Introduction

Excitatory synapses onto hippocampal CA1 pyramidal cells are a well-studied model system for understanding synaptic transmission and plasticity in the central nervous system [1]. These synapses contain two types of ionotropic receptors activated by the neurotransmitter glutamate: the fast α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor and the slower N-methyl-D-aspartate (NMDA) receptor [2]. The NMDA receptor (NMDAR) is permeable to Ca²⁺, which in turn drives multiple forms of synaptic plasticity [3–6] thought to underlie forms of learning and memory [1]. In addition to its role in plasticity, slow NMDAR currents help shape the dynamic activity of neurons and neural networks [7,8].

The NMDAR is a multimer, composed of two obligatory NR1 subunits and two (or more) NR2 subunits [9]. The NR2 subunit exists in multiple isoforms. In the mammalian forebrain, the majority of NR2 subunits are of the NR2A or NR2B subtype [9]. The expression levels of the NR2 subtypes are developmentally regulated [10]. At birth, the NR2B subtype is dominant and there is very little NR2A expression. During the course of development, NR2A expression gradually rises to adult levels. NR2A- and NR2B-containing receptors may exhibit differences in their spatial localization [11–14] and may also vary in relative numbers across synapses [15–17]. NR2 subunit identity can also confer very different biophysical properties onto the NMDA receptor [9,18]. Differences in subunit composition could thus have important consequences for synaptic plasticity and neuronal function. Notwithstanding the importance of these issues, fundamental questions concerning the activation properties of NMDA receptors containing different NR2 subtypes remain open.

One question concerns the fidelity with which NMDARs of distinct subunit composition at the synapse respond to glutamate release. Some studies have suggested that the open probabilities of NR2A- and NR2B-containing receptors are similar [19], while others indicate that they are dramatically different [20,21]. Pharmacological isolation of each receptor subtype could potentially resolve this issue, but the available drugs for blocking NR2A-containing receptors are not specific enough [22–24]. A second question concerns the number of functional NMDARs at a synapse. Measurement of NMDAR activation at single spines using two-photon glutamate uncaging and calcium imaging has begun to address this issue [17,25,26]. However, it is not known how these measurements would be affected by differences in NR2 subunit...
composition. Other open questions concern the spatial distribution of NMDARs containing different NR2 subtypes in and out of synapses and the response of receptors at different locations to glutamate release. These may have particular bearing on experiments that suggest spontaneously released vesicles activate a different population of synaptic receptors from those activated by vesicles released due to action potentials [13,27]. The role of NR2 subunit identity in long-term potentiation is also an open question, as the experimental evidence is contradictory [23,28].

Computational models with parameters well-constrained by experimental measurements can shed light on these issues. Previous models examining the role of the NMDA channel in synaptic transmission [29–31] have not included NR2 subtype differences and therefore have been unable to address how different subtypes could contribute to the synaptic response. To better understand the potential role of differential NR2 subunit-dependent NMDAR kinetics in synaptic transmission and plasticity, we have constructed a biophysically-realistic model of a CA1 excitatory synapse, incorporating glutamate release, diffusion and binding, and NMDAR opening and closing. We then used this model to address each of the open questions mentioned above. The model allowed us to interpret and integrate previous experimental results, as well as to suggest experiments to address remaining open questions.

**Results**

**NR2A-Containing NMDARs Open More Reliably and More Rapidly Than NR2B-Containing NMDARs**

Previous models of NMDA receptors [30–33] have been based on the kinetic schemes derived from recordings by Lester and Jahr [29]. Since then, genetic techniques have allowed for a more detailed understanding of the biophysics of NMDA channel gating [21,34,35]. Erreger et al. [21] measured single-channel NMDAR kinetics of recombinant diheteromeric NMDARs by expressing NR1 with either NR2A or NR2B. These recordings were used to fit kinetic schemes for each receptor type (Figure 1E) and predicted the average behavior of the channels in response to brief glutamate pulses. The surprising result from this study was that the kinetics of previous models of NMDA receptors were similar to those observed for NR2B-containing receptors, with slow opening, closing and glutamate unbinding. The NR2A-containing receptors, on the other hand, showed markedly faster kinetics, with an open probability 10 times higher, the early decay component time constant was four times faster, and the probability of success a receptor opening in response to glutamate release (P(success)) was three times greater for NR2A-containing receptors than for NR2B-containing receptors. 

**Figure 1. Receptor kinetics and open probability.** (A) The kinetic scheme used to model the receptors. O is the open state; D1 and D2 are desensitized; 0, 1, 2, C1 and C2 are closed. Rate constants, taken from Erreger et al. [21], and adjusted for temperature, are listed in Table 2. (B,C) Time course of free glutamate concentration in the synaptic cleft. Glutamate concentration peaked and decayed rapidly; the initial t of decay was −60 μsec. (D,E) Examples of single receptor opening and closing in response to glutamate release show that NR2A-containing receptors fail less often, and open and close more rapidly than NR2B-containing receptors. Note that the simulations were carried out at 33°C, so the receptor kinetics are significantly faster than those observed by Erreger et al. [21]. (F,G) Peak open probability was 10 times higher, the early decay component time constant was four times faster, and the probability of success a receptor opening in response to glutamate release (P(success)) was three times greater for NR2A-containing receptors than for NR2B-containing receptors.

doi:10.1371/journal.pcbi.1000208.g001
on-rate constant for glutamate (\(3\times10^7/\text{M} \cdot \text{sec}\)) similar to that of the AMPA receptor (\(2\times10^7/\text{M} \cdot \text{sec}\)). These results also implied that NR2A-containing receptors were better suited to sense rapid glutamate transients in the synaptic cleft and would open with a high probability, while NR2B receptors appeared to be tuned to sense ambient levels of glutamate and would open with much lower probability. However, the study used simplified models of glutamate concentration in the cleft and therefore may not inform us about how NMDA channels open in response to synaptically released glutamate.

We have previously developed a stochastic model of AMPA receptor transmission [36] using standard Monte Carlo techniques [31,37,38], which accounted for channel structure, activation and desensitization [39–42]. By implementing the kinetic schemes fit for NMDA receptors [21] within this biophysically-realistic Monte Carlo framework, we were able to simulate individual NMDA receptor responses to realistic glutamate signals. The model tracked the diffusion of individual glutamate molecules in a structurally-constrained model of the synapse. The simulated synapse had dimensions corresponding to an average nonperforated synapse on a mushroom or stubby spine [43]. Glutamate was released from a vesicle and diffused through a fusion pore into the cleft, where it diffused out into the extrasynaptic space, potentially interacting with the receptors on its way. Parameters used for the simulation of glutamate diffusion are listed in Table 1. The temperature-adjusted rate constants of the NMDAR model are listed in Table 2.

NR2A-containing receptors (NR2A-NMDARs) were about three times as likely as NR2B-containing receptors (NR2B-NMDARs) to open in response to the release of a single vesicle (\(P=0.73\) vs. 0.25). NR2A-NMDARs opened and closed much more quickly, and their peak open probability was more than 10 times greater (0.34 vs. 0.03, Figure 1B–E). On the other hand, NR2B-NMDARs closed more slowly than NR2A-NMDARs (\(t_d=14.4\) vs. 130 msec, \(t_1=12.7\) vs. 47.9 msec, \(t_2=505\) vs. 964 msec, Figure 2A and 2B). The weighted time constant of decay, \(t_w\), was calculated by taking an average of the two time constants (\(t_1\) and \(t_2\)) derived from a double exponential fit, weighted by their coefficients in that fit. When NR2B-containing NMDARs opened, they spent twice as much time open (8.0 vs. 16 msec) as NR2A-containing receptors. Thus, the overall time open and average open probability were only about 50 percent greater for NR2A-NMDARs (time open = 5.9 vs. 4.1 msec, Figure 2D).

**Table 1.** Parameters used in simulation of glutamate diffusion.

| Parameter                  | Value             | Notes                  | References |
|----------------------------|-------------------|------------------------|------------|
| Temperature                | 33°C              |                        |            |
| \(Q_10\) (diffusion)       | 1.4/10°C          |                        |            |
| \(Q_10\) (rate constants) | 2.2/10°C          | [110]                  |            |
| Diffusion coefficient      | 5.0 cm²/s         | 7.6 cm²/s in free solution | [106]     |
| Cleft width                | 15 nm             | [38]                   |            |
| Synapse area               | 0.12 μm²          | [43]                   |            |
| Glutamate in vesicle       | 2000 μM           | 200 mM [119]           |            |
| Transporter membrane       | 0.1 10000/μm²     | [111]                  |            |
| Transporter binding rate   | 3.2 \(\times10^7/\text{M} \cdot \text{sec}\) | [112]                  |            |
| Transporter unbinding rate | 3016/sec          | [112]                  |            |
| Transporter transport rate | 905/sec           | [112]                  |            |

**Table 2.** Rate constants for NMDAR models.

| Parameter                  | NR2A-NMDAR | NR2B-NMDAR | Triheteromer |
|----------------------------|------------|------------|--------------|
| \(k_{on}\)                 | 50.6 \(\times10^7/\text{M} \cdot \text{sec}\) | 4.53 \(\times10^7/\text{M} \cdot \text{sec}\) |              |
| \(k_{off}\)                | 3046/sec   | 115/sec    |              |
| \(k^+\)                    | 9469/sec   | 8553/sec   | 9011/sec     |
| \(k^-\)                    | 694/sec    | 145/sec    | 443/sec      |
| \(k_{on}^-\)               | 525/sec    | 528/sec    | 526/sec      |
| \(k_{off}^-\)              | 537/sec    | 694/sec    | 591/sec      |
| \(k_{on}^+\)               | 257/sec    | 1659/sec   | 932/sec      |
| \(k_{off}^+\)              | 694/sec    | 338/sec    | 516/sec      |
| \(k_{on}^-\)               | 89.6/sec   | 245/sec    | 194/sec      |
| \(k_{off}^-\)              | 3.05/sec   | 2.74/sec   | 2.94/sec     |

*doi:10.1371/journal.pcbi.1000208.t001*

**Figure 2.** Time open and receptor failure. (A,B) Upon opening, NR2B-containing receptors stayed open much longer than NR2A-containing receptors. The late decay component time constant was twice as slow, and the weighted time constant of decay (\(t_w\)) was 10 times slower. (C) The probability of at least one receptor opening, given the number of receptors at the synapse, shows that very few NR2A-containing receptors are needed to provide near-perfect fidelity. (D) Total time open, given the number of receptors. Despite the fact that NR2A-containing receptors open three times as often, NR2B-containing receptors stay open longer, so total time open is only about 50 percent greater for NR2A-containing receptors. *doi:10.1371/journal.pcbi.1000208.g002*
currents from nucleated patches in response to brief applications of glutamate and MK-801, a very high affinity open-channel NMDAR blocker. The decrease in current over successive stimulations, which reflected the open probability during the previous applications, was essentially the same in the control and in both of the overexpression conditions. This is an indirect measurement of open probability however, and the lack of observed differences between conditions could be explained by a number of other factors. First, the affinities of MK-801 with channels containing different subtypes are not the same. Dravid et al. [44] reported that the IC$_{50}$ for NR2A-containing NMDA receptors was 4.5 times greater than for NR2B-containing receptors, while fitted kinetic schemes showed similar off-rate constants for the two receptor types. Thus, the on-rate constant should be about 4.5 times faster for NR2B-NMDARs, so they will be blocked faster for the same open probability. Second, the measure of open probability chosen to quantify the response, peak current, will produce a measurement that disproportionately reflects the opening of NR2A-containing receptors, due to their much faster opening and higher peak open probability.

To examine how these factors may have affected the results of Prybylowski et al. [19], we constructed a simulation of their experiment (Figure 3), using a modified version of our NMDAR kinetic schemes and the kinetic parameters from Dravid et al. [44] for the block of NMDARs by MK-801. The kinetic scheme was essentially a doubled version of the eight-state kinetic scheme, with a blocked and unblocked version of each of the eight states and a single, reversible connection between the blocked and unblocked open states. When MK-801 is bound to the receptor and the receptor is no longer in the open state, MK-801 becomes trapped, so both blocking and unblocking are glutamate-dependent. We used IC$_{50}$ values (18 and 4 nM for NR2A- and NR2B-NMDARs, respectively) for resting membrane voltage [44], and ran the simulation at 23°C [19]. We applied a single, 4 msec pulse of 1 mM glutamate, followed ten pulses of 200 μM MK-801 and 1 mM glutamate [19], spaced 10 seconds apart. We set the single free parameter, the off-rate constant for MK-801 (0.25/sec), so as to produce a block after the first stimulation similar to what was observed experimentally. The simulation was deterministic, and reproduced the probabilistic time evolution of receptor state. As expected, the open probabilities were quite different, with peak open probabilities of 0.42 for NR2A-NMDARs and 0.11 for NR2B-NMDARs (Figure 3C). The average percent block from one stimulation to the next was also quite different (19% vs. 8.4%)

When plotted relative to the peak open probability of the control stimulus, the slope of the change in peak open probability is initially higher for NR2A-containing receptors, but tapers off to a level similar to that of NR2B-containing receptors (Figure 3D). To approximate the overexpression cases of Prybylowski et al. [19], we considered the case of 80 percent NR2A-NMDARs and 20 percent NR2B-NMDARs versus 80 percent NR2B-NMDARs and 20 percent NR2A-NMDARs. The relative expression of the different subunit types in the experiment were unknown, so these cases were chosen to represent high and low expression cases; other choices yielded similar results. The normalized decline in peak open probability was very similar in the two cases (Figure 3H). As in the experimental results, the slope of the decline was initially steeper for the NR2A “overexpression” case (~0.11 vs. ~0.086/stimulation for stimuli 1–4), but similar later (~0.039 vs. ~0.038 for stimuli 5–10). The one feature observed by Prybylowski et al. [19] that our simulations did not reproduce was a larger relative block of NR2B-containing receptors after the first stimulation, impossible given the steeper initial decline for NR2A-containing receptors. However, Monte Carlo simulations of the experiment showed a high degree of trial-to-trial variability, so it is possible this feature was simply due to random trial-to-trial variation in the experiments (Prybylowski et al. [19] did not report the number of trials or show error bars for their data).

Figure 3. Estimating open probability using MK-801 block. Simulation of an experiment that used brief pulses of glutamate and MK-801 to estimate the open probability of receptors given different NR2A/NR2B ratios [19]. The average behavior of NR2A and NR2B-containing receptors under this protocol was simulated using a probabilistic model. (A,B) The responses of NR2A and NR2B-containing receptors alone, showing the responses to glutamate alone (Control) and to the 1st and 5th stimulations. (C,D) The peak open probability upon successive stimulations, unnormalized (C) and normalized relative to the response to glutamate alone (D), showing that NR2A-containing receptors had a higher open probability, and were blocked more rapidly. (E–H) Same as above, but for two mixed populations of receptors. A population containing 80 percent NR2A-containing receptors had a higher open probability and was blocked more rapidly than a population containing 80 percent NR2B-containing receptors. However, when plotted relative to control (H), the block appeared very similar in the two cases. Similar results were observed for other mixed populations.

doi:10.1371/journal.pcbi.1000208.g003
Multivesicular Release and NMDAR Saturation

Multiple lines of evidence have shown that multivesicular release, the release of more than one glutamate vesicle in response to a single action potential, may occur at central synapses [25,45,46]. However, little is known about the consequences for neural function. We have previously shown that AMPA receptors can respond in a nearly linear fashion to multivesicular release [36]. In order to extend these results to NMDA receptors, and compare the response of receptors with different subunit composition, we simulated multivesicular release by allowing glutamate to diffuse out of two vesicles. Results at this spatial and temporal spacing were representative of a variety of spacings; changing these parameters did not alter the results significantly. Figure 4A and 4B summarize the results. NR2B-NMDARs responded linearly, with the probability of success for two vesicles 2.1 times what it was for one (0.52 vs. 0.25), while NR2A-NMDARs showed only a modest increase in success probability (0.87 vs. 0.73, ratio = 1.2). Success probability was still 67 percent greater for NR2A- than for NR2B-NMDARs, but time open was 15 percent longer for NR2B-NMDARs (Figure 4B). Chavis and Westbrook [47] found a population of synapses expressing NR2B-containing NMDARs early in development that showed a high probability of NMDA receptor activation. These responses may have been due to an increased probability of multivesicular release at these synapses, or to an increase in the number of glutamate molecules per release event [48], typically denoted as the quantal size, q.

The reason NR2B-containing receptors are able to respond linearly to multivesicular release and NR2A-NMDARs are not is relatively intuitive. Since 75 percent of NR2A-NMDARs open in response to a single vesicle release, not many receptors are available to respond to the additional glutamate. NR2A-NMDARs, on the other hand, have a low probability of opening, so there are enough receptors available to produce a graded response. This may seem counterintuitive given the higher affinity of NR2B-NMDARs, but the conditions simulated are far from steady state, so the dynamic properties of the receptors, rather than their steady-state properties, determine their behavior. To further explore how receptor saturation shaped the NMDAR responses, we released a glutamate from a single vesicle and varied q from 1000 to 20000 molecules. While this is not a physiological value for q, it does illustrate how NR2B-NMDAR responses saturate at much higher glutamate levels. Figure 4C and 4D show how success probability and time open increase with the number of glutamate molecules released. NR2A-containing NMDAR success probability reaches 90 percent of its saturated level with only 4000 glutamate released, but NR2B-containing receptors do not reach 90 percent until 10000 glutamate, or the equivalent of

![Figure 4. Spatial pattern of receptor opening.](https://www.ploscompbiol.org/article/f1000208.g004)
five vesicles, are released. This is in agreement with experimental results showing that NMDA responses were not saturated by single release events [49] and previous simulations where only a single receptor subtype (NR2B) was considered [31]. Our results suggest that at synapses where multivesicular release can occur, or even where the glutamate content of single vesicles is variable, NR2B-containing NMDARs could be very important for the transduction of graded glutamate signals.

Location Dependence of NMDAR Opening

We next studied the location-dependence of NMDAR activation relative to the site of glutamate release. When glutamate is released from a vesicle in the active zone, a very short-lived, high-concentration "hot spot" is produced in the synaptic cleft. AMPA receptors are very sensitive to this, and their probability of opening shows a similar hot spot around the site of release [36]. We would expect NMDARs, which have a higher affinity and lower desensitization, to open in response to more distant glutamate release, but we would also expect distinct differences between receptors containing different NR2 subtypes, due to their different kinetics. To investigate this, we compared the response of receptors in our model located close to the release site with the response of those located farther away. We held the site of release constant, close to the center of the synapse, and randomly varied the locations of the receptors. Figure 4E and 4F show the probability of receptor opening as a function of position in the cleft. NR2B-containing receptors showed an activation hot spot similar to that of AMPA receptors, while NR2A-NMDARs were almost indifferent to location. The NR2B-NMDARs closest to the release site (mean distance = 44 nm, $P = 0.46$) were more than three times as likely to open as those farthest away (mean distance = 229 nm, $P = 0.14$). For NR2A-NMDARs, the difference was less than 10 percent. The intuitive explanation of these response properties depends, again, on the dynamic properties of the receptors. While under steady-state conditions NR2B-NMDARs respond to lower concentrations of glutamate, under the conditions of a short-lived, high concentration glutamate signal the fast on-rate constant of NR2A-NMDARs that allows them to respond to lower concentrations of glutamate. Given the observed differences in location-dependence, regulation of the location of NR2B-containing receptors could have a profound effect on NMDAR transmission and synaptic plasticity. Indeed, an electrophysiological study in knockout mice showed that the location of NR2B-NMDARs may indeed be developmentally regulated [13].

NMDA receptors, primarily of the NR2B-containing subtype, can also be found extrasynaptically [50]. Recent studies have shown that the extrasynaptic pool of NMDARs activate signaling pathways that are distinct from and even opposite to the ones activated by the synaptic pool [51]. We simulated the effect of extrasynaptic glutamate release on NMDA receptors located outside of the synaptic cleft but adjacent to the synapse, at distances of 300–750 nm from the release site. The results are summarized in Figure 4G. The probability of success of extrasynaptic NR2A-NMDARs fell off rapidly at the edge of the synapse, but was still 0.3 at 750 nm. Extrasynaptic NR2A-containing receptors have been reported recently [14], but they are probably quite rare, and a function has not been proposed for them. Our results suggest that if they are located in the vicinity of synapses, they should be fairly sensitive to single release events. NR2B-containing receptors, on the other hand, already had a low probability of opening at the edge of the synapse, which dropped to 0.04 at 750 nm. Individual extrasynaptic NR2B-NMDARs would be unlikely to open in response to glutamate release, and significant activation of extrasynaptic receptors would require that glutamate diffuse over an area of membrane large enough to contain a number of receptors. A number of studies have shown evidence of extrasynaptic NMDAR activation [33,32,53], suggesting that this may be the case. Our estimates of NR2B activation by efflux of glutamate from the cleft after the release of a single vesicle are similar to previous simulations [31,54] but somewhat lower than other models where glutamate diffusion occurs in a neuropil modeled as a porous medium [33,55]. This difference is due to assumptions about the amount of glutamate released by a single vesicle.

The Number of NMDARs at Hippocampal Synapses

The number of NMDARs at individual synapses has been estimated from studies using microscopy studies [56]. Moreover, a tissue preparation technique which provides near one-to-one labeling of receptors present has provided approximate lower and upper bounds (10 and 100) for this number [57]. However, these anatomical techniques cannot distinguish functional receptors [58]. Physiological measurements [2] can in principle yield the number of active NMDAR by comparing miniature EPSCs to single channel currents. However, dendritic spines are far too small to record from individually, and techniques such as minimal stimulation do not reliably isolate single synapses [59], so an alternative approach must be used. Two-photon glutamate uncaging [17,60,61] or calcium imaging [17,26] can be used to record synaptic activity at single dendritic spines.

Nimchinsky et al. [26] used calcium imaging to estimate the number of NMDARs at hippocampal synapses. They measured the frequency of synaptic failures in the presence and in the absence of D-CPP, a competitive NMDA antagonist, and calculated $m$, the number of NMDARs present at the synapse (see Methods). However, they did not differentiate between receptors containing different NR2 subtypes. We ran our simulations at 30°C [26] and determined the probabilities of opening for NR2A-NMDARs (0.70) and NR2B-NMDARs (0.20). Using the values of vesicle release probability, mean number of receptors opening and failure rate from Nimchinsky et al. [26], and measurements of NR2 subtype-dependent D-CPP block from Lozovaya et al. [62], we estimated $M_{NR2A}$ and $M_{NR2B}$, the average number of receptors per synapse containing NR2A and NR2B subtypes (see Methods). We arrived at estimates of 0.63 NR2A- and 11 NR2B-NMDARs, on average, per synapse.

Because these estimates depend on opening probability, and because the probability of opening of NR2B-containing NMDARs varies so dramatically with location, assumptions about the distribution of receptors will have a strong impact on the results. We calculated the number of receptors as above, but under the assumption that NR2B-NMDARs were located either near the release site, or at the periphery of the synapse. At 30°C the success probability for NR2B-containing receptors close to the release site was 0.40, and our calculations yielded an average of 0.44 NR2A- and 4.9 NR2B-NMDARs. If NR2B-containing receptors were located at the periphery, as proposed by Tovar and Westbrook [50], probability of opening dropped to 0.11 and the number of receptors rose to 0.69 NR2A- and 19.2 NR2B-NMDARs. Our estimates compare well with the limits placed by structural [56] and two-photon imaging measurements [26]. Moreover, given the differences in $P_{\text{open}}$ for the two subtypes, our models predict that blocking NR2B receptors would result in a mean reduction of 50 percent in peak current, which is consistent with experimental data [17].

Simulations of Triheteromeric NMDARs Using a Kinetic Model

Our kinetic models so far have been restricted to channels that exclusively contain NR2A or NR2B subunits. However, multi-

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October 2008 | Volume 4 | Issue 10 | e1000208
meric channels that contain both subunits are known to be present at hippocampal synapses [12,63], although a recent study indicated that the majority of receptors are diheteromeric [64].

Up to this time, no kinetic model exists for a triheteromeric channel, as isolation of these channels for recording would be extremely difficult, though it may be possible by exploiting differential sensitivity to antagonists such as ifenprodil and zinc [12,63,66]. Without a kinetic scheme, it is difficult to estimate the number of these channels at synapses. As a first-pass approximation, we constructed a kinetic scheme derived from the schemes of the diheteromeric channels (Figure 5A). We assumed that glutamate bound and unbound from each subunit independently, so there were two single-bound states (1A and 1B). The rate constants for these steps were the same as the rates for NR2A and NR2B in the diheteromeric models. For the other kinetic transitions, which are proposed to be due to conformational changes in the NR1 subunits, we set the forward and reverse rate constants of each state transition such that the ratio between these rates and the sum of the magnitudes of the rates were the mean of those in the NR2A- and NR2B-NMDAR models.

We used this kinetic scheme in our model to estimate the kinetics and probability of opening of these simulated triheteromeric receptors (Figure 5B). The triheteromeric kinetics were intermediate between those of NR2A- and NR2B-NMDARs, but closer to those of NR2A-NMDARs. The weighted time constant of decay was 19.0 msec ($\tau_2 = 15.8$ msec, $\tau_2 = 695$ msec), the probability of success was 0.41, and the time open given a success was 7.8 msec, yielding an overall time open of 3.1 msec. If triheteromeric receptors behave similarly to those in our model, then in mature animals, where NR2A expression levels are high, a significant fraction of the NMDA receptors would have kinetics significantly faster than those assumed by most previous models, even if the majority of NR2A subunits were incorporated in triheteromeric receptors. This would have significant consequences for the results of many previous models.

Using the results from this simulation, we repeated the calculation of the mean number of receptors per synapse from the data of Nimchinsky et al. [26] (Figure 5C). We assumed that inhibition constant for D-CPP for the triheteromeric channel was the geometric mean of those for the diheteromers. To solve for the number of receptors of three species ($M_{NR2A}$, $M_{NR2B}$, $M_{NR2AB}$), we needed an additional constraint. We calculated solutions under four different assumptions. The first, that only NR2A and NR2B receptors were present, is what we calculated earlier ($M_{NR2A} = 0.63$, $M_{NR2B} = 11$). The second, that only triheteromeric and NR2B receptors were present, yielded $M_{NR2AB} = 3.0$ and $M_{NR2B} = 0.3$. Under the assumption that only NR2A and NR2A/B receptors were present there was no positive solution. Finally, we assumed that all three types were present and that they combined randomly (that is, $M_{NR2AB} = \sqrt{M_{NR2A}M_{NR2B}}$). Under these conditions, $M_{NR2A} = 0.29$, $M_{NR2AB} = 1.7$ and $M_{NR2B} = 9.4$. Overall, these results indicate that NR2A subunits made up somewhere between 5 and 15 percent of the total NMDA subunits present in functional receptors. This is not particularly surprising given the developmental age of the animals, but a similar approach could be used to estimate the number of receptors present at different developmental time points. Such estimates would be valuable in understanding the role of NMDARs in adult synapses, and in understanding the role of the developmental NR2 subunit switch.

### Effects of NR2 Subtype on CaMKII Activation

Synaptic NMDARs are incorporated into multiprotein complexes and are in close proximity to many calcium-sensitive enzymes, such as Ca$^{2+}$-calmodulin-dependent kinase II (CaMKII) [67] and protein phosphatases [68]. These enzymes are ideally positioned to detect the time-varying calcium concentration changes due to influx through NMDARs and transduce these signals by altering the phosphorylation state of their various substrates. The ensuing signaling events can lead to rapid alteration in the number of AMPARs in the synapse [61,69–71], activation of protein synthesis machinery in dendrites [72] and gene transcription in nucleus important for long-term maintenance of neuronal plasticity [73]. A number of recent studies have suggested that NR2A- and NR2B-containing NMDA receptors selectively induce potentiation and depression, respectively, of hippocampal synapses [28,74,75]. However, other studies have suggested that either subtype can be sufficient for the induction of long-term potentiation [23,76], that NR2B-containing receptors

![Figure 5. Triheteromeric receptors and the number of receptors at a synapse.](image)
can drive LTP [77,78], or that either subtype can drive long-term depression [79]. In many of these studies, differences were seen depending on developmental age and induction protocol. The differences in the ability of the receptor subtypes to induce plasticity could arise due to their distinct kinetics, which result in distinct spatiotemporal pattern of calcium concentration in the postsynapse. The rapid, reliable opening of NR2A-containing NMDA receptors, would produce large rapid increases in internal Ca\(^{2+}\) concentrations, which has been shown to selectively lead to LTP [80]. On the other hand, the much longer-lived activation of NR2B-containing NMDARs could lead to enhanced potentiation in situations where depolarization occurs over a long period of time, such as during bursts. On average, NR2B-containing NMDA receptors let in as much or more Ca\(^{2+}\) than NR2A-containing NMDARs, but they also fail much more often. Therefore, the variability in the Ca\(^{2+}\) signal through NR2B-containing NMDARs is very high. This variability could have significant effects on LTP induction. We next explored these questions by coupling our model of NMDAR activation to a postsynaptic model of LTP.

We calculated calcium influx from receptor opening data from our Monte Carlo simulation and used it as the input to a model of a CaMKII switch [81]. This latter model is deterministic, and assumed that all reactions are taking place in a single, well-stirred compartment. Each molecular species was represented by a single, time-varying concentration, and the model was a system of differential equations relating those concentrations. In the model, CaMKII activation was bistable between an unphosphorylated state and an activated state where a large fraction of CaMKII subunits are phosphorylated (see Methods). Its activation is set by the balance of the rates of calcium-dependent phosphorylation and autophosphorylation with the rate of dephosphorylation by Protein Phosphatase 1 (PP1). Under baseline conditions, dephosphorylation is faster than phosphorylation and activity tends towards a low level. Once calcium-dependent phosphorylation pushes the level of activation above a threshold, autophosphorylation begins to out-compete dephosphorylation, and CaMKII activity tends towards a high level.

Calcium current was determined by a simple model, based on the Goldman-Hodgkin-Katz equations [26]. Conductance and calcium permeabilities were the same in NR2A- and NR2B-NMDARs, as has been measured experimentally [82,83]. We assumed the block of NMDARs by Mg\(^{2+}\) was an instantaneous, voltage-dependent process, and modeled by fitting a sigmoidal curve to fractional block versus voltage data [83]. Our LTP induction protocol (Figure 6A and 6B) consisted of a train of 100 stimuli delivered at 100 Hz. The synapse had 60 percent release failure and both facilitation and depression were modeled [84], based on measured values [85,86]. We modeled postsynaptic voltage using a simple, single exponential approximation of the results of a detailed simulation [87]. For each stimulus, the voltage exponentially approached \(-10\) mV for 1 msec with a time constant of 0.1 msec and fell back towards the resting voltage with a time constant of 9 msec.

NR2A-containing NMDARs let in more calcium per receptor than NR2B-NMDARs (Figure 6C–F), and were more effective at driving LTP (Figure 7A–E). The probability of a synapse to potentiate after tetanic stimulation exceeded 99 percent with only 3 NR2A-NMDARs present, while the same required 9 NR2B-NMDARs (Figure 7C). Even if we set the number of receptors such that the total time open was the same, NR2A-NMDARs showed a greater rate of potentiation. This is because the time they spent open was mostly right after glutamate release, while the postsynaptic cell was depolarized. The total time open during the one second tetanic stimulation period, however, predicted the probability of potentiation well (Figure 7D and 7E). This quantity was about three times longer per receptor for NR2A-NMDARs than for NR2B-NMDARs (87.4 vs. 26.1 msec). We ran the simulation using our hypothetical kinetic scheme for triheteromeric receptors. Again, the behavior of the NR2A/B receptors

![Figure 6](https://www.ploscompbiol.org/static/images/1000208/006.png)

**Figure 6.** Calcium influx in response to tetanic stimulation. (A,B) LTP was induced by 100 Hz tetanic stimulation with a duration of 1 sec. The postsynaptic voltage (A) and stochastic glutamate release (B) were both modulated synaptic facilitation and depression. (C–F) Example postsynaptic calcium concentration traces (C,D) from simulations with 5 NR2A-containing or 5 NR2B-containing NMDARs, and mean calcium concentration in the spine in for three different numbers of receptors (E,F) show that NR2A-NMDARs drove spine calcium concentration much higher, per receptor, than did NR2B-NMDARs.

doi:10.1371/journal.pcbi.1000208.g006
was intermediate between the diheteromers but more similar to that of NR2A-NMDARs (Figure 7F). Reaching a 99 percent probability of potentiation required 4 receptors, and the time open during the tetanus also predicted the probability of potentiation well.

The precise timing of postsynaptic spikes relative to presynaptic glutamate release can have drastic effects on the magnitude and direction of synaptic potentiation [88]. Because the opening of NR2A- and NR2B-containing NMDARs have very different time courses, they may show great differences in this kind of precise timing-dependent plasticity. To test this, we paired 50 presynaptic glutamate release events with 50 postsynaptic voltage spikes, and varied the relative timing between them (Figure 7G). NR2B-NMDARs showed a much broader window in which paired stimuli could still drive LTP, while NR2A-NMDARs required relatively precise timing. The width at half height for NR2B-NMDARs was twice that of NR2A-NMDARs (36 vs. 18 msec). This suggests that the NR2 subunit may play an important role in determining the spike timing-dependent properties of LTP.

Discussion

In this study, we considered the role of NMDA receptor NR2 subunit on synaptic transmission and synaptic plasticity. We used a kinetic model of NMDA receptors [21] in a model of a central nervous system excitatory synapse whose parameters were well constrained by experimental measurements. We explicitly modeled the release of the neurotransmitter glutamate from a synaptic vesicle, its diffusion in and out of the cleft, its binding to NMDA receptors, and the opening and closing of the receptors. We showed that NR2 subunit composition dramatically affects the probability and the spatiotemporal pattern of synaptic receptor activation which can have significant effects on the activation of signal transduction events downstream of receptor activation.

Fidelity and Kinetics of Receptor Opening

We found that NR2A-containing receptors were about three times as likely as NR2B-containing receptors to open in response to a single glutamate vesicle release. This is in agreement with previous, in vitro studies [20,21]. In addition, when NR2B receptors opened, their total time open was about twice as long on average as NR2A-containing receptors, so the trial-to-trial variability in time open was much greater. The kinetics of the NR2B-containing receptors were much slower, however, and receptor opening was spread out over a much longer time. The peak open probability was more than 10 times greater for NR2A-containing receptors than for NR2B-containing receptors, while the weighted time constant of decay was almost 10 times slower. This distinction between different measures of open probability is important for the interpretation of experimental results. For example, a number of studies have used progressive blockade of NMDAR excitatory post-synaptic currents (EPSCs) by MK-801 to estimate open probability [19,33,47]. MK-801 is an irreversible open channel blocker, so progressive blockade reflects prior NMDAR opening. Typically, it is interpreted to indicate success probability, or even the number of receptors activated. However, blockade is usually measured relative to a baseline, and blockade is not instantaneous, so in the case of a chronic application of MK-801, blockade is actually indicative of mean time open. Thus, our results agree with Scimemi et al. [33], who showed that MK-801 blocked NR2B-containing NMDARs faster than NR2A-containing NMDARs. In a study where MK-801 is applied briefly [19],
blockade should better indicate peak open probability, although it may not if washout is incomplete.

Our results were based on receptor kinetics measured in a heterologous system. However, it has proved much more difficult to determine the properties of NMDA receptor subtypes natively expressed by neurons. One study that attempted to do so [19] implied that there was no difference in average open probability between NR2A- and NR2B-containing receptors. However, other studies suggest that the differences in activation kinetics between the two receptor subtypes measured in situ were similar to those measured in vitro. Our simulations of the Prybylowski et al. [19] experiment predicts that this result could potentially have arisen despite a difference in open probability, due to differences in antagonist affinity and the way receptor block was quantified. The presence of trimeric receptors could further complicate this situation. To finally resolve the question of the open probabilities and kinetics of NMDA receptors in synapses, direct measurements will have to be made. However, this has proved elusive, primarily due to the lack of a selective blocker for NR2A-containing receptors. However, genetic methods can be used to isolate receptor subpopulations [66,89]. In combination with two-photon uncaging and/or imaging [17,26], these methods should allow the properties of NMDA receptors to be measured in situ and the predictions of our model to be tested.

The slower opening kinetics of NR2B-containing receptors could have important consequences for calcium influx during miniature excitatory postsynaptic events. Due to the small size of the dendritic spine and the high resistance of the spine neck [90], AMPA mEPSCs should be sufficient to depolarize the spine head and relieve the NMDAR Mg²⁺ block. As the peak open probability of the NR2A-containing receptor is much greater than that of the NR2B-containing receptor, the Ca²⁺ influx during the brief AMPA mEPSC would be much greater. It has been shown that NMDA miniature excitatory currents can stabilize synaptic strength [72]. One prediction of our model is that the homeostatic stabilization of AMPA receptors at the synapse is preferentially mediated by NR2A-containing NMDARs. This prediction could be tested by studying whether homeostatic stabilization in the presence of ifenprodil (which blocks NR2B-containing receptors) differs from that in NR2A knockout animals.

## Location and Number of Receptors

We studied the differences in receptor activation as a function of the amount of neurotransmitter released. Such differences can arise either due to variation in the amount of neurotransmitter contained in vesicles [31,91] or differences in the number of vesicles released [25,45,46]. We found that synapses with predominantly NR2A-containing receptors were nearly insensitive to differences in the amount of neurotransmitter, while those with predominantly NR2B-containing receptors responded in graded fashion (Figure 3). We next considered the impact of distance from the release site on the activation of NMDA receptors containing different subtypes. While NR2A-containing receptors responded about equally regardless of where they were in the synapse, NR2B-containing receptors were highly location-sensitive, with the receptors located closest to the release site opening three times as often as the receptors located farthest away. Receptors located perisynaptically, outside the synapse but less than 1 μm from the release site, showed a very low probability of opening.

These results have important implications for the hypothesis that, over the course of development, NR2B-containing receptors at the center of synapses are displaced by NR2A-containing receptors, such that NR2B-containing receptors end up preferentially located at the periphery of synapses. This idea is based on the finding that miniature excitatory post-synaptic currents (mEPSCs) progressively declined with age in NR2A knockout animals, while evoked activity, which could result in multivesicular release, could still produce an NMDA current [13]. Our results suggest that NR2B-containing receptors located at the periphery of synapses would be very unlikely to open, even under evoked activity. This is difficult to reconcile with the experiments of Townsend et al. [13].

One potential explanation is that the mEPSCs of single NR2B-containing receptors are difficult to distinguish from noise, due to the extended, rapidly opening and closing nature of their activation. In the knockout experiments, the number of NR2B-NMDARs decreased over development. If the number of NR2B-NMDARs per synapse is relatively low, spontaneous release would be likely to open only one, or zero, NR2B-containing receptors, making mEPSCs nearly impossible to detect. On the other hand, action potential evoked release, might be multivesicular, leading to the activation of a detectable number of receptors (Figure 4). Once again, further experiments are needed to test the hypothesis.

Our simulations suggest one such experimental test. NR2B-NMDAR exhibit a sharp location-dependence of opening probability, implying that they require a very high concentration of glutamate to open. NR2A-NMDARs are essentially location-independent, suggesting their response is essentially saturated at low concentrations. Thus, a low-affinity antagonist such as L-AP5 or D-AA would have a much more dramatic effect on NR2B-containing receptors. Similarly, the antagonist would be much more effective at blocking NR2B-NMDARs located at the periphery of the synapse. If, later in development, NR2B-containing receptors are not just decreasing in number but are preferentially located at the periphery, we would expect a proportionally much stronger block of evoked activity when applying a low-affinity antagonist. A related prediction of our model is that in addition to the progressive decline in the spontaneous NMDA current in the knockout animal, the variance of the evoked NMDA response should increase.

It has also been shown that glutamate spillover from adjacent synapses can activate NMDA receptors [33,52,92]. Whether or not this happens depends upon the activity of glutamate transporters, the rate of glutamate diffusion, temperature, the geometry of the extracellular space and the extent of sheathing of synapses by glia. We did not address those factors here, but they have been considered elsewhere [31,93]. However, the very low open probability of perisynaptic NR2B-containing receptors in our simulations suggests that the excitation of these NMDA receptors by spillover from a single vesicle is quite difficult. Thus, if significant activation by spillover does occur, glutamate must diffuse far enough to potentially interact with a large number of receptors. We note that our model of the NR2B-containing receptors are similar to those used in Scimemi et al. [33], and our results on activation probabilities of these receptors are similar. However, it remains to be shown whether under normal in vivo conditions glutamate release at a single synapse (the conditions we simulate) can cause significant activation of extrasynaptic NMDARs. It could be that these extrasynaptic NR2B-containing NMDARs instead detect changes in ambient glutamate concentration related to average synaptic activity over longer timescales or to events that cause large amounts of glutamate to be released. Another possible function for these receptors could be to detect signals originating extrasynaptically, such as glutamate release by astrocytes, which may play a role in synchronizing hippocampal pyramidal cell activity [94].

## The Effect of Subunit Composition on CaMKII Activation

We studied the potential impact of NMDAR subunit composition on postsynaptic long-term potentiation by coupling our...
model of receptor opening driven by tetanic stimulation to a model of activation of calcium-sensitive enzymes in the postsynapse
known to be critical for induction of LTP. We found that either
NR2A- or NR2B-containing receptors could drive persistent
CaMKII autophosphorylation, but more NR2B receptors were
required to reliably drive autophosphorylation. Similarly, the
majority of experimental studies in adult animals using concen-
trations of NMDA receptor blockers small enough to be selective
for NR2 subtype have shown that either receptor type can drive
LTP [23,95,96], though some reports contradict this [28]. That
either receptor type could drive LTP stands to reason, as the
conductance and calcium permeability of both types would allow
large Ca\(^{2+}\) currents to enter the postsynaptic cell while it was
depolarized. We note that while our simulations suggest that given
nearly equal Ca\(^{2+}\) permeabilities of the two receptor-subtypes
[10,97], NR2A-containing receptors let in more calcium than
NR2B-containing NMDARs. This is compatible with experimental
findings [17] which suggest that synaptic NR2B-containing
receptors can have greater or smaller fractional calcium current
due to post-translational modifications depending on synapse size
and history [58].

In our simulation, the most important variable for determining
the probability of LTP was the total time open during the period
of tetanic stimulation, which was more than three times greater for
NR2A-containing receptors. We would expect the advantage of
NR2A-containing receptors in driving LTP to diminish in a low
frequency pairing protocol where the postsynaptic cell is held at a
depolarized voltage for the entire period of receptor opening,
during which NR2A-containing receptors are only open for about
50 percent longer. Still, the advantage of NR2A-containing
NMDARs in driving LTP is surprising, considering that NR2B-
NMDARs are the dominant receptor type during early, critical
periods of development [10]. It could be that early in development
other forms of synaptic plasticity are dominant, or that
multivesicular release is more common, there is a posttranslational
modification that allows more calcium to enter [17,58], or there is
a difference in postsynaptic biochemical signaling [98]. Barria
and Malinow [78] showed that in slices taken from young animals,
LTP was dependent upon interaction between NR2B subunit
intracellular C tails and CaMKII. This interaction may be
important in allowing NR2B-containing receptors to drive LTP
despite their slow kinetics. It is also interesting to note that Harris
and Teyler [99] were first able to observe hippocampal LTP at P7,
and that LTP was maximal at P15, time points which correspond
well with the expression of NR2A.

We also found a distinct difference between the receptor types
in a protocol in which glutamate release was paired with
depolarization of the postsynaptic cell at different temporal offsets,
similar to experiments used to assess spike timing-dependent
plasticity [88]. When presynaptic glutamate release was nearly
concurrent with or preceded postsynaptic depolarization by a
small offset, calcium entered the postsynapse and CaMKII
was autophosphorylated. But, the range of temporal offsets over which
CaMKII phosphorylation occurred was about twice as wide for
NR2B- as for NR2A-containing receptors, suggesting that the
temporal properties of spike timing-dependent plasticity (STDP)
could vary greatly with subunit composition. STDP is a
competitive plasticity mechanism and could play a role in the
formation and refinement of neuronal networks [100,101].

The more permissive temporal filter of NR2B-containing
receptors could allow potentially informative connections to be
strengthened and stabilized initially. Later, as the network settles
into a more mature state, the more precise temporal filtering of
NR2A-containing receptors would allow the circuit to be refined,
the cleft. The vesicle was a 25 nm cube connected to the cleft by a fusion pore 8 nm wide and 15 nm long. Before release, the glutamate molecules were randomly placed within the vesicle. At release, they were simply allowed to begin diffusing.

The diffusion coefficient of glutamate in the neuropil has been recently estimated to be $\sim 3 \times 10^{-6}$ cm$^2$/s at the mossy fiber-granule cell synapse [105], which is 3x lower than the measured value in free solution [106]. This value was estimated by measuring the reduction of the slow AMPA-mediated EPSC, presumably activated by glutamate spillover from neighboring synapses when slices are loaded with high molecular weight dextran, a crowding agent. This reduction was then fit to a battery of glutamate receptor kinetic models to extract the best fit value of $D$ that matched the observed reduction. We note that this number is an estimate, that depends on the particular kinetic model used, the amount of glutamate released per vesicle and the geometry. Direct measurements of glutamate diffusion have not been made at hippocampal synapses. Therefore, we used a similar procedure to estimate the diffusion constant of glutamate. As a constraint, we used the waveforms of sucrose-evoked AMPA miniature EPSCs measured close to the synapse [107]. We then simulated a battery of kinetic models of AMPAR activation at hippocampal synapses [36,42,108] in response to synaptic release of glutamate and matched the observed amplitudes, rise-time and decay times of the mEPSC.

The AMPAR model of Jonas et al. [108] assumed that the binding of two glutamate molecules to the receptor was sufficient to activate the receptor and postulated that the binding was cooperative. This has been ruled out by subsequent experiments [39,40], but we included it since it is the only published model of hippocampal AMPARs. The AMPAR model of Raghavachari and Lisman [36] was based on validated fits of fast glutamate application to AMPARs to outside-out patches pulled from CA1 pyramidal neurons [109]. The model of [42], although originally formulated for cerebellar AMPARs, was included for completeness as it is the only kinetic scheme that accounts for multiple glutamate binding and conductance states of the receptor.

The free variables were the diffusion constant of glutamate and the number of glutamate molecules in a vesicle. The best fit values for these parameters resulted in a diffusion coefficient of $5.0 \times 10^{-6}$ cm$^2$/sec at 37°C. We note that these values lie at the upper end of the estimates of Nielsen et al. [105]. Moreover, the higher values of $D$ in that study correlated with independent subunit models of AMPAR activation. Since our model of AMPAR activation is also an independent subunit model with multiple sub-conductance states [36], our estimate of $D$ is slightly higher than that reported. Varying this value by 20 percent did not affect the simulation results qualitatively. We used a fixed time step of 0.01 ms.

Receptors were represented by discrete 10 nm square patches on the postsynaptic membrane. When a particle hit one of these patches, a random number was generated to determine whether or not it would bind to the receptor. The probability of binding for a collision was determined by dividing $k_{on}$, the number of binding events per second per M of ligand, by the expected number of collisions per second, given a ligand concentration of 1 M. The expected number of collisions per time step $d$ is half the number of particles in the volume defined by the area of the receptor and the mean step size for a particle in one dimension. So, the probability of binding was equal to $k_{on} \left( 0.5 \times 10^6 \times 0.01^2 \times 0.67 \sqrt{2Ddt} \right)$.

### NMDAR Activation and Glutamate Transporters

The kinetic scheme for the NMDA receptors was as in Erreger et al. [21] (Figure 1A). There were eight states: zero bound (0), one bound (1), two bound (2), two desensitized states (D1, D2), two intermediate closed states (C1, C2) and one open state (O). The rate constants were taken from Erreger et al. [21] and adjusted for temperature (Table 2). We scaled the rate constants using $Q_0 = 1.4/10^6$ for diffusion-limited processes and $Q_0 = 3/10^6$ for non-diffusion-limited processes [110]. Our simulations were conducted at room temperature. A simulation where we fixed the temperature to 23°C, and applied a glutamate pulse of 1–4 mM for 1 msec, exactly reproduced the results of Erreger et al. [21], as it was, in fact, the same model.

Extra synaptic membranes contained glutamate transporters. They did not have a fixed location on the membrane. Instead, we assumed the density of transporters available to bind glutamate was $10000/\mu m^2$ [111] and assumed that the fraction of extrasynaptic membrane was 0.1 so that when a particle hit the extrasynaptic membrane it collided with a transporter with probability 0.1. Upon collision, the probability of binding was calculated as above. On subsequent time steps a bound particle could either unbind or be transported and removed from the simulation. The rate constants of the binding, unbinding and transport steps were as in Grewer et al. [112], adjusted for temperature (Table 1).

The opening and closing of individual NMDARs was independent of the other receptors in the synapse, as assessed by varying the number of NMDARs included. The average success probability and time open of the receptors was the same whether there was one receptor present or 20. We normally included 20 receptors, and combined the receptors from each simulation into a single pool for analysis.

### Calculating the Number of Receptors at a Synapse

We calculated the average number of receptors of each per synapse based on the data of Nimchinsky et al. [26], using their equations

\[
f = (1 - P_r) + P_r (1 - p_{on})^M\]

\[
f' = (1 - P_r) + P_r (1 - p_{on} f / I)^M\]

where $f$ is failure probability with no antagonist, $f'$ is failure probability in the presence of the antagonist, $P_r$ is the probability of neurotransmitter release, $p_{on}$ is the probability a receptor will open given neurotransmitter release, $f$ is the ratio of the NMDA current amplitude in the presence of the antagonist to the amplitude in the absence of the antagonist, and $M$ is the total number of receptors present at the synapse. They could not measure $P_r$ directly, so they used the approximation $(1 - P_{on})^M \approx e^{-P_{on} M}$ and solved

\[
\frac{1 - f}{1 - e^{-n}} - \frac{1 - f'}{1 - e^{-nk}} = 0
\]

numerically, where $n = M P_{on}$.

Nimchinsky et al. [26] report that $n = 3.1$, and by inspecting their data, we can observe that their mean $f = 0.43$, $f' = 0.58$, and $f / f' = 0.41$, and that the concentration of D-CPP that will produce an inhibition constant ($K_i$) for NR2A- and NR2B-containing NMDARs are 41 and 270 nM. We then ran our simulation at 30°C, the temperature used by Nimchinsky et al. [26], and
determined that \( P_{\text{ro}} \) for NR2A- and NR2B-containing NMDARs were 0.70 and 0.20. We then solved for \( M_{\text{NR2A}} \) and \( M_{\text{NR2B}} \), the number of NR2A- and NR2B-containing NMDARs at the synapse, by

\[
f = (1 - P_{\text{ro}}) + P_{\text{ro}} M_{\text{NR2A}} \frac{f_{\text{NR2A}}}{M_{\text{NR2A}}} + P_{\text{ro}} M_{\text{NR2B}} \frac{f_{\text{NR2B}}}{M_{\text{NR2B}}}
\]

(4)

\[
f' = (1 - P_{\text{ro}}) + P_{\text{ro}} M_{\text{NR2A}} \frac{f'_{\text{NR2A}}}{M_{\text{NR2A}}} + P_{\text{ro}} M_{\text{NR2B}} \frac{f'_{\text{NR2B}}}{M_{\text{NR2B}}}
\]

(5)

where \( f_{\text{NR2A}} = 1 - P_{\text{ro}} \) and \( f_{\text{NR2B}} = 1 - P_{\text{ro}} I' / (1 + \frac{D - CPP}{K_C}) \), where D-CPP is 240 nM. The equations could also be solved for the case where three receptor species were present, given an additional equation to constrain the number of each species present.

**Receptor Blockade by MK-801**

We calculated the average effect of applying 4 msec pulses of 1 mM glutamate and 200 nM MK-801 to NR2A or NR2B-containing receptors using a deterministic, explicit model. We used a doubled version of our NMDAR model, with the second set of states representing having MK-801 bound to the receptor. There was a single, reversible transition between the open and closed state representing having MK-801 bound to the receptor. A given equation to constrain the number of each species present.

**LTP Induction**

We simulated the effect of applying tetanic stimulation to a synapse containing either NR2A- or NR2B-containing receptors. It was assumed that the presynaptic cell was firing at a rate of 100 Hz for 1 second, but that vesicle release at the synapse was stochastic, with an adapting release probability, modeled using the method of Maass and Zador [84]. For each presynaptic spike, the probability of release, \( P_{\text{release}} = 1 - e^{-C_2 t} \). The value of \( C_2 \), the facilitation parameter, was initially set to \( C_0 \). After every presynaptic spike \( C_2 \) was incremented by \( \Delta C_2 \) and then decayed back towards \( C_0 \) exponentially with time constant \( \tau_C \). The depletion parameter, \( V \), was initially set to \( V_0 \). Every time a vesicle was released \( V \) was decreased by 1, or, if \( V<1 \), set to 0. \( V \) then decayed back towards \( V_0 \) with time constant \( \tau_W \). The parameters \( C_0 \), \( V_0 \), \( \tau_C \), and \( \tau_W \) were set to 0.26, 3.5, 20 msec, 50, 50, and 0.25, respectively, based on recordings in hippocampal slices using minimal stimulation [85,86]. For these simulations, rather than simulate the trajectories of 120,000 glutamate particles, we computed the time-varying average collision rate at each of the 121 possible receptor locations following a single vesicle release by counting collisions and averaging 1000 Monte Carlo runs, and used these averages to randomly determine whether a collision occurred at each time step. Receptor open probability using this technique was indistinguishable from that of the full Monte Carlo simulation (reduced \( \gamma^2 \) was 1.004 for NR2A- and 0.9617 for NR2B-NMDARs, and \( p \) values for a paired t test were 0.9950 and 0.9983, respectively), but the simulations ran \~\times 2000 \) times faster.

We modeled internal calcium concentration using the same parameters and model as Nimchinsky et al. [26]. Current was calculated by the Goldman-Hodgkin-Katz equation and concentration showed fast buffering and single exponential decay kinetics. We added a voltage-dependent \( M_{\text{GK}^{+}} \) block, fitted to the data of Monyer et al. [83]. Conductance, \( G_C \), was equal to \( G_0 / (1 + e^{-0.081V/20}) \), where \( G_0 \) is the conductance at 0 mV \( M_{\text{GK}^{+}} \) and \( V \) in voltage in mV. The conductance was half-maximal at \( -20 \) mV and increased from 10 to 90 percent over a 54.9 mV range. Calcium concentration was calculated by

\[
I_{\text{Ca}} = \frac{4V_{\text{M}}(P_{\text{Ca}}/P_{\text{M}})(C_{\text{ex}}/M)}{1 + e^{-0.08(V+20)}} \\
\frac{e^{2V_{\text{F}}/RT}}{1 - e^{2V_{\text{F}}/RT}}
\]

(6)

\[
dCa/dt = \frac{(I_{\text{Ca}}/2F_{\text{V}}) - \kappa(Ca - C_{\text{Ca}e})}{1 + \kappa}
\]

(7)

where \( I_{\text{Ca}} \) is calcium current, \( G_{\text{M}} \) is the conductance of the channel in the presence of 2 mM \( \text{Ca}^{2+} \) (46 pS), \( P_{\text{Ca}}/P_{\text{M}} \) is the ratio of NMDAR permeability to calcium to permeability to monovalent ions (3.6), \( C_{\text{ex}} \) is external calcium concentration (2 mM), \( M \) is the concentration of monovalent ions (130 mM), \( F \) is Faraday’s constant, \( R \) is the gas constant, \( T \) is temperature, \( Ca \) is internal calcium concentration, \( \nu \) is spine volume (0.08 fL), \( \kappa \) is the decay rate constant of internal calcium concentration (1.6 msec\(^{-1} \)), \( C_{\text{Ca}e} \) is the resting internal calcium concentration (0.1 \muM) and \( \kappa \) is the buffer capacity (20). \( I_{\text{Ca}} \) was maximal at \(-14.2 \) mV. The postsynaptic spine was modeled as a single compartment with uniform concentration throughout. Conductance and calcium permeability were assumed to be the same for NR2A- and NR2B-NMDARs [10,82,97].

The calcium influx through NMDARs leads to the activation of CaMII which is a significant component of the PSD [113]. The enzyme is activated by the binding of Calcium-Calmodulin (Ca-CaM). When two adjacent subunits bind Ca-CaM, the subunits become autophosphorylated. The kinase then becomes autonomous, that is it retains enzymatic activity even after CaM unbinding. CaMKII is dephosphorylated by Protein phosphatase 1 (PP1) in a calcium-dependent manner. High levels of calcium can trigger the autophosphorylation of all 12 holoenzymes, which can overcome phosphatase action to dephosphorylate the enzyme. It has been proposed that the dynamics of CaMKII phosphorylation could then function as a bistable “switch”, and that this switch could underly long-term synaptic potentiation [67]. Introduction of active CaMKII in hippocampal neurons mimics LTP [114], and animals with genetic mutations of CaMKII show severe deficits in learning and memory [67]. Based on this evidence, experimental and theoretical efforts have focused on understanding the properties of the CaMKII switch [81,115–118].

We used a bistable model of \( \text{Ca}^{2+}/\text{Calmodulin-dependent kinase II} \) (CaMKII) activation [81] as a model of LTP. This was a single-compartment, deterministic model of several interacting chemical processes, driven by the free calcium concentration. For details of the model, and parameter values, see Miller et al. [81]. Essentially, there are two, competing calcium-dependent processes, which phosphorylate and dephosphorylate CaMKII. If the phosphorylation process outcompetes the dephosphorylation process, [CaMKII]\(^{+}\) moves towards a high, stable value. We denoted such synapses as potentiated. We ran the simulation for 30 minutes, after which point it was possible to separate the potentiated synapses from those that were not by simply checking whether [CaMKII]\(^{+}\) was above a threshold (84 \muM).
Author Contributions
Conceived and designed the experiments: DMS SR. Performed the experiments: DMS SR. Analyzed the data: DMS SR. Wrote the paper: DMS SR.

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