Temporal Regulation of the Expression Locus of Homeostatic Plasticity

Corette J. Wierenga, Michael F. Walsh, and Gina G. Turrigiano
Department of Biology and Center for Complex Systems, Brandeis University, Waltham, Massachusetts

Wierenga, Corette J., Michael F. Walsh, and Gina G. Turrigiano.
Temporal regulation of the expression locus of homeostatic plasticity.
J Neurophysiol 96: 2127–2133, 2006. First published June 7, 2006;
doi:10.1152/jn.00107.2006. Homeostatic plasticity of excitatory synapses plays an important role in stabilizing neuronal activity, but the mechanism of this form of plasticity is incompletely understood. In particular, whether the locus of expression is presynaptic or postsynaptic has been controversial. Here we show that the expression locus depends on the time neurons have spent in vitro. In visual cortical cultures ≤14 days in vitro (DIV), 2 days of TTX treatment induced an increase in miniature excitatory postsynaptic current (mEPSC) amplitude onto pyramidal neurons, without affecting mEPSC frequency. However, in cultures ≥18 DIV, the same TTX treatment induced a large increase in mEPSC frequency, whereas the amplitude effect was reduced. The increased mEPSC frequency was associated with an increased density of excitatory synapses and increased presynaptic vesicle release in response to electrical stimulation. This indicates a shift from a predominantly postsynaptic response to TTX in ≤14 DIV cultures, to a coordinated pre- and postsynaptic response in ≥18 DIV cultures. This shift was not specific for cortical cultures because a similar shift was observed in cultured hippocampal neurons. Culturing neurons from older animals showed that the timing of the switch depends on the time the neurons have spent in vitro, rather than their postnatal age. This temporal switch in expression locus can largely reconcile the contradictory literature on the expression locus of homeostatic excitatory synaptic plasticity in central neurons. Furthermore, our results raise the intriguing possibility that the expression mechanism of homeostatic plasticity can be tailored to the needs of the network during different stages of development or in response to different challenges to network function.

INTRODUCTION

Neurons can adapt to prolonged changes in synaptic drive in a homeostatic manner, so that firing rates remain relatively stable (Burrone et al. 2002; Turrigiano et al. 1998). Such homeostatic plasticity likely plays an important role in preventing the destabilizing effects of developmental and learning-related changes in synapse number or strength (Davis and Bezprozvanny 2001; Turrigiano 1999; Turrigiano and Nelson 2004). One important form of homeostatic plasticity in vertebrate central neurons is the activity-dependent regulation of excitatory synapses, where prolonged (1 to 2 days) elevation of activity reduces all of a neuron's excitatory synaptic strengths and vice versa (Turrigiano and Nelson 2004). Although homeostatic plasticity of excitatory central synapses has now been identified in a number of preparations (Turrigiano and Nelson 2004), the cellular and molecular mechanisms of this plasticity remain largely obscure. In particular, the expression locus has been controversial, with some studies supporting exclusively postsynaptic changes and others supporting both

pre- and postsynaptic changes. Here we show that the expression locus of homeostatic plasticity is temporally regulated and suggest that this regulation can comprehensively account for the differences in expression mechanism reported in the literature.

In young neocortical neurons, 2 days of tetrodotoxin (TTX) treatment increases miniature excitatory postsynaptic current (mEPSC) amplitude, but not frequency. This arises predominantly, if not exclusively, from postsynaptic changes: the number of postsynaptic AMPA receptors and amplification of dendritic currents by Na^+ channels are increased (Wierenga et al. 2005). Changes in postsynaptic receptor accumulation after activity manipulations have also been observed in cultured spinal (O'Brien et al. 1998) and young hippocampal (Liao et al. 1999; Lissin et al. 1998) neurons, cerebellar slices (Liu and Cull-Candy 2000, 2002), and the ventral nerve cord of *Caenorhabditis elegans* (Grunwald et al. 2004). In addition, reduced sensory drive in vivo increases excitatory synaptic strength onto visual cortical neurons without affecting mEPSC frequency and with only a minor effect on short-term plasticity, suggesting mainly postsynaptic changes (Desai et al. 2002; Maffei et al. 2004).

In contrast, other studies have suggested a presynaptic locus of homeostatic plasticity. At the *Drosophila* neuromuscular junction, an increase in presynaptic release probability was observed after postsynaptic activity blockade (Paradis et al. 2001) and, in older hippocampal cultures, activity blockade induced a modest increase in mEPSC amplitude and a large increase in mEPSC frequency (Bacci et al. 2001; Burrone et al. 2002; Thiagarajan et al. 2002). This was associated with an increased size of the presynaptic terminal and increased release probability (Murthy et al. 2001; Thiagarajan et al. 2005). Thus even within similar experimental preparations from different labs (i.e., hippocampal cultures), the locus of change has been controversial. To date, there has not been any satisfactory explanation for these contradictory findings (Burrone and Murthy 2003; Turrigiano and Nelson 2004).

Besides inherent differences between brain regions, one major difference between many of the culture studies mentioned above is the time the neurons were kept in vitro. We show here that the time neurons have spent in vitro—rather than neuronal age—is a key factor determining the expression locus of homeostatic plasticity. Our data suggest that there exists an array of potential expression sites for homeostatic synaptic plasticity, and which particular mechanism is recruited depends either on the age of the synapse or on other factors that change with time in vitro.

METHODS

Cultures and mEPSC recordings

Visual cortical and hippocampal cultures were prepared from P3 rats, as previously described (Turrigiano et al. 1998; Watt et al. 2000). Culture medium was changed three times per week during the first week and once a week thereafter. Cell density declined with time in vitro: 47 ± 3 neurons/mm^2 at DIV 7, 35 ± 17 neurons/mm^2 at DIV 14, and 22 ± 6 neurons/mm^2 at DIV 21. The 2-day TTX treatment did not affect cell density ($P > 0.1$; paired t-test). Cultures from P9 rats were prepared in the same way, but were plated at a twofold higher initial density. The initial survival rate of neurons from P9 rats was lower than that from P3 rats, but after the first week in vitro cell density stayed fairly constant (21 ± 4 neurons/mm^2 at DIV 8 and 22 ± 8 neurons/mm^2 at DIV 21).

Recordings of mEPSCs were done at room temperature with a KMeSO$_4$-based pipette solution (Wierenga et al. 2005). Data points in Fig. 1 were obtained from cultures that were kept 7–9 (DIV 8), 13–16 (DIV 14), 17–18 (DIV 18), and 21–24 (DIV22) days in vitro.

FM1-43 labeling

Presynaptic terminals were labeled with 15 μM FM1-43 by either superfusion with 40 mM K$^+$ solution or by electrical stimulation, in either case in the presence of 10 μM DNQX, 50 μM APV, and 20 μM bicuculline, as described previously (Wierenga et al. 2005). All experiments were done at room temperature. In most cases random puncta were selected, representing a combination of excitatory (roughly 70%) and inhibitory (roughly 30%) terminals. In a few cases, we selected only FM1-43 puncta on distal apical dendrites of pyramidal neurons (labeled with DsRed transfection; three control and three TTX neurons). These puncta are primarily excitatory (Wierenga et al. 2005). Results were qualitatively the same for puncta on pyramidal neurons and randomly selected puncta and we have pooled data from all puncta in Fig. 3.

To estimate the destaining kinetics of individual FM1-43 puncta, the puncta intensity at each time point was measured relative to the local background and then normalized to the total intensity loss during the stimulation. For the stimulated destaining experiments, only puncta that destained to <50% of their initial intensity were selected.

For the spontaneous destaining experiments, only puncta that were destained by a subsequent 5-min 40 mM K$^+$ solution application were selected for analysis.

Immunostaining

Immunostaining experiments were performed as previously described (Wierenga et al. 2005). Measurements of cell densities were done as described previously (Rutherford et al. 1997), with the difference that anti-NeuN labeling (1:500, Chemicon) was used to visualize neuronal nuclei.

Statistics

Statistical analyses were performed using unpaired Student's t-test, unless indicated otherwise. For testing differences between distributions the Kolmogorov–Smirnov (K-S) test was used.

RESULTS

Miniature EPSCs

Two days of activity blockade induces homeostatic changes in excitatory synaptic transmission. In neocortical, spinal, or hippocampal cultures <14 days in vitro (DIV), activity blockade increases mEPSC amplitude, with no effect on mEPSC frequency (Ju et al. 2004; O'Brien et al. 1998; Turrigiano et al. 1998), whereas in hippocampal neurons >14 DIV, activity blockade results in an increase in mEPSC frequency, with only a small increase in amplitude (Burrone et al. 2002; Thiagarajan et al. 2002). We examined whether the difference in time in vitro could explain these contradictory findings.

Neurons from visual cortex from 3-day-old (P3) rat pups were kept in culture for 8, 14, 18, and 22 DIV and for each time point half of the dishes were treated with 0.5 μM TTX for 2 days before the experiments. The frequency of mEPSCs in pyramidal neurons increased only slightly during the first 2 wk in vitro and reached steady state after 14 DIV. The amplitude
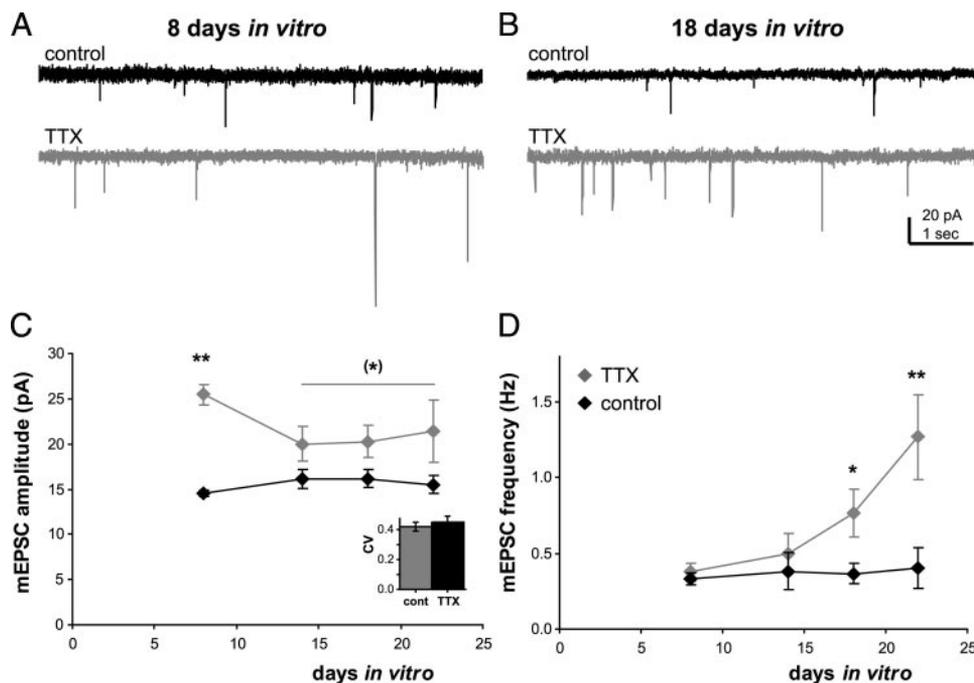

FIG. 1. Effect of a 2-day tetrodotoxin (TTX) treatment depends on time in vitro. *A* and *B*: recordings of miniature excitatory postsynaptic currents (mEPSCs) in control and TTX-treated pyramidal neurons at 8 and 18 days in vitro (DIV). *C* and *D*: amplitude and frequency of mEPSCs in control and TTX-treated neurons as a function of DIV. *Inset* in *C*: CV of mEPSC amplitudes at DIV 22. Data from 8, 18, 18, and 13 control neurons and 10, 21, 25, and 12 TTX-treated neurons at DIV 8, 14, 18, and 22, respectively. Asterisks indicate significant differences (*$P < 0.05$; **$P < 0.01$; ANOVA). (*) indicates that data were pooled over DIV 14–22.

of mEPSCs remained fairly constant over this time period (Fig. 1).

In young neocortical cultures (8 DIV), blocking activity for 2 days resulted in an increase in mEPSC amplitude, without an effect on mEPSC frequency, as previously described (Turrigiano et al. 1998; Watt et al. 2000; Wierenga et al. 2005). In neurons that were kept ≥14 DIV TTX treatment also induced an increase in mEPSC amplitude, but the magnitude of the increase was reduced from nearly 100% at 8 DIV to approximately 25% at ≥14 DIV (Fig. 1, *A–C*). The coefficient of variation (CV) of mEPSC amplitudes was similar in all experimental groups, indicating that the shape of the amplitude distribution was not significantly affected by the TTX treatment at any age, consistent with an activity-dependent scaling up of mEPSC amplitude (Turrigiano et al. 1998). TTX treatment did not affect mEPSC frequency in neurons that were kept in vitro for ≤14 days, but after 18 DIV the same 2-day TTX treatment induced a robust increase in mEPSC frequency (Fig. 1*D*). Resting membrane potentials and mEPSC kinetics were not significantly affected by the TTX treatment at any time point (mean resting membrane potential, -65 ± 1 mV; mean rise time, 1.3 ± 0.02 ms; mean decay time constant, 5.0 ± 0.2 ms). However, input resistance was increased in TTX-treated cultures ≥14 DIV (control: 266 ± 25 MΩ; TTX: 434 ± 25 MΩ; $P < 0.01$). There was no correlation between input resistance and mEPSC amplitude or frequency (Pearson's r values of 0.18 and 0.03, respectively), suggesting that the increase in input resistance after TTX treatment reflects additional changes induced by TTX treatment, which are unrelated to the effect on mEPSCs.

The observed increase in mEPSC frequency in neocortical cultures ≥18 DIV is in good agreement with the results obtained in hippocampal cultures of the same age (Burrone et al. 2002; Murthy et al. 2001; Thiagarajan et al. 2005). To test whether the switch we observed in our visual cortical cultures is a general phenomenon of central neurons, we recorded mEPSCs in hippocampal neurons and compared the effect of a 2-day TTX treatment at 10 and 18 DIV. As in the visual cortical cultures (Fig. 1), TTX-treated hippocampal neurons showed an increase in mEPSC amplitude at 10 DIV, whereas

at 18 DIV both mEPSC frequency and amplitude were increased after TTX treatment (Fig. 2). This suggests that the observed shift in response to activity blockade with time in vitro is not specific for neocortical cultures but may be a general response to prolonged time in vitro. This strongly suggests that the apparent contradiction between previous experimental reports on the locus of homeostatic plasticity can be explained chiefly by differences in the time the neurons have spent in vitro.

FM1-43 labeling and destaining

Our previous results indicated no change in presynaptic release properties after TTX treatment in neocortical cultures ≤14 DIV (Wierenga et al. 2005). To begin to address the mechanism underlying the increase in mEPSC frequency induced by TTX in cultures ≥18 DIV, we examined presynaptic vesicle recycling in DIV 18 cultures by labeling terminals with the fluorescent styryl dye FM1-43. When presynaptic vesicle turnover was induced by superfusion for 2 min with a HEPES-buffered solution containing 40 mM K^+, FM1-43 puncta intensities were higher in TTX-treated cultures than in control cultures [mean intensity was $122 \pm 6\%$ of control ($>1,000$ puncta per condition); $P < 0.001$, K-S test; Fig. 3, *A* and *B*]. The increased puncta intensity was also observed when presynaptic vesicle turnover was induced by electrical stimulation [600 stimuli at 20 Hz; mean TTX-treated puncta intensity was increased to $139 \pm 7\%$ of control (144 control, 239 TTX-treated puncta); $P < 0.001$, K-S test].

To examine whether the kinetics of destaining was altered by TTX treatment, we monitored the destaining time course of individual FM1-43 puncta every 10 s during application of 1,500 stimuli at 10 Hz. FM1-43 puncta in TTX-treated cultures showed faster destaining kinetics than that of control puncta (Fig. 3, *C* and *D*). These experiments were performed in the presence of synaptic blockers, but action potentials were not blocked to allow us to study evoked release. As a consequence, destaining reflects not only exocytosis of vesicles in response to electrical stimulation, but also release of synaptic vesicles by spontaneous action potentials and stochastic release. Poten-
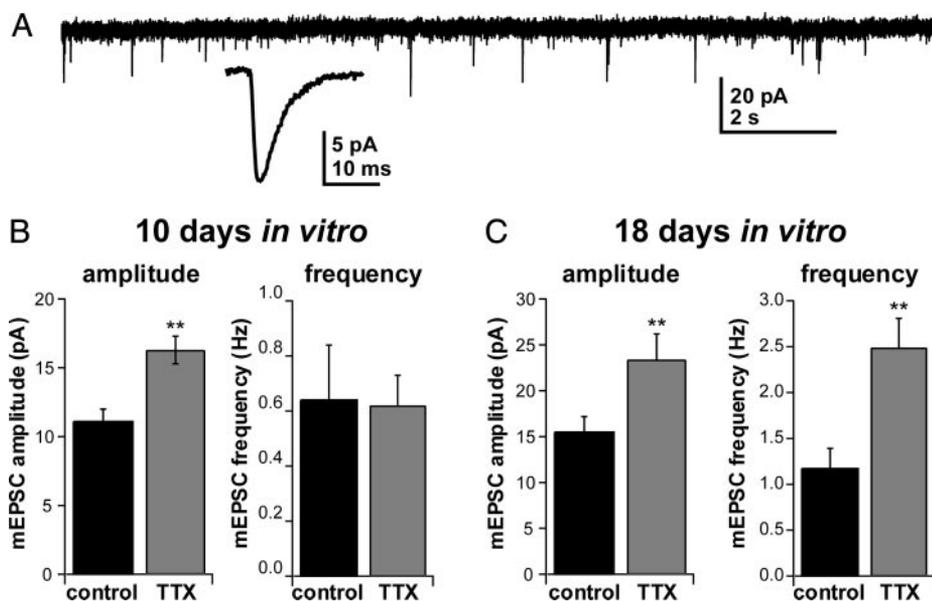

FIG. 2. A similar shift occurs in hippocampal cultures. *A*: recordings of mEPSCs in control hippocampal neurons at 10 DIV. *Inset*: average mEPSC. *B*: summary of mEPSC amplitudes and frequency in hippocampal cultures at 8–12 DIV (10 DIV). Data from 7 control and 8 TTX-treated neurons. *C*: summary of mEPSC amplitudes and frequency in hippocampal cultures at 18–19 DIV (18 DIV). Data from 9 control and 10 TTX-treated neurons. Asterisks indicate significant differences (**$P < 0.01$).
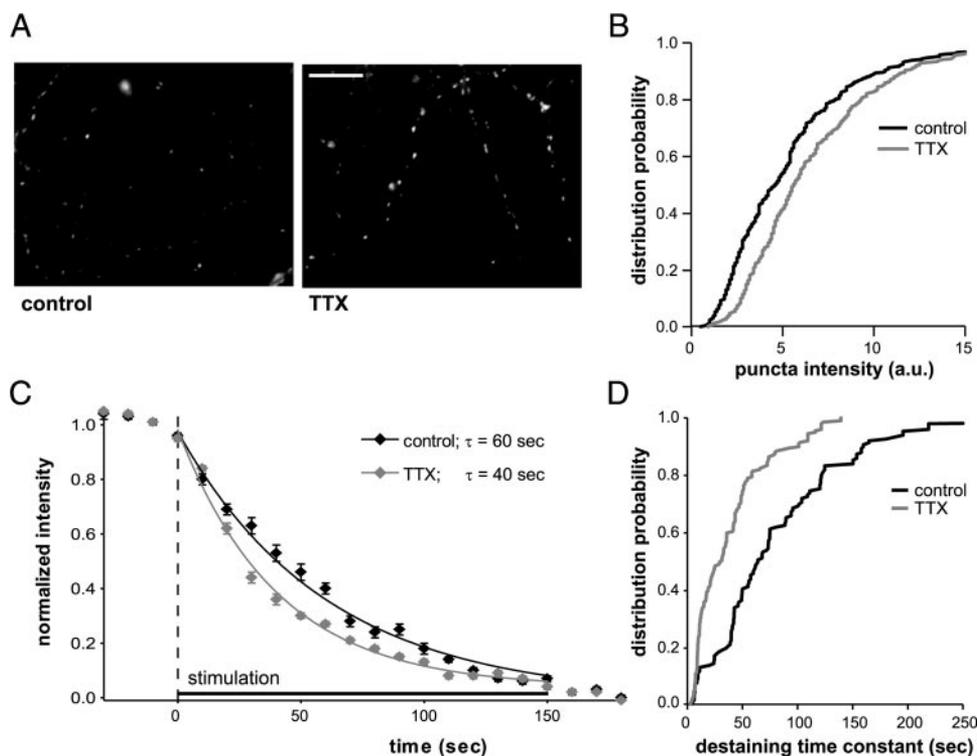
FIG. 3. TTX treatment increases FM1-43 labeling in DIV 18 neocortical cultures. *A*: examples of FM1-43 puncta in control and TTX-treated visual cortical cultures. Presynaptic terminals were labeled with superfusion of a 40 mM K$^+$ solution. Scale bar = 10 μm. *B*: distribution of puncta intensities labeled with high K$^+$. Data from 1,348 puncta, 21 images (control) and 1,484 puncta, 20 images (TTX-treated). *C*: destaining time course in control and TTX-treated cultures, normalized to the total loss of fluorescence during the stimulation. Puncta were labeled with 600 stimuli at 20 Hz and stimulated destaining was induced by applying 1,500 stimuli at 10 Hz. Single exponential fits are shown with time constants indicated. *D*: distribution of destaining time constants of individual puncta ($P < 0.001$, Kolmogorov–Smirnov test). Data from 132 puncta, 7 images (control) and 197 puncta, 9 images (TTX-treated).

tially, the observed difference in destaining kinetics could be explained by differences in spontaneous release, rather than differences in evoked release. We therefore measured the spontaneous destaining rates in control and TTX-treated cultures by repeating the destaining experiments while omitting the destaining stimulation. The average spontaneous destaining was well fit with a linear function. Spontaneous destaining rates were similar in control and TTX-treated cultures [control: -0.17 ± 0.004%/s (197 puncta) and TTX: -0.16 ± 0.003%/s (169 puncta); data not shown]. This indicates that the difference in destaining kinetics between TTX-treated and control FM1-43 puncta arises from a different response to the electrical stimulation.

We also fit the individual destaining time courses of FM 1–43 puncta to obtain the distribution of destaining time constants (Fig. 3*D*). TTX-treated cultures showed a larger fraction of fast destaining puncta, although fast and slowly destaining FM1-43 puncta are present in both conditions. These experiments suggest that, in contrast to young (DIV 7–10) cultures, where the same experimental protocol did not induce any changes in destaining kinetics (Wierenga et al. 2005), activity blockade in DIV 18 cultures increases vesicle release and/or recycling in presynaptic terminals.

Number of excitatory synapses

Changes in mEPSC frequency could reflect increased rates of spontaneous vesicle fusion, changes in the number of functional synapses, or both. We previously showed that TTX treatment in cultures ≤14 DIV does not affect the number of excitatory synapses (Wierenga et al. 2005). To determine whether this is also true after DIV 18–20 when TTX treatment increases mEPSC frequency, we performed triple immunolabeling experiments against excitatory synaptic markers. Excitatory synapses on apical dendrites of pyramidal neurons were

visualized using antibodies directed against two postsynaptic markers [the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR) subunit GluR1 and scaffolding protein PSD95] and one presynaptic marker [the vesicular glutamate transporter 1 (Vglut1); Fig. 4*A*]. GluR1 labeling was performed before permeabilization so that only surface receptors were labeled (Wierenga et al. 2005).

Two days of TTX treatment in DIV 18–20 cultures induced an increase in the density of puncta for all three excitatory synaptic markers (Fig. 4*B*). The density of puncta that had any combination of two or three of the markers was increased about twofold (Fig. 4*C*). However, the percentage of puncta that were co-localized with other synaptic markers was not different in control and TTX-treated cultures (Fig. 4*D*), suggesting that the number of excitatory synapses was increased, whereas the proportion of synaptic and extrasynaptic puncta was maintained.

Time in vitro versus postnatal age

The results described above indicate that the response to a period of activity blockade depends on time in vitro. In older cultures, where both mEPSC amplitude and frequency are increased after TTX treatment, neurons are both older and have spent more time in vitro than in the younger cultures, where only mEPSC amplitude is increased. To distinguish between the effect of postnatal age of the neurons and the time spent in vitro we prepared visual cortical cultures from P9 rats. After 2 wk in vitro, cultured neurons from P9 rats have the same postnatal age as that of neurons from a P3 rat that have been in vitro for 20 days, whereas they have spent the same time in vitro as 14 DIV neurons from a P3 culture (Fig. 5*A*). If the postnatal age of the neurons determines the response to activity blockade, one would expect to find an increased mEPSC frequency after TTX treatment in P9/DIV 14 cultures. How-
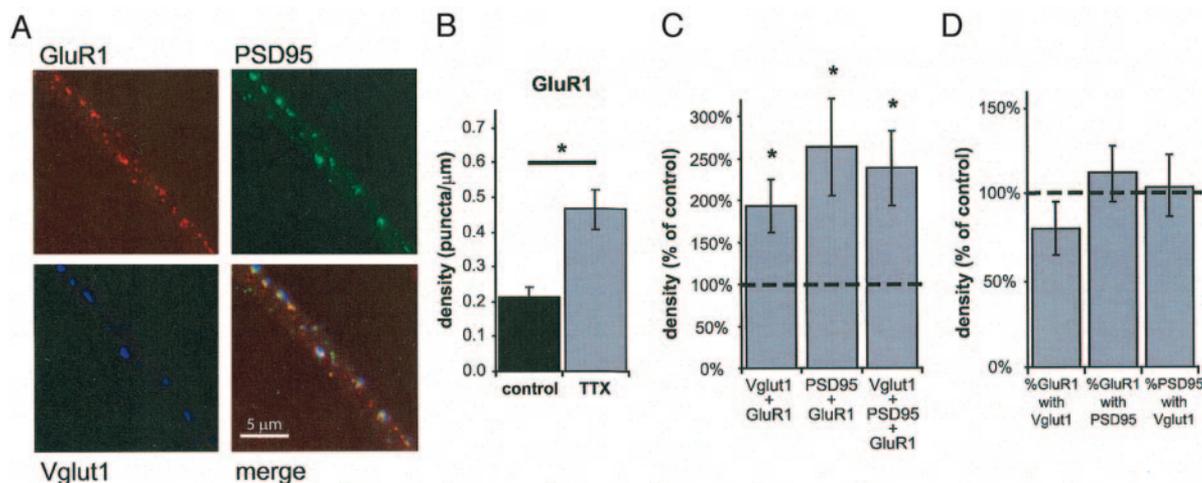
FIG. 4. TTX treatment increases the number of excitatory synapses in DIV 18–20 neocortical cultures. *A*: excitatory synapses on the apical dendrite of a pyramidal neuron at DIV18 were labeled by postsynaptic markers GluR1 and PSD95, and presynaptic marker Vglut1. *B*: density of GluR1 puncta in control and TTX-treated visual cortical cultures. *C*: density of puncta that were labeled with double or triple markers in TTX-treated cultures as percentage of control. *D*: co-localization between the 3 markers was not affected by the TTX treatment. Data from 7 control and 8 TTX-treated neurons. Asterisks indicate significant differences ($P < 0.05$).

ever, mEPSC frequency was not affected by TTX treatment in these cultures (Fig. 5*B*). Consistent with our results from P3/DIV 14 cultures (Fig. 1*C*), mEPSC amplitude was slightly but significantly increased in P9/DIV 14 cultures after TTX treatment [control: 17.9 ± 1.0 pA (19 neurons) and TTX: 19.0 ± 1.1 pA (20 neurons); $P < 0.05$, paired *t*-test]. To rule out the possibility that cultures derived from P9 animals generally lack the ability to respond to activity deprivation by altering mEPSC frequency, we kept P9 cultures in vitro for another week (DIV 21). In P9/DIV 21 cultures, the same TTX treatment induced a significant increase in mEPSC frequency (Fig. 5*B*, $P < 0.05$). Taken together these experiments indicate that the time neurons have spent in vitro, rather than their postnatal age, determines their response to activity blockade.
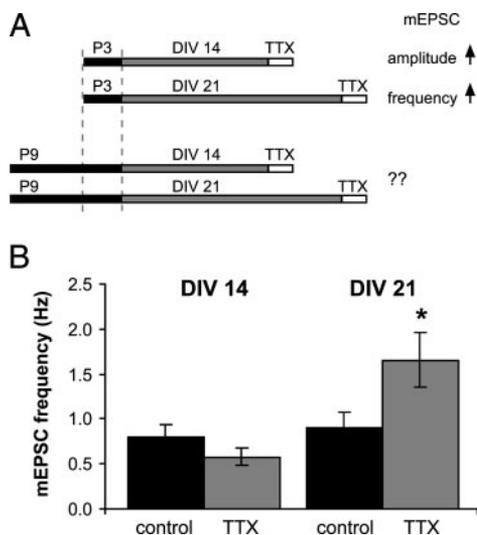

FIG. 5. Time spent in culture, rather than postnatal age of neurons, determines response to TTX treatment. *A*: cultures of visual cortical neurons from P9 rats that are kept in vitro for 14 days have the same postnatal age as DIV 20 neurons from P3 rats, and have spent the same time in vitro as DIV 14 neurons from P3 rats. *B*: frequency of mEPSCs in pyramidal neurons from a P9 culture. DIV 14: 19 control and 20 TTX-treated neurons; DIV 21: 12 control and 11 TTX-treated neurons. Asterisk indicates significant difference ($P < 0.05$).

DISCUSSION

Synaptic scaling of excitatory synapses is an important form of homeostatic plasticity that operates both in vitro and in vivo (Turrigiano and Nelson 2004). Understanding when and how this form of plasticity is expressed has important consequences for the function of cortical circuits because a purely postsynaptic expression locus will leave the short-term dynamics and filtering properties of synapses unaffected, whereas presynaptic changes in release probability will fundamentally change these short-term dynamics (Abbott et al. 1997; Markram et al. 1998; Wierenga et al. 2005). Here we show that the expression locus of excitatory homeostatic plasticity depends on the time the neurons have spent in vitro. In young cultures (≤ 14 DIV), mEPSC amplitude is increased by activity blockade, whereas mEPSC frequency is not affected. However, in neurons that were kept in vitro for longer (≥ 18 DIV), activity blockade increased both mEPSC amplitude and frequency. This increase in mEPSC frequency was accompanied by a doubling in the density of excitatory synapses and increased evoked release from presynaptic terminals. Finally, we found that this change in response to activity blockade depends not on the postnatal age of the neurons, but on the time the neurons have spent in vitro. These data indicate that the same synapses can use different homeostatic plasticity mechanisms under different circumstances.

In the literature different effects of activity blockade have been reported, even within the same experimental preparation. In postnatal hippocampal neurons, it has been controversial whether activity blockade affects only mEPSC amplitude (Lissin et al. 1998; Stellwagen and Malenka 2006) or primarily affects mEPSC frequency with a smaller effect on amplitude (Burrone et al. 2002; Thiagarajan et al. 2002, 2005). Our data indicate that the discrepancy between these results can be explained by a shift in expression locus with time in vitro because the former studies were done in cultures at <15 DIV, whereas the latter were performed after 2–4 wk in vitro.

The finding that hippocampal and neocortical neurons in culture show a similar shift in expression locus of homeostatic plasticity does not necessarily mean that the detailed expres-

sion mechanisms are identical. There may be region-specific differences in the underlying AMPAR trafficking mechanisms that adjust mEPSC amplitude and/or differences in presynaptic vesicle recycling (Ju et al. 2004; Thiagarajan et al. 2005; Virmani et al. 2006).

Our data suggest that, at cortical synapses, a major contributor to the increase in mEPSC frequency is the doubling in the density of excitatory synaptic contacts induced by activity blockade. In addition, we found an increased synaptic vesicle release in response to electrical stimulation. Higher puncta intensities in 18 DIV TTX-treated cultures are consistent with an increased size of presynaptic terminals and larger vesicle pools, as reported in hippocampal cultures (Murthy et al. 2001). The faster destaining kinetics we observed after activity blockade could reflect an increased release probability (Murthy et al. 2001; Thiagarajan et al. 2005), although neither our data nor previous reports can exclude the possibility that increased excitability of TTX-treated neuronal terminals (possibly reflected in the observed increase in input resistance) also contributed to the faster destaining kinetics (Desai et al. 1999).

It is not clear what determines the change in expression locus of homeostatic plasticity. Our results indicate that the postnatal age of cultured neurons is not crucial, but rather that the expression locus is determined by the time the neurons have spent in vitro. The lower cell density at longer times in vitro is not a determining factor for the response to activity blockade because the P9/DIV14 cultures had densities similar to those of P3/DIV 20 cultures, but responded differently to activity blockade. One possibility is that the altered response to activity blockade with time in vitro arises from prolonged exposure to the (artificial) in vitro environment. Alternatively, the response to activity blockade may be determined by the maturational state of the synapses because neurons that have spent longer times in vitro have more mature synaptic contacts. Maturation of synapses is associated with a multitude of pre- and postsynaptic changes, including increased vesicle pool size (Mohrmann et al. 2003), a change in composition of postsynaptic glutamate receptors (Gomperts et al. 2000), increased scaffolding proteins together with a decreased dependency on F-actin (Zhang and Benson 2001), a change in vesicular glutamate transporters (de Gois et al. 2005; Wilson et al. 2005), and altered expression of α- and β-CaMKII (Fink et al. 2003; Thiagarajan et al. 2002). Each of these changes has the potential to alter the effect of activity blockade on synaptic transmission. A recent report suggests that astrocytes are involved in mediating homeostatic changes in cultures <15 DIV (Stellwagen and Malenka 2006), raising the possibility that changes in neuron–astrocyte interactions could contribute to this time-dependent switch. The existence of multiple forms of excitatory homeostatic plasticity raises the general possibility that the expression mechanism may be tailored to the needs of the network during different stages of development or in response to different challenges to network function.

REFERENCES

Abbott LF, Varela JA, Sen K, and Nelson SB. Synaptic depression and cortical gain control. *Science* 275: 220–224, 1997.

Bacci A, Coco S, Pravettoni E, Schenk U, Armano S, Frassoni C, Verderio C, De Camilli P, and Matteoli M. Chronic blockade of glutamate receptors enhances presynaptic release and downregulates the interaction between synaptophysin-synaptobrevin-vesicle-associated membrane protein 2. *J Neurosci* 21: 6588–6596, 2001.

Burrone J and Murthy VN. Synaptic gain control and homeostasis. *Curr Opin Neurobiol* 13: 560–567, 2003.

Burrone J, O'Byrne M, and Murthy VN. Multiple forms of synaptic plasticity triggered by selective suppression of activity in individual neurons. *Nature* 420: 414–418, 2002.

Davis GW and Bezprozvanny I. Maintaining the stability of neural function: a homeostatic hypothesis. *Annu Rev Physiol* 63: 847–869, 2001.

de Gois S, Schäfer MK-H, Defamie N, Chen C, Ricci A, Weihe E, Varoqui H, and Erickson JD. Homeostatic scaling of vesicular glutamate and GABA transporter expression in rat neocortical circuits. *J Neurosci* 25: 7121–7133, 2005.

Desai NS, Cudmore RH, Nelson SB, and Turrigiano GG. Critical periods for experience-dependent synaptic scaling in visual cortex. *Nat Neurosci* 5: 783–789, 2002.

Desai NS, Rutherford LC, and Turrigiano GG. Plasticity in the intrinsic excitability of cortical pyramidal neurons. *Nat Neurosci* 2: 515–520, 1999.

Fink CC, Bayer KU, Myers JW, Ferell JE Jr, Schulman H, and Meyer T. Selective regulation of neurite extension and synapse formation by the β but not the α isoform of CaMKII. *Neuron* 39: 283–297, 2003.

Gomperts SN, Carroll RC, Malenka RC, and Nicoll RA. Distinct roles for ionotropic and metabotropic glutamate receptors in the maturation of excitatory synapses. *J Neurosci* 20: 2229–2237, 2000.

Grunwald ME, Mellen JE, Strutz N, Maricq AV, and Kaplan JM. Clathrin-mediated endocytosis is required for compensatory regulation of GLR-1 glutamate receptors after activity blockade. *Proc Natl Acad Sci USA* 101: 3190–3195, 2004.

Ju W, Morishita W, Tsui J, Gaietta G, Deerinck TJ, Adams SR, Garner CC, Tsien RY, Ellisman MH, and Malenka RC. Activity-dependent regulation of dendritic synthesis and trafficking of AMPA receptors. *Nat Neurosci* 7: 244–253, 2004.

Liao D, Zhang X, O'Brien RJ, Ehlers MD, and Huganir RL. Regulation of morphological postsynaptic silent synapses in developing hippocampal neurons. *Nat Neurosci* 2: 37–43, 1999.

Lissin DV, Gomperts SN, Carroll RC, Christine CW, Kalman D, Kitamura M, Hardy S, Nicoll RA, Malenka RC, and von Zastrow M. Activity differentially regulates the surface expression of synaptic AMPA and NMDA glutamate receptors. *Proc Natl Acad Sci USA* 95: 7097–7102, 1998.

Liu SJ and Cull-Candy SG. Synaptic activity at calcium-permeable AMPA receptors induces a switch in receptor subtype. *Nature* 405: 454–458, 2000.

Liu SJ and Cull-Candy SG. Activity-dependent change in AMPA receptor properties in cerebellar stellate cells. *J Neurosci* 22: 3881–3889, 2002.

Maffei A, Nelson SB, and Turrigiano GG. Selective reconfiguration of layer 4 visual cortical circuitry by visual deprivation. *Nat Neurosci* 7: 1353–1359, 2004.

Markram H, Wang Y, and Tsodyks M. Differential signaling via the same axon of neocortical pyramidal neurons. *Proc Natl Acad Sci USA* 95: 5323–5328, 1998.

Mohrmann R, Lessmann V, and Gottmann K. Developmental maturation of synaptic vesicle cycling as a distinctive feature of central glutamatergic synapses. *Neuroscience* 117: 7–18, 2003.

Murthy VN, Schikorski T, Stevens CF, and Zhu Y. Inactivity produces increases in neurotransmitter release and synaptic size. *Neuron* 32: 673–682, 2001.

O'Brien RJ, Kamboj S, Ehlers MD, Rosen KR, Fischbach GD, and Huganir RL. Activity-dependent modulation of synaptic AMPA receptor accumulation. *Neuron* 21: 1067–1078, 1998.

Paradis S, Sweeney ST, and Davis GW. Homeostatic control of presynaptic release is triggered by postsynaptic membrane depolarization. *Neuron* 30: 737–749, 2001.

Rutherford LC, DeWan A, Lauer HM, and Turrigiano GG. Brain-derived neurotrophic factor mediates the activity-dependent regulation of inhibition in neocortical cultures. *J Neurosci* 17: 4527–4535, 1997.

Stellwagen D and Malenka RC. Synaptic scaling mediated by glial TNF-α. *Nature* 440: 1054–1059, 2006.

Thiagarajan TC, Lindskog M, and Tsien RW. Adaptation to synaptic inactivity in hippocampal neurons. *Neuron* 47: 725–737, 2005.

Thiagarajan TC, Piedras-Renteria ES, and Tsien RW. α- and βCaMKII: inverse regulation by neuronal activity and opposing effects on synaptic strength. *Neuron* 36: 1103–1114, 2002.

Turrigiano GG. Homeostatic plasticity in neuronal networks: the more things change, the more they stay the same. *Trends Neurosci* 22: 221–227, 1999.

Turrigiano GG, Leslie KR, Desai NS, Rutherford LC, and Nelson SB. Activity-dependent scaling of quantal amplitude in neocortical neurons. *Nature* 391: 892–896, 1998.

Turrigiano GG and Nelson SB. Homeostatic synaptic plasticity in the developing nervous system. *Nat Rev Neurosci* 5: 97–107, 2004.

Virmani T, Atasoy D, and Kavalali ET. Synaptic vesicle recycling adapts to chronic changes in activity. *J Neurosci* 26: 2197–2206, 2006.

Watt AJ, van Rossum MCW, MacLeod KM, Nelson SB, and Turrigiano GG. Activity coregulates quantal AMPA and NMDA currents at neocortical synapses. *Neuron* 26: 659–670, 2000.

Wierenga CJ, Ibata K, and Turrigiano GG. Postsynaptic expression of homeostatic plasticity at neocortical synapses. *J Neurosci* 25: 2895–2905, 2005.

Wilson NR, Kang J, Hueske EV, Leung V, Varoqui H, Murnick JG, Erickson JD, and Liu G. Presynaptic regulation of quantal size by the vesicular glutamate transporter VGLUT1. *J Neurosci* 25: 6221–6234, 2005.

Zhang W and Benson DL. Stages of synapse development defined by dependence on F-actin. *J Neurosci* 21: 5169–5181, 2001.