Dark Rearing Alters the Development of GABAergic Transmission in Visual Cortex

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We studied the role of sensory experience in the maturation of GABAergic circuits in the rat visual cortex. Between the time at which the eyes first open and the end of the critical period for experience-dependent plasticity, the total GABAergic input converging into layer II/III pyramidal cells increases threefold. We propose that this increase reflects changes in the number of quanta released by presynaptic axons. Here, we show that the developmental increase in GABAergic input is prevented in animals deprived of light since birth but not in animals deprived of light after a period of normal experience. Thus, sensory experience appears to play a permissive role in the maturation of intracortical GABAergic circuits.

Key words: synaptic inhibition; critical period; IPSC; EPSC; plasticity; sensory experience

Sensory experience during the postnatal critical period is essential for the normal maturation of visual cortical circuits and function (Hubel and Wiesel, 1962). Although many studies have been devoted to the modification of excitatory circuits (Beaver et al., 1999; Rittenhouse et al., 1999; Di Cristo et al., 2001), there are indications that GABAergic circuits also change (Winfield, 1983). Indeed, one hypothesis is that the maturation of inhibitory circuits plays an important role in timing the critical period for the modifications of excitatory connections (Komatsu, 1983; Kirkwood and Bear, 1994; Hensch et al., 1998; Huang et al., 1999).

Evidence for the slow cortical maturation of inhibitory mechanisms derives primarily from anatomical studies. In rodents, the numbers of inhibitory synapses (Blue and Parnavelas, 1983; Miller, 1986) and levels of GABA-synthesizing enzymes increase postnatally until week 5 of age (Huang et al., 1999), which is close to puberty for these animals. The role of visual experience in this process has remained elusive. Although recordings *in vivo* suggest a weakened synaptic inhibition in animals deprived of light since birth (Benevento et al., 1992), the anatomical data are inconclusive. Perhaps this is because different approaches have been used to target different aspects of inhibitory function. For example, visual deprivation from birth reportedly decreases GABA immunoreactivity (Benevento et al., 1996; Gordon et al., 1997) and GABAergic synapses (Gabbott and Stewart, 1987) but does not affect the levels of GABA-synthesizing enzymes (Mower and Guo, 2001).

Direct intracellular analyses of the maturation of intracortical inhibition have focused primarily on aspects of the $GABA_A$ response that change before the eyes open, such as the shift in reversal potential (Agmon et al., 1996; Owens et al., 1999) and the increase in the response kinetics associated with changes in $GABA_A$ receptor subunit composition (Dunning et al., 1999). Thus, although inhibitory responses can be evoked in most cortical cells by postnatal week 3 (Luhmann and Prince, 1991; Komatsu and Iwakiri, 1993), very little is known about the changes that might take place beyond that age. The aim of this study was to understand the changes in the maturation of inhibitory circuits that might mediate the timing of the critical period. We report that in the time between when the eyes open (2 weeks) and the end of the critical period (5 weeks), the total GABAergic input converging into pyramidal cells undergoes a threefold increase. Furthermore, the enhancement of inhibition requires visual experience and involves an increase in the number of release sites per individual input.

MATERIALS AND METHODS

Coronal slices (300 μm) of visual cortex from 2- to 8-week-old rats were prepared as described previously (Kirkwood and Bear, 1994). Briefly, after sectioning in ice-cold oxygenated (95% O_2/5% CO_2) dissection buffer (in mM: 212.7 sucrose, 5 KCl, 1.25 NaH_2PO_4, 3 $MgCl_2$, 1 $CaCl_2$, 26 $NaHCO_3$, 10 dextrose, and 10 kynurenate), slices were transferred to a storage chamber containing recording buffer for at least 1 hr before recording. In the recording buffer, sucrose is replaced by 124 mM NaCl, $MgCl_2$ is lowered to 1 mM, $CaCl_2$ is raised to 2 mM, and kynurenate is omitted.

Whole-cell voltage clamp in visually identified layer II/III pyramidal cells was performed using an Axopatch 1D (Axon Instruments, Foster City, CA) and a Warner Instruments (Hamden, CT) PC-505A amplifier. Patch pipettes (2–4 MΩ) were filled with (in mM): 130 Cs-gluconate, 8 KCl, 10 EGTA, 10 HEPES, and 10 lidocaine *N*-ethyl bromide at a pH of 7.4 and 275–285 mOsm. In some experiments (see Figs. 5 and 6), 130 mM Cs-gluconate was replaced by 140 mM CsCl. The junction potential (typically <5 mV) was compensated. Only cells with membrane potentials more negative than −65 mV, access resistance <20 MΩ (8–18 MΩ, compensated at 80%), and input resistance >100 MΩ (130–410 MΩ) were studied. The cells were discarded if the input or the access resistance changed >15%. All recordings were done at 28–30°C.

Synaptic responses were evoked with 0.2 msec current pulses delivered with a bipolar stimulating electrode (200 μm diameter; FHC, Bowdoinham, ME) placed in the middle of the cortical thickness (approximately the boundary of layers V and IV). Interstimulus intervals were >10 sec to minimize depression resulting from high-frequency stimulation. Compound IPSCs were recorded at 0 mV, and EPSCs were recorded at −60 mV, the reversal potential of the IPSC (see Fig. 4). This value (−60 mV) is more positive than the predicted Nernst potential of Cl^- because of the non-negligible permeability to gluconate of the $GABA_A$ channels (Barker and Harrison, 1988). In some experiments (described in Figures

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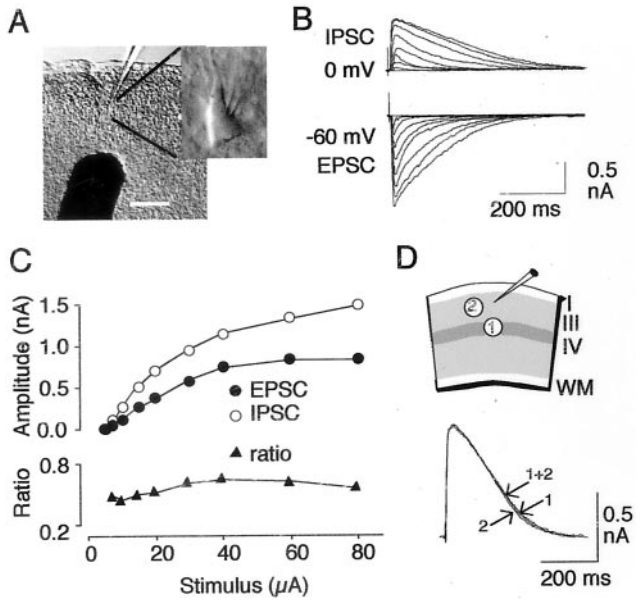

Figure 1. Determination of the maximal IPSCs in layer II/III cells. *A*, Stimulus recording configuration. Scale bar, 200 μm. *B, C*, Examples of IPSCs and EPSCs evoked by a stimulus series of increasing intensity. Both the IPSCs and the IPSC/EPSC reached saturation at a stimulus intensity of 40 μA. *D*, Saturation of the IPSCs does not depend on the stimulation site. *Top*, Stimulus recording configuration. *Bottom*, Maximal IPSCs evoked by the indicated stimulating electrode. *WM*, White matter.

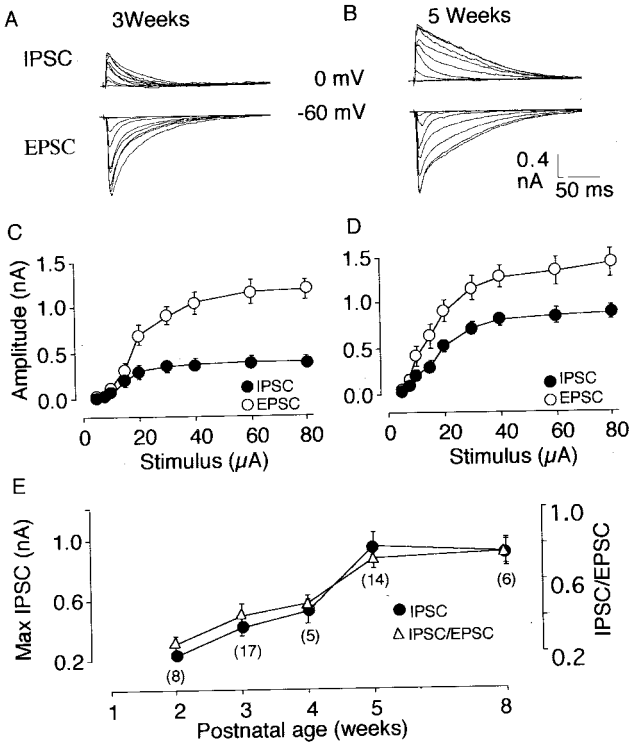

Figure 2. Developmental changes in the balance of excitation and inhibition. *A, B*, Examples of responses evoked by a stimulus series of increasing intensity in a cell from a 3-week-old rat (*A*) and a 5-week-old rat (*B*). The input–output relationship for the IPSC (*solid symbols*) and EPSC (*open symbols*) is shown in *C* for 3-week-old rats and in *D* for 5-week-old rats. *E*, Developmental changes in the maximal IPSC (*solid symbols*) and in the IPSC/EPSC ratio (*open symbols*). The number of cells included in each *data point* is given in *parentheses*.

1 through 4), the stimulus intensity was varied systematically (5, 7.5, 15, 20, 40, 60, and 80 μA). At least four to six responses at each intensity were averaged to compute the EPSC and IPSC. Responses were digitized at 10 kHz and analyzed using IGOR (WaveMetrics Inc., Lake Oswego, OR). Spontaneous miniature IPSCs (mIPSCs) were recorded in the presence of (in μM): 1 tetrodotoxin (TTX), 100 2-amino-5-phosphonovaleric acid (APV), and 20 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) and analyzed with the Mini Analysis program (Synaptosoft, Decatur, GA). We considered all recorded events in a single experiment for the determination of rates (between 800 and 3000) but excluded "bursts" with highly superimposed events in the determination of amplitudes. The decay constant, conversely, was calculated using the average of the first 100 isolated events. In all cases, the data were fitted with a single exponential. Minimal stimulation experiments were done using the same "regular" electrode placed in layer IV, which gave results similar to those using smaller glass pipettes closer to the target cell. Two major criteria for acceptance were a single latency of the response and a sharp threshold (Gil et al., 1999). Seventy to 300 responses were recorded in these experiments. Statistical significance was assessed using *t* tests or two-way repeated-measures ANOVAs followed by the Student–Newman–Keuls *post hoc* test. CNQX, APV, and bicuculline methiodide (BMI) were purchased from Sigma/RBI (St. Louis, MO).

RESULTS

We investigated how age and experience regulate the strength of synaptic inhibition in layer II/III of the rat visual cortex using the magnitude of the maximal IPSC to quantify the total inhibitory input converging onto a given cell (Ling and Benardo, 1998, 1999). As shown in Figure 1, the amplitude of IPSCs evoked with layer IV stimulation saturates as the stimulus intensity is increased. The IPSC/EPSC ratio also saturates, and its maximal value is used to compare the balance of inhibition and excitation between different cells. To confirm that stimulation of layer IV recruited nearly all of the fibers capable of evoking measurable responses in layer II/III cells, we placed an additional stimulating electrode ~100 μm lateral to the recorded cells. Stimulation of either pathway or both of them together evoked comparable maximal IPSCs ($n = 3$), indicating that the maximal IPSC is an adequate measure of the total inhibitory input converging in layer II/III cells.

Developmental changes in magnitude of the maximal IPSC

It has been proposed that changes in the strength of synaptic inhibition set the timing of the critical period for experience-dependent plasticity (Komatsu, 1983; Kirkwood and Bear, 1994; Hensch et al., 1998; Huang et al., 1999), which in rodents peaks at 3 weeks and ends by week 5 (Maffei et al., 1992; Fagiolini et al., 1994). Therefore, we decided to investigate how the magnitude of the maximal IPSC changes at 3 and 5 weeks of age. As shown in Figure 2, the magnitude of the maximal IPSC nearly doubled in this period [from 420 ± 55 pA ($n = 17$) at 3 weeks to 955 ± 58 pA ($n = 14$) at 5 weeks] and plateaued thereafter. The magnitude of the EPSC at the saturating intensity barely changed during this period. As a result, the balance of inhibition/excitation was dramatically altered during the first postnatal weeks (Fig. 2*C*). These developmental changes in the potency of synaptic inhibition mirror the decline of plasticity observed in the rat visual cortex.

Effects of sensory experience on the potency of inhibitory inputs

Many aspects of the functional maturation of the visual cortex can be delayed by sensory deprivation (Fagiolini et al., 1994). To examine the role of visual experience in the development of synaptic inhibition, we studied the maximal IPSC in 5-week-old

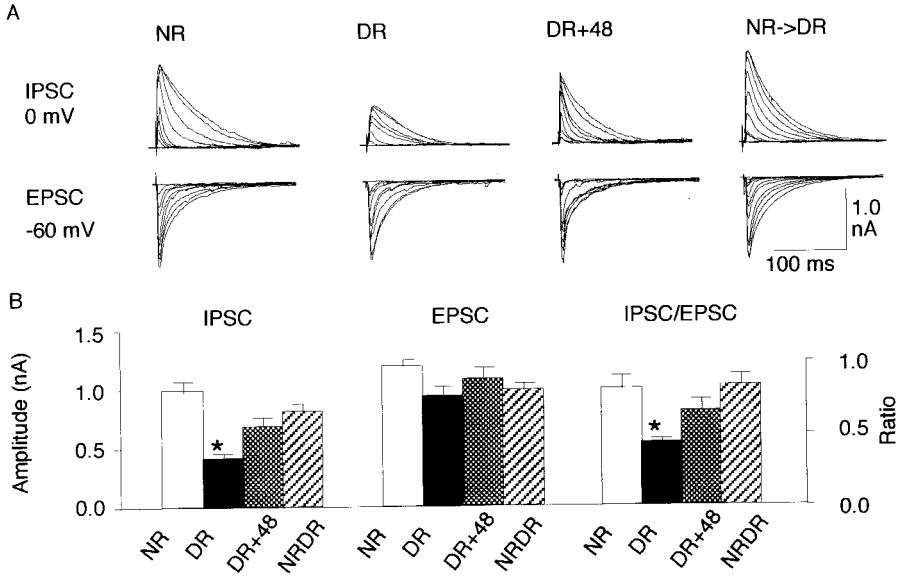

Figure 3. Visual experience triggers the developmental increase in the maximal IPSC. *A*, *Traces* are examples of responses evoked by stimulus series of increasing intensity in cells from rats reared in the indicated conditions. *B*, Average magnitude of the maximal IPSC, EPSC, and IPSC/EPSC ratio from rats reared in the indicated conditions. *Asterisks* denote values significantly different ($p < 0.005$) from controls. *NR*, Normally reared; *DR*, dark reared; *DR+48*, dark reared plus 2 d normally reared; *NR→DR/NRDR*, normally reared–dark reared.

rats reared under different conditions. One group of animals was reared in normal light/dark cycles (5 week normally reared rats: 7 rats, 15 cells), and another group was reared in complete darkness (5 week dark-reared rats: 6 rats, 16 cells). In addition, some dark-reared animals were exposed to light for 2 d before the experiments (5 week dark-reared + 48 rats: 5 rats, 10 cells). A third group was reared normally for 3 weeks and subsequently placed in the dark for the remaining 2 weeks [normally reared–dark reared (5 week normally reared → dark reared): 8 rats, 17 cells]).

The results, summarized in Figure 3, indicate that sensory experience affects the development of synaptic inhibition profoundly. The maximal IPSC was reduced by nearly half in dark-reared cells (462 ± 43 pA) compared with normally reared cells (939 ± 90 pA). The reduction in the IPSC magnitude was substantially reversed after 2 d of exposure to light (5 week normally reared → dark reared, 778.6 ± 55.6 pA). In contrast, 2 weeks of sensory deprivation after normal rearing (5 week normally reared → dark reared) did not affect the maximal IPSC (804 ± 75 pA). The results from a two-way ANOVA indicated that these differences were significant ($F_{(3,71)} = 10.05$; $p < 0.001$), and a Student–Newman–Keuls *post hoc* test confirmed that the IPSC was significantly smaller in dark-reared cells ($p < 0.001$). The EPSC, on the other hand, was affected less by the rearing conditions. Although the maximal EPSC was somewhat smaller in dark-reared cells (980 ± 71 pA) compared with the other groups (normally reared, 1197 ± 76 pA; 5 week dark reared + 48, 1137 ± 81 pA; 5 week normally reared → dark reared, 1009 ± 69 pA), the differences did not reach statistical significance ($F_{(3,71)} = 1.87$; $p = 0.1430$). Finally, as expected, the IPSC/EPSC ratio was significantly reduced ($F_{(3,71)} = 6.143$; $p = 0.009$) in dark-reared cells (0.471 ± 0.027) compared with the other groups (normally reared, 0.789 ± 0.071; 5 week dark reared + 48, 0.729 ± 0.058; 5 week normally reared → dark reared, 0.818 ± 0.091). To confirm the effects of sensory experience, we compared IPSCs and EPSCs from cells recorded from dark-reared and normally reared animals in a blinded study. The maximal IPSC was significantly ($p = 0.04$) smaller in 5 week dark-reared (643 ± 163 pA; $n = 8$) than in 5 week normally reared (1280 ± 152 pA; $n = 8$) animals. The IPSC/EPSC ratio was also smaller (0.52 ± 0.11 in 5 week dark reared; 0.95 ± 0.10 in 5 week normally reared; $p < 0.001$). Together, the results support the idea that sensory experience is necessary to trigger the maturation of GABAergic circuits in the cortex.

To test the possibility that the age- and experience-related changes observed in the IPSCs were simply a result of rearrangements of polysynaptic connections, we studied monosynaptic IPSCs isolated by applying 100 μM APV and 20 μM CNQX in a bath (Fig. 4). Under these conditions, the maximal IPSCs were still significantly larger ($F_{(2,21)} = 10.14$; $p = 0.0008$) in 5 week normally reared cells (730 ± 60 pA; four rats, seven cells) than in 3 week normally reared cells (321 ± 13; five rats, seven cells) or 5 week dark-reared cells (432 ± 32 pA; five rats, 10 cells). Similarly, the peak conductance underlying the maximal IPSC, estimated from a linear fit of the *I–V* plots shown in Figure 4*C*, was also larger in 5 week normally reared cells (11.09 ± 0.253 nS; $n = 5$ and 9) than in 3 week normally reared cells (5.89 ± 0.147 nS; $n = 3$ and 5) or 5 week dark-reared cells (7.73 ± 0.217 nS; $n = 3$ and 6). In contrast, age or experience did not significantly affect the passive properties of the cells, such as the input resistance [347 ± 27 MΩ ($n = 21$) in 3 week normally reared cells, 348 ± 23 MΩ ($n = 21$) in 5 week normally reared cells, and 361 ± 19 MΩ ($n = 20$) in 5 week dark-reared cells] or capacitance (80 ± 7, 79 ±0.7, and 75 ± 7 pF in 3 week normally reared, 5 week normally reared, and 5 week dark-reared cells, respectively). These results confirm that age and experience directly affect the magnitude of the evoked IPSCs.

Effects of age and sensory deprivation on unitary IPSCs

Changes in the maximal IPSC may result from several factors, including the number of inhibitory inputs, the number of release sites at each input, the magnitude of the unitary response, and the probability of release. To explore the latter possibility, we studied paired-pulse depression (PPD) evoked with a 20 msec interval. The magnitude of PPD, computed as the ratio of the second response to the first, is commonly used to assess changes in the probability of release. We found that this ratio increases with age, from 0.49 ± 0.04 at 3 weeks ($n = 12$) to 0.87 ± 0.07 at 5 weeks ($n = 20$). Furthermore, dark rearing (0.57 ± 0.03) prevents this increase. These differences in PPD were significant ($F_{(2,44)} = 13.288$; $p < 0.0001$) and are consistent with the hypothesis that

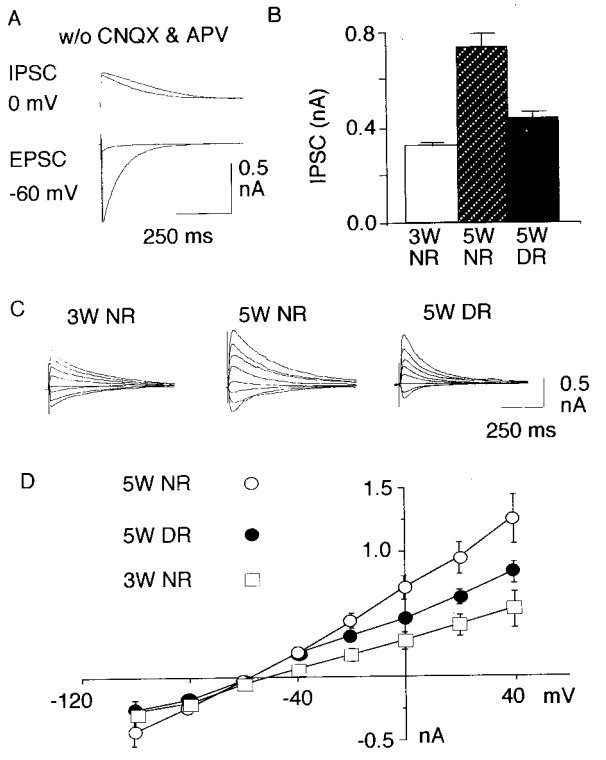

Figure 4. Effects of age and experience on the maximal monosynaptic IPSC. *A*, A 20 μM concentration of CNQX and a 100 μM concentration of APV abolish the responses recorded at −60 mV but barely affect the responses recorded at 0 mV. *B*, Average amplitude of monosynaptic maximal IPSCs recorded in 3-week-old (*3W*) cells, 5-week-old (*5W*) cells, and dark-reared (*DR*) cells. *C, D*, *I–V* relationship for the maximal IPSCs. *C*, Examples of IPSC recorded at different membrane potentials in 3-week-old cells, 5-week-old cells, and dark-reared cells. *D*, *I–V* plot of the peak amplitude of the maximal IPSCs. *NR*, Normally reared.

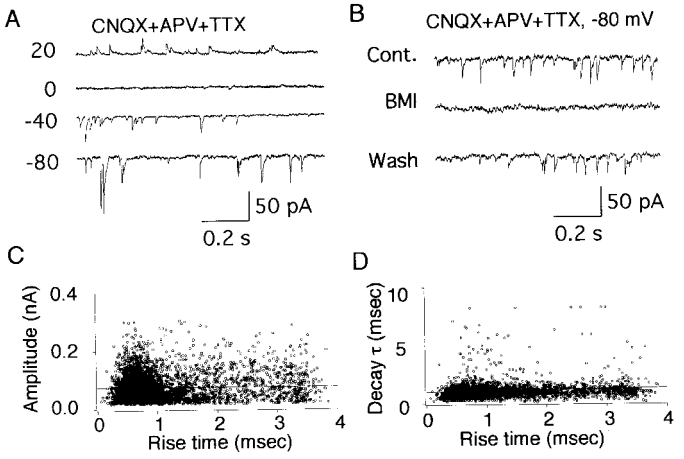

Figure 5. Miniature IPSCs recorded in 20 μM CNQX, 100 μM APV, and 1 μM TTX in layer II/III cells. *A*, Reversal potential of mIPSCs. The current traces were recorded at the indicated holding potential. *B*, A 1.0 μM concentration of BMI reversibly eliminates mIPSCs. *C, D*, Examples showing the relationship between the 10–90% rise time and the amplitude (*C*) and the decay (*D*) for all of the mIPSCs recorded in a 5-week-old cell. The *solid lines* indicate the best linear fit of the data.

there is a higher probability of release in cells from young and dark-reared rats. Hence, changes in the probability of release are unlikely to account for the regulation of the maximal IPSC.

To investigate a possible regulation of the magnitude of the unitary responses, we studied the release of spontaneous mIPSCs in cells from 3- and 5-week-old normal-reared rats and 5-week-old dark-reared rats (see Materials and Methods). The recordings were done at −80 mV because sustained postsynaptic depolarization might reduce the release of GABA from presynaptic terminals (Pitler and Alger, 1992; Wilson and Nicoll, 2001). To facilitate the detection of mIPSCs at this potential, the recording pipette contained the same [Cl^-] as the external buffer. Under those conditions, the mIPSCs reversed at 0 mV (Fig. 5*A*) ($n = 3$) and were reversibly abolished by 1 μM BMI (Fig. 5*B*) ($n = 3$). To minimize biases introduced by dendritic filtering of events originating far away from the soma, we adopted the standard criterion of analyzing only those cases in which the rise time did not show a negative correlation with the amplitude of the events (Fig. 5*C*).

Figure 6 summarizes the results obtained in cells prepared from 3-week-old rats ($n = 19$) and 5-week-old rats reared normally ($n = 15$) or in the dark ($n = 16$). The decay kinetics of the mIPSCs (Fig. 6*A*), which in all cases was fitted with a single exponential, was similar in the three groups ($F_{(2,47)} = 0.163$; $p = 0.85$). The average decay constant (see Materials and Methods) was 10.3 ± 0.7 msec in 3 week normally reared cells, 9.7 ± 0.8 msec in 5 week normally reared cells, and 10.2 ± 0.9 msec in 5 week dark-reared cells. This similarity is intriguing, considering the twofold developmental decrease in decay constant (from 43 to 17 msec) that has been reported in cells grown in culture (Dunning et al., 1999). The discrepancy might reflect a slower maturation in cultured cells. Under similar experimental conditions, the decay constant has decreased to 11 msec in slices from 21-d-old animals yet it is still 35 msec in 23-d-old cultured cells (Dunning et al., 1999).

The average mIPSC amplitude (Fig. 6*C*) was significantly different among the three groups ($F_{(2,47)} = 3.916$; $p = 0.023$). However, unlike the maximal compound IPSCs, the largest mIPSCs were recorded in 5 week dark-reared cells (53.3 ± 4.1 pA), and there was no effect of age on the amplitude of mIPSCs (38.7 ± 3.4 pA in 3 week normally reared cells; 40 ± 4.4 pA in 5 week normally reared cells). In contrast, the mIPSC frequency (Fig. 6*D*) showed an age-dependent increase (from 10.3 ± 0.7 Hz in 3 week normally reared cells to 17.0 ± 2.4 Hz in 5 week normally reared cells) that was reduced by dark rearing (10.2 ± 0.9 Hz in 5 week dark-reared cells). These differences were significant ($p = 0.004$). In summary, the results indicate that age barely affects the shape of the unitary IPSCs, whereas sensory deprivation increases their amplitude. Thus, the unitary IPSCs and the maximal IPSCs are affected in opposite directions by age and sensory experience. The changes in the frequency of mIPSCs, on the other hand, are consistent with changes in the number of synapses.

Effects of age and sensory deprivation on responses evoked with minimal stimulation

The results described above suggest that the developmental increase in the compound IPSCs is more likely to result from changes in the total number of GABAergic synapses. In turn, this could result from an increased number of GABAergic inputs and/or an increase in the average number of synaptic contacts made by each input. To explore the latter possibility, we quantified the responses of unitary GABAergic inputs using a minimal stimulation protocol (Gil, 1999). In these experiments, the recording conditions were similar to those described in Figure 5, except that TTX was not included in the bath. The stimulation

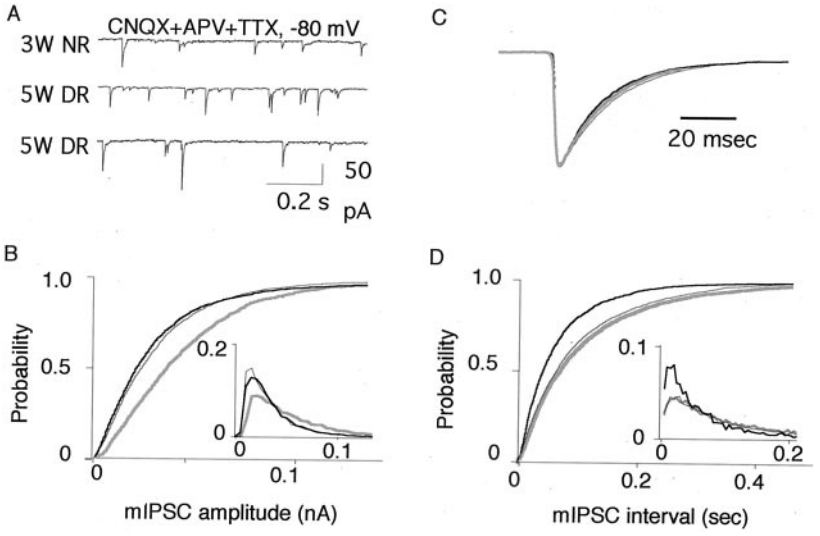

Figure 6. Effects of age and sensory deprivation on layer II/III mIPSCs. *A*, Examples of current traces recorded in a 3-week-old (*3W*) cell, a 5-week-old (*5W*) cell, and a dark-reared (*DR*) cell. *NR*, Normally reared. *B*, Superimposed normalized averages of the mIPSCs recorded in all 3-week-old cells (*thin line*), 5-week-old cells (*black thick line*), and dark-reared cells (*gray thick line*). For each cell, the average mIPSC was computed using the first 100 isolated events aligned by their rise time and normalized by their amplitude. Subsequently, these averages were further averaged across ages and rearing conditions. *C*, Cumulative probability distribution of the mIPSC amplitude for 3-week-old cells (*thin line*), 5-week-old cells (*black thick line*), and dark-reared cells (*gray thick line*). The first 300 events from each cell were used in this computation. *Inset*, Histograms of the amplitude distribution for all events recorded in all cells. The bin size was 5 pA. *D*, Cumulative probability distribution of the mIPSC interval. Conventions and calculations are as in *C*. *Inset*, Histograms of the interval distribution for all events recorded in all cells. The bin size was 10 msec.

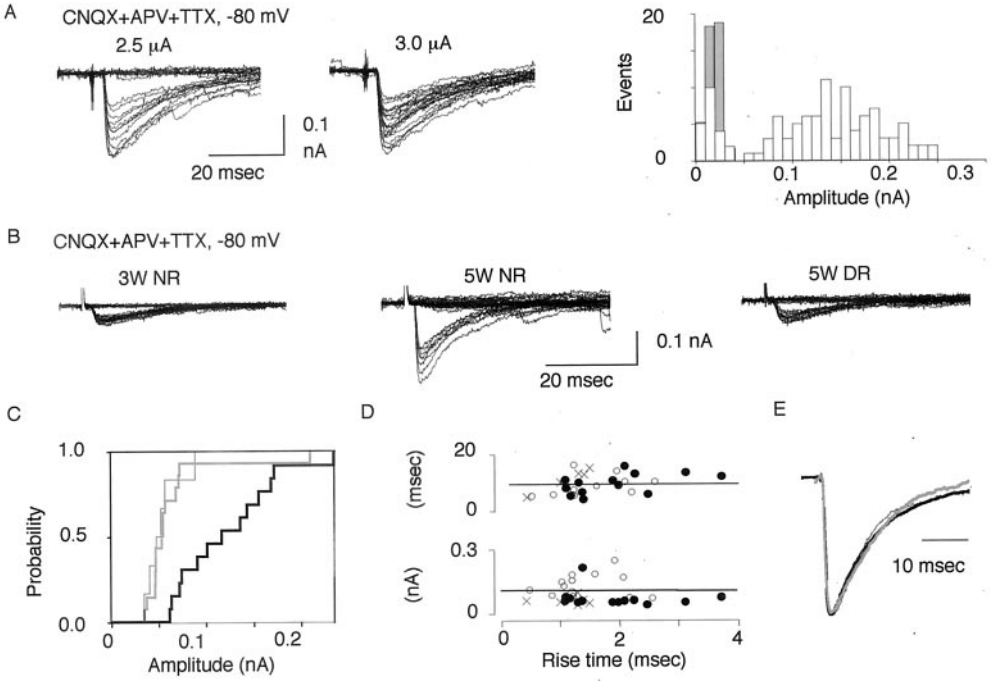

Figure 7. Effects of age and sensory deprivation on the responses evoked with minimal stimulation. All responses were recorded at −80 mV in the presence of 20 μM CNQX and 100 μM APV. *A*, Examples of responses evoked with minimal stimulation. The *superimposed traces* are 20 consecutive responses evoked with two stimulation intensities, 2.8 μA (*left*) and 3.0 μA (*right*). In each case, the *thick traces* correspond to the average of the successful responses. The amplitude histogram of the responses is displayed on the *right*. The noise level, calculated from the prestimulus baseline, is also plotted (in *gray*), but at a different scale (2×). *B*, Examples of responses evoked with minimal stimulation in a 3-week-old cell (*3W*, *left*), a 5-week-old cell (*5W*, *center*), and a 5-week-old dark-reared cell (*5W DR*, *right*). In each case, 20 consecutive responses are *superimposed*. *C*, Probability distribution of the average amplitude responses (excluding failures) evoked in 3-week-old cells (*thin line*), 5-week-old cells (*thick line*), and dark-reared cells (*thick gray line*). *D*, Relationship between the 10–90% rise time and the decay constant (*top graph*) and amplitude (*bottom graph*) for the responses evoked in 3-week-old cells (×), 5-week-old cells (○), and dark-reared cells (●). *Solid lines* correspond to the best linear fit of the data. *E*, Normalized averaged responses recorded in all 3-week-old cells (*thin line*), 5-week-old cells (*black thick line*), and dark-reared cells (*gray thick line*). Each of the *superimposed traces* represents the average of all different cell responses normalized by their amplitude and aligned by their rise time.

intensity was minimized until it elicited events in an all-or-none manner. All-or-none events with similar amplitude (Fig. 6*B*, *left*) are usually presumed to originate from the activation of single axons. However, the events were often variable in amplitude (Fig. 6*A*), consistent with multiple release sites in a single input (Tamas et al., 1997; Gupta et al., 2000). To confirm that a single input was activated, we varied the stimulation intensity in small increments. At each intensity, we measured the probability of evoking a response and the average amplitude of the elicited responses (excluding failures). If multiple recruiting occurs, then one would expect that increasing the stimulus intensity will recruit more axons, thus increasing not only the probability of observing a response but also the average response amplitude. Conversely, when only one axon is recruited, increasing stimulation intensity will increase the probability of firing but should not affect the size of the evoked responses. Therefore, we considered only those cases in which changing the stimulus intensity affected the probability of evoking a response but not the average amplitude of the evoked responses (Fig. 6*A*) (27% of the cells did not fulfill this criterion).

Figure 7 summarizes the results obtained with minimal stimulation in cells from 3-week-old ($n = 6$) and 5-week-old rats reared normally ($n = 14$) or reared in the dark ($n = 11$). The most dramatic effect of age and experience was on the amplitude of the evoked events (Fig. 7*B,C*), which was significantly larger ($F_{(2,27)} = 6.323$; $p = 0.0046$) in 5 week normally reared cells (122.0 ± 13.5 pA) than in 3 week normally reared cells (53.3 ± 7.7 pA) or in 5 week dark-reared cells (66.4 ± 14.8 pA). These differences in

amplitude are not likely to be a result of differences in dendritic filtering, because there was no correlation between the rise time and the response amplitude in any of these experimental groups (Fig. 7*D*). The decay phase of the response (Fig. 7*E*), which was fitted by a single exponential (like the mIPSCs), was somewhat larger in 3 week normally reared cells (11.17 ± 1.49 msec) than in 5 week normally reared cells (8.83 ± 1.09 msec) or 5 week dark-reared cells (8.83 ± 1.09 msec). However, these differences were not significant ($F_{(2,27)} = 1.182$; $p = 0.3221$). Finally, age and experience did not affect the rise time (10–90% of the peak) of the minimally evoked responses (1.58 ± 0.19 msec in 3 week normally reared cells, 1.46 ± 0.165 msec 5 week normally reared cells, and 1.87 ± 0.27 msec in 5 week dark-reared cells; $F_{(2,27)} = 2.22$; $p = 0.141$). Together, these results suggest that the potency of individual GABAergic inputs onto layer II/III pyramidal cells might experience a developmental increase that can be prevented or reduced by dark rearing.

DISCUSSION

We found that inhibitory circuits continue to mature after birth in the visual cortex, and that there is a dramatic postnatal increase in the total GABAergic input converging onto layer II/III pyramidal cells. The time course of this enhancement of synaptic inhibition mirrors the decline of the critical period for experience-dependent plasticity. Like the decline of plasticity, the increase in synaptic inhibition is postponed by visual deprivation. The effects of deprivation were rapidly reversed by visual experience, but deprivation after normal experience did not affect the potency of synaptic inhibition. Thus, visual experience appears to play a permissive role and is required to trigger the normal postnatal enhancement of GABAergic inhibition. We propose that these changes result from a developmental increase in the number of release sites per individual input.

We used the maximal IPSC to assess the total GABAergic inputs onto a given cell. Because the majority of GABAergic contacts occur on the soma or on the proximal dendrites (Beaulieu and Colonnier, 1985), it is unlikely that our measurements of the IPSCs were distorted because of problems of space clamping. The absence of dendritic filtering agrees with previous findings (Ling and Benardo, 1999) and also suggests that most of the GABA synapses are close to the soma. Thus, the maximal IPSC provides a useful estimate of the total potency of the GABAergic inputs converging onto a given cell.

The developmental increase of the maximal IPSC is unlikely to result from changes in the strength of individual synapses, which is regulated in the opposite direction by age and experience. Our results indicate that the release probability declines with age and increases with sensory deprivation, which also increases the amplitude of unitary responses (mIPSCs). Thus, sensory deprivation appears to upregulate the strength of GABAergic synapses, perhaps through compensatory mechanisms similar to those described for excitatory synapses (Turrigiano et al., 1998; Murthy et al., 2001).

We propose that the developmental changes in the maximal IPSC reflect an increase in the number of GABAergic synapses. The mIPSC frequency changed in parallel with the maximal IPSC and was reduced by sensory deprivation. Although multivesicular release (Ling and Benardo, 1999; Llano et al., 2000) may also contribute, it is worth pointing out that the differences in mIPSC frequency are usually attributed to differences in the number of synapses (Salin and Prince, 1996). This interpretation is also consistent with anatomical data indicating that there is a developmental increase in the total number of GABAergic synapses (Blue and Parnavelas, 1983; Miller, 1986). Recent evidence suggests that activity-dependent release of BDNF may be one of the signals that triggers the increase in the number of GABAergic synapses (our unpublished observations).

GABAergic cells make multiple contacts with target cells (Tamas et al., 1997; Gupta et al., 2000). Using minimal stimulation, we found that the potency of putative individual GABA inputs increases during development in an experience-dependent manner. Although the interpretation of these results is somewhat limited by methodological biases (electrical stimulation might activate severed axons and preferentially recruit lower-threshold, large-caliber axons), it is worth pointing out that the same biases were applied at all ages and rearing conditions. Therefore, it seems reasonable that at least in some inputs, the number of quanta released per axonal branch increases postnatally in an experience-dependent manner. The exact contribution of these changes to the developmental increase in the maximal IPSC remains to be determined. Another open issue is the identity of the GABAergic inputs that might change during development. Inhibitory interneurons are a highly diverse group in terms of connectivity, molecular markers, and functional properties (Kawaguchi and Kubota, 1997; Parra et al., 1998; Somogyi et al., 1998; Gibson et al., 1999; Gupta et al., 2000). The magnitude of the developmental increases (twofold to threefold) in the maximal IPSC suggests that these changes might affect a large proportion of the GABAergic cells.

The strength of inhibitory circuits is considered to be important for sharpening and tuning several aspects of the cortical response to visual stimulation (Douglas, 1991; Somers and Sur, 1995). In addition, a growing body of evidence indicates that the maturation of synaptic inhibition controls the timing of the critical period for visual cortical plasticity (Kirkwood and Bear, 1994; Hensch et al., 1998; Huang et al., 1999). Visual cortical plasticity is impaired by genetic manipulations that reduce (Hensch et al., 1998) or enhance (Huang et al., 1999) the efficacy of GABAergic inhibition. Interestingly, these manipulations also impair the plasticity of excitatory connections *in vitro* (Huang et al., 1999; Choi et al., 2002). The exact mechanisms by which GABAergic inhibition might control plasticity are unknown. According to one view (Hensch et al., 1998), a minimum inhibitory strength is necessary to trigger the critical period for plasticity, which in turn develops with its own time course, independent of any additional change in GABAergic circuits. Alternatively, we have proposed that the synaptic modifications might require a precise range of inhibitory strengths. Below or above that range, visual cortical plasticity may not occur. A slow and progressive maturation of GABAergic inhibition then determines the precise critical period for synaptic modifications before and after which visual cortical plasticity may not occur (Rozas et al., 2001). The developmental changes in the GABAergic circuitry that we describe here fit into that scenario, although one must bear in mind that the number of GABAergic synapses is not the only factor determining the strength of inhibitory circuits. In addition to a comparable developmental time course for the increase in the IPSC and the critical period (Fagiolini et al., 1994), we found that sensory experience plays a similar role in triggering both processes. First, it prevents or delays both processes (Cynader and Mitchel, 1980). In addition, it has long been known that sensory deprivation cannot affect the critical period when preceded by normal rearing (Mower et al., 1983; Mower and Christen, 1985). Similarly, we found that the number of GABAergic synapses does not change when the ani-

mals are sensory deprived after a period of normal rearing. In contrast, the effect of dark rearing on the maximal IPSCs was rapidly reversed by a brief exposure to light. This provides a way of testing the idea that the increase in GABAergic potency determines the end of the critical period. We predict that the critical period will terminate rapidly in dark-reared animals that are briefly exposed to light.

Together, our results show that GABAergic circuitry in the visual cortex is plastic and can be shaped by experience. Unlike the remodeling of excitatory circuitry that is primarily refinement caused by pruning of exuberant inputs, plasticity affecting inhibitory circuits appears to involve strengthening of pre-existing connections. Perhaps the two distinct modes of maturation reflect differences in functional demands of excitatory and inhibitory circuits in cortical processing. Maturation of excitatory circuitry allows increased specificity, and the mechanisms for inhibitory circuit maturation provide a powerful way to control the output of a network.

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