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Synaptic and Extrasynaptic NMDA Receptors Are Gated by Different Endogenous Coagonists

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SUMMARY

N-methyl-D-aspartate receptors (NMDARs) are located in neuronal cell membranes at synaptic and extrasynaptic locations, where they are believed to mediate distinct physiological and pathological processes. Activation of NMDARs requires glutamate and a coagonist whose nature and impact on NMDAR physiology remain elusive. We report that synaptic and extrasynaptic NMDARs are gated by different endogenous coagonists, D-serine and glycine, respectively. The regionalized availability of the coagonists matches the preferential affinity of synaptic NMDARs for D-serine and extrasynaptic NMDARs for glycine. Furthermore, glycine and D-serine inhibit NMDAR surface trafficking in a subunit-dependent manner, which is likely to influence NMDARs subcellular location. Taking advantage of this coagonist segregation, we demonstrate that long-term potentiation and NMDA-induced neurotoxicity rely on synaptic NMDARs only. Conversely, long-term depression requires both synaptic and extrasynaptic receptors. Our observations provide key insights into the operating mode of NMDARs, emphasizing functional distinctions between synaptic and extrasynaptic NMDARs in brain physiology.

INTRODUCTION

N-methyl-D-aspartate receptors (NMDARs) are glutamate ionotropic receptors implicated in multiple aspects of brain physiology and cognitive functions, such as learning and memory (Bliss and Collingridge, 1993). They are also central to the pathogenesis of various neurological and psychiatric diseases, including neurodegenerative disorders (Arundine and Tymianski, 2003), chronic pain (Woolf and Salter, 2000), and schizophrenia (Lin et al., 2012). NMDARs located at synaptic and extrasynaptic sites may constitute two functionally distinct pools of receptors. Synaptic NMDARs are responsible for inducing the most common forms of synaptic plasticity found in the brain, namely, long-term potentiation (LTP) and long-term depression (LTD). Whether specific subsets of synaptic NMDARs mediate LTP or LTD (Liu et al., 2004; Massey et al., 2004; Morishita et al., 2007; Berberich et al., 2005; Weitlauf et al., 2005) and whether extrasynaptic receptors also play a role in these processes (Rusakov et al., 2004) is controversial. Extrasynaptic NMDARs contribute to neuronal synchronization (Angulo et al., 2004; Fellin et al., 2004), but have mostly been implicated in neurodegenerative disorders, including stroke and Huntington's and Alzheimer's diseases (Arundine and Tymianski, 2003; Milnerwood et al., 2010; Bordji et al., 2010). Recent evidence also suggests that synaptic NMDARs are neuroprotective, whereas extrasynaptic receptors promote cell death (Hardingham and Bading, 2010). Thereby, NMDARs are crucial players in both physiological and pathophysiological processes, and as such, they have been studied extensively, generating massive clinical interest as potential therapeutic targets (Hardingham and Bading, 2010; Tsai et al., 2004).

Yet, basic information is still lacking, particularly regarding the endogenous gating of their glycine-binding site. To be activated, NMDARs require the binding of glutamate and of another agonist whose identity and control over NMDAR-mediated functions in situ is still unclear. Glycine was first proposed to serve as a coagonist of NMDARs (Forsythe et al., 1988, Johnson and Ascher, 1987; Kleckner and Dingledine, 1988), but evidence for its endogenous implication has only been obtained in regions where it is particularly abundant, such as the spinal cord (Ahmadi et al., 2003), the retina (Kalbaugh et al., 2009), and the nucleus tractus solitarius (Panatier et al., 2006). Another amino acid, D-serine, was reported to serve as an endogenous ligand at the

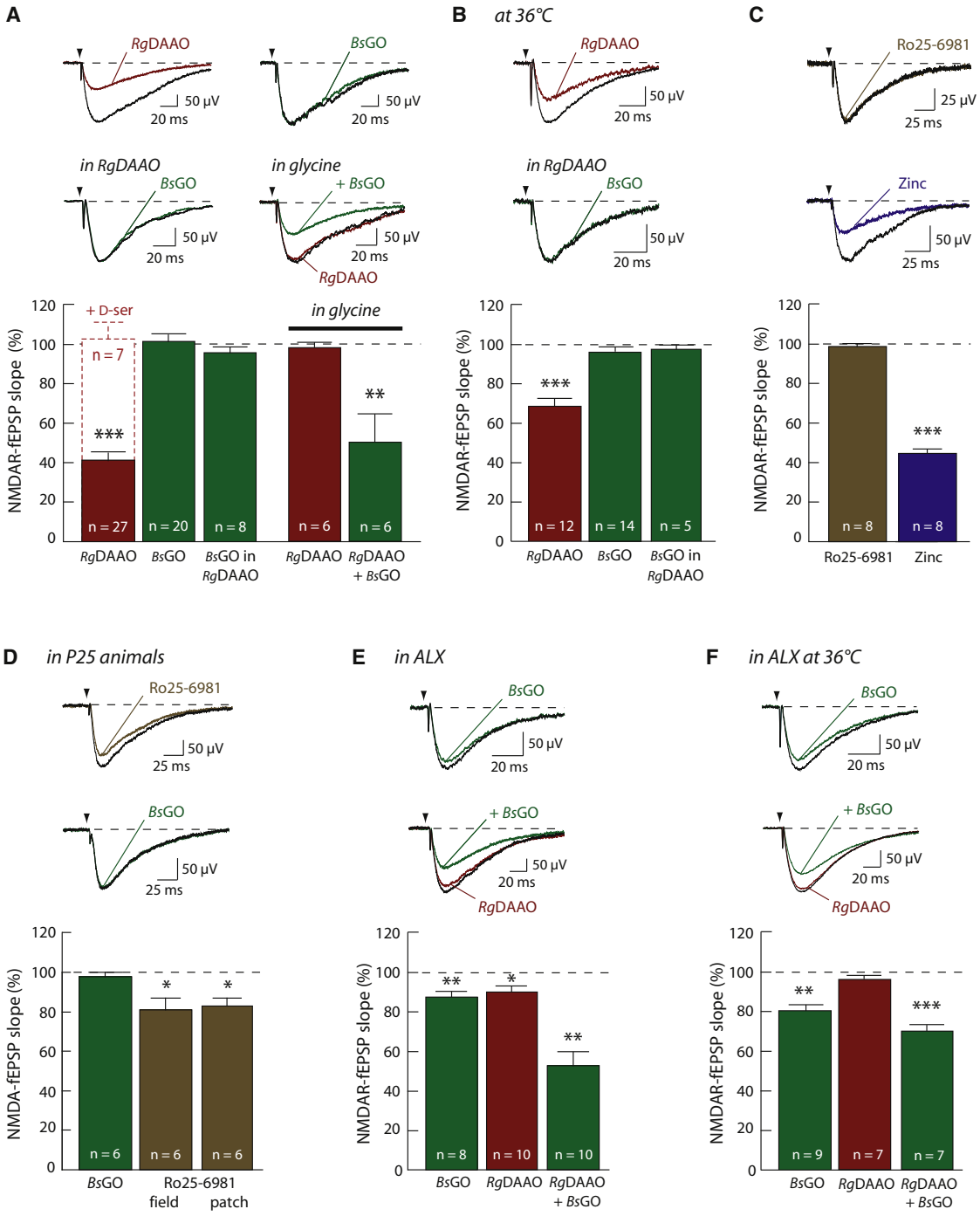

Figure 1. Glycine Is Not an Endogenous Coagonist of NMDARs at CA3-CA1 Synapses

(A) (Top) NMDAR-fEPSPs recorded at room temperature, under different experimental conditions. *Rg*DAAO inhibited these responses (42.4% ± 4.1% of control, n = 27, $p < 10^{-5}$; top-left trace), an effect that could be rescued with D-serine (101.9% ± 8.9%, n = 7 out of 18 experiments). *Bs*GO had no effect either under control conditions (101.3% ± 3.1%, n = 20, p = 0.674, top-right trace) or in *Rg*DAAO-treated slices (94.7% ± 2.2%, n = 8, p = 0.104, bottom-left trace). In glycine (0.1–0.5 mM), *Rg*DAAO no longer affected NMDAR-fEPSPs (97.2% ± 2.2%, n = 6, p = 0.413), while subsequent application of *Bs*GO induced a pronounced inhibition of these responses (50.6% ± 14.4% of control, n = 6, p < 0.01, bottom-right traces). (Bottom) Bar graphs summarizing the experiments depicted above. Error bars show mean ± SEM. *p < 0.05; **p < 0.01; ***p < 0.001; arrows represent stimulation artifact that was removed for clarity reasons. Regarding specificity and efficacy of *Rg*DAAO and *Bs*GO in our experimental conditions, see Figures S1, S2, and S3.

(B) (Top) Examples of NMDAR-fEPSPs recorded as in A at 36°C. Application of *Rg*DAAO still produced a strong inhibition (68.6% ± 4.0% of control, n = 12, p < 0.001; top trace) and *Bs*GO was still without effect (95.7% ± 3.0%, n = 14, p = 0.113) even in *Rg*DAAO-treated slices (97.4% ± 2.1%, n = 5, p = 0.171, bottom trace). (Bottom) Summarizing bar graphs.

so-called glycine-binding site of NMDAR at several CNS synapses, such as the hippocampus (Henneberger et al., 2010; Mothet et al., 2000; Schell et al., 1995; Yang et al., 2003) the hypothalamus (Panatier et al., 2006), the cortex (Fossat et al., 2012), the retina (Stevens et al., 2010), and the spinal cord (Wake et al., 2001). Therefore, the relative contribution of endogenous glycine and D-serine for synaptic NMDAR activity remains to be established. Moreover, the nature of the endogenous coagonist that gates extrasynaptic NMDARs is unknown.

To assess the respective contributions of endogenous glycine and D-serine at synaptic and extrasynaptic NMDARs, we performed electrophysiological recordings in the CA1 region of the hippocampus where NMDARs and NMDAR-mediated processes have been extensively studied in the context of learning, memory, and neurodegeneration. Using specific enzymes that degrade either D-serine or glycine, we demonstrate that D-serine is the coagonist at synaptic NMDARs, whereas glycine is the coagonist at extrasynaptic NMDARs. We show that such functional compartmentalization arises from glycine and D-serine availability that matches the preferential affinity of NMDARs for these two coagonists. In addition, glycine and D-serine differentially impact NMDAR surface diffusion, which is likely to contribute to this segregation. We then provide evidence that LTP and NMDA-mediated excitotoxicity depend on synaptic NMDARs, whereas LTD requires both synaptic and extrasynaptic receptors.

RESULTS

D-Serine Is an Endogenous Coagonist of Synaptic NMDARs

To identify the endogenous coagonist(s) of NMDARs at CA3-CA1 synapses, we first monitored the influence of D-amino acid oxidase (*Rg*DAAO; Molla et al., 1998, 2000; Pollegioni et al., 1992), on NMDAR-mediated field excitatory post-synaptic potentials (NMDAR-fEPSPs; Figure S1A available online). Degradation of extracellular D-serine by *Rg*DAAO (0.2 U/ml) produced a consistent reduction of NMDAR-fEPSP slope (Figures 1A and S1B) at room temperature. At 36°C, an inhibitory action of *Rg*DAAO was observed as well (Figure 1B), although its magnitude was smaller consistent with the compromised stability of the enzyme at such temperatures (Pollegioni et al., 1992; see Experimental Procedures). No inhibition was observed with an inactive variant of the enzyme, ΔDAAO (Molla et al., 2000; Experimental Procedures), ruling out side effects of *Rg*DAAO vehicle solution and a direct deleterious interaction between the enzyme and NMDARs (Figure S1C). *Rg*DAAO application did not affect the paired-pulse ratio or the afferent fiber volley (Figure S1D), arguing against an effect on glutamate release probability and axonal excitability. Consistently, AMPA receptor-mediated transmission was not affected by *Rg*DAAO either (Figure S1D), demonstrating that *Rg*DAAO inhibitory effect was confined to NMDARs. Because D-serine degradation is accompanied by the production of byproducts such as NH_4^+ and H_2O_2 that could impair NMDAR function, in particular through local disturbance of pH, we checked whether exogenous applications of coagonist were sufficient to rescue NMDAR-fEPSPs. Under continuous perfusion of the enzyme, D-serine (0.1–0.5 mM) could reverse the inhibition of NMDAR-fEPSPs mediated by *Rg*DAAO (Figure 1A). Alternatively, incubating slices in 0.1–0.5 mM glycine before applying *Rg*DAAO prevented the inhibitory action of the scavenger (Figure 1A). These experiments demonstrate that the inhibitory action of *Rg*DAAO was a direct result of a decreased occupancy of the NMDAR glycine-binding site because of D-serine degradation. Altogether, these findings confirm previous observations that identified D-serine as a major endogenous coagonist of synaptic NMDARs (Henneberger et al., 2010; Mothet et al., 2000; Schell et al., 1995; Yang et al., 2003).

Glycine Is Not an Endogenous Coagonist of Synaptic NMDARs

Although the persistence of NMDAR-fEPSPs in the presence of *Rg*DAAO can be explained by incomplete degradation of D-serine (see Experimental Procedures), another explanation could be that glycine acts as an endogenous coagonist at some synaptic NMDARs. Therefore, we assessed the contribution of endogenous glycine in gating NMDARs at CA3-CA1 synapses by using *Bs*GO (0.2 U/ml) that selectively degrades free extracellular glycine (Job et al., 2002). Unlike *Rg*DAAO, *Bs*GO did not affect the slope of NMDAR-fEPSPs over several hours of perfusion either at room (Figure 1A) or physiological temperature (Figure 1B), suggesting that endogenous glycine might not serve as a coagonist of synaptic NMDARs. The lack of inhibition by *Bs*GO could simply reflect the fact that endogenous D-serine, which is left intact in these experiments, masks the effect of degrading glycine. We therefore repeated these experiments on slices that had been treated with *Rg*DAAO beforehand, to lower endogenous D-serine levels. Under these conditions, *Bs*GO applications still had no effect on NMDAR-fEPSPs both at room and physiological temperature (Figures 1A and 1B).

To rule out the possibility that *Bs*GO does not efficiently scavenge glycine in our experimental conditions, we carried out several control experiments. First, when 0.1–0.5 mM glycine

(C) (Top) Examples of NMDAR-fEPSPs challenged with Ro25-6981 or zinc. While Ro25-6981 had no detectable effect (98.6% ± 1.5%, n = 8, p = 0.168; top trace), zinc induced a dramatic reduction of NMDAR-fEPSPs (44.8% ± 2.0% of control, n = 8, $p < 10^{-4}$; bottom trace). (Bottom) Summarizing bar graphs.
(D) (Top) Examples of NMDAR-fEPSPs obtained from P25-old animals. While Ro25-6981 inhibited synaptic NMDAR responses (80.8% ± 5.9% of control, n = 6, p < 0.05; top trace) *Bs*GO produced no significant effect (97.8% ± 2.3%, n = 6, p = 0.334; bottom trace). (Bottom) Summarizing bar graphs.
(E and F) (Top) in the presence of the GlyT1 inhibitor ALX (1 μM), NMDAR-fEPSPs were slightly inhibited by *Bs*GO both at room (86.8% ± 2.3% of control, n = 8, p < 0.01, top trace) and physiological temperature (80.2% ± 2.3%, n = 9, p < 0.01). Interestingly, applications of *Rg*DAAO in the presence of ALX no longer affected the field potentials (RT: 91.7% ± 2.6%, n = 10, p < 0.05; 36°C: 96.4% ± 2.1%, n = 7, p = 0.101, bottom traces). Subsequent application of *Bs*GO on top of *Rg*DAAO strongly reduced NMDAR-fEPSPs (RT: 53.1% ± 6.6%, n = 10, p < 0.01; 36°C: 70.9% ± 2.5%, n = 7, p < 0.001). (Bottom) Bar graphs summarizing the experiments depicted above.
See also Figures S1, S2, and S3.

was added to the bath, a significant increase of NMDAR-fEPSPs slope was observed (Figure S2), as expected if the NMDAR glycine site was not fully occupied. Subsequent applications of *Bs*GO reduced the slope of NMDAR-fEPSPs back to its initial value (Figure S2), suggesting an efficient degradation of exogenous glycine. Second, in the experiments in which exogenously applied glycine prevented the inhibitory action of *Rg*DAAO, addition of *Bs*GO on top of *Rg*DAAO significantly impaired NMDAR-fEPSPs (Figure 1A). Together these data indicate that *Bs*GO efficiently degrades glycine in acute hippocampal slices and its inability to inhibit NMDAR-fEPSPs supports the conclusion that endogenous glycine is not a coagonist of NMDARs at CA3-CA1 synapses of adult rats (Figure S3).

Synaptic NMDARs' Subunit Composition Favors D-Serine over Glycine

That D-serine but not glycine gates synaptic NMDARs could be the consequence of a preferential affinity of synaptic NMDARs for D-serine. Indeed, the GluN2-subunit composition of NMDARs is known to impact their affinity for D-serine versus glycine (Matsui et al., 1995; Madry et al., 2007; Priestley et al., 1995). GluN2B-heterodimers subtype of NMDARs (GluN2B-NMDARs) bind glycine with a 10-fold better affinity than do GluN2A-NMDARs, and they exhibit a stronger affinity for glycine than for D-serine (EC_{50} ~0.057 versus 0.15 μM, respectively) (Priestley et al., 1995; Madry et al., 2007). On the contrary, GluN2A-NMDARs exhibit a slightly stronger affinity for D-serine over glycine (EC_{50} ~0.22 versus 0.53 μM respectively, Priestley et al., 1995; Matsui et al., 1995; but see Wafford et al., 1995). Thus, the presence of GluN2A-NMDARs at synapses would favor gating by D-serine over glycine. We characterized the composition of NMDARs at CA3-CA1 synapses in slices obtained from adult rats and found that the GluN2B-NMDARs antagonist Ro25-6981 (2 μM) did not affect NMDAR-fEPSPs slope (Figure 1C). Conversely, 250 nM of free zinc (Experimental Procedures), a highly specific allosteric inhibitor of GluN2A-NMDARs that acts as a partial antagonist (Paoletti et al., 1997; Paoletti and Neyton, 2007), strongly reduced synaptic NMDAR-fEPSPs (Figure 1C). These results indicate that synaptic receptors are predominantly composed of GluN2A-NMDARs.

While favoring a role for D-serine over glycine, the differences in affinity of the NMDAR-subtypes for either coagonist are unlikely to allow full discrimination between D-serine and glycine. We further tested this possibility by performing experiments in hippocampal slices obtained from younger animals (P25) at an age where GluN2B-NMDARs are known to be present at CA3-CA1 synapses (Kirson and Yaari, 1996; Morishita et al., 2007; Harris and Pettit, 2007). As expected, NMDAR-fEPSPs were significantly inhibited by Ro25-6981 at P25 (Figure 1D), but no effect of *Bs*GO on NMDAR-fEPSPs could be detected (Figure 1D), suggesting that even when GluN2B-NMDARs participate in the synaptic responses, endogenous glycine does not gate these receptors.

Glycine Transporters Prevent Glycine Access to Synaptic NMDARs

Clearance of glycine from the synaptic space may explain why endogenous glycine does not gate NMDARs at CA3-CA1 synapses. Glycine transporters, predominantly GlyT1 (Zafra et al., 1995; Cubelos et al., 2005), are known to modulate glycine concentrations in the hippocampus (Berger et al., 1998; Bergeron et al., 1998). To test whether GlyT1 lowers synaptic glycine levels to ineffective concentrations, we inhibited the activity of these transporters with the specific blocker ALX 5407 (ALX, 1 μM) (Atkinson et al., 2001). Applying *Bs*GO on slices pre-incubated with ALX led to a small but consistent decrease of the slope of NMDAR-fEPSPs both at room temperature (RT) and 36°C (Figures 1E). In these experiments, the presence of endogenous D-serine is likely to compete with glycine, thereby minimizing the effect of *Bs*GO on synaptic NMDARs. Accordingly, in the presence of ALX, *Rg*DAAO inhibitory action appeared largely compromised (Figure 1E) whereas subsequent addition of *Bs*GO yielded a larger reduction of NMDAR-fEPSPs (Figure 1E). These results confirm that endogenous glycine would contribute to NMDARs activity in the absence of active uptake (Berger et al., 1998; Bergeron et al., 1998) and further demonstrate that *Bs*GO efficiently degrades endogenous glycine. More importantly, they point to an active process maintaining glycine levels within the synaptic cleft at values that do not significantly impact NMDAR activity.

Glycine and D-Serine Degradation Both Reduce NMDA-Evoked Responses

NMDARs are present not only at synaptic but also at extrasynaptic sites. According to the literature, the levels of glycine in the extracellular space (2–10 μM) should be sufficient to act on NMDARs located outside the synapses (Yamamoto et al., 2010, Horio et al., 2011). We thus determined the contribution of D-serine and glycine at gating extrasynaptic NMDARs. To this end, we switched to whole-cell patch clamp to record from CA1 pyramidal neurons held at +40 mV while evoking responses with local puff-applications of NMDA (1 mM, 20–200 ms, Figure 2A). Such agonist applications yielded long-lasting outward currents which resulted from the activation of both synaptic and extrasynaptic NMDARs (Harris and Pettit, 2007; Mothet et al., 2000). These agonist-evoked currents were stable for roughly 35 min and blocked by D-AP5 (Figure 2A). Consistent with its effect on synaptic receptors, *Rg*DAAO induced a reliable inhibition of NMDA-evoked current amplitude that could be reversed with exogenous glycine (Figure 2A). *Bs*GO also caused a significant reduction of NMDA-evoked excitatory postsynaptic currents (EPSCs) at room and physiological temperatures, which was rescued with exogenous D-serine. Because *Bs*GO did not affect synaptic NMDARs, these results suggest that a significant number of extrasynaptic NMDARs on CA1 neurons are gated by ambient glycine. Accordingly, we found that *Rg*DAAO and *Bs*GO had an additive effect when applied together (Figure 2A), as expected if the two enzymes were affecting two distinct populations of receptors by degrading either glycine or D-serine.

Glycine, but Not D-Serine, Controls NMDAR-Mediated Tonic Current

To test directly the role of endogenous glycine at gating extrasynaptic NMDARs, we attempted to study a process purely mediated by these receptors. It was previously reported in CA1

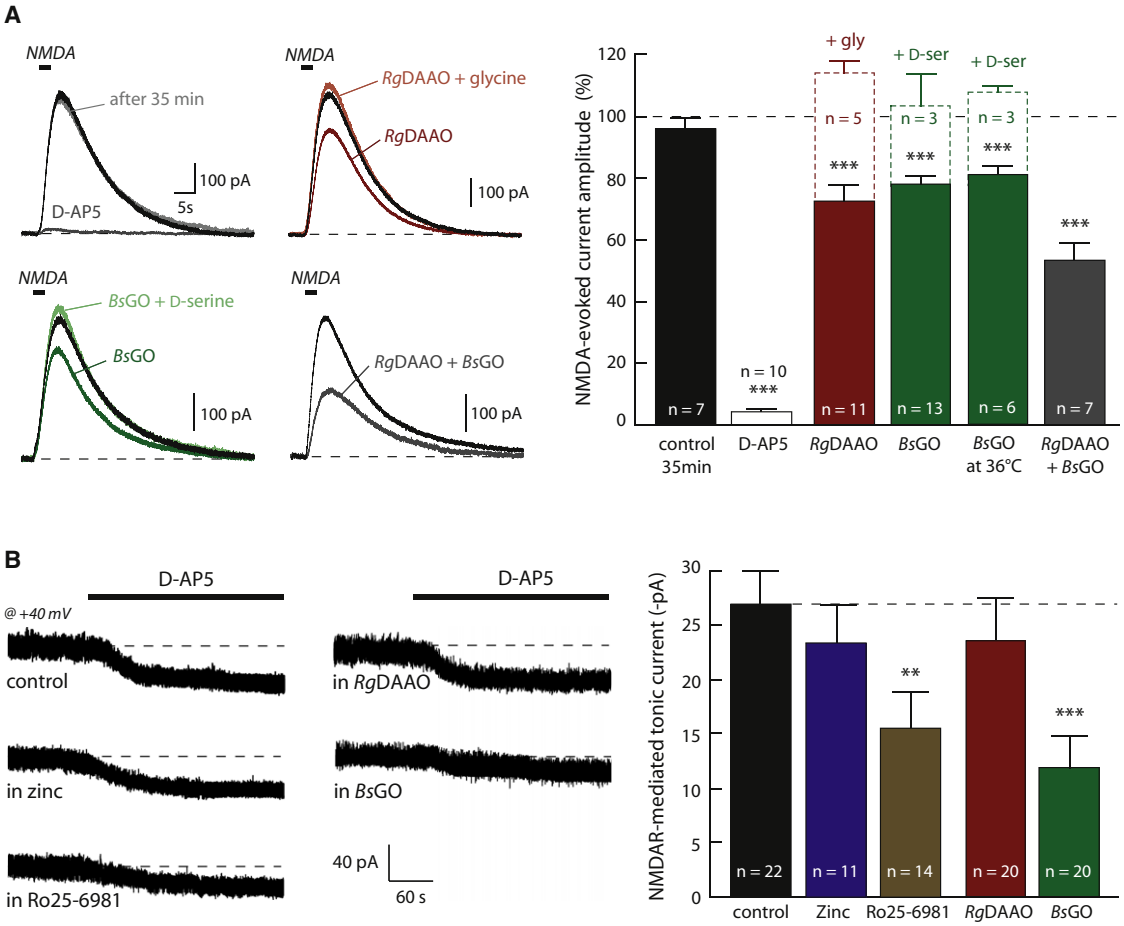

Figure 2. *Bs*GO Acts on Extrasynaptic NMDARs

(A) (Left) Example of responses evoked by local applications of NMDA at +40 mV, which were stable for 35–40 min (97.5% ± 2.4% of control, n = 7, p = 0.264) and sensitive to D-AP5 (4.1% ± 0.7%, n = 10, $p < 10^{-5}$, top-left traces). These responses were significantly inhibited with *Bs*GO (RT: 77.5% ± 2.0%, n = 13, $p < 10^{-4}$, bottom-left trace; 36°C: 81.0% ± 2.5%, n = 6, p < 0.01) and *Rg*DAAO (72.2% ± 3.2% of control, n = 11, $p < 10^{-4}$; top-right trace). Both inhibitory effects were reversed by adding either D-serine (102.6% ± 9.8%, n = 3 at RT and 108.8% ± 1.2%, n = 3 at 36°C) or glycine (113.8% ± 4.0%, n = 5) in the bathing solution. Coapplication of *Bs*GO and *Rg*DAAO led to an additive inhibition of NMDA-evoked responses (58.6% ± 3.4%, n = 7, $p < 10^{-4}$, bottom-right trace). (Right) Summarizing bar graphs (mean ± SEM).

(B) (Left) Examples of the shift in holding current observed at +40 mV in response to D-AP5 (26.8 ± 3.1 pA, n = 22), revealing the tonic activation of extrasynaptic NMDARs at rest. This shift was strongly reduced in slices incubated with either Ro25-6981 (15.7 ± 3.0 pA, n = 14, p < 0.01) or *Bs*GO (12.1 ± 2.9 pA, n = 20, p < 0.001), whereas it was not affected by zinc (23.2 ± 2.4 pA, n = 11, p = 0.440) or *Rg*DAAO (23.8 ± 4.0 pA, n = 20, p = 0.382). (Right) Summarizing bar graphs (mean ± SEM).

pyramidal neurons that ambient glutamate generates a tonic NMDAR-mediated current (Sah et al., 1989) carried by extrasynaptic receptors (Le Meur et al., 2007). In agreement with these studies, we found that 50 μM D-AP5 caused a 26.8 ± 3.1 pA inward current in CA1 neurons held at +40 mV (Figure 2B). Interestingly, the magnitude of this current was not significantly affected by zinc, whereas it was reduced in presence of Ro25-6981 (Figure 2B). Given the effects of zinc and Ro25-6981 on synaptic receptors (Figure 1C), these results are consistent with the involvement of extrasynaptic NMDARs (Le Meur et al., 2007). We then tested whether such tonic NMDAR activity was gated by D-serine or glycine. Lowering D-serine levels with *Rg*DAAO did not significantly affect the magnitude of the tonic current (Figure 2B) whereas it was reduced by 54.8% in *Bs*GO-slices. These results indicate that glycine, but not D-serine, serves as an endogenous coagonist of extrasynaptic NMDARs. However, because those receptors are tonically activated by ambient glutamate, it is very unlikely that they contributed to the extrasynaptic responses generated by the local applications of NMDA (Figure 2A).

Preferential Action of Glycine on GluN2B-NMDARs

To confirm the hypothesis that glycine serves as an endogenous coagonist at extrasynaptic receptors recruited during NMDA-puff applications, we took advantage of the difference in subunit composition between synaptic and extrasynaptic NMDARs that prevails in our experimental conditions. As already shown for NMDAR-fEPSPs, challenging NMDAR-EPSCs with Ro25-6981

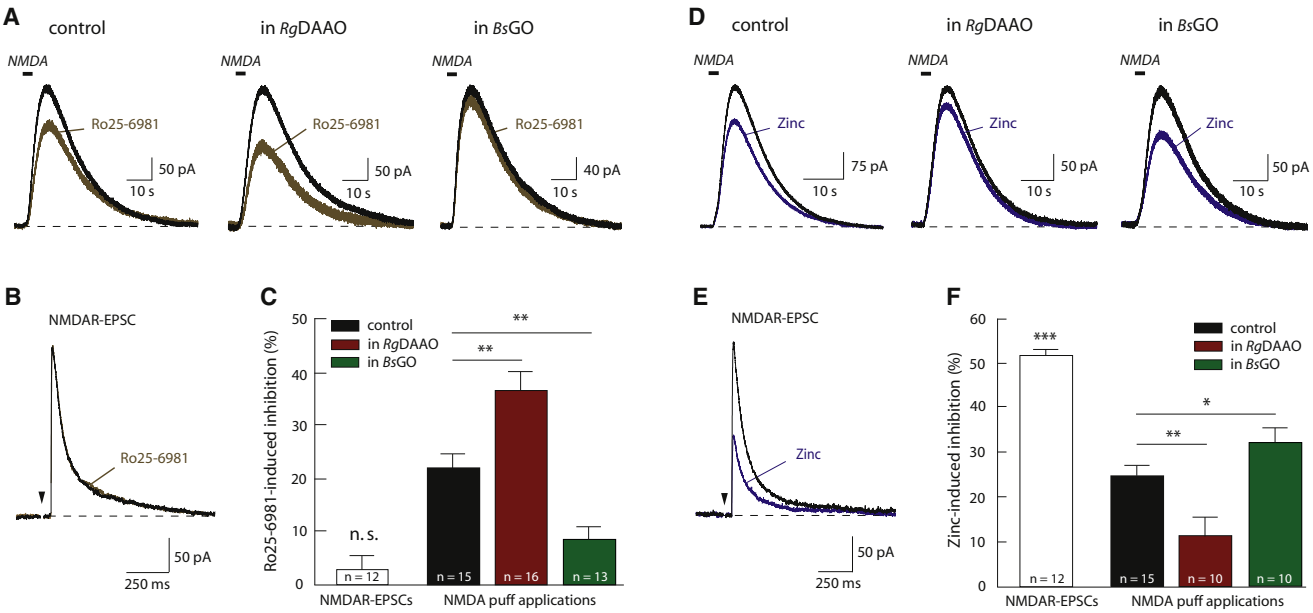

Figure 3. Glycine and D-Serine Serve as Endogenous Coagonist at Extrasynaptic GluN2B- and Synaptic GluN2A-NMDARs, Respectively

(A) Typical traces illustrating the inhibitory effect of Ro25-6981 on NMDA-evoked responses at +40 mV (21.5% ± 2.7%, n = 15, $p < 0.001$, left trace). This inhibition was enhanced in *Rg*DAAO-treated (36.6% ± 3.1%, n = 16, $p < 0.01$, middle trace) and occluded in *Bs*GO-treated (6.7% ± 3.1%, n = 13, $p < 0.001$, right trace) slices.

(B) Example illustrating the lack of effect of Ro25-6981 on NMDAR-mediated EPSCs (2.8% ± 3.4%, n = 12, p = 0.285) obtained at CA3-CA1 synapses, indicating that GluN2B-NMDARs are not present at synaptic sites.

(C) Bar graphs (mean ± SEM) summarizing the experiments depicted in (A) and (B).

(D) Traces illustrating the inhibitory effect of zinc on NMDA-evoked responses under control conditions (24.2% ± 2.0%, n = 15, $p < 10^{-5}$, left trace), in *Rg*DAAO- (11.2% ± 3.9%, n = 10, $p < 0.01$ versus control effect, middle trace) and in *Bs*GO-treated (31.9% ± 3.0%, n = 10, $p < 0.05$, right trace) slices.

(E) Example illustrating the strong inhibitory action of zinc on NMDAR-mediated EPSCs (50.2% ± 1.9%, n = 12, $p < 10^{-5}$), indicating that synaptic NMDARs are mainly composed of GluN2A-NMDARs.

(F) Bar graphs (mean ± SEM) summarizing the experiments depicted in (D) and (E).

had no effect (Figures 3B and 3C) whereas it significantly reduced NMDA-evoked currents (Figures 3A and 3C), in line with the extrasynaptic location of GluN2B-NMDARs. If endogenous glycine is serving as a coagonist at extrasynaptic NMDARs, then the inhibitory effect of the GluN2B-antagonist on NMDA-induced responses should be compromised once glycine has been degraded. Accordingly, inhibition by Ro25-6981 was dramatically reduced in *Bs*GO-treated slices (Figures 3A and 3C). These data also suggest that even in the absence of glycine, D-serine does not act as a coagonist at extrasynaptic GluN2B-receptors. D-serine degradation should then impair the activity of synaptic NMDARs, but leave intact the GluN2B-extrasynaptic pool. As a consequence, the contribution of extrasynaptic GluN2B-receptors to the initial NMDA-evoked response should be increased under conditions where D-serine has been already degraded. Indeed, Ro25-6981-mediated inhibition on NMDA-evoked currents was enhanced in *Rg*DAAO-treated slices (Figures 3A and 3C). Altogether, these results suggest that glycine, but not D-serine, is an endogenous coagonist of extrasynaptic GluN2B-NMDARs on CA1 pyramidal neurons.

Given the strong contribution of GluN2A-NMDARs to synaptic (Figure 1C) but not to extrasynaptic pool (Figure 2B), we performed the mirror experiment using zinc. It strongly reduced NMDAR-EPSCs (Figures 3E and 3F) but had a modest inhibitory effect on NMDA-evoked responses (Figures 3D and 3F) likely reflecting the contribution of synaptic receptors to these currents. Consistently, the effect of zinc was reduced in *Rg*DAAO-treated slices (Figures 3D and 3F), whereas it was slightly enhanced once glycine was degraded with *Bs*GO. Such observations are in agreement with a role for D-serine, but not glycine, at GluN2A-NMDARs that are mostly, if not only, synaptic.

Glycine Acts at Extrasynaptic Receptors

We attempted to isolate more specifically responses mediated by extrasynaptic receptors during NMDA puffs, using the activity-dependent open-channel NMDAR blocker MK-801 (Hessler et al., 1993; Rosenmund et al., 1993; Harris and Pettit, 2007, 2008). NMDAR-EPSCs and NMDA-evoked responses were monitored from the same cell (Figure 4B). Then, NMDA puffs were stopped and MK-801 was applied to selectively silence synaptic NMDARs under stimulation at 0.1 Hz (Hessler et al., 1993; Rosenmund et al., 1993). Once synaptic blockade was maximal, a subsequent single application of NMDA was used to monitor the remaining, extrasynaptic NMDARs-enriched response (Harris and Pettit, 2007, 2008). This response was 36.7% ± 2.9% of the initial NMDA-evoked current amplitude (Figures 4A and 4B). We repeated this procedure to assess the extrasynaptic NMDAR-enriched responses at different levels of

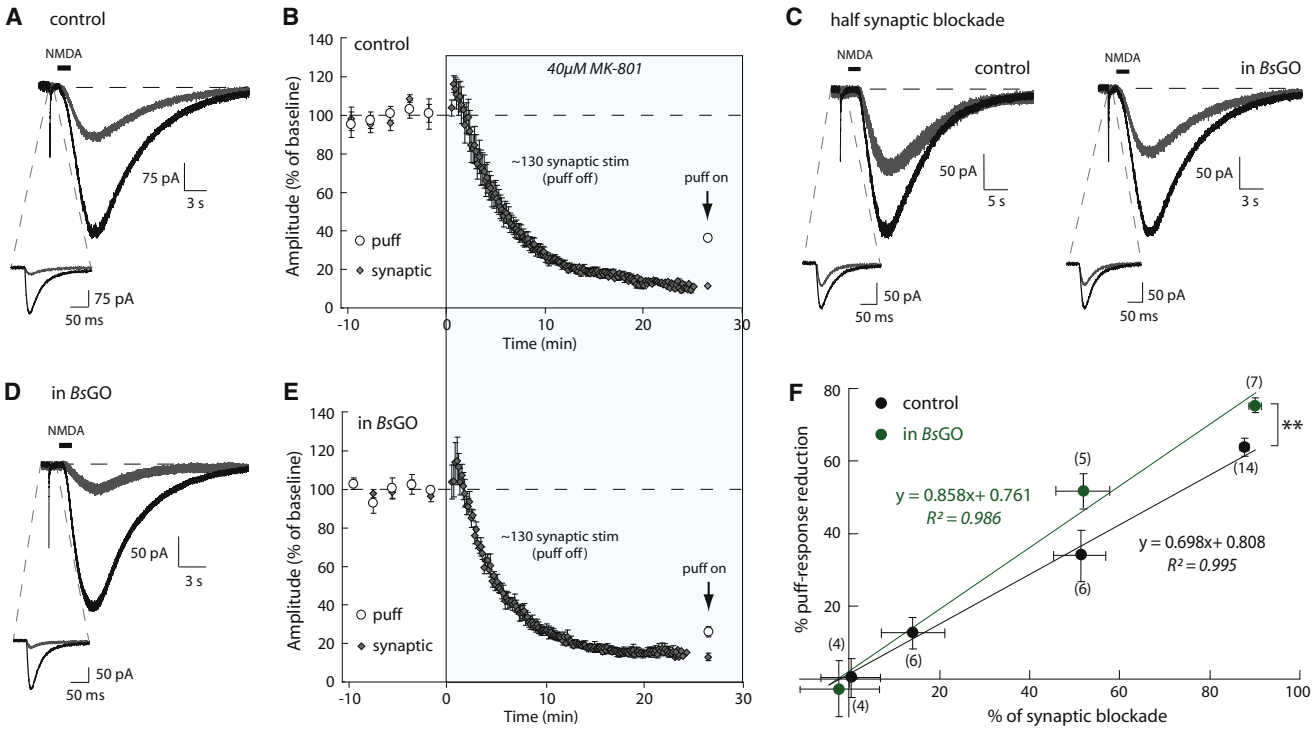

Figure 4. Extrasynaptic NMDARs Are Gated by Endogenous Glycine

(A) Example of responses evoked in a CA1 neuron by synaptic stimulation (inset) and local applications of NMDA, before (black traces) and after (gray traces) blockade of synaptic NMDARs with MK-801.

(B) Once a baseline was obtained for both synaptic and NMDA-evoked currents, MK-801 was applied (40 μM, blue area), NMDA-evoked stimulations were stopped (puff off) and the frequency of stimulations increased to 0.1 Hz. Once the synaptic blockade was optimal (~130 stim, 25 min), puff stimulation was turned on again.

(C) Representative examples of NMDA-evoked responses under conditions in which synaptic NMDARs were only inhibited by 50% in control (left trace) or in *Bs*GO-treated (right trace) slices.

(D and E) same as in (A) and (B) in *Bs*GO-treated slices.

(F) Graph summarizing the average inhibition of the NMDA-evoked responses (control: 63.3% ± 2.9%, 33.7% ± 7.3%, 12.4% ± 4.9%, 0.7% ± 7.2%; *Bs*GO: 74.9% ± 2.3% p = 0.007 compared to blockade in control 50.6% ± 5.8%, −3.9% ± 9.6%) achieved after different degrees of synaptic-current blockade (control: 87.8% ± 1.1%, 50.9% ± 6.9%, 14.1% ± 6.3%, 0.3% ± 6.6%; *Bs*GO: 90.1% ± 1.3%, 51.7% ± 4.9%, −2.8% ± 9.1%), in control (black dots) and *Bs*GO (green dots) slices. Numbers of cells are indicated. Regression equations and coefficients are displayed for control (0.698, $R^2 = 0.995$) and *Bs*GO condition (0.858, $R^2 = 0.986$). For further details, see Extended Experimental Procedures and Figure S4.

synaptic blockade, and the corresponding values were plotted to provide a linear relationship between synaptic blockade and inhibition of NMDA-evoked responses (Figures 4F and S4). The slope obtained from these experiments (Figure 4F) reflected a contribution of 69.8% of synaptic receptors to NMDA-evoked responses, which is remarkably consistent with previous findings (Harris and Pettit, 2007; Petralia et al., 2010). Accordingly, reducing the contribution of extrasynaptic receptors to NMDA-evoked response should shift the slope toward higher values. When repeating this protocol in *Bs*GO-treated slices (Figures 4C–4F and S4) the slope of the linear regression (Figure 4F) indicated that the total response was derived from at least 85% of synaptic receptors. This result strengthens our proposal that glycine gates extrasynaptic NMDARs.

Glycine and D-Serine Have a Different Impact on NMDARs' Lateral Diffusion

Because the nature of the endogenous coagonist used by synaptic and extrasynaptic NMDARs is unlikely to be dictated by receptors' subunit composition (see above), one possible alternative is that the subunit composition at a given location directly results from the coagonist availability. This could be accounted for by a differential impact of glycine and D-serine on surface trafficking of NMDAR-subtypes. We used the single nanoparticle approach (Quantum Dot [QD]; Groc et al., 2007) to investigate the effect of either coagonist on the surface diffusion of GluN2A- or GluN2B-containing single NMDARs in cultured hippocampal neurons. (Figures 5A and S5). In agreement with the literature (Groc et al., 2006; Bard et al., 2010), we observed that GluN2A- and GluN2B-NMDARs diffused at the neuronal surface, and that GluN2A-NMDARs were more stable than GluN2B-NMDARs due to their trapping at synapses (Figure S5). Strikingly, we observed that GluN2A-NMDARs diffusion was not affected in presence of exogenous D-serine (30 μM, Figure 5C) but was slowed down by glycine (30 μM). On the contrary, glycine did not affect the trafficking of GluN2B-NMDARs, which diffused less in the presence of D-serine. These changes in surface diffusion were mostly due

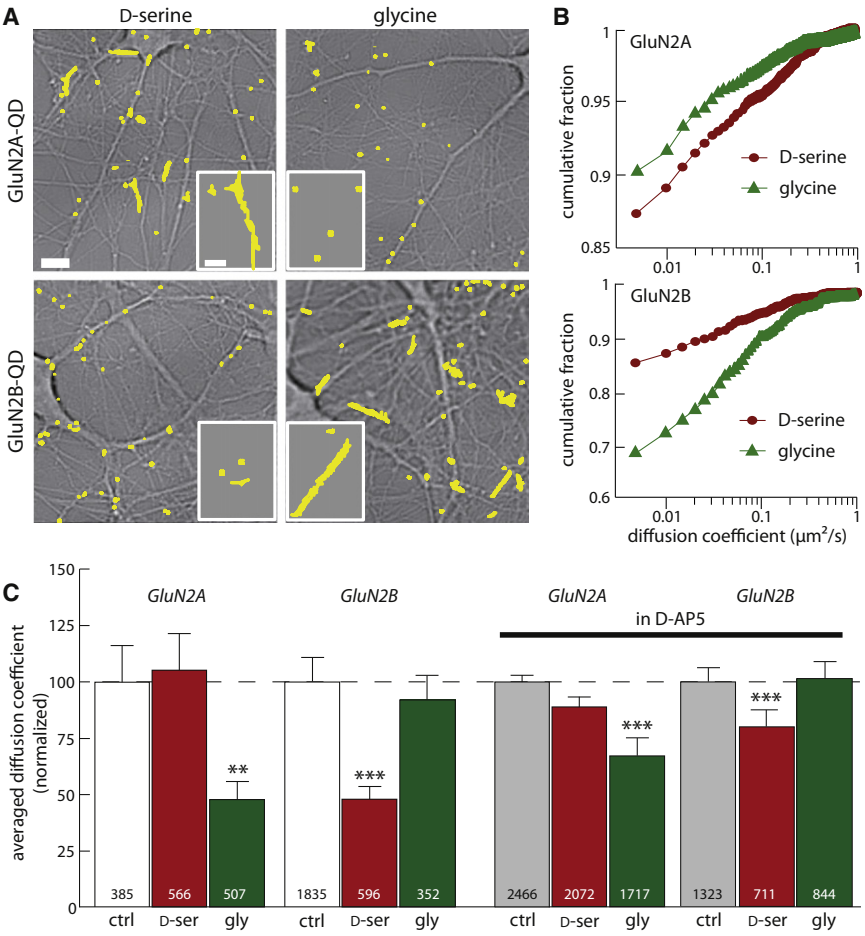

Figure 5. D-Serine and Glycine Differentially Regulate the Surface Trafficking of GluN2A- and GluN2B-NMDARs

(A) Representative trajectories of surface GluN2A- and GluN2B-NMDARs (yellow lines, 800–1000 frames, 50 ms acquisition) imaged over time using the single Quantum Dot tracking approach at the surface of cultured hippocampal neurons in presence of either D-serine (30 μM) or glycine (30 μM). Enlarged trajectories are shown in the insets. Scale bar = 4 μm, scale bar inset = 1 μm.

(B) Cumulative distributions of the instantaneous diffusion coefficient of surface GluN2A- or GluN2B-NMDAR in the presence of either D-serine (GluN2A: n = 385 trajectories, GluN2B: n = 596 trajectories) or glycine (GluN2A: n = 507 trajectories, GluN2B: n = 352 trajectories). Bin size = 0.005 $\mu m^2/s$.

(C) Bar graphs (mean ± SEM) summarizing the effect of D-serine or glycine on the averaged surface diffusion of GluN2A- and GluN2B-NMDARs, in control (GluN2A: D-serine: 105.4% ± 16.1% of control diffusion, p = 0.804; glycine: 47.4% ± 9.0% of control, $p < 0.01^{-4}$; GluN2B: D-serine: 47.9% ± 6.0% of control, p < 0.001; glycine: 91.8% ± 10.2% of control, p = 0.742) or in the presence of D-AP5 (GluN2A: D-serine: 87.4% ± 2.8% of control, p = 0.744; glycine: 67.3% ± 5.3% of control, $p < 10^{-4}$; GluN2B: D-serine: 79.8% ± 8.0% of control, $p < 10^{-4}$; glycine: 101.0% ± 7.9% of control, p = 0.908). Numbers indicate n values.

See Extended Experimental Procedures and Figure S5 for control experiments performed with FLAG-NMDARs.

to a change in the fraction of immobile receptors (diffusion coefficient <0.005 $\mu m^2/s$) rather than a change in the characteristics of diffusion itself (Figure 5B), in line with a rapid (minute range) coagonist-induced change in NMDAR anchoring efficacy. These data thus demonstrate that D-serine and glycine differentially modulate the surface behavior of GluN2A- and GluN2B-NMDARs and that they could actively contribute to the spatial segregation of NMDAR subtypes. Remarkably, such phenomenon persisted in the presence of D-AP5, a NMDAR antagonist acting on the glutamate-binding site (Figure 5C), suggesting that this process is mostly independent of the activity of the receptor.

Contribution of Synaptic and Extrasynaptic NMDARs to Synaptic Plasticity

We next took advantage of the gating of synaptic and extrasynaptic NMDARs by distinct endogenous coagonists to tackle the respective role of these receptors in mediating synaptic plasticity. LTP was induced at CA3-CA1 synapses using high-frequency stimulation. This protocol yielded a long-lasting enhancement of AMPAR-fEPSP slope (Figures 6A1 and 6C) that was inhibited in *Rg*DAAO-treated slices as previously reported (Yang et al., 2003) or in the presence of zinc (Figures 6A2 and 6C). Conversely, degrading glycine with *Bs*GO or inhibiting GluN2B-NMDARs with Ro25-6981 did not affect LTP. These findings indicate that synaptic, but not extrasynaptic, NMDARs are essential for LTP induction. We next assessed the involvement of those receptors in LTD induced by a low frequency stimulation protocol (Figures 6B1 and 6C). As previously reported (Zhang et al., 2008), LTD was impaired in *Rg*DAAO-treated slices, an effect that could be rescued with exogenous glycine (Figures 6B1 and 6C). Interestingly, LTD was also abolished in *Bs*GO-treated slices and rescued with D-serine (Figures 6B2 and 6C). In agreement with these findings, both zinc and Ro25-6981 abolished LTD (Figures 6B3 and 6C). These results establish that both synaptic and extrasynaptic NMDARs are required for LTD induction.

Contribution of Synaptic and Extrasynaptic NMDARs to Neurotoxicity

We finally tested the respective contribution of synaptic and extrasynaptic NMDARs to neurotoxicity (Katsuki et al., 2004; Léveillé et al., 2008; Shleper et al., 2005). To this end, acute hippocampal slices were exposed to 50 μM NMDA for 30 min before assessing neuronal death by Nissl staining in the pyramidal layer of the CA1 area (Figure 7A). NMDA applications reduced cell viability to 64.0% ± 2.8% of control values, an effect prevented by D-AP5 (Figure 7B). We observed that *Rg*DAAO had

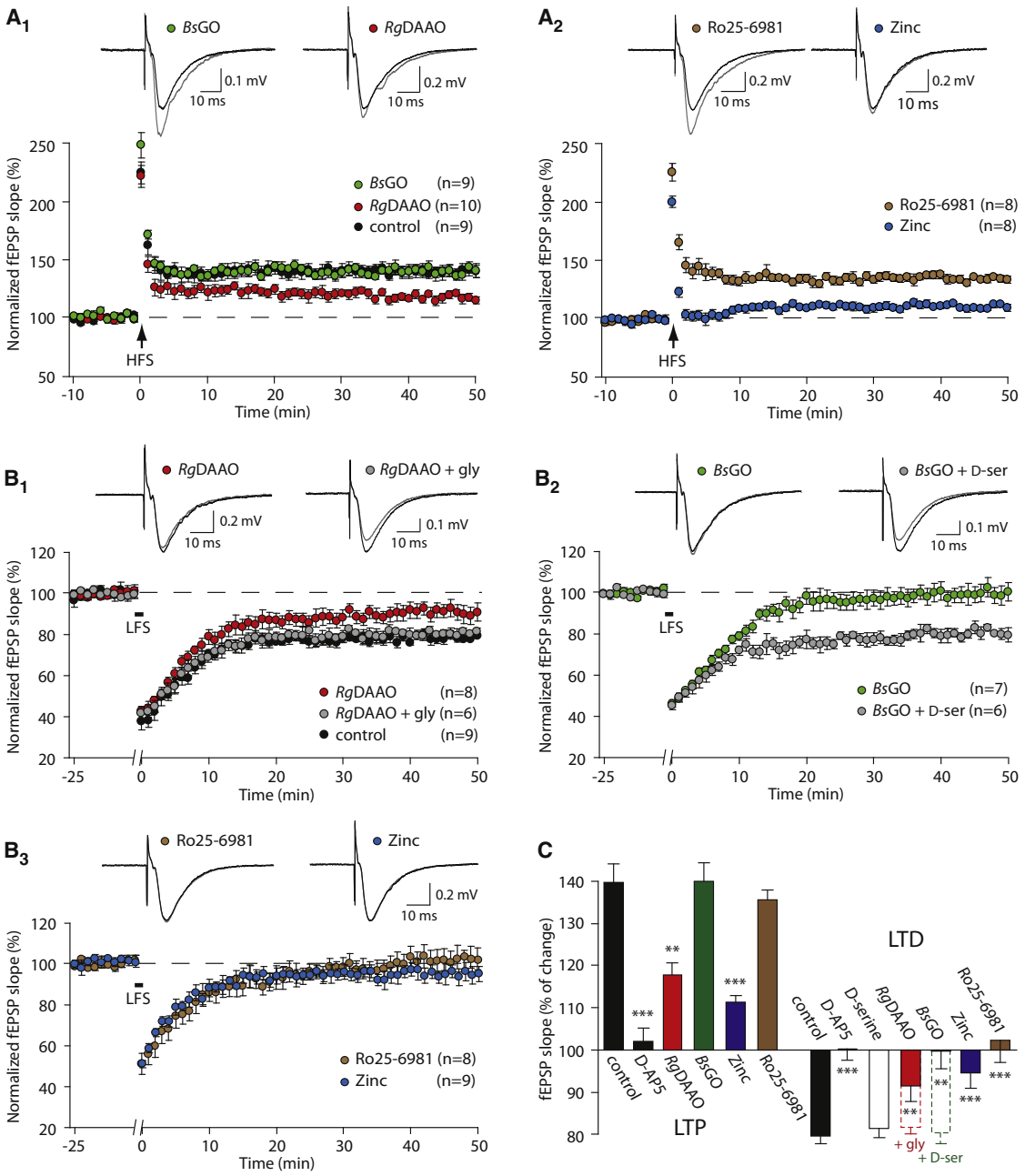

Figure 6. Contribution of Synaptic and Extrasynaptic NMDARs to LTP and LTD

(A_1) LTP (139.6% ± 5.0% of baseline, n = 9, $p < 10^{-4}$, black dots) induced by high frequency stimulation (HFS) was significantly impaired in *Rg*DAAO- (117.8% ± 2.7%, n = 10, $p < 0.01$, red dots) but not in *Bs*GO-treated slices (140.0% ± 4.7%, n = 9, p = 0.953, green dots). Insets represent average fEPSPs obtained during baseline (black trace) and 40–50 min after induction of synaptic plasticity (gray trace), here and throughout the figure. (A_2) Same experiments as in (A_1) conducted in the presence of zinc (111.3% ± 1.4%, n = 8, $p < 0.001$) or Ro25-6981 (135.5% ± 2.4%, n = 8, p = 0.487).

(B) LTD (79.5% ± 1.9% of baseline, n = 9, $p < 10^{-5}$, black dots) induced by low frequency stimulation (LFS) was impaired in *Rg*DAAO- (B_1: 91.3% ± 3.5%, n = 8, $p < 0.01$ versus control, red dots) and *Bs*GO-treated slices (B_2: 99.6% ± 4.4%, n = 7, p = 0.902, $p < 0.001$, green dots), effects that were prevented in the presence of glycine (81.5% ± 1.5%, n = 6, p = 0.967, $p < 0.05$ versus *Rg*DAAO) and D-serine (80.3% ± 2.4%, n = 6, p = 0.766, $p < 0.01$ versus *Bs*GO), respectively (gray dots). (B_3) LTD was abolished in the presence of zinc or Ro25-6981 (94.6% ± 3.8%, n = 9, p = 0.199, $p < 0.01$, and 95.8% ± 4.4%, n = 6, p = 0.707, $p < 0.01$, respectively).

(C) Bar graphs (mean ± SEM) summarizing LTP (left) and LTD (right) magnitudes in different experimental conditions. Statistical significance is assessed compared to control condition.

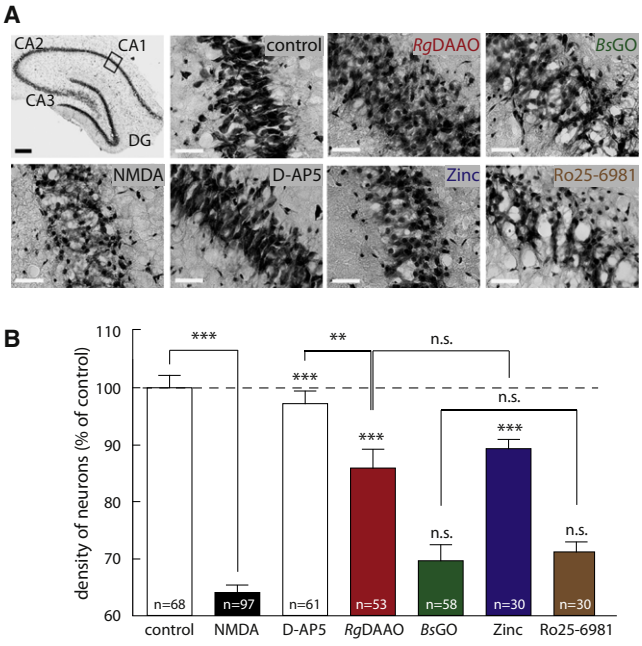

Figure 7. NMDA-Induced Neurotoxicity Is Mediated by Synaptic, but Not Extrasynaptic, NMDARs

(A) Representative images illustrating Nissl staining of a hippocampal slice (top left) to reveal healthy nuclei, and of high magnification images obtained from the stratum pyramidale of the CA1 region in control condition or in the presence of NMDA with or without D-AP5, *Rg*DAAO, *Bs*GO, Ro25-6981 or zinc (other panels). Scale bars: black 400 μm, white 40 μm.

(B) Bar graphs (mean ± SEM) summarizing the density of healthy CA1 pyramidal neurons in the different conditions. NMDA: 64.0% ± 2.8% of control, $p < 0.001$; D-AP5: 97.3% ± 4.5%, $p > 0.05$; *Rg*DAAO: 86.0% ± 7.1%, $p < 0.001$; *Bs*GO: 69.6% ± 6.4%, $p > 0.05$; Zinc: 89.3% ± 3.3%, $p < 0.001$; Ro25-6981: 71.2% ± 3.7%, $p > 0.05$.

See also Figure S6.

a strong protective effect (Figure 7B) as already reported in brain slices (Shleper et al., 2005; Katsuki et al., 2004) or suggested by in vivo experiments (Inoue et al., 2008; Mustafa et al., 2010). Conversely, excitotoxicity was not significantly inhibited by *Bs*GO. In agreement with these results, zinc but not Ro25-6981 had a significant neuroprotective action. These data indicate that NMDARs located at synaptic rather than extrasynaptic sites mediate the neurotoxic effect of NMDA on CA1 pyramidal neurons in our experimental conditions.

DISCUSSION

We provide evidence that synaptic and extrasynaptic NMDARs located on CA1 pyramidal cells are gated by distinct endogenous coagonists. Whereas D-serine is the coagonist at synaptic receptors, glycine acts at extrasynaptic NMDARs. Furthermore, we provide insights into the roles of synaptic and extrasynaptic NMDARs in synaptic plasticity and in excitotoxicity.

The widely accepted idea that glycine is the coagonist of NMDARs was mainly based on the demonstration that exogenous glycine potentiates NMDAR-mediated responses (Johnson and Ascher, 1987; Kleckner and Dingledine, 1988; Forsythe et al., 1988; Berger et al., 1998; Bergeron et al., 1998; Tsai et al., 2004). While these data indicated that increasing ambient glycine concentrations was sufficient to enhance NMDAR activity, they did not demonstrate that endogenous glycine contributed to NMDARs activity under basal conditions. Furthermore, this idea was significantly challenged during the last decade by reports showing that reducing D-serine levels strongly impaired NMDAR-mediated processes in many structures, including in the CA1 region of the hippocampus (Basu et al., 2009; Henneberger et al., 2010; Mothet et al., 2000; Yang et al., 2003). Here, we show that directly reducing glycine levels with *Bs*GO did not affect synaptic NMDAR activity, even under conditions where D-serine was depleted, demonstrating that glycine does not serve as an endogenous coagonist at these receptors.

GluN2A-NMDARs, which have a better affinity for D-serine than for glycine (Matsui et al., 1995), appear to compose 70%–75% of the NMDAR population at CA3-CA1 synapses in the adult rat. In contrast, GluN2B-NMDARs that are more sensitive to glycine were not detected, suggesting that the 20%–25% remaining synaptic NMDARs might be GluN2A-GluN2B triheteromers. This profile agrees with previous studies (Harris and Pettit, 2007; Kirson and Yaari, 1996; Stocca and Vicini, 1998; Tovar and Westbrook, 1999) and indicates that NMDARs at CA3-CA1 synapses in adult rats are well suited to disregard low levels of glycine while efficiently detecting D-serine. Concomitantly, our results demonstrate that glycine transporters actively maintain glycine concentrations within the synaptic cleft at levels too low to coagonize NMDARs, arguing against previous conclusions that these transporters were tuning glycine levels within the cleft at concentrations enabling NMDARs activation (Berger et al., 1998; Bergeron et al., 1998).

One of the important observations of our study is that glycine, but not D-serine, gates NMDARs located at extrasynaptic sites. These receptors which contain GluN2B-subunits (Angulo et al., 2004; Fellin et al., 2004, Köhr, 2006) were largely silenced by *Bs*GO but not by *Rg*DAAO, indicating that endogenous glycine controls the activity of extrasynaptic NMDARs that are activated tonically by ambient glutamate, as well as those that can be recruited phasically by NMDA applications. Conversely, D-serine did not significantly contribute to these responses, even when glycine was depleted, confirming that the action of D-serine is most likely restricted to the synaptic cleft. Together, our results suggest that the nature of the endogenous coagonist gating NMDARs in and outside the synapses is dictated by D-serine and glycine availability at those locations. This is paralleled by differences in GluN2-subunit composition that confer preferential affinities to synaptic and extrasynaptic NMDARs for the two coagonists. However, GluN2 subunits content does not seem to account for the nature of the local endogenous NMDAR coagonist. Indeed, our recordings obtained in the CA1 of young rats, in agreement with previous findings obtained at other central synapses (Panatier et al., 2006; Fossat et al., 2012), show that endogenous glycine does not gate synaptic receptors even though they contain some GluN2B-NMDARs. Another explanation could be that the presence of D-serine within the synapse and glycine outside the synapse influences the subunit composition of NMDARs at these locations. This is supported

by lateral diffusion experiments showing that glycine strongly and rapidly inhibits the mobility of GluN2A-NMDARs, whereas D-serine preferentially slows down lateral diffusion of GluN2B-NMDARs. Unexpectedly, we found this effect to be independent of the activity of NMDARs, meaning that the coagonist site of GluN2A- and GluN2B-NMDARs distinguishes glycine from D-serine and directly impacts the lateral mobility and/or recycling of the receptors (Nong et al., 2003). This could occur through conformational changes of the receptor, impacting the interaction with extracellular and/or intracellular partners. Because these interactions shape the surface dynamics and distribution of NMDARs, it is likely that the sole binding of D-serine on GluN2B-NMDARs and glycine on GluN2A-NMDARs could prevent their diffusion in and out of synapses, respectively, leading to a confinement of GluN2A-NMDARs at synapses and an enrichment of GluN2B-NMDARs at extrasynaptic location. We thus propose that in the hippocampus coagonists regionalization actively contributes to segregate NMDARs at specific locations and delineates distinct functional pools of surface NMDAR subtypes with their associated intracellular pathways (Figure S6).

Degrading glycine or D-serine was used to assess the role of these receptors in different cellular processes. We provide evidence that LTP is mediated by synaptic NMDARs while extrasynaptic NMDARs do not contribute to this form of plasticity. This indicates that extrasynaptic NMDARs are not recruited by glutamate spillover and/or that the intracellular pathway activated by synaptic receptors prevails over that recruited by extrasynaptic NMDARs, during LTP protocol. Conversely, both synaptic and extrasynaptic NMDARs have to be activated to induce LTD. These results, that were confirmed by blocking GluN2A- and GluN2B-NMDARs, add to the debate regarding the specific role of GluN2A and GluN2B-NMDARs in mediating LTP and LTD, respectively (Ge et al., 2010; Liu et al., 2004; Massey et al., 2004; Zhao and Constantine-Paton, 2007; but see Morishita et al., 2007; Berberich et al., 2005). Our data indicate that the location of NMDARs, rather than subunit-composition, might be the relevant parameter for their specific involvement in these processes, as proposed earlier (Rusakov et al., 2004).

We also found that silencing synaptic NMDARs offers neuroprotection against NMDA-induced excitotoxicity. More surprisingly, inhibiting extrasynaptic NMDARs did not have any protective effect, arguing against a role for these receptors in neurotoxicity. These findings confirm previous reports about the role of D-serine in mediating cell death in slices (Shleper et al., 2005; Katsuki et al., 2004) and in vivo (Inoue et al., 2008; Mustafa et al., 2010). They are, however, in strong disagreement with those obtained in cultured neurons (Hardingham et al., 2002; Hardingham and Bading, 2003; Léveillé et al., 2008) reporting a preferential role for extrasynaptic NMDARs in excitotoxicity. Although our results do not rule out a possible role for extrasynaptic NMDARs in other excitotoxic conditions, such as hypoxia or long-lasting glutamate applications, they show that cell death can result from the sole activation of synaptic NMDARs.

Changes in glycine concentrations in the extracellular space and of D-serine levels within the synapse are likely to obey completely different rules and dynamics. Glial cells, through their supply of D-serine to synapses (Henneberger et al., 2010) and removal of glycine from the cleft, appear to be key controller of synaptic NMDAR functions. Conversely, they may provide glutamate to extrasynaptic NMDARs (Angulo et al., 2004; Fellin et al., 2004; Le Meur et al., 2007) that are coagonized by ambient glycine. Such disparities in the nature and supply of endogenous agonists and coagonists, on top of the differential impact of coagonists on NMDARs subtypes mobility, is likely to be of paramount importance for the activity of synaptic and extrasynaptic NMDARs and, consequently, for the different physiological and pathophysiological processes in which these receptors are involved.

EXPERIMENTAL PROCEDURES

Slice Preparation

Experiments were carried out on acute hippocampal slices (300 μm) obtained from adult Wistar rats (2–3 months old) as described in Extended Experimental Procedures. Electrophysiological experiments were performed in the presence of 50 μM picrotoxin and 10 μM strychnine. NMDAR-mediated responses were isolated with 10 μM NBQX to block AMPA/Kainate receptors. All experiments were conducted with respect to European and French directives on animal experimentation (authorization no. 33 0004).

Field Recordings

Schaffer collaterals fibers were electrically stimulated at 0.05 Hz and evoked fEPSPs were recorded using a glass electrodes placed in the stratum radiatum. NMDAR-mediated fEPSPs were studied in low Mg^{2+} (0.2 mM). LTP was induced using 1 s trains of 100 Hz stimulation repeated three times at 20 s intervals. LTD was induced with a low frequency stimulation protocol (15 min at 1 Hz).

Patch-Clamp Recordings

Pyramidal CA1 neurons were identified visually using infrared DIC microscopy (Olympus BX50). Patch-clamp recording pipettes (2–4 MΩ) were filled with (in mM): 150 caesium methane-sulfonate; 1.3 $MgCl_2$; 1 EGTA; 10 HEPES; 0.1 $CaCl_2$ (adjusted to pH ~7.2 with CsOH, 290–296 $mOsm.kg^{-1}$). Access resistance and holding current were monitored throughout the experiment. For NMDA puffs, a glass pipette containing 1 mM NMDA diluted with the bathing solution was connected to a picospritzer air-pressured system and placed in the stratum radiatum at a distance of ~200 μm from the cell soma. Synaptic NMDARs were defined as those recruited during afferent stimulation at low frequency (<0.1 Hz), and extrasynaptic NMDARs as those not activated during such stimulation.

Data Analysis

The data were recorded with a Multiclamp 700A amplifier (Axon Instruments, Inc.), sampled at 20 kHz, filtered at 2 kHz, and analyzed using pClamp9 software (Axon Instruments, Inc.). Average EPSCs and fEPSPs traces were obtained from at least 10 min of stable recordings. Data, reported as mean ± SEM, were compared using paired or unpaired Student's t test. Significance was assessed at $p < 0.05$. Symbols used are $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$ throughout the manuscript.

Single Quantum Dot Tracking

Hippocampal cultures, containing neurons and glial cells, were prepared from E18 rat embryos and grown on glass coverslips as previously described (Bard et al., 2010). Imaging sessions were performed on hippocampal 8–10 div neurons in glutamate- and serum-free aSCF. Quantum Dot (QD) labeling and microscopy was performed as previously described in Bard et al. (2010). A recording session typically lasted 60 s. The instantaneous diffusion coefficient was calculated for each trajectory, from linear fits of the first four points of the mean-square-displacement versus time function using $MSD(t) = < r^2 > (t) = 4Dt$.

Neurotoxicity Experiments

Neurotoxicity was performed and assessed as described by Katsuki et al. (2004) with minor modifications. Briefly, slices similar to those used for electrophysiological recordings were treated with NMDA (50 μM) for 30 min, fixed and then cut to 30 μm thick sections before mounting onto glass and exposed to Cresyl violet (10 min) for Nissl staining. Positively stained cell bodies in an area of 54.5 × 54.5 μm^2 within the CA1 region of individual slices were counted.

Enzymes

Recombinant wild-type *Rhodotorula gracilis* D-amino acid oxidase (*Rg*DAAO, EC 1.4.3.3) was overexpressed in *E. coli* cells and purified as reported earlier (Molla et al., 1998). An inactive form of *Rg*DAAO (ΔDAAO) that does not dehydrogenate D-amino acids was generated by substitution of Arg285 with an alanine (Molla et al., 2000). Recombinant wild-type *Bacillus subtilis* glycine oxidase (*Bs*GO, EC 1.4.3.19) was overexpressed in *E. coli* cells as well (Job et al., 2002). The final *Rg*DAAO and *Bs*GO preparations had a specific activity of ~75 U/mg protein on D-serine as substrate and 1.1 U/mg protein on glycine as substrate, respectively. The ΔDAAO specific activity was ~0 U/mg protein. Further details are provided in Extended Experimental Procedures.

Drugs

The drugs used were picrotoxin 50 μM, strychnine hydrochloride 10 μM, NBQX salt 10 μM (NBQX), glycine 0.1–0.5 mM, D-serine 10–500 μM, D-AP5 50 μM, ALX 5407 hydrochloride 1 μM (ALX), Ro 25-6981 maleate 2 μM (Ro25-6981), N-methyl D-aspartate 1 mM (NMDA), $ZnCl_2$ 250 nM used in Tricine 10 mM with the relation $[Zinc]_{free} = [Zinc]_{applied}/200$ (Paoletti et al., 1997; Paoletti and Neyton, 2007) and MK-801 maleate 40 μM (MK-801). For electrophysiological experiments, drugs were all bath-applied except NMDA (see above). Cresyl violet paraformaldehyde was obtained from Electron Microscopy Science, Tricine from Sigma, and all other drugs were purchased from Tocris.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Extended Experimental Procedures and six figures and can be found with this article online at http://dx.doi.org/10.1016/j.cell.2012.06.029.

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T.P. designed, performed, and analyzed the electrophysiological experiments and wrote the paper. L.L. and J.R. contributed equally to this work. L.L. performed and analyzed the QD experiments. J.R. designed, performed, and analyzed the excitotoxicity experiments. S.S. produced the enzymes and analyzed for interferencies. M.L. and M.H. performed excitotoxicity experiments. L.G. supervised the QD experiments. L.P. engineered the enzymes and supervised their production. J.-P.M. supervised the excitotoxicity experiments, the use of the enzymes in the entire study and also designed experiments. S.H.R.O. supervised the study and wrote the paper.

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