Mu-opioid Receptors Facilitate the Propagation of Excitatory Activity in Rat Hippocampal Area CA1 by Disinhibition of all Anatomical Layers

A. Rory McQuiston and Peter Saggau
Division of Neuroscience, Baylor College of Medicine, Houston, Texas 77030

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McQuiston, A. Rory and Peter Saggau. MORs facilitate the propagation of excitatory activity in rat hippocampal area CA1 by disinhibition of all anatomical layers. J Neurophysiol 90: 1936–1948, 2003. First published May 15, 2003; 10.1152/jn.01150.2002. Hippocampal μ-opioid receptors (MORs) have been implicated in memory formation associated with opiate drug abuse. MORs modulate hippocampal synaptic plasticity acutely, when chronically activated, and during drug withdrawal. At the network level, MORs increase excitability in area CA1 by disinhibiting pyramidal cells. The precise inhibitory interneuron subtypes affected by MOR activation are unknown; however, not all subtypes are inhibited, and specific interneuron subtypes have been shown to preferentially express MORs. Here we investigate, using voltage-sensitive dye imaging in brain slices, the effect of MOR activation on the patterns of inhibition and on the propagation of excitatory activity in rat hippocampal CA1. MOR activation augments excitatory activity evoked by stimulating inputs in stratum oriens [i.e., Schaffer collateral and commissural pathway (Scc) and antidromic], stratum radiatum (i.e., Scc), and stratum lacunosum-moleculare (Slm; i.e., perforant path and thalamus). The augmented excitatory activity is further facilitated as it propagates through the CA1 network. This was observed as a proportionately larger increase in amplitudes of excitatory activity at sites distal from where the activity was evoked. This facilitation was observed for excitatory activity propagating from all three stimulation sites. The augmentation and facilitation were prevented by GABAA receptor antagonists (bicuculline, 30 μM), but not by GABAA receptor antagonists (CGP 55845, 10 μM). Furthermore, MOR activation inhibited IPSPs in all layers of area CA1. These findings suggest that MOR-induced suppression of GABA release onto GABA receptors augments all inputs to CA1 pyramidal cells and facilitates the propagation of excitatory activity through the network of area CA1.

INTRODUCTION

The sole receptor subtype responsible for the addictive properties of opioids appears to be the μ-opioid receptor (MOR) (Matthes et al. 1996). Inhibition of MORs, but not other opioid receptor subtypes, decreases self-administration of opiates and conditioned place preference for opiate administration in laboratory animals (Matthes et al. 1996; Negus et al. 1993). Interestingly, hippocampal ablation also disrupts conditioned place preference for systemically administered morphine (Olmstead and Franklin 1997a), and morphine injected directly into the hippocampus can induce conditioned place preference in rats (Corrigall and Linseman 1988). Together, these findings suggest that the activation of MORs in the hippocampus may contribute to the formation of associations between environmental cues and drug use, and that MORs in the hippocampus may play an important role in the expression of addictive behavior. Therefore it is important to understand how MOR activity affects neuronal network function in the hippocampus.

Inhibitory interneurons (primarily GABAAergic) are very diverse in structure and function (Freund and Buzsaki 1996). MORs are found almost exclusively on inhibitory interneurons in area CA1 (Arvidsson et al. 1995; Bausch and Chavkin 1995; Drake and Milner 1999, 2002; Mansour et al. 1995), but not all subtypes of interneurons express MORs (Drake and Milner 2002). Autoradiographic studies have localized MORs to the pyramidal cell layer, suggesting a selective action of MORs on synaptic inhibition onto the somata of pyramidal cells (Crain et al. 1986; Mansour et al. 1987; McLean et al. 1987). However, immunohistochemistry has localized MOR1 to interneuron subtypes that synapse on the perisomatic region and on the distal dendrites of CA1 pyramidal cells (Drake and Milner 2002), suggesting that MOR activation may modulate synaptic inhibition onto both pyramidal cell bodies and their distal dendrites. Therefore MOR activation in CA1 of the hippocampus appears to modulate synaptic inhibition onto pyramidal cell bodies, although their function in the dendrites is less clear.

The differential expression of MORs on selective subtypes of interneurons is supported by physiological studies. Activation of MORs has been shown to hyperpolarize interneurons (Madison and Nicoll 1988; Svoboda and Lupica 1998; Svoboda et al. 1999; Wimpey and Chavkin 1991) and presynaptically inhibit the release of GABA (Capogna et al. 1993; Cohen et al. 1992; Lupica 1996; Lupica et al. 1992; Masukawa and Prince 1982; Nicoll et al. 1980; Rekling 1993). More specifically, MOR-induced hyperpolarizations were more frequently observed in stratum oriens (SO) interneurons that targeted the pyramidal cell body layer than in interneurons projecting to their dendrites (Svoboda et al. 1999). These authors interpreted this observation to be consistent with previous extracellular electrophysiological studies that described an MOR-induced increase in the excitability of pyramidal cells’ somata, with no concomitant effect on excitatory inputs to their dendrites (Corrigall and Linseman 1980; Dingledine 1981; Haas and Ryall 1980; Lee et al. 1980; Lynch et al. 1981; Martinez et al. 1979; Ziegglansberger et al. 1979). Furthermore, discrete application of opioid agonists to the cell body region of CA1 produced a
larger and more consistent increase in pyramidal cell body excitability, than application to the apical dendrites (Dingledine 1981; Robinson and Deadwyler 1981). Taken together, previous physiological studies suggest that MORs act to selectively inhibit the release of GABA onto CA1 pyramidal cell somata, resulting in an increase cell body excitability and output, while having little influence on inputs and synaptic integration in their dendrites.

Although most physiological studies suggest that MORs act to selectively inhibit the release of GABA onto pyramidal cell somata, the anatomical data are less consistent as to the relative effect of MORs on pyramidal cell somata versus their distal dendrites. To determine if MOR activation significantly affects synaptic inhibition in the dendrites, we investigated the influence of MOR activity on the network properties, excitatory inputs, synaptic inhibition, and synaptic integration in hippocampal area CA1 using voltage-sensitive dye imaging.

METHODS

Young male Sprague–Dawley rats (3–7 wk old) were anesthetized with halothane, followed by an ip injection of ketamine/xylazine. Once the animals were deeply anesthetized, they were transcardially perfused through the left ventricle with ice cold saline (30°C) consisting of (in mM) 230 sucrose, 2.5 KCl, 1 CaCl2, 4 MgCl2, 1 NaHPO4, 25 NaHCO3, and 10 glucose. The animals were then decapitated and their brains were removed. The brain was hemisected, and the most rostral portion of the brain cut and glued down to the stage of a Vibratome 1000 plus (TPi, St. Louis, MO). Coronal sections (350 μm) of the hemisected brain were cut in sucrose saline, held in a holding chamber at 30°C for 30 min, and allowed to adjust to room temperature. Brain slices were maintained in a holding solution consisting of (in mM) 125 NaCl, 3.0 KCl, 2 CaCl2, 2 MgCl2, 1 NaHPO4, 25 NaHCO3, and 10 glucose, until used in experiments. Harvesting of brain tissue was performed in accordance with the guidelines of the National Institutes of Health, as approved by the animal care and use committee of Baylor College of Medicine.

Slices from the mid- to temporal hippocampus were used in these experiments. Individual slices were transferred to a staining chamber containing 25 μM of the voltage-sensitive dye (VSD) RH-795 diluted in the holding solution. This dilution was made from a 2.5 mM stock solution of RH-795, dissolved in water, and stored at 4°C for less than a week. The brain slices were stained for 30 min, placed back in the holding chamber for 5 min, then perfused in the recording chamber for 15 min before performing experiments to permit the removal of excess dye.

The stained hippocampal slice was imaged from beneath through the glass coverslip bottom of the recording chamber that was mounted on the stage of a Zeiss IM35 inverted microscope (Thornwood, NY). The transilluminated real-time image of the slice was captured with a frame grabber connected to a CCD camera and displayed on a computer monitor. This permitted the placement of tungsten bipolar stimulating electrodes (FHC, Bowdoinham, Maine) either in the SO, stratum radiatum (SR), or stratum lacunoso-moleculare (SLM) to evoke synaptic potentials from specific anatomical inputs. In some experiments, cuts in the slices were made to isolate specific inputs to the CA1 pyramidal cells. Synaptic events were evoked by 100-μs current pulses of 20–100 μA. Stimulation strength was adjusted to produce approximately a 50% maximal response. Stimulation occurred 60 and 110 ms after the beginning of every record. Individual records lasted 300 ms. During experiments, brain slices were continuously perfused in a submerged recording chamber with saline consisting of (in mM) 125 NaCl, 3.0 KCl, 2 CaCl2, 2 MgCl2, 1 NaHPO4, 25 NaHCO3, and 10 glucose, either at room temperature or 30°C. All examples in the results are taken from experiments performed at room temperature (approximately 22°C).

Details of the VSD imaging have been described previously (Colom and Saggau 1994; Sinha et al. 1995; for review: Sinha and Saggau 1999) and will be mentioned here briefly. Brain slices stained with RH-795 were illuminated with a tungsten-halogen light source (12 V, 100 W, Xenophot HLX Osram-Sylvania, Danvers, MA) using a stable power supply (Kepco ATE 75–85W, Flushing, NY). A 10 × 0.5 NA objective lens (Nikon, Melville, NY) focused the excitation light on the region of interest of hippocampal area CA1. Excitation light passed through a 555/50 nm band-pass filter and was reflected by a 605-nm dichroic beam splitter into the back focal aperture of the objective to illuminate the slice. Emitted fluorescent light was collected by the same objective, passed through the dichroic beamsplitter, and through a 610-nm long pass filter. The filters and dichroic beam splitter were purchased from Chroma (Brattleboro, CT). An image of the region of interest was focused on a photodiode array (10 × 10 elements; MD-100, Centronics, Newbury Park, CA), and a custom-made 100-channel current-to-voltage converter and amplifier was used to quantitatively measure the fluorescent light. The data were displayed as the change in fluorescence divided by the resting fluorescence (ΔF/F). This eliminates variations in the signal due to differences in dye concentration, illumination intensity, sensitivity of the photodiode elements, and bleaching that occurred as the experiments progressed. The signals were sampled at 2 kHz per channel and filtered with a 10-point moving box filter. A depolarization of membrane potential produces a reduction in ΔF/F, or a downward deflection, therefore to be consistent with electrophysiological conventions. ΔF/F signals were inverted so that depolarizations were displayed as upward deflections. Optical signals were either shown as traces (averages of adjacent photodiode signals that collected signals from the same anatomical layer) or color-coded images of the ΔF/F signals from the entire photodiode array. VSD signals were quantified by measuring the peak amplitudes of the first and second excitatory postsynaptic potential (EPSP) or inhibitory postsynaptic potential (IPSP) and the area (relative to baseline) of both synaptic events. To make comparisons of a drug’s effect between anatomical layers, the changes in amplitudes and areas were normalized to the control values for each layer [i.e., (Drug – Control)/Control]. Values were reported as mean ± SE. For data with paired measurements, statistical significances were determined using a repeated measures ANOVA. For unpaired data, statistical significances were examined using a one-way ANOVA.

RESULTS

We used optical imaging with a VSD and a photodiode array to examine the modulatory effect of MOR activation on the propagation of excitatory activity in the neuronal network of rat hippocampal area CA1. To do this, we placed bipolar stimulating electrodes in three different excitatory afferent pathways of CA1: in the SLM, to determine the effect of MOR activation on inputs from the entorhinal cortex and thalamus; in the SR, to examine the effect of MOR activation on the Schaffer collateral and commissural (SCC) inputs from CA3 onto CA1 pyramidal cell apical dendrites; and in the SO, to examine the effect of MOR activation on the SCC inputs onto the CA1 pyramidal cell basal dendrites and back propagating action potentials (AP) into the apical dendrites of CA1 pyra-
midal cells. Stimulation consisted of paired current pulses (100 μs, 20–100 μA) delivered 60 and 110 ms after the beginning of an individual trace (300 ms duration). Due to the nature of VSDs, stimulation resulted in optical signals that are similar to EPSPs and IPSPs measured by intracellular electrophysiological methods (Fig. 1) (Jin et al. 2002; Sinha et al. 1995). Consistent with the VSD signals arising from synaptic potentials, the VSD signals were inhibited by ionotropic glutamate and GABA receptor antagonists (Fig. 1C). Occasionally fast depolarizing VSD signals were observed (approximately 6 ms in duration at the base) in the presence of glutamate and GABA receptor blockers, and this presynaptic activity was discernible from the postsynaptic events (Fig. 1A). The fast signals could be blocked by tetrodotoxin (1 μM; data not shown). Although glutamatergic antagonists inhibited the depolarizing signals, suggesting that they arise from the activation of EPSPs, these signals may include contributions from the activation of voltage-dependent ion channels and asynchronous action potentials. Therefore rather than calling these synaptically evoked depolarizations EPSPs, we shall refer to these depolarizations as excitatory activity or excitatory events. The hyperpolarizing potentials on the other hand were completely blocked by GABA<sub>α</sub> antagonists, suggesting that they were IPSPs. In addition to EPSP-like excitatory activity and IPSPs, stimulation in SLM produced a slow delayed depolarization that was confined to SLM (Fig. 1Aii). This delayed depolarization appeared to result from the activation of a number of different types of receptors. The delayed depolarization was inhibited by both glutamatergic and GABA<sub>α</sub> receptor antagonists suggesting that activation of both of these receptor types contributed to the depolarization (Fig. 1Aii). Interestingly, the pseudo-color image shows that the delayed depolarization persisted in SLM while IPSPs were observed in other portions of CA1 (SO, stratum pyramidale (SP), and SR; Fig. 1B). Thus consistent with previous studies, these observations suggest that we measured synaptically driven membrane potential changes from a population of nerve cells imaged onto the photodiode detectors (Jin et al. 2002; Sinha et al. 1995; for review see Zochowski et al. 2000). We investigated the effect of MOR activation on the different types of excitatory input to CA1 and how MOR activity affected the spread of excitatory activity in the network of CA1. Only results in which the MOR antagonists naloxone (5 μM) or naloxonazine (5 μM) reversed the effects of MOR agonists at the end of each experiment were included for analysis.

**MOR activation facilitates the propagation of excitatory activity from SLM inputs**

Paired-pulse stimulation of afferents in SLM (perforant path and thalamus; Fig. 2Ai) produced VSD signals reminiscent of EPSPs (Fig. 2Aii). This excitatory activity degraded in ampli-

![Image](http://jn.physiology.org/10.1152/jn.00820.2003)
FIG. 2. SLM-evoked excitatory activity is increased in all anatomical layers of CA1. Ai: bright field image of the hippocampal slice. Arrowhead points to stimulation site in SLM. Color-coded boxes in different layers show individual photodiodes used for ΔF/ΔF signals in Ai and Cii. SO, green; SP, blue; SR, red; SLM, gold. Box dimensions were 141 × 141 μm². Ai: representative VSD ΔF/ΔF traces for each layer. Excitatory postsynaptic potential (EPSP) amplitudes (evoked at 60 and 110 ms) decreased as EPSPs propagated from SLM to SO (black). DAGO (2 μM) increased amplitude of EPSPs in all layers (blue). Difference between control EPSP and EPSP in DAGO (DAGO — Control) are shown in orange. Note, late depolarization in SLM was not augmented, but was inhibited by DAGO (arrow). Naloxone (5 μM) inhibited the effect of DAGO (yellow). Scale bars: vertical 0.01% ΔF/ΔF, horizontal 50 ms. B: pseudo-color images of difference between control EPSPs and DAGO EPSPs. (DAGO — Control). Thin lines indicate borders of individual anatomical layers of Ai, dot shows stimulation site. Asterisks signify time of stimulation. Initially, DAGO (2 μM) produced an augmentation of EPSP (yellow/red) around stimulation site (small dot), followed by an augmentation in output layers SP/SO. Note later (>190 ms), DAGO inhibited late depolarization in SLM (blue) and simultaneously augmented activity (yellow/red) in output layers (SO/SP). C: normalization of DAGO-induced augmentation of EPSPs, [(DAGO — Control)/Control], for each individual photodiode in the array, in the different anatomical layers. Cii: note, DAGO increased EPSPs to a relatively greater extent as they moved from site of initiation (SLM, gold), through network (SR, red), to output regions (SO, green/SP, blue). Scale bars: vertical 23% of control, horizontal 50 ms (Cii—Civ). Histograms of DAGO-induced augmentation of EPSPs in different anatomical layers, expressed as percentage of control EPSPs [100 × (DAGO — Control)/Control]. DAGO — Control). DAGO-induced augmentation of 1st EPSP amplitude (Ciii), 2nd EPSP amplitude (Civ), and total EPSP area (Cii) showed a significant difference between anatomical layers [repeated measures ANOVA, P < 0.01 (amplitude 1), P < 0.05 (amplitude 2), P < 0.05 (area)]. Post hoc analysis showed a significant trend to a larger percent augmentation of EPSPs as they moved away from stimulation site (SLM) to output layers of CA1 (SO/SP) post-test for linear trend, P < 0.001 (amplitude 1), P < 0.01 (amplitude 2), P < 0.01 (area)]. D: pseudo-color image of normalized DAGO-induced augmentation of EPSPs. Normalization of change in EPSP shows largest relative DAGO-induced augmentation (red) of EPSP occurred away from stimulation site, near proximal apical dendrites in SR, SP, and SO.

The pseudo-color images illustrate the spatial and temporal effects of DAGO on excitatory activity arising from inputs in SLM (Fig. 2B). DAGO produced an increase in the amplitude of the first excitatory event amplitude in SLM (60 ms) and in the layers that it propagated through (SR, SP, and SO). DAGO also increased the amplitude of the second excitatory event (110 ms) in all layers. Interestingly, while there was an inhibition of the late depolarization in SLM, the excitatory activity continued to be augmented in the outputs layers (SP and SO; at 190–250 ms; Fig. 2B).

To compare the effect of DAGO on the excitatory activity across anatomical layers, the difference between the control signals and the signals in DAGO were normalized for each

tude as it propagated away from the site of stimulation (from SLM via SR and SP to SO). IPSPs were also observed in some layers (Fig. 2Aii, SP and SO). The amplitude of the excitatory activity was increased in all anatomical layers by the MOR agonist DAGO (Fig. 2Aii). The amount of augmentation of the excitatory activity amplitude is best observed in the subtraction of the control signal from the signal in DAGO (Fig. 2Aii). In addition to fast excitatory events, stimulation in the SLM produced a delayed depolarization that was restricted to SLM. In contrast to the augmentation of the initial excitatory events, DAGO inhibited the delayed depolarization in SLM (Fig. 2Aii, amplitude −33 ± 5%, P < 0.001; area −28 ± 2%, P < 0.0001, n = 9).
photodiode [i.e., (DAGO – Control)/Control; Fig. 2C]. Normalization of the effect of DAGO showed a larger relative augmentation of excitatory activity at sites distal (SP/SO) to the site of stimulation (SLM). To quantify the relative differences in the augmentation of excitatory activity between anatomical layers, we measured the amplitude of the excitatory event produced by the first stimulation (amplitude 1; Fig. 2Ci), the amplitude of the excitatory event produced by the second stimulation (amplitude 2; Fig. 2Civ), and the total area of the two excitatory events combined (area; Fig. 2Ci) in the absence and presence of DAGO. The amount of DAGO-induced augmentation was then expressed as a percentage of the control [i.e., 100 × (DAGO – Control)/Control]. Changes in the amplitude of the excitatory activity reflects an effect on the excitability and propagation of the excitatory activity between layers. A change in area reflects not only a change in excitability but is also a measure of a change in inhibition. This is because in some situations inhibition may not affect the amplitude of the excitatory events, but instead may only affect their kinetics (rise and decay time) and thus area. However, the effect of DAGO on the excitatory event amplitudes and area were similar. Excitatory event amplitudes and area all showed a significant increase in the amount of augmentation as the excitatory activity propagated away from the site of stimulation (SLM) to the output layers (SP/SO; Fig. 2C, ii–iv). The pseudo-color image also showed that the excitatory activity was augmented by a larger relative amount at sites distal to the stimulation location (Fig. 2D). Therefore not only does MOR activity augment the excitatory input in SLM, but MOR activity also facilitates the propagation of the excitatory activity through the dendritic tree of CA1 pyramidal cells to the output layers of CA1.

MOR activation facilitates the propagation of excitatory activity from SR inputs

We also tested whether MOR activity modulated SCC afferents in SR. Similar to the observations in SLM, excitatory activity generated in SR (Fig. 3Ai) diminished in amplitude as it propagated away from the site of stimulation (from SR to SLM and SR to SP/SO). In addition to excitatory activity, IPSPs were observed in some layers (Fig. 3Aii, SO). Excitatory activity in all layers was increased in amplitude by the application of DAGO. Subtraction of the control signals from the signals in DAGO illustrates the amount of augmentation of the excitatory activity (Fig. 3Aii).

As for the stimulation in SLM, pseudo-color images show the spatial and temporal extent of the DAGO-induced augmentation of the excitatory activity generated in SR (Fig. 3B). Both of the excitatory events generated in SR (60 and 110 ms) were augmented by DAGO, and this augmentation was also observed in the output layers (SP and SO). However, little activity was observed in SLM in the color plot because of the relatively small amount of propagation of excitatory activity into SLM (see Fig. 3Aii).

The relative amount of augmentation increased as the excitatory activity propagated from the site of stimulation (SR) into SLM and into the output layers (SP/SO; Fig. 3Ci). Normalization of the effect of DAGO on excitatory activity amplitudes (Fig. 3C, iii and iv) showed a significant increase in the amount augmentation as the excitatory activity propagated from the site of stimulation (SR) into SLM and into the output layers (SP/SO). There was no significant difference in the amount that the excitatory activity area was augmented between anatomical layers, although there was a trend to larger changes in the area of excitatory activity at sites distal from the stimulation location. The normalized change in excitatory activity amplitudes is illustrated in the pseudo-color images (Fig. 3D). These images also show that the excitatory activity was augmented to a larger relative extent as it propagated away from the stimulation site (SR) into proximal SLM and to the output layers (SP/SO). Therefore like the afferents in SLM, the SCC excitatory input to SR is augmented by MOR activity. Also similar to SLM, MOR activation facilitated the propagation of excitatory activity away from the site of stimulation in SR.

MOR activation facilitates the propagation of excitatory activity from SO inputs

To complete the study of the effect of MOR activity on excitatory activity in CA1, we examined the effect of DAGO on excitatory activity generated in SO. Stimulation of afferents in SO (Fig. 4Ai) produced excitatory activity that decreased in amplitude as it propagated away from the site of stimulation (from SO through SP and SR to SLM; Fig. 4Aii). As in SLM and SR, IPSPs were also observed (Fig. 4Aii). The excitatory activity was augmented in all layers by DAGO (Fig. 4Aiii). The difference between the excitatory events in the presence and absence of DAGO is shown in Fig. 3Aii (orange traces).

Spatially and temporally, DAGO augmented the first (60 ms) and second (110 ms) excitatory events, both of which subsequently propagated to the SR/SLM border (Fig. 4B). However, because of the relatively small propagation into SLM, little activity was observed in this layer. Normalization of the DAGO effect showed that MOR activation produced a larger relative augmentation of excitatory activity, as it propagated from the site of stimulation (SO) to SR and SLM (Fig. 4Ci). Excitatory event amplitudes and area all showed significant differences in the amount of DAGO-induced augmentation between anatomical layers (Fig. 4C). This is shown spatially and temporally in the pseudo-color images (Fig. 4D). Therefore like SLM and SR, excitatory activity in SO is augmented by MOR activation. Furthermore, MOR activation facilitates the propagation of excitatory activity from its site of generation in SO to other anatomical layers.

MOR does not facilitate excitatory activity in the presence of GABA<sub>A</sub> and GABA<sub>B</sub> antagonists

Previous work has shown that in hippocampal CA1, MORs are not localized to pyramidal neurons or excitatory synaptic terminals, but are instead localized to inhibitory interneurons and inhibitory terminals (Bausch and Chavkin 1995; Drake and Milner 1999, 2002; Mansour et al. 1995). Furthermore, studies have shown that MOR activation can hyperpolarize interneurons and inhibit both GABA<sub>A</sub> and GABA<sub>B</sub> IPSPs in pyramidal cells (Capogna et al. 1993; Cohen et al. 1992; Lupica 1995; Lupica et al. 1992; Masukawa and Prince 1982; Nicoll et al. 1980; Rekling 1993). Therefore we wanted to determine if blockade of GABA<sub>A</sub> and GABA<sub>B</sub> receptors could prevent the DAGO-induced augmentation of excitatory activity. On occasions, blockade of inhibition produced epileptiform activity, and
these data were not included for analysis. However, the slicing procedure often produced brain slices in which inputs from CA3 were not intact. This often occurred in coronal slices taken from the mid-hippocampus. In general, these hippocampal slices did not produce epileptiform activity in the presence of GABA receptor antagonists.

Even in the presence of GABA_A and GABA_B receptor antagonists [bicuculline (BIC), CGP 55845, respectively], excitatory activity continued to diminish in amplitude as it propagated away from the stimulation site (SLM) through SR to the output layers SP/SO (Fig. 5). The delayed depolarization previously observed in SLM (Figs. 1 and 2) was not observed in the presence of GABA receptor antagonists (compare with Fig. 5iv). Application of DAGO had no effect on the excitatory activity (Fig. 5). Under the same conditions, stimulating the afferents in SR and SO produced similar results (data not shown). Thus in the presence of GABA antagonists, DAGO produced no significant change in the amplitudes or area of the excitatory activity, regardless of the stimulation site. This suggests that DAGO augments excitatory activity in CA1 through an inhibition of GABA function.

MOR facilitation of excitatory activity involves inhibition of GABA release onto GABA_A receptors

Since the combination of GABA_A and GABA_B receptor antagonists prevented an effect of MOR activation on excitatory activity, we next determined which GABA receptor sub-

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**FIG. 3.** SR-evoked EPSPs are increased in all anatomical layers of CA1. A: image of hippocampal slice. Arrowhead points to stimulation site in SR. Color-coded boxes in different layers show position of individual photodiodes used for ΔF/ΔF signals in Aii and Cii. SO, green; SP, blue; SR, red; SLM, gold. Box dimensions were 141 × 141 μm². Aii: representative VSD ΔF/ΔF traces for each layer. EPSP amplitudes (evoked at 60 and 110 ms) decreased as EPSPs propagated from SR in 2 directions, 1 toward SP/SO and 1 toward SLM (black). DAGO (2 μM) increased amplitude of EPSPs in all layers (blue). Difference between control EPSP and EPSP in DAGO (DAGO – Control) are shown in orange. Scale bars: vertical 0.017% ΔF/ΔF, horizontal 50 ms. B: pseudo-color image of difference between Control EPSPs and DAGO EPSPs, (DAGO – Control). Thin lines indicate borders of anatomical layers in A. Dot shows stimulation site. Asterisks signify time of stimulation. Initially, DAGO (2 μM) produced an augmentation of EPSP (yellow/red) around stimulation site (small dot), followed by an augmentation in distal layers (SO, SP). C: normalization of the change in EPSPs produced by DAGO (DAGO – Control)/Control. Ci: note, DAGO increased EPSPs to a relatively greater extent as they moved from site of initiation (SR, red) to other anatomical layers (SP blue/SO green, and SLM gold). Scale bars: vertical 29% of Control, horizontal 50 ms (Cii–Civ). Histograms of DAGO-induced augmentation of EPSPs in different anatomical layers, expressed as a percentage of control EPSPs [100 × (DAGO – Control)/Control]. DAGO-induced increase of 1st EPSP amplitude (Ci) and 2nd EPSP amplitude showed a significant difference between anatomical layers [repeated measures ANOVA, P < 0.01 (amplitude 1), P < 0.001 (amplitude 2)], whereas total area (Ci) did not show a statistical significant difference. Post hoc analysis showed a significant trend to a larger percent augmentation of EPSPs amplitudes as they moved away from stimulation site (SR) into other layers of CA1 (SO, SP, and SLM) [post-test for linear trend, P < 0.001 (amplitude 1), P < 0.001 (amplitude 2)]. D: pseudo-color image of normalized DAGO-induced augmentation of EPSPs. Normalization of the change in EPSPs shows that largest relative DAGO-induced augmentation (red) of EPSP occurred away from stimulation site, near output regions SO/SP, and proximal SLM.
types were involved in the disinhibition by DAGO. In the presence of the GABA_A receptor antagonist CGP55845, all GABAergic inhibition in hippocampal slices resulted from the activation of GABA_A receptors. Under these conditions, stimulation in SR (Fig. 6Bi) produced excitatory activity and IPSPs in all layers (Fig. 6A). The amplitude of the excitatory activity diminished as it propagated away from the site of stimulation. Subsequent application of DAGO increased the amplitude of the excitatory activity in all layers (Fig. 6Bi). Normalization of the DAGO-induced augmentation of excitatory activity illustrates that DAGO caused a relatively larger change in the excitatory activity in layers distal (SLM, SP, SO) from the site of stimulation (SR; Fig. 6Bi). Similar findings were observed when the stimulation site was in SO or SLM. In the presence of CGP 55845, the effects of DAGO on both the excitatory event amplitudes and area were similar to observations made in the absence of GABA_A receptor antagonists. When stimulated in SO, both excitatory event amplitudes and area showed significant increases in the amount augmentation in layers distal to the site of stimulation (Fig. 6Ci). When stimulating in SR, excitatory event amplitudes showed a significant increase in the amount augmentation at sites distal to the site of stimulation (Fig. 6Ci). However, the augmentation of excitatory activity area did not show a significant difference between layers, although there was a trend toward a larger amount of augmentation at locations distal to the stimulation site. When stimulating in SLM, both excitatory activity amplitudes and area all showed a significant increase in the relative amount...
augmentation as the excitatory activity propagated away from the site of stimulation (SLM; Fig. 6Cii). Thus in the presence of GABA_A receptor inhibition, CA1 excitatory activity is augmented by MOR activation. Therefore the mechanism for the DAGO augmentation of excitatory activity appears to involve the modulation of GABAergic synaptic transmission on to GABA_A receptors.

**Fig. 6.** GABA_B receptor antagonist (10 μM CGP 55845) does not affect DAGO-induced augmentation of EPSPs. A: representative VSD ΔF/ΔF traces for each layer. EPSP amplitudes (evoked at 60 and 110 ms, in the presence of CGP 55845; 10 μM) decreased as EPSPs propagated away from SR (black). DAGO (2 μM) increased amplitude of EPSPs in all layers (blue). Difference between control EPSP and DAGO EPSP (DAGO − Control) are shown in orange. Scale bars: vertical 0.015% ΔF/ΔF, horizontal 50 ms. Bi: image of hippocampal slice. Arrowhead points to stimulation site in SR. Color-coded boxes show photodiodes used for ΔF/ΔF signals in A and Bii. SO, green; SP, blue; SR, red; SLM, gold. Box dimensions were 141 × 141 μm². Bii: normalization of EPSPs augmented by DAGO ([DAGO − Control]/Control). Note, DAGO increased EPSPs to a relatively greater extent as they moved from site of initiation (SR, red). Scale bars: vertical 65% of Control, horizontal 50 ms. C: histograms of normalized effect of DAGO on EPSPs expressed as a percentage of control EPSPs [100 × (DAGO − Control)/Control]. Left: DAGO-induced increase of 1st EPSP amplitude. Middle: DAGO-induced increase of 2nd EPSP amplitude. Right: DAGO-induced increase of combined EPSP area. Ci: DAGO-induced augmentation of SO-stimulated EPSPs showed a significant difference between anatomical layers [repeated measures ANOVA, P < 0.05 (amplitude 1), P < 0.001 (amplitude 2), P < 0.05 (area)]. Post hoc analysis showed a significant trend to a larger percent augmentation of EPSP amplitudes as they moved away from stimulation site (SO) [post-test for linear trend P < 0.01 (amplitude 1), P < 0.0001 (amplitude 2)]. Cii: DAGO-induced augmentation of SR-stimulated EPSPs showed a significant difference between anatomical layers for EPSP amplitudes, but not area [repeated measures ANOVA, P < 0.001 (amplitude 1), P < 0.05 (amplitude 2)]. Post hoc analysis showed a significant trend to a larger percent increase of EPSPs amplitudes as they moved away from the stimulation site (SR) [post-test for linear trend P < 0.0001 (amplitude 1), P < 0.01 (amplitude 2)]. Ciii: DAGO-induced augmentation of SLM-stimulated EPSPs showed a significant difference between anatomical layers [repeated measures ANOVA, P < 0.0001 (amplitude 1), P < 0.0001 (amplitude 2), P < 0.05 (area)]. Post hoc analysis showed a significant trend to a larger percent augmentation of EPSPs as they moved away from stimulation site (SLM) [post-test for linear trend, P < 0.0001 (amplitude 1), P < 0.0001 (amplitude 2), P < 0.01 (area)].

**MOR modulation of propagation of excitation in CA1**

In an attempt to determine if the release of GABA onto GABA_B receptors played a role in the mechanism of the DAGO-induced augmentation of excitatory activity, we isolated GABA_B receptor inhibition in hippocampal slices by performing experiments in the presence of the GABA_B antagonist BIC. Under these conditions, stimulation in SLM produced excitatory activity that declined in amplitude as it propagated away from the site of initiation (SLM) to the output layers (SP/SO; Fig. 7). In contrast to previous observations (Figs. 1Aii and 2Aii), there was no clear late depolarization in SLM in the presence of BIC (Fig. 7Aii). In the presence of BIC, DAGO did not increase the size of the excitatory activity (Fig. 7). Similar findings were found when stimulating SO or SR. In the presence of BIC, DAGO did produce a slow late depolarization in some experiments (data not shown); however, the late depolarization was not completely captured within the 300-ms window. A similar observation was made with the GABA_B receptor antagonist CGP 55845, but since 300 ms was...
to compare the effect of DAGO on the IPSPs across anatomical layers, the changes in IPSPs were normalized similar to the normalization of the excitatory activity of previous sections. Unlike the excitatory activity, DAGO did not appear to inhibit the IPSPs by a larger relative amount when measured further away from the stimulation electrode (Fig. 8C). IPSP amplitudes and area all showed no significant difference in the amount DAGO-induced disinhibition between anatomical layers (Figs. 8C, ii–iv). This is also illustrated spatially and temporally in the pseudo color image (Fig. 8D). Therefore disinhibition of GABA synaptic transmission, by MOR activation, was equivalent in all layers of CA1.

**DISCUSSION**

The activation of MORs augmented all excitatory inputs to area CA1 of the rat hippocampus (SCC, perforant path, and thalamus). Regardless of the specific excitatory input stimulated (SO, SR, or SLM), the excitatory activity was further facilitated as it propagated through the CA1 network. More specifically, the amount of augmentation of the excitatory activity was larger at locations distant to the stimulation site. The augmentation was exclusively due to the inhibition of GABAergic synaptic transmission onto GABA_B receptors. Although MOR activation is known to inhibit GABA release onto GABA_B receptors, under the experimental conditions of this study the augmentation of excitatory activity by MOR activation did not involve GABA_B receptors. Interestingly, MOR activation reduced GABAergic synaptic transmission in all layers of CA1 by the same amount. We hypothesize that MOR activation continuously augmented excitatory activity as the excitatory event propagated and encountered reduced GABAergic inhibition throughout the CA1 neuronal network. This resulted in larger relative amounts of augmentation of excitatory events at sites distant from the site of stimulation, irrespective of the anatomical site of stimulation.

Although excitatory inputs in all layers of CA1 was augmented by MOR activation, the sources contributing to the synthetically evoked depolarization cannot be determined with certainty from these investigations. What we can say is that because the resultant depolarizations are inhibited by postsynaptic glutamatergic receptors antagonists, they must have contributions from EPSPs. Furthermore, they also have similar shapes and kinetics to EPSPs. However, in addition to EPSPs, we cannot discount the possible contribution of voltage-dependent ion channels or action potentials to the amplitude and kinetics of the observed depolarizations. The neuronal source for the depolarizing signals likely arise from excitatory activity produced in pyramidal cells of CA1, as they make up 80–90% of neurons in CA1 (Freund and Buzsaki 1996). Thus the most likely explanation for the MOR augmentation of the excitatory activity is a disinhibition of synthetically evoked excitation at all inputs to CA1 pyramidal cells.

Our findings may appear to be in contrast to previous studies that showed an increase of synthetically driven excitability at the cell body layer without an increase in the EPSP observed in the dendritic layer (Corrigall and Linseman 1980; Dingleidine 1981; Haas and Ryall 1980; Lynch et al. 1981; Martinez et al. 1979). However, these dendritic synaptic events were measured using extracellular electrode techniques. This technique does not measure membrane potential changes associated with

**FIG. 7.** GABA_A receptor antagonist prevents augmentation of EPSPs by DAGO. Extracellular stimulation in the SLM, in presence of bicuculline (30 µM), produced decrementing EPSPs (Δf/f, black) in all anatomical layers of CA1: (i) SO, (ii) SP, (iii) SR, (iv) SLM. Application of DAGO (2 µM; in continued presence of bicuculline) did not significantly affect EPSPs in any anatomical layer (blue). There was no significant difference between Control EPSPs and EPSPs in the presence of DAGO (DAGO — Control; orange; P > 0.05 for all stimulation sites and all layers, repeated measures ANOVA).

**FIG. 8.** GABA inhibits IPSPs in all layers of CA1

Because MOR density and differential expression on interneurons in CA1 (Drake and Milner 2002) may affect synaptic inhibition in a layer specific manner (Svoboda et al. 1999), we isolated IPSPs by stimulating afferents in the presence of glutamatergic antagonists (NBQX and APV). Unlike excitatory afferents, inhibitory axons cannot be isolated to an individual anatomical layer as a single axon often innervates more than one anatomical layer (Freund and Buzsaki 1996). Thus stimulation in any given anatomical layer will produce IPSPs in many, if not all CA1 hippocampal layers. Therefore data from stimulation in SO, SR, and SLM were analyzed together.

Stimulation in SLM in the presence of glutamate antagonists produced action potentials and IPSPs in all layers (Fig. 8Aii). IPSPs did not degrade in amplitude when measured further away from the stimulation site (Fig. 8Aiii). This is consistent with the idea that the IPSPs were not propagating but were due to the release of GABA in all layers. However, IPSPs in SLM were compounded by a late depolarization. Application of DAGO inhibited IPSPs (disinhibition) in all layers (Fig. 8Aiii), and inhibited the late depolarization in SLM (Fig. 8Aiv, amplitude = 42 ± 6%, P < 0.001; area −52 ± 1%, P < 0.01, n = 7). The portion of the IPSP and the late depolarization inhibited by DAGO is illustrated in the subtraction of the control IPSPs from the IPSPs in the presence of DAGO (Fig. 8Aiii).

The spatial and temporal pattern of the DAGO-induced inhibition of IPSPs is illustrated in the pseudo-color images in Fig. 8B. The first stimulation (60 ms) produced an inhibition of IPSPs in SR that was subsequently observed in the outer layers (SP and SO). This effect may be the result of a delay in propagation of presynaptic action potentials to their distal release sites. The second stimulation (110 ms) also produced an inhibition of IPSPs in all layers. Interestingly, while there was an inhibition of the late depolarization in SLM, there continued to be an inhibition of IPSPs in the SP and SO (190–250 ms).
the population EPSP, but instead measures the current flow created by the activation of ligand-gated ion channels. While it is sensitive to changes in the number of postsynaptic receptors and the amount of transmitter released, this technique is not sensitive to changes in input resistance or to evoked inhibitory synaptic transmission in the dendrites, both of which could alter EPSP amplitudes. There have been reports of EPSPs of increased duration (Dingledine 1981; Swearengen and Chavkin 1989) due to the unmasking of an N-methyl-D-aspartate (NMDA) component (Swearengen and Chavkin 1989). However, it is not clear that this was due to a disinhibition in the dendrites. The NMDA component occurred 5–10 ms after the population spike appeared in the cell body region and thus the unmasking of the NMDA component could be due to a back-propagating action potential produced in the soma that then relieved the voltage-dependent block of the NMDA receptors. Therefore it is unclear from previous physiological studies whether MOR activity can disinhibit the dendrites or affect synaptic inputs in the dendrites. However, our studies clarify this issue by demonstrating that MOR activation can increase the amplitude of excitatory synaptic events in the dendrites of CA1 pyramidal cells.

In addition to the EPSP-like excitatory events, stimulation of SLM inputs produced a slow delayed depolarization confined to SLM. This delayed depolarization appeared to result from the activation of a number of different processes. The delayed depolarization could be inhibited by both glutamate and GABA$_A$ receptor antagonists, but was inhibited by DAGO (arrow). Scale bars: vertical 0.005% $\Delta F/F$; horizontal 50 ms. B: pseudo-color image of difference between Control IPSPs and DAGO IPSPs (DAGO − Control). Thin lines show borders of individual anatomical layers shown in A and dot stimulation site. Asterisks signify stimulation. DAGO (2 $\mu M$) produced inhibition of IPSPs (yellow/red), which we call disinhibition, around the stimulation site (small dot), followed by a disinhibition in the output layers SPSO. Note, later (>190 ms), DAGO produced inhibition of late depolarization in SLM (blue), while simultaneously disinhibiting activity (yellow/red) in output layers (SO/SP). C: normalization of the DAGO-induced inhibition of IPSPs [(DAGO − Control)/Control]. Ci: note, DAGO inhibited IPSPs almost equivalently in all layers. For stimulation in SLM, amount of disinhibition was not measured due to overlapping depolarizing event. Scale bars: vertical 13% of Control, horizontal 50 ms (Ci−Civ). Histograms of DAGO-induced inhibition of IPSPs in different anatomical layers, expressed as a percentage of control IPSPs [100 × (DAGO − Control)/Control]. Histograms include data from all 3 stimulation sites (SLM, SR, SO). DAGO-induced inhibition of 1st IPSP amplitude (Ci), 2nd IPSP amplitude (Civ), and total IPSP area (Cii) showed no significant difference between anatomical layers ($P > 0.20$ for all stimulation sites and all layers, 1-way ANOVA, $n = 30$ (SO, SP, SR), $n = 20$ (SLM)). D: pseudo-color plot of normalized DAGO-induced inhibition of IPSPs. Normalized change in IPSPs shows DAGO-induced inhibition (red) of IPSP occurred uniformly throughout layers SR, SP, and SO. Relative amount of disinhibition occurring in SLM could not be judged in slices stimulated in SLM because of overlapping late depolarizing event. Later in recording, inhibition of depolarizing event in SLM occurred simultaneously with a continuing disinhibition in output layers (SP, SO).

FIG. 8. DAGO inhibits IPSPs in all layers of CA1. A: image of the hippocampal slice. Arrowhead points to stimulation site in SLM. Color-coded boxes show photodiodes used for $\Delta F/F$ signals in Ai and Ci. SO, green; SP, blue; SR, red; SLM, gold. Box dimensions were 141 × 141 $\mu m^2$. Ai: representative VSD $\Delta F/F$ traces for each layer. Evoked IPSPs were isolated from EPSPs by continuously superfusing slice with ionotropic glutamate receptor antagonists (NBQX, 30 $\mu M$; APV, 50 $\mu M$). IPSP amplitudes (black; evoked at 60 and 110 ms) were decreased in presence of 2 $\mu M$ DAGO (blue). Difference between control IPSP and IPSP in DAGO (DAGO − Control) are shown in orange. Note, late depolarization in SLM was not inhibited by ionotropic glutamate antagonists, but was inhibited by DAGO (arrow). Scale bars: vertical 0.005% $\Delta F/F$; horizontal 50 ms. B: pseudo-color image of difference between Control IPSPs and DAGO IPSPs (DAGO − Control). Thin lines show borders of individual anatomical layers shown in A and dot stimulation site. Asterisks signify stimulation. DAGO (2 $\mu M$) produced inhibition of IPSPs (yellow/red), which we call disinhibition, around the stimulation site (small dot), followed by a disinhibition in the output layers SPSO. Note, later (>190 ms), DAGO produced inhibition of late depolarization in SLM (blue), while simultaneously disinhibiting activity (yellow/red) in output layers (SO/SP). C: normalization of the DAGO-induced inhibition of IPSPs [(DAGO − Control)/Control]. Ci: note, DAGO inhibited IPSPs almost equivalently in all layers. For stimulation in SLM, amount of disinhibition was not measured due to overlapping depolarizing event. Scale bars: vertical 13% of Control, horizontal 50 ms (Ci−Civ). Histograms of DAGO-induced inhibition of IPSPs in different anatomical layers, expressed as a percentage of control IPSPs [100 × (DAGO − Control)/Control]. Histograms include data from all 3 stimulation sites (SLM, SR, SO). DAGO-induced inhibition of 1st IPSP amplitude (Ci), 2nd IPSP amplitude (Civ), and total IPSP area (Cii) showed no significant difference between anatomical layers ($P > 0.20$ for all stimulation sites and all layers, 1-way ANOVA, $n = 30$ (SO, SP, SR), $n = 20$ (SLM)). D: pseudo-color plot of normalized DAGO-induced inhibition of IPSPs. Normalized change in IPSPs shows DAGO-induced inhibition (red) of IPSP occurred uniformly throughout layers SR, SP, and SO. Relative amount of disinhibition occurring in SLM could not be judged in slices stimulated in SLM because of overlapping late depolarizing event. Later in recording, inhibition of depolarizing event in SLM occurred simultaneously with a continuing disinhibition in output layers (SP, SO).
Lambert et al. 1991; Michelson and Wong 1991; Perkins 1999; Perkins and Wong 1996; Staley et al. 1995; Taira et al. 1997) that in turn permitted the opening of NMDA receptors (Staley et al. 1995). However, sometimes a slow depolarization persisted in the presence of glutamate and GABA\textsubscript{A} receptor antagonists, suggesting another unknown contributor to the delayed depolarization. Interestingly, in contrast to the MOR augmentation of the EPSP-like excitatory events, MOR activation inhibited this delayed depolarization in SLM, and this delayed depolarization. This occurred at the same time other layers were disinhibited, suggesting that MOR activity can produce opposing effects in different layers of CA1 under certain conditions—more specifically, MOR activation can produce a late inhibition of excitatory activity in SLM while the other layers are being disinhibited.

In addition to augmenting excitatory inputs to CA1, MOR activation facilitated the propagation of excitatory activity away from the site of initiation. The simplest explanation for this facilitation is based on a passive electrical cable model of reduced inhibition in all layers of CA1. As the excitatory event propagates through the network, there is reduced inhibition in each anatomical layer of the network. At locations distal to the site of stimulation, the excitation is augmented by the reduced inhibition at the stimulation site and also by the reduced inhibition at all points along the neuronal network through which the signal has propagated. This results in a proportionately larger increase in excitation at distal sites due to the summed points of disinhibition. However, we cannot discount the possible contribution of voltage-dependent ion channels or action potentials to the observed increase in excitability in the different layers of CA1. Voltage-dependent ion channels are found throughout the somato-dendritic axis of CA1 pyramidal neurons (for reviews, see Haussuer et al. 2000; Magee 2000; Magee et al. 1998; Reyes 2001). During MOR activation, the augmented excitatory events may activate these voltage-dependent sodium or calcium channels, boosting the excitation amplitudes more at these distal sites than at the site of stimulation (Gillessen and Alzheimer 1997; Lipowsky et al. 1996). Even though the exact mechanisms contributing to the facilitation of the propagation of excitatory events cannot be determined from these experiments, it can be concluded that MOR activation augments excitatory events from all inputs and facilitates the propagation of excitatory activity between layers of CA1.

Under the conditions of these investigations, the activation of MORs augments CA1 excitatory inputs and facilitates the propagation of excitatory activity in CA1 pyramidal cells by inhibiting GABAergic synaptic transmission onto GABA\textsubscript{A} receptors. Furthermore, our studies suggest that MOR activation controls excitatory activity by inhibiting IPSPs in all layers of CA1 by an equal amount. These findings differ somewhat from the studies of Svoboda et al. (1999) who hypothesized that the primary function of MORs was to inhibit the output of the pyramidal cells, with a less significant effect on their inputs or dendrit integration. However, their study revealed that MOR-induced hyperpolarizations were also observed in a significant number of interneurons projecting to the dendritic layers (SR, 50%; SLM, 43%; SO, 38%) in addition to interneurons projecting to pyramidal cell somata (SP, 91%) (Svoboda et al. 1999). Furthermore, these studies do not exclude the possibility that MORs may be preferentially expressed on the terminals of some interneurons, where MORs have been shown to be located (Drake and Milner 1999). Thus it is possible that MOR activation produces a significant disinhibition in the dendritic layers that is not discernable from this previous electrophysiological study (Svoboda et al. 1999). This is consistent with more recent anatomical studies. MORs appear to be equivalently expressed on interneuron profiles (soma, axon or dendrite) that innervate the perisomatic region and the distal dendrites of pyramidal cells, whereas interneurons that selectively innervate other interneurons showed few cells expressing MOR (Drake and Milner 2002). This study (Drake and Milner 2002) did not examine the possibility that MOR is also located on interneurons that project to the proximal dendrites of pyramidal cells; however, Svoboda et al. (1999) showed that nearly half of these proximal dendritically projecting interneurons were hyperpolarized by MOR activation. From their own studies, Drake and Milner (2002) have suggested that MORs are expressed on heterogeneous groups of interneurons and likely have a more complicated effect on the neural network, including disinhibiting excitatory inputs onto CA1 pyramidal cell distal dendrites. Our findings are consistent with this (Drake and Milner 2002) and show that MOR inhibits GABAergic synaptic transmission in all layers of CA1. This suggests that MOR is functionally expressed on the terminals of interneuron subsets that project to all somato-dendritic regions of CA1 pyramidal cells, and that MOR activation can influence all inputs to CA1 pyramidal neurons, including integration within pyramidal cell dendrites, as well as influencing the output of pyramidal cells.

Opioid receptors expressed in the hippocampus may contribute to forming associations between environmental cues and drug intake (Corrigall and Linesman 1988; Olmstead and Franklin 1997a; Self and Stein 1993; Stevens et al. 1991; but see Olmstead and Franklin 1997b). Furthermore, opioids have been shown to alter synaptic plasticity and network oscillations in CA1 (Faulkner et al. 1998, 1999; Mansouri et al. 1997, 1999; Pu et al. 2002; Wagner et al. 2001; Whittington et al. 1998). Alteration of synaptic plasticity may affect the formation of associations between environment cues and drug intake, and the disruption of network oscillations may affect normal cognitive processing during drug use, as these oscillations have been correlated with perceptual tasks and attentive states. Our data suggest that activation of MORs in CA1 facilitates excitatory activity within the network, especially between distantly separated portions of the hippocampal network. This may facilitate interactions between spatially separated synaptic inputs in the same pyramidal neuron and contribute to the network changes involved in the formation of associations made by the hippocampus during drug intake.

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DISCLOSURES

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