Neuron, Vol. 47, 101–114, July 7, 2005, Copyright ©2005 by Elsevier Inc. DOI 10.1016/j.neuron.2005.05.024

Synapse-Specific Downregulation of NMDA Receptors by Early Experience: A Critical Period for Plasticity of Sensory Input to Olfactory Cortex

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Summary

Olfaction is required at birth for survival; however, little is known about the maturation of olfactory cortical circuits. Here we show that in vivo sensory experience mediates the development of excitatory transmission in pyramidal neurons of rat olfactory cortex. We find a postnatal critical period during which there is an experience-dependent increase in the contribution of AMPARs versus NMDARs to transmission at primary sensory synapses but not associational inputs. The shift in receptors underlying transmission is mediated by a strong activity-dependent downregulation of NMDARs and modest increase in AMPARs. Sensory activity leads to a loss of "silent" NMDAR-only synapses and an increase in threshold for inducing long-term plasticity. These results indicate the importance of early olfactory experience in the establishment of cortical circuits and could reflect mechanisms governing early olfactory "imprinting."

Introduction

Early sensory experience plays a crucial role in the refinement of developing thalamocortical circuits (Buonomano and Merzenich, 1998; Katz and Shatz, 1996). In the visual system, animals are born before complete maturation of the visual cortex, and these cortical circuits can be altered by sensory experience (Blakemore and Cooper, 1970; Wiesel and Hubel, 1963). Indeed, functional maturation of visual thalamocortical circuits depends upon patterned activity, both before and shortly after birth (Katz and Shatz, 1996). Experience-dependent modifications at afferent cortical inputs typically only occur during a postnatal "critical period," after which synaptic reorganization is difficult to induce (Hensch, 2004). It is presumed that the critical period is a permissive temporal window during which activity-dependent synaptic rearrangements occur and after which mature connectivity is established such that no further refinement is typically required.

NMDA receptors (NMDARs) are generally believed to play a critical role in the development of central synapses. In particular, NMDAR-mediated long-term potentiation (LTP) has been proposed to underlie the changes in synaptic strength that occur during the maturation and refinement of cortical circuits (Katz and Shatz, 1996). A currently prevailing view is that activity-dependent LTP reflects the recruitment of AMPA receptors (AMPARs) to synapses that previously contained only NMDARs ("silent synapses") (Malenka and Bear, 2004; Malinow and Malenka, 2002). It has been proposed that NMDAR-only synapses dominate during early development and are converted to fast-signaling, functional synapses via experience-dependent NMDAR-mediated insertion of AMPARs (Durand et al., 1996; Wu et al., 1996; Zhu et al., 2000; Zhu and Malinow, 2002). The conversion of silent synapses to functional ones has similarly been proposed to occur during the maturation of synaptic contacts in a number of cortical areas. For example, in visual and somatosensory cortex the relative contribution of NMDAR-only synapses decreases during development, and this coincides with the closure of a critical period for the induction of LTP (Isaac et al., 1997; Rumpel et al., 1998).

While these developmental changes in glutamatergic transmission and critical periods for experience-dependent plasticity have been well characterized in the visual and somatosensory systems, factors shaping the maturation of other cortical sensory synapses are less clear. For example, olfaction is a critical sensory modality in most newborn animals; however, the consequences of early olfactory experience in the CNS have not been well explored.

Newborn rodents rely on olfactory cues to initiate nipple attachment and suckling (Leon, 1992). Indeed, mice lacking the cyclic nucleotide-gated channels essential for sensory transduction in olfactory receptor neurons display a high degree of postnatal lethality (Brunet et al., 1996). In addition, neonatal animals (including humans) use olfactory information to form strong maternal attachments, and this "imprinting" to maternal odors is critical for survival in many species (Leon, 1992; Sullivan, 2003). These results indicate that, in contrast to most other sensory modalities, the olfactory system is both functional and necessary at birth. However, little is known about the developmental maturation of synaptic transmission at afferent cortical synapses in the olfactory system. Furthermore, it is unknown whether central olfactory circuits display enhanced plasticity during early postnatal development.

Layer II/III pyramidal cells in primary olfactory (piriform) cortex receive two distinct classes of glutamatergic synapses: one class conveys primary sensory input, while a different set of associational synapses mediate intra- and intercortical signaling. Olfactory information is conveyed ipsilaterally, from olfactory receptor neurons to glomeruli in the olfactory bulb, and then to primary olfactory cortex via the axons of olfactory bulb mitral cells in the lateral olfactory tract (LOT). This afferent sensory input from the LOT occurs at synaptic contacts on the distal dendritic tufts of pyramidal cells (Haberly, 1998). Associational (ASSN) fibers from a variety of cortical regions form synaptic contacts on the proximal dendrites of the same pyramidal cells.

Within olfactory cortex, integration of sensory LOT inputs in pyramidal neurons is a key step underlying the discrimination and perception of olfactory information in the brain. Furthermore, genetic tracing experiments indicate that LOT inputs and their postsynaptic pyrami-

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dal cells represent a stereotyped sensory map in olfactory cortex (Zou et al., 2001). Given the possibility that LOT inputs are "hardwired" for the coding of olfactory information, it does not seem obvious that these sensory synapses should represent an important site for long-lasting activity-dependent plasticity. Consistent with this idea, previous studies of LOT synapses in slices of adult rat olfactory cortex reported that NMDAR-mediated LTP is not reliably induced (Jung et al., 1990) or results in only a relatively modest increase in synaptic strength (Jung et al., 1990; Kanter and Haberly, 1990, 1993). In contrast, ASSN excitatory synapses are poised to regulate the salience of olfactory information in pyramidal cells, and NMDAR-mediated LTP of these inputs is thought to provide a basis for olfactory memory and learning throughout adulthood (Kanter and Haberly, 1990; Quinlan et al., 2004).

In this study, we explore the role of early sensory experience in shaping excitatory synaptic transmission in rat primary olfactory cortex. We find that during the first several weeks of life there is an increase in the relative contribution of AMPARs versus NMDARs at LOT synapses but not ASSN inputs of pyramidal neurons. The shift in the contribution of AMPA and NMDARs at LOT synapses can be greatly delayed by reducing early olfactory experience in vivo. The activity-dependent change in glutamatergic signaling at sensory olfactory inputs can be largely accounted for by a marked downregulation of synaptic NMDARs. We find that the experience-dependent downregulation of NMDARs coincides with a selective loss of NMDAR-only silent synapses at LOT but not ASSN inputs to pyramidal cells. Furthermore, the role of olfactory experience in regulating LOT synaptic NMDARs is confined to a critical period that does not extend past the first few postnatal weeks. During this critical period, we show that the experience-dependent reduction in NMDARs raises the threshold for induction of LTP at LOT synapses. These results indicate the importance of early sensory experience in the maturation and plasticity of olfactory cortical circuits that play a crucial role in newborn mammals.

Results

Pyramidal cells in the olfactory cortex receive anatomically segregated inputs on their apical dendrites. ASSN inputs are formed on proximal dendrites in layer Ib, while afferent inputs from mitral cell axons in the LOT make synapses on distal dendrites in layer Ia (Figure 1A). The different laminae can be clearly resolved under DIC optics (Figure 1B).

We first performed experiments to verify that we could selectively evoke excitatory postsynaptic responses from LOT and ASSN synapses. We recorded field excitatory postsynaptic potentials (fEPSPs) in slices of anterior piriform cortex by placing an extracellular recording electrode in layer Ia. Putative afferent and associational synapses were alternately evoked via stimulating electrodes (~0.5–1 mm apart) in the LOT and layer II/III, respectively. Using this recording configuration, stimulation of layer II/III produced an upward-deflecting fEPSP, while stimulation of the LOT produced a downward-deflecting fEPSP (Figure 1C_1), indicating activation of distinct sets of synapses in the different laminae of olfactory cortex (Jung et al., 1990; Kanter and Haberly, 1993; Patil et al., 1998; Tang and Hasselmo, 1994).

It has previously been reported that activation of presynaptic $GABA_B$ receptors strongly depresses ASSN transmission with no effect on LOT-evoked responses (Tang and Hasselmo, 1994). Although $GABA_B$-mediated presynaptic inhibition is widespread in the CNS, this finding suggests that $GABA_B$ receptors are not expressed on LOT nerve terminals. We next tested the action of the $GABA_B$ receptor agonist baclofen on responses evoked alternately from the two pathways in olfactory cortex slices. Indeed, we found that baclofen (50 μM) caused a marked reduction in fEPSPs evoked by stimulation in layer II/III, while LOT-evoked responses were unaffected (Figure 1C_1). Washout of baclofen in the presence of the $GABA_B$ antagonist CGP 55845 (10 μM) restored transmission at ASSN synapses without affecting LOT inputs, and subsequent application of the AMPAR antagonist NBQX (10 μM) abolished both inputs. The selective $GABA_B$-mediated inhibition of the ASSN fEPSP provides strong evidence that we can selectively activate these two distinct pathways.

We further explored the ability to independently activate LOT and ASSN inputs using whole-cell recordings of layer II pyramidal cells with the same placement of stimulating electrodes. At –70 mV, stimulation of each pathway evoked fast EPSCs. As found for fEPSPs, baclofen (50 μM) markedly reduced the amplitude of EPSCs evoked by stimulation in layer II/III but did not affect LOT-evoked responses (Figure 1C_2). This selective block was observed in fEPSP recordings from postnatal day (P) 16–22 animals (n = 9; Figure 1D_1) as well as whole-cell voltage-clamp recordings performed in P8–P10 (n = 8) or P15–P19 (n = 8) animals (Figure 1D_2). In all cases, the strong reduction in ASSN EPSC amplitude was accompanied by a marked increase in the paired-pulse ratio (PPR, $EPSC_2/EPSC_1$, 50 ms ISI), indicating a presynaptic locus for $GABA_B$ receptor modulation. Consistent with a lack of presynaptic $GABA_B$ receptors at LOT synapses, baclofen had no effect on the PPR of LOT inputs (Figure 1E).

Due to their different electrotonic locations on layer II pyramidal cells (Haberly, 1998), somatic recordings of ASSN and LOT EPSCs could also be distinguished by their kinetics (Figure 1F). On average, both the 10%–90% rise time and decay of LOT inputs were significantly slower than ASSN inputs (rise: ASSN, 1.0 ± 0.08 ms; LOT, 2.2 ± 0.17 ms, n = 12, $p < 0.001$ [Student's t test]; decay: ASSN, 4.3 ± 0.34 ms; LOT, 6.6 ± 0.46 ms, n = 12, $p < 0.001$). These clear differences in EPSC kinetics, as well as the distinct expression of presynaptic $GABA_B$ receptors and anatomical separation of ASSN and LOT synapses, allowed us to unambiguously study these two inputs onto pyramidal cells independently.

We next examined the glutamate receptors mediating transmission at LOT and ASSN synapses. We determined the relative contribution of AMPA and NMDARs at these synapses by alternately stimulating the LOT and ASSN pathways at +40 mV. At this depolarized potential, both inputs displayed a slowly decaying EPSC (Figure 2A). Application of the NMDAR antagonist D-APV (100 μM) abolished the slow component of the

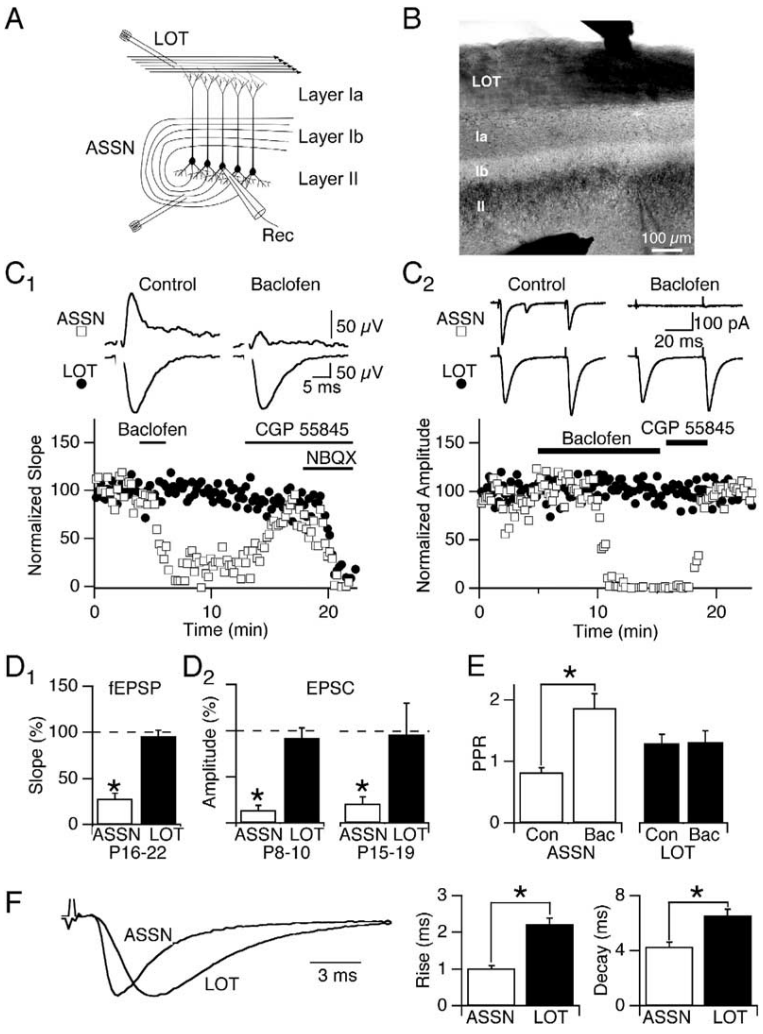

Figure 1. Selective Activation of LOT and ASSN Synapses

(A) Schematic of the olfactory cortex.

(B) The different layers of anterior piriform cortex viewed under DIC optics. Stimulating electrodes are in the LOT and layer II/III. A whole-cell electrode is in layer II.

(C) Baclofen reduces excitatory transmission at ASSN but not LOT inputs. (C_1) Representative experiment showing fEPSPs recorded in layer Ia evoked by stimulation of LOT and ASSN inputs. Baclofen (50 μM) decreases transmission at ASSN inputs (open squares) without affecting LOT inputs (filled circles). (C_2) Plot of the peak amplitudes of EPSCs at −70 mV evoked by stimulation of LOT (filled circles) and ASSN inputs (open squares) in the same pyramidal cell. Traces represent responses from each pathway to paired-pulse stimulation (50 ms ISI).

(D) Summary of the effects on (D_1) fEPSPs and (D_2) EPSCs from P8–P10 and P15–P19 animals.

(E) Baclofen increases PPR in ASSN but not LOT inputs.

(F) Normalized EPSCs evoked at LOT and ASSN inputs are shown from a representative cell. Both the 10%–90% rise-time and the decay of ASSN EPSCs were significantly faster than EPSCs evoked in the LOT.

Error bars indicate ± SEM.

EPSC, and the remaining fast component was blocked by NBQX (10 μM, Figure 2A). In separate experiments, the AMPAR components of ASSN and LOT EPSCs had linear I/V relationships and reversed at ~0 mV (n = 6, data not shown). We calculated the AMPAR/NMDAR ratio at LOT and ASSN synapses by measuring the peak amplitudes of the NBQX- and APV-sensitive components of the EPSCs. We observed a marked difference in the relative contribution of these two receptor types: while the peak amplitudes of the AMPA and NMDAR components at LOT synapses were similar, NMDARs dominated synaptic transmission at ASSN synapses (AMPAR/NMDAR ratio: LOT, 1.0 ± 0.16; ASSN, 0.31 ± 0.033; n = 24, $p < 0.0001$ [paired Student's t test]; age, P15–P19; Figure 2B).

We were intrigued by the large difference in the contribution of AMPA and NMDARs at two distinct inputs onto the dendrites of the same cell. The relative contribution of AMPARs increases during development in both visual (Rumpel et al., 1998) and somatosensory cortex (Crair and Malenka, 1995; Isaac et al., 1997; Mierau et al., 2004). Does the dramatic difference in the AMPAR/NMDAR ratio at LOT and ASSN synapses reflect different rates of maturation of the two inputs in olfactory cortex? To address this question, we compared the relative contribution of AMPARs and NMDARs at LOT and ASSN synapses of P8–P9 and P22 rats. Consistent with other afferent cortical synapses, the AMPAR/NMDAR ratio increased dramatically with age at LOT synapses (P8–P9: 0.64 ± 0.074, n = 8; P22: 1.2 ± 0.14, n = 9; $p < 0.005$ [unpaired t test]; Figures 2C and 2D). However, we found no change in the AMPAR/NMDAR ratio at ASSN synapses during the same developmental period (P8–P9: 0.39 ± 0.072, n = 7; P22: 0.36 ± 0.085, n = 8; p = 0.80; Figures 2C and 2D). Changes in the AMPAR/NMDAR ratio were not accompanied by a marked change in the decay kinetics of NMDAR currents (Figure 2E). These results indicate that there is a developmental increase in the relative contribution of AMPARs to excitatory transmission at LOT synapses. In contrast, NMDARs continue to dominate transmission at ASSN synapses over the same period.

The developmental increase in the AMPAR/NMDAR ratio could reflect an upregulation of synaptic AMPARs or a downregulation of NMDARs. We first considered whether the increase in AMPAR/NMDAR ratio could be

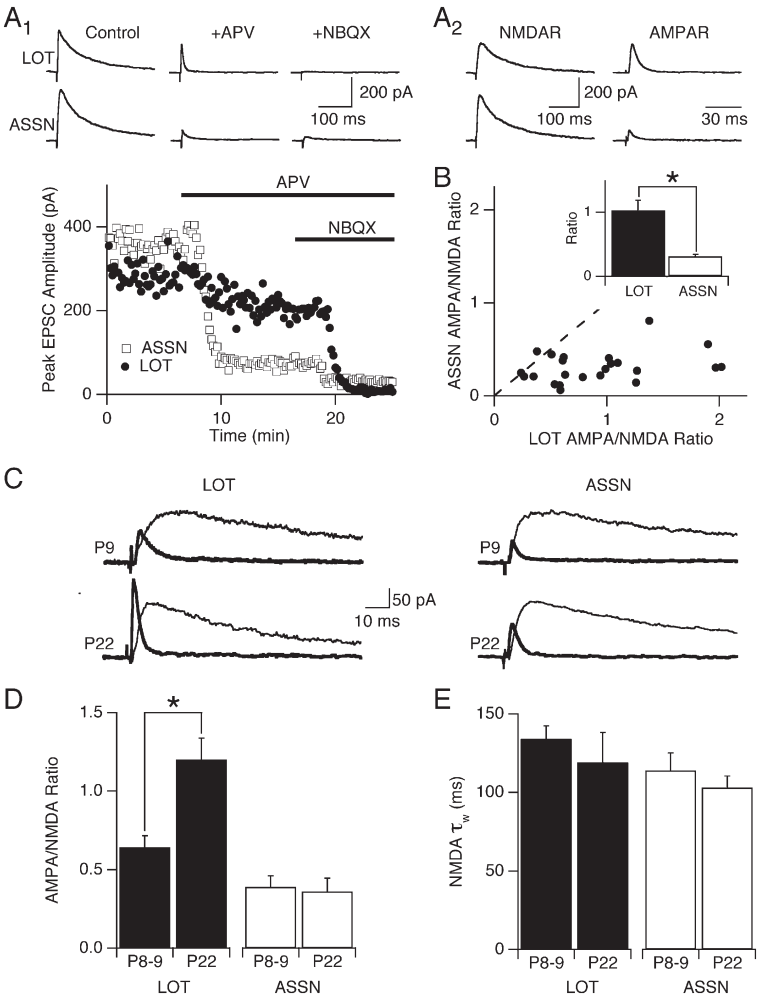

Figure 2. Different Relative Contributions of AMPA and NMDA Receptors at LOT and ASSN Synapses

(A_1) Representative experiment showing the action of APV (100 μM) and NBQX (10 μM) on the peak amplitudes of LOT- (circles) and ASSN- (squares) evoked EPSCs. (A_2) Digital subtraction of the responses in APV and NBQX reveals the NMDAR and AMPAR components of the EPSCs in the two pathways.

(B) Summary of the AMPAR/NMDAR ratio at ASSN synapses versus the AMPAR/NMDAR ratio at LOT synapses in the same cells (P15–P19, n = 21). (Inset) Average AMPAR/NMDAR ratio at LOT and ASSN synapses.

(C) The AMPAR/NMDAR ratio increases during early development at LOT but not ASSN synapses. (Left) Representative AMPAR and NMDAR components of EPSCs at LOT synapses from a P9 and P22 rat. (Right) Same components at ASSN synapses.

(D) Summary of AMPAR/NMDAR ratios at P8–P9 and P22 for LOT and ASSN synapses.

(E) Summary of NMDAR decay kinetics at P8–P9 and P22 at LOT and ASSN synapses. Error bars indicate ± SEM.

accounted for by a general increase in the expression of AMPARs at LOT synapses. We examined the quantal amplitude of AMPAR-mediated EPSCs evoked by LOT stimulation in the presence of Sr^{2+} (4 mM) to desynchronize transmitter release (Xu-Friedman and Regehr, 2000). However, we found no evidence for a developmental increase in the amplitude of AMPAR quantal events (P9: 12.8 ± 1.7 pA, n = 9; P17–P18: 12.9 ± 1.1 pA, n = 10; Figures 3A and 3B). These experiments rule out a general upregulation of AMPARs at LOT synapses during the developmental period in which the AMPAR/NMDAR ratio increases dramatically.

We next considered the possibility that the difference in the AMPAR/NMDAR ratio at LOT and ASSN inputs reflected a change in the contribution of NMDAR-only synapses. We alternately evoked synaptic AMPAR responses from ASSN and LOT inputs at –80 mV using minimal stimulation. Under these conditions we could clearly resolve failures from successful responses (Figure 3C). Stimulus intensity was adjusted so that successes occurred in ~50% of trials. Without changing stimulus parameters, synaptic responses were then recorded at +40 mV to relieve the Mg^{2+} block of NMDARs. Relief of the Mg^{2+} block dramatically increased the fraction of successes at ASSN synapses, but not at LOT synapses. On average, we observed a marked number of silent synapses at ASSN inputs (success rate: 0.37 ± 0.064 [–80 mV] versus 0.69 ± 0.094 [+40 mV]; n = 10; p < 0.005; P15–P17; Figure 3D). In these same recordings, we found no evidence for silent synapses at LOT inputs in the same cells (success rate: 0.52 ± 0.048 [–80 mV] versus 0.53 ± 0.066 [+40 mV]; n = 10; Figure 3E). In contrast to these results, LOT stimulation at +40 mV evoked significantly more successes (0.73 ± 0.075) than stimulation at –80 mV in younger (P7–P8) animals (0.57 ± 0.062; n = 6; p < 0.05; Figure 3F). These results indicate a reduction in the contribution of silent synapses that coincides with the developmental increase in the AMPAR/NMDAR ratio at LOT inputs. However, silent synapses persisted at ASSN synapses, which do not show a developmental change in the relative contribution of AMPA and NMDARs.

The apparent developmental decrease in NMDAR-only synapses in the LOT could possibly reflect a maturation of glutamate uptake. If uptake mechanisms are insufficient in younger animals (Diamond, 2005), low concentrations of glutamate, diffusing from neighboring synapses, may activate high-affinity NMDARs, but

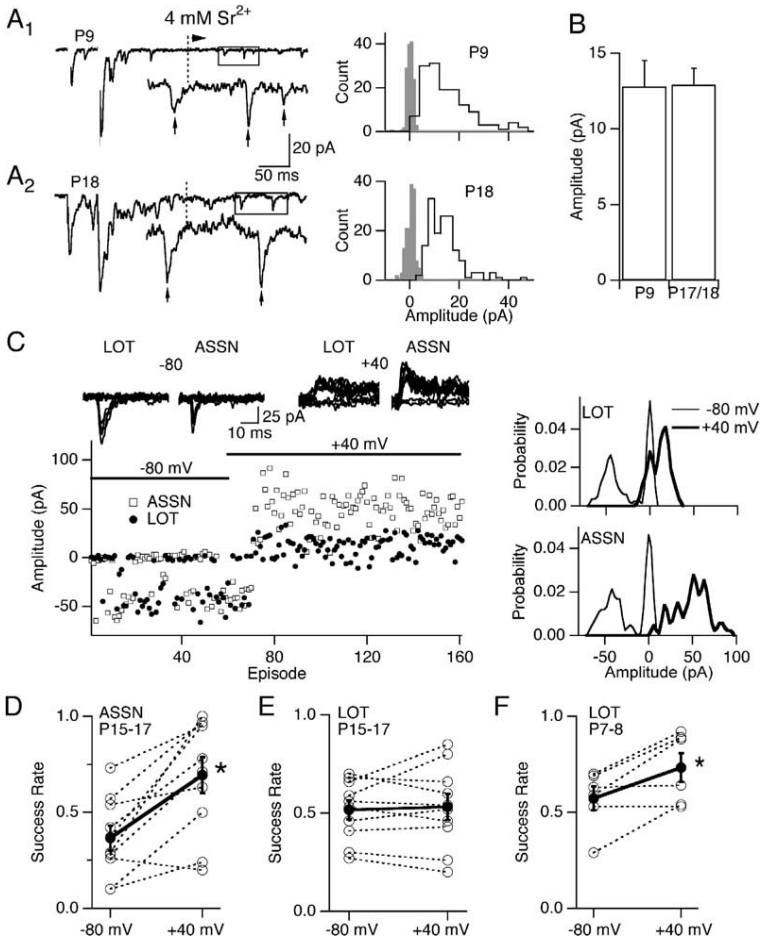

Figure 3. The Developmental Increase in AMPAR/NMDAR Ratio at LOT Inputs Is Associated with a Decrease in NMDAR-Only "Silent" Synapses but No Change in AMPAR Quantal Size

(A) (Left) Representative single traces of AMPAR-mediated quantal events at LOT synapses from (A_1) P9 and (A_2) P18 rats. Dotted lines indicate the onset of time window in which mEPSCs were collected. Insets show boxed region of traces on an expanded scale. Arrows indicate quantal synaptic responses. (Right) Corresponding distribution of mEPSC amplitudes. Gray distributions indicate baseline noise. (B) Summary data of AMPAR quantal amplitude at P9 and P17–P18. (C) Representative "minimal stimulation" experiment showing interleaved EPSCs evoked at LOT (filled circles) and ASSN (open squares) inputs from a P16 animal. (Right) Distribution of EPSC amplitudes recorded at –80 mV (thin line) and +40 mV (thick line) at LOT and ASSN. Summary plots of success rates at –80 mV and +40 mV in P15–P17 rats reveal a significant fraction of NMDAR-only synapses at ASSN inputs (D), but not at LOT inputs (E) onto the same cell; * indicates a significant difference between success rates at –80 mV and +40 mV. (F) In contrast, NMDAR-only synapses are present at LOT inputs of P7–P8 animals. Error bars indicate ± SEM.

not lower-affinity AMPARs (Kullmann and Asztely, 1998). To address this possibility, we determined the effect of blocking glutamate uptake on LOT NMDAR EPSCs in young (P8–P9) and older (P19–P23) animals. We recorded NMDAR EPSCs at +40 mV in the presence of picrotoxin (100 μM), NBQX (20 μM), baclofen (50 μM), and the mGluR antagonist MCPG (400–500 μM). Application of the glutamate transport blocker TBOA (50 μM) led to a small and variable increase in the amplitude of NMDAR EPSCs in P8–P9 animals (peak: 105% ± 6%; n = 8; p = 0.52). However, in these same cells, the uptake blocker caused a marked prolongation of the EPSC decay (charge: 236% ± 40%; n = 8; p = 0.01; Figures 4A and 4C). In older animals (P19–P23), TBOA had no effect on the EPSC amplitude (peak: 98% ± 8%; n = 10; p = 0.94), but also prolonged the EPSC decay (charge: 132% ± 13%; n = 10; p = 0.03; Figures 4B and 4C). To ensure that the more modest effect of the uptake blocker on NMDAR EPSCs in mature animals was not due to a reduced sensitivity to TBOA, we tested the effect of the uptake blocker on exogenously applied glutamate. In these same cells, TBOA caused a marked increase in the amplitude of NMDAR-mediated responses evoked by flash photolysis of caged glutamate (50 μM) in layer Ia (Figure 4B).

We determined the uptake-sensitive component of responses to synaptically released glutamate by subtracting the normalized NMDAR EPSCs under control conditions from those in TBOA (Figure 4D). The TBOA-sensitive component had a very slow rise and a prolonged decay in both P8–P9 (rise, 123 ± 31 ms; decay, 352 ± 75 ms; n = 8) and P19–P23 animals (rise, 47 ± 8.5 ms; decay, 442 ± 242 ms; n = 7). These uptake-sensitive currents were far slower than the "successes" evoked at +40 mV by minimal stimulation in the LOT in P8–P9 (rise, 2.8 ± 0.78 ms; decay, 42 ± 7.0 ms; n = 5) and P15–P17 (rise, 3.2 ± 0.68 ms; decay, 89 ± 23 ms; n = 9) animals. Together, these data indicate that glutamate uptake mechanisms are functional by the end of the first postnatal week. Furthermore, the kinetics of the uptake-sensitive NMDAR component were too slow to contaminate our measured success rates in minimal stimulation experiments. Therefore it is unlikely that a maturation of glutamate uptake underlies an apparent developmental decrease in NMDAR-only synapses at LOT inputs.

The uptake-sensitive component of the NMDAR EPSCs was markedly larger in P8–P9 (55% ± 12% of control) than P19–P23 (21% ± 8%) animals. What underlies this difference in NMDAR activation? The enhancement of NMDAR EPSCs by uptake blockers has been proposed to reflect either a pooling of transmitter

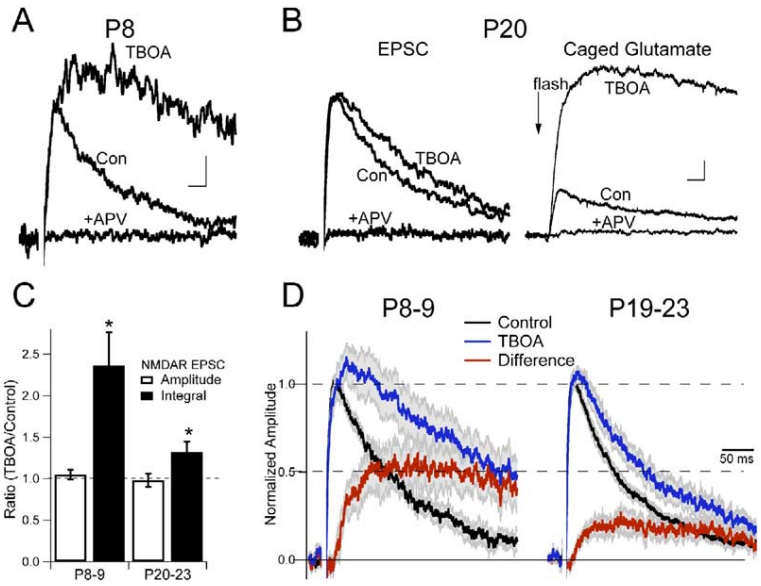

Figure 4. Efficient Glutamate Uptake at LOT Synapses in Both Young and Older Animals

(A) Representative experiment showing the effect of TBOA on an LOT-evoked NMDAR EPSC in a P8 rat.

(B) Action of TBOA on an LOT-evoked NMDAR EPSC (left) and response to flash photolysis of caged glutamate (right) in a P20 rat. Scale bars: EPSCs, 10 pA; glutamate uncaging, 20 pA; 20 ms.

(C) Summary of effects of TBOA on NMDAR EPSC amplitude and charge.

(D) Normalized, average EPSCs evoked by LOT stimulation before (black) and after (blue) uptake blockade. Difference currents (red) reveal the uptake-sensitive component in P8–P9 (n = 8) and P19–P23 (n = 10) animals. Gray shaded regions indicate standard error.

Error bars indicate ± SEM.

from coactivated synapses (Arnth-Jensen et al., 2002) or spillout of glutamate onto extrasynaptic NMDARs (Diamond, 2001). The actions of TBOA on EPSCs in the LOT are consistent with either interpretation.

Over the developmental period (P7–P22) in which there is a marked increase in AMPAR/NMDAR ratio, we observe a loss of NMDAR-only synapses and no change in the quantal amplitude of AMPAR EPSCs. Together, these findings suggest two possible cellular mechanisms to account for the developmental increase in the AMPAR/NMDAR ratio at LOT inputs. In one case, all synapses would have either zero or a fixed ("quantal") number of AMPARs. During development, a quantum of AMPARs would have to be inserted at previously silent, NMDAR-only synapses. Alternatively, there may be no change in AMPAR expression at all, but rather a developmental downregulation of synaptic NMDARs.

One way to resolve absolute changes in the contribution of AMPA and NMDARs to synaptic transmission is to measure synaptic input/output (I/O) relationships. A simple approach is to use extracellular recording to compare the relationship between the numbers of active inputs (fiber volley) versus the magnitude of the synaptic response (fEPSP slope; Hsia et al., 1998; Saura et al., 2004). However, given the laminar structure of the olfactory cortex, this assay is quite sensitive to the position of the recording electrode relative to the LOT. The large changes in cortex size during early development precluded comparisons of synaptic I/O relationships between young and old animals. We thus developed a different approach to probe the maturation of glutamatergic signaling in olfactory cortex.

Early sensory experience has been shown to play an important role in the maturation of synaptic transmission in other brain structures. To address the role of sensory experience in the maturation of transmission in olfactory cortex, we used unilateral naris occlusion (Meisami, 1976) to deprive one hemisphere of olfactory sensory input beginning at P1–P3. Because the projection from the nasal epithelium, through the olfactory bulb, to the olfactory cortex is wholly ipsilateral (Haberly, 1998), the effects of activity can be directly assessed by comparing developmental changes in synaptic transmission in spared and deprived slices from the same animal.

If sensory experience governs the developmental maturation of olfactory cortical synapses, one would expect that unilateral sensory deprivation would alter the properties of excitatory transmission of deprived versus spared hemispheres. All experiments and analyses of spared versus deprived synapses were performed blind. We first examined the role of sensory experience in the developmental shift in the contribution of AMPA and NMDARs at LOT synapses (Figure 5A). The AMPAR/NMDAR ratio in cells from spared hemispheres was 0.59 ± 0.05 (n = 20 cells/3 animals) by the first postnatal week and increased to 1.31 ± 0.29 (n = 8/3) by P35 (Figure 5B), essentially identical to that observed in control animals (Figure 2D). However, we found that the developmental change in the LOT AMPAR/NMDAR ratio was markedly altered by sensory deprivation. In the first postnatal week, the AMPAR/NMDAR ratios in deprived and spared hemispheres were similar (deprived, 0.63 ± 0.06; n = 19/3; p = 0.59). However, between the second and fourth postnatal week the AMPAR/NMDAR ratio in deprived hemispheres was significantly smaller than that in spared hemispheres. At P14–P21, the AMPAR/NMDAR ratio of LOT synapses in spared hemispheres (1.14 ± 0.08, n = 73/21) was 50% greater than those from deprived hemispheres (0.77 ± 0.04, n = 76/21; p < 0.001; Figure 5B). By the fifth postnatal week, the AMPAR/NMDAR ratio in deprived hemispheres had increased to similar levels to that observed in spared hemispheres. The developmental increases in the AMPAR/NMDAR ratio of spared and deprived synapses were well fit by sigmoidal curves, with a 1.7 week difference in half-width (spared, 2.5; deprived, 4.2 weeks) and a 2-fold difference in rate

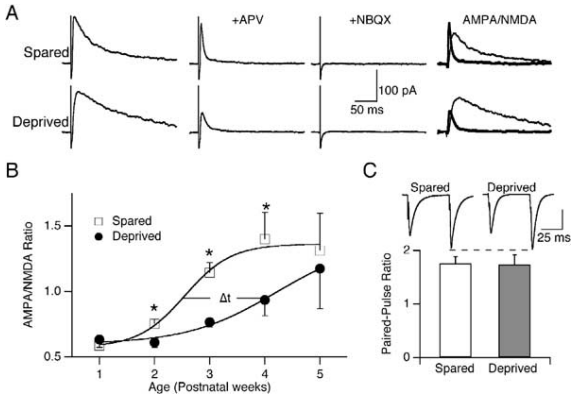

Figure 5. Olfactory Deprivation Changes the Relative Contribution of AMPARs and NMDARs at LOT Synapses

(A) Representative interleaved experiments showing isolation of APV- (thin line) and NBQX-sensitive (thick line) currents in spared and deprived slices (P17).

(B) Developmental timecourse of AMPAR/NMDAR ratios at LOT synapses from spared (squares) and deprived (circles) hemispheres. Solid lines are sigmoidal fits to the data, with a half-width difference (Δt) of 1.7 weeks.

(C) Paired-pulse facilitation of fEPSPs normalized to the amplitude of the first pulse, in representative, interleaved experiments from a spared and deprived slice. (Bottom) Summary of PPR for nine slices spared, nine slices deprived, from three animals (P14–P16). Scale bar, spared 1 mV, deprived 0.8 mV; 25 ms.

Error bars indicate ± SEM.

(spared, 0.42; deprived, 0.81). These data indicate that early olfactory experience accelerates the maturation of LOT inputs. In contrast, and consistent with the absence of a developmental change at ASSN synapses, sensory deprivation did not alter the AMPAR/NMDAR ratio at ASSN synapses (spared: 0.37 ± 0.054, n = 12; deprived: 0.44 ± 0.056, n = 19; age: P15–P25; p = 0.45).

To determine whether sensory deprivation was associated with changes in release probability, we measured the PPR of LOT-evoked fEPSPs. We found no difference in paired-pulse facilitation in slices from spared (1.76 ± 0.13, n = 9/3; P14–P16) and deprived (1.74 ± 0.18, n = 10/3, p = 0.93) hemispheres (Figure 5C), ruling out any obvious changes in presynaptic transmitter release. Together, these results indicate that the developmental increase in the relative contribution of AMPARs to synaptic transmission at LOT synapses is governed by early sensory experience and that sensory deprivation delays the postsynaptic maturation of afferent sensory synapses in the olfactory cortex.

Does an increase in the number of synaptic AMPARs underlie the activity-dependent increase in the AMPAR/NMDAR ratio at LOT synapses? To address this possibility, we again measured the quantal amplitude of AMPAR-mediated EPSCs evoked by LOT stimulation in the presence of Sr^{2+}. As before, there was no significant change in the quantal amplitude of AMPAR EPSCs at spared (12.0 ± 0.98 pA; n = 9/3; Figures 6A_1 and 6A_3) versus deprived synapses (10.8 ± 0.64 pA; n = 9/3; Figures 6A_2 and 6A_3; p = 0.33, unpaired t test; P16–P22). These results confirm that sensory experience does not cause a marked increase in the number of AMPARs at "functional" synapses.

These results do not rule out the possibility of an experience-dependent conversion of silent synapses by the insertion of a quantum of AMPARs. If this were true, there should be a greater fraction of functional LOT synapses, and on average, a greater AMPAR response per LOT release site, in spared versus deprived hemispheres. To address this, we determined synaptic I/O relationships by plotting the initial slope of LOT-evoked AMPAR fEPSPs versus fiber volley amplitude, an indicator of afferent fiber recruitment, over a range of stimulus intensities (Figures 6B_1 and 6B_2). This approach allowed us to directly compare the relative number of AMPARs at LOT synapses from spared and deprived hemispheres of age-matched animals. We observed only a small and highly variable difference in I/O relationships of AMPAR fEPSPs between spared and deprived hemispheres, whether the data were pooled and averaged across slices (spared: 4.3 ± 0.31 ms^{-1}, n = 33; deprived: 3.6 ± 0.37 ms^{-1}; n = 32, P14–P21, p = 0.12, unpaired t test; Figure 6B_3) or compared within animals (spared: 4.4 ± 0.31 ms^{-1}; deprived: 3.7 ± 0.40 ms^{-1}; n = 16, p = 0.12, paired t test; Figure 6B_4). Together, these data indicate that the developmental increase in the AMPAR/NMDAR ratio governed by sensory experience cannot be accounted for solely by an increase in the expression of synaptic AMPARs.

We next considered that marked changes in the AMPAR/NMDAR ratio could reflect an experience-dependent decrease in synaptic NMDARs. To explore this possibility, we determined I/O relationships of NMDAR-mediated transmission by isolating NMDAR fEPSPs in low Mg^{2+} and NBQX (10 μM; Figures 7A_1 and 7A_2). The I/O relationship of NMDAR-mediated transmission was ~2-fold greater in deprived than spared hemispheres when grouped either by slice (spared: 0.27 ± 0.04 ms^{-1}, n = 17; deprived: 0.52 ± 0.06 ms^{-1}; n = 18; P14–P21; p < 0.005; Figure 7A_3) or by animal (spared, 0.29 ± 0.04 ms^{-1}; deprived, 0.52 ± 0.07 ms^{-1}; n = 6, p < 0.05; Figure 7B_4). These data indicate that a marked activity-dependent decrease in NMDAR-mediated transmission contributes to the difference in AMPAR/NMDAR ratio between deprived and spared hemispheres in the third postnatal week (i.e., Figure 5B).

A global, activity-dependent downregulation of NMDARs should be reflected by a loss of NMDAR-only synapses. In support of this hypothesis, minimal stimulation experiments in spared and deprived hemispheres showed a clear, experience-dependent difference in the number of NMDAR-only synapses. As expected, there was no evidence for silent synapses in spared hemispheres (success rate: 0.55 ± 0.025 [–80 mV] versus 0.59 ± 0.32 [+40 mV]; n = 12/6; p = 0.20; Figure 7B_1). In deprived synapses, however, the success rate increased significantly from –80 mV (0.52 ± 0.026) to +40 mV (0.70 ± 0.039; n = 11/6; p < 0.003; Figure 7B_2). These data indicate that sensory experience regulates the loss of NMDAR-only synapses.

At thalamocortical synapses in the somatosensory (Crair and Malenka, 1995; Feldman et al., 1998) and visual systems (Kirkwood et al., 1995), synaptic plasticity is largely constrained to an early postnatal period. Is there a critical period for the activity-dependent regulation of LOT NMDARs? To address this question, we performed unilateral naris occlusion on older rats (P28).

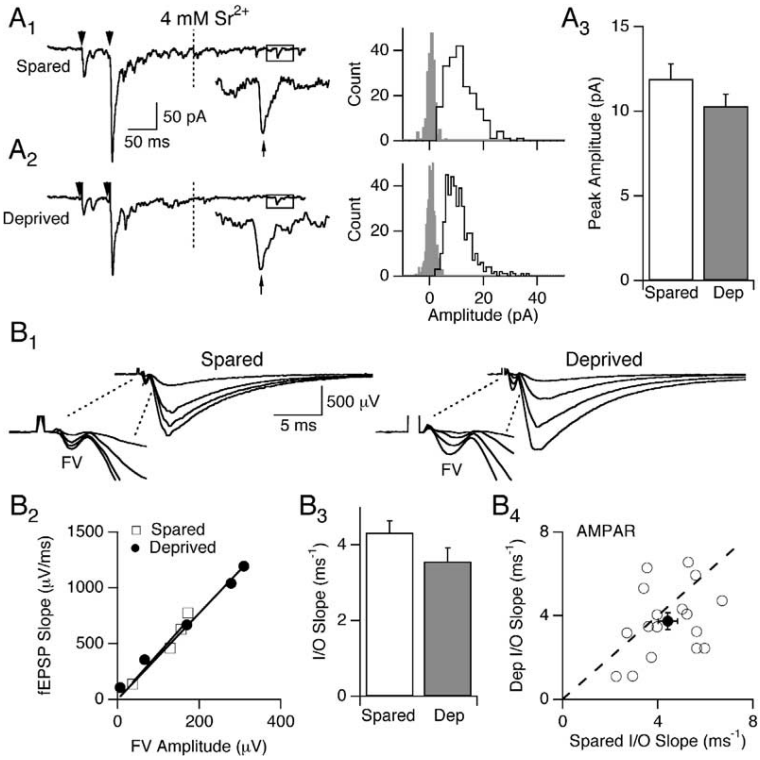

Figure 6. Sensory Experience Does Not Markedly Alter the Expression of Synaptic AMPARs

(A) (Left) Representative traces of AMPAR-mediated quantal events at LOT synapses from (A_1) spared and (A_2) deprived rats. Arrowheads indicate time of stimuli. Dotted line indicates the onset of the time window in which mEPSCs were collected. Insets show boxed region of traces on an expanded scale. Arrows indicate quantal synaptic responses. (Right) Corresponding distribution of mEPSC amplitudes. Gray distributions indicate baseline noise. (A_3) Summary data of AMPAR quantal amplitude in spared and deprived animals.

(B_1) AMPAR-mediated fEPSPs in slices from spared and deprived hemispheres of the same animal (P20). (Insets) Blowup showing stimulus artifacts, fiber volleys (FV), and fEPSPS. (B_2) Corresponding I/O relationships: spared, $y = 4.1x$, $r^2 = 0.96$, open squares; deprived, $y = 3.9x$, $r^2 = 0.99$, filled circles. (B_3) AMPAR I/O slopes averaged across all slices. (B_4) Average AMPAR I/O slopes in spared versus deprived hemispheres for each animal (open circles) and the average I/O slope for all animals averaged together (filled circle). Dashed line denotes unity.

Error bars indicate ± SEM.

Following 14–23 days of deprivation in these animals, there was no difference in the NMDAR I/O relationship between spared and deprived hemispheres, whether the data were pooled and averaged across slices (spared: 0.11 ± 0.019, n = 19; deprived: 0.11 ± 0.021, n = 18; p < 0.99; Figure 8$_1$) or compared within animals (spared: 0.12 ± 0.030; deprived: 0.11 ± 0.019; p = 0.66; n = 6; Figure 8A$_2$). The experience-dependent downregulation of NMDARs was therefore confined to a postnatal critical period of less than 4 weeks

Early sensory experience increases the relative proportion of $NR2_A$ versus $NR2_B$ subunits of NMDARs in the visual cortex (Philpot et al., 2003; Philpot et al., 2001; Quinlan et al., 1999a). It has been suggested that the greater fraction of $NR2_B$ versus $NR2_A$ subunits in younger animals increases the capacity for plasticity in developing circuits (Quinlan et al., 1999a; Tang et al., 1999; Yoshimura et al., 2003) and may underlie the cortical critical period. We therefore examined whether olfactory experience altered the relative contribution of $NR2_B$ subunits at LOT synapses when the greatest difference in NMDAR expression at spared and deprived slices was observed (P17 ± 1). Application of the $NR2_B$-selective antagonist ifenprodil (3 μM) reduced the amplitude of LOT NMDAR EPSCs by 28% ± 7.0% in spared (n = 7/4) and 33% ± 5.3% in deprived hemispheres (n = 7/4; p = 0.51; Figure 8B), indicating no difference in the relative expression of $NR2_B$ at spared and deprived synapses. In agreement with these results, there was no difference in the decay time constant of NMDAR EPSCs in spared (109 ± 6.0 ms, n = 7/4) and deprived (106 ± 11 ms, n = 7/4) hemispheres. These experiments show that the critical period of NMDAR plasticity at LOT synapses is not associated with changes in the relative contribution of $NR2_B$ subunits.

NMDAR-dependent plasticity is often proposed to underlie the development of cortical circuits. We first determined whether we could induce a long-lasting, synapse-specific and NMDAR-dependent potentiation of LOT transmission in P16–P18 rats. We recorded field EPSPs in layer Ia that were alternately evoked by two stimulating electrodes placed in the LOT, on either side of the recording electrode. In the presence of APV (50 μM), a theta-burst stimulus (TBS) to one pathway (test pathway) had no effect on fEPSPs evoked by either the test (98% ± 3.3% of baseline) or the control (95% ± 4.7%; n = 9; Figure 9A) pathway. Following APV washout, TBS induced a long-lasting enhancement of the test pathway (127% ± 7.2%), while the control pathway was unaltered (99% ± 6.5%). These data indicate that LTP at LOT inputs is synapse specific and confirm a role for NMDARs in long-term synaptic plasticity at these synapses (Jung et al., 1990; Kanter and Haberly, 1990).

We next considered whether LTP at afferent synapses in the olfactory cortex is regulated by early sensory experience. In slices from the spared hemispheres of rats (P16–P23) that had undergone postnatal naris occlusion, a TBS induced a long-lasting potentiation of fEPSPs (135% ± 5.0%, n = 25/9). Deprived hemispheres from the same rats showed markedly greater LTP (157% ± 4.9%, n = 26/9; p < 0.005; Figure 9B).

The larger magnitude of LTP in deprived versus spared hemispheres could reflect either a difference in the amount of LTP that could be expressed or in the ability to induce LTP. To distinguish between these possibilities, we followed the first, weak TBS with a

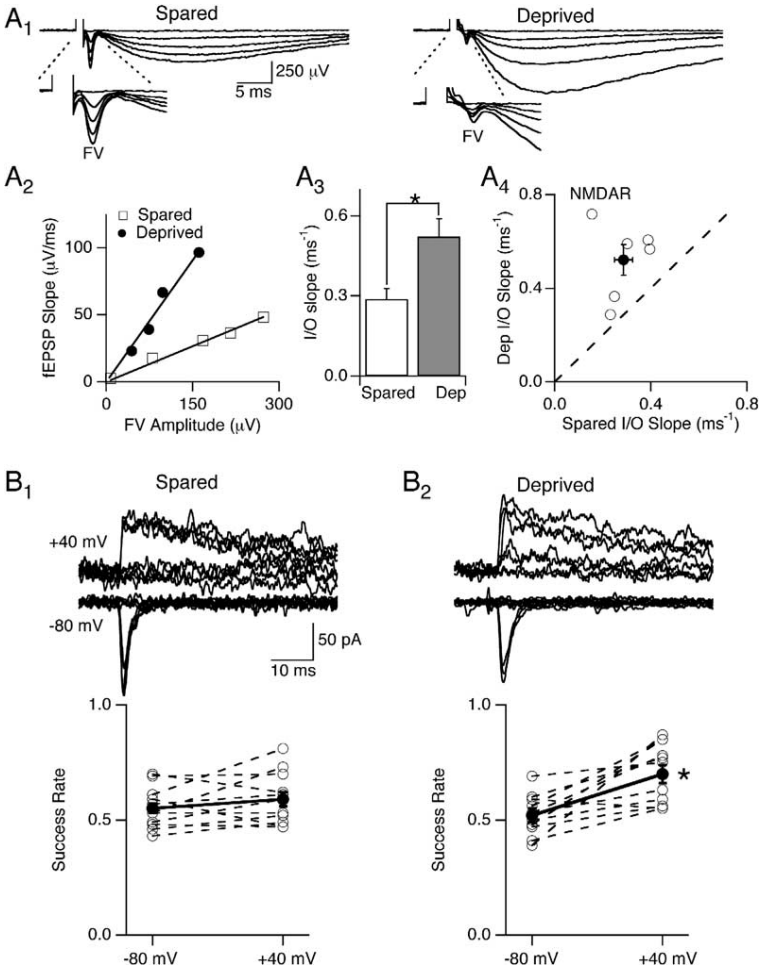

Figure 7. Sensory Experience Downregulates Expression of Synaptic NMDARs at LOT Inputs

(A_1) Representative NMDAR-mediated fEPSPs. (Insets) Blowup of region. (A_2) Corresponding I/O relationships (P20). Spared, y = 0.18x, r^2 = 0.99, open squares; deprived, y = 0.60x, r^2 = 0.98, filled circles. (A_3) Average NMDAR I/O for all slices. (A_4) Average NMDAR I/O slopes of spared versus deprived hemispheres for each animal (open circles), and the average I/O slope for all animals averaged together (filled circle). Dashed line denotes unity.

(B) Representative experiments at –80 mV and +40 mV (top) and summary data (bottom) showing the loss of NMDAR-only LOT synapses in spared hemispheres (B_1), but their persistence in deprived hemispheres (B_2). Error bars indicate ± SEM.

stronger TBS to approach the maximal amount of LTP that can be expressed at LOT synapses. Strong tetanic stimulation further potentiated spared and deprived synapses to similar levels (spared: 179% ± 12%, n = 11/5; deprived: 204% ± 16%, n = 11/5 p = 0.23; Figure 9B). In a separate set of experiments, we induced LTP using only a strong TBS (16×). Under these conditions, spared and deprived slices were similarly potentiated (spared: 196% ± 20%; n = 7/3; deprived: 186% ± 15%; n = 7/3; p = 0.68; Figure 9C). These data indicate that olfactory experience raises the threshold for LTP induction without affecting its expression.

In these experiments examining the effect of sensory deprivation on synaptic plasticity, the age of the animals corresponded to the developmental window in which we observed the greatest effects of naris occlusion on the contribution of NMDARs to synaptic transmission (P16–P23; Figure 5B). In older rats (P26–P32), TBS induced significantly less LTP than that in spared synapses of younger rats (P26–P32: 120% ± 3.8% of baseline, n = 10 versus P16–P23: 135% ± 5.0%). Taken together, these results indicate that early olfactory experience progressively raises the threshold for the induction of LTP. This is consistent with the hypothesis that an activity-dependent downregulation of NMDARs raises the induction threshold for plasticity at afferent olfactory cortical inputs.

To demonstrate directly the role of NMDAR expression in setting the threshold for LTP induction, we next determined the concentration of APV that blocked ~50% of NMDARs at LOT synapses. Application of 5 μM APV decreased the amplitude of LOT NMDAR EPSCs to 49% ± 6.8% of baseline (n = 5; Figure 9D). We next determined the effect of reducing the fraction of functional NMDARs on LTP induction at LOT synapses. Control experiments were interleaved with slices bathed in 5 μM APV. A weak TBS resulted in significantly less LTP in the presence of 5 μM APV than in control slices (150% ± 6.9%; n = 7 versus 112% ± 2.9%; n = 6; p < 0.001; Figure 9D_2). A subsequent strong TBS further potentiated both control and APV-treated synapses to similar levels (control: 194% ± 18%, n = 7; 5 μM APV: 165% ± 7.8%, n = 6; p = 0.19). These findings show that both a fractional block and the experience-dependent downregulation of NMDARs had virtually identical effects on LTP induction. Thus, early sensory experience downregulates the expression of synaptic NMDARs in olfactory cortex, which raises the threshold for LTP induction at LOT synapses.

Discussion

In this study, we examined the development of synaptic transmission in the primary olfactory cortex. Over the

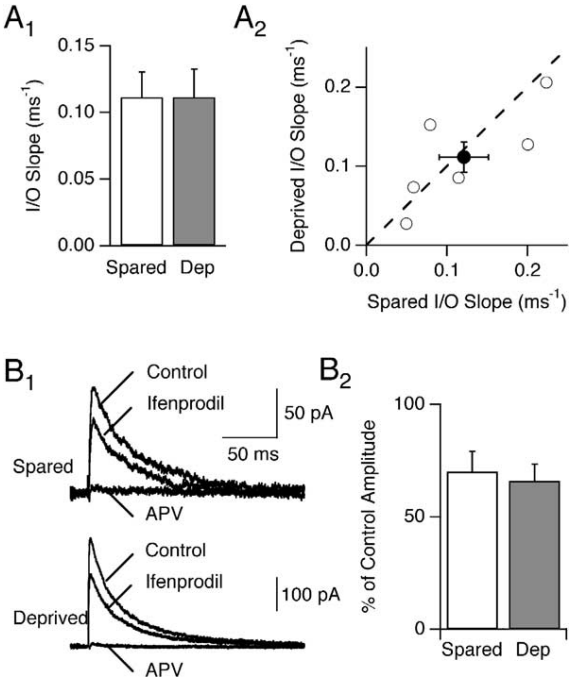

Figure 8. A Critical Period for Experience-Dependent Plasticity of NMDARs

(A) I/O relations of spared and deprived NMDAR-mediated fEPSPs in older animals (occluded at P28) were identical when data were compared by either (A_1) slice or (A_2) animal.

(B_1) Representative experiments showing the effect of ifenprodil on NMDAR-mediated EPSCs in spared and deprived slices from animals occluded at P1–P3. (B_2) The fractional block of NMDAR-mediated EPSCs by ifenprodil was similar in spared and deprived slices.

Error bars indicate ± SEM.

first postnatal month, we find a developmental increase in the relative contribution of AMPARs versus NMDARs at sensory LOT synapses that is regulated by early sensory experience. In contrast, NMDARs dominate over AMPARs at associational synapses on the same cells, and the relative contribution of the two receptor types to transmission is unaltered by early sensory experience. The developmental change in signaling at LOT synapses is associated with a marked activity-dependent downregulation of NMDARs and relatively modest increase in AMPAR-mediated transmission. The activity-dependent downregulation of NMDARs at sensory synapses occurs during a critical period confined to the first postnatal month. During this period, sensory experience raises the threshold for the induction of long-term synaptic plasticity. These results suggest a critical period for synaptic plasticity at sensory synapses in olfactory cortex.

We used unilateral naris occlusion to selectively decrease neuronal input to one hemisphere of the olfactory cortex. Naris occlusion produces an immediate and sustained attenuation of background and odor-evoked activity in ipsilateral olfactory bulb mitral cells (Philpot et al., 1997), whose axons project to the ipsilateral olfactory cortex via the LOT. Unilateral naris occlusion therefore selectively decreases neuronal activity at ipsilateral LOT synapses and allows use of the contralateral hemisphere as an internal control for the effects of sensory deprivation. We show that naris occlusion greatly delayed, but did not block, the maturation of glutamatergic signaling at LOT synapses. This result is consistent with a role for spontaneous activity in the maturation of neural circuits (Zhu et al., 2000). Alternatively, odor-evoked responses may only be strongly attenuated rather than completely blocked by naris occlusion due to trans-septal passage of odors (Philpot et al., 1997). Previous studies have reported a decrease in the width of the LOT following early sensory deprivation (Wilson et al., 2000) and a reduction in the maximal amplitude of LOT-evoked fEPSPs in vivo (Best and Wilson, 2003). These results are consistent with a role for neuronal activity in regulating mitral cell survival (Fiske and Brunjes, 2001). However, these effects of naris occlusion may reflect a reduction in the number of axons from olfactory bulb to cortex rather than an effect on postsynaptic signaling.

Pyramidal cells in the olfactory cortex receive associational inputs from other olfactory pyramidal cells, as well as many other brain regions (Haberly, 1998). Associational synapses are believed to play a crucial role in the synthetic processing of complex mixtures of odorants and are also thought to contribute to the formation of olfactory memories (e.g., association of odors with reward (Haberly and Bower, 1989; Quinlan et al., 2004; Wilson and Stevenson, 2003). Consistent with this role of associational synapses in adult olfactory learning, we see no evidence for developmental or experience-dependent changes in the receptors governing transmission at these inputs. The large contribution of NMDARs at these synapses presumably persists through adulthood and endows them with a high capacity for plasticity.

It is generally believed that NMDARs contribute more to synaptic transmission than AMPARs during early development and that AMPARs play a progressively greater role in transmission during the maturation of central circuits. A popular view is that immature, silent synapses contain only NMDARs and that AMPARs are then incorporated into the synapse in an activity-dependent manner. The cellular mechanisms mediating activity-dependent increases in AMPAR-mediated transmission are presumed to be identical to those mediating the expression of NMDAR-dependent LTP. Indeed, silent synapses have been described in the developing hippocampus (Durand et al., 1996; Liao et al., 1995; but see Groc et al., 2002; Xiao et al., 2004), somatosensory cortex (Isaac et al., 1997), visual cortex (Rumpel et al., 1998; Rumpel et al., 2004), and frog optic tectum (Wu et al., 1996), where age- and activity-dependent increases in the AMPAR/NMDAR ratio of transmission are found. More recently, it has been shown that sensory experience drives the insertion of AMPARs to synapses in somatosensory cortex (Takahashi et al., 2003).

Does a developmental/activity-dependent insertion of AMPARs explain the experience-dependent changes at sensory LOT synapses in olfactory cortex? Several of our results seem consistent with this model. (1) There is a developmental, activity-dependent increase in the AMPAR/NMDAR ratio. (2) There is a decrease in NMDAR-

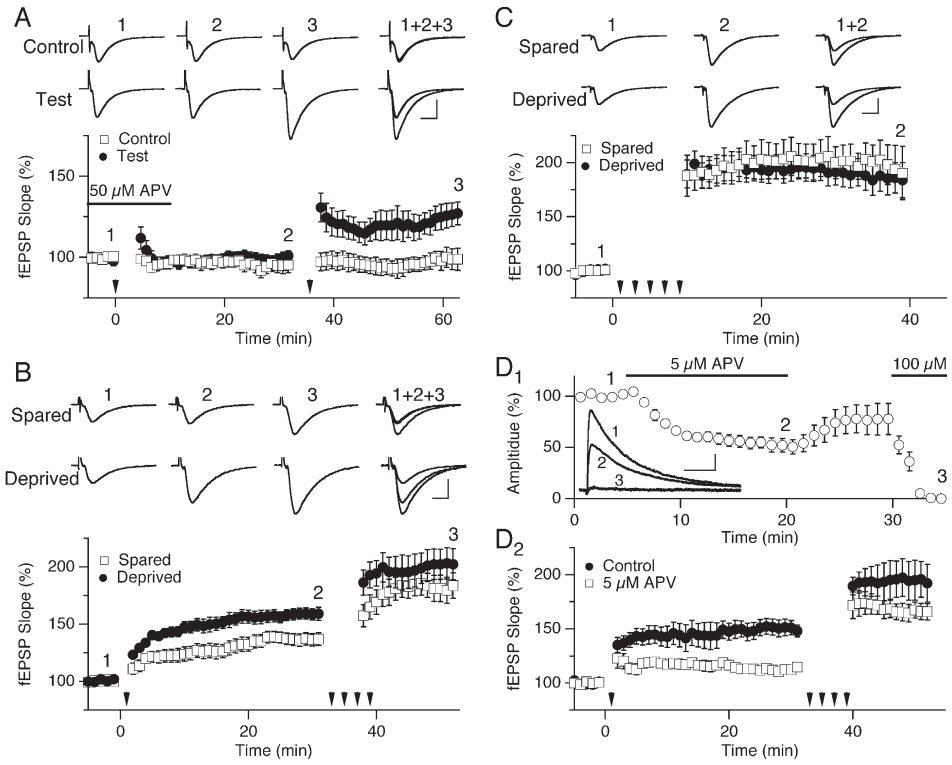

Figure 9. Experience-Dependent Downregulation of Synaptic NMDARs Raises the Threshold for Induction of LTP in Olfactory Cortex

(A) Summary plot of fEPSP slope evoked at separate test (filled circles) and control (open squares) LOT pathways in the same slices (n = 10). A theta-burst stimulus (TBS, arrowhead) was presented to the test pathway in the presence of APV (50 μM) and after washout of the antagonist. Scale bar, 500 μV, 5 ms. (Top) Representative experiment showing traces collected at times indicated on summary plot.

(B) Summary plot showing the time course of LTP following weak (single arrowhead) and strong (multiple arrowheads) TBS in spared (open squares) and deprived (filled circles) hemispheres.

(C) In naive spared (open squares) and deprived slices (closed circles), strong TBS induces similar levels of LTP.

(D_1) Partial blockade by 5 μM APV decreases the amplitude of NMDAR-mediated EPSCs by ~50%. (Inset) Representative NMDAR EPSC from the time points marked on graph. Scale bar, 50 pA, 5 ms. (D_2) Summary plot showing greater LTP in control slices (filled circles) compared to slices treated with 5 μM APV after weak TBS. The levels of potentiation converged following a strong TBS.

Error bars indicate ± SEM.

only, silent synapses. (3) LTP is greater at deprived versus spared synapses. However, for a number of reasons, our results are inconsistent with a model in which an activity-dependent insertion of AMPARs alone governs changes in LOT synapses driven by olfactory experience.

In contrast to a simple AMPAR-insertion model, our data indicate that the expression of synaptic NMDARs is downregulated by early experience at LOT synapses. Addition of AMPARs at functional LOT synapses should result in an increase in the average quantal amplitude of AMPAR-mediated currents. However, we found neither a developmental nor an activity-dependent change in the quantal amplitude of LOT synapses. If AMPARs were inserted at synapses in a fixed, "quantal" number, this would not be revealed in our measurements of quantal amplitude. However, any addition of AMPARs would be revealed by synaptic I/O relationships. We did see a small (~20%) but variable difference in AMPAR I/O relationships between spared and deprived synapses. However, this increase in AMPAR-containing synapses alone is insufficient to account for the ~50% change in AMPAR/NMDAR driven by sensory experience. In contrast, the I/O relationship of NMDAR-mediated transmission was significantly smaller (~45%) in spared versus deprived synapses. Furthermore, an ~50% reduction in NMDARs is consistent with the developmental changes we observed in the amplitude of the uptake-sensitive component of NMDAR EPSCs. Together, these data are most consistent with a strong activity-dependent decrease in NMDAR expression and only a weaker increase in AMPAR expression.

One might also expect that an activity-dependent insertion of synaptic AMPARs driven by olfactory experience would occlude LTP at LOT inputs. Indeed, we found that LTP was more robustly expressed at deprived versus spared synapses after weak tetanic stimulation. However, the magnitude of LTP in spared and deprived synapses converged with strong tetanic stimulation. This result is inconsistent with occlusion of LTP at spared synapses. Moreover, we could mimic the effects of sensory experience on the induction of LTP by acute, partial block of NMDARs. Together, our findings demonstrate that the threshold for LTP induction is

raised by an activity-dependent downregulation of NMDARs.

If LTP reflects the conversion of silent synapses to functional AMPAR-containing sites at LOT inputs, it could be argued that the experience-dependent loss of NMDAR-only inputs should also lead to a reduction in the maximal amount of LTP. We may have underestimated the maximal level of LTP in our experiments if even "strong" TBS did not saturate LTP at LOT inputs. Also, the experience-dependent reduction in NMDARs could occur in a graded rather than an all-or-none fashion at silent LOT synapses. At the developmental period that we examined, we cannot exclude the possibility that the "loss" of silent synapses in our recordings also reflects individual NMDAR-only synapses with so few NMDARs that they escape detection. The minimal number of NMDARs required for the conversion of a silent synapse to a functional one during LTP has yet to be established. It may be that activation of as few as one or two NMDARs (Nimchinsky et al., 2004) is sufficient to trigger the insertion of AMPARs at silent synapses during LTP.

The downregulation of NMDARs at LOT synapses could reflect either a selective loss of NMDAR-only inputs or a global reduction of NMDARs at all sensory synapses. The dramatic (~2-fold) difference in NMDAR I/O relationships at spared and deprived synapses appears more substantial than the difference we observed in the numbers of NMDAR-only synapses. Together, our data suggest an activity-dependent downregulation of NMDARs at all LOT synapses, although we cannot exclude a more complex model.

Activity-dependent rearrangements of afferent cortical circuits are largely constrained to a postnatal critical period. Critical periods for anatomical and synaptic plasticity have been demonstrated in the visual (Wiesel and Hubel, 1963), somatosensory (Woolsey and Wann, 1976), and auditory cortices (Zhang et al., 2001). Indeed, we find that activity-dependent downregulation of NMDARs at LOT synapses in olfactory cortex does not occur after the first month. The relative expression of $NR2_A$ versus $NR2_B$ subunits may be a major determinant of the critical period in other cortical regions (Erisir and Harris, 2003; Mower and Chen, 2003; but see Barth and Malenka, 2001; Lu et al., 2001), and sensory deprivation, which extends the critical period, is associated with a delay in the subunit switch (Philpot et al., 2001; Quinlan et al., 1999a, 1999b). A recent study showed that olfactory learning regulated the subunit composition of NMDARs and was accompanied by a reduction in LTP at ASSN synapses (Quinlan et al., 2004). In contrast, naris occlusion did not regulate the subunit composition of NMDARs at LOT synapses in our study. These findings suggest that different cellular mechanisms may be employed by LOT and ASSN synapses during maturation and adult learning.

Individual pyramidal cells in the olfactory cortex appear to receive overlapping inputs from different receptors, allowing for a spatial olfactory code (Zou et al., 2001). We speculate that there are two types of LOT synapses that arise from individual mitral cell axons in the developing cortex. One type contains both AMPA and NMDARs and is responsible for the transmission of sensory information into the olfactory cortex. Axons from the same mitral cells may also give rise to NMDAR-only synapses onto the same pyramidal cells. NMDAR-only synapses at LOT inputs would be ideal for generating synaptic plasticity that contributes to maternal imprinting.

Neonatal rats must learn their mother's odor to survive, and during early development strong maternal attachments are imprinted via the olfactory system. Furthermore, a variety of studies indicate that memories of early olfactory experience persist throughout adulthood (Leon, 1992). Maturation of the locus coeruleus and amygdala are thought to underlie closure of the sensitive period for maternal imprinting (Sullivan, 2003). Plasticity at LOT synapses might contribute to the persistence of early olfactory memories. Indeed, a recent study found that pyramidal cells in the anterior piriform cortex participate in neonatal olfactory preference learning (Roth and Sullivan, 2005).

We suggest a scenario in which repeated exposure to maternal odors would lead to strong activation of a unique subset of mitral cells and their inputs to olfactory cortex. During early development, NMDAR-dependent LTP would convert silent synapses from these inputs to functional ones via AMPAR insertion. This potentiation would thus selectively increase the strength of a relatively restricted set of cortical sensory inputs activated by early experience. Furthermore, the activity-dependent downregulation of synaptic NMDARs would impart greater salience to early olfactory experiences by decreasing the capacity for long-term enhancement of inputs activated by odors later in development.

Experimental Procedures

Experiments followed approved national and institutional guidelines for animal use. Rats (Sprague-Dawley, P7–P53) were anesthetized with pentobarbital (400 mg/kg) and decapitated. Parasagittal cortical slices (350 μm) were cut using a vibrating slicer (Vibratome) in ice-cold artificial CSF (aCSF) containing 83 mM NaCl, 2.5 mM KCl, 0.5 mM $CaCl_2$, 3.3 mM $MgSO_4$, 1 mM NaH_2PO_4, 26.2 mM $NaHCO_3$, 22 mM glucose, and 72 mM sucrose equilibrated with 95% O_2 and 5% CO_2. Slices were maintained at room temperature until they were transferred to a microscope equipped with DIC optics (BX50; Olympus, Tokyo, Japan). All experiments were conducted at 30°C–32°C.

For patch-clamp recordings, slices were superfused with aCSF containing 119 mM NaCl, 5 mM KCl, 4 mM $CaCl_2$, 4 mM $MgSO_4$, 1 mM NaH_2PO_4, 26.2 mM $NaHCO_3$, 22 mM glucose, and 0.1 mM picrotoxin, equilibrated with 95% O_2 and 5% CO_2. For fEPSP experiments, aCSF was identical except for 2.5 mM KCl, 2.5 mM $CaCl_2$, and 1.3 mM $MgSO_4$. Baclofen (50 μM) was added to the aCSF when isolated LOT inputs were examined. Patch electrodes (3–5 MΩ) contained 130 mM D-Gluconic acid, 130 mM CsOH, 5 mM mM NaCl, 10 mM HEPES, 12 mM phosphocreatine, 3 mM MgATP, 0.2 mM NaGTP, and 10 mM EGTA. Series resistance, which was <20 MΩ, was compensated at 80%–95%. Responses were recorded with a Multiclamp 700A amplifier (Axon Instruments, Foster City, CA), filtered at 2 kHz and digitized at 10 kHz. (ITC-18; Instrutech, Mineola, NY). Data were collected and analyzed using Axograph (Axon Instruments) and IGOR Pro (Wavemetrics, Lake Oswego, OR). Unless stated otherwise, traces represent the average of five to ten sequential trials.

Developmental changes in AMPAR/NMDAR ratio were fit using $y(t) = A_{Base} + A_{Max}/[1 + \exp -((t - T_{Half})/(T_{Rate}))]$, where y(t) is the ratio after t weeks, A_{Base} and A_{Max} are the initial and maximal increase in ratio, respectively, T_{Half} is the half-time, and T_{Rate} is the rate.

For experiments in which asynchronous release was examined, 4 mM Sr^{2+} was substituted for 4 mM Ca^{2+}. Quantal events were

detected and captured within a 200 ms window beginning 200 ms after LOT stimulation using a sliding template algorithm. To test for silent synapses, we waited >10 min after rupture of the cell membrane. This period of washout, along with high intracellular Ca^{2+} buffer, prevented induction of LTP. Typically, ~100 stimuli were presented at 0.2–0.5 Hz at both –80 and +40 mV. Reponses and failures were sorted by eye. To ensure stability, ensemble averages of the first and second halves of each stimulus epoch were divided by the average amplitude of successful responses during the corresponding trials, yielding success rate. Cells in which there was a >10%–20% difference in success rates (7/59) were excluded. There was no change in the success rates recorded in the presence of APV at LOT (0.51 ± 0.083 [–80 mV] versus 0.47 ± 0.14 [+40 mV]; n = 5) and ASSN synapses (0.50 ± 0.056 [–80 mV] versus 0.52 ± 0.64 [+40 mV]; n = 5) confirming that differences in success rate were mediated by NMDAR-only synapses.

To examine the effect of glutamate uptake blockade on responses to exogenously applied glutamate, a UV flashlamp (T.I.LL. Photonics, Gräfelfing, Germany) was used to photolyze caged glutamate (50 μM, Sigma). The UV pulse was focused through a 60× objective onto an ~350 μm diameter region centered in layer Ia.

For fEPSPs, a recording electrode was placed halfway between the border of the LOT and layer Ib. I/O relationships were measured by isolating AMPAR- and NMDAR-mediated synaptic transmission by recording in the presence of D-APV (100 μM) or low Mg^{2+} (100 μM) and NBQX (5 μM), respectively.

For LTP experiments, fEPSPs were evoked (0.067 Hz) using a stimulus strength that yielded responses that were 1/3 of maximal. LTP was induced by theta-burst stimulation (TBS, 5 bursts at 5 Hz, burst = 5 pulses at 100 Hz), and the stimulus was set to yield a response that was 2/3 of maximal. "Weak" and "strong" tetani consisted of 4 or 12 TBS trains every 30 s. Four fEPSPs were averaged for each time point. The magnitude of LTP was determined 25–30 min after induction.

Naris occlusion was performed on neonatal rats (P1–P3) anesthetized by hypothermia. One nares was cauterized, covered with antibiotic (Fougera), and the pups returned to their dam. Some rats were maintained in an odor-enriched environment by placing a tea ball into their cages containing odors (sage oil). However, we did not observe differences between control animals and those exposed to odors, and results were pooled. For deprivation in older animals, rats (P28) were anesthetized with ketamine/xylazine (100:6 mg/kg) and occluded by cauterization or insertion of a nasal plug (Cummings and Brunjes, 1997) into one nostril. Naris closure was always confirmed prior to slice preparation. Brain slices from the deprived and control hemispheres were separated and relabeled so as to be blind to the experimenter.

Summary data are presented as mean ± SEM. Statistical significance was determined at $p \leq 0.05$.

Acknowledgments

We thank Gabe Murphy and Massimo Scanziani for helpful discussions. This work was supported by NIDCD R01 DC04682, a McKnight Scholar award, and a Klingenstein Fellowship.

Received: January 19, 2004
Revised: April 21, 2004
Accepted: May 17, 2005
Published: July 6, 2005

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