PERGAMON

Neuropharmacology 37 (1998) 1393–1398

NEURO PHARMACOLOGY

No change in NMDA receptor-mediated response rise-time during development: evidence against transmitter spillover

K. Haas, H. Cline, R. Malinow *

Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724, USA

Accepted 5 August 1998

Abstract

Glutamatergic transmission was examined in tadpole optic tectum to test the possibility that transmitter concentration reaching *N*-methyl-D-aspartate (NMDA) receptors increases over development. Pharmacologically isolated NMDA receptor-mediated transmission was monitored with whole-cell recordings. Synaptic responses were recorded from cells at different locations in the optic tectum, corresponding to different stages of development. Rise-times and decay-times of NMDA currents were analyzed. We found no significant correlation between rise-time and developmental stage. As NMDA rise-times can correlate with concentration for glutamate concentrations below 200 μM, these results argue that, if there is developmental variation in transmitter concentration, this occurs for values greater than 200 μM. Furthermore, we found a correlation between rise-times and decay-times, consistent with a model in which transmitter concentration is high and rise-time is controlled by channel closings. These results argue against synaptic models in which low concentrations of transmitter (as by spillover from nearby release sites) selectively activates NMDA receptors.

Keywords: Development; Glutamatergic; Kinetics; NMDA receptor; Spillover; Synaptic transmission

1. Introduction

Retinotectal transmission in *Xenopus* tadpoles provides a useful system to examine development of glutamatergic synapses since tectal neurons along the rostrocaudal axis range in their maturational state from newly differentiated at the caudal proliferative zone to more mature as they move rostrally. Postsynaptic tectal neurons are also electrophysiologically compact and monosynaptic single fiber transmission can be readily elicited. Previous studies have examined physiological and morphological changes that occur over development by obtaining measurements at different locations along the rostrocaudal axis (Wu et al., 1996). These studies have led to a model whereby retinotectal synapses initially transmit only with *N*-methyl-D-aspartate (NMDA) receptors, and add an α-amino-3-hydroxy-5-methyl-4-isoxazol-epropionic component with maturation. That is, a large fraction of synapses in immature regions transmit only with NMDA receptors, while in mature regions most synapses show both AMPA and NMDA components.

Pure NMDA-mediated transmission has been observed in a number of preparations (Liao et al., 1995, Durand et al., 1996, Wu et al., 1996, Isaac et al., 1997), and several models have been proposed to explain such responses. One possibility is that some synapses contain only NMDA receptors. Another possibility is that synapses contain both AMPA and NMDA receptors, but some synapses receive only low concentrations of transmitter, sufficient to activate NMDA but not AMPA receptors (Kullmann and Siegelbaum, 1995, Kullmann et al., 1996). This is theoretically possible given the considerably higher affinity of NMDA receptors for glutamate (Patneau and Mayer, 1990). Low concentrations could reach synapses either if there is a low amount of transmitter in vesicles or because the release site is anatomically distant from the receptors.

Previous studies in isolated patches (Clements and Westbrook, 1991) have shown that for low agonist concentrations (glutamate < 200 μM) NMDA receptor activation time course is related to transmitter concentration. For high concentration values (glutamate

* Corresponding author. Tel.: +1 516 3678416; e-mail: malinow@cshl.org.

0028-3908/98/$ - see front matter
PII: S0028-3908(98)00137-3

>200 μM), NMDA receptor activation time course is independent of concentration and is controlled by channel closings. Modeling studies (Rusakov and Kullmann, 1998) suggest that low concentrations of glutamate that activate predominantly NMDA receptors will produce NMDA currents with slower rise-times.

We have thus examined NMDA rise-times for transmission onto neurons at different maturational states, recorded at different positions along the rostrocaudal axis. We find that rise-times are not correlated with developmental progression and on average correlate well with decay-times. Both findings support the view that NMDA responses are produced by exposure to high concentration of transmitter, and argue against spillover as a mechanism for pure NMDA responses in optic tectum.

2. Methods

Albino *Xenopus* tadpoles (stage 47–48) were anesthetized in 0.02% MS222 in Steinberg's solution and their brains were rapidly dissected out (Wu et al., 1996). The tectum was filleted along the dorsal midline and laid out in a recording chamber with the ventricular surface facing up. The brain was held in place with fine wire weights and continuously perfused with a bath solution composed of 115 mM NaCl, 4 mM KCl, 3 mM $CaCl_2$, 3 mM $MgCl_2$, 5 mM HEPES, 10 μM glycine, 100 μM picrotoxin and 10 mM glucose (pH 7.2 with NaOH). Whole-cell patch-clamp recordings of tectal neurons at different maturational states were performed by visually assisted patching on cells at different distances from the caudomedial proliferative zone. The internal patch pipette solution contained 80 mM cesium methane sulfonate, 10 mM EGTA, 20 mM tetraethylammonium chloride (TEA), 5 mM $MgCl_2$, 2 mM ATP, 0.3 mM GTP, and 20 mM HEPES, pH 7.2 with CsOH. All recordings were performed at room temperature (20–23°C). Neurons had input resistances in the range 1–4 GΩ and series resistances were less than 50 MΩ. A bipolar tungsten wire electrode was placed in the optic chiasm to elicit synaptic currents from retinal ganglion cell axons. The optic nerve was stimulated at 0.1 Hz at a voltage (4–26 V) adjusted to limit polysynaptic excitatory postsynaptic currents. Pure NMDA receptor-mediated retinotectal currents were recorded by holding the membrane potential at $+60$ mV in the presence of NBQX (3 μM) to block AMPA receptors. No outward current was observed at a holding potential of 0 mV, indicating that GABAergic inhibition was completely blocked. Approximately 50 responses, at 0.1 Hz, were recorded at sample durations of 60 ms and 1 s to measure NMDA current rise-times and decay rates, respectively. Responses were filtered (2 kHz) and amplified with an Axopatch 1D, digitized (3–10 kHz) and stored on a computer for later analysis. Following recording, a low magnification brightfield image of the tectum was taken to determine location of the recorded cell.

3. Results

Whole-cell recordings were obtained from tadpole optic tectum cell bodies positioned at different locations along the rostrocaudal axis. Pharmacologically isolated NMDA synaptic responses (see Section 2) are shown in Fig. 1A. Sample responses recorded from neurons in caudal (left trace) and rostral (middle trace) tectum are shown and compared (right, superimposed). There is little difference in their activation time course. We quantified activation time course by measuring the time required to go from 10% to 90% of peak value (10–90% rise-time), from 20% to 80% of peak (20–80% rise-time) and from 30% to 70% of peak (30–70% rise-time). We found no correlation between any of these measures and cell body position along the rostrocaudal axis (Fig. 1B–D).

As noted by Clements and Westbrook (1991), if ligand concentration is high (>200 μM), then NMDA receptor activation time course is expected to be controlled by the channel closing rate since the closing rate is much faster than the opening rate. NMDA receptor decay time course is also likely to be controlled by channel closings. Hence, if transmitter concentration is high, activation and decay time course would be expected to be correlated. We quantified the NMDA receptor-mediated synaptic decay time course by measuring the time required to decay to 50% of peak value ($t_{50\%}$). Fig. 2 shows a plot of synaptic NMDA receptor decay-times versus 10–90% rise-time. We found a significant correlation ($R = 0.49$, $P < 0.01$) between these values.

4. Discussion

Previous studies on several brain preparations have identified synaptic responses mediated purely by actions on NMDA receptors (Isaac et al., 1995, Liao et al., 1995, Durand et al., 1996, Wu et al., 1996). One proposal has been that such responses are due to release of transmitter at synapses that contain only NMDA receptors. These have been termed 'silent synapses' since the absence of AMPA receptor-mediated depolarization to remove Mg^{2+} blockade of NMDA receptors leaves these synapses non-functional at resting membrane potentials. An alternative proposal is that such responses result from low concentrations of transmitter reaching the postsynaptic surface caused by reduced glutamate release, longer intrasynaptic dis-

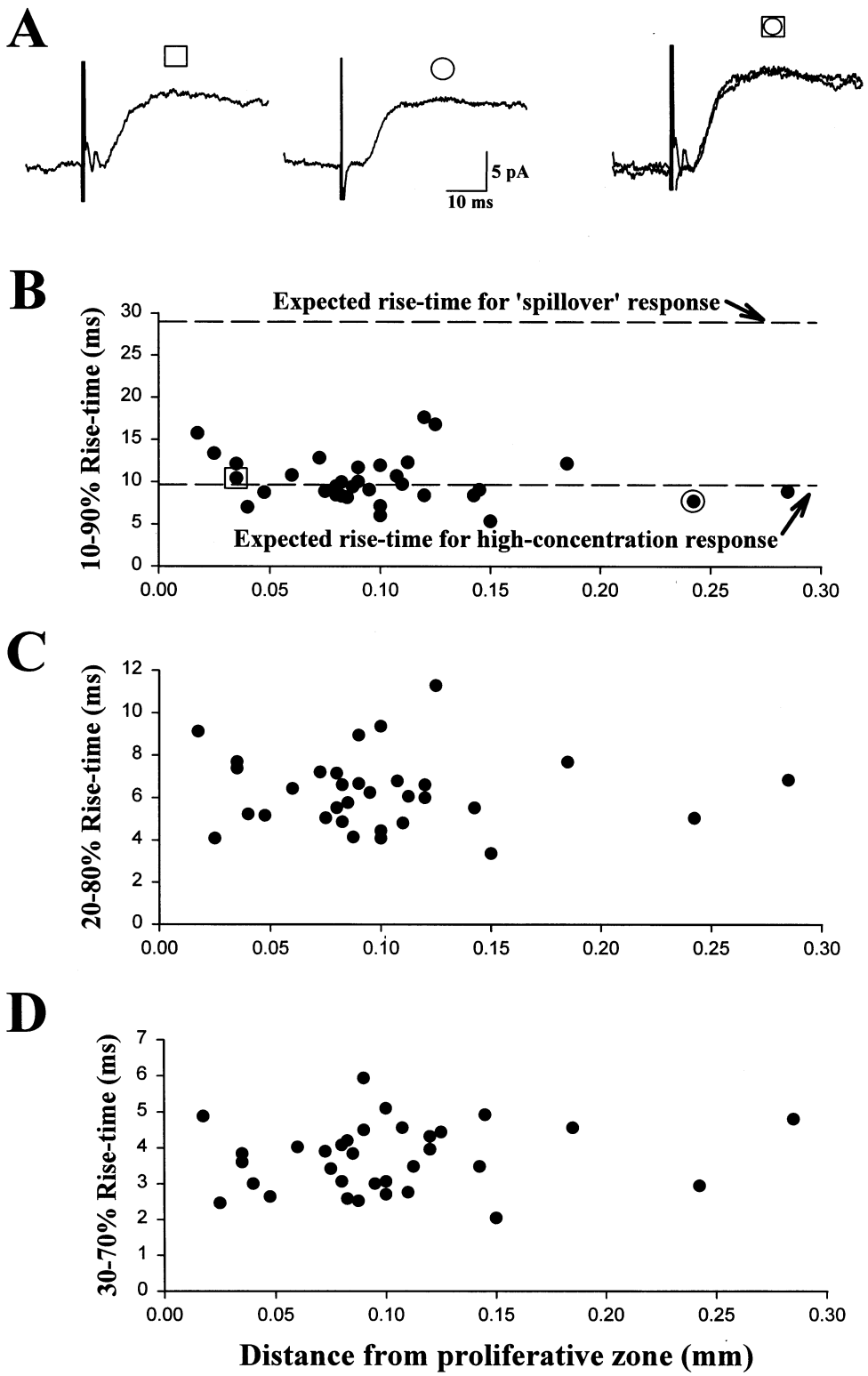

Fig. 1. Rise-times of retinotectal NMDA EPSCs in tectal cells do not vary with maturational states. (A) Traces of retinotectal evoked NMDA currents from an immature neuron (left) in the caudal tectum near the proliferative zone, and from a more mature cell in the rostral tectum (middle). Traces are averages of 50 samples. At the right, normalized traces are superimposed, demonstrating similarity in rise-times. (B) Rise-times (10–90%) of NMDA EPSCs plotted against each neuron's distance from the caudal proliferative zone. Square and circle denote cells presented in (A). Top dotted line indicates predicted rise-times for low concentrations of glutamate expected from spillover models (Rusakov and Kullmann, 1998). Lower dotted line denotes rise-time demonstrated for levels of glutamate >200 μM in outside-out patches (Clements and Westbrook, 1991). (C, D) Rise-times, 20–80% and 30–70%, respectively, of NMDA currents from the same cells as (B).

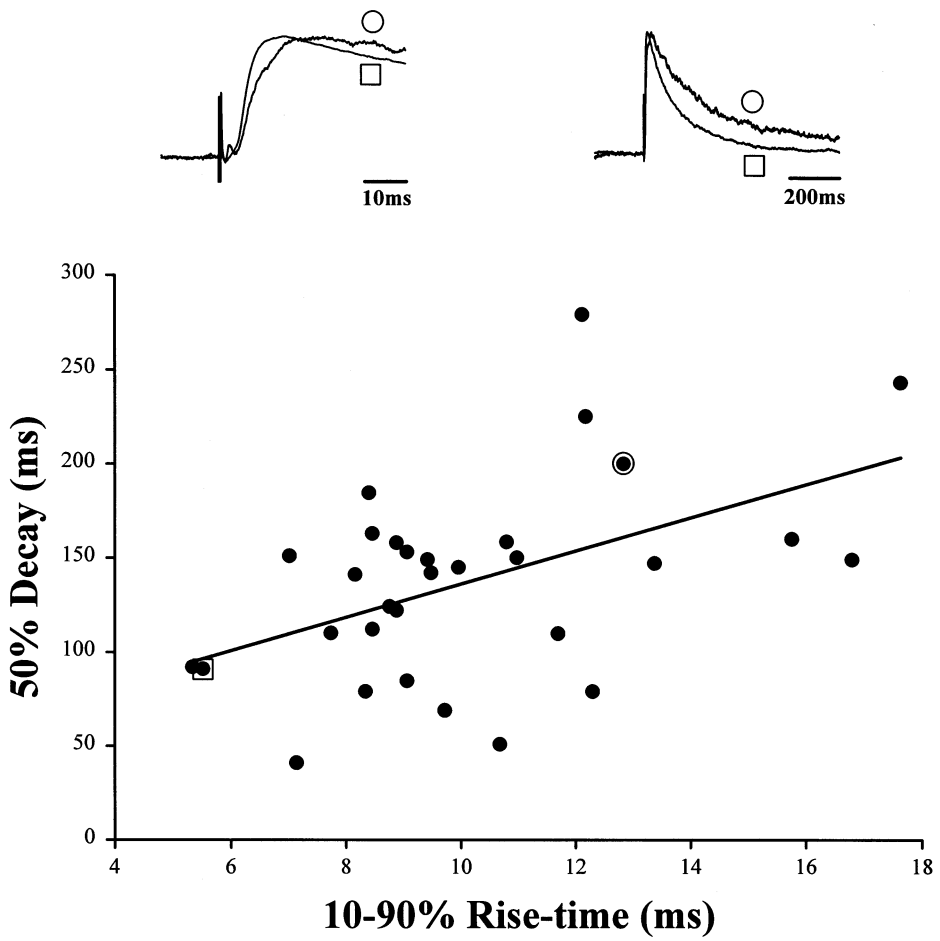

Fig. 2. Rise-times (10–90%) of tectal cell NMDA EPSCs are significantly correlated ($R = 0.49$, $P < 0.01$) with decay rates (time to decay to 50% of peak) in the same cells used in Fig. 1. A correlation between rise-times and decay rates suggests that both are controlled by channel closing rates and supports models in which glutamate concentrations at all tadpole tectal synapses are high. Traces at top demonstrate corresponding change in rise-time (left) with decay rate (right). Circles and squares correspond to indicated points on plot. The amplitudes of NMDA receptor-mediated currents in these cells are 186 pA (square) and 32 pA (circle).

tances, or 'spillover' of glutamate from neighboring synapses (Kullmann et al., 1996). Due to differences in affinity, low concentrations of transmitter could selectively activate NMDA receptors, leaving AMPA receptors quiescent (Patneau and Mayer, 1990). One way to distinguish between these possibilities is to examine neuronal preparations showing pure NMDA responses with immunogold electron microscopy (Esteban et al., 1998, Petralia et al., 1998).

In this study, we have investigated the biophysical mechanisms responsible for silent synapses by examining the kinetics of such responses. The kinetics of NMDA-mediated responses has been previously characterized using measurements from patches of membranes containing NMDA receptors responding to fast perfusion of agonist (Clements and Westbrook, 1991). Such experiments demonstrate that the activation rise-time is controlled by different factors depending on whether the agonist concentration is high (> 200 μM) or low (< 200 μM). For low agonist concentrations the activation time course is sigmoidal and the rise-time depends on agonist concentration (Clements and Westbrook, 1991). For high agonist concentrations the 10–90% rise-time is ≈ 10 ms (as measured from Fig. 3 of Clements and Westbrook, 1991) and is determined by channel closing rate. The channel closing rate is also expected to control the decay of the current. One recent modeling study suggests that the rise-time of NMDA responses from synapses receiving 'spillover' amounts of transmitter will be slower than the rise-time of NMDA responses at synapses receiving transmitter from opposing release site (Rusakov and Kullmann, 1998). This particular model resulted in a 'spillover' NMDA response with 10–90% rise-time of ≈ 29 ms (as measured from Fig. 8C3 of Rusakov and Kullmann, 1998). These properties of NMDA receptors can be used to shed light on the biophysical mechanisms underlying silent synapses. If silent synapses are due to a low concentration of transmitter as modeled by Rusakov and Kullmann (1998), then their 10–90%

rise-time should be ≈ 30 ms and their rise-times should be slower than for synapses producing mixed AMPA and NMDA responses. If silent synapses are due to high concentrations of glutamate released at sites containing only functional NMDA receptors, then the rise-time should correlate with decay-time.

To compare synapses producing pure NMDA responses and those producing mixed AMPA and NMDA responses, we chose to record from different cells along the rostrocaudal axis in tadpole optic tectum. This region has previously been shown to have a gradient of both morphologic development and synaptic responses (Wu et al., 1996). Transmission onto cells at the caudal border, near the proliferative zone, produce responses that are largely (80–100%) composed of pure NMDA responses. Alternatively, responses obtained from more mature cells at the rostral end show 80–100% mixed AMPA and NMDA responses.

Several lines of evidence in this study support the view that silent synapses in tadpole optic tectum are due to action of high concentration of transmitter. First, the rise-time shows no correlation with position. Thus, for regions where 80–100% of responses have been shown to arise from silent synapses, the rise-time of NMDA responses is comparable to that seen in regions where 80–100% of responses originate from mixed synapses. Second, the average rise-time is ≈ 10 ms, comparable to that seen when high concentrations of transmitter are delivered to patches of membrane containing NMDA receptors. Third, no synaptic response gave a rise-time even close to that predicted for a pure NMDA response resulting from low glutamate concentrations. The slowest response we saw had a 10–90% rise-time of 17 ms. For a spillover NMDA response producing a rise-time of 16 ms, the amplitude of the spillover AMPA response was 26% of the NMDA amplitude (as measured from Figs 8C2 and 8C3 of Rusakov and Kullmann, 1998). Our typical quantal NMDA responses at silent synapses are > 5 pA (see Figs 3 and 4 of Wu et al., 1996). A 1-pA AMPA response would readily be observable. The fourth piece of evidence comes from the prediction that with high agonist concentrations, the rise-time should be related to the decay-time. Variable NMDA channel kinetics which would influence both rise-time and decay rates may be expected from differences in receptor phosphorylation states (Lieberman and Mody, 1994, Wang et al., 1994) or different combinations of NMDA receptor subunits that may be present in different cells (Monyer et al., 1994, Kirson and Yaari, 1996, Flint et al., 1997). Indeed, plots of rise-time versus decay-times gave a significant correlation. These considerations argue against the view that low concentrations of glutamate are responsible for the pure NMDA responses we observe.

Two important qualifications must be stated. First, our data argue against a particular quantitative model that has been described to produce spillover NMDA responses (Rusakov and Kullmann, 1998). It is possible that other biophysical models (using different agonist concentration and time course, different diffusion and uptake properties, different AMPA and NMDA kinetics) could yield 'spillover' responses without slow rise-times. Indeed, our results should challenge those investigators maintaining a 'spillover' mindset to come up with such a model. Second, the responses on isolated patches (Clements and Westbrook, 1991) from which we compared rise-times were due to activation of NMDA receptors exposed to concentration steps rather than concentration transients. The latter should mimic more accurately what occurs at synapses and it is possible that responses to transients behave differently than responses to steps. In conclusion, our data argue against particular models of transmitter 'spillover' but a definitive resolution requires further experimentation.

References

Clements, J.D., Westbrook, G.L., 1991. Activation kinetics reveal the number of glutamate and glycine binding sites on the *N*-methyl-D-aspartate receptor. Neuron 7, 605–613.

Durand, G.M., Kovalchuk, Y., Konnerth, A., 1996. Long-term potentiation and functional synapse induction in developing hippocampus. Nature 381, 71–75.

Esteban, J.A., Petralia, R.S., Wang, Y.-X., Wenthold, R.J., Malinow, R., 1998. Estimating silent synapse prevalence from immunogold data using low detection correction. Soc. Neurosci. Abstr. 24, 338.

Flint, A.C., Maisch, U.S., Weishaupt, J.H., Kriegstein, A.R., Monyer, H., 1997. NR2A subunit expression shortens NMDA receptor synaptic currents in developing neocortex. J. Neurosci. 17, 2469–2476.

Isaac, J.T., Nicoll, R.A., Malenka, P.C., 1995. Evidence for silent synapses: implications for the express of LTP. Neuron 15, 427–434.

Isaac, J.T., Crair, M.C., Nicoll, R.A., Malenka, R.C., 1997. Silent synapses during development of thalamocortical inputs. Neuron 18, 269–280.

Kirson, E.D., Yaari, Y., 1996. Synaptic NMDA receptors in developing mouse hippocampal neurones: functional properties and sensitivity to ifenprodil. J. Physiol. 497, 437–455.

Kullmann, D.M., Siegelbaum, S.A., 1995. The site of expression of NMDA receptor-dependent LTP: new fuel for an old fire. Neuron 15, 997–1002.

Kullmann, D.M., Erdemli, G., Asztely, F., 1996. LTP of AMPA and NMDA receptor-mediated signals: evidence for presynaptic expression and extrasynaptic glutamate spill-over. Neuron 17, 461–474.

Liao, D., Hessler, N.A., Malinow, R., 1995. Activation of postsynaptically silent synapses during pairing-induced LTP in CA1 region of hippocampal slice. Nature 375, 400–404.

Lieberman, D.N., Mody, I., 1994. Regulation of NMDA channel function by endogenous Ca^{2+}-dependent phosphatase. Nature 369, 235–239.

Monyer, H., Burnashev, N., Laurie, D.J., Sakmann, B., Seeburg, P.H., 1994. Developmental and regional expression in the rat brain and functional properties of four NMDA receptors. Neuron 12, 529–540.

Patneau, D.K., Mayer, M.L., 1990. Structure–activity relationships for amino acid transmitter candidates acting at *N*-methyl-D-aspartate and quisqualate receptors. J. Neurosci. 10, 2385–2399.

Petralia, R.S., Esteban, J., Wang, Y.-X., Partridge, J.G., Weaver, D., Malinow, R., Wenthold, R.J., 1998. Immunocytochemical evidence of silent synapses in the hippocampus during development. Soc. Neurosci. Abstr. 24, 338.

Rusakov, D.A., Kullmann, D.M., 1998. Extrasynaptic glutamate diffusion in the hippocampus: ultrastructural constraints, uptake, and receptor activation. J. Neurosci. 18, 3158–3170.

Wang, L.-Y., Orser, B.A., Brautigan, D.L., MacDonald, J.F., 1994. Regulation of NMDA receptors in cultured hippocampal neurons by protein phosphatases 1 and 2A. Nature 369, 230–232.

Wu, G., Malinow, R., Cline, H.T., 1996. Maturation of a central glutamatergic synapse. Science 274, 972–976.