Presynaptic cholinergic neuromodulation alters the temporal dynamics of short-term depression at parvalbumin-positive basket cell synapses from juvenile CA1 mouse hippocampus

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Lawrence JJ, Haario H, Stone EF. Presynaptic cholinergic neuromodulation alters the temporal dynamics of short-term depression at parvalbumin-positive basket cell synapses from juvenile CA1 mouse hippocampus. J Neurophysiol 113: 2408–2419, 2015. First published January 28, 2015; doi:10.1152/jn.00167.2014.—Parvalbumin-positive basket cells (PV BCs) of the CA1 hippocampus are active participants in theta (5–12 Hz) and gamma (20–80 Hz) oscillations in vivo. When PV BCs are driven at these frequencies in vitro, inhibitory postsynaptic currents (IPSCs) in synaptically connected CA1 pyramidal cells exhibit paired-pulse depression (PPD) and multiple-pulse depression (MPD). Moreover, PV BCs express presynaptic muscarinic acetylcholine receptors (mAChRs) that may be activated by synaptically released acetylcholine during learning behaviors in vivo. Using acute hippocampal slices from the CA1 hippocampus of juvenile PV-GFP mice, we performed whole cell recordings from synaptically connected PV BC-CA1 pyramidal cell pairs to investigate how bath application of 10 μM muscarine impacts PPD and MPD at CA1 PV BC-pyramidal cell synapses. In accordance with previous studies, PPD and MPD magnitude increased with stimulation frequency. mAChR activation reduced IPSC amplitude and transiently reduced PPD and MPD, but MPD was largely maintained. Consistent with a reduction in IPSC decay time constant, presynaptic mACHR activation increased both the coefficient of variation of IPSC amplitudes and the fraction of failures. Using variance-mean analysis, we converted MPD trains to p_i functions and developed a kinetic model that optimally fit six distinct p_i conditions. The model revealed that vesicular depletion caused MPD and that recovery from depression was dependent on calcium. mAChR activation reduced the presynaptic calcium transient fourfold and initial p_i twofold, thereby reducing PPD. However, mAChR activation slowed calcium-dependent recovery from depression during sustained repetitive activity, thereby preserving MPD. Thus the activation of presynaptic mAChRs optimally protects PV BCs from vesicular depletion during short bursts of high-frequency activity.

GABA; gamma; hippocampus; inhibition; muscarinic acetylcholine receptor

PARVALBUMIN-POSITIVE (PV) basket cells (BCs) are a major subclass of hippocampal neurons that provide GABAergic inhibition to the perisomatic regions of principal cells and are among the most abundant and well characterized of PV cell types in vitro (Buhl et al. 1994; Bartos et al. 2007; Cea-del Rio et al. 2010) and in vivo (Sik et al. 1995; Klausberger et al. 2003; Tukker et al. 2007; Klausberger and Somogyi 2008; Hu et al. 2014). Their intrinsic membrane properties are distinguished by their low input resistance, short membrane time constant, fast-spiking phenotype, dendritic potassium conductances, and cellular resonance properties (Pike et al. 2000; Bartos et al. 2001; Jonas et al. 2004; Zemankovic et al. 2010). Excitatory and inhibitory postsynaptic events are therefore short-lived due to the short membrane time constant (Jonas et al. 2004; Bartos et al. 2007). More recent studies have drawn attention to the capacity of PV BCs to elicit highly synchronous synaptic release (Daw et al. 2009; Eggermann et al. 2012; Eggermann and Jonas 2012). Collectively, these intrinsic membrane and synaptic properties enable PV neurons to participate in gamma (50–80 Hz)-frequency oscillatory activity (Bartos et al. 2007; Tukker et al. 2007). Optogenetic methods have recently determined that the generation of gamma oscillations requires excitation of PV neurons (Cardin et al. 2009; Sohal et al. 2009).

In the hippocampus (Kraushaar and Jonas 2000; Maccarelli et al. 2000; Szabó et al. 2010) and cortex (Galaretta and Hestrin 1998; Kruglikov and Rudy 2008; Pafundo et al. 2013), the presynaptic terminals of PV BCs onto their postsynaptic targets exhibit synaptic depression, a form of short-term plasticity (STP) in which GABAergic transmission is reduced upon the arrival of subsequent action potentials (APs) at presynaptic terminals (Fioravante and Reggehr 2011). Synaptic depression is a property common to all PV BC synapses onto their principal cell targets in the dentate gyrus (DG) (Kraushaar and Jonas 2000; Bartos et al. 2001; Hefft et al. 2002), CA3 (Szabó et al. 2010), and CA1 (Maccarelli et al. 2000). Coefficient of variance analysis indicated a presynaptic mechanism (Kraushaar and Jonas 2000; Bartos et al. 2001; Hefft et al. 2002). Synaptic depression is less pronounced or completely absent from PV bistratified or oriens-lacunosum moleculare interneuron subtypes (Maccarelli et al. 2000; Szabó et al. 2010). Therefore, synaptic depression may be a specific synaptic specialization that endows PV BCs with the capacity to encode “onset” information during hippocampal processing (Pouille and Scanziani 2004).

Electrical stimulation of CA1 stratum pyramidale (SP) evokes inhibitory postsynaptic currents (IPSCs) that are subject to cholinergic modulation (Pitler and Alger 1992; Seeger et al. 2004), raising the possibility that mAChR activation presynaptically inhibits GABA transmission from PV BCs. As shown...
by whole cell recordings from synaptically connected pairs, IPSCs from PV BCs are specifically inhibited by mACHR activation in the DG (Hefft et al. 2002; Chiang et al. 2010), CA3 (Szábo et al. 2010), and cortex (Krulikov and Rudy 2008; Pafundo et al. 2013). While presynaptic M2 mACHRs have been identified on PV terminals in the perisomatic regions in CA1 (Hajós et al. 1998) and M2 mACHR mRNA was identified in the cytoplasm of a subset of CA1 PV BCs (Cea-del Rio et al. 2010), no study has yet systematically examined how presynaptic mACHRs modulate BC-PV transmission in area CA1. Given that CA1 PV BCs exhibit a broad range of firing frequencies in vivo (Klausberger et al. 2003; Klausberger and Somogyi 2008), which are thought to be driven in part by cholinergic transmission from the medial septum-diagonal band of Broca (Yi et al. 2014), we investigated the frequency dependence of PV BC transmission in the CA1 hippocampus and determined the extent that these cells undergo presynaptic mACHR neuromodulation.

Here, we employed paired whole cell recordings (Miles and Poncer 1996), variance-mean (VM) analysis (Silver et al. 1998; Clements and Silver 2000), and mathematical modeling (Dittman et al. 2000: Lee et al. 2009; Stone et al. 2014) to investigate the frequency dependence of cholinergic neuromodulation at CA1 PV BC-pyramidal cell (PC) synapses. To facilitate targeting PV BCs for patch-clamp recording, we employed transgenic mice in which GFP was driven by the parvalbumin promoter (Ango et al. 2008; Klausberger et al. 2003; Klausberger and Somogyi 2008), which showed post hoc axonal arborizations restricted largely to SP, suggesting that they were largely PV BCs (Cea-del Rio et al. 2010). The axons occasionally strayed to the SO/SP border; therefore, we cannot rule out that a subset of these PV cells were axo-axonic cells (Baude et al. 2017). Electodes were pulled to a 3- to 5-MΩ tip impedance with a two-stage PC10 vertical puller (Narishige, East Meadow, NY) for current-clamp recordings from PV BCs, the intracellular solution (ICS) consisted of the following (in mM): 110 K-glucinate, 0.1 EGTA, 4 MgATP, 0.3 NaGTP, 10 HEPES, 10 phosphocreatine, and 0.2% bicocytin, adjusted to pH 7.3 with KOH. Postsynaptic IPSCs were selected within 5–20 μm of the presynaptic PV BC cell at a similar depth. For PC recordings, the ICS was the following (in mM): 110 CsCl, 10 TEA, 5 EGTA, 4 MgATP, 0.3 GTP, 30 HEPES, 10 phosphocreatine, 1 QX314, and 0.2% bicocytin, adjusted to pH 7.3 with CsOH. Whole cell data were acquired with a Multichannel 700A amplifier (Molecular Devices, Sunnyvale, CA), low-pass filtered at 4 kHz (LP02 8-pole Bessel; Frequency Devices, Ottawa, IL), and digitized at 20 kHz (Digidata 1322A) with pClamp9.2 software (Molecular Devices) running on a PC. Bridge balance was employed for recordings from PV-GFP neurons. PV-GFP cells were initially screened with a 1-s long, 500-pA current step from 0 mV to +10 mV and then tested at 10–20 mV steps. Trains of presynaptic APs were evoked using a 1-ms long, 500-pA current step from –60 mV to confirm a FS phenotype. Trains of presynaptic APs were evoked using a 1-ms long, suprathreshold stimulus consisting of 3- to 4-nA amplitude somatic current steps from –60 mV. Traces in which current steps failed to elicit APs throughout the train were not included in analyses. Evoked IPSCs were recorded in voltage-clamp mode from –60 mV. Access resistance was monitored with a 100-mS, –5-mV step seal test from –60 mV at the beginning of each protocol. Input resistance was measured in the last 20 ms of the –5-mV step.

Morphological identification. After whole cell recording, to facilitate visualization of the neuronal membrane, the electrode was withdrawn until an outside-out patch was formed. Hippocampal slices were then binned in 10- to 25-pA increments of current. The VM relationship was fit with a simple parabolic function (Variance-Mean Analysis Programs, AxographX):

\[ \text{VM} = \text{MPD} + \text{PPD} \times \frac{(2 \times \text{MPD})}{(\text{MPD} + \text{PPD})} \]

Materials and Methods

Transverse hippocampal slice preparation. All experiments were conducted in accordance with animal protocols approved by the National Institutes of Health. PV-GFP (“B13 line”) mice in the juvenile age range of postnatal day (P)15-P20 were anesthetized by isoflurane inhalation and decapitated (Daw et al. 2009; Cea-del Rio et al. 2010). The brain was quickly removed and immersed in an oxygenated, ice-cold partial sucrose-based cutting ringer (SBR) containing the following (in mM): 75 NaCl, 24 NaHCO3, 25 glucose, 75 sucrose, 2.5 KCl, 1.25 NaH2PO4·H2O, 1.5 ascorbic acid, 2 pyruvate, and 1 mM CaCl2. To block glutamatergic transmission, the AMPA receptor antagonist DQNX (25 μM) and NMDA receptor antagonist dl-APV (50 μM) was added to the ECS. The ECS was continuously superfused with 95% O2-5% CO2. The brain was quickly removed and immersed in an oxygenated, ice-cold partial sucrose-based cutting ringer (SBR) containing the following (in mM): 75 NaCl, 24 NaHCO3, 25 glucose, 75 sucrose, 2.5 KCl, 1.25 NaH2PO4·H2O, 1.5 ascorbic acid, 2 pyruvate, and 1 mM CaCl2. To block glutamatergic transmission, the AMPA receptor antagonist DQNX (25 μM) and NMDA receptor antagonist dl-APV (50 μM) was added to the ECS. The ECS was continuously superfused with 95% O2-5% CO2.

Whole cell patch-clamp recordings. Experiments were performed on a Zeiss FS2 upright microscope equipped with manipulators (Infrapatch; Luigs and Neumann, Ratingen, Germany). Hippocampal slices were imaged using a CCD-cooled tube camera (Hamamatsu, Bridgewater, NJ) and IR Dodt contrast. To visualize PV-GFP cells, a long-pass GFP emission filter (Chroma Technology, Bellows Falls, VT) was used to scan hippocampal slices under fluorescence with and without IR Dodt contrast (SPOT RT; Diagnostic Instruments, Sterling Heights, MI). Although PV-GFP cells were sparse in CA1, PV-GFP cells with cell bodies in SP that had detectable vertically oriented dendrites invariably possessed a fast-spiking (FS) phenotype and showed post hoc axonal arborizations restricted largely to SP, suggesting that they were largely PV BCs (Cea-del Rio et al. 2010). The VM rule out that a subset of these PV cells were axo-axonic cells (Baude et al. 2007). Electodes were pulled to a 3- to 5-MΩ tip impedance with a two-stage PC10 vertical puller (Narishige, East Meadow, NY) for current-clamp recordings from PV BCs, the intracellular solution (ICS) consisted of the following (in mM): 110 K-glucinate, 0.1 EGTA, 4 MgATP, 0.3 NaGTP, 10 HEPES, 10 phosphocreatine, and 0.2% bicocytin, adjusted to pH 7.3 with KOH. Postsynaptic IPSCs were selected within 5–20 μm of the presynaptic PV BC cell at a similar depth. For PC recordings, the ICS was the following (in mM): 110 CsCl, 10 TEA, 5 EGTA, 4 MgATP, 0.3 GTP, 30 HEPES, 10 phosphocreatine, 1 QX314, and 0.2% bicocytin, adjusted to pH 7.3 with CsOH. Whole cell data were acquired with a Multichannel 700A amplifier (Molecular Devices, Sunnyvale, CA), low-pass filtered at 4 kHz (LP02 8-pole Bessel; Frequency Devices, Ottawa, IL), and digitized at 20 kHz (Digidata 1322A) with pClamp9.2 software (Molecular Devices) running on a PC. Bridge balance was employed for recordings from PV-GFP neurons. PV-GFP cells were initially screened with a 1-s long, 500-pA current step from –60 mV to confirm a FS phenotype. Trains of presynaptic APs were evoked using a 1-ms long, suprathreshold stimulus consisting of 3- to 4-nA amplitude somatic current steps from –60 mV. Traces in which current steps failed to elicit APs throughout the train were not included in analyses. Evoked IPSCs were recorded in voltage-clamp mode from –60 mV. Access resistance was monitored with a 100-mS, –5-mV step seal test from –60 mV at the beginning of each protocol. Input resistance was measured in the last 20 ms of the –5-mV step.
\[ \sigma^2 = (1 + CV_1^2)qI - \frac{I^2}{N} \]

where \( \sigma^2 \) is the variance, \( I \) is the mean, \( q \) is the average quantal amplitude, \( N \) is the number of independent functional release sites, and \( CV_1 \) is the intrasite quantal coefficient of variation. In muscarine conditions, the fit was often linear, which enabled only \( q \) to be determined.

To reduce the number of free parameters, we assumed that \( N \) was frequency independent, \( p_1 \) was uniform, GABA concentration (and hence \( q \)) was stable and nont saturating, and conduction failures did not occur. The number of free parameters was further reduced by setting \( C V_1 = 0.3 \) (Clements and Silver 2000), which does not change the estimate of \( N \); however, if the \( C V_1 \) ranges from 0.2– 0.4 (Kraushaar and Jonas 2000), the error associated with fixing the intrasite CV at 0.3 is <5% for \( q \) and <5–15% for \( p_{\text{max}} \), with an understimation of \( C V_1 \) leading to an understimation of \( p_{\text{max}} \). We felt that these assumptions were reasonably justified given the properties of PV synapses described in the DG (Kraushaar and Jonas 2000) and cortex (Szabadics et al. 2007; Connelly and Lees 2010). Frequency-dependent changes in average \( p_1 (p_2) \) were obtained from the parabolic fit under control conditions.

Overlaying all events within a pulse number group, with attention to the precise onset of large-amplitude synchronous IPSCs, allowed apparent failures in synaptic release to be unambiguously detected by eye (see Fig. 3, A and B). Plots of cumulative failure probability were obtained by calculating the cumulative fraction of failures for pulse (P) numbers 1–25 using custom analysis code in AxographX (Sydney, Australia).

**Mathematical model of synaptic depression at PV-BC-PC synapses.** As developed in an earlier model (Lee et al. 2009), equations that described depression (due to depletion of vesicles), facilitation (from an increase in presynaptic calcium concentration), and CDR (Dittman and Regehr 1998; Yang and Xu-Friedman 2008) were included. Details on the construction of this kinetic model, including model reduction and analysis, are provided in a companion article (Stone et al. 2014).

**RESULTS**

**mACHr receptor activation inhibits PV-BC-CA1 PC transmission and reduces PPD onto CA1 PCs.** To investigate GABAergic transmission specifically between PV BCs and CA1 PCs, we used acute hippocampal slices from PV-GFP mice. In a representative experiment, a presynaptic PV-GFP cell was initially visualized under epifluorescence (Fig. 1A) and IR-Dodt contrast (Fig. 1B). In a synaptically connected pair from the PV-GFP and a neighboring CA1 PC, APs were elicited in the PV-GFP cell during a 1-s long, +500-pA depolarizing current step, revealing well-resolved, time-locked postsynaptic IPSCs in the postsynaptic CA1 PC, which exhibited synaptic depression after the initial IPSC (Fig. 1C). Post hoc processing of biocytin-filled cells identified the presynaptic neuron as a PV BC (Fig. 1D), as indicated by the axonal arborization in SP (Fig. 1D, inset) (Buhl et al. 1994; Freund and Buzsáki 1996).

To determine the effect of mACHr activation on PPD of the postsynaptic IPSCs in CA1 PCs, pairs of APs were elicited in the presynaptic PV-GFP cell 20-ms apart every 20 s before and during bath application of 10 μM muscarine. In the same representative experiment as in Fig. 1, A–D, muscarine depressed the initial IPSC amplitude (P1; Fig. 1E) and induced an inward current at −60 mV (Fig. 1F) over the course of 4 min, consistent with concomitant presynaptic M2 mAChR-mediated inhibition of GABA release (Hájos et al. 1998) and M1 mAChR-mediated activation of a cationic conductance (Fisahn et al. 2002; Yamada-Hanff and Bean 2013). In a population of 11 morphologically identified PV BCs, the initial IPSC was reduced from −252 ± 73 pA to −87 ± 22 pA (P = 0.017; n = 11; Fig. 1G), accompanied by a −43 ± 18 pA increase in holding current (\( I_{\text{hold}} \); P = 0.035; n = 11; Fig. 1H). This mAChR-induced inhibition of PV BC IPSCs was similar to earlier observations in the DG (Hefft et al. 2002) and CA3 (Szabó et al. 2010).

We then examined PPD by examining the first IPSC (P1) and second IPSC (P2) elicited by pairs of APs at a 20-ms interval. The average IPSC recorded at the PV BC-CA1 PC synapses exhibited PPD (Fig. 1I, blue). During the application of 10 μM muscarine, both P1 and P2 were reduced. Normalizing to P1, it was apparent that PPD was reduced in the presence of muscarine (Fig. 1I, red). In a population of 11 synaptically connected PV BC-CA1 PC pairs, the amplitude of P1 (−252 ± 73 pA) was significantly larger than P2 (−143 ± 47 pA; P = 0.003), indicating that all PV BCs consistently exhibited PPD in control conditions [paired-pulse ratio (PPR) = 0.55 ± 0.06]. This finding indicates a high \( p_1 \) at PV BC synapses. However, in the presence of 10 μM muscarine, P1 (−79 ± 21 pA) was not significantly different than P2 (−70 ± 20 pA; P = 0.50; PPR = 1.29 ± 0.32), with 10 of 11 of PV BC pairs exhibiting either a reduction in PPD (from 0.53 ± 0.10 to 0.89 ± 0.07; n = 6) or paired-pulse facilitation (PPF) (from 0.58 ± 0.07 to 2.29 ± 0.62; n = 4; Fig. 1J). An investigation of the CV of P1 amplitudes revealed that the CV increased from 0.22 ± 0.03 to 0.32 ± 0.03 (n = 11; Fig. 1K). Moreover, the reduction in P1 amplitude was not accompanied by a significant mAChR-induced change in input resistance in the CA1 PC (from 226 ± 23 MΩ in control to 220 ± 40 MΩ in muscarine; P = 0.82). These results are consistent with a presynaptic mechanism of mAChR activation at CA1 PV BC synapses.

The magnitude of MPD depends on stimulation frequency at CA1 PV-BC-PC synapses. To more systematically investigate how stimulation frequency alters CA1 PV BC transmission, we introduced trains of 25 APs at 5, 50, and 100 Hz into visually identified PV-GFP neurons while recording IPSCs from an adjacent, synaptically connected CA1 PC (Fig. 2, blue). Similarly to previous observations (Fig. 1, I and J), PPD was observed at the onset of the train (Fig. 2A, blue; PPR = 0.45). Normalizing to the P1 peak amplitude indicated that all subsequent IPSCs (P3–P25) were reduced (Fig. 2A, blue; range of −64 to −143 pA; PPR = 0.30–0.76). In a population of 11 paired recordings in which the presynaptic PV-GFP neuron was morphologically confirmed to be a PV BC, PPD was observed (P1: −294 ± 90 pA; P2: −194 ± 58 pA; P = 0.014; n = 11; Fig. 2B, blue). During trains of 5-Hz stimuli, average IPSC amplitudes at P2–P25 were consistently reduced compared with the average P1 amplitude (P < 0.05; n = 11; Fig. 2B, blue), indicating that PV BC terminals undergo MPD (Fig. 2C, blue).
mACHr activation eliminates PPD but preserves MPD at CA1 PV BC-PC synapses. To investigate how mACHr activation altered the frequency dependence of MPD, we reintroduced trains of 25 APs at 5 and 50 Hz after bath application of 10 μM muscarine (Fig. 2, red). Consistent with earlier observations (Fig. 1, G and J), mACHr activation decreased the average P1 amplitude and relieved PPD at the onset of 5 Hz (Fig. 2A, red; P1: 37% of control; PPR 0.45 to 0.93) and 50 Hz (Fig. 2D, red; P1: 33% of control; PPR 0.68 to 0.82) trains. mACHr activation appeared to relieve MPD in some cells (Fig. 2D, I) but was not a consistent observation. In a population of 11 synaptically connected PV BC-CA1 PC pairs, mACHr activation reduced P1 amplitude at the onset of 5 Hz (Fig. 2B, red; from −294 ± 90 to −96 ± 28 pA; P = 0.03; n = 11) and 50 Hz (Fig. 2E, red; from −275 ± 91 to −98 ± 31 pA; P = 0.033; n = 10) trains. Steady-state (average of P16–P25) amplitudes during 5 Hz (from −146 ± 45 to −50 ± 14 pA; P = 0.038; n = 11) and 50 Hz (from −84.3 ± 26.7 to −43.8 ± 12.7 pA; P = 0.030; n = 10) were also reduced by mACHr activation. mACHr activation eliminated PPD at 5 Hz (Fig. 2B; P1: −96 ± 28 pA; P2: −71 ± 17 pA; P = 0.08; n = 10) and 50 Hz (P1: −98 ± 31 pA; P2: −76 ± 22 pA; P = 0.11; n = 10). However, at 5 Hz, MPD remained largely intact for P3–P25 (P < 0.05; n = 10; Fig. 2C). At 50 Hz (Fig. 2E),
average P1 amplitude (−98 ± 31 pA) was not significantly different than average P2 (−76 ± 22; P = 0.11) or P3 (−61 ± 16 pA; P = 0.08) amplitudes (n = 10). However, MPD (P4–P25) remained intact (P < 0.05; n = 10). In contrast to control conditions, steady-state (average P16–P25) amplitudes were not significantly different between 5- and 50-Hz trains (P = 0.08; n = 9), suggesting that mAChR activation had a normalizing effect on the frequency dependence of MPD (Pafundo et al. 2013).

Finally, in a subset of a subset of 4 synaptically connected CA1 PV BC-PC pairs, we also delivered a 100-Hz train of 25 pulses. Similarly to 5- and 50-Hz trains, the average P2 amplitude (−113 ± 7 pA) was reduced relative to the average P1 amplitude (−312 ± 36 pA; P = 0.002; n = 4), indicating powerful PPD. Also, 100-Hz trains resulted in MPD (average P16–P25, −55 ± 9 pA; P = 0.003; n = 4). mAChR activation depressed P1 (from −312 ± 36 to −130 ± 26 pA; P = 0.005) but eliminated PPD (P1: −130 ± 26 pA; P2: −87 ± 10 pA; P = 0.12; n = 4) while keeping MPD intact (average P1 vs. P3-25; P < 0.05; n = 4). In summary, our results, which were consistent across 5-, 50-, and 100-Hz frequencies, indicated that mAChR activation reduced PPD without altering MPD.

Synaptic depression is presynaptic in origin. Strongly depressing synapses may indicate a high $p_r$ upon calcium influx (Fioravante and Regehr 2011). For GABAergic synapses that have multiple release sites and high $p_r$, such as CA1 PV BC-PC (Buhl et al. 1994) or DG PV BC-granule cell (GC; Kraushaar and Jonas 2000) synapses, evoked IPSCs would be expected to exhibit no synaptic failures in release and large, regular evoked IPSCs with little variability in amplitude. At the opposite extreme, near-complete depletion would generate mostly synaptic failures and the occasional fusion of one or a few vesicles, generating consistently small amplitude events with low variability in IPSC amplitude. At intermediate $p_r$, a broad range of IPSC amplitudes would be possible, including the possibility of synaptic failures.

Alterations in the CV (SD/mean) have been demonstrated to indicate changes in presynaptic release (Malinow and Tsien 1990). Therefore, during a train of evoked IPSCs in which rates of vesicle depletion are faster than rates of replenishment of the RRP, one would predict that the CV initially be low, then increase after the first IPSC. Indeed, at PV BCs that exhibit strong PPD and MPD, initial P1 amplitudes during a 5-Hz train exhibited a CV of 0.073 (Fig. 3A; −296 ± 22 pA). Later in the

Fig. 2. Multiple-pulse depression (MPD) and mAChR activation at CA1 PV BC-PC synapses. Representative trains of 25 IPSCs [pulse number 1–25 (P1–25)] at 5 Hz in (blue) control and (red) after bath application of 10 μM muscarine (A). A, right inset: expanded region illustrating P1 and P2 responses. Below, IPSCs are normalized to P1. Average peak IPSC amplitude for each pulse for 5 Hz (B; n = 11) and normalized to P1 (C). D–F are as in A–C but for a 50-Hz train (n = 10).
5-Hz train, P4 amplitudes were reduced (−220 ± 31 pA), concomitant with a doubling in CV (0.14; Fig. 3A). At 50 Hz, the CV of the initial P1 was 0.068 (−185 ± 13 pA), which greatly increased (CV = 0.64) at P4 later in the train, including an apparent failure in release (Fig. 2B, black). In a population of nine synaptically connected PV BC-PC pairs, relative to the initial P1 amplitude (0.21 ± 0.04), the CV was significantly higher at 5 Hz in subsequent P2–P25 amplitudes (CV = 0.39–0.64; P < 0.05; n = 9; Fig. 3C). At 50 Hz, relative to the initial P1 amplitude (0.23 ± 0.04), the CV was significantly higher for subsequent P2–P25 amplitudes (CV = 0.59–0.92; P < 0.01; n = 10; Fig. 3D).

We also examined the incidence of apparent failures in synaptic release by quantifying the cumulative failure probability over the course of 5- and 50-Hz trains. Consistent with previous work in the DG (Kraushaar and Jonas 2000; Hefft et al. 2002), we observed no failures in initial P1 amplitude under control conditions for 5-, 50-, and 100-Hz trains, indicating that PV BCs have high initial $p_r$ with no apparent synaptic or axonal failures. At the end of the 5-Hz train, despite a significant increase in CV from P2–P25, the incidence of synaptic failures at 5 Hz was rare, with the average cumulative failure probability being only 0.058 ± 0.026, equating to only 10.1 ± 4.5 failures of 175 possible events (P = 0.055; n = 9; Fig. 3E, blue). During 50-Hz trains, failures accumulated more rapidly; by P4, the cumulative incidence of failures was statistically significant (0.007 ± 0.002; P = 0.027) and remained significant from P5 to P25 (P < 0.05; n = 10; Fig. 3F). At P25, the cumulative probability was 0.095 ± 0.029, or 9.5% of 175 IPSCs delivered during the 50-Hz train (P = 0.0092). Consistent with the frequency dependence of PV BC transmission, we observed that the steady-state CV (average of P16–P25) was higher for 50-Hz trains (0.66 ± 0.06) than for 5-Hz trains (0.49 ± 0.06; P = 0.0094; n = 9). Moreover, the cumulative fraction of failures was higher during 50-Hz trains (0.100 ± 0.032) than 5-Hz trains (0.058 ± 0.026; P = 0.0019; n = 9).

**Fig. 3.** Synaptic depression and mAChR activation are presynaptic in origin. Examples of individual IPSCs at P1 and P4 during a 5-Hz (A) or 50-Hz (B) train. Blue and red denote control and 10 μM muscarine, respectively. Coefficient of variation (CV) plotted as a function of train number for n = 5 cells at 5 Hz (C) or 50 Hz (D). E: cumulative fraction of failures at 5 Hz. F: cumulative fraction of failures at 50 Hz.
Thus the activity-dependent increase in CV and failures during 5- and 50-Hz trains is consistent with a presynaptic mechanism.

mAChR modulation of IPSCs from PV BCs is presynaptic in origin. We then applied the same analysis to 5- and 50-Hz trains after bath application of 10 µM muscarine (Fig. 3, red). In a representative synaptically connected PV BC-PC pair (Fig. 3A, red), mAChR activation modestly increased P1 CV for 5 Hz (from 0.07 to 0.09) and 50 Hz (0.07 to 0.23) trains. By P4, mAChR activation increased CV above control conditions for 5 Hz (from 0.14 to 0.80) and 50 Hz (from 0.64 to 0.75). In addition, failures appeared at P4 under conditions of mAChR activation (Fig. 3A, black). As a population, mAChR activation increased CV for the initial P1 (from 0.20 to 0.21) and failures during 5-Hz trains (from 0.04 to 0.49). As a population, mAChR activation increased CV above control conditions for 5 Hz (from 0.20 ± 0.03 to 0.63 ± 0.15; P = 0.009; n = 9) and at steady state, relative to control conditions (from 0.66 ± 0.06 to 0.93 ± 0.10; P = 0.004; n = 9; Fig. 3D, red). As a population, mAChR activation increased the cumulative fraction of failures during 5-Hz trains (from 0.058 ± 0.026 to 0.21 ± 0.07; P = 0.009; n = 9; Fig. 3E, red) and 50-Hz trains (from 0.095 ± 0.029 to 0.25 ± 0.058; P = 0.002; n = 10; Fig. 3F, red). However, in the presence of mAChR activation, the cumulative fraction of failures were not significantly different for 5- and 50-Hz trains (P = 0.15; n = 9), suggesting that the mAChR effect on failures is occluded by the frequency effect on MPD.

VM analysis reveals that CA1 PV BC-PC synapses possess multiple release sites and high p_r. To obtain quantal parameters for CA1 PV BC-PC transmission, we employed VM analysis (Fig. 4) (Silver et al. 1998; Reid and Clements 1999; Clements and Silver 2000). We varied p_r by evoking trains of 25 IPSCs at 5, 50, and 100 Hz. Means and variances for each of these conditions are plotted in Fig. 4A. P1 at 5 Hz (Fig. 4A, blue), 50 Hz (Fig. 4A, red), and 100 Hz (Fig. 4A, green) was observed to have low variance and high mean, consistent with each release event having high p_r. At 50 and 100 Hz, most subsequent p_r conditions were clustered in a region of low mean and low variance, suggesting that the synapse had rapidly achieved a depleted state dominated by synaptic failures (Fig. 4A). At 5 Hz, subsequent release conditions fell into zone with intermediate mean and relatively high variance, consistent with a broad range of IPSC amplitudes possible. After grand means.

Fig. 4. Variance-mean (VM) analysis reveals that CA1 PV BC-PC synapses possess multiple release sites and high p_r. The peak mean (M) and variance (V) was computed for each of the 25 pulses in each train in control (A) or 10 µM (B) muscarine conditions. In A and B, 25 points are shown for (blue) 5-Hz (red), 50-Hz, and (green) 100-Hz conditions, with each condition representing the M and V of 7 individual IPSCs. VM plots for control (C) and 10 µM (D) muscarine conditions, binned in 25- or 10-pA increments, respectively. In the presence of muscarine, the fit was linear. p_r for any mean (I) during the train was obtained by dividing by Nq, p_r functions for 5-Hz (E; n = 9), 50-Hz (F; n = 9), and 100-Hz (G; n = 3) trains; ○: control; •, 10 µM muscarine.
Table 1. Quantal parameters for 9 PV BC-PC pairs

<table>
<thead>
<tr>
<th>Pair No.</th>
<th>N</th>
<th>( p_i )</th>
<th>( q_{cont} ) pA</th>
<th>( I_{max} ) pA</th>
<th>( q_{inact} ) pA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6.7</td>
<td>0.60</td>
<td>57.4</td>
<td>382</td>
<td>65.3</td>
</tr>
<tr>
<td>2</td>
<td>5.9</td>
<td>1.04</td>
<td>37.9</td>
<td>222</td>
<td>31.2</td>
</tr>
<tr>
<td>3</td>
<td>12.7</td>
<td>1.03</td>
<td>26.8</td>
<td>340</td>
<td>26.9</td>
</tr>
<tr>
<td>4</td>
<td>11.2</td>
<td>0.76</td>
<td>30.5</td>
<td>342</td>
<td>29.0</td>
</tr>
<tr>
<td>5</td>
<td>10.6</td>
<td>0.98</td>
<td>24.5</td>
<td>260</td>
<td>24.7</td>
</tr>
<tr>
<td>6</td>
<td>10.6</td>
<td>0.92</td>
<td>49.7</td>
<td>527</td>
<td>57.4</td>
</tr>
<tr>
<td>7</td>
<td>3.3</td>
<td>0.97</td>
<td>21</td>
<td>70</td>
<td>19.5</td>
</tr>
<tr>
<td>8</td>
<td>14.1</td>
<td>0.59</td>
<td>12.5</td>
<td>176</td>
<td>13.6</td>
</tr>
<tr>
<td>9</td>
<td>38.4</td>
<td>0.65</td>
<td>43.5</td>
<td>1670</td>
<td>37.6</td>
</tr>
</tbody>
</table>

Means ± SE 12.6 ± 3.4 0.84 ± 0.06 33.8 ± 4.8 443 ± 159 33.9 ± 5.7

PV BC, parvalbumin-positive basket cell; PC, pyramidal cell; N, number of independent functional release sites; \( p_i \), release probability; \( q_{cont} \), average quantal control amplitude; \( I_{max} \), maximum mean (N\(^q\)); \( q_{inact} \), average quantal amplitude in muscarine.

and variances were obtained from 25-pA bins, quantal parameters were obtained from Eq. 1 (see MATERIALS AND METHODS). For this synaptic connection, \( N = 11, p_i = 0.98, \) and \( q = 24.5 \) pA. These quantal parameters are consistent with earlier work from PV BC-PC synapses in rat CA1 (Buhl et al. 1994).

A similar VM analysis was performed under conditions of 10 \( \mu \)M muscarine (Fig. 4, C and D). In this case, variances and means for all release conditions fell along a linear continuum, consistent with the notion that mAChR activation reduced \( p_i \) such that a high mean, low variance condition was not achieved. The linear slope of the VM plot indicated a \( q \) of 24.7, comparable to that achieved under control conditions, suggesting that mAChR activation did not alter \( q \). This observation is consistent with earlier data that indicate that mAChR activation induces a reduction in IPSC amplitude through presynaptic mechanisms (Fig. 3) (Hefft et al. 2002).

In a population of nine recordings from synaptically connected CA1 PV BC-PC pairs, VM analysis yielded 3–38 active release sites, maximum \( p_i \) of 0.84 ± 0.06, and an average quantal amplitude of 33.8 ± 4.8 pA (Table 1). The quantal amplitude obtained under 10 \( \mu \)M muscarine conditions (33.9 ± 5.7 pA) was not significantly different than that obtained under control conditions (\( P = 0.92 \)). \( p_i \) for each condition, obtained by dividing the IPSC amplitude in the train by \( N_q \), was then calculated during 5 Hz (Fig. 4E), 50 Hz (Fig. 4F), and 100 Hz (Fig. 4G) trains. These \( p_i \) functions obtained under both control and muscarine conditions were utilized as the basis of the kinetic model.

Kinetic modeling indicates that the frequency dependence of synaptic depression requires CDR. Starting with a kinetic model of STP that unified short-term facilitation and depression (Dittman and Regehr 1998; Lee et al. 2009), we developed a model at PV BC-PC synapses that reproduced the frequency dependence of \( p_i \) functions at 5, 50, and 100 Hz under both control conditions and in the presence of mAChR activation (Stone et al. 2014). The parameters are listed in Table 2. An illustration of how the underlying variables in the model dynamically change during 50-Hz trains in both control (blue) and muscarine (red) conditions is shown in Fig. 5. Consistent with the PV BC synapse being a depressing synapse, the calcium transient is very brief (Fig. 5A, blue), prohibiting the accumulation of calcium even at very short intervals of stimulation. A fast rate of decay without appreciable calcium accumulation is consistent with the general observation of PPD (Galarreta and Hestrin 1998; Maccarelli et al. 2000) and MPD at PV synapses (Kraushaar and Jonas 2000; Bartos et al. 2001; Hefft et al. 2002). This rise in calcium results in a transient elevation in \( p_i \) (Fig. 5B, blue), inducing massive vesicular depletion of all but 15% of the fraction of the RRP (Fig. 5C, blue). In the presence of calcium, the rate of replenishment of the RRP is rapid and then decays to the minimal rate. However, full recovery cannot occur before the P2 stimulus causes another incidence of release even at 5 Hz. After four stimuli, an equilibrium is established between the rate of release and replenishment in which the synaptic response asymptotes at ~22% of the initial amplitude.

The kinetic model explains how mAChR activation reduces PPD without disrupting MPD. The model predicts that the effect of mAChR activation can be described by an 80% reduction in peak calcium concentration (Fig. 5A, red). The consequence is a twofold reduction in initial \( p_i \) (Fig. 5B, red).

The RRP is protected from depletion in the short term, creating the appearance that PPD is transiently relieved relative to the strong PPD observed under control conditions. However, the decay of the calcium transient is unaltered with mAChR activation (Fig. 4A, red; Table 2). Therefore, the underlying mechanism cannot be described as mAChR activation “unmasking” of PPF or multiple-pulse facilitation. mAChR-induced reduction in calcium entry reduces both initial \( p_i \) and the rate of calcium-dependent recovery from depression, which causes the equilibrium value at steady state to be lower than under control conditions (Fig. 5D).

**DISCUSSION**

In this study, employing paired whole cell recordings in mouse CA1 hippocampus, we examined the frequency dependence and mAChR modulation of inhibitory synaptic transmission between PV BCs and PCs. We gained insights into the underlying mechanisms through VM analysis and kinetic modeling.

**Classical depletion of the RRP explains both PPD and MPD at CA1 PV BC synapses.** We found that PV BCs exhibited PPD (Fig. 1) and MPD (Fig. 2), consistent with previous work in CA1 (Maccarelli et al. 2000), CA3 (Szabó et al. 2010), and DG (Kraushaar and Jonas 2000; Bartos et al. 2001; Hefft et al. 2002; Hefft and Jonas 2005). GABA release from fast-spiking, presumably PV, interneurons in the cortex also exhibit synaptic depression (Galarreta and Hestrin 1998; Gupta et al. 2000; Reyes 2011; Ma et al. 2012; Pafundo et al. 2013). The wide...

Table 2. Parameters for the kinetic model of CA1 PV BC-PC transmission

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>Means ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \Delta )</td>
<td>Relative increase in calcium concentration from the base value</td>
<td>0.53 ± 0.01</td>
</tr>
<tr>
<td>( p_{max} )</td>
<td>Maximum ( p_i )</td>
<td>From Fig. 4, E–G</td>
</tr>
<tr>
<td>( K )</td>
<td>Half calcium concentration value for ( p_i ) function</td>
<td>0.67 ± 0.01</td>
</tr>
<tr>
<td>( k_{min} )</td>
<td>Minimum rate of recovery of release sites</td>
<td>0.0013 ± 0.00011</td>
</tr>
<tr>
<td>( \Delta [Ca] / K )</td>
<td>Absolute difference between ( k_{max} ) and ( k_{min} ) divided by the half calcium concentration value for rate of recovery function</td>
<td>0.057 ± 0.009</td>
</tr>
<tr>
<td>( \tau_{C_s} )</td>
<td>Decay constant for calcium</td>
<td>4.0 ± 0.5</td>
</tr>
</tbody>
</table>
spread exhibition of PPD and MPD at PV synapses across cortex and hippocampus implies that synapse-specific principles govern STP at inhibitory synapses. In accordance with a presynaptic mechanism, we observed that PPD and MPD were associated with an increase in the CV of IPSC amplitudes and the fraction of failures (Fig. 3). Presynaptic mechanisms of PPD and MPD are consistent with previous studies in DG PV BCs (Kraushaar and Jonas 2000; Bartos et al. 2001; Hefft et al. 2002) and at other GABAergic synapses (Wilcox and Dichter 1994).

VM analysis (Fig. 4; Table 1) yielded values for the estimated number of release sites of PV synapses onto principal cells that was strikingly similar to anatomical PV terminal counts in rat CA1 (Buhl et al. 1994) \( n = 10 - 12 \) and electrophysiologically derived counts in rat DG (Kraushaar and Jonas 2000) \( n = 3 - 8 \). Using a kinetic model that unified synaptic depression, facilitation, and CDR (Stone et al. 2014), we found that both PPD and MPD could be explained by a classic mechanism of depletion from the RRP. Although Kraushaar and Jonas (2000) found that even though P2 increased CV relative to P1, consistent with a presynaptic mechanism, there was no correlation between P1 and P2. While this discrepancy might be explained by the confounding presence of spurious PPF (Kim and Alger 2001), a failure of P1 to achieve a sufficiently high \( p_r \), or other intrinsic differences in PV BC synapses between DG and CA1, classic vesicular depletion was sufficient to explain PPD and MPD at CA1 PV BC-PC synapses. Moreover, fixed rates of vesicle depletion and replenishment could not describe the frequency dependence of both PPD and MPD; it was only when both depletion and CDR processes were incorporated that the model was able to fully capture the frequency dependence of PPD and MPD (Fig. 5). Thus this study shows that CDR, a mechanism that previously has been considered only in the context of glutameric synapses (Dittman and Regehr 1998; Dittman et al. 2000), also can be applied to GABAergic synapses.

Given the short calcium transient and high \( p_r \) at these synapses (Eggermann et al. 2012), it is unlikely that calcium accumulates appreciably to induce short-term facilitation even at high (100 Hz) frequencies (Fig. 5). However, Markov chain Monte Carlo sensitivity analysis predicts that an exceedingly short calcium transient, as predicted from the opening of presynaptic calcium channels during APs (Bucurenciu et al. 2009; Eggermann et al. 2012), does not adequately describe the kinetic model (Table 2), suggesting that a slower calcium transient may be needed to optimize CDR (Fig. 5 C). The frequency dependence of MPD depends exclusively on depletion and CDR. Although our model cannot differentiate between the classical depletion mechanism and alternative mechanisms of refractoriness, such as inactivation of presynaptic calcium channels or desensitization of the calcium sensor, a simple depletion mechanism adequately describes PPD and MPD at CA1 PV BC synapses. By contrast, previous studies at DG BC-GC synapses found that PPD and MPD remained intact in the presence of reduced external calcium or during pharmacological block of presynaptic calcium channels with cadmium (Kraushaar and Jonas 2000; Hefft et al. 2002). There are likely many factors that contribute to the calcium sensitivity of PPD and MPD, including the initial \( p_r \), density and spatial localization of presynaptic calcium channels, as well as other calcium-sensitive proteins in the presynaptic terminal that regulate both release and synaptic vesicle recycling. To fully elucidate the specific molecular mechanisms by which presynaptic calcium dynamics control PPD and MPD at CA1 PV BC and other synapses, future experiments that manipulate calcium entry and/or genetically ablate specific presynaptic calcium binding proteins are necessary. Finally, a multicompartment model of the presynaptic terminal may be required to

![Fig. 5. A kinetic model explains MPD and mAChR activation at CA1 PV BC-PC synapses.](http://jn.physiology.org/)

A: model output for a train of 10 pulses at 50 Hz in (blue) control conditions or (red) after mAChR activation. A: Ca concentration ([Ca]). B: \( p_r \). C: R. D: \( P^*R \) functions are illustrated.
fully capture the spatiotemporal dimensions of the presynaptic calcium transient and CDR processes.

Presynaptic modulation by mAChRs at CA1 PV BC-PC synapses limits depletion of the RRP but does not "unmask" short-term facilitation. In addition to elucidating mechanisms of PPD and MPD, we also examined mAChR-induced modulation at CA1 PV BC-PC synapses. Similar to other studies in DG (Hefft et al. 2002), CA3 (Szabó et al. 2010), prefrontal cortex (Pafundo et al. 2013), and neocortex (Kruglikov and Rudy 2008), we observed that mAChR activation inhibited IPSCs from PV interneurons. The mechanism is consistent with the activation of presynaptic G_{i} -coupled M2 mAChRs (Wess et al. 2007) on PV terminals (Hajos et al. 1998; Fukudome et al. 2004). However, in a previous study, a subset of PV BCs contained mRNA transcript for the M4 mAChR (Cea-del Rio et al. 2010), suggesting that M4 mAChRs may also be expressed on PV BC terminals. A resolution to the pharmacology awaits the availability of M4 mAChR-specific antibodies (Jositsch et al. 2009) and/or recordings from M2/M4 double transgenic mAChR knockout mice (Wess et al. 2007).

Consistent with a presynaptic mechanism (Gonzalez et al. 2013), mAChR activation was associated with an increase in CV, an increase in the fraction of failures (Fig. 4), and linearization of the VM plot (Fig. 5). Moreover, the average q, as extracted from VM analysis, did not change in the presence of mAChR activation (Table 1). Although postsynaptic mAChR activation does induce an inward current in principal cells (Fisahn et al. 2002), postsynaptic mAChRs and GABA_{A} receptors do not appear to interact (Kruglikov and Rudy 2008). These observations converge on a mAChR-induced reduction in p_{r}. A similar mechanism has been proposed at PV BC synapses in DG (Hefft et al. 2002) and CA3 (Szabó et al. 2010), yet mAChR activation reduces PPD and MPD at CA3 synapses but not DG synapses.

Regarding the underlying mechanism at CA1 PV BC synapses, PPD and MPD during mAChR activation could be explained in the kinetic model by an 80% reduction in peak calcium concentration (with no change in the decay of the calcium transient) and twofold reduction in p_{r} (Fig. 5). Although not shown specifically at PV BC synaptic boutons, this prediction was recently substantiated at PV septohippocampal synapses by observing a reduction in the peak calcium transient upon administration of the GABA_{B} receptor agonist baclofen (Kaifosh et al. 2013). Therefore, it seems unlikely that mAChR activation would unmask a classical short-term facilitation mechanism by promoting the accumulation of calcium. The presynaptic mechanism simply preserves the RRP through decreased depletion. This mechanism is consistent with the capacity of mAChRs to inhibit P/Q-type calcium currents (Hefft and Jonas 2005) through a G_{i, o}-coupled presynaptic mechanism (Hefft et al. 2002; Gonzalez et al. 2013). However, a presynaptic contribution of G-protein coupled Kir3 channels is possible (Bartos et al. 2007).

Regarding how mAChR activation impacts CDR, one study in cell culture suggested that mAChR activation accelerates recovery from depression (Gonzalez et al. 2013). However, this finding is inconsistent with our model; since CDR is required for PPD and MPD, reduced calcium entry via mAChR activation would be expected to reduce CDR. Indeed, we find that for the mAChR conditions, the model adequately fitted MPD at three different frequencies without altering the kinetics of CDR. The mAChR-induced reduction in both p_{r} and CDR is apparent especially at gamma frequencies, in which reduced p_{r} reduces depletion, yet is counterbalanced by reduced CDR. These opposing forces act to reduce PPD yet maintain MPD, stabilizing IPSCs at lower steady-state values than in control conditions (Fig. 5D).

Potential roles of presynaptic mAChR modulation of PV BCs during information processing, network oscillations, and hippocampal learning. Within the hippocampus, repetitive activation of the Schaeffer collaterals induces MPD onto PV BCs (Losonczy et al. 2002; Pouille and Scanziani 2004). In turn, PV BCs exhibit synaptic depression onto the perisomatic regions of CA1 PCs (Maccarelli et al. 2000). The result of short-term depression at glutamatergic and GABAergic synapses results in PV BCs primarily encoding the onset of a train of synaptic stimuli (Pouille and Scanziani 2004). Therefore, PV BCs are specialized to encode timing information whereas other interneurons that exhibit short-term facilitation of excitatory potentials, such as O-LM cells, participate in encoding rate. This leads to a dynamic redistribution of synaptic weights from perisomatic to dendritic regions during repetitive stimulation of glutamate afferents (Pouille and Scanziani 2004). These observations reinforce the concept that there are rules governing STP at both excitatory and inhibitory synapses that are important for information processing (Reyes et al. 1998). Indeed, molecular determinants have now been identified that could potentially explain cell type-specific STP at excitatory synapses (Sylwestrak and Ghosh 2012). Inhibitory synapses may utilize similar molecular determinants; genetic manipulation of STP could yield insights into the role of synaptic depression at PV BC synapses during feedforward inhibition (Kruglikov and Rudy 2008) and the modulation of gain control (Rothman et al. 2009).

Through our experimental conditions in vitro, we are able to examine p_{r} across a range of firing frequencies both in control and muscarine conditions. However, this in vitro experimental design maximizes IPSC amplitude by beginning with the maximum RRP size, a condition that is unlikely to occur in vivo (Sik et al. 1995). PV BCs are spontaneously active (Sik et al. 1995), especially during theta and gamma oscillations (Klausberger et al. 2003; Tukker et al. 2007; Varga et al. 2012). During 5-Hz trains, PV BCs exhibit a wide range of IPSC amplitudes that are associated with a large variance (Fig. 4). mAChR modulation of PV BCs occurs both presynaptically and postsynaptically (Cea-del Rio et al. 2010; Yi et al. 2014). Presynaptic mAChR activation reduces p_{r}, thereby normalizing IPSC amplitude and reducing depletion of the RRP (Fig. 5). Simultaneously, postsynaptic mAChRs depolarize PV BCs (Cea-del Rio et al. 2010; Szabó et al. 2010; Yi et al. 2014), leading to increased spontaneous AP frequency, threshold to spike, and use-dependent MPD of PV BC output. Taken together, presynaptic and postsynaptic mAChR modulation of PV BCs likely serves to promote the emergence of coherent gamma oscillations. This hypothesis could be tested by the implementation of the kinetic model into CA1 network models.

At a behavioral level, CA1 PV cells have been shown to be involved in working memory (Murray et al. 2011). Therefore, it is likely that perisomatic inhibition from PV BCs is important for this form of memory. Interestingly, M2 mAChR knockout mice exhibit working memory deficits (Seeger et al.
2004). Consistent with a prominent role of M2 mAChRs in modulation of PV cells (Hajós et al. 1998), M2 mAChR activation had larger effects on evoked IPSCs than EPSCs (Seeger et al. 2004). Therefore, it is possible that the lack of M2 mAChR modulation of PV BCs contributes to behavioral impairments in learning observed in M2 KO mice. This behavioral deficit is striking because it occurs despite a compensatory increase in ACh release from the elimination of M2 mAChR-mediated feedback inhibition on cholinergic terminals (Tzavara et al. 2003). Future experimental and modeling work will be able to determine the exact role of presynaptic cholinergic neuromodulation of CA1 PV BC synapses in learning and memory.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


REFERENCES


