Receptor saturation controls short-term synaptic plasticity at corticothalamic synapses

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Sun Y-G, Beierlein M. Receptor saturation controls short-term synaptic plasticity at corticothalamic synapses. J Neurophysiol 105: 2319–2329, 2011. First published February 16, 2011; doi:10.1152/jn.00942.2010.—Glutamatergic synapses of layer 6 corticothalamic (CT) neurons form a major excitatory input onto thalamic relay cells, allowing neocortex to continuously control sensory information processing in thalamic circuits. CT synapses display both short- and long-term forms of use-dependent synaptic enhancement, mediated at least in part by increases in the probability of transmitter release. At some synapses, such increases in release probability are accompanied by a higher degree of multivesicular release (MVR) and larger glutamate transients at individual release sites, resulting in the saturation of postsynaptic receptors. The extent to which MVR and postsynaptic saturation interact and control short-term plasticity at CT synapses is not known. Here we examined two distinct presynaptic forms of short-term enhancement, facilitation and augmentation, at CT synapses contacting relay neurons in the ventrobasal nucleus of the mouse thalamus. We found that, in the presence of the low-affinity antagonist y-aminobutyric acid (GABA) receptor saturation, the magnitude of facilitation and augmentation increased. Whereas receptor saturation was prominent for both AMPA and N-methyl-D-aspartate receptors, desensitization of AMPA receptors did not significantly alter short-term plasticity. Our results suggest that at CT synapses the activity-dependent increase in synaptic strength is controlled by postsynaptic receptor saturation.

desensitization; facilitation; receptor occupancy; low-affinity antagonist; augmentation

SYNAPSES IN THE MAMMALIAN BRAIN undergo activity-dependent changes in strength over short time scales, lasting from milliseconds to tens of seconds (Magleby 1979; Zucker and Regehr 2002). Both pre- and postsynaptic mechanisms underlie short-term plasticity (Blitz et al. 2004). Changes in the amount of transmitter released can be mediated by changes in release probability P. In turn, changes in P can be accompanied by changes in the number of simultaneously released vesicles at a given release site, resulting in changes in transmitter concentration in the synaptic cleft (Bender et al. 2002; Foster et al. 2005; Foster et al. 2002; Higley et al. 2009; Li et al. 2009; Oertner et al. 2002; Singer et al. 2004; Wadiche and Jahr 2001). These forms of presynaptic plasticity can interact with the properties of postsynaptic receptors, such as receptor desensitization (Jones and Westbrook 1996; Trussell et al. 1988) or saturation (Foster et al. 2002; Harrison and Jahr 2003; Tong and Jahr 1994; Wadiche and Jahr 2001). Short-term plasticity determines how a neuron activates its postsynaptic targets and therefore enriches information processing in neuronal circuits (Abbott and Regehr 2004; Dittman et al. 2000).

In sensory thalamic nuclei such as the lateral geniculate nucleus or the ventrobasal nucleus, neurons receive two types of glutamatergic inputs with dramatically different properties. Sensory afferents elicit large-amplitude, reliable postsynaptic responses, which display short-term depression (Castro-Alamancos 2002; Chen and Regehr 2000). On a given neuron, sensory synapses are greatly outnumbered by corticothalamic (CT) feedback projections formed by neurons in cortical layer 6 (Deschenes et al. 1998; Jones 2009). The role of CT feedback for sensory processing is not well understood. Growing evidence suggests that CT inputs, rather than simply modulating excitability of thalamic networks, can rapidly and precisely control the gain of sensory-evoked responses in individual thalamic neurons (Briggs and Usrey 2007; Temereanca and Simons 2004), implying that information transfer at CT synapses is highly reliable. However, our understanding of the cellular mechanisms mediating CT synaptic transmission remains incomplete.

CT synapses display various forms of short- and long-term enhancement (Castro-Alamancos and Calcagnotto 1999; Granseth et al. 2002; Scharfman et al. 1990; Turner and Salt 1998), mediated in part by an increase in release probability. The extent to which postsynaptic receptor saturation can control short-term enhancement is not known. Saturation could occur if the concentration of synaptically released glutamate increases along with changes in release probability. For a number of central nervous system synapses, such use-dependent changes in transmitter transients have been attributed to the release of multiple vesicles at individual release sites (Christie and Jahr 2006; Foster et al. 2005; Foster et al. 2002; Higley et al. 2009; Oertner et al. 2002; Tong and Jahr 1994; Wadiche and Jahr 2001).

Here we examine this issue for CT synapses contacting relay cells in the ventrobasal nucleus of the thalamus using the low-affinity DL-α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptor (AMPAR) antagonist y-d-glutamylglycine (DGG) to relieve receptor saturation. We find that the magnitude of two distinct forms of short-term enhancement, facilitation and augmentation, is controlled by receptor desensitization (Jones and Connor 1991). Briefly, C57 BL/6 mice (P13-P17) were anesthetized with isoflurane and decapitated. Slices were cut in ice-cold, oxygenated solution containing (in mM) 111 Choline Cl, 2.5 KCl, 1.25 NaH2PO4, 10 MgSO4, 26
NaHCO$_3$, 10 glucose, and 0.5 CaCl$_2$, saturated with 95% O$_2$, 5% CO$_2$, using a vibratome (Leica VT1200S, Leica, Wetzlar, Germany). Slices were incubated at 34°C for 40 min in saline solution containing (in mM) 126 NaCl, 2.6 NaHCO$_3$, 2.5 KCl, 1.25 NaH$_2$PO$_4$, 10 glucose, 2 CaCl$_2$, and 2 MgCl$_2$, and then held at room temperature prior to recordings. All procedures were conducted according to established guidelines, approved by the UTHSC-Houston Animal Welfare Committee.

**Electrophysiology.** Slices were trimmed to remove neocortex, placed on glass coverslips coated with poly-t-lysine (Sigma, St. Louis, MO), and submerged in a recording chamber (Warner Instruments, Hamden, CT). All recordings were performed at near-physiological temperatures (32–34°C), unless stated otherwise, using an in-line heater (Warner Instruments) while perfusing the recording chamber with solution at 3–4 ml/min using a Minipulse 3 pump (Gilson, Middleton, WI). Whole cell recordings from relay cells in the ventrobasal nucleus of the thalamus were obtained using infrared differential interference contrast (IR-DIC) visualization using an Olympus BX51WI microscope (Olympus Optical, Tokyo, Japan) and a CCD camera (Hamamatsu, Hamamatsu City, Japan). Recording pipettes (2–3 MΩ) contained (in mM): 120 CsMeSO$_4$, 11 CsCl, 10 HEPES, 11 EGTA, 1 MgCl$_2$, 1 CaCl$_2$, 2 Mg-ATP, 0.3 Na-GTP, and 1 QX-314, adjusted to 295 mOsM and pH 7.3. Cells were held in voltage clamp at −70 mV using a Multiclamp 700B amplifier (Molecular Devices, Sunnyvale, CA), at access resistances of 8–15 MΩ. Access resistance and leak current were continually monitored, and experiments were discarded if either changed significantly. CT axons were stimulated with brief pulses (200 μs, 10–100 μA) using platinum/iridium electrodes (Frederick Haer, Bowdoin, ME) or glass electrodes filled with artificial cerebrospinal fluid placed in the internal capsule. To eliminate disinvasive inhibitory inputs mediated by neurons in the reticular nucleus, picrotoxin (50 μM) and CGP55845 (5 μM) were added to the bathing solution to block GABA$_A$ and GABA$_B$ receptors, respectively. AMPAR-mediated excitatory postsynaptic currents (EPSCs) were recorded, including 3-((R)-2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid (R-CPP; 5 μM) in the bath to block N-methyl-D-aspartate (NMDA) receptors (NMDAR).

In some cases, NMDAR EPSCs were recorded at a holding potential of +40 mV, in the presence of 2.3 dioxyo-6-nitro-1,2,3,4-tetrahydrobenzo (f)quinoxaline-7-sulfonamide (NBQX; 5 μM). All drugs were purchased from Tocris (Ellisville, MO).

**Data acquisition and analysis.** Recordings were filtered at 2–4 kHz and digitized at 20 kHz with a 16-bit analog-to-digital converter (Digidata 1440A; Molecular Devices). Data were acquired using pClamp software (Molecular Devices). Data analysis was performed with custom macros written in Igor Pro (WaveMetrics, Lake Oswego, OR). Statistical tests were performed with unpaired or paired Student’s t-test. Differences are considered to be significant at P < 0.05. Data are expressed as means ± SE.

**RESULTS**

**Postsynaptic saturation of AMPARs at CT synapses.** To study short-term plasticity at CT synapses, we obtained whole-cell recordings from relay neurons in the ventrobasal nucleus of the thalamus and activated synaptic inputs formed by layer 6 CT neurons, using a stimulus electrode placed in the internal capsule. CT neurons contact the distal dendrites of relay cells via small (<1 μm in diameter) terminals that contain a single vesicle release site (Bourassa et al. 1995; Graziano et al. 2008). When activated repetitively, CT synapses display short-term facilitation (Castro-Alamancos and Calcagnotto 1999; Granseth et al. 2002; McCormick and von Krosigk 1992; Reichova and Sherman 2004; Scharfman et al. 1990; Turner and Salt 1998), mediated at least in part by an increase in release probability P. At facilitating synapses in the cerebellum and hippocampus, such activity-dependent increases in P are accompanied by enhanced glutamate transients at individual release sites (Christie and Jahr 2006; Foster et al. 2005; Oertner et al. 2002), most likely due to a higher likelihood of MVR. In turn, MVR can result in postsynaptic AMPAR saturation. Here we define saturation as the sublinear increase in receptor occupancy and EPSC amplitude in response to increases in glutamate concentration (Foster et al. 2005). Importantly, under this terminology, receptor occupancy does not need to be close to 100% to observe saturation. To test whether short-term facilitation leads to postsynaptic AMPAR saturation at CT synapses, we measured AMPAR-mediated responses evoked by paired pulses, before and following bath application of the low-affinity competitive AMPAR antagonist DGG (2 mM). Because of its low affinity, DGG rapidly unbinds from postsynaptic AMPARs and effectively competes with synthetically released glutamate, thereby preventing receptor saturation (Chanda and Xu-Friedman 2010; Foster et al. 2005; Foster et al. 2002; Liu et al. 1999; Wadiche and Jahr 2001). The blockade of AMPARs by DGG largely depends on the glutamate concentration in the synaptic cleft, with the blockade being less effective for higher synaptic glutamate concentrations. Therefore, if postsynaptic saturation is prominent during paired-pulse facilitation, DGG should lead to a smaller reduction in amplitude of EPSC$_2$. Consistent with this hypothesis, we found that DGG application reduced the amplitude of both EPSCs, with the percentage block of EPSC$_2$ being significantly less than the block of EPSC$_1$, resulting in an increase in paired-pulse ratio (Fig. 1, A and B). On average, EPSC$_1$ was reduced to 25.1 ± 1.6%, and EPSC$_2$ was reduced to 29.0 ± 1.7% in 2 mM external calcium (Ca$_2+$, Fig. 4B), compared with control. Paired-pulse ratio increased from 2.15 ± 0.15 to 2.54 ± 0.15 (Fig. 1E, 20 Hz, P < 0.01, n = 9). These data indicate that, during paired-pulse facilitation, postsynaptic receptor saturation is prominent at CT synapses. To exclude possible artifacts associated with poor voltage control, we repeated these experiments using the noncompetitive AMPAR antagonist NBQX (Fig. 1C). Because NBQX has much slower dissociation kinetics compared with DGG, glutamate does not compete with NBQX for binding at AMPARs. NBQX (400 mM) reduced the EPSC amplitude to a comparable level as 2 mM of DGG (Fig. 1, A and C, 25.1 ± 1.6% and 21.5 ± 2.6% in DGG and NBQX, respectively, P = 0.24, n = 6–9). In contrast to the actions of DGG, NBQX blocked EPSC$_1$ and EPSC$_2$ to a similar degree (Fig. 1, D and F) and therefore did not significantly change paired-pulse ratio (2.10 ± 0.10 and 2.10 ± 0.13 in control and NBQX, respectively; Fig. 1F, n = 6).

Taken together, these data show that the concentration of synthetically released glutamate increases in an activity-dependent manner, resulting in more pronounced postsynaptic receptor saturation.

Glutamate spillover does not contribute to changes in glutamate concentration at CT synapses. Increases in synaptic glutamate concentration associated with increases in release probability are most likely mediated by the simultaneous release of multiple vesicles from individual release sites (Bender et al. 2009; Christie and Jahr 2006; Foster et al. 2002). However, in principle such increases could also occur following the spillover and pooling of glutamate from closely spaced activated synapses (Carter and Regehr 2000; Marcaggi and...
with an increase in paired-pulse ratio (2.10 ± 0.14 and 2.40 ± 0.14 in control and TBOA, respectively, \( P < 0.05, n = 6 \)), suggesting that partially blocking glutamate uptake leads to an increase in extracellular glutamate levels and the constitutive activation of metabotropic glutamate receptors, which are known to be expressed at CT synaptic terminals (Alexander and Godwin 2005). Consistent with this idea, TBOA application did not change either amplitude (97.5 ± 5.2% of control, \( P = 0.47, n = 8 \)) or paired-pulse ratio (1.94 ± 0.09 and 1.92 ± 0.10 in control and TBOA, respectively, \( P = 0.41, n = 8 \)), in experiments performed in the presence of the broad-spectrum metabotropic glutamate receptor antagonist LY341495 (10 \( \mu M \), Fig. 2, C and D). In the presence of LY341495, TBOA significantly increased the charge transfer during the decay

Fig. 1. \( \gamma \)-Glutamylglycine (DGG) increases paired-pulse (PP) ratio of corticothalamic (CT) excitatory postsynaptic currents (EPSCs) by blocking receptor saturation. A and C: \( \alpha \)-amino-3-hydroxy-5-methylisoxazole-propionic acid (AMPA) receptor (AMPAR) EPSCs were evoked with paired stimuli (\( \Delta t = 50 \) ms) every 12 s, and the effects of bath application of either 2 mM DGG (A) or 400 nM 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[f]quinoxaline-7-sulfonamide (NBQX) (C) on EPSC amplitude and paired-pulse ratio are plotted as a function of time. B and D: top: EPSCs (average of 20–30 individual trials) elicited by paired stimuli, in control (gray) and following application of DGG (2 mM, black, B), or NBQX (400 nM, black, D). Bottom: same responses as shown above, normalized to the peak of EPSCs. E and F: summary data show paired-pulse ratio for each cell (\( * \)) in control and following application of DGG (E) or NBQX (F). Mean values are shown (\( * \)). **\( P < 0.01, n = 9 \), paired Student’s t-test for DGG (E), \( P = 0.93, n = 6 \), paired Student’s t-test for NBQX (F). Data are expressed as means ± SE.

Attwell 2005). To directly examine this possibility at CT synapses, we performed experiments using low concentrations of the glutamate transporter blocker threo-\( \alpha \)-benzyloxyaspartate (TBOA) to partially block glutamate uptake into astrocytes (Christie and Jahr 2006). If glutamate spillover and pooling of glutamate contributes to an increase in glutamate transients during paired-pulse facilitation at this synapse, enhanced spillover following TBOA application should lead to a further increase in paired-pulse ratio when saturation is eliminated with DGG.

We first tested the effectiveness of subsaturating concentrations of TBOA (10 \( \mu M \)) at CT synapses (Fig. 2, A and B). Bath application of TBOA led to a consistent decrease in CT EPSC amplitude (68.8 ± 3.4% of control, \( P < 0.05, n = 6 \)), along
phase of EPSC$_2$ ($236.8 \pm 25.7\%$ of control, $P < 0.001, n = 8$), a result consistent with a temporal increase of the evoked synaptic glutamate transient (Fig. 2, C and D). However, with presynaptic metabotropic glutamate receptors blocked, in the presence of DGG, application of TBOA had no effect on paired-pulse ratio ($2.34 \pm 0.20$ and $2.32 \pm 0.21$ in control and TBOA, respectively, $P = 0.68, n = 5$, Fig. 2, E and F). Therefore, any possible increases in the degree of spillover at CT synapses do not appear to lead to significant increases of peak glutamate levels, leaving the effectiveness of the DGG block of the EPSC unchanged.

**Short-term plasticity at CT synapses is not influenced by AMPAR desensitization.** Upon binding of synaptically released glutamate, AMPARs can undergo rapid desensitization (Edmonds et al. 1995; Trussell et al. 1988). The comparatively slow recovery from desensitization can significantly influence synaptic plasticity during repetitive activity (Chen et al. 2002; Rozov et al. 2001; Trussell et al. 1993). The extent to which AMPAR desensitization contributes to synaptic plasticity at CT synapses is not known. Several studies have shown that DGG, because of its rapid dissociation kinetics, can keep a fraction of synaptic AMPARs in a blocked state, thereby protecting them from desensitization (Chanda and Xu-Friedman 2010; Crowley et al. 2009; Wong et al. 2003). At facilitating synapses, both postsynaptic desensitization and saturation would act to decrease the amount of paired-pulse facilitation. Thus, in principle, the DGG-induced increases in paired-pulse plasticity described above could be explained by a block of AMPAR desensitization, rather than saturation. To test whether desensitization contributes to short-term plasticity at CT synapses, we measured paired-pulse ratio before and following bath application of cyclothiazide (CTZ), a drug that prevents conformational changes in AMPARs that lead to desensitization (Partin et al. 1993; Yamada and Tang 1993). CTZ (50 $\mu$M) increased the amplitude and prolonged the decay of the AMPAR EPSC but did not change paired-pulse ratio at either 20 or 50 Hz ($2.07 \pm 0.11$ and $1.98 \pm 0.12$ at 20 Hz, $2.27 \pm 0.18$ and $2.25 \pm 0.13$ at 50 Hz, for control and CTZ, respectively, Fig. 3, A and B). The actions of CTZ strongly depend on the AMPAR subtype, with “flop” splice variants showing low sensitivity to a block of desensitization (Wong et al. 2003). To confirm that CTZ is effective at CT AMPARs, we repeated the experiments at room temperature, in an effort to improve the conditions for detecting desensitization. Indeed, CTZ significantly increased paired-pulse ratio at 50 Hz ($1.61 \pm 0.16$ in control vs. $1.85 \pm 0.16$ in CTZ, Fig. 3, C and D) under those conditions, confirming its effectiveness at CT AMPAR subtypes. In addition to blocking postsynaptic desensitization, CTZ can have nonspecific effects on presynaptic release at certain synapses (Bellingham and Walmsley 1999; Diamond and Jahr 1995; Ishikawa and Takahashi 2001), potentially complicating the interpretation of our experiments. To test for potential presynaptic effects of CTZ, we examined NMDAR EPSCs. Bath application of CTZ had no effect on the amplitude ($339.8 \pm 46.3$ pA in control vs. $346.0 \pm 57.6$ pA in CTZ) or the paired-pulse ratio ($1.63 \pm 0.09$ in control vs. $1.57 \pm 0.12$ in CTZ) of CT NMDAR EPSCs (Fig. 3, E and F), suggesting that, at the concentration used in our experiments, CTZ did not influence presynaptic release probability.

Taken together, these data suggest that, at near-physiological temperatures, AMPAR desensitization does not contribute to short-term plasticity at CT synapses, at paired-pulse frequencies of 50 Hz or lower. Instead, saturation is the sole postsynaptic mechanism controlling short-term plasticity.

**Saturation depends on release probability.** Our results so far indicate that the degree of postsynaptic saturation increases during paired-pulse facilitation, suggesting that the degree of postsynaptic saturation is closely tied to release probability. We further examined this issue, by comparing the effect of DGG on paired-pulse responses evoked in different C$_{ae}$ concentrations. We found that DGG reduced EPSC$_1$ amplitude to a greater extent in 1 mM C$_{ae}$, compared with 1.5, 2.0, and 3.0 mM C$_{ae}$ ($19.4 \pm 1.0\%$, $23.1 \pm 0.7\%$, $25.7 \pm 1.2\%$, and $26.1 \pm 1.3\%$ for 1, 1.5, 2.0, and 3.0 mM C$_{ae}$, respectively, Fig. 4B), indicating that saturation already exists for synaptic responses evoked by single stimuli, at physiological levels of C$_{ae}$ (1.5 mM). Additionally, in 1 mM C$_{ae}$, DGG inhibited EPSC$_1$ to the
same extent as EPSC₂, and, as a result, paired-pulse plasticity did not change under those conditions (Fig. 4, A and C). These data indicate that, under conditions of lowered release probability, paired-pulse facilitation reflects the recruitment of additional release sites, with little contribution of use-dependent increases in glutamate transients at a given site. Furthermore, the lack of change in paired-pulse plasticity in 1 mM Ca²⁺ following DGG application confirms that under our conditions this antagonist does not act nonspecifically to alter transmitter release. By contrast, for higher Ca²⁺ concentrations, DGG consistently blocked EPSC₁ to a larger extent compared with EPSC₂ suggesting that, for Ca²⁺ levels higher than 1 mM, release evoked by a second stimulus leads to larger glutamate transients and a higher degree of receptor saturation (Fig. 4B). As a consequence, paired-pulse plasticity significantly increased in DGG (Fig. 4, A and C). Interestingly, in the presence of DGG, paired-pulse ratios remained constant over the range of Ca²⁺ concentrations tested (Fig. 4C), suggesting that the differences in paired-pulse plasticity observed under control conditions are almost entirely attributable to postsynaptic receptor saturation.

**Postsynaptic saturation is prominent during stimulus trains.** Sustained activation of CT neurons in the γ-range (20–40 Hz) powerfully controls network activity in thalamic networks (Steriade 1997, 2004). To examine the role of postsynaptic saturation for more prolonged levels of synaptic activation, we stimulated CT inputs with brief trains (10 stimuli, 20 Hz) and measured AMPA EPSCs. In control conditions, such trains led to short-term facilitation, with a maximal enhancement at the end of the train (EPSC₈₋₁₀/EPSC₁ = 2.10 ± 0.13, n = 6). Following bath application of DGG (2 mM), short-term facilitation increased to 2.61 ± 0.18 (Fig. 5A), indicating that saturation is prominent during sustained levels of synaptic activation.

In addition to activity-dependent changes in P, transmitter release can be controlled by a number of neuromodulators that bind to presynaptic receptors, ultimately resulting in an increase, or more commonly, a decrease in P. At CT synapses, transmitter release is strongly attenuated by activation of group II metabotropic glutamate receptors (Alexander and Godwin 2005). We found that following bath application of a subsaturating concentration of the group II mGlur agonist 4-aminopyrrolidine-2,4-dicarboxylate (APDC) (10 μM), EPSC amplitude was reduced to 38.8 ± 3.0% of control, and short-term facilitation increased from 1.83 ± 0.16 to 2.71 ± 0.19 (EPSC₈₋₁₀/EPSC₁, n = 6, Fig. 5B).

Does postsynaptic receptor saturation play a role under conditions of reduced release probability? Our data above suggest that postsynaptic saturation following paired-pulse stimulation is not prominent when release probability is decreased by lowering Ca²⁺ concentration to 1 mM. Similarly, we found that, in the presence of 10 μM APDC, bath application of DGG (2 mM) only led to a small increase in paired-pulse ratio (2.00 ± 0.06 in APDC vs. 2.13 ± 0.04 in APDC + DGG, n = 7), indicating that presynaptic neuromodulators can regulate the degree of postsynaptic saturation. However, DGG application still led to a significant increase in short-term facilitation (EPSC₈₋₁₀/EPSC₁ = 2.52 ± 0.15 in APDC, 2.90 ±
0.12 in APDC + DGG, n = 7, Fig. 5, E and F), suggesting that, even under conditions of reduced release probability, sustained levels of presynaptic activity can lead to increases in P that are sufficient to cause postsynaptic receptor saturation. 

**Augmentation at CT synapses.** At many synapses, sustained, high-frequency synaptic activity can lead to presynaptic forms of synaptic enhancement such as augmentation or posttetanic potentiation (PTP), which last tens of seconds to minutes (Magleby 1979; Zucker and Regehr 2002). These forms of short-term enhancement are triggered by elevations in presynaptic calcium concentrations in the hundreds of nanomolar range (Delaney et al. 1989; Habets and Borst 2005; Korogod et al. 2005; Lee et al. 2008; Zucker and Regehr 2002), resulting in the activation of protein kinase C, the vesicle priming factor Munc13, and possibly other targets (Alle et al. 2001; Beierlein et al. 2007; Brager et al. 2003; Korogod et al. 2007; Lee et al. 2007; Wierda et al. 2007), ultimately leading to an increase in P. We characterized posttetanic short-term enhancement at CT synapses, by measuring AMPAR EPSCs before and following a stimulus train of 1-s duration, at 50 Hz. Facilitation at the end of the induction train (EPSC_{48-50}/EPSC_{1}) was 1.48 ± 0.14, compared with control (n = 6). As shown in Fig. 6A, initial EPSC amplitude following the train (∆t = 3 s after beginning of train) was 3.05 ± 0.07 and decayed to baseline level over ~60 s (τ_{Decay} = 26.0 ± 1.9 s). We found that varying the length of the induction train from 5 to 50 stimuli led to a progressive increase in the peak amplitude of the enhancement, whereas the time course of decay remained roughly constant (Fig. 6B). These properties characterize augmentation, a form of short-term enhancement originally described at the neuromuscular junction (Magleby and Zengel 1976). By contrast, PTP typically shows a progressive increase in the time course of decay with increases in the length of the induction train (Korogod et al. 2005; Magleby and Zengel 1975). In keeping with this terminology, we will refer to the train-induced enhancement at CT synapses as augmentation.

If augmentation is mediated presynaptically by an increase in release probability, paired-pulse plasticity is expected to be reduced following a stimulus burst, with a time course similar to the increase in synaptic strength. We found that paired-pulse ratio changed from 2.65 ± 0.23 under baseline conditions to 1.12 ± 0.07 (∆t = 3 s) following the train (Fig. 6C), indicating a presynaptic expression of augmentation. Phorbol esters potently enhance transmitter release at synapses (Malenka et al. 1986) (Fig. 6D). Application of the phorbol ester phorbol 12,13-dibutyrate (PDBu) (1 μM) led to an average increase in EPSC amplitude of 3.30 ± 0.98 compared with control and occluded augmentation (2.28 ± 0.15 in control vs. 1.01 ± 0.26 in PDBu, p < 0.001, Fig. 6, E and F), confirming a presynaptic expression.

**Postsynaptic saturation of both AMPARs and NMDARs during augmentation.** Having established the presynaptic expression of augmentation at CT synapses, we tested whether this form of synaptic enhancement is controlled by postsynaptic receptor saturation. In principle, augmentation could in large part be mediated via the recruitment of previously silent release sites. Thus glutamate transients at a given release site would not show any significant increases, and as a conse-
quence postsynaptic saturation would not be enhanced during augmentation. However, we found that, following bath application of DGG (2 mM), the amplitude of AMPAR augmentation was dramatically increased (2.65 ± 0.16 in control vs. 4.16 ± 0.30 in DGG, P < 0.01, n = 6, Fig. 7, A and D). This indicates that during augmentation a large fraction of release sites generate increased glutamate transients, resulting in stronger postsynaptic AMPAR saturation. We further examined the relative importance of saturation in controlling augmentation evoked by stimulus trains of different frequencies (Fig. 7B). In control conditions, augmentation was prominent following stimulus trains of as low as 5 Hz and its peak amplitude progressively increased for higher frequencies. Following the application of DGG, augmentation significantly increased at each frequency tested, but this increase was more pronounced at higher frequencies, demonstrating that postsynaptic saturation strongly limits the frequency dependence of augmentation.

High-frequency stimulus trains could lead to a partial saturation of glutamate uptake, resulting in prolonged synaptic glutamate transients and postsynaptic receptor desensitization. Thus the large increase in AMPAR augmentation following DGG application might be partly due to a block of desensitization. However, bath application of CTZ had no significant effect on the amplitude of AMPAR augmentation evoked by a 50-Hz stimulus train (2.65 ± 0.16 in control vs. 3.01 ± 0.17 in CTZ, P = 0.35, n = 5, Fig. 7D), suggesting that synaptic enhancement following the stimulus train is not significantly influenced by desensitization.

Thus far we have focused on the role of saturation in shaping AMPAR-mediated short-term plasticity. At CT synapses, glutamate release leads to activation of both AMPARs and NMDARs (Scharfman et al. 1990; Turner and Salt 1998). Assuming that NMDARs and AMPARs are distributed uniformly in the subsynaptic membrane, NMDARs should be similarly exposed to changes in glutamate transients associated with changes in P during augmentation. To test whether postsynaptic NMDARs experience saturation during augmentation, we pharmacologically isolated NMDAR EPSCs and measured NMDAR augmentation, by holding neurons at +40 mV. In control, augmentation for NMDAR EPSCs was 2.02 ± 0.14 (Fig. 7, C and D), slightly lower than that of AMPAR EPSCs (2.65 ± 0.16). We then bath applied 2-amino-5-phosphonopentanoic acid (L-AP5) (1 mM), a competitive low-affinity NMDAR antagonist, to reduce any existing saturation at NMDARs. L-AP5 attenuated NMDAR EPSC amplitudes to 21.8 ± 2.3% of control and significantly increased augmentation to 3.29 ± 0.20 (Fig. 7, C and D, n = 5). We conclude that, at CT synapses, enhanced glutamate release associated with increases in P will saturate both AMPARs and NMDARs to a similar degree.

**DISCUSSION**

In this study we examined the involvement of two distinct postsynaptic mechanisms, receptor saturation and desensitization, in shaping short-term synaptic enhancement at CT syn-
Fig. 7. Postsynaptic saturation attenuates frequency-dependence of augmentation. A: summary of the time course of AMPAR-mediated EPSCs in control conditions (open symbols) and following bath application of DGG (2 mM, solid symbols). The inset shows representative EPSCs before and following the stimulus train, in control (gray) and DGG (black), normalized to the baseline EPSC in the respective condition. B: dependence of augmentation amplitude on the frequency of the stimulus train (50 stimuli). Graph plots the average EPSC amplitude (normalized to respective baseline) elicited 2 s following the end of the stimulus train (5, 10, 20, and 50 Hz), in control (open symbols) and following application of DGG (solid symbols). C: summary of the time course of NMDAR EPSC, in control conditions (open symbols) and after bath application of 2-amino-5-phosphonopentanoic acid (t-AP5) (1 mM, solid symbols). Inset shows representative EPSCs in control (gray) and t-AP5 (black) at 3 different time points. EPSCs are normalized to the baseline EPSC in the respective condition. D: summary data for AMPAR and NMDAR augmentation. AMPAR augmentation was significantly increased in DGG (**P < 0.01, n = 6, paired Student’s t-test), whereas CTZ (50 μM) did not change augmentation significantly (P = 0.35, n = 5, paired Student’s t-test). t-AP5 significantly increased NMDAR augmentation (**P < 0.001, n = 5, paired Student’s t-test). Data are expressed as means ± SE.

Our data indicate that spillover and pooling of glutamate are unlikely to account for the significant changes in the peak glutamate concentration detected by changes in DGG block. Spillover and pooling of neurotransmitter is prominent at glomerular or calyceal synapses with closely spaced release sites that are poorly isolated from another by glial processes (Xu-Friedman and Regehr 2004). However, CT axons of layer 6 neurons form small (<1 μm in diameter) bouton-like terminals that are juxtaposed to individual postsynaptic densities, reflecting the presence of a single vesicle release site (Graziano et al. 2008). Spillover could also occur if extracellular stimulation activates a beam of tightly packed axons, resulting in the activation of synapses in close proximity to one another (Carter and Regehr 2000; Marcaggi and Attwell 2005). However, upon entering the thalamus CT axons do not run next to one another and boutons on individual axons tend to be well isolated from one another by glial processes. In summary, neither the synaptic ultrastructure nor the organization of the axonal systems favor glutamate pooling and spillover at CT synapses, leaving MVR as the most likely mechanism for the enhancement of glutamate transients during changes in P.

Our results extend previous findings made at cerebellar parallel fiber synapses (Bender et al. 2009; Foster et al. 2005) and hippocampal Schaffer collateral–CA1 terminals (Christie and Jahr 2006), which display low initial release probability but show MVR and postsynaptic receptor saturation following use-dependent increases in P. However, across different types of synapses, no defining principles have emerged that link the existence of MVR and saturation to other functional properties.

Pos
tsynaptic saturation and MVR. Our results show that the inhibition of postsynaptic AMPARs by DGG was reduced following increases in P, either by manipulating Cae or via induction of facilitation or augmentation. The most parsimonious explanation for this result is that during short-term synaptic enhancement peak glutamate levels increase at individual release sites and thus compete more effectively with DGG for binding at postsynaptic AMPARs. In turn, the enhancement of glutamate transients is most readily explained by an increase in the number of simultaneously released vesicles at a given site (i.e., MVR). Ultrastructural studies of CT terminals have demonstrated the existence of multiple closely spaced vesicles near individual release sites (Graziano et al. 2008), supporting at least in principle the possibility of MVR. We found that MVR and saturation were prominent even under conditions of low initial P induced by a presynaptic neuromodulator. Thus MVR and saturation are likely to be important under in vivo conditions, where initial P tends to be significantly lower than in brain slices (Borst 2010).
such as initial $P$. For example, MVR and saturation can exist at some high $P$ synapses (Chanda and Xu-Friedman 2010; Foster et al. 2002; Wadiche and Jahr 2001), while others do not show evidence for it (Murphy et al. 2004; Silver et al. 2003). Furthermore, MVR can occur without leading to receptor saturation (Higley et al. 2009; Li et al. 2009). At excitatory synapses in neocortex, MVR and saturation depend on the postsynaptic target (Watanabe et al. 2005) and on cortical area (Huang et al. 2010). Thus, rather than being merely a byproduct of the stochastic nature of transmitter release, the ability of synapses to release either single or multiple vesicles and the degree of postsynaptic receptor saturation appear to be tightly regulated and are expressed independently from other synaptic properties.

We found that postsynaptic saturation was most prominent during augmentation, indicating that the gain in synaptic strength following persistent synaptic activation is tightly controlled. This suggests that the magnitude of similar forms of short-term enhancement at other synapses such as cerebellar parallel fibers (Beierlein et al. 2007) or the calyx of held (Habets and Borst 2005) might also be strongly limited by postsynaptic saturation. In addition to expressing augmentation, CT synapses can undergo a presynaptic form of long-term potentiation (Castro-Alamancos and Calcagnotto 1999), and our data predict that saturation will become more prominent following long-term potentiation induction, similar to what has been observed at cerebellar parallel fibers (Bender et al. 2009).

Our results differ from a recent study at corticogeniculate synapses, which, using the low-affinity antagonist kynurenic acid to partially block postsynaptic AMPARs, did not find any evidence for postsynaptic saturation following a train-evoked increase in synaptic strength (Granstedt and Lindstrom 2004). It is possible that CT synapses show differences in MVR and saturation, depending on the thalamic nucleus they target.

Under our experimental conditions, both AMPARs and NMDARs experienced a similar degree of saturation during augmentation, suggesting that changes in $P$ do not significantly change the relative contribution of the NMDA and AMPA EPSC to the synaptic response. Whereas the steady-state affinity for glutamate is much higher at NMDARs compared with AMPARs, their forward binding rates are similar. Assuming a homogenous distribution in the subsynaptic membrane, AMPAR and NMDARs should experience a similar degree of saturation following increases in glutamate transients, consistent with our findings. However, because NMDARs have much slower dissociation rates compared with AMPARs, transmitter release following trains of stimuli will lead to a progressive decrease in the number of available NMDARs (Franks et al. 2002). Saturation of NMDARs following paired pulses or trains has been demonstrated at a number of synapses (Chen et al. 2002; Wang 2000; Yang and Xu-Friedman 2008) but was not further investigated here.

*Physiological consequences of MVR and postsynaptic saturation.* CT synapses play a critical role in controlling the transfer of sensory-evoked activity in the thalamus (Briggs and Usrey 2007; Temereanca and Simons 2004). MVR at CT synapses might ensure reliable synaptic transmission and therefore allow for a rapid and precise control of thalamic firing, particularly at high frequencies. At the same time, postsynaptic saturation will limit the frequency-dependent gain in cortical feedback and might therefore reduce the possibility of pathological forms of hypersynchrony in thalamic networks (Beenhakker and Huguenard 2009).

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the authors.

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