Published in final edited form as:

Brain Res. 2015 October 5; 1622: 22–35. doi:10.1016/j.brainres.2015.06.013.

Facilitated glutamate release at Schaffer collateral to CA1 synapses has access to an exclusive population of NMDA receptors

Chessa S. Scullin^{1,2}, Adrian R.B. Schiess^{1,3}, and L. Donald Partridge¹

¹Dept. of Neurosciences, Univ. of New Mexico, Albuquerque, NM 871311

²Biosciences, Sandia National Labs, Albuquerque NM 87123

³Physical Biosciences, Lawrence Berkeley National Laboratories, Berkeley, CA 94720

Abstract

In order to explore short-term facilitation of the Schaffer collateral to CA1 synapse in mouse hippocampal brain slices, we measured the time course of the decay of the peak amplitude of successive EPSCs during progressive MK-801-dependent block (PMDB) of NMDAR responses to paired (R1 and R2) stimuli. We made the unexpected observation that the R2 response exhibited a slower PMDB decay constant than that of the R1 response. This indicated that the facilitated R2 response engages release sites with NMDARs that are protected from opening and consequent MK-801 block during the basal R1 response. We then utilized conditions that affect synaptic glutamate distribution to dissect the components of the distinct PMDB decay constants of the first and second of paired pulses. While extra-synaptic NMDARs and glutamate transporters appear to play only minor roles in the differences of the PMDB decay constant, we showed important roles for the R1 response itself and for glutamate diffusion in determining the PMDB decay constant of R2. We used a simple computational model with realistic parameters that allowed us to predict the time course of R2 decay based on the R1 decay time course.

Keywords

Mouse; Synaptic transmission and plasticity; modeling; hippocampal slice; NMDA

1. Introduction

Short-term synaptic plasticity is an ubiquitous property of central nervous system synapses (Fioravante and Regehr, 2011) that affects synaptic efficacy on a timescale relevant to the encoding of information in spike trains (Dittman et al., 2000) with important implications

Corresponding Author: Donald Partridge, Department of Neurosciences, University of New Mexico School of Medicine, Albuquerque, New Mexico 87131, USA, FAX: (505) 272-8082, dpartridge@salud.unm.edu.

None of the authors has any conflict of interest with any material included in this study.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

for working memory (Deng et al., 2011; Mongillo et al., 2008). Paired-pulse facilitation (PPF), commonly seen in synapses with a low probability of release (P_r), is characterized by enhanced postsynaptic responses for a period up to about 300 ms following an initial presynaptic stimulus. (Zucker and Regehr, 2002). In many synapses, PPF is predominately a presynaptic phenomenon initiated by a residual increase in Ca^{2+} concentration ($[\text{Ca}^{2+}]_{\text{res}}$) in the presynaptic terminal (Zucker and Regehr, 2002), which permits release from additional sites. In this study we have addressed the important question of whether these additional facilitated release sites differ from those activated during basal release; such a difference could have important implications in the ability to differentially modulate facilitated transmission.

The P_r of glutamatergic synapses can be assessed by measuring the progressive MK-801 dependent block (PMDB) of NMDA receptor (NMDAR) EPSCs or fEPSPs following successive stimuli in the presence of the noncompetitive open-channel NMDAR antagonist MK-801 (50 μM) (Bagley and Westbrook, 2012; Hessler et al., 1993; Schiess and Partridge, 2005). At Schaffer collateral to CA1 synapses, PMDB of the response to single pulses typically follows a double exponential decay time course that has been attributed to either release sites with two distinct P_r s (Hessler et al., 1993; Rosenmund et al., 1993), or to release sites with a continuous distribution of P_r s (Huang and Stevens, 1997).

In addition to using the PMDB technique to measure P_r following a single pulse, the technique has been used for measurements during PPF (Castro-Alamancos and Connors, 1997; Huang and Stevens, 1997), although these studies did not independently assess the time course of PMDB decay during the basal (R1) and facilitated (R2) responses. If a single vesicle release pool is activated, the time course of PMDB during the R1 and R2 responses should be equivalent; on the other hand, if the R1 and R2 responses are associated with distinct release pathways, then the PMDB decay constants may differ between R1 and R2. Our preliminary experiments (Partridge et al., 2008) showed that, while the PMDB of the R1 fEPSP of paired-pulses occurred with the same decay constant as that of a single pulse (P1), the PMDB of the R2 fEPSP occurred with a slower decay constant. This difference in decay constants was unexpected and can be interpreted to indicate that the facilitated R2 response engaged release sites with postsynaptic NMDARs that were protected from opening and consequent PMDB during R1. These findings raise the interesting possibility that paired-pulse facilitation recruits a population of release sites that were not accessed during basal release.

In this study we have followed up on the preliminary studies and used conditions that affect synaptic glutamate distribution, to dissect the components of the distinct R1 and R2 PMDB processes. We then used a simple computational model to make general predictions about the dependence of R2 on R1.

2. Results

The progressive MK-801-dependent block (PMDB) technique is a straightforward and powerful technique that is especially suited to comparing the characteristics of synaptic glutamate release pools (Bagley and Westbrook, 2012; Hessler et al., 1993; Schiess and

Partridge, 2005). In this study, we have used this technique to investigate the possibility that PPF recruits a population of release sites and post-synaptic NMDARs during the second, facilitated EPSC that are distinct from those accessed during first, basal EPSC of the pair. We measured the responses (R1 and R2) of CA1 pyramidal neurons to paired stimuli to the Schaffer collaterals at a 50 ms inter-pulse interval with a stimulus intensity ($< \frac{1}{2}$ max) that produced consistent paired-pulse facilitation (PPF) and assessed the time course of PMDB of the R1 and R2 responses. We found that PMDB of the facilitated R2 response exhibits a slower time course than that of the basal R1 response indicating that a portion of the facilitated release pool is independent from the pool accessed during basal release. These findings suggest the presence of a site for modulation of short term facilitation that is downstream from presynaptic Ca^{2+} .

2.1. During PPF, PMBD of the R2 response exhibits a slower decay constant than that of the R1 response

Figure 1A shows every second EPSC for both R1 and R2 at an interpulse interval of 50 ms in a representative neuron during the bath application of MK-801 while Figure 1B gives the time course of PMDB of the average normalized peak amplitude of the R1 and R2 EPSCs for 9 neurons. Figure 1B also includes the time course of progressive block of normalized EPSCs following a single stimulus (P1) under these same conditions in 8 additional neurons. These PMDB data were fit to a double exponential process (equation 1) using a least squares regression and Figure 1C shows the averaged parameters from the individual fits for each neuron. (The independent variable in these fits was successive stimuli, but for simplicity we have represented the resultant decay constants as τ s.) In each case, the parameters from the fits to R1 and P1 were not significantly different. Facilitation of the R2 EPSC resulted in an increase in the scaling factor of both the fast (A) and slow (B) components of the double exponential fit, although the relative contributions of these two components to the total current did not change significantly between R1 and R2. Surprisingly, the PMDB of the R2 EPSC occurred more slowly than that of the R1 (or P1) EPSC as demonstrated by the significant increase in the decay constant of the fast component (τ_1) and the trend toward an increase in the decay constant of the slow component (τ_2).

To demonstrate the consistency of this finding across the time course of short-term facilitation, we repeated these PMDB experiments by measuring fEPSPs in *stratum radiatum* following Schaffer collateral stimulation at 40 ms and 70 ms interpulse intervals. Consistent with the 50 ms interpulse interval data shown in Figure 1, we found a significantly slower R2 PMDB decay at both intervals (40 ms interpulse interval: R1 $\tau_1 = 6.03$, R2 $\tau_1 = 12.10$, $p = 0.022$; R1 $\tau_2 = 231.80$, R2 $\tau_2 = 486.21$, $p = 0.016$. 70 ms interpulse interval: R1 $\tau_1 = 5.58$, R2 $\tau_1 = 11.67$, $p = 0.012$; R1 $\tau_2 = 118.99$, R2 $\tau_2 = 352.69$, $p = 0.007$)

We also used a least squares regression to fit the PMDB data for R1 and R2 with single and triple term exponential equations and with a continuous function (equation 2) and the coefficients of determination for the respective fits are given in Table 1. As has been previously noted (e.g. (Rosenmund et al., 1993)), the poor fit for a single exponential suggests that activation of NMDARs is not mediated by glutamate release with a uniform P_r .

Adding a third term to the exponential equation led to a slight improvement in the fit, but we followed the generally accepted approach and fit our data as a double exponential process. While this choice of a double exponential is not intended to imply that there are necessarily two discrete release sites (e.g. (Huang and Stevens, 1997)), the double exponential fits shown in Figure 1 suggest that facilitation is not simply the result of a shift between existing fast and slow basal release pathways, which would be expected to produce a change in the relative contribution of the fast component (scaling factor A in equation 1) with no change in the τ_1 or τ_2 decay constants. One interpretation of the PMDB time course, which is especially apparent in the decay of R2 in Figure 1B, is that EPSC amplitudes approach an asymptote suggestive of the presence of a population of MK-801-resistant NMDARs. However, fitting the R2 data with an additional additive constant (equation 3) did not improve the fit and the asymptote term (C) for the fit to the R2 EPSC was highly variable. This argues against a contribution from MK-801-resistant NMDARs, but suggests either low access of glutamate to or low affinity of a pool of NMDARs during R1 that are predominately accessed during R2.

2.2. The PMDB characteristics of short-term facilitation and are not shared by short-term depression

We next sought to determine whether the observed distinction between the time course of the PMDB of the R1 and R2 EPSCs was a unique feature of short term facilitation. To this end, we repeated our paired-pulse recordings under conditions that generated paired-pulse depression (PPD). As is common at many synapses, when the amplitude of the presynaptic stimulus was increased appreciably above one half of maximum, short-term facilitation was consistently replaced with short-term depression (Debanne et al., 1996; Schiess et al., 2006). Figure 2A shows every 2nd EPSC during the decay of both R1 and R2 in a representative neuron following the bath application of MK-801. Figure 2B gives the time course of PMDB of R1 and R2 EPSCs in 9 neurons in which there was an average initial PPD of about 70%. The characteristic increases in τ s and scaling factors seen in fits to the PPF data (Figure 1C) were absent in double exponential fits to the PPD data and the initial depression of R2 resulted most markedly from a reduction in the contribution of the fast component (scaling factor A in equation 1) of the double exponential fit (Figure 2C).

2.3. Synaptic glutamate modulators distinctly modify PPF

If glutamate release during R1 and R2 activates the same pool of NMDARs, then PMDB of R1 and R2 EPSC amplitudes should occur with the same decay constants. Thus the differing time constants shown in Figure 1 would occur only if some of the NMDARs activated during R2 are part of a separate pool that is protected from MK-801-dependent block during R1. Some of the factors that might contribute to this partial independence of the R2 NMDAR pool are the efficacy of glutamate diffusion; contributions from glutamate uptake transporters; and/or the effects of glutamate acting at extra- or peri-synaptic NMDARs. In an attempt to determine the possible contribution of these factors to short term plasticity, we measured paired EPSCs following bath application of: 5% w/v of 40,000 M_r dextran to decrease solute diffusivity (Nielsen et al., 2004); 30 μ M DL-TBOA to inhibit glial GLT1 and GLAST and neuronal EAAT1 glutamate transporters (Cattani et al., 2007); or 5 μ M memantine, to block extra-synaptic NMDARs (Wroge et al., 2012; Xia et al., 2010). Figure

3A shows the effect of bath application of dextran, DL-TBOA, or memantine on the amplitudes of R1 and R2 EPSCs. We have included in this figure an estimation of the time course for the change of bath concentration of these drugs, which is based on the measured bath exchange rate and is clearly consistent with the change of refractive index that occurs during bath application of dextran. Figures 3B and C summarize the effects on paired EPSCs when these modulators had reached stable bath concentrations. Dextran caused a marked increase in the R1 EPSC with a smaller increase in the R2 EPSC, which led to a shift from PPF to PPD. A small, but not significant increase in both the R1 and R2 EPSCs were observed in DL-TBOA while a small, but not significant decrease in both the R1 and R2 EPSCs were observed in memantine; however, neither of these drugs significantly altered PPF. The depression in the R2 EPSC amplitude, near the end of the application of DL-TBOA, is likely to have resulted from a depletion of presynaptic glutamate stores.

In the experiments shown in Figure 3, we measured the R2 EPSC amplitude as an increment in the whole-cell current, which in some instances had not entirely decayed to the pre-stimulus baseline in the 50 ms following the R1 EPSC. As noted above, when measured in this way, dextran eliminated PPF and in most instances produced PPD (Figure 3C). This PPD could be the result of a differential effect of dextran on the access of glutamate to the basal and facilitated postsynaptic sites that contribute to the R2 EPSC. Alternatively, since increasing the amplitude of the R1 EPSC leads to a transition from PPF to PPD in these synapses (Debanne et al., 1996; Schiess et al., 2006), elimination of PPF by dextran could simply have been the result of the increased R1 amplitude. To test for this latter possibility, in a separate series of experiments we measured the paired-pulse ratio (PPR) as a function of the amplitude of the R1 EPSC over a range of stimulus intensities in the presence of the synaptic glutamate modulators (Figure 3D). Interestingly, all three modulators caused a transition from PPF to PPD at amplitudes of the R1 EPSC that would have still produced PPF in ACSF(NMDA) suggesting that the PPR is not determined solely by the amplitude of the R1 EPSC.

2.4. PPF persists when the postsynaptic response to R1 is minimized

Short-term facilitation at the Schaffer collateral to CA1 pyramidal neuron synapse is primarily a presynaptic phenomenon (Zucker and Regehr, 2002) that has been attributed to an increased vesicular release (Christie and Jahr, 2006). Consistent with this, minimal stimulation experiments show that the success rate of R2 quantal release is greater than the success rate of R1 quantal release and this results in facilitation of the R2 event even in the absence of a postsynaptic R1 event (Schiess et al., 2010). Since the PMDB technique uses postsynaptic NMDAR block as a proxy for presynaptic release probability, we sought to measure R2 facilitation under conditions when the R1 EPSC had been eliminated. To do this, we designed two voltage clamp protocols to be used in the presence of high Mg^{2+} ACSF where EPSCs are blocked at negative, but not positive holding potentials. Neurons were held at -70 mV and in protocol A were ramped to $+40$ mV before the application of presynaptic R1 and R2 stimuli. In protocol B, neurons were stepped to -100 mV during the R1 stimulus and then ramped to $+40$ mV before application of the R2 stimulus (Figure 4A1). Protocols A and B were applied in random order in each neuron and all EPSCs were normalized to the R1 EPSC that was measured in protocol A.

Figure 4A2 shows the currents recorded in a representative neuron for protocols A and B and Figure 4A3 shows the conductances that were calculated with the NMDAR $E_{rev} = 7.2$ mV that was determined from I-V curves averaged for all experiments. As can be seen in the average current and conductance data in Figure 4B, when normalized to the R1 EPSC measured in protocol A, the R2 EPSC shows significant facilitation even when it follows the minimal postsynaptic R1 response that is generated by protocol B. This is consistent with our minimal stimulation experiments and indicates that under the conditions used here, R2 facilitation is predominately a presynaptic phenomenon. For simplicity, we will thus refer to R2 EPSCs generated using protocol B as the “no R1” condition.

2.5. Glutamate diffusion and the postsynaptic R1 response are important determinants of the time course of PMDB

As we showed in Figure 1 in ACSF(NMDA), the PMDB of the R2 EPSC followed a slower time course than that of the R1 or P1 EPSC suggesting the involvement of NMDARs in the R2 EPSC, which were not accessed during the R1 EPSC. To better understand this phenomenon, we measured the PMDB of paired EPSCs under conditions where synaptic glutamate was modulated as shown in Figures 3 and 4. First, we made a comparison of the time course of PMDB on single (P1) EPSCs in the presence of dextran, DL-TBOA, and memantine by fitting the decay curves using a least squares regression to a double exponential process (equation 1, data not shown). None of the three glutamate modulators had a significant effect on any of the parameters of these fits (one-way ANOVA: A, $F_{(3,24)} = 0.5451$ $p = 0.6562$; τ_1 , $F_{(3,24)} = 2.0876$ $p = 0.1285$; τ_2 , $F_{(3,24)} = 0.3325$ $p = 0.8019$; B, $F_{(3,24)} = 0.8543$ $p = 0.4781$). An equivalent control experiment was not possible for the “no R1” condition. As was the case for the single pulse P1 EPSCs, none of the three glutamate modulators had a significant effect on any of the parameters for the least squares fit of a double exponential (equation 1) to the time course of PMDB of the R1 EPSC, although there were changes in τ_1 that approached significance resulting from an increase in this term in dextran and a decrease in both memantine and TBOA (one-way ANOVA: A, $F_{(3,31)} = 0.275$ $p = 0.843$; τ_1 , $F_{(3,31)} = 2.854$ $p = 0.053$; τ_2 , $F_{(3,31)} = 1.337$ $p = 0.279$; B, $F_{(3,31)} = 1.529$ $p = 0.227$). Again, an equivalent experiment was not possible for the “no R1” condition.

Although these manipulations only minimally affected the basal P1 and R1 responses, several of them produced pronounced effects on the facilitated R2 response. Dextran had a striking effect on the time course of PMDB of the R2 EPSC (Figure 5A and B) when compared to that in ACSF(NMDA) (Figure 1B and C) that was apparent as a decrease in the contribution of the fast (A) component of the double exponential fit (Figure 5B, column a of Table 3). This reduction allowed the slow (B) component of the double exponential fit to predominate with the result that the time course of the PMDB of the R2 EPSC in the presence of dextran was almost equally well fit with a single as with a double exponential (dextran, $R^2 = 0.9285$ double exponential, $R^2 = 0.9205$ single exponential). A similar reduction in the fast (A) component and predominance of the slow (B) component of the double exponential fit is also apparent in the results of the PPD protocol (Figure 2B and C) ($R^2 = 0.9812$ double exponential, $R^2 = 0.9427$ single exponential). In contrast to the effects of dextran and the PPD protocol, neither DL-TBOA nor memantine had a pronounced effect

on the overall pattern of the PMDB of the R2 EPSC when compared with that observed in NMDA(ACSF) (Figures 1B and C, 5A and B, and column a of Table 3).

We also tested PMDB of the R2 EPSC in the “no R1” condition where, in contrast to the procedure used in Figure 4, protocols A (for R1) and B (for R2) were necessarily tested in different neurons. The MK-801-dependent block of NMDA currents has been shown to be effective when NMDA currents are outward and, although the recovery of block under these conditions is faster (Huettnner and Bean, 1988), it is still several times slower than the 15 s interval between our stimulus pairs. When we fit the time course of the PMDB of the R2 EPSC with a double exponential, there was a decrease in the contribution of the slow (B) component (Figure 5B, column a of Table 3). This reduction allowed the fast (A) component of the double exponential fit to predominate with the result that the time course of the PMDB of the R2 EPSC in protocol B was almost equally well fit with a single as with a double exponential ($R^2 = 0.9609$ double exponential, $R^2 = 0.9495$ single exponential).

For each of the 5 conditions described above, we also fit the PMDB data with a continuous distribution (equation 2, (Huang and Stevens, 1997)). As in the case with the ACSF(NMDA) data (Table 1), these least squares fits were not as good as those obtained with a double exponential (Figure 5C). However, the continuous distribution provides a direct determination of a value proportional to P_r and these values are given in Table 2. The P_r values determined from fits to the ACSF(NMDA), DL-TBOA, and memantine data all exhibit a reduction during R2 to about 2/3 of that during R1. The dextran and to a lesser extent the PPD data, show a greater reduction of P_r during R2 while the “no R1” condition yielded a P_r during R2 that was greater than that during R1 (see Column b Table 3).

2.6. A computational model, incorporating a facilitated pathway with a decreased P_r , accurately predicts the PMDB time course R2 based on that of R1

In order to further explore the dependency of the R2 EPSC on the R1 EPSC during their simultaneous PMDBs, we developed a simple computational model that is based on separate basal and facilitated release pathways with terms for the putative Ca^{2+} -dependent generation of the facilitated release state, the probability of release over basal and facilitated pathways, and the probability of MK-801-dependent block of the basal and facilitated pathways (see Supplementary Material). We used the MatLab curve fitting tool with the Trust-Region algorithm to fit normalized R2 EPSC amplitudes as a function of normalized R1 EPSC amplitudes at successive stimuli during PMDB for the average ACSF(NMDA) data and parameters for these fits are given in Table S1 of the Supplementary Material. Notably, our computational model indicates a decreased P_r of the facilitated pathway (P_{rF}) over that of the basal pathway (P_{rB}) with little change in the effectiveness of MK-801 block (P_{bF} vs. P_{bB}) between the two pathways. We then fixed these parameters and subsequently calculated the relative contributions of the basal and facilitated pathways in the presence of the other 5 experimental conditions and these values are given in Table S2 of the Supplementary Material while Table 3 column c presents the resultant change of P_r for R1 and R2. Figure 6A shows the resultant fits of R2 as a function of R1 at successive stimuli for these 6 conditions and Figure 6B shows the R2 vs R1 projection of these fits superimposed on the experimental data. Also indicated in the latter plots is a unity slope dotted line that demarks

PPF (above) and PPD (below). The response in ACSF(NMDA), TBOA, and memantine are seen to exhibit PPF throughout the course of PMDB. Consistent with the predominance of the fast component of the double exponential fit in the “no R1” condition, the R2 vs. R1 data show PPF early in the period of PMDB (large values of R1) that progresses toward PPD later in the process. In contrast, the R2 vs. R1 data in dextran and with the PPD protocol, exhibit pronounced PPD early in the period of PMDB that gradually wanes later in the process.

3. Discussion

3.1. During PPF, PMDB predicts a lower P_r for the facilitated R2 response than for basal release R1 response

We measured the progressive MK-801-dependent block (PMDB) for each of the paired pulses during PPF at the hippocampal Schaffer collateral to CA1 pyramidal neuron and made the unexpected observation that the R2 response exhibits a slower PMDB decay constant than the R1 response. This indicated that the facilitated R2 response engages release sites with NMDARs that are protected from opening and consequent MK-801 block during the basal R1 response. We repeated these measurements under conditions designed to modulate the effectiveness of postsynaptic glutamate distribution and binding and then constructed a simple computational model that included basal and facilitated release pathways to predict R2 EPSC amplitude as a function of R1 EPSC amplitude at successive stimuli. We fit these PMDB data to simple mathematical relationships and then with the computational model. The former procedure relied on separate 2-dimensional least squares fits to R1 and R2 data as a function of stimulus number while the latter procedure was based on a 3-dimensional least squares fit to the R2 data as a function of the corresponding R1 data at successive stimuli. Consistent with both of these approaches is the conclusion that facilitated glutamate release during the facilitated R2 EPSC accesses a pool of NMDARs that were not accessible during the basal R1 EPSC. The accepted interpretation of the fits of the simple mathematical relationships to our ACSF(NMDA) data, which is supported by our simple computational model, gives an estimate of the relative P_r of R2 that is lower than the P_r of R1. We will discuss this unexpected conclusion as the basis of a more detailed mechanistic model at the end of this section.

Consistent with the majority of published reports, we found that the decay of EPSC amplitudes during the PMDB was not accurately fit with a single exponential process. This has been attributed to either multiple discrete release sites (Hessler et al., 1993; Rosenmund et al., 1993) or to a continuum of release processes (Huang and Stevens, 1997)). In either case, release is predicted not to occur with a single uniform P_r . Presynaptic $[Ca^{2+}]_{res}$ during R2 is generally accepted to play a central role in short term facilitation at Schaffer collateral terminals and this could expand the size of the active zone at a single synaptic terminal, cause release from synaptic boutons that had been silent during R1, or lead to release from vesicles within a heterogeneous pool that are associated with SNARE proteins with differing Ca^{2+} affinities. Importantly, it is clearly an oversimplification to interpret the PMDB of NMDA responses as a definitive indication of presynaptic P_r . Thus, while we have interpreted differences in PMDB decay constants between those of the R1 (or P1) EPSC and

the R2 EPSCs to indicate differences in P_r between R1 and R2, it could equally indicate a postsynaptic effect in which glutamate released during R2 binds to a pool of NMDARs that differs in part from the pool accessed during R1 and that includes NMDARs with different MK-801 binding affinities.

3.2. The facilitated R2 EPSC accesses NMDARs that are protected from MK-801 block during basal R1 EPSC

There are several mechanisms by which some of the NMDARs activated during the facilitated R2 release could have been protected from MK-801 block during the R1 release. These include: diffusion of an increased synaptic glutamate concentration during R2 to more distant synaptic, peri-synaptic, or extra-synaptic NMDARs (Hardingham and Bading, 2010; Harris and Pettit, 2007; Harris and Pettit, 2008; Newpher and Ehlers, 2008); saturation of glutamate transporters during R2 that had buffered the access of glutamate to a pool of NMDARs during R1 (Christie and Jahr, 2006; Harney et al., 2008); release from facilitated release sites during R2 that were not release competent during R1 (Chamberland et al., 2014; Freneau et al., 2004; Manabe and Nicoll, 1994), spillover during facilitated R2 glutamate release to presynaptic GluN2B-containing NMDARs (McGuinness et al., 2010); or contributions of NMDARs that were in a desensitized state following R1 (Korinek et al., 2010; Lester and Jahr, 1992). We will consider here several of these possibilities.

3.2.1. Neither extra-synaptic NMDARs nor glutamate transporters make a significant contribution to facilitation of the R2 EPSC—Extra-synaptic and peri-synaptic glutamate receptors are clearly potential targets of an increased glutamate release during R2 and their contribution could perhaps explain the apparent differences in P_r seen during PMDB of R2 EPSCs. Memantine is reported to be a selective antagonist of extra-synaptic and peri-synaptic NMDARs, although it is a rather non-selective drug that also blocks other neurotransmitter receptors. In our studies, memantine caused only a small reduction in R1 and R2 EPSC amplitudes and had a minimal effect on either the short term plasticity or on the PMDB parameters, suggesting that extra-synaptic and peri-synaptic NMDARs make a minimal contribution to R2 facilitation.

In addition to the role of glutamate diffusion, uptake by neuronal and glial transporters is important in shaping the spatial and temporal bounds of the synaptic glutamate signal (Arnth-Jensen et al., 2002; Christie and Jahr, 2006; Zheng et al., 2008). DL-TBOA, a non-transportable competitive blocker of both glial and neuronal glutamate uptake transporters, has been reported to block glutamate uptake without causing glutamate release or acting as an agonist of glutamate receptors (Cattani et al., 2007). We observed a small increase in the R1 and R2 EPSC amplitudes (Figure 3A2), and no change of PPF (Figure 3C) when DL-TBOA was bath applied. Interestingly, there was a consistent decrease in the amplitude of both the R1 and R2 EPSCs near the end of the DL-TBOA application that persisted following return to ACSF(NMDA), which could have resulted from a decrease in presynaptic glutamate uptake and a consequent reduction of vesicle refilling (Kondratskaya et al., 2010). As was observed with memantine, DL-TBOA had a minimal effect on the parameters of the fit to the PMDB decay. Our interpretation of these observations is that

regulation of glutamate concentration by cellular transporters makes only a small contribution to short term facilitation in our experimental setting.

3.2.2. Neither presynaptic NMDARs nor NMDAR desensitization make a significant contribution to facilitation of the R2 EPSC—Presynaptic NMDARs could potentially make a contribution to the facilitation of the R2 EPSC through a feedback increase in glutamate release following R1. We do not believe that this is a major factor, since when we bath applied 3 μ M ifenprodil to block GluN2B-containing presynaptic NMDARs (Luccini et al., 2007), there was not a significant change in the amount of PPF (ACSF(NMDA) = 92.55%; ifendropil = 100.97%; unpaired *t*-test *p* = 0.521). The generally accepted reaction scheme for NMDAR activation is that, following binding of 2 agonist molecules, the NMDAR enters a state where it can repeatedly open or desensitize before ligand dissociation (Korinek et al., 2010; Lester and Jahr, 1992; Thomas et al., 2006). Thus the time course of the PMDB of open NMDARs should be determined by a combination of ligand binding to unbound NMDARs and the opening of previously desensitized NMDARs, which are insensitive to block by MK-801 (Dzubay and Jahr, 1996). The forward and reverse rate constants of desensitization (Lester and Jahr, 1992) suggest that most NMDARs that were desensitized during R1 would remain so throughout the 50 ms inter-pulse interval and thus would be protected from MK-801-dependent block during R2. These desensitized NMDARs would, however, return to the pool of available receptors during R1 of the next pulse pair. Since Mg²⁺, in addition to its role in voltage-dependent block of the channel, enhances NMDAR desensitization (Kampa et al., 2004), any difference in the time course of PMDB of R1 and R2 EPSCs between our ACSF(NMDA) (0 Mg²⁺) and “no R1” (2 mM Mg²⁺, protocol A) data (Figure 5) could be in part attributed to differences in the degree of desensitization protection from MK-801 block of NMDARs during the R2 EPSCs. When these two data sets were fit using a least squares regression to our computational model with an added contribution from postsynaptic desensitization (see Supplementary Material), there were no appreciable changes in the fitting parameters and only a small improvement in the goodness of the fit ($R^2_{\text{no desensitization}} = 0.985$, $R^2_{\text{desensitization}} = 0.993$) in the 0 Mg²⁺ (ACSF(NMDA)) conditions of the majority of our experiments. In the 2 mM Mg²⁺ conditions of the “no R1” experiments, there was marked improvement in the goodness of fit when desensitization was included in the computational model ($R^2_{\text{no desensitization}} = 0.811$, $R^2_{\text{desensitization}} = 0.998$). Importantly, the computational model predicted only a very small contribution of desensitization to the ACSF(NMDA) data, but a larger contribution to the “no R1” data (see Supplementary Material). To the extent that our computational model accurately reflects the desensitization process, this would suggest that protection of NMDARs during the R2 response does not play a major role in the process of PMDB under the 0 Mg²⁺ conditions in which the majority of our experiments were carried out. This conclusion is consistent with measurements made at single synapses in neonatal rats (Hanse and Gustafsson, 2001).

3.2.3. Limiting synaptic glutamate diffusion restricts its access to the facilitated pool of receptors—Glutamate, released from presynaptic sites, rapidly diffuses to adjacent postsynaptic receptors and the diffusion path and diffusion coefficient of the peri-synaptic space determine the extent of this diffusion (Franks et al., 2002;

Santamaria et al., 2011). One approach to studying glutamate diffusion is by the bath application of high molecular weight dextran, which has little effect on osmolarity or the activity of glutamate (Nielsen et al., 2004), while significantly affecting the glutamate diffusion coefficient (Min et al., 1998; Nielsen et al., 2004). We compared fits to a Hill function (equation 4) of EPSC input-output relationships in ACSF(NMDA) and with added dextran and found only a large increase in the $Y_{\max}$ for the normalized R1 EPSC (ACSF, $Y_{\max} = 1.06$; dextran, $Y_{\max} = 2.90$, one-way ANOVA with Bonferroni post hoc $F_{(3,23)} = 3.549$, $p < 0.05$), but no significant change in $Y_{\max}$ for the R2 EPSC or for the K_D or Hill constant for either the R1 or R2 EPSC. This is consistent with the interpretation that dextran limits synaptic glutamate diffusion to a single population of NMDARs. One possible explanation for the disparity in the effect of dextran on the amplitudes of the R1 and R2 EPSCs is that, since the R2 EPSC was measured as an increment in current above the slowly decaying tail of the R1 EPSC, the R2 EPSC could be limited by the ability of the neuron to generate additional NMDAR current. However, Figure 3D indicates that there is a decrease in the R2 EPSC amplitude in dextran regardless of the amplitude of the R1 EPSC suggesting that, when diffusion is limited, the facilitated R2 glutamate release does not have access to NMDARs that are normally activated during R2.

3.2.4. Glutamate released during R1 preferentially accesses sites contributing to the fast component of PMDB decay—

We have previously shown, using minimal stimulation, that the adult phenotype of facilitation is characterized by an unreliable basal synaptic efficacy and a highly reliable facilitated synaptic efficacy that results primarily from an increased R2 success rate of release and secondarily from an increased R2 synaptic potency (Schiess et al., 2010). Importantly, these characteristics of R2 release do not require a postsynaptic response to R1. Using our “no R1” protocol, we demonstrate here a similar independence of R2 facilitation from a postsynaptic R1 response (Figure 4). Interestingly, PMDB of the R2 EPSC under the “no R1” protocol occurred with a considerable reduction in the relative contribution of the slow component of a double exponential fit and an increase in P_r as predicted by a continuous fit to the decay of successive EPSCs (Figure 5, Table 3). Contrasting these results to those obtained in dextran and during PPD in which the relative contribution of the fast component of the exponential fit was decreased, suggests that the fast component of the double exponential fit represents postsynaptic sites that are more susceptible to block during R1. Thus when R1 glutamate release is increased in PPD or physically constrained with dextran, there are fewer fast component sites available during R2. Likewise, in the “no R1” condition, when fewer of these sites are blocked during R1, more fast component sites are available during R2.

3.3. Modeling parameters indicates that facilitation of the R2 EPSC results from the additional contribution from sites with a lower P_r that were not available during the R1 EPSC

In Table 3 we summarize results from fitting the PMDB data that were generated under the 6 different experimental conditions with simple mathematical relationships and with the computational model. Column a compares the relative contributions of the fast component of the double exponential fit (equation 1) to the PMDB data, column b compares the ratio of the P_r of R2 to that of R1 as determined by fitting the PMDB data with the continuous

function (equation 2) and column c compares the values of P_r2/P_r1 as predicted by our computational model. Importantly, the parameters in columns a and b of Table 3 arise from independent 2-dimensional fits of the R1 and R2 PMDB data in which presynaptic P_r was the implicit or explicit variable while the parameters in column c arise from single 3-dimensional fits of the R2 PMDB data in which presynaptic P_r was determined from the ACSF(NMDA) trials and the postsynaptic effect of the computed basal and facilitated pathways were the variables in the remaining 5 conditions. All three parameter sets indicate a marked difference of the “no-R1” and dextran and to a lesser extent PPD data from the other three conditions; however, the differences among the columns highlight the limitations of the standard PMDB analysis when it is used to compare R2 with R1 data. In particular, typical use of the PMDB analysis would make the assumption that R2 is independent of R1 and that differences result solely from changes in presynaptic processes while our simple computational model assumes that R2 is a function of R1 and allows changes in postsynaptic processes. In spite of these inconsistencies, the comparisons in Table 3 allow us to draw the following conclusions about facilitated release: (1) Facilitated release accesses NMDARs that were not accessed during R1. (2) R2 release occurs from a mix of basal and facilitated release sites. (3) Facilitated release includes sites that have a lower P_r than some basal release sites. (4) The fast (A) and slow (B) components of the double exponential fit to PMDB data do not uniquely represent basal and facilitated release sites, but rather a consistent combination of the two.

Our double exponential (equation 1) fits to the PMDB data are based on alternating R1 and R2 stimuli. The resultant R2 PMDB decay constants of $\tau_1 = 6.3$ and $\tau_2 = 109.7$ (Figure 1C) make the tacit assumption of an overlapping pool of NMDARs accessed during R1 and R2. If the R2 PMDB data were fit using sequential stimuli, then the decay constants would be halved yielding $\tau_1 = 3.2$ and $\tau_2 = 54.8$. This value of τ_1 is similar to the R1 $\tau_1 = 3.3$ (Figure 1C), while the value of τ_2 is shorter than the R1 $\tau_2 = 77.9$ (Figure 1C). One interpretation suggests an additional release pool during R2 with lower P_r than that of the basal release sites. This suggestion will be explored further in the mechanistic description below.

Our simple computational model allowed us to determine R2 as a function of R1 and stimulus number and to compare fitting parameters for each of our experimental conditions. In particular it made predictions about the P_r s that underlie the R1 and R2 responses. Based on the fits to our PMDB data and the predictions of our simple computational model, we propose a mechanistic description for short-term facilitation that is based on basal glutamate release from presynaptic active zones with separate low and high synaptic P_r s that act on closely opposed postsynaptic NMDARs. Short term facilitation of the R2 response would include release from facilitated sites with a P_r that is intermediate between that of the high and low P_r basal sites. Neither the low nor the high P_r basal release sites would typically activate extra- or peri-synaptic NMDARs in an adjacent postsynaptic spillover area (Hardingham and Bading, 2010). For high basal P_r release sites, recruitment to the facilitated state during R2 would have nominal impact and the P_r during R2 would be approximately the same as that during R1. These release sites would predominate during R1 and hence their NMDARs would be the predominant ones blocked by MK-801. Because of this high proportion of R1 MK-801 block, NMDARs associated with high basal P_r release sites would thus account for a smaller portion of the MK-801 block that occurs during R2.

Recruitment to a facilitated state during R2 would have a larger impact on the low basal P_r release sites. While NMDARs associated with these low P_r release sites would only contribute minimally to R1 and hence to MK-801 block, they would contribute significantly to the MK-801 block during R2 thereby significantly slowing the PMDB decay rate during R2. In the “no-R1” condition there would be less block of the high basal P_r release sites and hence they would be available to make a larger contribution to the PMDB decay rate during the R2 response. Conversely, in the presence of dextran, the higher local postsynaptic glutamate concentration would lead to more block of the high P_r sites during R1 and hence allow a larger contribution of the low P_r release sites to the R2 response. Finally, due to an increase in the R2 quantal success rate and potency (Schiess et al., 2010), spillover to extra- and peri-synaptic NMDARs during R2 is expected to be somewhat greater than that during R1. Although our memantine data suggest that this is not a large contribution, it could further contribute to the slowing of the PMDB decay rate during R2. Application of this mechanistic description to the PMDB data in Figure 1 implies that the ratio of high to low basal P_r active zones is approximately 2 to 1.

4. Experimental Procedure

4.1 Slice preparation

All experiments were approved by the Institutional Animal Care and Use Committee at the University of New Mexico Health Sciences Center and conformed with NIH guidelines. Experiments were performed in coronal hippocampal slices prepared from a total of 79 P21 – 28 C57BL/6 mice (*Mus musculus*) as previously described (Scullin and Partridge, 2010). Briefly, animals were deeply anaesthetized by i.p. injection of 250 mg kg⁻¹ ketamine (Fort Dodge Animal Health, Fort Dodge, IA, USA), brains were rapidly removed, and slices were cut at 300 μ m with a vibroslicer (Pelco 101, St Louis, MO, USA) in an ice bath with a cutting solution containing (in mM): 220 sucrose, 3 KCl, 1.2 NaH₂PO₄, 26 NaHCO₃, 12 MgSO₄, 0.2 CaCl₂, 10 glucose and 0.01 mg ml⁻¹ ketamine equilibrated with 95%O₂–5%CO₂. Slices were then transferred to a bath containing artificial cerebrospinal fluid (ACSF) containing (in mM): 124 NaCl, 5 KCl, 1.25 NaH₂PO₄, 1.3 MgSO₄, 26 NaHCO₃, 2.5 CaCl₂ and 10 glucose equilibrated with 95%O₂–5%CO₂ at 30°C for 1 h and then maintained at room temperature (~ 22°C) until transfer to a temperature-controlled recording chamber (Warner Instruments, Hamden, CT, USA), which was maintained at 32°C and continuously perfused at 2 ml min⁻¹ with ACSF saturated with 95%O₂–5%CO₂. NMDAR EPSCs were isolated in Mg²⁺-free ACSF containing (in mM): 124 NaCl, 5 KCl, 1.25 NaH₂PO₄, 26 NaHCO₃, 3.8 CaCl₂ and 10 glucose equilibrated with 95%O₂–5%CO₂ with 10 μ M CNQX and 50 μ M picrotoxin or 20 μ M bicuculline, which for simplicity we will refer to as ACSF(NMDA). Experiments shown in Figure 4 were carried out in high Mg²⁺ with the constituents of normal ACSF, but with Mg²⁺ increased to 2 mM.

4.2 Whole cell patch recordings

Whole-cell voltage clamp recordings were made from hippocampal CA1 pyramidal neurons following stimulation of the CA3 Schaffer collaterals using a Multiclamp 700B amplifier, and a Digidata 1322A interfaced with pCLAMP 10 software (all from Molecular Devices, Sunnyvale, CA). EPSCs, recorded from CA1 *Stratum pyramidale* neurons, were digitized at

500 kHz, sampled at 100 kHz, and digitally filtered at 1 kHz. The K⁺ patch electrode solution contained (in mM): 135 KCl, 10 HEPES, 0.5 EGTA, 0.2 MgCl₂, 5 MgATP, 1 NaGTP, 1 QX314; experiments summarized in Figure 3 used a Cs⁺ patch electrode solution that contained (in mM) 130 CsCl, 10 HEPES, 4 NaCl, 0.2 EGTA, 10 creatine PO₄, 4 MgATP-Mg, 0.3 NaGTP, 6 QX314, both solutions had an osmolarity of 285 and a pH of 7.25 and were aliquoted and frozen until use. Neurons were voltage clamped at -70 mV, membrane properties were monitored, and only recordings in which the access resistance changed by less than 20% and the holding current was stable and less negative than -100 pA were accepted.

4.3 Stimulation

Paired constant current square pulses (150 μs duration) were applied to Schaffer collateral fibers with an Iso-Flex constant current stimulator (AMPI Instruments, Jerusalem, Israel) and a concentric bipolar electrode (25 μm inner pole, 125 μm outer cylinder diameter, FHC, Bowdoinham, ME, USA) at an inter-pulse interval of 50 ms once every 15 seconds at an amplitude adjusted to produce consistent paired-pulse facilitation. Because the high Ca²⁺ low Mg²⁺ conditions of our ACSF(NMDA) tended to decrease PPF, it was generally necessary to use stimulus amplitudes well below 1/2 max on the input-output curve. The paired-pulse depression in Figure 2 was produced by increasing the stimulus amplitude. For simplicity, we have used the abbreviation R1 for the first response and R2 for the second response to a pair of stimuli and P1 for the response to a single stimulus. The amplitude of the R2 EPSC was measured as an increment in whole cell current which in some instances had not decayed entirely to base following the R1 EPSC. Data were post-filtered with a Gaussian low-pass filter at 1 kHz, which removed the majority of the high frequency noise intrinsic to the CV-7B Headstage.

4.4 Curve fitting

Progressive MK-801-dependent block (PMDB) data were fit using a non-linear least squares regression to equations 1 – 3:

$$normEPSC = Ae^{-sn/\tau_1} + Be^{-sn/\tau_2} \quad (1)$$

where sn is the stimulus number, A and B are scaling constants, C is an asymptote, τ₁ and τ₂ are decay constants (equivalent to time constants and hence represented with τs), and r is inversely proportional to Pr (Huang and Stevens, 1997).

Input-output relationship data in dextran experiments were fit using a least squares regression to a Hill function:

$$normEPSC = \frac{1}{(1 + \frac{sn}{r})} \quad (2)$$

$$normEPSC = Ae^{-sn/\tau_1} + Be^{-sn/\tau_2} + C \quad (3)$$

$$normEPSC = \frac{Y_{max}}{1 + \left(\frac{K_D}{I_{stim}}\right)^n} \quad (4)$$

where Y_{max} is the maximum normalized EPSC, I_{stim} is the stimulus current intensity, K_D is the stimulus intensity that produces a half maximum amplitude EPSC, and n is the Hill coefficient. All fits employed the user defined feature of ProStat (v 6.5, Poly Software International, Pearl River NY) with either the Levenberg-Marquardt or Simplex methods.

4.5 Drugs

Dextran (40,000 M_r) was from MP Biochemicals, (Solon, OH); CNQX (6-Cyano-7-nitroquinoxaline-2,3-dione), picrotoxin, DL-TBOA (DL-*threo*- β -Benzyloxyaspartic acid), (+)-MK-801 Maleate ((5*S*,10*R*)-(+)-5-Methyl-10,11-dihydro-5*H*-dibenzo[*a,d*]cyclohepten-5,10-imine maleate), (+)-Bicuculline, Memantine hydrochloride, and (+)-Tubocurarine chloride were from Tocris Bioscience (Bristol, England); all other chemicals were from Sigma Aldrich (Milwaukee, WI).

4.6 Statistical analysis

Statistical analyses were calculated using either ProStat (v 6.5, Poly Software International, Pearl River NY) or SPSS (Sun Microsystems, Chicago IL). Significance is indicated with: $P < 0.05$ *, $P < 0.01$ **, $P < 0.005$ ***, $P < 0.001$ ****.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported in part by National Institutes of Health; Grant number: MH070386. The authors would like to thank Drs. Fernando Valenzuela and Russell Morton for technical assistance and critically reading the manuscript.

Abbreviations

R1,R2	first and second of paired stimuli
PMDB	progressive NMDA-dependent block
ACSF(NMDA)	0 Mg^{2+} ACSF with 10 μM CNQX and 50 μM picrotoxin or 20 μM bicuculline
P_r	synaptic release probability

References

- Arnth-Jensen N, Jabaudon D, Scanziani M. Cooperation between independent hippocampal synapses is controlled by glutamate uptake. *Nature neuroscience*. 2002; 5:325–31. [PubMed: 11896395]
- Bagley EE, Westbrook GL. Short-term field stimulation mimics synaptic maturation of hippocampal synapses. *The Journal of physiology*. 2012; 590:1641–54. [PubMed: 22351628]

- Castro-Alamancos MA, Connors BW. Distinct forms of short-term plasticity at excitatory synapses of hippocampus and neocortex. *Proceedings of the National Academy of Sciences of the United States of America*. 1997; 94:4161–6. [PubMed: 9108122]
- Cattani AA, Bonfardin VD, Represa A, Ben-Ari Y, Aniksztejn L. Generation of slow network oscillations in the developing rat hippocampus after blockade of glutamate uptake. *Journal of neurophysiology*. 2007; 98:2324–36. [PubMed: 17634340]
- Chamberland S, Evstratova A, Toth K. Interplay between Synchronization of Multivesicular Release and Recruitment of Additional Release Sites Support Short-Term Facilitation at Hippocampal Mossy Fiber to CA3 Pyramidal Cells Synapses. *The Journal of neuroscience: the official journal of the Society for Neuroscience*. 2014; 34:11032–11047. [PubMed: 25122902]
- Christie JM, Jahr CE. Multivesicular release at Schaffer collateral-CA1 hippocampal synapses. *The Journal of neuroscience: the official journal of the Society for Neuroscience*. 2006; 26:210–6. [PubMed: 16399689]
- Debanne D, Guerineau NC, Gahwiler BH, Thompson SM. Paired-pulse facilitation and depression at unitary synapses in rat hippocampus: quantal fluctuation affects subsequent release. *The Journal of physiology*. 1996; 491(Pt 1):163–76. [PubMed: 9011608]
- Deng PY, Sojka D, Klyachko VA. Abnormal presynaptic short-term plasticity and information processing in a mouse model of fragile X syndrome. *The Journal of neuroscience: the official journal of the Society for Neuroscience*. 2011; 31:10971–82. [PubMed: 21795546]
- Dittman JS, Kreitzer AC, Regehr WG. Interplay between facilitation, depression, and residual calcium at three presynaptic terminals. *The Journal of neuroscience: the official journal of the Society for Neuroscience*. 2000; 20:1374–85. [PubMed: 10662828]
- Dzubay JA, Jahr CE. Kinetics of NMDA channel opening. *The Journal of neuroscience: the official journal of the Society for Neuroscience*. 1996; 16:4129–34. [PubMed: 8753874]
- Fioravante D, Regehr WG. Short-term forms of presynaptic plasticity. *Current opinion in neurobiology*. 2011; 21:269–74. [PubMed: 21353526]
- Franks KM, Bartol TM Jr, Sejnowski TJ. A Monte Carlo model reveals independent signaling at central glutamatergic synapses. *Biophysical journal*. 2002; 83:2333–48. [PubMed: 12414671]
- Freneau RT Jr, Kam K, Qureshi T, Johnson J, Copenhagen DR, Storm-Mathisen J, Chaudhry FA, Nicoll RA, Edwards RH. Vesicular glutamate transporters 1 and 2 target to functionally distinct synaptic release sites. *Science*. 2004; 304:1815–9. [PubMed: 15118123]
- Hanse E, Gustafsson B. Paired-pulse plasticity at the single release site level: an experimental and computational study. *The Journal of neuroscience: the official journal of the Society for Neuroscience*. 2001; 21:8362–9. [PubMed: 11606624]
- Hardingham GE, Bading H. Synaptic versus extrasynaptic NMDA receptor signalling: implications for neurodegenerative disorders. *Nature reviews. Neuroscience*. 2010; 11:682–96. [PubMed: 20842175]
- Harney SC, Jane DE, Anwyl R. Extrasynaptic NR2D-containing NMDARs are recruited to the synapse during LTP of NMDAR-EPSCs. *The Journal of neuroscience: the official journal of the Society for Neuroscience*. 2008; 28:11685–94. [PubMed: 18987204]
- Harris AZ, Pettit DL. Extrasynaptic and synaptic NMDA receptors form stable and uniform pools in rat hippocampal slices. *The Journal of physiology*. 2007; 584:509–19. [PubMed: 17717018]
- Harris AZ, Pettit DL. Recruiting extrasynaptic NMDA receptors augments synaptic signaling. *Journal of neurophysiology*. 2008; 99:524–33. [PubMed: 18057106]
- Hessler NA, Shirke AM, Malinow R. The probability of transmitter release at a mammalian central synapse. *Nature*. 1993; 366:569–72. [PubMed: 7902955]
- Huang EP, Stevens CF. Estimating the distribution of synaptic reliabilities. *Journal of neurophysiology*. 1997; 78:2870–80. [PubMed: 9405507]
- Huettnner JE, Bean BP. Block of N-methyl-D-aspartate-activated current by the anticonvulsant MK-801: selective binding to open channels. *Proceedings of the National Academy of Sciences of the United States of America*. 1988; 85:1307–11. [PubMed: 2448800]
- Ireland DR, Abraham WC. Mechanisms of group I mGluR-dependent long-term depression of NMDA receptor-mediated transmission at Schaffer collateral-CA1 synapses. *Journal of neurophysiology*. 2009; 101:1375–85. [PubMed: 19109458]

- Kampa BM, Clements J, Jonas P, Stuart GJ. Kinetics of Mg²⁺ unblock of NMDA receptors: implications for spike-timing dependent synaptic plasticity. *The Journal of physiology*. 2004; 556:337–45. [PubMed: 14754998]
- Kondratskaya E, Shin MC, Akaike N. Neuronal glutamate transporters regulate synaptic transmission in single synapses on CA1 hippocampal neurons. *Brain research bulletin*. 2010; 81:53–60. [PubMed: 19665527]
- Korinek M, Sedlacek M, Cais O, Dittert I, Vyklicky L Jr. Temperature dependence of N-methyl-D-aspartate receptor channels and N-methyl-D-aspartate receptor excitatory postsynaptic currents. *Neuroscience*. 2010; 165:736–48. [PubMed: 19883737]
- Le Meur K, Galante M, Angulo MC, Audinat E. Tonic activation of NMDA receptors by ambient glutamate of non-synaptic origin in the rat hippocampus. *The Journal of physiology*. 2007; 580:373–83. [PubMed: 17185337]
- Lester RA, Jahr CE. NMDA channel behavior depends on agonist affinity. *The Journal of neuroscience: the official journal of the Society for Neuroscience*. 1992; 12:635–43. [PubMed: 1346806]
- Luccini E, Musante V, Neri E, Raiteri M, Pittaluga A. N-methyl-D-aspartate autoreceptors respond to low and high agonist concentrations by facilitating, respectively, exocytosis and carrier-mediated release of glutamate in rat hippocampus. *Journal of neuroscience research*. 2007; 85:3657–65. [PubMed: 17671992]
- Manabe T, Nicoll RA. Long-term potentiation: evidence against an increase in transmitter release probability in the CA1 region of the hippocampus. *Science*. 1994; 265:1888–92. [PubMed: 7916483]
- McGuinness L, Taylor C, Taylor RD, Yau C, Langenhan T, Hart ML, Christian H, Tynan PW, Donnelly P, Emptage NJ. Presynaptic NMDARs in the hippocampus facilitate transmitter release at theta frequency. *Neuron*. 2010; 68:1109–27. [PubMed: 21172613]
- Min MY, Rusakov DA, Kullmann DM. Activation of AMPA, kainate, and metabotropic receptors at hippocampal mossy fiber synapses: role of glutamate diffusion. *Neuron*. 1998; 21:561–70. [PubMed: 9768842]
- Molinari F, Cattani AA, Mdzomba JB, Aniksztejn L. Glutamate transporters control metabotropic glutamate receptors activation to prevent the genesis of paroxysmal burst in the developing hippocampus. *Neuroscience*. 2012
- Mongillo G, Barak O, Tsodyks M. Synaptic theory of working memory. *Science*. 2008; 319:1543–6. [PubMed: 18339943]
- Newpher TM, Ehlers MD. Glutamate receptor dynamics in dendritic microdomains. *Neuron*. 2008; 58:472–97. [PubMed: 18498731]
- Nielsen TA, DiGregorio DA, Silver RA. Modulation of glutamate mobility reveals the mechanism underlying slow-rising AMPAR EPSCs and the diffusion coefficient in the synaptic cleft. *Neuron*. 2004; 42:757–71. [PubMed: 15182716]
- Papouin T, Ladepeche L, Ruel J, Sacchi S, Labasque M, Hanini M, Groc L, Pollegioni L, Mothet JP, Oliet SH. Synaptic and Extrasynaptic NMDA Receptors Are Gated by Different Endogenous Coagonists. *Cell*. 2012; 150:633–46. [PubMed: 22863013]
- Partridge LD, Scullin CS, Wilson MC. Changes in hippocampal presynaptic Ca buffering assessed with a Snap25 mutant mouse. *Society for Neuroscience Abstracts*. 2008; 34(2)
- Rosenmund C, Clements JD, Westbrook GL. Nonuniform probability of glutamate release at a hippocampal synapse. *Science*. 1993; 262:754–7. [PubMed: 7901909]
- Santamaria F, Wils S, De Schutter E, Augustine GJ. The diffusional properties of dendrites depend on the density of dendritic spines. *The European journal of neuroscience*. 2011; 34:561–8. [PubMed: 21771115]
- Schiess AR, Partridge LD. Pregnenolone sulfate acts through a G-protein-coupled sigma1-like receptor to enhance short term facilitation in adult hippocampal neurons. *European journal of pharmacology*. 2005; 518:22–9. [PubMed: 15996654]
- Schiess AR, Scullin CS, Partridge LD. Neurosteroid-induced enhancement of short-term facilitation involves a component downstream from presynaptic calcium in hippocampal slices. *The Journal of physiology*. 2006; 576:833–47. [PubMed: 16931546]

- Schiess AR, Scullin C, Partridge LD. Maturation of Schaffer collateral synapses generates a phenotype of unreliable basal evoked release and very reliable facilitated release. *The European journal of neuroscience*. 2010; 31:1377–87. [PubMed: 20384768]
- Scullin CS, Partridge LD. Contributions of SERCA pump and ryanodine-sensitive stores to presynaptic residual Ca^{2+} Cell calcium. 2010; 47:326–38. [PubMed: 20153896]
- Thomas CG, Krupp JJ, Bagley EE, Bauzon R, Heinemann SF, Vissel B, Westbrook GL. Probing N-methyl-D-aspartate receptor desensitization with the substituted-cysteine accessibility method. *Molecular pharmacology*. 2006; 69:1296–303. [PubMed: 16377766]
- Thomas CG, Tian H, Diamond JS. The relative roles of diffusion and uptake in clearing synaptically released glutamate change during early postnatal development. *The Journal of neuroscience: the official journal of the Society for Neuroscience*. 2011; 31:4743–54. [PubMed: 21430173]
- Wroge CM, Hogins J, Eisenman L, Mennerick S. Synaptic NMDA Receptors Mediate Hypoxic Excitotoxic Death. *The Journal of neuroscience: the official journal of the Society for Neuroscience*. 2012; 32:6732–42. [PubMed: 22573696]
- Xia P, Chen HS, Zhang D, Lipton SA. Memantine preferentially blocks extrasynaptic over synaptic NMDA receptor currents in hippocampal autapses. *The Journal of neuroscience: the official journal of the Society for Neuroscience*. 2010; 30:11246–50. [PubMed: 20720132]
- Zheng K, Scimemi A, Rusakov DA. Receptor actions of synaptically released glutamate: the role of transporters on the scale from nanometers to microns. *Biophysical journal*. 2008; 95:4584–96. [PubMed: 18689452]
- Zucker RS, Regehr WG. Short-term synaptic plasticity. *Annual review of physiology*. 2002; 64:355–405.

Highlights

Progressive MK-801 block is slower for facilitated than for basal EPSCs.

These data are consistent with an exclusive population of facilitated NMDA receptors.

Extra-synaptic receptors are not a major contributor to short term facilitation

Short term facilitation selectively recruits low P_r basal release sites.

Figure 1.

Progressive MK-801-dependent block of paired NMDAR EPSCs in ACSF(NMDA). A. Representative traces showing every 2nd EPSC during PMDB. (R1, black; R2, gray). B. Normalized EPSCs during single and paired-pulse PMDB. Paired-pulse: R2 normalized to initial value of R1 and plotted as a function of every other stimulus. Least squares fits of double exponential decay (equation 1) to mean values. (Paired-pulse, R1, (black, solid) $R^2 = 0.976$, R2 (gray, solid) $R^2 = 0.980$, $n=9$; Single pulse, P1 (black, dotted) $R^2 = 0.992$, $n=8$ (mean $\pm$ sem). C. Individual experiments were fit with a least squares regression to equation 1 and resultant parameters were averaged. Comparisons by one-way ANOVA with Bonferroni post hoc (A: $F_{(2,23)} = 4.629$ $p = 0.020$; τ_1 : $F_{(2,23)} = 7.144$ $p = 0.004$; τ_2 : $F_{(2,23)} = 2.184$ $p = 0.135$; B: $F_{(2,23)} = 4.320$ $p = 0.026$; A/(A+B): $F_{(2,23)} = 0.156$ $p = 0.857$).

Figure 2.

Paired-pulse depression A. PPD in response to increased strength of presynaptic stimulus. A. Representative traces showing every 2nd EPSC during the initial period of PMDB. (R1, black; R2, gray). B. Normalized EPSCs during paired-pulse PMDB. Paired-pulse, R2 normalized to initial value of R1 and plotted as a function of every other stimulus.. Least squares fits of double exponential decay (equation 1) to mean values. (R1, black, $R^2 = 0.984$; R2, gray, $R^2 = 0.981$; $n = 9$.) (mean $\pm$ sem). C. Individual experiments were fit to equation 1 and resultant parameters were averaged. Comparisons by *t*-test.

Figure 3.

Response of paired EPSCs to modulators of synaptic glutamate. A. Time course of R1 (black) and R2 (gray) EPSC amplitudes during bath application of dextran (A1), DL-TBOA (A2), and memantine (A3). Calculated estimate of bath concentration is shown in pink. Average R1 and R2 NMDAR EPSCs (B) and PPF (C). ACSF(NMDA) (10 μ M CNQX, 50 μ M picrotoxin) determined from average of all drug trials measured before drug application (120 – 300 s). Unpaired *t*-test showed no difference between R1 and R2 values in ACSF(NMDA) vs. ACSF(NMDA) + curare ($p = 0.591$) so memantine control values were combined with those from dextran and DL-TBOA; drug trials were measured after bath exchange (375 – 525 s): dextran (5% w/v dextran, $n=13$), DL-TBOA (30 μ M DL-TBOA, $n=6$), and memantine (5 μ M memantine + 10 μ M curare, $n= 14$). Comparisons by one-way ANOVA with Bonferroni post hoc: B. $F_{(9,176)} = 92.201$ $p < 0.001$ probabilities shown only for R1 and R2 against respective values in ACSF(NMDA). C. $F_{(4,88)} = 13.856$, $p < 0.001$ probabilities shown only for comparison to ACSF(NMDA). D. PPR = R2 EPSC/R1 EPSC measured in separate experiments. Respective transitions from PPF to PPD are marked with a vertical arrow. (ACSF(NMDA), red, $n = 18$; dextran, blue, $n = 10$; TBOA, violet, $n = 15$; memantine, orange, $n = 15$).

Figure 4.

Facilitated R2 following reduced postsynaptic R1. A1. Clamp protocols: Protocol A (thick line), $E_h = -70$ mV, $E_c = +40$ mV; Protocol B (thin line, “no R1”), $E_h = -70$ mV, $E_{c-R1} = -100$ mV, $E_{c-R2} = +40$ mV; Cs^+ internal solution, E_m based on junction potential compensation of 6.1 mV. A2. Representative whole cell current measured in response to R1 (black) and R2 (gray) stimuli with protocols A (thick line) and B (thin line). A3. Conductances for currents shown in A2. For determination of NMDAR $E_{rev} = 7.2$ mV and transient membrane conductances, R_s compensation was calculated from access resistance as determined by ClampEx and measured currents at steady state clamp potentials in the absence of presynaptic stimuli. B. Average currents (left) and conductances (right) normalized to R1 for protocol A ($R1_A$). Comparisons by one-way ANOVA with Bonferroni post hoc: norm I : $F_{(3,44)} = 33.335$, $p < 0.0001$, norm g $F_{(3,44)} = 24.027$, $p < 0.0001$. ($n = 12$).

Figure 5.

PMDB of paired NMDAR EPSCs in drugs affecting synaptic glutamate. A. Paired-pulse: R2 (gray) normalized to initial value of R1 (black) and plotted as a function of stimulus number (R1 even, R2 odd). Least squares double exponential fits of equation 1 to mean values. TBOA Slices were maintained in ACSF(NMDA) and 30 μ M DL-TBOA for 5 min then 50 μ M MK-801 was added for 10 minutes with no stimulus before recordings were begun. (n=11). Memantine Slices were maintained in ACSF(NMDA) with 5 μ M memantine and 10 μ M curare for 5 minutes then 50 μ M MK-801 was added for 10 minutes with no stimulus before recordings were begun (n=9). Dextran Slices were maintained in ACSF(NMDA) for 5 min then 50 μ M MK-801 was added for 8 minutes with no stimulus and then for an additional 2 minutes in 50 μ M MK-801 + 5% w/v 40,000 M_r dextran with no stimulus before recordings were begun (n=10). "no R1" Slices were maintained in ACSF(NMDA) for 5 minutes and control protocol A and B records obtained then 50 μ M MK-801 was added for 10 minutes with no stimulus before recordings were begun. R1 records are from subsequent protocol A recordings and R2 records are from subsequent protocol B recordings both normalized to the protocol A R1 control record. (n = 5). B. Least squares fit to equation 1 of individual experiments with resultant parameters averaged. Comparisons by paired *t*-test for TBOA, memantine, and dextran and un-paired *t*-test for "no-R1." C. Least squares fit of data in A with a continuous distribution (equation 3, (Huang and Stevens, 1997)). *P*'s calculated from these fits are given in Table 2.

Figure 6.

Fits of $R2 = f(R1, n)$ for average data from 6 experimental conditions. Non-linear least squares fits generated by MatLab curve fitting tool using Trust-Region algorithm to the computational model equations described in the Supporting Information for the average data generated during PMDB for ACSF(NMDA), red, $R^2 = 0.986$. Fitting parameters (F_{Ca} , P_{rB} , P_{rF} , P_{bB} , and P_{bF}) were fixed. R2 basal site scaling factor, K , was determined for each of the 6 conditions. Basal and facilitated pathway contributions (F_B and F_F) were variables in fitting the remaining experimental conditions. Resultant best fit curves (A) and projection onto the R2 vs R1 axes with average data superimposed (B). PPD, light blue, $R^2 = 0.899$; memantine, orange, $R^2 = 0.933$; TBOA, purple, $R^2 = 0.919$; "no R1," green, $R^2 = 0.937$; and dextran, dark blue, $R^2 = 0.792$.

Table 1

Coefficients of determination for non-linear least squares fits to PMDB in ACSF(NMDA).

	R1	R2
1 exponential term	$R^2 = 0.737$	$R^2 = 0.799$
2 exponential terms (equation 1)	$R^2 = 0.992$	$R^2 = 0.997$
3 exponential terms	$R^2 = 0.992$	$R^2 = 0.997$
continuous function (equation 2)	$R^2 = 0.893$	$R^2 = 0.892$

Table 2

Probability of release from non-linear least squares fit to a continuous function (equation 2). Values proportional to P_r for R1 (P_{r1}) and R2 (P_{r2}) calculated as the reciprocal of r for data shown in Figure 5C. ACSF(NMDA) data are from fits to data in Figure 1B (fit not shown) (mean $\pm$ sem).

	ACSF	TBOA	memantine	dextran	“no RI”	PPD
P_{r1}	0.22 ± 0.05	0.19 ± 0.03	0.28 ± 0.17	0.360 ± 0.10	0.11 ± 0.03	0.24 ± 0.01
P_{r2}	0.13 ± 0.02	0.12 ± 0.04	0.13 ± 0.05	0.133 ± 0.03	0.20 ± 0.05	0.09 ± 0.02

Table 3

Summary of selected fitting parameters. Column a. Ratio of fast (A) component from fit of R2 data with a double exponential equation (equation 1) to that of an equivalent fit to R1 data $\{A_{R2}/A_{R1} = [R2A/(R2A + R2B)] \div [R1A/(R1A + R1B)]\}$ (Figure 1C, 3C, 6B). (one-tailed *t*-test with respect to a parent mean of 1.0: “no R1,” $p < 0.005$; dextran, $p = 0.0517$; PPD, $p < 0.05$; mean $\pm$ sem). Column b. Ratio of R2 to R1 P_r as determined from fits with a continuous function (equation 2) (Figure 5C) (one-tailed *t*-test with respect to a parent mean of 1.0: ACSF, $p < 0.05$; memantine, $p < 0.05$; TBOA, $p < 0.05$; “no R1,” $p < 0.05$; dextran, $p < 0.005$; PPD, $p < 0.005$; mean $\pm$ sem). (For “no R1” experiments in column a & b, R1 amplitudes from control protocol A measurements preceeding protocol B PMDB experiments were used to match R1 and R2 data.) Column c. Effective P_r2/P_r1 as predicted by the computational model. ACSF(NMDA) values are based on fits given in table A1. Values for the remaining 5 conditions used these parameters and the variables given in table A2.

	a. norm $A_{R2}/\text{norm } A_{R1}$	b. P_r2/P_r1	c. model P_r2/P_r1
ACSF (NMDA)	0.98 ± 0.06	$0.68 \pm 0.11^*$	0.89
memantine	0.94 ± 0.12	$0.75 \pm 0.12^*$	0.67
TBOA	1.11 ± 0.25	$0.63 \pm 0.15^*$	0.66
dextran	0.72 ± 0.16	$0.47 \pm 0.12^{***}$	0.14
PPD	$0.75 \pm 0.13^*$	$0.53 \pm 0.09^{***}$	0.32
“no R1”	$2.24 \pm 0.52^{***}$	$2.26 \pm 0.56^*$	1.90