Pergamon

Neuropharmacology 41 (2001) 762–770

NEURO PHARMACOLOGY

www.elsevier.com/locate/neuropharm

Effect of transgenic overexpression of NR2B on NMDA receptor function and synaptic plasticity in visual cortex

Benjamin D. Philpot [a], Michael P. Weisberg [a], Margarita S. Ramos [a], Nathaniel B. Sawtell [a], Ya-Ping Tang [b], Joe Z. Tsien [b], Mark F. Bear [a,*]

[a] *Howard Hughes Medical Institute, Department of Neuroscience, Brown University, Box 1953, Providence, RI 02912, USA*
[b] *Department of Molecular Biology, Princeton University, Princeton, NJ 08544 USA*

Received 23 July 2001; received in revised form 21 August 2001; accepted 21 August 2001

Abstract

The NMDA receptor (NMDAR) is a heteromer comprised of NR1 and NR2 subunits. Mice that overexpress the NR2B subunit exhibit enhanced hippocampal LTP, prolonged NMDAR currents, and improved memory (Tang et al., 1999). In the current study, we explored visual cortex plasticity and NMDAR function in NR2B overexpressing transgenic mice. Unlike the hippocampus, in vitro synaptic plasticity of the visual cortex was unaltered by NR2B overexpression. Consistent with the plasticity findings, NMDAR excitatory postsynaptic current (EPSC) durations from layer 2/3 pyramidal cells were similar in wild-type (wt) and transgenic (tg) mice. Furthermore, temporal summation of NMDAR EPSCs to 10, 20, and 40 Hz stimulation did not differ between cells from wt and tg mice. Finally, although in situ studies clearly demonstrate overexpression of NR2B mRNA in visual cortex, we failed to observe a significant elevation in the synaptic expression of NR2B protein. We conclude that the synaptic ratio of NR2B over NR2A in the NMDA receptor complex in the visual cortex is not significantly influenced by the transgene overexpression. These data suggest that mRNA availability is not a limiting factor for the synthesis of NR2B protein in the visual cortex, and support the hypothesis that levels of NR2A, rather than NR2B, normally determine the subunit composition of NMDARs in visual cortex.

Keywords: NR2B; Cortical plasticity; Metaplasticity; LTP; NMDA receptor; Critical period

1. Introduction

During a critical period of early postnatal development, visual experience refines synaptic connections such that a stable and functional network of neurons is established. Visual deprivation studies during this formative period suggest that synapses are modified by specific patterns of pre- and postsynaptic activity (Rittenhouse et al., 1999; Wiesel and Hubel, 1965), thus allowing neurons to acquire ocular and orientation specificity. Notably, reverse occlusion studies have shown that the receptive field properties of cortical neurons can also be bidirectionally modified during early life (Blakemore and vanSluyters, 1974; Mioche and Singer, 1989). These types of experience-dependent modifications are thought to require both the strengthening and weakening of synapses. Activation of NMDARs is thought to be necessary for the experience-dependent strengthening and weakening of synapses during receptive field development, because NMDAR activation is required for receptive field plasticity as well as the induction of long-term potentiation (LTP) and long-term depression (LTD) (Bear et al., 1990; Bear and Malenka, 1994; Roberts et al., 1998).

Recent data suggest that composition and function of the NMDAR can be modified by experience, but the functional implications and mechanisms of this change remain unclear. The NMDAR is a heteromer that consists of an obligatory NR1 subunit and NR2 subunits that impart functional properties (McBain and Mayer, 1994; Monyer et al., 1992). Early in postnatal life, the NR2B subunit is predominant in visual cortex and NMDAR-mediated currents decay slowly (Carmignoto and Vicini,

* Corresponding author. Tel.: +1-401-863-2070; fax: +1-401-863-1997.
E-mail address: mark_bear@brown.edu (M.F. Bear).

0028-3908/01/$ - see front matter
PII: S0028-3908(01)00136-8

1992; Quinlan et al., 1999a). With development, there is an experience-dependent inclusion of NR2A-containing NMDARs that shortens NMDAR currents (Carmignoto and Vicini, 1992; Flint et al., 1997; Quinlan et al., 1999b). Recent data demonstrate that visual experience can bidirectionally regulate the composition and function of NMDARs (Philpot et al., 2001). Specifically, visual experience decreases the proportion of NR2B- to NR2A-containing receptors, shortens NMDAR current durations, and reduces the summation of NMDAR currents during high-frequency stimulation (Philpot et al., 2001; Quinlan et al., 1999a,b). Visual deprivation exerts an opposite effect. An important unanswered question is how these experience-dependent modifications in NMDAR function contribute to receptive field plasticity.

Two alternative hypotheses exist regarding the role of experience-dependent modifications of NMDAR function during receptive field organization and plasticity. The first suggests that the long NMDAR current durations found early in development enable synaptic plasticity and permit the dynamic organization of receptive fields (Chen et al., 2000; Nase et al., 1999). This theory suggests that the shortening of NMDAR current duration stabilizes synapses and brings an end to the critical period for receptive field plasticity. The second hypothesis suggests that stimulus selectivity and synaptic stability arise, in part, due to experience-dependent regulation of the induction of NMDAR-dependent forms of synaptic plasticity (Bear, 1995; Bear et al., 1987; Bienenstock et al., 1982). Several lines of evidence now suggest that the bidirectional experience-dependent regulation of NR2A/NR2B is a mechanism by which the induction of synaptic plasticity can be modified (Carmignoto and Vicini, 1992; Philpot et al., 1999, 2001; Quinlan et al., 1999a,b). For example, a low ratio of NR2A/NR2B correlates with enhanced LTP and diminished LTD, while a high NR2A/NR2B ratio correlates with diminished LTP and enhanced LTD (Kirkwood et al., 1996; Philpot et al., 2001; Quinlan et al., 1999a,b).

The advent of genetic technology has afforded us the opportunity to explore the role of NMDAR composition in visual cortical plasticity. Tang et al. (1999) have recently developed a transgenic mouse that overexpresses the NR2B subunit throughout the forebrain. Hippocampal pyramidal cells from transgenic mice have prolonged NMDAR currents, and transgenic mice exhibit improved learning and memory. Notably, NR2B overexpression enhances hippocampal LTP, supporting the possibility that the induction of synaptic plasticity might be controlled, at least in part, by the composition and function of NMDARs. In the present study, we tested whether overexpression of NR2B alters synaptic plasticity in primary visual cortex.

2. Methods

2.1. Subjects

NR2B transgenic (tg) mice and wild-type (wt) littermate controls were provided by Ya-Ping Tang and colleagues (Tang et al., 1999) or bred in a colony kept at Brown University. Mice were used at postnatal day (P) 25–90. Subjects were fed ad libitum and reared in normal lighting conditions (12/12 light/dark cycle). Some subjects were raised in complete darkness.

2.2. Cortical slice preparation

Dissections were consistently performed between 8:30 am and 10:30 am. Following 0.1 cc Nembutal (i.p.), mice were decapitated upon disappearance of corneal reflexes in compliance with the US Department of Health and Human Services and Brown University guidelines. The brain was rapidly removed and immersed in ice-cold dissection buffer (composition in mM: sucrose, 212.7; KCl, 2.6; NaH_2PO_4, 1.23; $NaHCO_3$, 26; dextrose, 10; $MgCl_2$, 3; and $CaCl_2$, 1) bubbled with 95% O_2 and 5% CO_2. The visual cortex was rapidly removed and 350–400 μM coronal slices were cut using a DSK microslicer (Ted Pella, Redding, CA). Slices were allowed to recover for 1 h at 31°C in a submersion chamber containing oxygenated artificial cerebrospinal fluid (ACSF), and then moved to room temperature until use. ACSF contained 124 mM NaCl, 5 mM KCl, 1.25 mM Na_2PO_4, 26 mM $NaHCO_3$, 1 mM $MgCl_2$, 2 mM $CaCl_2$, and 10 mM dextrose, saturated in 95% O_2, 5% CO_2.

2.3. Extracellular electrophysiology

Slices were transferred to an interface recording chamber and perfused with 30°C ACSF at a rate of 2.5 ml/min. Extracellular glass electrodes (filled with ACSF; 1.0 MΩ) placed in layer 2/3 were used to monitor field excitatory postsynaptic potentials (fEPSPs) evoked with a stimulating electrode (concentric bipolar tungsten) placed in layer 4. The magnitude of responses was monitored by the amplitude of the field potential. Stable baseline responses were elicited by a 200 μs pulse given every 30 s. The resulting signals were filtered between 0.1 Hz and 3 kHz, amplified 1000 times, and captured at 10 kHz on an IBM-compatible computer using Experimenter's Workbench software (DataWave Technologies Corp.). Stimulation intensity was adjusted to evoke a response 50% of the maximal value. After a 15 minute stable baseline, a stimulation protocol consisting of either a high-frequency stimulation (HFS) at 100 Hz for 1 s or a low-frequency stimulation (LFS) at 1 Hz for 15 min was given. Field potential amplitudes were recorded every 30 s for 45 minutes following the cessation of the

stimulation protocol. The data were normalized, averaged, and reported as means ±SEM.

2.4. Voltage-clamp recordings

Slices were placed in a submersion chamber, maintained at 30°C, and perfused at 2 ml per min with oxygenated ACSF containing 4 mM $MgCl_2$, 4 mM $CaCl_2$, 1 μM glycine, 50 μM picrotoxin (Fluka), and 20 μM CNQX. Layer 2/3 pyramidal cells were identified using an Axioskop microscope (Zeiss) combined with infrared differential interference contrast optics. Patch pipettes were pulled from thick-walled borosilicate glass. Open tip resistances were 4–8 MΩ when pipettes were filled with the internal solution containing 102 mM cesium gluconate, 5 mM TEA-chloride, 3.7 mM NaCl, 20 mM Hepes, 0.3 mM sodium guanosine triphosphate, 4 mM magnesium adenosine triphosphate, 0.2 mM EGTA, 10 mM BAPTA, and 5 mM QX-314 chloride (Alomone Labs, Jerusalem, Israel) with pH adjusted to 7.2 and osmolarity adjusted to ~300 mmol/kg with sucrose or ddH_2O. Cells were voltage-clamped at +40 mV in the whole cell configuration using a patch-clamp amplifier (Axoclamp 1D, Axon Instruments), and data were acquired and analyzed using a system from DataWave Technologies Inc. (Boulder, CO). Pipette seal resistances were typically >1 GΩ, and pipette capacitive transients were minimized prior to breakthrough. Series resistance was measured manually at the start of the experiment and was monitored throughout the experiment by giving a test pulse and measuring the amplitude of the capacitive current filtered at 30 kHz. Only cells with series resistance <30 MΩ were included in this study. No series resistance compensation was applied. Input resistance was monitored throughout the experiment by measuring the amplitude of the steady-state current, filtered at 2 kHz, evoked from a test pulse. Input resistances did not differ between experimental groups and averaged 130±6 MΩ. EPSCs were evoked from a stimulating electrode (concentric bipolar stimulating; ~200 μM tip separation) placed in layer 4, and stimulation was given for 200 μs every 6 s. To examine functional changes in the summation of NMDA-mediated currents, 11 pulses of 10, 20, or 40 Hz trains of stimulation were given every 6 s for 3 min.

2.5. Biochemical analysis

Synaptoneurosomes were prepared as previously described (Quinlan et al., 1999a). Briefly, animals were given a lethal dose of Nembutal (0.2 cc) and decapitated upon suppression of the tail pinch reflex. The brain was quickly removed into ice cold dissection buffer (212.7 mM sucrose, 2.6 mM KCl, 1.23 mM NaH_2PO_4, 26 mM $NaHCO_3$, 10 mM dextrose, 1 mM $MgCl_2$, 0.5 mM $CaCl_2$, 0.02 mM CNQX, 0.1 mM AP5) saturated with 95% O_2, 5% CO_2. Visual cortices and hippocampi were bilaterally removed and separately placed in homogenization buffer consisting of 10 mM HEPES, 2 mM EDTA, 2 mM EGTA, 0.5 mM DTT, 0.1 mM PMSF, 10 mg/l leupeptin, and 100 nM microcystin. The samples were homogenized using two ml glass–glass tissue homogenizers (Kontes, Vineland, New Jersey), then filtered through a double layer of 105 μm pore nylon mesh filter, and finally passed through a 5 μm pore filter paper. Homogenized tissue was centrifuged at 35000 rpm for 10 minutes at 4°C. The sediment containing a high density of synaptic protein was then resuspended in 1% boiling SDS and vortexed. A portion of the supernatant, containing non-synaptic material was saved and dissolved in 10% SDS. The supernatant and the resuspended pellet were placed in a boiling water bath for 10 minutes and stored at −20°C.

An optical densitometric assay (BCA Assay, Pierce) was used to measure the concentration of synaptic proteins. Protein concentrations across samples were normalized and preserved from decay with sample buffer. 15 μg of protein were loaded per lane and samples were run on 8% polyacrylamide gels using BioRad mini-protean II and III cells. A master homogenate mixture was included in every gel to standardize for transfer, blotting, and developing conditions. Gels were then transferred to nitrocellulose membranes and probed against NR2B (Upstate Biotechnology, polyclonal; 1:500), tubulin (Sigma, monoclonal; 1:200), and PSD-95 (Upstate Biotechnology, monoclonal; 1:1000) antibodies. Samples were next probed with secondary antibody (α mouse for tubulin, PSD-95 and α rabbit for NR2B) in Tris-buffered saline (pH 7.4) containing 0.1% Triton X-100. To visualize the immunoblots, enhanced chemiluminescence (Amersham ECL) was used with autoradiographic film (Amersham Hyper ECL). Developed autoradiographs were scanned using Alpha Imager software. Immunoblot bands were quantified by densitometric analysis using NIH Image software.

2.6. In vivo recordings

Visual evoked potentials (VEPs) were recorded as previously described (Porciatti et al., 1999). Briefly tg mice and wt littermate controls were anesthetized with an intraperitoneal injection of 20% urethane (Sigma, 8 ml/kg) and mounted in a modified stereotaxic apparatus. The skull overlying the visual cortex was thinned with a dental drill and a portion of bone (3×3 mm) was lifted away leaving the dura intact. VEPs were recorded with a tungsten microlelectrode with tip impedance of 0.5 MΩ. In most experiments the electrode was placed on the dural surface 2.6–3.2 mm lateral to the intersection between the sagittal and lambdoid sutures. To insure proper placement of the electrode within the binocular zone of the visual cortex, VEPs were recorded from mul-

tiple sites until an optimal ipsilateral VEP was recorded. In addition, after many experiments electrolytic lesions (10–20 μA, 5 s) were made to mark recording sites, and lesions were located within primary visual cortex using Nissl-stained sections, according to established cytoarchitectonic criteria (Caviness, 1975). Electrical signals were amplified, filtered (0.1 and 3.0 kHz), digitized at 20 kHz, and averaged (50–100 events per block) in synchrony with the stimulus contrast reversal using an IBM compatible computer running Experimenter's Workbench (DataWave Systems Corp., Boulder, CO). Transient VEPs in response to abrupt contrast reversal (1 Hz) were analyzed in the time domain by measuring the amplitude and latency of the positive peak of the major response component. Visual stimuli consisted of vertical and horizontal sinusoidal gratings of different spatial frequencies and contrasts presented on a computer monitor (DataWave, SVGA) positioned 15 cm from the mouse's eyes, in a darkened room. Monocular deprivation was performed by eyelid suture. Mice were anesthetized at P49 with inhalated isoflurane (*IsoFlo* 2–3%) and placed under a surgical microscope. The area around the eye was swabbed with iodophor (1% iodine). Lid margins were trimmed and three to five mattress stitches were placed using 7-0 silk, opposing the full extent of the trimmed lids. Animals were checked daily to be sure the sutures remained closed and the eye remained uninfected. Animals whose eyelids did not fully seal shut were excluded from the experiment. Four days later the deprived eye was opened and VEP recordings were made in the hemisphere contralateral to the deprived eye. In order to assess cortical ocular dominance, a low spatial frequency visual stimulus was presented alternately to each eye and the amplitude ratio of contralateral versus ipsilateral eye responses was calculated.

2.7. Drugs

Unless otherwise noted, drugs were purchased from Sigma.

2.8. Statistical analyses

ANOVA or *t* tests were used to test for significance ($p<0.05$).

3. Results

To investigate the possibility that NR2B overexpression might prolong the critical period, we performed a pilot study of ocular dominance plasticity. Wild-type (wt; $n=5$) and transgenic (tg; $n=5$) animals were monocularly deprived by lid suture starting at P49, approximately 2 weeks after the end of the critical period as defined in earlier studies (Gordon and Stryker, 1996; Huang et al., 1999). After 4 days of monocular deprivation, the mice were anesthetized and visually evoked potentials in the binocular segment of visual cortex were measured in response to stimulation of the deprived and non-deprived eyes. These experiments revealed no difference between tg and wt mice; in both, the contralateral eye continued to dominate the response, regardless of whether it was deprived or not (data not shown). Therefore, we elected to abandon further study of in vivo plasticity, and turned to slices of visual cortex in vitro to better understand the consequences of the transgenic overexpression of NR2B on NMDAR-dependent synaptic transmission and plasticity.

3.1. LTD and LTP

Because NR2B overexpression in the hippocampus does not alter LTD induced by 1 Hz stimulation but favors the induction of LTP at higher frequencies of stimulation, we examined whether synaptic plasticity was similarly modified in visual cortex. We recorded layer 2/3 fEPSPs evoked by layer 4 stimulation in slices of visual cortex from mice with an average age of P52. The half-maximal fEPSP was similar in wt and tg cortical slices (~1.0 mV). Like the hippocampus, NR2B transgene overexpression did not alter LTD induced by 1 Hz stimulation (Fig. 1(A); wt: fEPSP=92±3% baseline, $n=20$; tg: fEPSP=88±5% baseline, $n=8$; $p=0.45$). However, in striking contrast to the hippocampus, LTP induced by 100 Hz stimulation was similar in visual cortex of wt and tg mice (Fig. 1(B); wt: fEPSP=114±3% baseline, $n=17$; tg: fEPSP=111±2%, $n=23$; $p=0.46$).

While NR2B transgene expression favors the induction of LTP in the hippocampus (Tang et al., 1999), these data demonstrate that 1 and 100 Hz stimulation protocols similarly affect visual cortical responsiveness in wt and tg mice. Thus, NR2B transgene expression differentially affects hippocampal and visual cortical plasticity.

3.2. NMDA receptor current duration

Several studies suggest that NR2B-containing receptors exhibit long currents, while the inclusion of NR2A shortens NMDA receptor current duration (Flint et al., 1997; Roberts and Ramoa, 1999). The failure to observe enhanced LTP in visual cortex might be attributed to ineffectiveness of the NR2B transgene to substantially alter NMDAR currents in visual cortex. To examine this possibility, we measured the kinetics of NMDAR-mediated currents from visually identified layer 2/3 pyramidal cells, identified by their size, pyramidal shape, and prominent apical dendrite, in visual cortex slices from wt and tg mice. NMDAR-mediated currents were pharmacologically isolated as previously described (Philpot et al., 2001). Briefly, slices were bathed in

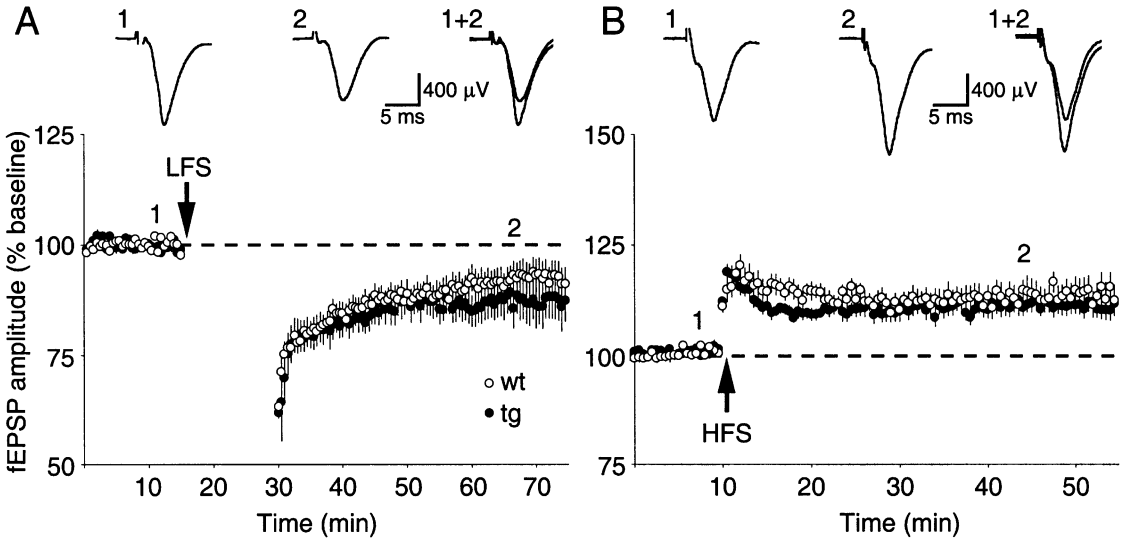

Fig. 1. In vitro visual cortex plasticity does not differ in wild-type (wt) and transgenic (tg) mice. (A) 1 Hz low-frequency stimulation (LFS) in layer 4 produces similar long-term depression recorded from layers 2/3 in slices from wt and tg animals. (B) Long-term potentiation induced by 100 Hz high-frequency stimulation (HFS) does not differ in magnitude in slices from wt and tg animals.

ACSF containing CNQX to block non-NMDARs, picrotoxin to block $GABA_A$ receptors, saturating levels of the NMDAR co-agonist glycine, and high divalent cation concentrations to minimize polysynaptic activity. Cells were voltage-clamped at +40 mV to remove Mg^{2+} block of the NMDAR ionophore. To describe NMDAR EPSC decay, 60 evoked EPSCs were averaged and the current decays were fit (Fig. 2(A)) using the following formula:

$$I(t)=I_f \exp(-t/\tau_f)+I_s \exp(-t/\tau_s)$$

A weighted time constant (τ_w) was used for quantification purposes (Rumbaugh and Vicini, 1999) and is described by the following formula:

$$\tau_w=\tau_f(I_f/(I_f+I_s))+\tau_s(I_s/(I_f+I_s))$$

We initially examined NMDAR current duration during an early period of NR2B transgene expression (P32-36). In these juvenile animals, NMDAR current durations were similar between wt and tg animals (Fig. 2(B); wt: τ_w=95.3±28.4 ms, n=10; tg: τ_w=127.2±20.0 ms, n=16; p=0.24). Because the NR2B transgene may not be fully expressed in these young animals, we examined the possibility that NMDAR currents might be altered in older tg animals, when the transgene is fully expressed. However, NMDAR EPSC decay in pyramidal cells from P69-83 mice was similar between wt and tg (Fig. 2(B); wt: τ_w=103.2±11.3 ms, n=21; tg: τ_w=97.5±12.7 ms, n=24; p=0.72).

These data demonstrate that the expression of the NR2B transgene fails to alter NMDAR-mediated currents. A failure to detect differences in NMDAR current duration due to space clamp difficulties is unlikely for two reasons. First, we have had previous success observing small differences in NMDAR EPSC duration in cortical slices (Philpot et al., 2001). Second, although most of the developmental reduction in EPSC duration occurs before 4 weeks of age, we were still able to observe a developmental trend in older animals for reduced NMDAR current durations (Fig. 2(C)) over a period when pyramidal size and arborizations are, if anything, increasing (Juraska, 1982).

3.3. Temporal summation of NMDAR-mediated currents

Although we were unable to detect differences in NMDAR current duration between wt and tg, we tested the possibility that the synaptic dynamics of NMDAR currents might be modified by undetected differences in current duration or receptor function. We have previously demonstrated that even modest differences in NMDA EPSC duration can be manifested in profound differences in the temporal dynamics of synaptic NMDAR-mediated currents (Philpot et al., 2001). Thus, we used 10, 20, or 40 Hz trains of 11 stimulation pulses, given every 6 seconds for 3 minutes, to test the possibility that NR2B transgene expression alters the summation of NMDAR-mediated currents. In both juvenile and adult visual cortex pyramidal cells, the temporal summation of NMDA-mediated EPSCs to 10, 20, and 40 Hz stimulation is nearly identical between wt and tg (Fig. 3; $p>0.05$). These data demonstrate that the short-term NMDAR current dynamics are remarkably similar in visual cortex of wt and tg animals. Notably, we observed a strong positive correlation between current duration and temporal summation, as previously described (Philpot et al., 2001), demonstrating that

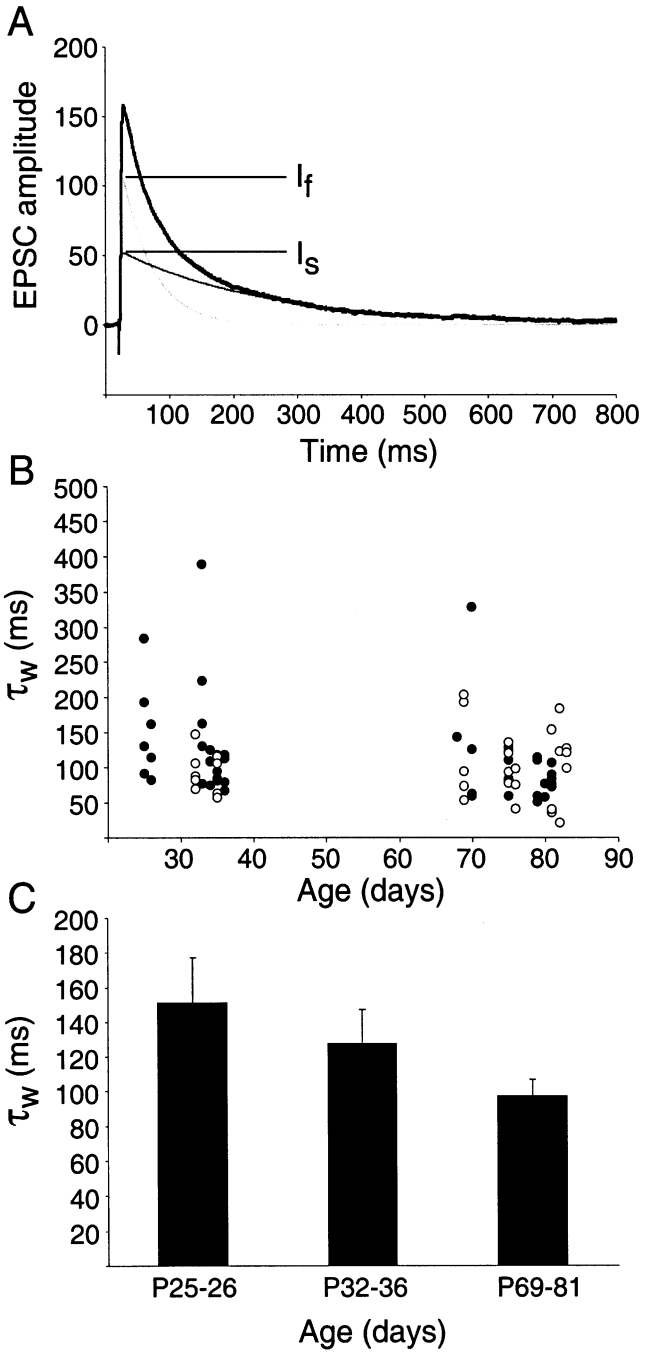

Fig. 2. NMDA excitatory-postsynaptic current (EPSC) kinetics are similar in visual cortex pyramidal cells from wild-type and transgenic animals. (A) A sample averaged current demonstrating that the NMDA EPSC decay is accurately fit by the sum of two exponentials. I_f=peak of the fast exponential, I_s=peak of the slow exponential. (B) NMDA EPSC decay, quantified by the weighted time constant (τ_w; see Results), does not differ in layer 2/3 pyramidal cells from wild-type (open circles) and transgenic (filled circles) mice. (C) Transgenic mice exhibit a developmental trend towards faster EPSC currents. Note that the most dramatic reductions in NMDAR current typically occur before P26 (Carmignoto and Vicini, 1992).

NMDAR current duration is a strong predictor of the degree of temporal summation (Fig. 4; r=0.62, $p<0.001$).

3.4. NR2B protein expression in synaptoneurosomes

Although in situ hybridization clearly demonstrates expression of the NR2B transgene in visual cortex (Tang et al., 1999), the lack of transgenic effects on plasticity or NMDAR function in visual cortex might be attributed to a failure of the transgene to significantly influence NR2B protein levels in this area of the brain. Alternatively, NR2B protein might be overexpressed without significantly influencing current duration, as a recent study in somatosensory cortex suggests that synaptic NR2B protein levels do not always correlate with NMDA receptor current duration (Barth and Malenka, 2001). To examine these possibilities, we enriched for synaptic proteins by making synaptoneurosomes from the hippocampus and visual cortex of P90 transgenic and wild-type dark-reared animals, and immunoblotted for NR2B. While transgenics exhibited the expected elevation of synaptic NR2B levels in the hippocampus ($p<0.05$, one-tailed t-test), we did not observe any differences between wt and tg in synaptoneurosome NR2B levels in visual cortex ($p>0.05$; Fig. 5). These data further suggest that synaptic NR2B levels are regulated in visual cortex by mechanisms other than transcript availability, and that the hippocampus and visual cortex are differentially affected by the transgenic expression of NR2B under the CaMKII promoter.

4. Discussion

While overexpression of the NR2B transgene alters NMDAR function and synaptic plasticity in hippocampus (Tang et al., 1999) and insular cortex (Wei et al., 2001), here we demonstrate that expression of the transgene does not alter NMDAR function or synaptic plasticity in visual cortex. Specifically, synaptically evoked NMDAR-mediated EPSCs have similar decay kinetics in visual cortex layer 2/3 pyramidal cells from wt and tg mice. Moreover, the temporal summation of NMDA currents to repetitive stimulation are similar in cells from wt and tg mice. The temporal summation data support our conclusion that NMDA currents are unaffected by the transgene, because temporal summation of NMDA currents is an extremely sensitive measure to detect even subtle differences in EPSC decay kinetics. In addition, two NMDA-mediated forms of synaptic plasticity in the visual cortex, LFS-induced LTD and HFS-induced LTP, are similar in magnitude in slices from wt and tg mice. Finally, transgenics have increased synaptic NR2B protein levels in the hippocampus but not the visual cortex. Collectively, these data strongly suggest that over-

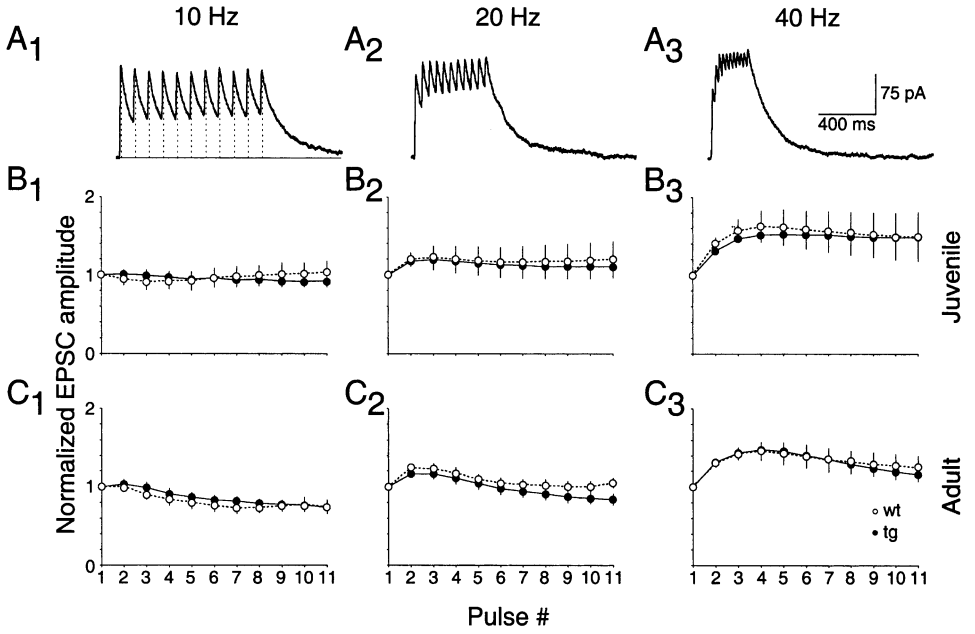

Fig. 3. The temporal summation of NMDAR currents is not modified by the NR2B transgene. (A) Examples of NMDA-mediated EPSCs evoked by 10, 20, or 40 Hz stimulation. (B) Normalized average temporal summation evoked by 10, 20, or 40 Hz stimulation in cells from juvenile wild-type (wt) and transgenic (tg) mice. (C) Temporal summation in cells from adult animals.

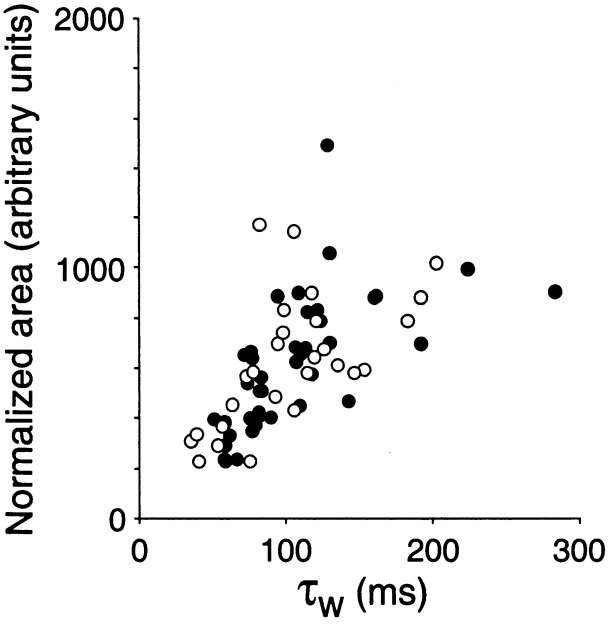

Fig. 4. The magnitude of temporal summation (area) and NMDAR current duration are tightly correlated in pyramidal cells from both wild-type (open circles) and transgenic (filled circles) animals.

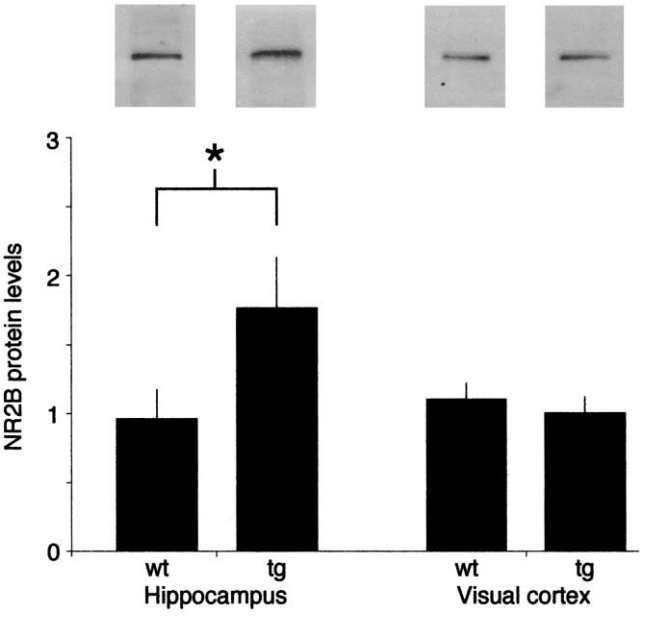

Fig. 5. Synaptic NR2B protein levels are increased in hippocampus ($p<0.05$) but not visual cortex of transgenic mice.

expression of NR2B under the CaMKII promoter is not sufficient to alter NMDAR-mediated function in visual cortex.

What could account for the different responses that hippocampal and visual cortical pyramidal cells have to transgenic expression of NR2B? It is possible that despite overexpression of NR2B protein in the soma, NR2B-containing receptors fail to be incorporated into the synaptic membrane in visual cortex. The mechanisms for NMDAR trafficking and targeting might differ between the visual cortex and hippocampus. An alternative hypothesis is that NR2B mRNA levels are endogenously saturated in visual cortex, thus making it difficult to observe changes in plasticity or current duration without a concomitant change in NR2A levels. Notably, we have previously demonstrated that NR2B protein levels continue to rise in the visual cortex after they have

reached a plateau in other regions of the brain (Quinlan et al., 1999b). Additionally, there are numerous examples of dichotomies between mRNA and protein expression (e.g., Pollock et al., 2001). Regardless of the mechanism, it is apparent that the ratio of NR2B over NR2A protein in the NMDA receptor complex at visual cortical synapses is not significantly influenced by the transgene overexpression.

The failure of NR2B overexpression to alter NMDAR kinetics and plasticity lends new insights into developmental and experience-dependent regulation of NMDARs. We have previously demonstrated that visual experience can bidirectionally regulate the composition and function of NMDARs in visual cortex (Philpot et al., 2001), such that visual experience increases the ratio of NR2A- to NR2B-containing NMDARs and shortens EPSC current duration. However, the mechanism that regulates the ratio of NR2A:NR2B remains unknown. Our present data suggest that the expression level of NR2B does not significantly influence the ratio of NR2A:NR2B at the synapse, suggesting that either the expression of NR2A is important for regulating the subunit composition of receptors expressed at the synapse and/or that synaptic trafficking of receptors is tightly regulated. Because we have previously demonstrated that NR2A levels are dramatically altered by visual experience while NR2B levels remain unchanged (Quinlan et al., 1999a,b), we suggest that the synaptic ratio of NR2A:NR2B might be dictated by the availability of NR2A subunits.

Acknowledgements

Special thanks to E. Quinlan for assistance with the synaptoneurosome preparation, and to E. Sklar and S. Meagher for assistance. This work was supported in part by grants from the NIH, NSF, HFSP, and NIMH Grant #MH 58880.

References

Barth, A.L., Malenka, R.C., 2001. NMDAR EPSC kinetics do not regulate the critical period for LTP at thalamocortical synapses. Nature Neuroscience 4, 235–236.

Bear, M.F., 1995. Mechanism for a sliding synaptic modification threshold. Neuron 15, 1–4.

Bear, M.F., Cooper, L.N., Ebner, F.F., 1987. A physiological basis for a theory of synaptic modification. Science 237, 42–48.

Bear, M.F., Kleinschmidt, A., Gu, Q., Singer, W., 1990. Disruption of experience-dependent synaptic modifications in striate cortex by infusion of an NMDA receptor antagonist. Journal of Neuroscience 10, 909–925.

Bear, M.F., Malenka, R.C., 1994. Synaptic plasticity: LTP and LTD. Current Opinion in Neurobiology 4, 389–399.

Bienenstock, E.L., Cooper, L.N., Munro, P.W., 1982. Theory for the development of neuron selectivity: Orientation specificity and binocular interaction in visual cortex. Journal of Neuroscience 2, 32–48.

Blakemore, C., VanSluyters, R.C., 1974. Reversal of the physiological effects of monocular deprivation in kittens: Further evidence for a sensitive period. Journal of Physiology (London) 237, 195–216.

Carmignoto, G., Vicini, S., 1992. Activity-dependent decrease in NMDA receptor responses during development of the visual cortex. Science 258, 1007–1011.

Caviness, V.S. Jr., 1975. Architectonic map of neocortex of the normal mouse. Journal of Comparative Neurology 164, 247–263.

Chen, L., Cooper, N.G., Mower, G.D., 2000. Developmental changes in the expression of NMDA receptor subunits (NR1, NR2A, NR2B) in the cat visual cortex and the effects of dark rearing. Molecular Brain Research 78, 196–200.

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. Journal of Neuroscience 17, 2469–2476.

Gordon, J.A., Stryker, M.P., 1996. Experience-dependent plasticity of binocular responses in the primary visual cortex of the mouse. Journal of Neuroscience 16, 3274–3286.

Huang, Z.J., Kirkwood, A., Pizzorusso, T., Porciatti, V., Morales, B., Bear, M.F., Maffei, L., Tonegawa, S., 1999. BDNF regulates the maturation of inhibition and the critical period of plasticity in mouse visual cortex. Cell 98, 739–755.

Juraska, J.M., 1982. The development of pyramidal neurons after eye opening in the visual cortex of hooded rats: A quantitative study. Journal of Comparative Neurology 212, 208–213.

Kirkwood, A., Rioult, M.G., Bear, M.F., 1996. Experience-dependent modification of synaptic plasticity in visual cortex. Nature 381, 526–528.

McBain, C.J., Mayer, M.L., 1994. N-methyl-D-aspartic acid receptor structure and function. Physiological Reviews 74, 723–760.

Mioche, L., Singer, W., 1989. Chronic recordings from single sites of kitten striate cortex during experience-dependent modifications of receptive-field properties. Journal of Neurophysiology 62, 185–197.

Monyer, H., Sprengel, R., Schoepfer, R., Herb, A., Higuchi, M., Lomeli, H., Burnashev, N., Sakmann, B., Seeburg, P.H., 1992. Heteromeric NMDA receptors: Molecular and functional distinction of subtypes. Science 256, 1217–1221.

Nase, G., Weishaupt, J., Stern, P., Singer, W., Monyer, H., 1999. Genetic and epigenetic regulation of NMDA receptor expression in the rat visual cortex. European Journal of Neuroscience 11, 4320–4326.

Philpot, B.D., Bear, M.F., Abraham, W.C., 1999. Metaplasticity: the plasticity of synaptic plasticity. In: Katz, P.S. (Ed.), Beyond Neurotransmission: Neuromodulation and its importance for information flow. Oxford University Press, Oxford.

Philpot, B.D., Sekhar, A.K., Shouval, H.Z., Bear, M.F., 2001. Visual experience and deprivation bidirectionally modify the composition and function of NMDA receptors in visual cortex. Neuron 29, 157–169.

Pollock, G.S., Vernon, E., Forbes, M.E., Yan, Q., Ma, Y.T., Hsieh, T., Robichon, R., Frost, D.O., Johnson, J.E., 2001. Effects of early visual experience and diurnal rhythms on BDNF mRNA and protein levels in the visual system, hippocampus, and cerebellum. Journal of Neuroscience 21, 3923–3931.

Porciatti, V., Pizzorusso, T., Maffei, L., 1999. The visual physiology of the wild type mouse determined with pattern VEPs. Vision Research 39, 3071–3081.

Quinlan, E.M., Olstein, D.H., Bear, M.F., 1999a. Bidirectional, experience-dependent regulation of N-methyl-D-aspartate receptor subunit composition in the rat visual cortex during postnatal development. Proceedings of the National Academy of Sciences of the USA 96, 12876–12880.

Quinlan, E.M., Philpot, B.D., Huganir, R.L., Bear, M.F., 1999b. Rapid,

experience-dependent expression of synaptic NMDA receptors in visual cortex in vivo. Nature Neuroscience 2, 352–357.

Rittenhouse, C.D., Shouval, H.Z., Paradiso, M.A., Bear, M.F., 1999. Monocular deprivation induces homosynaptic long-term depression in visual cortex. Nature 397, 347–350.

Roberts, E.B., Meredith, M.A., Ramoa, A.S., 1998. Suppression of NMDA receptor function using antisense DNA block ocular dominance plasticity while preserving visual responses. Journal of Neurophysiology 80, 1021–1032.

Roberts, E.B., Ramoa, A.S., 1999. Enhanced NR2A subunit expression and decreased NMDA receptor decay time at the onset of ocular dominance plasticity in the ferret. Journal of Neurophysiology 81, 2587–2591.

Rumbaugh, G., Vicini, S., 1999. Distinct synaptic and extrasynaptic NMDA receptors in developing cerebellar granule neurons. Journal of Neuroscience 19, 10603–10610.

Tang, Y.P., Shimizu, E., Dube, G.R., Rampon, C., Kerchner, G.A., Zhuo, M., Liu, G., Tsien, J.Z., 1999. Genetic enhancement of learning and memory in mice. Nature 401, 63–69.

Wei, F., Wang, G.D., Kerchner, G.A., Kim, S.J., Xu, H.M., Chen, Z.F., Zhuo, M., 2001. Genetic enhancement of inflammatory pain by forebrain NR2B overexpression. Nature Neuroscience 4, 164–169.

Wiesel, T.N., Hubel, D.H., 1965. Comparison of the effects of unilateral and bilateral eye closure on cortical unit responses in kittens. Journal of Neurophysiology 28, 1060–1072.