pole} without changing the S_{pole}, and shifted the phase profile towards the D-AD (blue). Increasing inhibition at both the soma and D-AD decreased the D-AD_{pole} without changing the S_{pole}, and shifted the phase profile towards the soma (grey).

Figure 12.

Simulating an increase in tonic inhibitory current. **A**, The phase profile of the θ -filtered simulated LFP was shifted towards the D-AD with decreased impedance, corresponding to increase in tonic inhibition (grey) traces, compared to control 3-input simulation (black trace). **B**, The amplitude of the CSD signal decreased at both the somatic and D-AD sites with decreasing impedance.

Table 1
Effects of midazolam and atropine on peak frequency

Frequency - Hz Mean $\pm$ Std.Dev.	Pre-injection Saline-control	Saline-control	Pre-injection Treatment	Treatment
Midazolam	8.2 $\pm$ 0.2 n=6	8.3 $\pm$ 0.2 n=6	8.2 $\pm$ 0.3 n=7	7.0 $\pm$ 0.5 n=7
Atropine	8.3 $\pm$ 0.3 n=4	8.2 $\pm$ 0.3 n=4	8.2 $\pm$ 0.3 n=5	8.2 $\pm$ 0.3 n=45

Peak θ frequency in exploring mice before and after injection of midazolam or atropine, and time-matched saline controls. Midazolam decreased the frequency of θ by ~ 1 Hz compared to both pre-injection baseline (treatment) and saline-control ($p < 0.001$ one-way ANOVA).

Table 2
Average phase difference between D-AD_{pole} and S_{pole}

Phase Difference-degrees Mean $\pm$ S.D.	Pre-injection Saline-control	Saline-control	Pre-injection Treatment	Treatment
Midazolam	191.0 $\pm$ 6.0 n=4	189.0 $\pm$ 6.1 n=4	187.6 $\pm$ 4.7 n=6	194.0 $\pm$ 3.8 n=6
Atropine	189.5 $\pm$ 4.9 n=4	189.3 $\pm$ 5.5 n=4	188.2 $\pm$ 5.3 n=5	190.0 $\pm$ 5.1 n=5

Neither midazolam nor atropine significantly altered the relative timing of these CSD peaks.