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RAPID PUBLICATION

NMDA Receptor-Mediated Currents are Prominent in the Thalamocortical Synaptic Response Before Maturation of Inhibition

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SUMMARY AND CONCLUSIONS

1. The *N*-methyl-D-aspartate subtype of glutamate receptor (NMDAR) is thought to underlie synaptic plasticity in both adult and developing CNS; however, its involvement in the thalamocortical synapse has not yet been directly demonstrated.

2. Whole-cell, thalamus-evoked synaptic currents were recorded from layer IV cells in slices of immature mouse somatosensory cortex.

3. Earlier than *postnatal day 9* the majority of responses were monosynaptic and purely excitatory, with both non-NMDAR and NMDAR-mediated glutamatergic components.

4. In older animals, disynaptic inhibitory currents summated with the excitatory ones and lowered the reversal potential of the response to voltages at which the NMDAR conductance is mostly blocked.

5. These findings suggest a cellular basis for the transient plasticity observed in layer IV during early postnatal development.

INTRODUCTION

Electrical activity plays a major role in sculpting synaptic circuits in the nervous system. Among the more striking examples are segregation of thalamocortical terminals into eye-specific stripes in cat and monkey visual cortex (LeVay et al. 1978, 1980) and into whisker-specific clusters, associated with structures called barrels, in rodent somatosensory cortex (Killackey et al. 1990). Each of these processes is dependent on normal sensory input during a time-window of plasticity restricted to early postnatal development (Jeanmonod et al. 1981; Mower et al. 1985). Analogy with other systems (Constantine-Paton et al. 1990) suggests that these phenomena are dependent on activation of the *N*-methyl-D-aspartate subtype of glutamate receptor (NMDAR). One would therefore expect NMDAR-mediated activity in the thalamocortical system to be time-correlated with the period of plasticity. Indeed, evoked single-unit responses in layer IV of kitten visual cortex are NMDAR-dependent initially but lose this dependency after the end of thalamocortical terminal segregation (Fox et al. 1989); other studies in cat (Hagihara et al. 1988) and turtle (Larson-Prior et al. 1991) suggest that thalamocortical responses in the adult visual cortex are mediated mainly by non-NMDA receptors. The cellular basis for the apparent loss of NMDAR-mediated activity in the adult animal remains unknown because the only reported loss of NMDAR binding sites in layer IV of cat visual cortex occurs several weeks after terminal segregation (Bode-Greuel and Singer 1989), and no loss has been reported in layer IV of the rodent barrel cortex (Jaarsma et al. 1991). We utilized a novel slice preparation of the mouse thalamocortical system (Agmon and Connors 1991) to follow, at the intracellular level, the developing roles of both NMDA and non-NMDA receptors in thalamocortical neurotransmission during early postnatal life.

METHODS

Thalamocortical slices, 400-μm thick, were prepared as described (Agmon and Connors 1991) from C57BL/6 mice (Simonsen) and were continuously superfused at room temperature with artificial cerebrospinal fluid (ACSF). ACSF composition was (in mM) 126 NaCl, 3 KCl, 1.25 NaH_2PO_4, 1.3 $MgSO_4$, 2.5 $CaCl_2$, 26 $NaHCO_3$, and 10 dextrose. Thalamocortical responses were evoked by 0.2-ms, 3- to 10-V cathodal pulses delivered at 0.1 Hz (or slower) to the lateral border of the ventrobasal nucleus of the thalamus (VB) by monopolar tungsten microelectrodes. Whole-cell recordings were obtained from layer IV as described (Blanton et al. 1989). Pipette solution included (in mM) 135 CsF, 1 $CaCl_2$, 2 $MgCl_2$, 11 ethylene glycol-bis(β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid (EGTA, dissolved in KOH), 10 *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES), titrated with KOH to pH 7.4 and adjusted to 270–280 mosM. In some experiments, 2–4 mM ATP were added to the pipette solution. Transillumination of slices clearly revealed cytoarchitectonic structures unique to layer IV (barrels), as well as the boundaries of VB, allowing placement of recording and stimulating microelectrodes under direct visual control.

RESULTS

Whole-cell synaptic currents evoked by electrical stimulation of the thalamus were recorded from 39 layer IV neurons of somatosensory (barrel) cortex in slices taken from 22 mouse pups, 3–17 days old. Responses were recorded as early as *postnatal day 3* (*P3*, *P0* being the first 24 h after birth), the day on which layer IV differentiates and barrels are formed (Rice and Van der Loos 1977). Responses could be elicited in >75% of cells sampled in neonatal (*P3–P8*) animals and in virtually all cells in juvenile (*P9–P17*) animals. Responses representative of neonatal animals are shown in Fig. 1. The synaptic current response (Fig. 1*A*) was monophasic at all holding potentials and reversed at a

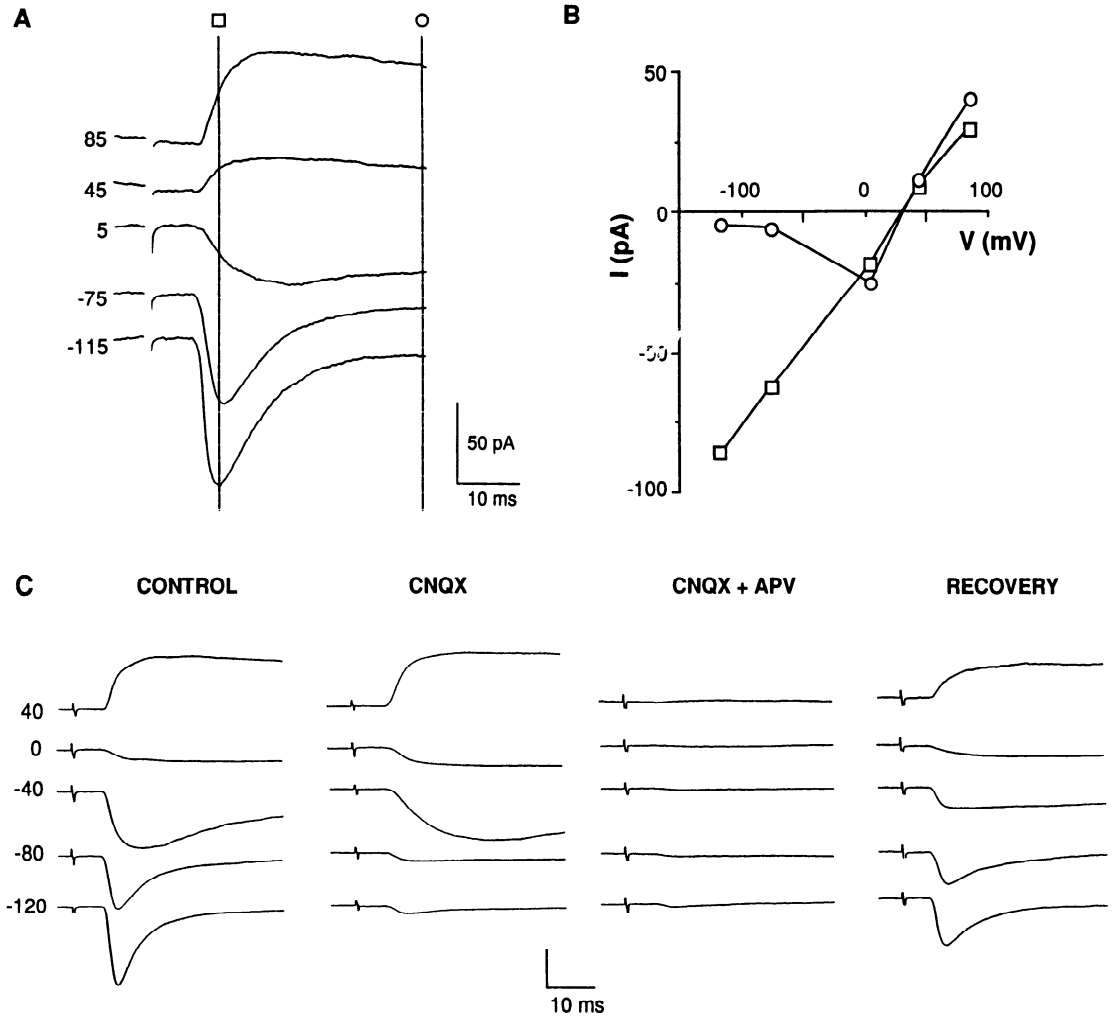

FIG. 1. Pure excitatory postsynaptic currents (EPSCs) in neonatal cells. *A*: voltage-clamped synaptic currents in *postnatal day 4* (*P4*) cell; holding potentials (in mV) indicated to the left of each trace. Each trace is a 5-point smoothed average of 8 sweeps. Dashed lines indicate time points for which current-voltage (*I-V*) plots in *B* were calculated. *B*: *I-V* plot calculated from the traces in *A*. Note that *I-V* curve is linear at 3 ms but has a zone of pronounced negative slope at 35 ms after response onset. *C*: pharmacological analysis of an EPSC in a *P7* cell. The fast component, which dominated the two most negative potentials in control artificial cerebrospinal fluid (ACSF), was blocked by 2.5 μM 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX). The remaining slow component, which was activated at depolarized potentials, was blocked by 50 μM DL-2-amino-5-phosphonovalerate (APV). Both components partially recovered 1 h after return to control ACSF. Vertical scale is 200 pA for control, 100 pA for the other panels.

positive potential, identifying it as an excitatory postsynaptic current (EPSC). Its short and invariant latency indicated that it was a direct (monosynaptic) thalamocortical response (Agmon and Connors 1992). The current-voltage (*I-V*) relationship of the response (Fig. 1*B*) was linear at early times after response onset (□) but exhibited a region of negative slope conductance typical of NMDAR-mediated currents (Hestrin et al. 1990) at late time points (○). The involvement of both NMDA and non-NMDA receptors in the neonatal response was tested pharmacologically in six cells (Fig. 1*C*). In all tested cells 6-cyano-7-nitroquinoxaline-2,3-dione (RBI) (CNQX), a specific non-NMDAR antagonist, blocked the fast component of the response, and DL-2-amino-5-phosphonovaleric acid (RBI) (APV), a specific NMDAR antagonist, blocked the remaining slow component. We conclude that, like various other monosynaptic pathways in the CNS (e.g., Hestrin et al. 1990), the neonatal thalamocortical response is a dual-component EPSC, mediated by both non-NMDA and NMDA receptors.

The majority (62%) of neonatal cells exhibited a pure excitatory response. In contrast, none of the juvenile cells exhibited responses that were exclusively excitatory. Rather, 65% of them, as well as the remaining 38% of neonatal cells, exhibited a composite, biphasic response. This response consisted of two distinct components, the onset of the second delayed by 2–3 ms relative to the first and thus identified as disynaptic (Fig. 2*A*, arrows). The reversal potential of the monosynaptic component of the composite response [18 ± 20 (SD) mV, $n = 22$] was not significantly different from that of the neonatal EPSC (13 ± 11 mV, $n = 8$) and did not show any age dependency. The reversal potential at the disynaptic peak, however, was significantly more negative, indicating activation of an inhibitory post-

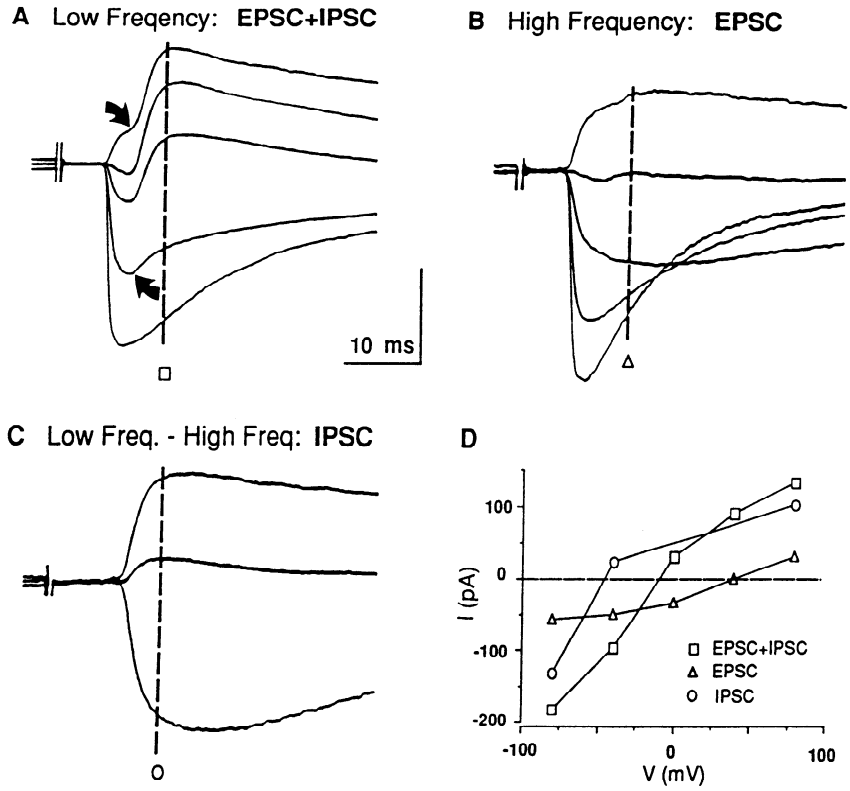

FIG. 2. Composite response [excitatory postsynaptic current-inhibitory postsynaptic current (EPSC-IPSC)] in a juvenile (*P9*) cell. *A*: low-frequency (0.08 Hz) stimulation elicited a biphasic response. Arrows denote onset of disynaptic inhibitory component. *B*: increasing stimulation frequency to 2 Hz resulted in suppression of the IPSC, uncovering a slow voltage-dependent component. The slow component was abolished by 50 μM APV (not shown). *C*: digitally isolated inhibitory component, calculated from the difference between the low-frequency and high-frequency response in the presence of 50 μM APV. High-frequency traces were scaled up before subtraction to match the peak of the monosynaptic response at low frequency. *D*: *I-V* plots for *A–C*, calculated at the time points designated by the dashed lines. Current traces shown were recorded at −80, −40, 0, 40, and 80 mV (*A* and *B*), and −80, −40, and 80 mV (*C*). Vertical scale bar is 100 pA (*A*), 33 pA (*B*), and 90 pA (*C*). All traces are averages of 8 sweeps. For abbreviations, see Fig. 1 legend.

synaptic current (IPSC). In animals younger than *P10*, the *I-V* curve of the composite response exhibited a negative slope at late time points (not shown), suggesting that the disynaptic IPSC was superimposed on the NMDAR-mediated component of the monosynaptic EPSC. Attempts to isolate this component by blocking GABAergic neurotransmission were unsuccessful because they triggered polysynaptic events (Luhmann and Prince 1990a,b) that occluded any underlying monosynaptic EPSC. This component was, however, demonstrated directly in the cell shown in Fig. 2. Increasing the frequency of stimulation suppressed the IPSC and revealed a nearly pure EPSC with a pronounced voltage-dependent component (Fig. 2*B*) that was blocked by APV (not shown). Digital subtraction of the high-frequency from the low-frequency response revealed the inhibitory component in isolation (Fig. 2*C*). The reversal potential at the disynaptic peak (Fig. 2*D*, □) was intermediate between the reversal potentials of the pure excitatory (△) and pure inhibitory (○) components. We conclude that the juvenile thalamocortical response is a composite synaptic response, consisting of a monosynaptic dual-component EPSC followed by a disynaptic IPSC and that the time course of the IPSC overlaps considerably with that of the NMDAR-mediated component of the EPSC.

The reversal potential at the disynaptic peak exhibited pronounced age-dependent changes. Figure 3 shows the reversal potential at the disynaptic peak for all 22 cells with a composite response (■) and, for comparison, the reversal potential at equivalent time points for all 8 cells with a pure EPSC (□). In *P9* and younger animals the disynaptic reversal potential values were widely scattered around a low negative average value [−9 ± 26 (SD) mV, n = 11]. A steep negative shift occurred between *P9* and *P11*, bringing the values to −58 ± 14 mV (n = 11) in *P11* and older animals. Because of the voltage-dependent blockade of the NMDAR, at voltages below −60 mV less than 10% of the total NMDAR-mediated conductance is available for activation (Hestrin et al. 1990). Since under physiological conditions inhibitory inputs in the neocortex tend to bring the membrane potential of the cell very close to the inhibitory equilibrium potential (Connors et al. 1988), we conclude that in *P11* and older animals activation of the thalamocortical synapse will result in very little recruitment of NMDAR-mediated currents in layer IV.

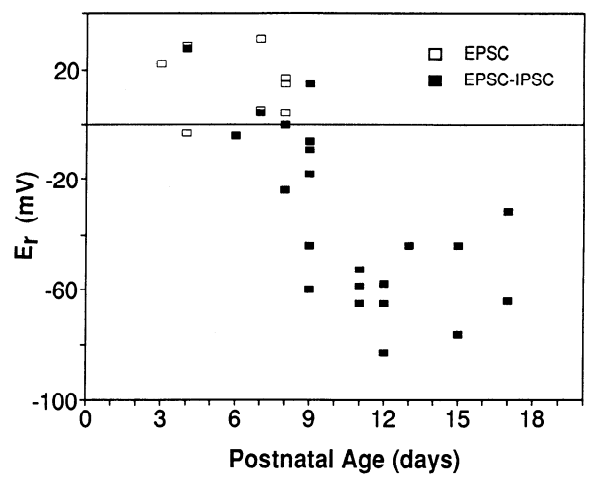

FIG. 3. Age-dependent change in the reversal potential at the disynaptic peak. Reversal potentials were measured at the disynaptic peak for all 22 cells that exhibited a composite response (■) and at equivalent time points after response onset for all 8 cells with a pure monosynaptic response (□). Nine additional juvenile cells exhibited a pure or nearly pure inhibitory postsynaptic current and are not included.

DISCUSSION

Our results indicate that thalamocortical synaptic responses can be elicited in mouse layer IV neurons as early as *P3*, and in deeper layers as early as *P0* (Agmon and O'Dowd 1990), a week earlier than single-unit responses were previously recorded in the rat (Armstrong-James 1975). These responses have both NMDAR and non-NMDAR-mediated components. NMDAR-mediated spontaneous and evoked activity was previously reported in neonatal rat neocortex (LoTurco et al. 1991; Yuste and Katz 1991), and sensory stimulation evokes NMDAR-dependent responses in kitten visual cortex (Fox et al. 1989; Tsumoto et al. 1987). Our data localize the NMDAR-mediated activity to the thalamocortical synapse and provide intracellular evidence that thalamocortical neurotransmission is mediated by both major types of excitatory amino acid receptors. The NMDAR-mediated component of this synapse is most prominent during the first postnatal week and is thus present at the appropriate place and time to participate in segregation of thalamocortical terminals and in morphogenesis of barrels.

Consistent with previous reports from cat (Komatsu 1983) and rat (Luhmann and Prince 1991) neocortex, we found that maturation of inhibitory synaptic responses was delayed relative to excitatory ones; however, in layer IV we encountered immature disynaptic IPSCs as early as *P4*, again ~1 wk earlier than previously reported in the rat. The IPSCs apparently mature in the first one-half of the second postnatal week, as can be judged by the steep negative shift in the reversal potential of the disynaptic peak. Part of this shift could have been due to developmental changes in the inhibitory equilibrium potential (Luhmann and Prince 1991); however, the major factor was most likely an increase in inhibitory conductance, due to establishment and/or maturation of corticocortical inhibitory synapses (Lund and Harper 1991; Miller 1986). By mid second postnatal week, IPSCs were strong enough to bring the reversal potential of the thalamocortical response below threshold for NMDAR activation and thus presumably reduce the capacity of the thalamocortical synapse to undergo anatomic and physiological reorganization. Indeed at least one study (Seo and Ito 1987) suggests that the barrel cortex loses its capacity for reorganization around *P10*, coincident with the maturation of inhibition described here. A link between maturation of inhibition and loss of polysynaptic NMDAR-mediated activity in upper layers of rat neocortex has previously been proposed (Luhmann and Prince 1990a,b); our data suggest that a similar process occurs, ~1 wk earlier, in the thalamocortical synapse in layer IV. One corollary of this hypothesis is that even beyond the age range studied here, any process that reduces the efficacy of inhibition may cause unblocking of the NMDAR-mediated component and thus potentially restore synaptic plasticity in the adult animal.

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