Layer 2/3 Synapses in Monocular and Binocular Regions of Tree Shrew Visual Cortex Express mAChR-Dependent Long-Term Depression and Long-Term Potentiation

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McCoy P, Norton TT, McMahon LL. Layer 2/3 synapses in monocular and binocular regions of tree shrew visual cortex express mAChR-dependent long-term depression and long-term potentiation. *J Neurophysiol* 100: 336–345, 2008. First published May 14, 2008; doi:10.1152/jn.01134.2007. Acetylcholine is an important modulator of synaptic efficacy and is required for learning and memory tasks involving the visual cortex. In rodent visual cortex, activation of muscarinic acetylcholine receptors (mAChRs) induces a persistent long-term depression (LTD) of transmission at synapses recorded in layer 2/3 of acute slices. Although the rodent studies expand our knowledge of how the cholinergic system modulates synaptic function underlying learning and memory, they are not easily extrapolated to more complex visual systems. Here we used tree shrews for their similarities to primates, including a visual cortex with separate, defined regions of monocular and binocular innervation, to determine whether mAChR activation induces long-term plasticity. We find that the cholinergic agonist carbachol (CCh) not only induces long-term plasticity, but the direction of the plasticity depends on the subregion. In the monocular region, CCh application induces LTD of the postsynaptic potential recorded in layer 2/3 that requires activation of m3 mAChRs and a signaling cascade that includes activation of extracellular signal-regulated kinase (ERK) 1/2. In contrast, layer 2/3 postsynaptic potentials recorded in the binocular region express long-term potentiation (LTP) following CCh application that requires activation of m1 mAChRs and phospholipase C. Our results show that activation of mAChRs induces long-term plasticity at excitatory synapses in tree shrew visual cortex. However, depending on the ocular inputs to that region, variation exists as to the direction of plasticity, as well as to the specific mAChR and signaling mechanisms that are required.

INTRODUCTION

Long-term modulation of synaptic efficacy is believed to be a cellular correlate of learning and memory, and a role for acetylcholine (ACh) as a key modulator is well supported (Malenka and Bear 2004; Whitlock et al. 2006). In macaque monkeys and healthy adult humans, infusion of scopolamine, a muscarinic acetylcholine receptor (mAChR)-specific antagonist, results in impaired learning dependent on accurate processing of visual information, such as visuospatial paired-associate learning (Taffe et al. 2002), complex spatial learning (Soffie et al. 1986), visual recognition memory formation (Potter et al. 1992; Thiel et al. 2002), and visual attention tasks (Hao et al. 2005). In Alzheimer's disease (Ikonomovic et al.

2005) and schizophrenia (Dean et al. 2002), the degree of cholinergic denervation has been linked to the severity of memory deficits and visual impairments. Furthermore muscarinic-specific agonists play a neuroprotective role (Kemp et al. 2003) as well as rescue impaired learning in these disease states (Bodick et al. 1997; Dean 2004).

Despite the clear and critical role of ACh and mAChRs in normal visual system processing and learning and the impairments that ensue following cholinergic dysfunction, the identity of which mAChRs and signaling molecules involved at the synaptic level remain undefined. Furthermore, although a role for cholinergic modulation of developing visual cortical pathways has long been established, how it modulates synapses in mature visual cortex is still under investigation (Karmarkar and Dan 2006). In rat visual cortex, a form of long-term depression dependent on activation of mAChRs has been characterized (Kirkwood et al. 1999; McCoy and McMahon 2007). Although these studies expanded our knowledge of how the cholinergic system modulates synaptic function in visual cortex that could underlie learning and memory dependent on processing of visual information, they are not easily extrapolated to more complex visual systems. It is important to understand how the cholinergic system modulates synaptic function in both normal and disease states as it exists in more-developed, mature visual systems. For that purpose, we used tree shrew visual cortex for its closer phylogenetic relationship to primates (Luckett 1980).

Tree shrews (*Tupaia glis belangeri*) are small mammals that are closely related to primates (Luckett 1980), have excellent vision (Petry et al. 1984), including color vision (Jacobs and Neitz 1986). Tree shrew primary visual cortex is segregated into distinct regions that contain different ocular innervation: a monocular region that receives inputs originating from the contralateral eye and a binocular region, receiving inputs from both eyes. Within the binocular region, neurons receive input from both eyes but are more strongly activated by the contralateral eye (Humphrey et al. 1977). The boundary between the binocular and monocular regions is clearly defined in tree shrew (Kaas et al. 1972).

The goal of this study was to determine if synaptic plasticity dependent on mAChRs exists in tree shrew visual cortex and if there are differences in the cholinergic modification of synaptic responses in regions that anatomically receive inputs from either one eye or both eyes. Our results show that activation of

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mAChRs induces long-term plasticity in tree shrew visual cortex. However, the monocular and binocular regions differ in the direction of the plasticity as well as in the specific mAChR and signaling mechanisms that underlie this plasticity.

METHODS

Slice preparation

Visual cortical slices were prepared from nine adult (2–5 yr) and nine juvenile (3–5 mo) tree shrews using standard methods for rats (McCoy and McMahon 2007). Data were pooled because there was no difference between the two age groups. Animals were anesthetized with a ketamine/xylazine mixture (200 mg/kg, 0.5 mg/kg). After decapitation, coronal visual cortex slices (400 μm) were cut in artificial cerebrospinal fluid (ACSF) containing (in mM) 85 NaCl, 2.5 KCl, 4 $MgSO_4$, 0.5 $CaCl_2$, 1.25 NaH_2PO_4, 25 $NaHCO_3$, 25 glucose, 75 sucrose, 2 kynurenic acid, and 0.5 ascorbate and saturated with 95% O_2-5% CO_2 (pH 7.4). Slices were maintained at room temperature for ≤5 h in standard ACSF [containing (in mM) 119 NaCl, 2.5 KCl, 2.5 $CaCl_2$, 1.3 $MgSO_4$, 1 NaH_2PO_4, 26 $NaHCO_3$, and 10 glucose] plus 2 mM kynurenic acid, and continually oxygenated (95% O_2-5% CO_2).

Electrophysiology

EXTRACELLULAR FIELD RECORDINGS. Experiments were performed in a submersion recording chamber perfused (3–4 ml/min) with ACSF (no kynurenic acid) at 28–30°C. The binocular region was located on the dorsal surface and the monocular region located on the lower mesial surface (Fig. 1A). Layer 2/3 extracellular fPSPs (field postsynaptic potentials; 0.4–0.6 mV) were recorded (Axoclamp 2B amplifier) following stimulation in layer 4 (0.1 Hz, 100-μs duration) using standard methods (Fig. 1A) (McCoy and McMahon 2007). Following acquisition of a stable baseline (≥20 min), 50 μM carbachol (CCh) was bath applied (10 min). Antagonists were applied as indicated. Data were acquired using custom software written in Labview. Individual data points are a running average of five fPSPs, and percent change was measured 35 min post agonist washout. Statistical significance ($P < 0.05$) was determined using Student's t-test (means ± SE).

PATCH-CLAMP RECORDINGS. Recordings were obtained from somas of layer 2/3 pyramidal cells using the "blind" patch technique (input resistance: 120–160 MΩ; series resistance: 20–30 MΩ). Electrodes (5–8 MΩ) were filled with (in mM) 117 cesium gluconate, 0.6 EGTA, 2.8 NaCl, 5 $MgCl_2$, 2 ATP, 0.3 GTP, 20 HEPES, and 5 QX-314. A stimulating electrode was placed in layer 4 and the stimulus intensity (0.1 Hz, 100-μs duration) was set to elicit evoked excitatory postsynaptic currents (EPSCs) of 150–250 pA. Glutamatergic currents were isolated by blocking $GABA_A$ receptors ($GABA_A$Rs) with 100 μM picrotoxin, unless stated otherwise. Following acquisition of a stable baseline (≥5 min), 50 μM CCh was bath applied (5 min). If either input or series resistance varied by >10%, the experiment was excluded. Signals were collected using an Axopatch (2B) amplifier in voltage clamp mode, at 1× gain, filtered at 2 kHz and acquired in software written in Labview. Statistical significance was determined using Student's t-test.

CHEMICALS. Drugs were prepared as 1,000× stocks in dd$H_2$0 or DMSO and diluted immediately before use. All drugs were obtained

A

Binocular region

Monocular region

Monocular region

B_1 **B_2**

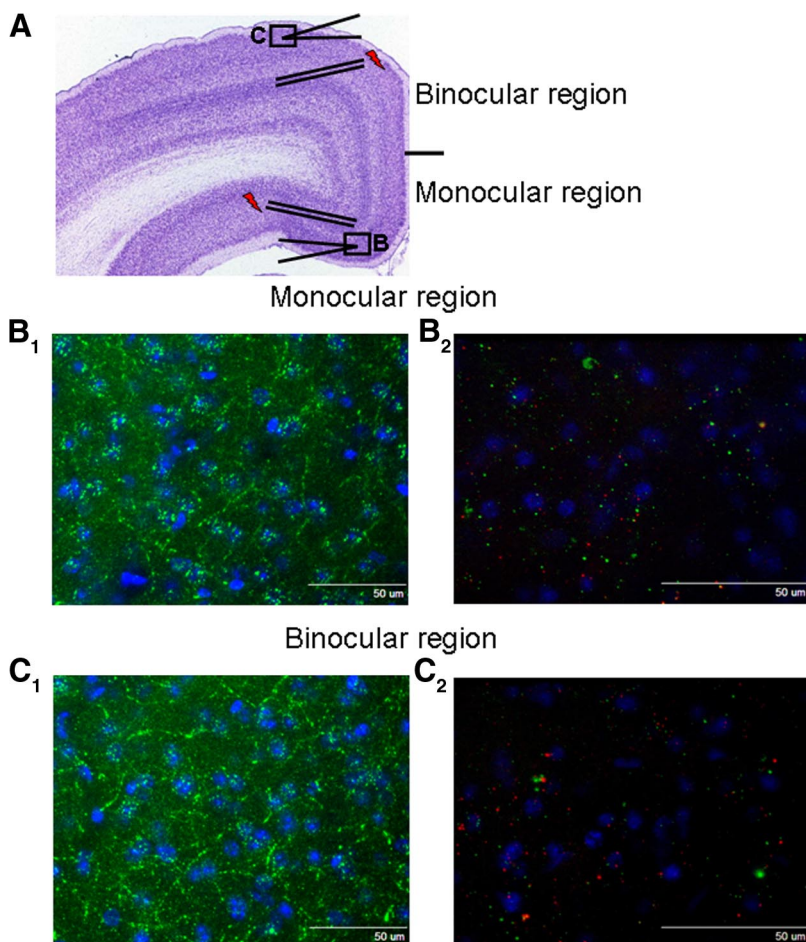**Binocular region**

C_1 **C_2**

FIG. 1. Robust cholinergic innervation exists in the monocular and binocular region. *A*: distinct bands of layer 4 neurons can be seen by cresyl violet stain. Two bands of layer 4 neurons, separated by a cell-sparse cleft, define the binocular region, while the monocular region has a single band of layer 4 neurons (located respectively above and below the dark bar). Recording-stimulation configuration is shown for both regions. *B1* and *C1*: immunostaining for cholinergic innervation in monocular and binocular regions (respectively; from *inset* boxes) shows dense cholinergic innervation [choline acetyl transferase (ChAT), green; 4′,6-diamidino-2-phenylindole (DAPI), blue]. Scale bar, 50 μM. *B2* and *C2*: immunostaining for m1 and m3 receptors reveals expression in the monocular and binocular regions (respectively; from *inset* boxes; m1, red; m3, green; DAPI, blue). Scale bar, 50 μM.

from Sigma (St. Louis, MO), except CCh, U0126, and U73122 (Calbiochem, La Jolla, CA). Because CCh is a nonselective cholinergic agonist, we have used selective antagonists to determine which receptor and signaling molecule are mediating the observed effects. Atropine is a nonselective mAChR antagonist at 1 μM (Richards 1991). Pirenzepine is selective for m1 mAChRs at 75 nM (Marino et al. 1998). 4-diphenylacetoxy-N-methyl-piperidine (4-DAMP) is selective for m3 mAChRs over other mAChR subtypes at 100 nM (Ehlert 1996). U0126 selectively inhibits activation of extracellular signal-regulated kinase (ERK) 1/2 over other kinases at 20 μM (Davies et al. 2000). U73122 preferentially inhibits phospholipase C (PLC) at 10 μM (Davies et al. 2000).

Immunohistochemistry

Eleven animals were transcardially perfused with 4% paraformaldehyde (PFA) in phosphate-buffered saline (PBS), and brains were postfixed in 4% PFA overnight. Sections (50 μm) were stained with cresyl violet or incubated free floating in goat anti-choline acetyl transferase (ChAT; 1:200, Chemicon, Temecula, CA), or goat anti-m3 mAChR, and rabbit anti-m1 mAChR, (both 1:300, Santa Cruz, Santa Cruz, CA) overnight at 4°C following a 1 h blocking incubation (10% normal donkey serum, 0.3% Triton X-100 in PBS) at 25°C. Slices were washed with PBS and incubated in donkey anti-goat Alexa 488, and anti-rabbit Alexa 594, (both at 1:300; Molecular Probes, Carlsbad, CA) for 1 h at 25°C. Cell bodies were stained with the nuclear stain 4′,6-diamidino-2-phenylindole (DAPI; 1:2,000; Sigma) 5 min at 25°C and mounted with Permafluor (Immunon, Pittsburgh, PA). Maximum projection confocal images (15 μm *z*-axis stack at 0.8 μm per step) were obtained using an Olympus DSU confocal microscope (Center Valley, PA).

RESULTS

mAChR activation induces LTP in the binocular region and LTD in the monocular region

In cresyl-violet-stained sections of tree shrew visual cortex, distinct bands of layer 4 neurons can be readily observed (Fig. 1*A*). Two bands of layer 4 neurons, separated by a cell-sparse cleft, define the binocular region, while the monocular region has a single band of layer 4 neurons (Kaas et al. 1972). Immunohistochemical staining using an anti-choline acetyl transferase (anti-ChAT) antibody to label cholinergic fibers reveals dense cholinergic innervation in layer 2/3 in both regions (Fig. 1, *B1* and *C1*). Staining for m1 and m3 mAChRs reveals abundant expression in both the monocular and binocular regions (Fig. 1, *B2* and *C2*).

A primary goal of this study was to determine if activation mAChRs induces long-term changes in synaptic efficacy in tree shrew visual cortex circuits. To investigate this, we used extracellular recordings of fPSPs in layer 2/3 in response to layer 4 stimulation and bath application of a broad-spectrum cholinergic agonist CCh (50 μM, 10 min) in acute slices of tree shrew visual cortex. In the binocular region, the superficial layer 4 band was stimulated to excite both the superficial layer 4 cells as well as the fibers of passage through this layer, originating from the underlying layer 4 neurons, while fPSPs were recorded in layer 2/3. In the monocular region, the single layer 4 band was stimulated and fPSPs were recorded in layer 2/3 (Fig. 1*A*).

Bath application of CCh reliably induced a long-lasting depression of the fPSP amplitude in the monocular region of visual cortex (62 $\pm$ 3% of baseline; n = 6 slices/4 animals,

P < 0.01; Fig. 2, *A1,* single experiment; *A2,* group data). Surprisingly, in the binocular region, after a transient depression of the fPSP during agonist application (69 $\pm$ 8% of baseline) a long-lasting potentiation was reliably induced (133 $\pm$ 8% of baseline; n = 7 slices/4 animals, P < 0.005; Fig. 2, *B1,* single experiment; *B2,* group data). Both the long-term depression (LTD) and potentiation (LTP) were prevented by the mAChR antagonist atropine (1 μM), indicating that mAChR activation is required for both plasticities (LTD: atropine 95 $\pm$ 4% of baseline; n = 3 slices/2 animals, compared with interleaved control 73 $\pm$ 8% of baseline; n = 3 slices/2 animals, P < 0.01; LTP: atropine 104 $\pm$ 4% of baseline; n = 3 slices/2 animals, compared with interleaved controls 131 $\pm$ 6% of baseline; n = 3 slices/2 animals, P < 0.05; Fig. 2, *A3* and *B3*). Thus we find that CCh application differentially induces mAChR-dependent long-term plasticity (mLTD or mLTP) in subregions of the visual cortex.

mLTD and mLTP require different mAChR subtypes and signaling cascades

To further define the cellular mechanisms underlying mLTD and mLTP, we used pharmacological tools to investigate which mAChR subtypes and signaling cascades are required for induction. We find that the m1 antagonist pirenzepine (75 nM) (Marino et al. 1998) has no effect on mLTD induction (73 $\pm$ 4% of baseline; n = 5 slices/4 animals, compared with interleaved controls 74 $\pm$ 3% of baseline; n = 5 slices/4 animals, P > 0.05; Fig. 3*A1*). However, the m3 antagonist 4-DAMP (100 nM), completely blocks it and unmasks a significant, long-lasting synaptic potentiation (113 $\pm$ 10% of baseline; n = 5 slices/5 animals, compared with interleaved controls 75 $\pm$ 4% of baseline; n = 6 slices/5 animals, P < 0.01; Fig. 3*A2*). Surprisingly, this unmasked potentiation is not dependent on m1 receptors. Coapplication of pirenzepine (75 nM) and 4-DAMP (100 nM) results in potentiation that is not different from that in 4-DAMP alone (123 $\pm$ 3% of baseline; n = 5 slices/3 animals, compared with interleaved 4-DAMP alone 120 $\pm$ 5% of baseline; n = 3 slices/3 animals, P > 0.05; Fig. 3*A3*), This implies that neither m1 nor m3 receptors underlie this LTP, but we know that it is still dependent on mAChRs because it is not induced when atropine is present (Fig. 2). In contrast, mLTP in the binocular region is prevented by blocking m1 receptors with pirenzepine (75 nM; 107 $\pm$ 7% of baseline; n = 6 slices/3 animals, compared with interleaved controls 133 $\pm$ 8% of baseline; n = 7 slices/4 animals, P < 0.05; Fig. 3*B1*), and was unaffected by the m3 receptor antagonist 4-DAMP (100 nM; 129 $\pm$ 3% of baseline; n = 4 slices/3 animals, compared with interleaved controls 127 $\pm$ 4% of baseline; n = 4 slices/3 animals, P > 0.05; Fig. 3*B2*). Thus different mAChRs subtypes are responsible for the induction of mLTD and mLTP.

Both m1 and m3 receptor subtypes couple to the G protein, Gαphaq (Offermanns et al. 1994), which canonically activates the PLC signaling pathway, leading to activation of the MAPK, ERK 1/2 (Budd et al. 2001). Gαphaq also couples to the Src family of tyrosine kinases that can stimulate activation of ERK1/2 via a PLC-independent mechanism (Peavy et al. 2001). In rat visual cortex, we recently reported that mLTD requires ERK 1/2 activation with only a partial requirement for PLC activation upstream (McCoy and McMahon 2007). Thus
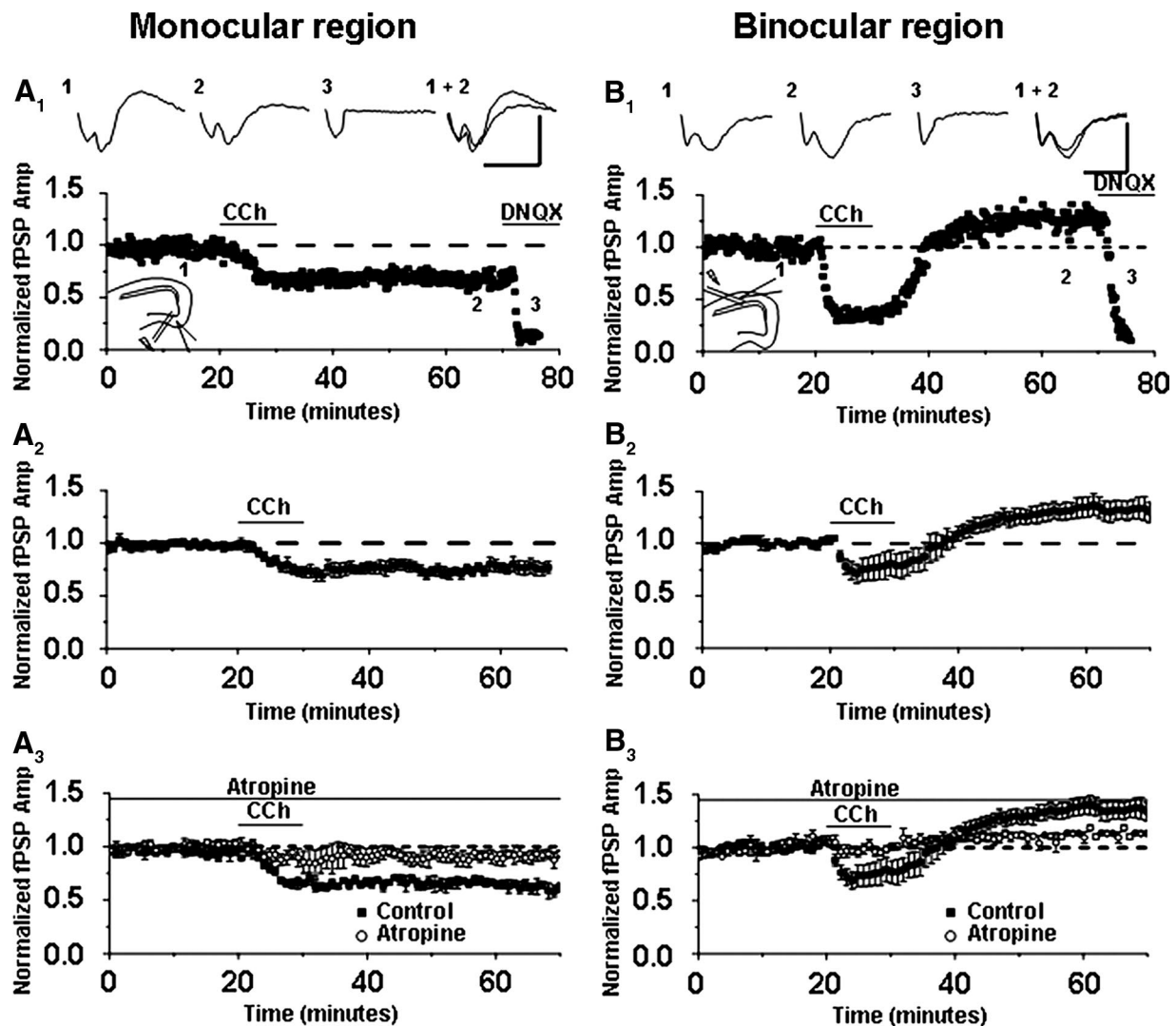
Monocular region

Binocular region

FIG. 2. The direction of plasticity induced by mAChR activation differs in the monocular and binocular regions. *A* and *B*: effects of a 10-min bath application of carbachol (CCh) on the field postsynaptic potential (fPSP) in the different regions of tree shrew visual cortex. Note: to ensure that the recording was a postsynaptic potential rather than a presynaptic fiber volley, 10 μM 6,7-dinitro-quinoxaline-2,3-dione (DNQX) was applied to block AMPARs at the experiment's conclusion. *A1*: representative example and summary data (*A2*) showing that application of CCh to the monocular region results in a long-lasting depression of layer 2/3 fPSPs. *B1*: representative example and showing the same application of CCh to the binocular region (*B2*) results in a long-lasting potentiation of layer 2/3 fPSPs. *A3* and *B3*: time course of CCh application in the presence and absence of 1 μM atropine, a nonselective muscarinic antagonist. The presence of atropine completely inhibits (*A3*) the depression of synaptic responses seen in the monocular region, (*B3*) and the potentiation of responses seen in the binocular region. Waveforms are averages of 20 events taken from 5 min before and 35 min after agonist application. Scale bar, 0.5 mV, 10 ms.

we next tested whether PLC and ERK1/2 activation are required for mLTD and mLTP at synapses in tree shrew visual cortex. Inhibition of ERK activation with U0126 (20 μM) not only blocked mLTD but unmasked a significant potentiation of the fPSP amplitude (130 $\pm$ 10% of baseline; $n = 5$ slices/3 animals, compared with interleaved controls 79 $\pm$ 2% of baseline; $n = 5$ slices/3 animals, $P < 0.001$; Fig. 4*A1*), similar to what was observed when m3 receptors were inhibited with 4-DAMP (Fig. 3*A2*). Inhibition of PLC with U73122 (10 μM) was without effect (76 $\pm$ 9% of baseline; $n = 5$ slices/3 animals, compared with interleaved controls 74 $\pm$ 9% of baseline; $n = 4$ slices/3 animals, $P > 0.05$; Fig. 4*A2*). In contrast, mLTP was unaffected by blocking ERK activation with U0126 (134 $\pm$ 8% of baseline; $n = 4$ slices/2 animals, compared with interleaved controls 130 $\pm$ 1% of baseline; $n = 2$ slices/2 animals $P > 0.05$; Fig. 4*B1*) but was completely prevented by blocking PLC with U73122 (99 $\pm$ 9% of base-

line; $n = 6$ slices/3 animals, compared with interleaved controls 142 $\pm$ 8% of baseline; $n = 3$ slices/3 animals $P < 0.05$; Fig. 4*B2*). Together these data show that two different mAChRs (m1 and m3), which are similarly coupled to Galphaq, stimulate different signaling molecules to induce long-term plasticity in opposing directions.

mLTP but not mLTD is associated with changes in PPR

Previously in rat visual cortex, mLTD induction was associated with a transient change in paired-pulse ratio (PPR) during CCh application that either remains elevated (Kirkwood et al. 1999) or returns to baseline during mLTD expression (McCoy and McMahon 2007). We wanted to determine if such changes in PPR were observed with mLTD and mLTP in tree shrew. Accordingly, we analyzed the PPR during induction and expression of each plasticity. We find that there is no signifi-

Monocular region

A₁

A₂

A₃

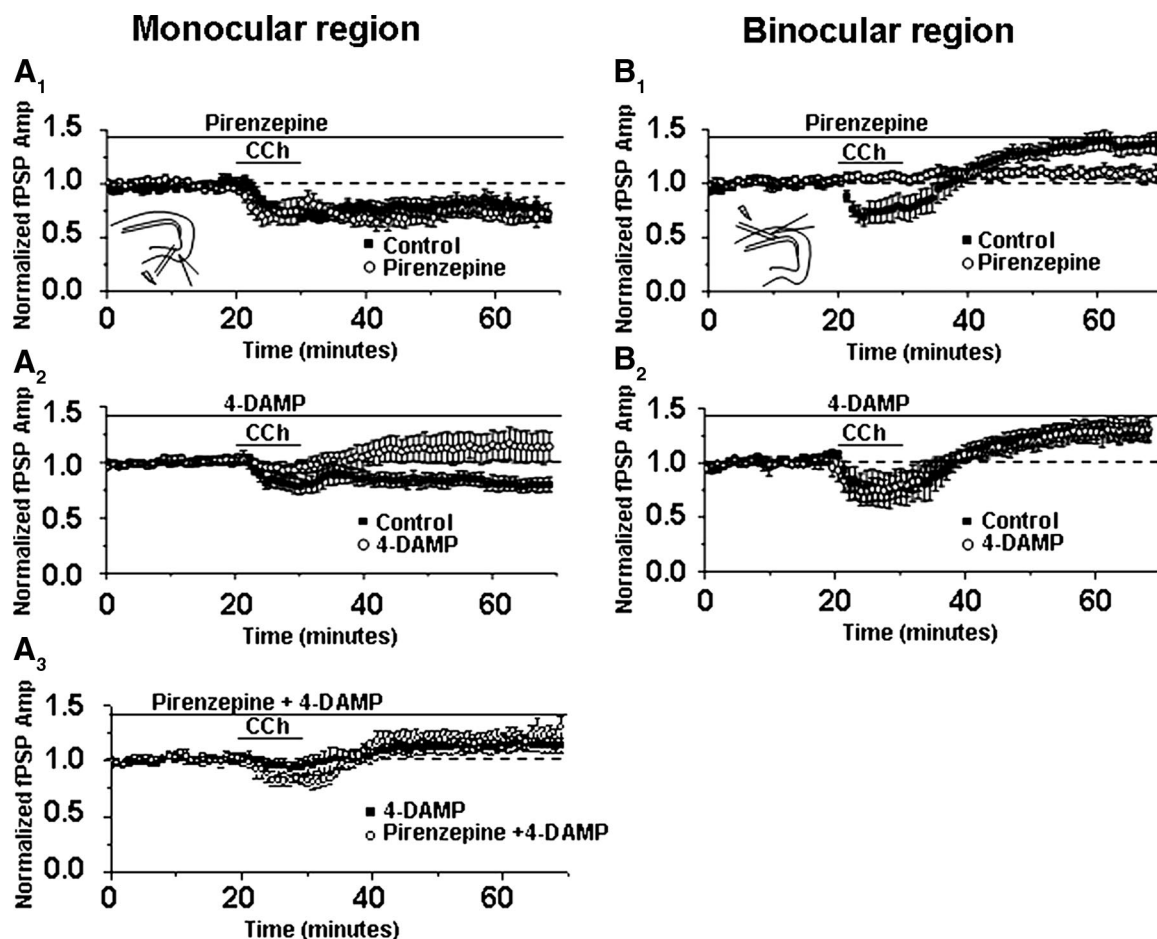## Binocular region

B₁

B₂

FIG. 3.   mLTD in the monocular region requires activation of m3 mAChRs and mLTP in the binocular region requires m1 receptors. *A1*: in the monocular region, 75 ηM pirenzepine, a concentration that is selective for m1 mAChRs, does not prevent mLTD. *A2*: 25 μM 4-diphenylacetoxy-*N*-methyl-piperidine (4-DAMP), an m3 mAChR-selective antagonist completely inhibits mLTD and results in potentiation of synaptic responses in the monocular region. *A3*: coapplication of pirenzepine and 4-DAMP results in a potentiation of responses. *B1*: bath application of pirenzipine prevents mLTP in the binocular region. *B2*: 4-DAMP does not prevent mLTP in the binocular region.

cant change in PPR in the monocular region at any time during the induction or expression of mLTD ($105 \pm 8\%$ of baseline; $n = 6$ slices/4 animals, $P > 0.05$; Fig. 5*A*). However, in the binocular region there is a transient increase in PPR during CCh application consistent with a presynaptic depression of glutamate release (Kimura and Baughman 1997; Mrzljak et al. 1993). Furthermore, during expression of mLTP, the PPR is significantly decreased ($87 \pm 3\%$ of baseline; $n = 7$ slices/4 animals, $P < 0.01$; Fig. 5*B1*). Interestingly, the change in PPR observed in the binocular region during mLTP induction and expression is also blocked by application of pirenzepine ($98 \pm 7\%$ of baseline; $n = 6$ slices/3 animals; Fig. 5*B2*). These data show a lack of a change in PPR during mLTD induction and expression in the monocular region and a significant increase in PPR during induction of mLTP followed by a long-term decrease during expression of mLTP in the binocular region.

Change in inhibition underlies mLTP but mLTD

A change in synaptic efficacy can result from either a direct change in glutamatergic transmission or an indirect effect via a change in GABAergic transmission. To investigate a potential role for inhibition in both mLTD and mLTP, we used whole cell patch-clamp recordings and blocked inhibition with 100

μM picrotoxin. Surprisingly, in both monocular (Fig. 5*C1*) and binocular (Fig. 5*D1*) regions, a 5-min application of CCh induced LTD ($64 \pm 2\%$ of baseline; $n = 7$ cells/4 animals, $P < 0.05$; and $58 \pm 6\%$ of baseline; $n = 6$ cells/4 animals, $P < 0.05$, respectively). These data imply that a change in inhibition is underlying the induction/expression of mLTP. If this is indeed the case, then mLTP should be induced when inhibition remains intact. This is precisely what we find. A slight but significant LTP was induced in whole cell recordings in the absence of picrotoxin ($113 \pm 8\%$ of baseline; $n = 7$ cells/5 animals, $P < 0.05$; Fig. 5*E1*), suggesting that an alteration in inhibitory transmission is responsible for mLTP and that mLTD can be unmasked when inhibition is blocked.

Interestingly, when we analyzed the PPR from the whole cell recordings, we again saw a change in PPR in binocular region during LTP induction and expression in the absence of picrotoxin, similar to what was observed in the extracellular recordings ($85 \pm 3\%$ of baseline; $P < 0.05$, $118 \pm 2\%$ of baseline; $n = 7$ slices/5 animals, $P < 0.05$, respectively; Fig. 5*E2*). Importantly, no change in PPR was observed when inhibition was blocked with picrotoxin ($99 \pm 4\%$ of baseline; $n = 6$ slices/4 animals, $P > 0.05$; Fig. 5*D2*). In whole cell recordings from pyramidal cells in the monocular region, no
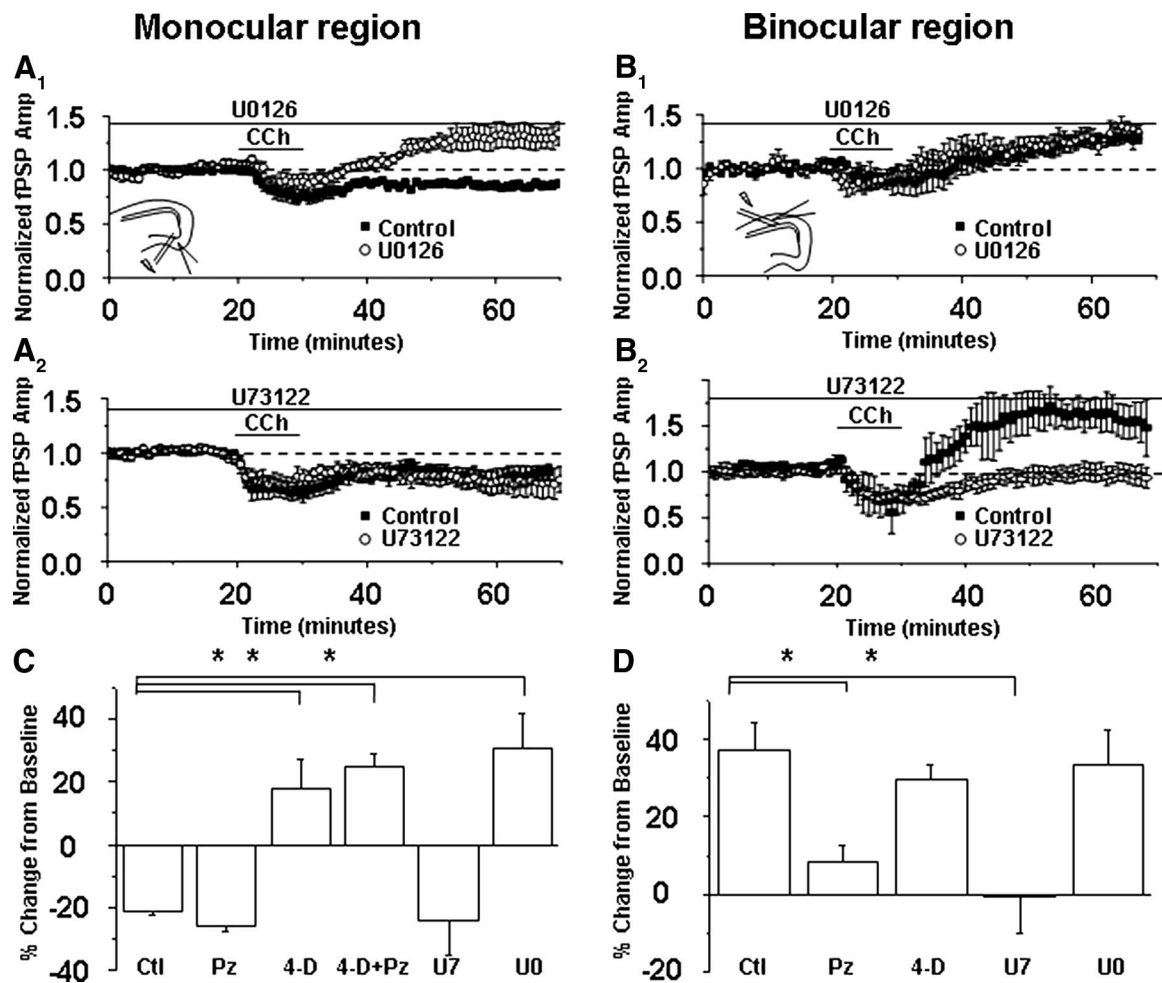
FIG. 4. mLTD in the monocular region requires activation of ERK 1/2, and mLTP in the binocular region requires PLC. *A1*: 20 μM U0126 (U0), an inhibitor of ERK 1/2 activation, inhibits mLTD and results in a potentiation in the monocular region. *A2*: in the monocular region 10 μM U73122 (U7), a PLC inhibitor, has no effect on mLTD. *B1*: 20 μM U0126, has no effect on mLTP in the binocular region. *B2*: 10 μM U73122, prevents mLTP in the binocular region. *C* and *D*: bar chart shows summarized data for the monocular and binocular regions, respectively. *, statistical significance, $P < 0.05$. Control, Ctl; pirenzepine, Pz; 4-DAMP, 4-D; U73122, U7; U0126, U0.

change in PPR was observed at any time during the experiment, similarly to what was observed in the extracellular recordings (98 ± 7% of baseline; $n = 7$ slices/4 animals, $P > 0.05$; Fig. 5C2). These data are consistent with the interpretation that GABA$_A$ receptor-mediated inhibition modulates the PPR in the binocular, but not the monocular, region during induction and expression of mLTP.

The model shown in Fig. 6 illustrates potential mechanisms underlying mLTD and mLTP following activation of mAChRs with CCh. Consistent with our data in the monocular region (*A*), m3 receptor activation leads to activation of ERK 1/2 and induction of mLTD. In the binocular region (*B*), activation of presynaptic m1 receptors, located on GABAergic interneurons, leads to increased release of GABA via a PLC-dependent mechanism, thereby leading to a decrease in inhibition and an increase glutamate release from the presynaptic terminal. These findings indicate that activation of mAChRs can bidirectionally induce plasticity depending on receptor location and the signaling cascade that is activated. This may be physiologically important to allow preferential transfer of information from the binocular region to higher order visual processing areas.

DISCUSSION

The ability of ACh, via mAChRs, to modulate synapses makes it primed for a key role in learning and memory. Here we report that in primary visual cortex, the same cholinergic agonist application induces LTD in the monocular region and LTP in the binocular region via different mAChRs and signaling mechanisms. This dual capability of the cholinergic system to bidirectionally induce plasticity is supported by recent studies demonstrating that endogenous ACh is capable of inducing LTD (Li et al. 2005) as well as LTP (Dringenberg et al. 2007). The ability of a single induction protocol to elicit opposing long-term plasticities in the visual cortex has been shown previously; however, in these studies, there were either differences in intrinsic calcium signals (Ismailov et al. 2004) or the state of basal transmission into the visual cortex (Tsanov and Manahan-Vaughan 2007). The idea that acetylcholine may bidirectionally induce plasticity is supported from work done with serotonin (5-HT). It has previously been reported that 5-HT paired with 1-Hz stimulation is capable of inducing LTP or LTD depending on the level of receptor expression in visual cortical subregions. Our results indicate that the direction of plasticity induced depends on the mAChR activated, but there
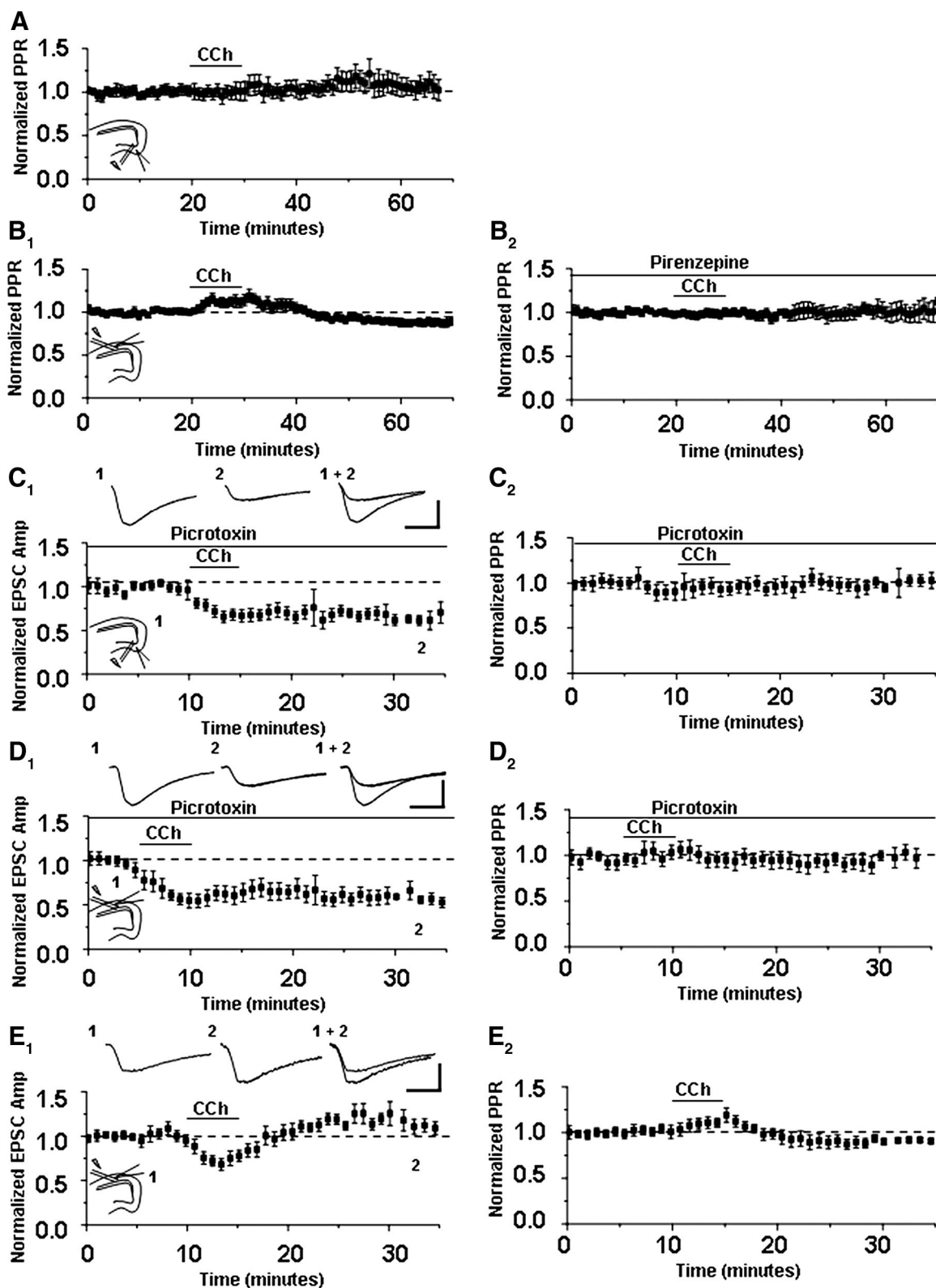
FIG. 5.   A change in PPR and GABA$_A$R-mediated inhibition underlie mLTP in the binocular region but not mLTD in the monocular region. *A*: in the monocular region, CCh application does not result in a change in the PPR. *B1*: in the binocular region, CCh application causes a transient increase in the PPR that returns to a stable decreased level as compared with baseline during mLTP expression. *B2*: application of pirenzepine abolishes the change in PPR seen in the binocular region. *C1*: in the monocular region CCh application (50 μM, 5 min) results in a depression of synaptic responses, when inhibition is blocked with 100 μM picrotoxin, that does not result in a change in the PPR (*C2*). *D1*: in the binocular region, CCh application results in a mLTD, when inhibition is blocked, that does not result in a change in the PPR (*D2*). *E1*: CCh application results in mLTP, when inhibition is not blocked, and causes an increase in the PPR that returns to a stable decreased level in the binocular region (*E2*). Representative traces taken from 3 min before and 15–25 min after agonist application. Scale bar, 150 pA, 50 ms.
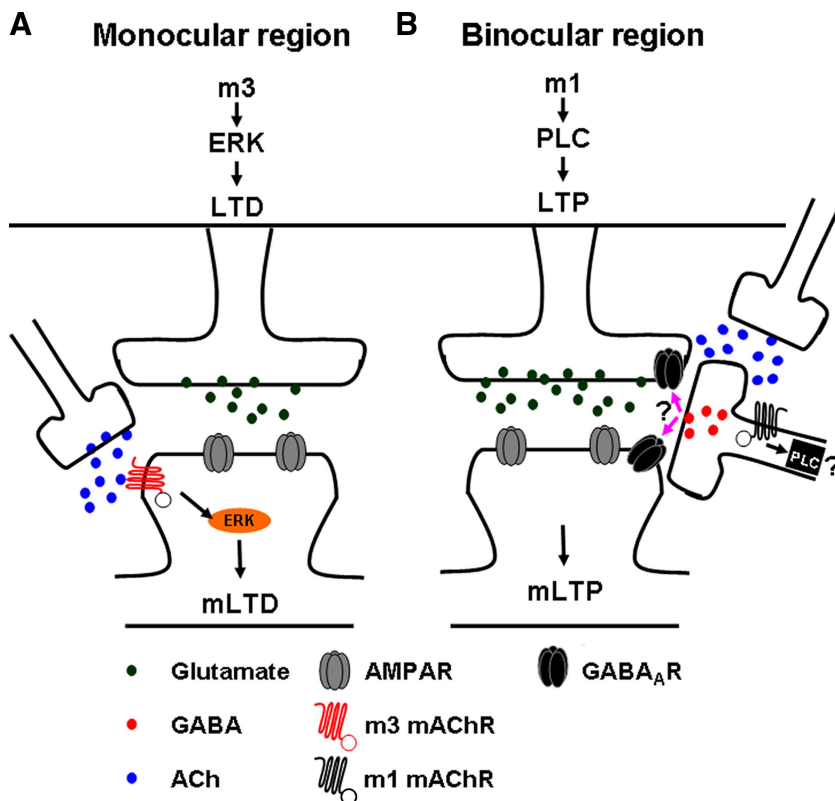
FIG. 6. Proposed model of mLTD and mLTP induction. Our data demonstrate in the monocular region (*A*) m3 receptor activation leads to activation of ERK 1/2 and induction of mLTD. In the binocular region (*B*), activation of presynaptic m1 receptors, located on GABAergic interneurons, leads to increased release of GABA via a PLC-dependent mechanism. Increased GABA release leads to increased glutamate release from the presynaptic terminal, resulting in LTP.

is no obvious difference in receptor localization between the two regions (Fig. 1).

A change in the PPR, the magnitude of which is inversely proportional to presynaptic release probability, is indicative of a presynaptic locus, while no change in the ratio is indicative of a postsynaptic locus (Dobrunz and Stevens 1997). The finding that the PPR is increased during CCh application to induce mLTP suggests that CCh activates presynaptic mAChRs that decrease presynaptic glutamate release. This mAChR-induced presynaptic depression has been reported previously in rat visual cortex and hippocampus by us and others (Kimura and Baughman 1997; Kirkwood et al. 1999; McCoy and McMahon 2007; Mrzljak et al. 1993; Scheiderer et al. 2006). However, during mLTP expression, the PPR is decreased, suggesting that the long-term synaptic potentiation results from an increase in glutamate release. In contrast, the PPR does not change at anytime during induction or expression of mLTD, suggesting that presynaptic mechanisms do not participate in this plasticity. The apparent difference in presynaptic modulation by mAChRs likely contributes to the differences in long-term plasticity that we observe.

This long-term presynaptic modulation of glutamate release by m1 receptors in the binocular region is likely to be an indirect effect on glutamate transmission rather than due to a direct activation of m1 receptors on glutamate terminals. This notion is supported by findings in whole cell voltage-clamp recordings of pyramidal cells in the binocular region, where changes in PPR of the evoked EPSC during induction (CCh application) and expression of mLTP were prevented in the presence of the GABA$_A$R antagonist picrotoxin. These findings suggest that m1 receptors are located on GABAergic interneurons the activation of which manipulates GABA release, which subsequently modulates glutamate transmission

leading to expression of mLTP. In fact, when GABA$_A$Rs are blocked, CCh application induces mLTD, rather than mLTP. Furthermore, in the presence of picrotoxin, the PPR does not change during CCh application or following its washout, suggesting that the increase and decrease in PPR that normally occur during induction and expression of mLTP, respectively, are a consequence of GABA$_A$R activation. Thus collectively, our data show that blockade of GABA$_A$Rs with picrotoxin removes mechanistic differences between the binocular and monocular regions such that CCh induces mLTD in both regions.

So where are the m1 receptors located that are responsible for the differential effects observed in the binocular region compared with the monocular region? Currently we are unable to determine this with the present results, and because m1 receptors are expressed both pre- and postsynaptically on pyramidal cells and interneurons, it is likely going to be difficult to determine conclusively. Given this, there are multiple scenarios one could draw that would explain mLTP expression. One such scenario supported by our results, places m1 receptors on presynaptic glutamate terminals and postsynaptic on interneurons. Presynaptic m1 receptors decrease release probability of glutamate, as previously mentioned, causing the transient decrease in transmission and increase in PPR seen during CCh application. Postsynaptic m1 receptors located on interneuron somas and dendrites increases interneuron excitability (Kimura and Baughman 1997; McQuiston and Madison 1999), resulting in an increase in GABA release that activates GABA$_A$Rs located on glutamate terminals, where the Cl$^-$ gradient is such that it causes a depolarization and subsequent facilitation of glutamate release (Koga et al. 2005). This scenario assumes that there is no appreciable shunting of excitation occurring by activation of postsynaptic GABA$_A$Rs

on the pyramidal cell. As we previously mentioned, there are other plausible scenarios that could explain our findings.

Our data suggest that there are competing mechanisms inducing LTP or LTD in the two regions. In the monocular region, LTP is uncovered when mLTD is blocked at the level of the mAChR (m3) or the signaling molecule (ERK 1/2 activation). These data indicate that the monocular LTP, while not requiring m3 receptors nor m1 receptors (Fig. 3), is still dependent on mAChRs because it is not unmasked when all mAChRs are blocked with atropine. Furthermore it is not dependent on ERK activation, because blockade of ERK unmasks LTP. This does not rule out the possibility that the final expression mechanisms for the mLTP unmasked in the monocular region are the same as that in the binocular region. It is likely that the presynaptic mechanism in the binocular region allows LTP to predominate while in the monocular region LTD predominates because of a lack of such a presynaptic component.

The functional implications for differential visual processing in tree shrews is unknown at this time; however, in rat, it has been shown that fibers of different origin, whether intracortical or thalamocortical, projecting into the visual cortex are differentially suppressed and excited (respectively) by ACh (Kimura et al. 1999). The bidirectional induction/expression of plasticity between the two regions could be a result of evolutionary adaptation. Classically, visual periphery (which is monocular) is used primarily to detect novel stimuli that need to be attended to by the higher-resolution central visual field, which is binocular. Constant changes in attention would cause multiple head and eye movements that would distract the animal from continuing to pay attention to important objects. Conversely, the binocular visual field includes the regions of central vision that are used to resolve and identify objects of importance. Because Ach is important for attention (Bentley et al. 2004; Blokland 1995; Himmelheber et al. 2000), bidirectional cholinergic plasticity could be a way to filter out objects in the visual field that need to be attended to. The depression induced in the monocular region would represent all objects outside of the central visual field, while the potentiation induced in the binocular region would represent objects in the central visual field. This suggests a mechanism exists whereby, given all objects in a field of view, the information encoding the objects the animal is attending to is preferentially relayed over nonnovel stimuli in the periphery. What mechanisms drive this segregation and modulation to occur are not completely understood, but the same mechanisms that help shape the architecture may serve as a platform for unique modulation of the synaptic circuits by acetylcholine and will be explored in tree shrews in future studies.

Reductions in the function/efficiency of the cholinergic system have been implicated in normal aging and disease states in association with alterations in learning and memory involving the visual system. Mimicking this cholinergic loss in animal studies results in impairment in visual attention (Balducci et al. 2003), visual discrimination (Barefoot et al. 2000), and spatial memory tasks (Caccamo et al. 2006), which can be rescued with mAChR-specific agonists. LTP is required for learning and memory (Malenka and Bear 2004; Pastalkova et al. 2006; Whitlock et al. 2006), and has been long hypothesized that the same is true for LTD (Bear 1999). The existence of an LTD and LTP dependent on the activation of mAChRs helps to explain the dependence of normal learning and memory on the cholinergic system. This may serve to further elucidate the mechanisms of loss of visual memory formation and help in understanding how to treat visual memory deficits.

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