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

ARIEL AGMON AND DIANE K. O'DOWD
Departments of Anatomy and Neurobiology, Developmental and Cell Biology, University of California, Irvine, California 92717

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.

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 NaH₂PO₄, 1.3 MgSO₄, 2.5 CaCl₂, 26 NaHCO₃, 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 CaCl₂, 2 MgCl₂, 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 5 (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. 1A) 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.

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. 2A, 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-
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. 2B) 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. 2C). The reversal potential at the disynaptic peak (Fig. 2D) 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.
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.

We are deeply grateful to Dr. Edward Jones for strong commitment and generous support for this study. We thank Drs. Barry Connors, Michael Gutnick, Leslie Henderson, Edward Jones, Martin Smith, and Richard Warren for comments on earlier versions of this manuscript and Dr. Joseph LoTurco for advice regarding the recording technique.

This study was supported by National Institute of Neurological Disorders and Stroke Grants NS08364 (A. Agmon), NS21377 (E.G. Jones), and NS27501 (D.K. O'Dowd), and by National Institute of Aging Training Grant AG-00096 (A. Agmon).

Received 21 February 1992; accepted in final form 2 April 1992.

REFERENCES

Agmon, A. and Connors, B. W. Thalamocortical responses of mouse somatosensory (barrel) cortex in vitro. Neuroscience 41: 363–379, 1991.
Agmon, A. and Connors, B. W. Correlation between intrinsic firing patterns and thalamocortical synaptic responses of neurons in mouse barrel cortex. J. Neurosci. 17: 519–539, 1992.
Agmon, A. and O'Dowd, D. K. Development of thalamocortical responses in barrel cortex of early postnatal mice. Soc. Neurosci. Abstr. 16: 631, 1990.
Armstrong-James, M. The functional status and columnar organization of single cells responding to cutaneous stimulation in neonatal rat somatosensory cortex SI. J. Physiol. Lond. 246: 501–538, 1974.
Blanton, M. G., LoTurco, J. J., and Kriegstein, A. R. Whole cell recording from neurons in slices of reptilian and mammalian cerebral cortex. J. Neurosci. Methods. 30: 203–210, 1989.
Bode-Greuel, K. M. and Singer, W. The development of N-methyl-D-aspartate receptors in cell visual cortex. Dev. Brain Res. 46: 197–204, 1989.
Connors, B. W., Malenka, R. C., and Stiver, I. R. Two inhibitory postsynaptic potential genes and GABA,, and GABA, receptor-mediated responses in neocortex of rat and cat. J. Physiol. Lond. 406: 443–468, 1989.
Constantine-Paton, M., Cline, H. T., and Debski, E. Patterned activity, synaptic convergence and the NMDA receptor in developing visual pathways. Ann. Rev. Neurosci. 13: 129–154, 1990.
Fox, K., Sato, H., and Daw, N. The location and function of NMDA receptors in cat and kitten visual cortex. J. Neurosci. 9: 2443–2454, 1989.
Hagiwara, K., Tsumoto, T., Sato, H., and Hata, Y. Actions of excitatory amino acid antagonists on geniculo-cortical transmission in the cat's visual cortex. Exp. Brain Res. 69: 407–416, 1988.
Hestrin, S., Nicoll, R. A., Perkel, D. J., and Sah, P. Analysis of inhibitory synaptic action in pyramidal cells using whole-cell recording from rat hippocampal slices. J. Physiol. Lond. 422: 203–225, 1990.
Jaarsma, D., Seden, J. B., and Korf, J. Localization of NMDA and AMPA receptors in rat barrel field. Neurosci. Lett. 133: 233–236, 1991.
Jeanmonod, D., Roff, F. I., and Van der Loos, H. Mouse somatosensory cortex: alterations in the barrelfield following receptor injury at different early postnatal ages. Neurosciences 6: 1301–1335, 1981.
Killackey, H. P., Jocquin, M. F., and Rhoades, R. W. Development of somatosensory system structures. In: Development of Sensory Systems in Mammals, edited by J. R. Coleman. New York: Wiley, 1990, p. 403–429.
Komatsu, Y. Development of cortical inhibition in kitten striate cortex investigated by a slice preparation. Dev. Brain Res. 8: 196–198, 1983.
Larson-Prior, L. J., Ultsch, P. S., and Slater, N. T. Excitatory amino acid receptor-mediated transmission in geniculocortical and intracortical pathways within visual cortex. J. Neurophysiol. 66: 293–306, 1991.
LeVay, S., Strayer, M. P., and Shatz, C. J. Ocular dominance columns and their development in layer IV of the cat's visual cortex: a quantitative study. J. Comp. Neurol. 179: 223–244, 1978.
LeVay, S., Wiesel, T. N., and Hubel, D. H. The development of ocular dominance columns in normal and visually deprived monkeys. J. Comp. Neurol. 191: 1–51, 1980.
LoTurco, J. J., Blanton, M. G., and Kriegstein, A. R. Initial expression and endogenous activation of NMDA channels in early neocortical development. J. Neurosci. 11: 792–799, 1991.
Luhmann, H. J. and Prince, D. A. Transient expression of polysynaptic NMDA receptor-mediated activity during neocortical development. Neurosci. Lett. 111: 109–115, 1990a.
NMDA CURRENTS IN THE IMMATURE THALAMOCORTICAL SYNAPSE

LUHMANN, H. J. AND PRINCE, D. A. Control of NMDA receptor mediated activity by GABAergic mechanisms in mature and developing rat neocortex. *Dev. Brain Res.* 54: 287–290, 1990b.

LUHMANN, H. J. AND PRINCE, D. A. Postnatal maturation of the GABAergic system in rat neocortex. *J. Neurophysiol.* 65: 247–263, 1991.

LUND, J. S. AND HARPER, T. R. Postnatal development of thalamic recipient neurons in the monkey striate cortex. III. Somatic inhibitory synapse acquisition by spiny stellate neurons of layer 4C. *J. Comp. Neurol.* 309: 141–149, 1991.

MILLER, M. W. Maturation of rat visual cortex. III. Postnatal morphogenesis and synaptogenesis of local circuit neurons. *Dev. Brain Res.* 25: 271–285, 1986.

MOWER, G. D., CAPLAN, C. J., CHRISTEN, W. G., AND DUFFY, F. H. Dark rearing prolongs physiological but not anatomical plasticity of the cat visual cortex. *J. Comp. Neurol.* 235: 448–466, 1985.

RICE, F. I. AND VAN DER LOOS, H. Development of the barrels and barrel field in the somatosensory cortex of the mouse. *J. Comp. Neurol.* 171: 343–360, 1971.

SEO, M. L. AND ITO, M. Reorganization of rat vibrissa barreelfield as studied by cortical lesioning on different postnatal days. *Exp. Brain Res.* 65: 251–260, 1987.

TSUMOTO, T., HAGIHARA, K., SATO, H., AND HATA, Y. NMDA receptors in the visual cortex of young kittens are more effective than those of adult cats. *Nature Lond.* 327: 513–514, 1987.

YUSTE, R. AND KATZ, L. C. Control of postsynaptic Ca\(^{2+}\) influx in developing neocortex by excitatory and inhibitory neurotransmitters. *Neuron* 6: 333–344, 1991.