Astrocytic iNOS-Dependent Enhancement of Synaptic Release in Mouse Neocortex

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INTRODUCTION

Nitric oxide (NO) has direct neural effects and has been defined as neurotransmitter (Boehning and Snyder 2003). It has been largely assumed that a neuronal Ca\(^{2+}\)/calmodulin-regulated NO synthase isoform (nNOS) consists the principal source of NO acting as a retrograde messenger in the brain to regulate synaptic release (e.g., Arancio et al. 1996; Micheva et al. 2003; Prast and Philippu 2001). Accordingly, the potentiation of certain excitatory synaptic pathways in the neocortex exhibits a NO-mediated presynaptic component (Feldman 2009; Hardingham and Fox 2006; Sjostrom et al. 2007). However, puzzling information points to lack of nNOS expression by pyramidal neurons in the neocortex as only a small specific subset of GABAergic neurons express this enzyme (Gonchar and Burkhalter 1997; Vruwink et al. 2001). Another Ca\(^{2+}\)/calmodulin-regulated NOS isoform is probably confined to endothelial cells (eNOS) to affect blood flow, though it has been proposed that NO produced by the cerebral vasculature affects nearby neurons (Garthwaite et al. 2006). A third NOS isoform is expressed only by glia cells (iNOS) and acts in a Ca\(^{2+}\)-independent manner. The involvement of this isoform in modulating neuronal activity has been largely ignored as it has been well accepted that this enzyme is regulated by gene induction following detrimental events such as ischemia or inflammation. Nevertheless, several studies indicate a low constitutive expression of iNOS in various brain regions (Chan et al. 2001; Starkey et al. 2001) including the neocortex (Buskila et al. 2005). Furthermore, a recent study using spinal-cord slice preparation showed that long-term potentiation (LTP) of the presynaptic afferents was mediated by NO released from glial cells via mGluR1 activation (Ikeda and Murase 2004). We suspected that a similar process may exist in the neocortex, namely that NO from glial sources participates in modulating synaptic transmission. In the following study, we examine this possibility and demonstrate that iNOS inhibition in acute slices indeed affects synaptic release.

METHODS

We used CD1 mice (14–21 days old). All animals were healthy and handled with standard conditions of temperature, humidity, and a 12-h light/dark cycle, free access to food and water, and without any intended stress stimulations. All experiments were approved by the Ben-Gurion University committee for the ethical care and use of animals in experiments.

NOS activity assay

NOS catalytic activity was assayed by measuring both the Ca\(^{2+}\)-dependent and the Ca\(^{2+}\)-independent conversion of \(^{[3}\text{H}]\)arginine to \(^{[3}\text{H}]\)citrulline (NOS activity assay kit, Cayman Chemicals, Ann Arbor, MI). In brief, the neocortex was homogenized in 1 ml buffer containing (in mM) 25 Tris-HCl (pH 7.4), 1 EDTA, and 1 EGTA. Samples were incubated at room temperature for 30 min in the presence of \(^{[3}\text{H}]\)-arginine (1 \(\mu\)Ci/\(\mu\)l; Amersham, UK) and cofactors. The reaction was terminated by the addition of stop buffer containing 50 mM HEPES (pH 5.5) and 5 mM EDTA. To determine the relative fraction of Ca\(^{2+}\)-independent NOS activity, calcium was omitted from the reaction mixture in some samples and EGTA (1 mM) was added. \(^{[3}\text{H}]\)-citrulline was quantified by liquid scintillation counting of the eluate, and the counts per minute for all samples were averaged and corrected with respect to the background radioactivity. NOS activity was expressed as pmol \(^{[3}\text{H}]\)L-citrulline/30 mm/mg protein.

Slice preparation and recording

Animals were deeply anesthetized with ketamine (100 mg/kg)/xylazine (10 mg/kg) mixture and decapitated, and their brains were quickly removed into cold (5°C) physiological solution. Brain slices (300 \(\mu\)m thick) were cut with a vibratome (Campden Instruments, London, UK) in the angle which maintains nucleus VB of the thalamus, the barrel cortex and intact axonal fibers between them (somatosensory thalamocortical slices) (Agmon and Connors 1991; Gil and Amitai 1996) and then transferred to a holding chamber, where they were kept at 36°C for \(\geq 1\) h before recording or any treatment. In the holding chamber, the slices were continuously bubbled with 95% \(\text{O}_2\)-5% \(\text{CO}_2\). The normal bathing and superfusing
solution contained (in mM) 124 NaCl, 3.5 KCl, 2 MgSO₄, 1.25 Na₂HPO₄, 2 CaCl₂, 26 NaHCO₃, 10 dextrose and was saturated with 95% O₂-5% CO₂ (pH 7.4).

The recording chamber was mounted on an upright fluorescent microscope equipped with IR/DIC optics (Nikon Physiostation EC-600). During recordings the slices were kept at 30–32°C and constantly superfused (2–3 ml/min) with oxygenated solution. Whole cell recordings were performed from layer 2/3 neurons in the somatosensory cortex with patch pipettes (3–5 MΩ) containing (in mM) 125 K-glucuronate, 2 MgCl₂, 10 HEPES, 5 NaCl, and 2 Na₂ATP, pH = 7.2, 280 mosM. For evoked-release experiments we blocked N-methyl-D-aspartate (NMDA) receptors with d-2-amino-5-phosphono-4-pentanoic acid (APV, 50 μM). Picrotoxin (1 μM) was added to the solution in LTP experiments. The NOS inhibitors N-(3-aminoethyl)benzyl acetamide (1400W), L-N6-(1-iminoethyl)lysine (L-NIL), and 1-NNL-nitroarginine (l-NNa) were bath applied unless otherwise noted. Voltages or currents were recorded using patch-clamp amplifier (AxoPatch 2B, Axon Instruments), digitally sampled at 10 kHz, filtered at 3 kHz, and analyzed off-line. Recordings were considered stable and suitable for analysis when the series and input resistances and resting membrane potential did not change >20% of their initial value.

ELECTRICAL STIMULATION. Stimulation was performed using theta capillaries (2–5 μm tips) filled with artificial cerebrospinal fluid (ACSF). The electrodes were connected to a Master-8 stimulator through an isolation unit and placed in layer 4 of the somatosensory cortex. Pyramidal cells in layers 2/3 were patched under visual guidance using a ×60 water-immersion objective (Nikon). Stimulation (250 μs duration) was set to produce an excitatory postsynaptic potential (EPSP) of 2–3 mV with a reversal potential around 0 mV [average from 20 representative neurons was 3.9 ± 2.2 (SD) mV]. When the reversal potential was estimated to be more negative than −10 mV, the neurons were not included in the analysis, assuming contamination by inhibitory conductance. EPSP amplitude was measured from the onset of the event to the peak. When temporal summation occurred, the error in the amplitude of the second EPSP in a pair was estimated by using the following equation: EPSP₂ = EPSP₁[1 + (Vₑ − Vᵦ)/(Vₑ − Vᵢ)], where EPSP₂ is the corrected amplitude of the second EPSP in a pair, EPSP₁ is the measured amplitude of the second EPSP from onset, Vₑ is the membrane voltage at EPSP₁ onset, Vᵦ is the resting membrane potential, and Vᵢ is the reversal potential. We found that the changes in driving force due to temporal summation of EPSPs did not significantly contribute to changes in amplitude.

Miniature excitatory postsynaptic currents (mEPSCs) were recorded in the whole cell voltage-clamp configuration at a holding potential of −70 mV to avoid NMDA conductance and in the presence of 1 μM tetrodotoxin (TTX) and 50 μM picrotoxin. mEPSCs were analyzed off-line using MiniAnalysis software (Synaptosoft, Decatur, GA). Periods of ≥5 min (>250 events) were used to calculate the frequency and amplitude of events.

SPIKE-TIMING-DEPENDENT PLASTICITY (STDP) PROTOCOL. The stimulating electrode was placed within the wall of a layer 4 barrel, and pyramidal cells were recorded in layer 2/3 in the same column. The electrical stimulation and postsynaptic action potentials were paired to induce long-term potentiation (LTP); an electrical stimulation evoked an EPSP, and a brief 10-ms-long somatic current pulse was timed to produce a postsynaptic spike at the end of that EPSP, such that the presynaptic stimulus precedes the postsynaptic spike by 10 ms. This protocol was repeated 100 times at a rate of 2/5 in two cycles with a 30-s interval between them. Only cells that showed a stable baseline during the first 10-min period were analyzed.

Statistical analysis

The Kolmogorov–Smirnov test was used to analyze the differences in probability distribution of mEPSCs. Data comparisons for non-paired data were done with Mann-Whitney test and the Wilcoxon signed-ranked test for paired data, unless otherwise noted. Data are expressed as means ± SE.

RESULTS

iNOS activity in mouse brain

Our previous study indicated that astrocytes in the brains of young healthy mice express basal levels of iNOS and suggested the existence of posttranslational regulation (Buskila et al. 2005). Because iNOS is the only isoform of which is not dependent on the elevation of intracellular Ca²⁺, the Ca²⁺-independent conversion of [³H]arginine to [³H]citrulline is considered specific to iNOS (Grandati et al. 1997; Lecanu et al. 1998). Measuring the Ca²⁺-independent fraction of NOS activity in the neocortex of young, healthy mice, we found that it constitutes a small but consistent fraction (7.8 ± 0.45%, n = 5) of the total NOS activity (Fig. 1A). Before performing electrophysiology in brain slices, we also verified...
that the sectioning process did not drastically affect iNOS activity. Performing the enzymatic assay on cortical slices at various times after the sectioning revealed that the Ca\(^{2+}\)-independent fraction was elevated 1 h after the slicing as compared with nonsectioned brains (147 ± 18.2% of baseline) but returned to baseline levels afterward (Fig. 1B). Statistical analysis supported the preceding observations: one-way ANOVA with repeated measures revealed a significant effect of the time postsectioning \(F(3,30) = 3.29, P = 0.034\). Bonferroni post hoc test confirmed a significant difference between iNOS activity 1 h postsectioning and nonsectioned brain \((P = 0.008)\), and no difference between any other time-related groups. These findings confirm basal level expression of functionally regulated iNOS in the tissue.

iNOS inhibition reduces spontaneous release

We hypothesized that the nitrosative presynaptic modulation of at least some cortical synapses is mediated by the astrocytic iNOS. Hence we examined the effects of the highly selective iNOS inhibitor 1400W (3 orders of magnitude higher affinity toward iNOS compared with the constitutive isoforms) (Garvey et al. 1997; Zhu et al. 2005) on synaptic function. mEPSCs were recorded from layer 2/3 pyramidal neurons in the somatosensory cortex. Inhibitory synaptic events were blocked by the GABA\(_A\) receptor blocker picrotoxin (50 \(\mu\)M), and the neurons were voltage clamped to −70 mV to avoid NMDA receptors activation. Bath application of 1400W (3 \(\mu\)M) resulted in a significant decrease in mEPSCs frequency \((1.67 ± 0.16 \text{ Hz before}, 0.97 ± 0.18 \text{ Hz after}; n = 7; P = 0.016)\) while their amplitude remained unaffected \((21.6 ± 2.0 \text{ pA before}, 20.7 ± 2.0 \text{ pA after}; P = 0.45, \text{Fig. 2})\). In the same neurons, the change in mEPSCs frequency was reversed upon application of the cGMP analogue 8-pCPT-cGMP (50 \(\mu\)M) \((1.60 ± 0.6 \text{ Hz}, n = 4, \text{Fig. 2, A, C, and D})\). Again the amplitude distribution of mEPSCs following the application of pCPT-cGMP was not different from control. An additional iNOS inhibitor, \(i\)-NIL, is considered less selective than 1400W, but nevertheless exhibits 30 times higher affinity to iNOS compared with nNOS (Moore et al. 1994). Bath application of \(i\)-NIL (50 \(\mu\)M) reduced the frequency of mEPSCs from 4.19 ± 1 to 2.68 ± 0.76 Hz \((P = 0.03, n = 6)\) without affecting their amplitude \((22.1 ± 0.9 \text{ pA before vs.} 22.4 ± 1 \text{ pA after}, \text{Fig. 2D})\). This selective change in mEPSCs frequency implies that both drugs modulated the presynaptic terminal, inhibiting the vesicular release process.

iNOS affects short-term synaptic dynamics

Changes in the short-term dynamics of synaptic events are typically caused by modulation of the vesicular release probability, where stronger synaptic depression is correlated with...
higher initial release (Debanne et al. 1996; Gil et al. 1999; Zucker 1989). Accordingly, we investigated the effects of iNOS inhibition on the dynamics of EPSPs evoked on layer 2/3 pyramidal neurons. Pairs of stimuli at 40-ms intervals were delivered to layer 4 of the same column as the recorded neuron, and their amplitude was measured before and after application of 1400W (Fig. 3A). The first EPSP in the pair exhibited a small but significant reduction in amplitude following drug application (2.88 ± 0.32 mV before, 2.36 ± 0.41 mV after, n = 13, P = 0.027, Fig. 3A), and the ratio between the amplitudes of the second and the first EPSPs (paired-pulse ratio, PPR) increased from 0.77 ± 0.07 before to 0.88 ± 0.07 (P = 0.002, Fig. 3B). Similarly, t-NIL reduced the amplitude of the first EPSP from 2.5 ± 0.3 to 1.62 ± 0.28 mV (n = 6, P = 0.03) and increased the PPR from 0.88 ± 0.08 to 1.14 ± 0.1 (n = 6, P = 0.03). Adding 8-pCPT-cGMP to the solution following 1400W treatment countered the effect of 1400W; the amplitude of the first EPSP increased by 41 ± 13% (n = 7, P = 0.022), and the PPR was reduced back to control levels (0.97 ± 0.07 before, 0.77 ± 0.06 after, n = 7, P = 0.016, Fig. 3B). The effect of iNOS inhibition on the PPR was similar for interstimulus intervals such as 25 ms (0.64 ± 0.07 before vs. 0.84 ± 0.06 after; n = 8; P = 0.03) or 100 ms (0.68 ± 0.07 before, 0.88 ± 0.05 after n = 8; P = 0.03, Fig. 3C).

Because iNOS activity was found to be elevated 1 h after tissue slicing, we compared the effect of iNOS inhibition on neurons recorded <2.5 h after slicing (“early neurons”), to neurons recorded >2.5 h afterward (“late neurons”). We found that the effect was identical (average change in PPR – 0.22 ± 0.06 in early neurons, n = 11, 0.25 ± 0.05 in late neurons, n = 8, P = 0.84; average change in 1st amplitude: −0.28 ± 0.09 in early neurons, n = 11, −0.25 ± 0.06 in late neurons, n = 8, P = 0.81), allowing data pooling regardless of the exact time of recording relative to the slicing.

**Effects of iNOS on LTP**

There are ample indications that NO mediates enhancement of synaptic release in some forms of LTP (reviewed by Feldman 2009; Garthwaite 2008). Considering the possibility that NO is released from the adjacent astrocyte, we examined whether iNOS inhibition has an effect on LTP. Spike-timing-dependent plasticity (STDP) was evoked by stimulating in layer 4 and recording from layer 2/3 neurons in the somatosensory cortex (Fig. 4A, see METHODS). Under control conditions, STDP protocol led to stable potentiation of the EPSP amounting to 158 ± 17% of the baseline EPSP amplitude, (average of the 30–40 min following STDP period, n = 7, P = 0.001, Fig. 4, B–D). Following 30-min preincubation with 1400W (3 µM), STDP protocol resulted in a potentiation amounting to 122 ± 18% (n = 7, P = 0.001, Fig. 4, B–D), but the magnitude of this potentiation was significantly reduced compared with potentiation in control slices (P = 0.001). The PPR under baseline conditions was 1.06 ± 0.19 and decreased

![Graph showing the effect of 1400W on synaptic transmission](http://jn.physiology.org/)

**Fig. 3.** Selective iNOS inhibition affects evoked synaptic release. *A:* example traces of paired extracellular stimuli before (black) and after (gray) bath application of 1400W. A current pulse following synaptic activation demonstrates the stability of membrane properties during 1400W application. *B:* summary graph of the changes in the paired-pulse ratio (PPR) and the amplitude of the first excitatory postsynaptic potential (EPSP) following bath application of 1400W (black, n = 13), t-NIL (gray, n = 6) and 8-pCPT-cGMP (white, n = 7). *C:* iNOS inhibition by 1400W resulted in a comparable increase in PPR for several inter-stimuli intervals (marked above). Data are presented for single neurons. The PPR changes of the example neuron displayed in *A* are marked in gray in the *middle panel*. A summary bar graph of the average change in PPR for each interval is presented in the *right panel*. Data presented as percentage of change compared with baseline; *P < 0.05, **P < 0.01.*
FIG. 4. Selective inhibition of iNOS reduces synaptic potentiation. Neurons were recorded in layer 2/3 and the stimulating electrode was placed in layer 4. A: example trace of spike-timing-dependent plasticity (STDP) paradigm. Potentiation was induced by pairing presynaptic stimulation (arrow marked as Pre) and postsynaptic spikes evoked by intracellular current pulse (beginning indicated by an arrow marked as Post), such that the presynaptic stimulus precedes the postsynaptic spike by 10 ms. B: potentiation over time plot. Each dot represents an average of 5 consecutive EPSPs. Black circles represent average peak EPSPs before STDP protocol (black), and 45 min afterward (gray), in control slices (top) and 1400W-treated slices (bottom). C: summary diagram of the changes in amplitude following STDP protocol, showing a similar potentiation in both control cells (black, \( P < 0.001, n = 7 \)) and L-NNA-treated cells (white, \( P = 0.001, n = 6 \)), and a concurrent increase in the PPR to its prepotentiation values (PPR: control, \( 1.23 \pm 0.19 \); 10 min post STDP, \( 0.88 \pm 0.13 \); 30 min post STDP +1400W, \( 1.12 \pm 0.07 \); \( P = 0.03 \), Fig. 4F). Hence the inhibition of iNOS reversed the presynaptic change, having a comparable effect on potentiated and nonpotentiated synapses.

DISCUSSION

Our previous work demonstrated a functional iNOS in astrocytes of neocortex based on the rapid response of these cells to the fluorescent NO-indicator DAF-2DA, which was sensitive to iNOS inhibitors, and Western blot analysis, which confirmed the presence of the protein (Buskila et al. 2005). We also found that the astrocytes of an iNOS mutant exhibit increased NO production via an alternative biochemical pathway (Buskila et al. 2007) and that the same knockout mouse is resistant to iNOS inhibitors, and Western blot analysis, which confirmed the presence of the protein (Buskila et al. 2005). We further verified the NOS isoform responsible for the presynaptic component in STDP by applying a selective nNOS inhibitor L-NNA (1 \( \mu \)M). In our hands, this dose of L-NNA effectively prevented neuronal staining with the NO indicator diaminofluorescein–2 diacetate (DAF-2DA) (Buskila et al. 2005). L-NNA-treated slices exhibited strong potentiation (172 \( \pm \) 18%, Fig. 4D), and the average PPR was reduced by 41 \( \pm \) 8% following the STDP protocol (1.14 \( \pm \) 0.19) before and 0.64 \( \pm \) 0.10 after STDP, \( n = 6, P = 0.03 \), Fig. 4E), similarly to control slices. In contrast, when the STDP protocol was carried out on 1400W-treated cells, the PPR was not significantly modified (0.92 \( \pm \) 0.23 before and 0.85 \( \pm \) 0.15 after STDP, \( P = 0.52, n = 5 \), Fig. 4E).

Together, these results imply that the enzyme responsible for NO production required for the presynaptic potentiation is the astrocytic iNOS and not the neuronal nNOS.

When 1400W is applied before LTP, the synapse is theoretically undergoing two contradicting processes: potentiation in which a presynaptic component results in increased release and decreased PPR and inhibition of NO production that reduces release and increased PPR. Hence these two processes could cancel each other. We next examined whether iNOS inhibition could affect in the same manner the synaptic potentiation when applied after the STDP protocol once a new value of PPR has been established. Similarly to the effect on evoked release, bath application of 1400W 10 min after the STDP protocol resulted in a minor reduction of the EPSP amplitude (from 135 \( \pm \) 5% of control value before 1400W application to 127 \( \pm \) 9% 30 min following drug application, \( P = 0.06 \)), and a concurrent increase in the PPR to its prepotentiation values (PPR: control, 1.23 \( \pm \) 0.19; 10 min post STDP, 0.88 \( \pm \) 0.13; 30 min post STDP +1400W, 1.12 \( \pm \) 0.07; \( P = 0.03 \), Fig. 4F). Hence the inhibition of iNOS reversed the presynaptic change, having a comparable effect on potentiated and nonpotentiated synapses.
terminals. The evidence for iNOS involvement in synaptic function is largely pharmacological. While this method is sometimes considered inconclusive due to lack of specificity, the following points strongly support our conclusion: 1) 1400W is highly selective to iNOS: steady-state IC$_{50}$ values of 1400W are estimated to be 0.23 $\mu$M for iNOS, 7.3 $\mu$M for nNOS, and 1,000 for eNOS (see table in Alderton et al., 2001). Thus the concentration we use (3 $\mu$M) is lower than needed to completely block nNOS. Moreover the dense slice tissue is likely to hamper the even distribution of the drug in the short time required to hold neurons through exchange of solutions. Hence the concentration affecting cellular components in the depth of the tissue is likely to be even lower. 2) Using NO imaging, we have previously demonstrated that these concentrations of 1400W and l-NIL abolished fluorescence in astrocytes (expressing iNOS) but did not affect nNOS-mediated neuronal fluorescence (Buskila et al. 2005). 3) We found no effect of the selective nNOS inhibitor l-NNA in our experimental paradigms. And 4) the binding of 1400W to iNOS is practically irreversible but quickly reversible to nNOS (Alderton et al. 2001). Hence one set of the LTP experiments was conducted after preincubation in 1400W, and the slices were then perfused with ACSF without drugs, conditions under which nNOS is unlikely to be affected.

Numerous studies have demonstrated the involvement of the NO/cGMP signaling pathway in regulating synaptic release. (Holscher 1997; Prast and Philippu 2001; Wang et al. 2005; recently reviewed by Feil and Kleppisch 2008), and there are (Holscher 1997; Prast and Philippu 2001; Wang et al. 2005; Wakatsuki et al. 1998). Yet the cellular source of NO has remained elusive. Electron microscopic study in hippocampal CA1 field established the presence of nNOS inside the postsynaptic membrane of excitatory synapses on pyramidal neurons (Burette et al. 2002). Such evidence is not available for the neocortex (Ding et al. 2004). In our hands, hippocampal pyramidal neurons indeed exhibit DAF-2DA fluorescence while neocortical pyramidal neurons do not (unpublished). Other studies suggested the involvement of eNOS in synaptic plasticity (Haul et al. 1999; O’Dell et al. 1994) based on the use of developmental knockout mice. Realizing the secondary changes that might occur in these genetically altered animals and the failure to confirm eNOS expression by neurons (Chiang et al. 1994) have both cast doubt on this possibility.

Measuring Ca$^{2+}$-independent NOS activity, we estimate that iNOS is responsible for $<$10% of the total NOS activity in the neocortex. The increase in the Ca$^{2+}$-independent NOS activity 1 h after slicing is compatible with our previous finding, which revealed a rapid, protein synthesis-independent increase in DAF-2DA fluorescence in astrocytes following glutamate excitotoxicity (Buskila et al. 2005) and suggested that iNOS was activated by neuronal death. Similarly, we suspect that the neuronal death caused by the slicing procedure results in short-term increase in iNOS activity. Because practically nothing is known about the posttranslational regulation of iNOS in the brain, we don’t know what this activation step might be. In addition, electrophysiological experiments were carried out on immature animals, a factor that might influence the magnitude of effect, but the expression profile of iNOS in developing animals is also unknown. Whereas the increase in enzyme activity post sectioning complicates the quantitative evaluation of the effect of neuronal activity (STDP) on the nitrosative component in the release process, it substantiates the existence of a functionally regulated protein in the tissue.

The role of astrocytes in modulating synaptic function has been discussed intensely in recent years. Typically, calcium signaling is considered the functional correlate for astrocytic activation (reviewed recently by Aquilhon et al. 2008; Fellin 2008). In the case of astrocytic nitrosative activity, intracellular calcium is not expected to rise as iNOS activity is Ca$^{2+}$-independent. Additional research will probably have to use direct quantitative NO imaging, which at this point, still suffers from multiple technical shortcomings. Fortunately, 1400W is considered a highly selective iNOS inhibitor, as it inhibits iNOS with 5,000- and 2,000-fold greater potency than eNOS and nNOS, respectively (Garvey et al. 1997; Zhu et al. 2005). At the concentrations used in this study, 1400W abolished completely the astrocytic DAF-2DA fluorescence while preserving the neuronal fluorescence (Buskila et al. 2005).

These data reveal a novel form of astrocytic modulation of neuronal function. The presynaptic effect of iNOS inhibition occurred under resting conditions (TTX) as well as under various activity regimes. It both prevented and reversed a presynaptic component of the STDP. Thus it seems that astrocytes produce NO tonically, a production that may be upregulated by neuronal activity, but we have no evidence for such a regulation, as the effect of iNOS inhibition on potentiated and nonpotentiated synapses was similar. The idea of tonic NO release affecting synaptic function has been put forward for hippocampal CA1 synapses, but it was suggested that the source of the NO is the endothelial isoform (Hopper and Garthwaite 2006). We propose that astrocytes, as part of the tri-partite synapse, are perfectly positioned to secret NO in a highly compartmentalized, focal and discrete manner, affecting synaptic function and being affected by it. Future research will have to uncover the details of these interactions.

GRANTS

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