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Mu opioid receptor activation normalizes temporo-ammonic pathway driven inhibition in hippocampal CA1

A. Rory McQuiston

Department of Anatomy and Neurobiology Virginia Commonwealth University Box 980709
Richmond, VA 23298 USA Tel: 804-828-1573 amcquiston@vcu.edu

Abstract

The hippocampus of the mammalian brain is important for the formation of long term memories. Hippocampal-dependent learning can be affected by a number of neurotransmitters including the activation of μ -opioid receptors (MOR). It has been shown that MOR activation can alter synaptic plasticity and network oscillations in the hippocampus, both of which are thought to be important for the encoding of information and formation of memories. One hippocampal oscillation that has been correlated with learning and memory formation is the 4-10 Hz theta rhythm. During theta rhythms, inputs to hippocampal CA1 from CA3 (Schaffer collaterals, SC) and the entorhinal cortex (perforant path) can integrate at different times within an individual theta cycle. Consequently, when excitatory inputs in the stratum lacunosum-moleculare (the temporo-ammonic pathway (TA), which includes the perforant path) are stimulated approximately one theta period before SC inputs, the TA can indirectly inhibit SC inputs. This inhibition is due to the activation of postsynaptic GABA_B receptors on CA1 pyramidal neurons. Importantly, MOR activation has been shown to suppress GABA_B inhibitory postsynaptic potentials in CA1 pyramidal neurons. Therefore, we examined how MOR activation affects the integration between TA inputs and SC inputs in hippocampal CA1. To do this we used voltage-sensitive dye imaging and whole cell patch clamping from acute hippocampal slices taken from young adult rats. Here we show that MOR activation has no effect on the integration between TA and SC inputs when activation of the TA precedes SC by less than one half of a theta cycle (< 75 ms). However, MOR activation completely blocked the inhibitory action of TA on SC inputs when TA stimulation occurred approximately one theta cycle before SC activation (> 150 ms). This MOR suppression of TA driven inhibition occurred in both the SC input layer of hippocampal CA1 (*stratum radiatum*) and the output layer of CA1 pyramidal neurons (*stratum pyramidale*). Thus MOR activation can have profound effects on the temporal integration between two primary excitatory pathways to hippocampal CA1 and subsequently the resultant output from CA1 pyramidal neurons. These data provide important information for understanding how acute or chronic MOR activation may affect the integration of activity within hippocampal CA1 during theta rhythm.

Keywords

voltage-sensitive dye; integration; interneuron; feed forward; theta

1. Introduction

During exploratory behaviors and learning and memory tasks the hippocampal neural network oscillates at a frequency of 4 to 12 Hz called the theta rhythm (Buzsaki, 2002; Hasselmo, 2005). In hippocampal region CA1, the Schaffer collaterals (SC) from hippocampal CA3 and the perforant path from the entorhinal cortex (PP) on average activate CA1 out of phase from each other during theta rhythms (Bragin et al., 2000; Buzsaki et al., 1986; Kamondi et al., 1998). However, individual CA3 pyramidal neurons do not always fire at the same phase of theta. CA3 pyramidal neurons in the dorsal hippocampus, which encode an animal's position in space (O'Keefe and Dostrovsky, 1971), produce action potentials at different phases of the theta rhythm depending on the animal's location (O'Keefe and Recce, 1993; Skaggs et al., 1996). Thus, inputs from CA3 and PP may integrate in hippocampal CA1 at varying time intervals within a single theta period.

The PP together with excitatory inputs from the reuniens nucleus of the thalamus form the major excitatory input to the stratum lacunosum-moleculare (SLM) of hippocampal CA1 called the temporo-ammonic pathway (TA). Previous *in vitro* studies have examined how the TA inputs affect SC excitation of CA1 pyramidal neurons (Dvorak-Carbone and Schuman, 1999; Empson and Heinemann, 1995; Enoki et al., 2001; Enoki et al., 2002). Simultaneous stimulation of the TA and SC resulted in nonlinear summation of the two EPSPs (Empson and Heinemann, 1995; Enoki et al., 2001; Enoki et al., 2002). However, if TA input stimulation occurred one theta period or longer before SC stimulation, SC EPSPs could be suppressed in both the dendrites and somata of CA1 pyramidal neurons (McQuiston, 2010) and action potential spiking could be prevented in CA1 pyramidal neurons (Dvorak-Carbone and Schuman, 1999) through a GABA_B receptor dependent mechanism. Interestingly, if the TA was stimulated half a theta period or shorter before SC stimulation, SC EPSPs and their propagation to CA1 pyramidal cell somata were unaffected by TA stimulation (McQuiston, 2010). Therefore, depending on the timing of inputs from the TA and SC, the TA may suppress incoming inputs from CA3 within an individual theta cycle.

Both hippocampal rhythmicity and memory performance can be altered by a number of neuromodulators, including endogenous and exogenous opioids. The activation of μ -opioid receptors (MOR) can alter synaptic plasticity in hippocampal area CA1 and impair spatial memory performance (Mansouri et al., 1997; Mansouri et al., 1999; Pourmotabbed et al., 1998; Pu et al., 2002; Wagner et al., 2001). In addition to altering synaptic plasticity, MOR activation has been shown to disrupt synchronous oscillations among populations of CA1 pyramidal cells, which is thought to be important for coding of information (Faulkner et al., 1998; Faulkner et al., 1999; Whittington et al., 1998). Furthermore, gating of sensory information in the rodent hippocampus has been shown to be disrupted during opioid dependence and facilitated during opioid withdrawal (Zheng et al., 2005). However, exactly how MOR receptor modulation of hippocampal neurons and synapses results in changes in network properties is incompletely understood.

Immunohistochemistry at both the light and electron microscope levels has shown that MORs are almost exclusively located on GABAergic inhibitory interneurons axons, terminals, dendrites and somata (Bausch et al., 1995; Drake and Milner, 1999; Drake and Milner, 2002; Kalyuzhny and Wessendorf, 1997). Consistent with their anatomical localization in the hippocampus, activation of MORs was shown to hyperpolarize CA1 inhibitory interneurons and presynaptically inhibit GABAergic inhibitory postsynaptic potentials (IPSPs) in CA1 pyramidal neurons (Capogna et al., 1993; Cohen et al., 1992; Lupica et al., 1992; Lupica, 1995; Lupica and Dunwiddie, 1991; Madison and Nicoll, 1988; Masukawa and Prince, 1982; Reikling, 1993; Svoboda et al., 1999; Svoboda and Lupica,

1998; Swearengen and Chavkin, 1989; Wimpey and Chavkin, 1991). MOR disinhibition was subsequently shown to facilitate EPSPs in all layers of hippocampal CA1 (McQuiston and Saggau, 2003) by presynaptically inhibiting the release of GABA onto both GABA_A and GABA_B receptors (McQuiston, 2007; McQuiston, 2008; McQuiston and Saggau, 2003). This MOR inhibition in GABA release resulted in a 50% suppression of monosynaptic GABA_B receptor IPSPs in all layers of hippocampal CA1 (McQuiston, 2007). Interestingly, it is through the activation of GABA_B receptors that inputs from the TA inhibit SC EPSPs in hippocampal CA1 (Dvorak-Carbone and Schuman, 1999). Therefore, the effects of MOR activation on synaptic plasticity and learning and memory may occur in part by altering the interaction of TA and SC inputs during theta rhythm activity. Thus, we investigated the effect of MOR activation on the integration of synaptic inputs from the TA and SC within an individual theta cycle. To do this we used voltage-sensitive dye imaging and whole cell patch clamp methods to measure the impact TA inputs have on SC EPSPs in the somatic and dendritic layers of hippocampal CA1. Here we show that MOR activation completely blocks TA driven feed forward inhibition of SC inputs in hippocampal CA1.

2. Methods

2.1. Preparation of hippocampal slices and staining with voltage-sensitive dye

Male Sprague Dawley rats (42 to 60 days old) were deeply anaesthetized with ketamine (200 mg/kg) and xylazine (20 mg/kg), administered by intraperitoneal injection. Once the animal's heart rate and respiration approached zero and the animals no longer responded to toe pinch, the animals were transcardially perfused with ice cold saline (consisting of (in mM): sucrose 230, KCl 2.5, CaCl₂ 2, MgCl₂ 6, NaHPO₄ 1, NaHCO₃ 25, glucose 25) and sacrificed by decapitation in adherence with ethical guidelines approved by the Institutional Animal Care and Use Committee of Virginia Commonwealth University (protocol AD20109). The brain was removed, hemi-sectioned, and horizontal slices containing the mid temporal hippocampus were cut at 350 μ m on a vibratome 3000 (Ted Pella Inc, Redding CA). Sections were incubated in a holding chamber kept at 36°C for 30 min and then allowed to return to room temperature. The holding chamber solution consisted of normal saline (in mM): NaCl 125, KCl 3.0, CaCl₂ 1.2, MgCl₂ 1.2, NaHPO₄ 1.2, NaHCO₃ 25, glucose 25 bubbled with 95% O₂ / 5% CO₂. Slices were stained for 30 to 60 min with the voltage-sensitive dye (VSD) NK3630 (0.02 to 0.05 mg/ml) prior to experimentation.

2.2. Voltage-sensitive dye imaging and electrophysiology

Following staining with NK3630, slices were submerged and continuously perfused in a glass bottom recording chamber with warmed normal saline. The recording chamber was mounted on a fixed stage under an Olympus BX51WI microscope equipped with differential interference contrast (DIC) optics. The image of the slice was collected using transmitted near infrared light (> 775 nm) with a 20x (0.95 NA) water immersion objective. The image was captured (Foresight I-50 frame grabber) with a DAGE-MTI IR1000 CCD camera with contrast enhancement.

For voltage-sensitive dye absorbance measurements, slices were illuminated with a tungsten-halogen 100 W lamp passed through a bandpass filter (705 $\pm$ 30 nm, Chroma Technology, Rockingham, VT). The transmitted light was collected with a Wutech H-469IV photodiode array that is part of the Redshirtimaging integrated Neuroplex II imaging system, mounted on the front port of the Olympus BX51WI microscope. The data were acquired, displayed and analyzed with Neuroplex software.

To evoke synaptic electrical activity in hippocampal CA1, bipolar tungsten or platinum/iridium stimulating electrodes (approx. 100 k Ω , FHC) were placed in *stratum radiatum* (SR)

to excite SC from CA3 pyramidal neurons, and in SLM (adjacent to or within subiculum) to excite inputs from the entorhinal cortex and reuniens nucleus of the thalamus (TA). In some experiments, a cut was made to the slice, near the subiculum, from the alveus down to the border of SR and SLM to ensure that only afferents in SLM were activated (Maccaferri and McBain, 1995). Electrical current was delivered (100 - 200 μ s, 10 to 100 μ A) by a stimulation isolation unit (NL800, Digitimer, Hertfordshire, England) to activate excitatory inputs in SR and SLM. Adjacent groups of photodiodes of the array that were perpendicularly oriented to the SP were selected to measure the voltage-sensitive dye (VSD) signals that propagated from their initiation site through SR and SP of hippocampal CA1. This increased the likelihood that the signals between anatomical layers in CA1 came from the same population of pyramidal cells. The signal from one photodiode was spatially averaged with its immediate surrounding photodiodes (nine photodiodes in total) to represent the electrical response in SR and SP of CA1. Furthermore, the VSD signals were an average of four measurements that were sampled at 1.6 kHz and low pass filtered at 126 Hz.

2.3. Electrophysiological Measurements

Whole cell patch clamp recordings at the soma of CA1 pyramidal neurons were used to examine the inhibitory effect of TA prepulses on the ability of SC inputs to drive action potential firing. Whole cell patch clamp recordings were performed using patch pipettes (2 to 5 M Ω) pulled from borosilicate glass (8250 1.65/1.0 mm) on a Narishige PP830 pipette puller filled with (in mM): KMeSO₄ 135, NaCl 8, MgATP 2, NaGTP 0.3, HEPES 10, BAPTAK₄ 0.1. Membrane potentials were measured with a Model 2400 patch clamp amplifier (A-M Systems, Port Angeles, WA) and further amplified and filtered using an 8 pole Bessel filter with a second stage amplifier (Brownlee 440, Brownlee Precision Co, San Jose, CA) to maximize the signal across a PCI-6040E A/D board (National Instruments, Austin, TX). WCP Strathclyde Electrophysiological Software was used to store and analyze membrane potential responses on a PC computer (courtesy of Dr. J Dempster, Strathclyde University, Glasgow, Scotland).

2.4. Activation of μ -opioid receptors

In order to activate MORs, we bath applied DAMGO (1 μ M) at a concentration required to produce maximal activation of MORs with little activity at other opioid receptor subtypes (Chieng and Christie, 1994; Law and Loh, 1999; Shen and Johnson, 2002; Zhao et al., 2003). To ensure that any effect resulted from the activation of MORs, we blocked the effect of DAMGO with the MOR antagonist CTOP (1 μ M) at a concentration selective for MORs (Kramer et al., 1989; Law and Loh, 1999).

2.5. Statistics and data analysis

Data were analyzed using WCP software for the electrophysiological measurements and Neuroplex II software for the VSD recordings. Statistics were performed using GraphPad Instat (GraphPad Software, San Diego, CA). In order to quantify the effect of a TA prepulse on the amplitude of SC elicited EPSPs, the amplitude of the SC-elicited VSD signals in SR and SP were measured using baseline signal values preceding the TA prepulse. The amplitude of SC EPSPs preceded by TA stimulation were normalized to SC EPSPs measured in the absence of a TA prepulse (Amplitude with TA prepulse/Amplitude SC stimulation alone) for statistical comparisons.

To quantify the excitability of CA1 pyramidal neurons, the probability that a SC-evoked EPSP produced an action potential was measured. For statistical comparisons, the probability of an action potential occurrence following a TA prepulse was normalized to the probability of action potential occurrence from SC stimulation alone. Statistical

significances were determined using repeated measures ANOVA or One-way ANOVA and Bonferroni post hoc tests. In some cases, paired t-tests were used. Differences were determined to be statistically significant for p values less than 0.05. All data was reported as the mean $\pm$ standard error of the mean (SEM).

2.6. Chemicals

All chemicals were purchased from VWR unless otherwise indicated. NK3630 was obtained from Hayashibara Co. (Japan). [D-Ala², NMe-Phe⁴, Gly-ol⁵]-enkephalin (DAMGO) and D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH₂ (CTOP) were purchased from Tocris Bioscience (Ellisville, Missouri).

3. Results

We have previously demonstrated that SC input to hippocampal CA1 can be inhibited by the TA when TA stimulation occurred approximately a single theta cycle (150 – 225 ms) before the activation of SC (McQuiston, 2010). Furthermore, the activation of cholinergic muscarinic receptors, which increases the amplitude of theta rhythms in the hippocampus (Lee et al., 1994), augmented the inhibitory effect of TA stimulation on SC inputs (McQuiston, 2010). Interestingly, previous studies have shown that rhythmicity in the hippocampus can be disrupted by the activation of MOR, likely through the suppression of interneuron function (Whittington et al., 1998). We have previously shown that inhibitory interneurons activated by TA inputs can be inhibited by the activation of MORs (McQuiston, 2008; McQuiston and Saggau, 2003). Therefore, we investigated the effect of MOR activation on the integrative properties of TA and SC inputs in hippocampal CA1 across various theta time periods. All experiments were performed in the presence of carbachol (1 μ M) to activate cholinergic receptors in an attempt to mimic conditions that may occur during theta rhythms.

3.1. MOR activation does not affect integration between temporo-ammonic and Schaffer collateral inputs in hippocampal CA1 when Schaffer collateral activation is delayed by twenty five milliseconds

In order to examine the effect of TA stimulation on SC inputs in hippocampal CA1, we placed bipolar stimulating electrodes in SLM and SR to activate TA and SC afferents respectively (Fig. 1A). We measured the effect of TA stimulation on SC evoked EPSP VSD signals in both the dendritic layer and the output layer of hippocampal CA1. In order to quantify the SC evoked EPSP magnitude, we spatially averaged VSD signals from 9 adjacent photodiodes overlying SR (Fig. 1A red traces) and SP (Fig. 1A blue traces). The amplitude of the averaged SC evoked EPSP (illustrated in Fig. 1B) was measured and the amplitude of SC evoked EPSP following TA stimulation was normalized to SC evoked EPSP amplitudes elicited in the absence of TA stimulation.

When TA stimulation preceded SC stimulation by 25 ms, the amplitude of the TA EPSP and SC EPSP in both SR and SP of hippocampal CA1 summated to produce larger VSD signals relative to signals measured following SC stimulation alone (Fig. 1B top). Bath application of DAMGO (1 μ M) did not significantly affect the summation of TA and SC EPSPs. These data are summarized in a histogram showing that the normalized amplitude of SC EPSPs that followed TA stimulation by 25 ms was not significantly affected by the application of DAMGO (Fig. 1C, repeated measures ANOVA $p = 0.66$, $n = 6$). Therefore, the activation of MORs did not affect the integration between TA inputs and SC inputs when TA input activation preceded SC activation by 25 ms.

3.2. MOR activation does not affect integration between temporo-ammonic and Schaffer collateral inputs in hippocampal CA1 when Schaffer collateral activation is delayed by seventy five milliseconds

We next examined whether the activation of MORs could alter the integration between TA and SC inputs in hippocampal CA1 when TA inputs were stimulated 75 ms before SC activation. We chose the 75 ms interval because it is equivalent to half a 7 Hz theta cycle or the conditions in which TA inputs and SC inputs in CA1 are out of phase with one another. This out of phase relationship between TA and SC inputs in CA1 has been observed when measuring population activity using current source density analysis (Bragin et al., 2000; Kamondi et al., 1998). Therefore, we investigated the effect of DAMGO on SC evoked EPSPs preceded 75 ms by stimulation of TA afferents.

When TA stimulation preceded SC stimulation by 75 ms, the amplitude of the TA EPSPs and SC EPSPs in both SR and SP of hippocampal CA1 did not summate to produce VSD signals that were significantly different in amplitude compared to signals measured following SC stimulation alone (Fig. 2A). Importantly, bath application of DAMGO (1 μ M) did not significantly affect the interaction between TA and SC EPSPs (Fig. 2B). The lack of effect of DAMGO on the interaction between TA and SC inputs was normalized and illustrated in figure 2C (repeated measures ANOVA $p = 0.51$, $n = 5$). Therefore, the activation of MORs did not affect the integration between TA inputs and SC inputs when TA input stimulation preceded SC activation by 75 ms.

3.3. MOR activation prevents temporo-ammonic input driven suppression of Schaffer collateral inputs in hippocampal CA1 when Schaffer collateral activation is delayed by 200 ms

We have previously shown that activation of TA afferents 200 ms before SC stimulation resulted in an inhibition of SC EPSPs in SR and SP of hippocampal CA1 (McQuiston, 2010). Because MOR activation partially blocks monosynaptic IPSPs in SR and SP of hippocampal CA1 (McQuiston, 2007; McQuiston, 2008; McQuiston and Saggau, 2003), we next examined the effect that the activation of MORs has on the integrative properties between TA and SC afferents in hippocampal CA1 when SC stimulation was delayed by 200 ms.

When TA was stimulated 200 ms before SC, the amplitude of EPSPs in SR (purple) and SP (dark blue) were smaller relative to EPSPs evoked by SC stimulation alone (SR – red, SP – light blue, Fig. 3A). This TA prepulse inhibition of SC inputs was completely blocked by the activation of MORs by DAMGO (1 μ M) (Fig. 3B). The amplitude of SC evoked EPSPs inhibited by a TA prepulse was normalized to the amplitude of SC evoked EPSPs measured in the absence of TA stimulation (Fig. 3C). On average SC evoked EPSPs were smaller when TA was stimulated 200 ms before SC (Fig. 3C grey bars, two-tailed t-test: SP $p < 0.0005$, SR $p < 0.0001$, $n = 10$). However, application of DAMGO completely blocked the inhibition produced by the TA prepulse (Fig. 3C red bars, repeated measures ANOVA $p < 0.0001$, Bonferroni post hoc test $p < 0.0005$ for SP and $p < 0.0001$ for SR for comparisons to control, paired t-test, $p > 0.05$ for comparisons to hypothetical value of 1, $n = 10$). Subsequent application of the MOR antagonist CTOP (1 μ M) reversed the effects of DAMGO on TA prepulse inhibition (Fig. 3C, blue bars, repeated measures ANOVA $p < 0.0001$, Bonferroni post hoc test $p < 0.01$ for SP, $p < 0.0001$ for SR, $n = 10$). Therefore, the activation of MORs completely prevents TA inhibition of SC inputs in hippocampal CA1.

3.4. MOR activation impairs temporo-ammonic prepulse gating of Schaffer collateral synaptically driven action potential firing

Our data has shown that the inhibition of subthreshold SC evoked EPSPs by TA prepulses was completely eliminated by MOR activation. In these studies we used small stimulation intensities to prevent the activation of postsynaptic action potentials. These small intensity stimuli permitted us to examine subthreshold integration of synaptic events between the TA and SC in hippocampal CA1. Next we examined how MOR activation and TA prepulse inhibition affected SC driven suprathreshold activity in hippocampal CA1 pyramidal neurons.

Whole cell patch clamp recordings were obtained from hippocampal CA1 pyramidal neurons in rat brain slices. The intensity of stimulation of SC inputs was increased relative to the preceding experiments so that SC EPSPs evoked action potentials on the majority of trials (Fig. 4A, black trace). Stimulation of the TA produced a small EPSP followed by an IPSP that suppressed the amplitude of the SC driven EPSP (Fig. 4A red trace). The IPSP resulted in a reduction in the number of trials that an SC driven EPSP produced an action potential. DAMGO (1 μ M) blocked the TA-evoked IPSP and increased the number of trials that SC stimulation produced an AP (Fig. 4B). The probability that SC synaptic stimulation produced an action potential in CA1 pyramidal neurons was quantified for each condition. TA prepulses (200 ms) significantly reduced the probability that an SC stimulus produced a postsynaptic action potential (Fig. 4C, dark bars, ANOVA $p < 0.0001$, Bonferroni post hoc test $p < 0.001$, $n = 8$). DAMGO application had no effect on the probability that a single SC stimulus produced an action potential; however, DAMGO did block the ability of a TA prepulse to reduce the probability of action potential firing following SC stimulation (Fig. 4C, Bonferroni post hoc test $p < 0.001$, $n = 8$). The effect of DAMGO was blocked by the MOR antagonist CTOP (Fig. 4C, Bonferroni post hoc test $p < 0.001$, $n = 5$). Therefore, the activation of MORs in hippocampal CA1 can completely block TA suppression of suprathreshold responses to SC inputs.

4. Discussion

These studies have shown that MOR activation impairs the integration between TA and SC inputs in hippocampal CA1. The effect of MOR activation was dependent on the timing between TA and SC stimulation. If TA was activated less than half a theta cycle before SC activation, MOR did not influence TA and SC integration. In contrast, if TA inputs were stimulated approximately one theta cycle before SC input, MOR activation completely eliminated any influence TA had on SC driven activity. Therefore, during theta rhythms, MOR activity is capable of completely disrupting the interaction by TA and SC activity in hippocampal CA1, depending on the timing of the two inputs.

Previous studies have shown that TA feed forward activation of interneurons resulted in the postsynaptic inhibition of SC inputs in CA1 pyramidal neurons through the activation of GABA_B receptors (McQuiston, 2010). Furthermore, MOR activation has been shown to inhibit monosynaptic GABA_B IPSPs in all layers of hippocampal CA1 by approximately 50% (McQuiston, 2007). In contrast to the monosynaptic studies, the present studies have shown that TA driven GABA_B IPSPs in CA1 pyramidal neurons were completely inhibited by MOR activation. This implies that the interneurons activated by TA input are profoundly inhibited by MOR activation, whereas other interneuron subtypes are less sensitive to MOR activation (McQuiston, 2007). This further suggests that TA regulation of SC inputs may be more sensitive to MOR modulation than other sites in the hippocampal CA1 network and that a primary site for MOR modulation of hippocampal network function maybe on the TA driven inhibition observed in both the SR and SP of hippocampal CA1. Thus MOR

activation should significantly influence both the integration of input and output of CA1 pyramidal neurons.

MOR activity in the hippocampus has affects on learning and memory (Corrigall and Linesman, 1988; Stevens et al., 1991; Self and Stein, 1993; but see Olmstead and Franklin, 1997b) and may also play a role in chronic morphine tolerance as well as dependence (Fan et al., 1999; Lu et al., 2000; Mitchell et al., 2000). Not surprisingly, MOR activation can modulate synaptic plasticity in hippocampal CA1 and spatial memory in rodents (Mansouri et al., 1997, 1999; Pourmotabbed et al., 1998; Pu et al., 2002; Wagner et al., 2001). The precise effect that MORs has on synaptic plasticity in CA1 depends on previous drug exposure. In drug naïve animals, exogenous or endogenous MOR activation facilitates long-term depression (LTD) (a long term decrease in the efficacy of synaptic transmission) (Wagner et al., 2001). In contrast, morphine-dependent animals showed a facilitation of long-term potentiation (LTP) (Mansouri et al., 1997; 1999; Pourmotabbed et al., 1998), whereas during morphine withdrawal LTP was inhibited in hippocampal CA1 (Pu et al., 2002). Although MOR activation can acutely and chronically affect synaptic plasticity, exactly how MOR modulation of hippocampal neurons and synapses results in changes in network properties responsible for impaired memory processing remains incompletely understood.

At the cellular and synaptic level, MOR activation has been shown to hyperpolarize CA1 inhibitory interneurons and presynaptically inhibit GABAergic inhibitory postsynaptic potentials (IPSPs) in CA1 pyramidal neurons (Capogna et al., 1993; Cohen et al., 1992; Lupica et al., 1992; Lupica, 1995; Lupica and Dunwiddie, 1991; Madison and Nicoll, 1988; Masukawa and Prince, 1982; Reklung, 1993; Svoboda et al., 1999; Svoboda and Lupica, 1998; Swearengen and Chavkin, 1989; Wimpey and Chavkin, 1991). MOR disinhibition was subsequently shown to facilitate EPSPs in all layers of hippocampal CA1 (McQuiston and Saggau, 2003) by presynaptically inhibiting the release of GABA onto both GABA_A and GABA_B receptors (McQuiston, 2007; McQuiston, 2008; McQuiston and Saggau, 2003). In addition to facilitating inputs, integration and output of CA1 pyramidal neurons, MOR activation was also shown to disrupt synchronous oscillations among populations of CA1 pyramidal cells, which is likely to be important for the coding of information required for memory formation (Whittington et al., 1998). Indeed, both acute and chronic activation of MORs has been shown to alter theta rhythms in experimental animal models (Sala et al., 1995; Yamamoto, 1985). Given that theta rhythms have been correlated with behaviors associated with learning and memory (Buzsaki, 2002), understanding how MOR activity alters network activity during theta rhythms is important for understanding the network basis for changes in memory formation. To this end we have shown that one site where MOR activation may affect hippocampal theta rhythm is through its influence on the temporal integration between the TA and SC in hippocampal CA1. The TA input can indirectly inhibit SC inputs when the TA precedes SC inputs by approximately on theta cycle (McQuiston, 2010). Furthermore, we have shown that the TA driven inhibition can be completely blocked by MOR activation. Thus the exogenous (opiates) activation of MORs by drug abusers will have a significant effect on the integration between the TA and SC during theta rhythms and the formation of long term memories.

In addition to the exogenous activation of MORs by drug users, MORs in hippocampal CA1 can be activated by the endogenous release of opioid peptides (Wagner et al., 1990; Wagner et al., 2001). These endogenous opioid peptides have been localized to inhibitory interneurons and terminals of the PP in CA1 (Blasco-Ibanez et al., 1998; Fuentealba et al., 2008; Gall et al., 1981). In order to elicit the release of opioid peptides, high frequency bursts of stimulation are required (Wagner et al., 1990; Wagner et al., 2001). However, the entorhinal cortical neurons that give rise to the PP rarely fire bursts of action potentials

making it unclear how or when the PP would release endogenous opioids (Chrobak and Buzsaki, 1998; Frank et al., 2001). In contrast, enkephalinergic interneurons in hippocampal CA1 have been shown to fire high frequency bursts of action potentials following sharp waves and possibly during theta rhythms (Fuentelba et al., 2008). Both of these neuronal population rhythms have been correlated with memory formation (Buzsaki, 2002; Diba and Buzsaki, 2007; Foster and Wilson, 2006). Therefore, it is possible that enkephalin release during sharp waves and theta rhythms suppress TA control of SC inputs thereby increasing the input and output of CA1 pyramidal neurons during neural network activities associated with learning and memory. Thus, the TA feed forward inhibition in hippocampal CA1 may be an important site for the modulation of hippocampal network function by both the endogenous and exogenous activation MORs.

Research Highlights

- Temporo-ammonic pathway activity inhibits Schaffer collateral (SC) input.
- Temporo-ammonic pathway activity inhibits CA1 pyramidal cell firing.
- μ -Opioid receptor activation blocks temporo-ammonic effects.

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Abbreviations

CA1	cornu ammonis 1
CA3	cornu ammonis 3
CTOP	D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH ₂
DAMGO	[D-Ala ² , NMe-Phe ⁴ , Gly-ol ⁵]-enkephalin
EPSP	excitatory postsynaptic potential
PP	perforant path
SC	Schaffer collateral
SLM	stratum lacunosum-moleculare
SP	stratum pyramidale
SR	stratum radiatum
TA	temporo-ammonic pathway

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Figure 1.

Activation of μ -opioid receptors has no effect on integration between temporo-ammonic and CA3 Schaffer collateral excitatory inputs in hippocampal CA1 when temporo-ammonic inputs are stimulated twenty five milliseconds before Schaffer collaterals. **A.** Image of VSD signals from a photodiode array superimposed on the hippocampal slice from which the signals were recorded. Stimulating electrodes in the stratum lacunosum-moleculare (SLM, yellow arrow) and the stratum radiatum (SR, red arrow) were used to excite the temporo-ammonic and Schaffer collateral inputs respectively. For quantitative analysis, the VSD signals from nine photodiodes were spatially averaged in SR (red) and stratum pyramidale (SP, blue). SO – stratum oriens. **B.** Traces from nine spatially averaged photodiodes in A. Top. Stimulation of temporo-ammonic afferents 25 ms preceding Schaffer collateral stimulation (paired) produced larger amplitude VSD signals in SR (dark blue) and SP (purple) when compared to VSD signal amplitudes produced by SC stimulation alone (unpaired, SR – light blue, SP – red). Bottom. In the presence of DAMGO (1 μ M), a preceding stimulation of entorhinal/thalamic afferents increased Schaffer collateral evoked VSD responses in SR (dark blue) and SP (purple) when compared to stimulation of Schaffer collaterals alone (SR – light blue, SP – red). **C.** Histogram of Schaffer collateral EPSP amplitudes following temporo-ammonic input stimulation (150 – 200 ms prepulses) normalized to EPSP amplitudes produced by Schaffer collateral stimulation alone. Application of DAMGO did not affect mean normalized VSD amplitudes when temporo-ammonic afferents were stimulated 25 ms before SC afferent stimulation.

Figure 2.

Activation of μ -opioid receptors has no effect on integration between temporo-ammonic and Schaffer collateral excitatory inputs in hippocampal CA1 when temporo-ammonic inputs are stimulated seventy five milliseconds before Schaffer collaterals. **A.** Traces from nine spatially averaged photodiodes. Stimulation of temporo-ammonic afferents 75 ms preceding Schaffer collateral stimulation (paired) produced VSD signal in SR (dark blue) and SP (purple) of similar amplitudes compared to VSD signals produced by SC stimulation alone (unpaired, SR – light blue, SP – red). **B.** In the presence of DAMGO (1 μ M), prestimulus of temporo-ammonic afferents 75 ms before Schaffer collateral stimulation produce VSD response in SR (dark blue) and SP (purple) with similar amplitudes to VSD responses produced by Schaffer collateral stimulation alone (SR – light blue, SP – red). **C.** Application of DAMGO did not affect mean normalized VSD amplitudes when temporo-ammonic afferents were stimulated 75 ms before SC afferent stimulation.

Figure 3.

The inhibition of Schaffer collateral inputs by preceding stimulation (150 – 200 ms) of temporo-ammonic inputs is blocked by μ -opioid receptor activation. **A.** Stimulation of temporo-ammonic inputs 200 ms before Schaffer collateral stimulation inhibits Schaffer collateral EPSP amplitudes (SP – dark blue, SR – purple) when compared to EPSPs generated by Schaffer collateral stimulation alone (SP – light blue, SR – red). **B.** Application of DAMGO (1 μ M) completely blocks inhibition of Schaffer collateral inputs produced by preceding stimulation of temporo-ammonic inputs (control: SP – light blue, SR – red; prepulses: SP – dark blue, SR – purple). **C.** Histogram of Schaffer collateral EPSP amplitudes following temporo-ammonic input stimulation (150 – 200 ms prepulses) normalized to EPSP amplitudes produced by Schaffer collateral stimulation alone. DAMGO (red) prevented the temporo-ammonic input prepulses from inhibiting Schaffer collateral EPSPs (dark grey) in both SP and SR. CTOP completely reversed the effect of DAMGO (blue).

Figure 4. Inhibition of Schaffer collateral evoked action potential firing by a preceding stimulation (150 – 200 ms) of temporo-ammonic inputs is blocked by μ -opioid receptor activation. **A.** Schaffer collateral stimulation (black) produced action potential firing in all four trials. Stimulation of temporo-ammonic inputs 200 ms before Schaffer collateral stimulation prevented action potential firing in 3 of 4 trials (red). Action potential amplitudes were clipped. **B.** In the presence of DAMGO, Schaffer collateral stimulation produced action potentials in all four trials when stimulated alone (black) and when Schaffer collateral stimulation was preceded (200 ms) by stimulation of temporo-ammonic afferents (red). **C.** Histogram of the probability that an action potential was produced by stimulation of Schaffer collateral inputs. Application of DAMGO (red) blocked the inhibitory effect of temporo-ammonic prepulses on Schaffer collateral evoked action potential firing (black). The effect of DAMGO was reversed by the MOR antagonist CTOP (blue).