Progression of Change in NMDA, non-NMDA, and Metabotropic Glutamate Receptor Function at the Developing Corticothalamic Synapse

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Golshani, Peyman, Richard A. Warren, and Edward G. Jones. Progression of change in NMDA, non-NMDA, and metabotropic glutamate receptor function at the developing corticothalamic synapse. J Neurophysiol. 80: 143 ± 154, 1998. The development of receptor function at corticothalamic synapses during the first 20 days of postnatal development is described. Whole cell excitatory postsynaptic currents (EPSCs) were evoked in relay neurons of the ventral posterior nucleus (VP) by stimulation of corticothalamic fibers in in vitro slices of mouse brain from postnatal day 1 (P1). During P1–P12, excitatory postsynaptic conductances showed strong voltage dependence at peak current and at 100 ms after the stimulus and were almost completely antagonized by DL-2-amino-5-phosphonopentoic acid (APV), indicating that N-methyl-D-aspartate (NMDA) receptor-mediated currents dominate corticothalamic EPSCs at this time. After P12, in 42% of cells, excitatory postsynaptic conductances showed no voltage-dependence at peak current but still showed voltage-dependence 100-ms poststimulus. This voltage-dependent conductance was antagonized by APV. The nonvoltage-dependent component was APV resistant, showed fast decay, and was antagonized by the non-NMDA antagonist, 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX). In the remaining 58% of cells after P12, excitatory postsynaptic conductances showed moderate voltage dependence at peak conductance and strong voltage dependence 100 ms after the stimulus. Analysis of EPSCs before and after APV showed a significant increase in the relative contribution of the non-NMDA conductance after the second postnatal week. From P1 to P16, there was a significant decrease in the time constant of decay of the NMDA EPSC but no change in the voltage dependence of the NMDA response. After P8, slow EPSPs, 1.5–30 s in duration and mediated by metabotropic glutamate receptors (mGluRs), could be evoked by high-frequency stimulation of corticothalamic fibers in the presence of APV and CNQX. Similar slow depolarizations could be evoked by local application of the mGluR agonist (±)-1-amino-cyclopentane-trans-1,3-dicarboxylic acid (t-ACPD) but from P0. Both conductances were blocked by the mGluR antagonist, (RS)-α-methyl-4-carboxyphenylglycine. Hence functional mGluR receptors are present on VP cells from birth, but their synaptic activation at corticothalamic synapses can only be detected after P8. In voltage clamp, the extrapolated reversal potential of the t-ACPD current, with potassium gluconate-based internal solution, was +12 ± 10 (SE) mV, and the measured reversal potential with cesium gluconate-based internal solution was 1.5 ± 9.9 mV, suggesting that the mGluR-mediated depolarization was mediated by a nonselective cation current. Replacement of NaCl in the external solution caused the reversal potential of the current to shift to −18 ± 2 mV, indicating that Na + is a charge carrier in the current. The current amplitude was not reduced by application of Cs +, Ba 2+, and Cd 2+, indicating that the t-ACPD current was distinct from the hyperpolarization-activated cation current (Ih) and distinct from certain other previously characterized mGluR-activated, nonselective cation conductances.

INTRODUCTION

The corticothalamic projection that arises from cells of layers V and VI of the cerebral cortex exerts its effects in the thalamus on both relay neurons and on neurons of the reticular nucleus (RTN) (Jones 1985). Ultrastructural, neurochemical, and physiological studies all indicate that the corticothalamic projection is excitatory and glutamatergic (Baughman and Gilbert 1981; Eaton and Salt 1966; Jones and Powell 1969; Kao and Coulter 1997). Although this projection constitutes the largest synaptic input onto thalamic relay neurons (Liu et al. 1995; Sherman and Koch 1986), its functional role remains unclear. Recent studies in the cat lateral geniculate nucleus (dLGN) suggest that the corticothalamic projection induces correlated firing in relay cells in response to oriented moving contours, increasing the gain of input for feature-linked events detected by the cortex (Sillito et al. 1994). Furthermore, cortical feedback onto dLGN cells has been shown to engage inhibitory mechanisms that reinforce surround mechanisms to engage low spatial frequency cutoffs in the dLGN (Cudeiro and Sillito 1996).

The corticothalamic projection also potentiates the genesis of spindle (7–14 Hz) (Contreras and Steriade 1996) and delta (1–4 Hz) (Steriade et al. 1991) oscillations and synchronizes spindle oscillations across large distances in the thalamus (Contreras et al. 1996, 1997). In addition, high-frequency stimulation of corticothalamic fibers in the adult dLGN in vitro can shift the firing mode of thalamocortical relay neurons from burst firing, which underlies spindling, to the tonic or relay mode of discharge through activation of metabotropic glutamate receptors (mGluRs) (McCormick and von Krosigk 1992), suggesting a neuromodulatory role for the corticothalamic projection.

While the development of receptor function at the thalamocortical synapse has been examined in several recent studies (Agmon and O’Dowd 1992; Agmon et al. 1996; Craig and Malenka 1995), no developmental studies of corticothalamic function have been made. The development of receptor function at the corticothalamic synapse may be important during the activity-dependent refinement of sensory maps at thalamic and cortical levels in the course of development.
and during the shaping of sensory receptive fields of neurons in the immature cortex and thalamus. The development of thalamic oscillations that result from reciprocal synaptic activation of RTN and relay neurons also may depend on the maturation of the corticothalamic system. These oscillations are evoked readily by stimulation of corticothalamic fibers in rodents older than ~2 wk (Warren et al. 1994) but cannot be elicited in the early postnatal period (Warren and Jones 1997). It has been hypothesized that oscillations cannot be evoked in the early postnatal period because of the immaturity of intrinsic membrane conductances in RTN and relay cells, in addition to the lower (more depolarized) reversal potential of \( \gamma \)-aminobutyric acid-A (GABA\(_A\))-mediated chloride currents at RTN-relay cell synapses (Warren and Jones 1997). Failure of immature corticothalamic synapses to effectively depolarize RTN and/or relay neurons also may play a substantial role in the lack of low-frequency oscillations in the early postnatal period.

This study characterizes developmental changes occurring in glutamatergic receptor function at the corticothalamic synapse in mouse ventroposterior nucleus (VP) neurons in vitro. The study attempts to answer the following questions: When do corticothalamic synapses become functional? What are the relative contributions of N-methyl-d-aspartate (NMDA) and non-NMDA receptors in generating synaptic currents at different stages of development? Can mGluR mediated excitatory postsynaptic potentials (EPSPs) be evoked by stimulation of corticothalamic fibers, and if so, at what developmental stage can the first mGluR EPSPs be observed? And which ionic conductances underlie the postsynaptic effects of mGluR activation in developing thalamic relay neurons?

**METHODS**

Postnatal day 0 (P0; the day of birth) to P20 ICR mice (Harlan-Sprague Dawley) were anesthetized by hypothermia (P0–P4) or with ether (P5–P20) and decapitated. The brain was quickly removed and put in chilled artificial cerebrospinal fluid (ACSF) containing (in mM) 126 NaCl, 3 KCl, 1.25 NaH\(_2\)PO\(_4\), 1.4 MgSO\(_4\), 2.5 CaCl\(_2\), 26 NaHCO\(_3\), and 20 dextrose (pH 7.4 when bubbled with 95% O\(_2\)-5% CO\(_2\), osmolarity 300–315 mOsm). Slices (400–500 \( \mu \)m thick) containing the somatosensory cortex, RTN, and VP were cut at an angle that preserves corticothermal and thalamocortical connectivity (Agmon and Connors 1991); the somatosensory cortex was later dissected from the slice. Slices were transferred to a submersion type chamber, superfused with ACSF aerated with 95% O\(_2\)-5% CO\(_2\), and allowed to recover for 1 h before recording. In one experiment in which four cells were recorded, NaCl was replaced by sucrose on an equiosmolar basis in the dissection solution and the slice recovered for 20 min while superfused with a 50.0:5.0 solution of NaCl/sucrose (Aghajanian and Rasmussen 1989). The results obtained from this experiment were not different from results obtained in the other experiments. Experiments were performed at room temperature (22–25°C) except for the investigation of the metabotropic response. These experiments were performed at 35°C to ensure that second-messenger systems were being activated at physiological temperatures.

Whole cell recording pipettes were pulled from borosilicate glass on a Narishige PP-83 two-stage puller and had resistances of 3–5 MΩ. Internal solutions (in mM) included the following: 1) 120 potassium gluconate, 10 N-2-hydroxyethylpiperezine-N‘-2-ethanesulfonic acid (HEPES), 1 ethylene glycol-bis(\( \beta \)-aminoethyl ether)-N,N,N’,N’-tetraacetic acid (EGTA), 2 MgCl\(_2\), 0.1 CaCl\(_2\), 20 NaCl, and 2 Na\(_2\)ATP, (0.5 NaGTP was added in certain experiments as indicated in RESULTS), pH adjusted to 7.2–7.4 with KOH; and 2) 120 CsOH, 120 d-gluconic acid, 10 HEPES, 1.1 EGTA, 2 MgCl\(_2\), 0.1 CaCl\(_2\), 20 NaCl, and 2 Na\(_2\)ATP (0.5 NaGTP and 3 QX-314 were added in certain experiments as indicated in RESULTS), pH adjusted to 7.2–7.4 with CsOH. Osmolarity was calibrated to 290±300 mOsm. Signals were recorded with an Axoclamp-2A or an Axopatch 200B amplifier (Axon Instruments, Foster City, CA) with bridge mode and continuous single electrode voltage clamp for current and voltage-clamp recordings, respectively. Access resistance was <30 MΩ and was compensated in bridge mode. Data were digitized via a CED 1401plus interface (Cambridge Electronic Design, Cambridge, UK) at 0.15–10 kHz and analyzed with CED software packages. All traces, except those obtained from the ACPD application experiments, are typically averages of two to four traces.

Corticothermal synaptic responses were evoked in VP neurons in the presence of bicuculline methochloride (BMC) by electrical stimulation of corticothalamic fibers in the internal capsule or in the RTN, using a monopolar tungsten microelectrode as previously described (Warren et al. 1994). In this preparation, no other afferents to the VP nucleus pass through the region stimulated, and because thalamocortical fibers have no intra-VP collaterals, excitatory synaptic responses in VP cells are due to corticothermal stimulation. For experiments investigating ionotropic responses, the stimulation paradigm consisted of single 0.1 ms cathodal stimuli delivered at 0.05–0.2 Hz. For experiments investigating mGluR responses, the stimulation paradigm consisted of trains of 0.1-ms stimuli (10) at 100 Hz delivered every 60 s.

The mGluR agonist, (\( \pm \))-1-aminoheptocyclopentane-trans,3,1-dicarboxylic acid (\( \pm \)-ACPD), was released locally onto VP neurons by applying a brief pulse of nitrogen gas (5–200 ms) to the back of a broken microelectrode (tip diameter 5–15 \( \mu \)m) or to the back of a patch pipette containing the drug in solution.

Drugs used included (in \( \mu \)M) 10 bicuculline methochloride (BMC), 50 \( \mu \)-d-2-amino-5-phosphonovaleric acid (APV), 20 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), 20–200 2-hydroxy-saclofen (2OH-S), 100 CGP-35348, 400 \( \alpha \)-methyl-4-carboxyphenylmethylglycine (MCPG), and 0.5–1.0 tetrodotoxin (TTX). BMC, CNQX, and 2OH-S were purchased from RBI (Natick, MA). TTX was purchased from Sigma (St. Louis, MO). APV was purchased from RBI and from Sigma. CGP-35348 was a gift from CIBA-Geigy (Basel). \( \pm \)-ACPD and MCPG were purchased from Tocris Cookson (Ballwin, MO).

**RESULTS**

This study is based on data collected from whole cell recordings of 254 VP neurons at P0–P16. Previous studies show that by P20, VP cells have acquired all the membrane properties of mature neurons (Warren and Jones 1997; Warren et al. 1994) All cells, except those recorded in the presence of TTX, were recorded in the presence of BMC to block GABA\(_A\)-receptor-mediated, inhibitory postsynaptic events evoked by activation of RTN. All cells recorded with a potassium gluconate-based solution in the pipette had membrane potentials more negative than ~60 mV and fired overshooting action potentials in response to depolarizing current pulses.

**NMDA-receptor-mediated currents dominate the corticothermal EPSC during early postnatal development**

To investigate the evoked ionotropic, corticothalamic excitatory postsynaptic current (EPSC), 92 cells were recorded...
At room temperature with cesium gluconate and QX-314 in the pipette, to facilitate voltage clamp and block GABA<sub>B</sub>-mediated inhibitory postsynaptic currents (IPSCs) (Nathan et al. 1990). No corticothalamic EPSCs could be elicited at P0 (n = 4) by cathodal stimulation of corticothalamic fibers, but they could be evoked on P1 and at later ages. Between P1 and P12, in 90% (47/52) of VP neurons, corticothalamic excitatory postsynaptic conductances showed strong voltage dependence at both the peak of the current and 100 ms after the stimulus, suggesting dominance of the EPSC by NMDA-receptor–mediated currents (Fig. 1A). At membrane potentials near the resting membrane potential, EPSCs consisted of a very low-amplitude inward current, which decayed within 100–300 ms. In some records, a small fast component could be observed preceding the slower component (Fig. 1A, →). Hyperpolarization of the membrane typically led to a decrease in the amplitude of the slow current and at times unmasked the small amplitude fast current. With depolarization of the membrane to −40 to −20 mV, the amplitude of the inward current increased dramatically, delimiting a zone of negative conductance on current versus voltage plots. The inward current reversed to an outward current at around 0 mV and showed a linear relationship to voltage at holding membrane potentials between −20 and +50 mV. At membrane potentials positive to +10 mV, outward currents were large in amplitude and decayed slowly during several hundred milliseconds.

In the remaining 10% (5/52) of VP neurons between P1 and P12, corticothalamic EPSCs were similar to those recorded in neurons from animals older than P12 described below.

Pharmacological analysis of the corticothalamic EPSC, between P1 and P12 (n = 9), confirmed that in almost all of the cells (8/9), the corticothalamic EPSC was mediated largely by NMDA receptors during this developmental period. Bath application of the NMDA receptor antagonist, APV, blocked 90–100% of the current at the peak of the EPSC (n = 8), and this APV-sensitive current returned after washout of the drug (n = 5; Fig. 2). The APV-insensitive current was typically very small in amplitude and was linearly related to holding membrane potential (Fig. 2, →). In contrast, application of the non-NMDA antagonist, CNQX, had no effect on the corticothalamic EPSC elicited in most cells between P1–P12 (n = 5). In two cells where an effect could be seen, it consisted of a blockade of the small amplitude fast current recorded at hyperpolarized membrane potentials.

In a large proportion of P13–P20 VP neurons (15/36;
but one case \( (n = 5; \text{Fig. 4}) \) In the one case, \( (\text{Fig. 4}) \), a low-amplitude current, reversing \( \sim 0 \text{ mV} \) was resistant to CNQX and APV. This current probably was mediated by the mGluR-activated cation conductance, discussed later. Blockade of the non-NMDA-mediated response was also reversible by washout of the drug \( (n = 2) \).

To quantify the relative increase in non-NMDA-mediated currents after P12, corticothalamic EPSCs were evoked at various membrane potentials between +40 and \( -80 \text{ mV} \) before and after application of APV. Total conductance at peak current was measured before APV by measuring the slope of the \( I-V \) curve at holding potentials between 0 and 40 \text{ mV} \), where all \( I-V \) relations are linear due to the voltage-dependent relief of \( Mg^{2+} \) block of the NMDA receptor. The non-NMDA conductance was measured by measuring the slope of the linear \( I-V \) curve after APV. The relative non-NMDA contribution was expressed as the non-NMDA conductance/total conductance and was plotted versus development age (Fig. 3). There was a significant increase in the relative non-NMDA contribution to the peak EPSC conductance after the second postnatal week \( (P < 0.05) \).

**Developmental changes in NMDA-receptor-mediated EPSCs at the corticothalamic synapse**

To characterize developmental changes in the voltage dependence and decay kinetics of the NMDA-receptor-mediated components of the corticothalamic EPSC, EPSCs were recorded from 40 P1–P16 VP neurons at room temperature with cesium and QX-314 in the pipette and BMC and CNQX in the bath. NMDA-receptor-mediated excitatory postsynaptic conductances recorded in neurons of all ages showed strong nonlinear voltage dependence, and no detectable changes in this parameter could be discerned in animals of increasing age (Fig. 5). The time constant of decay was determined at +40 mV for all cells. The decay phase of the NMDA EPSC could be fitted by a single exponential in almost all cases \( (37/40 \text{ cases}) \). Otherwise, the decay time constant was found by determining the time after peak at which the current had decayed to \( 1/e \) of its peak value. Linear regression analysis revealed a statistically significant decrease in the time constant of decay of corticothalamic currents \( (R^2 = 0.3689, P < 0.02, n = 40; \text{Fig. 6}) \). Access resistance of the recordings was not correlated with development \( (R^2 = 0.007, P = 0.608, n = 40) \). There was no significant change in the reversal potential of NMDA-receptor-mediated currents.

**Development of mGluR function at the corticothalamic synapse**

mGluR-mediated, evoked corticothalamic EPSPs and EPSCs were recorded with pipettes containing potassium gluconate-based internal solution plus ATP and GTP and in the presence of BMC at 35°C in 46 VP neurons. The NMDA receptor antagonist, APV, and the non-NMDA receptor antagonist, CNQX, also were present during recordings in 12 of these neurons. The GABA\(_A\) receptor antagonists, 2OH-S and CGP-35348, were present in two and four recordings, respectively.

All neurons recorded in BMC responded to single-pulse
stimulation of corticothalamic fibers with fast ionotropic EPSPs, and therefore absence of a metabotropic response in a percentage of these cells was not a result of lack of afferent input to the cell from fibers traversing the stimulation site. mGluR-mediated synaptic responses typically were evoked by stimulation of corticothalamic fibers with a train of 10 cathodal pulses at 100 Hz. mGluR-mediated EPSP(C)s could be evoked from P8 but were not observed in eight cells recorded in P3–P7 slices. The mGluR-mediated EPSP(C)s were found in 63% (24/38) of P8–P16 cells. EPSPs were typically 1–10 mV in amplitude and 3–30 s in duration. mGluR-mediated, slow EPSP(C)s could be evoked in the presence of the ionotropic glutamate receptor antagonists, APV and CNQX (n = 10; Fig. 7A), but were antagonized reversibly by the mGluR antagonist, MCPG (Fig. 7B; n = 2). The slow EPSP was at times preceded by a 1- to 2-s hyperpolarization, which decayed before commencement of the depolarization and probably resulted from activation of GABA_B receptors, either by direct stimulation of RTN neu-

FIG. 4. EPSCs recorded from a P16 neuron in response to stimulation of corticothalamic fibers before (control), during 6-cyano-7-nitroquinolinic acid (CNQX) and APV, and after (wash) the addition of CNQX and CNQX and APV to the perfusing medium. Note that CNQX blocks the large-amplitude, fast-decaying current recorded at hyperpolarized potentials, whereas APV blocks the remaining voltage-dependent, slow-decaying conductance, confirming that after P12, both NMDA and non-NMDA–receptor-mediated EPSCs can be evoked by stimulation of corticothalamic fibers. Also note that both NMDA and non-NMDA–receptor-mediated EPSCs almost completely recover after washout of APV and CNQX. All recordings were performed with BMC in the bath.

FIG. 5. NMDA-receptor–mediated corticothalamic EPSCs recorded in P4 (left) and P13 (right) VP neurons in the presence of CNQX and BMC in the bath and cesium gluconate and ATP in the pipette (top), and current voltage relationships (bottom). Note that NMDA-receptor–mediated EPSCs in both neurons showed similar voltage dependence.

FIG. 6. Scatter plot and linear regression of the decay time constant of corticothalamic NMDA-mediated currents in relation to age.
FIG. 7. A: slow EPSC-mediated by metabotropic glutamate receptors (mGluR) recorded in a P13 VP neuron and elicited by high-frequency stimulation of corticothalamic fibers (train of 10 stimuli at 100 Hz). Note that the slow EPSC is ~30 s in duration. A faster inhibitory postsynaptic current (IPSC), <1 s in duration and probably mediated by activation of γ-aminobutyric acid-B (GABA_B) receptors, precedes the mGluR-mediated EPSC. B: effects of (RS)-α-methyl-4-carboxyphenylglycine (MCPG) on a slow EPSP (→) mediated by mGluR recorded in a P16 VP neuron elicited by high-frequency stimulation of corticothalamic fibers (train of 10 stimuli at 100 Hz). Note that MCPG (500 μM) reversibly antagonizes the mGluR-mediated slow EPSP. Inhibitory postsynaptic potential (IPSP) preceding the excitatory postsynaptic potential (EPSP) probably results from activation of GABA_B receptors. C: mGluR-mediated EPSCs recorded in a P16 VP neuron in response to high-frequency stimulation (train of 10 stimuli at 100 Hz) of corticothalamic fibers. Note that the mGluR-mediated current is inward at all holding membrane potentials between −40 and −100 mV and tends to increase with hyperpolarization of the membrane, suggesting that the EPSC does not result from closing of leak K⁺ channels. Cell firing was evoked during the stimulation at −40 mV. IPSC preceding the slow EPSC probably result from indirect activation of GABA_A receptors. All recordings were performed with BMC, APV, and CNQX in the bath and with a potassium gluconate-based internal solution and ATP and GTP in the pipette.

ions or through synaptic activation of RTN cells by stimulation of corticothalamic and/or thalamocortical fiber collaterals in the internal capsule (Fig. 7B). In VP cells in which mGluR-mediated responses were sufficiently large to allow further characterization in voltage clamp, the mGluR-mediated EPSC was inward at all holding membrane potentials between −35 and −120 mV, and typically increased in amplitude with hyperpolarization of the membrane (Fig. 7C; n = 4), suggesting that the slow EPSC did not result from closure of leak K⁺ channels, as previously suggested in adult cat dorsal lateral geniculate nucleus (McCormick and von Krosigk 1992).

To facilitate the study of mGluR-mediated currents and to determine whether functional mGluR receptors unassociated with corticothalamic synapses are present on developing VP neurons before P8, the mGluR agonist, t-ACPD (400 μM), was applied locally to VP neurons recorded in the whole cell mode. All experiments were done at 35°C. Eighty-eight VP neurons were recorded with cesium gluconate, ATP, and GTP in the pipette. Puffs of the vehicle solution did not result in activation of current. Local application of t-ACPD to VP neurons evoked a slow depolarization as early as postnatal day 0 and at all later ages (Fig. 8A). Slow depolarizations were 5–20 mV in amplitude and 20–60 s in duration and, in some cells (5/26), led to a prolonged tonic barrage of action potentials crowning the slow depolarization (Fig. 8A). The t-ACPD–induced slow depolarization was reversibly blocked by bath application of the mGluR-specific antagonist, MCPG (500 μM; n = 2). Continuous hyperpolarizing voltage steps in voltage clamp demonstrated that two of six cells at P0–P3 and five of seven cells at P6–P9, showed increases in input conductance during the slow inward current, whereas the remainder showed no change in input conductance during the t-ACPD–induced current (Fig. 8B). In contrast, in two of four P13–P14 VP neurons, the t-ACPD–induced current was associated with a decrease in input conductance, in one of four with an increase in input conductance, and in the remaining one of four, there was no change in this parameter.
FIG. 8. A: slow depolarization elicited by focal application of (±)-1-aminocyclopentane-trans-1,3-dicarboxylic acid (t-ACPD; 400 μM) and crowned by a tonic barrage of action potentials recorded from a P9 VP neuron in the presence of BMC. Some action potentials appear truncated because of the low digitization frequency. B: voltage-clamp trace from the same neuron in which the membrane potential was stepped continuously from −60 to −75 mV for 500 ms every 1 s, before (1), during (2), and after (3) application of t-ACPD. Bottom traces: correspond to voltage steps numbered 1–3 with capacitative transients blanked. Note increase in input conductance associated with activation of mGluRs. C: inward currents recorded from the same cell elicited by focal application of t-ACPD in the presence of BMC in the bath and potassium gluconate, ATP, and GTP in the pipette (top) and current voltage relationships (bottom). t-ACPD–evoked current has a linear relationship to holding membrane potential, and linear regression analysis reveals an extrapolated reversal potential close to +20 mV, suggesting that a mixed cation conductance underlies the t-ACPD–evoked current. A low-amplitude outward current (−) reversing to an inward current at −95 mV precedes the slow inward current and either results from polysynaptic GABA₉ receptor activation or from direct postsynaptic activation of K⁺ channels by mGluRs. D: currents recorded in a P0 VP neuron elicited by focal application of t-ACPD in the presence of BMC in the bath and cesium gluconate, ATP, and GTP in the pipette. Note that the t-ACPD elicited current reverses from an inward current to an outward current at +10 mV, confirming that the t-ACPD current arises from activation of a cation conductance.
The t-ACPD–induced inward current was observed in the presence of TTX (n = 26), confirming that the current resulted from direct postsynaptic activation of mGluR receptors.

In 12% of cells (4/34), a small amplitude hyperpolarization, 1–2 s in duration preceded the slow depolarization (Fig. 8A). This slow hyperpolarization appeared as a small outward current preceding a slow inward current in voltage clamp, and in the one cell where a clear voltage relationship could be seen, was seen to reverse at −90 mV, i.e., around the K+ equilibrium potential (Fig. 8C). The outward current was never observed in the presence of intracellular cesium (n = 22) or in the presence of TTX (n = 26).

The voltage dependence of the t-ACPD–induced depolarization was characterized in voltage clamp. The slow depolarization appeared as a slow inward current at all holding membrane potentials between −35 and −120 mV, and currents appeared to increase in amplitude with hyperpolarization of the membrane (Fig. 8C). When a clear current/voltage relationship could be established, linear regression analysis revealed that the t-ACPD–induced depolarization reversed at an extrapolated reversal potential of +12 ± 10 mV (n = 3), near the cation reversal potential (Fig. 8C).

To ascertain the reversal potential of t-ACPD responses at positive membrane potentials, recordings were performed with cesium gluconate, ATP, and GTP in the pipette solution. At holding membrane potentials close to the resting membrane potential (−60 mV) of the cell, t-ACPD application induced a slow inward current. The current typically increased in amplitude at more hyperpolarized potentials and decreased at more depolarized holding potentials. The t-ACPD–induced current reversed to an outward current at 1.5 ± 9.9 mV (n = 10), at a potential not significantly different from that obtained through extrapolation when the intracellular solution contained potassium gluconate, confirming the fact that t-ACPD activates a cation current in developing mouse thalamic neurons (Fig. 8D). Substitution of the NaCl with choline chloride in the ACSF (extracellular Na+ = 27 mM) caused the reversal potential of the t-ACPD–induced current to shift by almost 20 mV to −18 ± 2 mV (n = 4), indicating that Na+ is a charge carrier in the current. Furthermore, the t-ACPD–induced current was still present in a nominally Ca2+-free ACSF and reversed at +4.5 ± 6.4 mV (n = 2), a reversal potential not significantly different from the reversal potential obtained in normal ACSF, implying that Ca2+ is not a charge carrier in the current (data not shown).

The sensitivity of the t-ACPD–induced current to the bath application of Cs+ (2 mM), Ba2+ (2 mM), and Cd2+ (100 μM) was examined. The amplitude of the t-ACPD–induced current at a holding membrane potential of −60 mV was not significantly reduced by any of the three ions tested. The amplitude of the t-ACPD current was 95.5 ± 9.9% of control in the presence of Cs+ (n = 3), 99.3 ± 50.9% of control in the presence of Ba2+ (n = 3), and 100.7 ± 11.8% of control in the presence of Cd2+ (n = 3; data not shown).

**Discussion**

This study describes the development of receptor function at the corticothalamic synapse during the first 16 days of postnatal life. During this time, VP cells are acquiring their definitive physiological and morphological characteristics (Warren and Jones 1997). Functional corticothalamic synapses first became detectable on P1, indicating that the time period examined is likely to coincide with the period when corticothalamic synapses are being generated and stabilized. NMDA-receptor–mediated currents dominated the corticothalamic EPSC during the first 12 postnatal days in almost all cells, but late in the second postnatal week, and thereafter, large non-NMDA–mediated currents were recorded in a large proportion of cells in addition to the NMDA component. During the developmental period studied, there was a significant decrease in the time constant of decay of the NMDA EPSC, but no changes in the voltage dependence of the NMDA response were observed. Only after the first postnatal week, slow EPSPs mediated by metabotropic glutamate receptors could be evoked by high-frequency stimulation of corticothalamic fibers, although similar slow depolarizations could be evoked by local application of the mGluR agonist t-ACPD as early as P0. This suggests that either functional mGluR receptors are present on VP cells from birth but only become concentrated at corticothalamic synapses after the first postnatal week or that corticothalamic terminals release insufficient glutamate to affect postsynaptic mGluR receptors. Depolarizations evoked by t-ACPD application at all ages were shown to be mediated at least in part by the activation of a nonselective cation current.

**NMDA-mediated currents dominate the EPSC during the first two postnatal weeks**

Corticothalamic fibers in the rat enter the ventrobasal complex by P1, where they form rudimentary branches (Frassoni et al. 1995). We have shown that functional corticothalamic synapses are formed on mouse VP neurons by P1, and if the timing of corticothalamic innervation is similar to that in the rat, there is little delay between arrival of afferents and formation of functional synapses. The present study demonstrates that NMDA-receptor–mediated currents dominate the EPSC during the first 12 postnatal days, whereas large non-NMDA–mediated currents also can be evoked in a majority of cells toward the end of the second postnatal week. Predominance of NMDA-receptor–mediated EPSCs during early development has been shown at the thalamocortical synapse (Crair and Malenka 1995), and this dominance was shown to correlate well with the period when the thalamocortical synapse is susceptible to long-term potentiation. Corticothalamic synapses show the same early predominance of NMDA-receptor–based events as thalamocortical synapses. Corticothalamic synaptic activity during the period when thalamocortical fibers are innervating their targets in layer IV of the cortex (P4–P12; Agmon et al. 1993) may be in a position to modulate the formation and stabilization of thalamocortical synapses by increasing the gain of thalamocortical excitation. The strong voltage dependence of the corticothalamic NMDA response, presumably the result of Mg2+ blockade of the NMDA ion channel pore (Ascher and Nowak 1988; Mayer and Westbrook 1987; Nowak et al. 1984), indicates that corticothalamic EPSPs will be largest and most effective at depolarizing a VP neuron to firing threshold if they occur simultaneously with EPSPs generated...
by other inputs. In the VP nucleus, the most effective additional excitatory synaptic input is likely to arise from lemniscal fibers. During development, detection by VP neurons of coincident inputs from lemniscal and corticothalamic fibers would be facilitated by the relatively long time course of the dominant NMDA-mediated EPSPs and could play an important role in establishing the finer somatotopy of the thalamocortical representation. Large non-NMDA EPSCs were observed in a few young cells and a large proportion of cells after the end of the second postnatal week. The prevalence of the non-NMDA response after the first two postnatal weeks suggests that the function of the corticothalamic projection changes from a modulatory one to a fast excitatory one after the stabilization of synaptic connections.

Involvement of NMDA receptor activation in the response of thalamic neurons to corticothalamic stimulation previously has been shown to be evoked by the activation of GluR2 and/or GluR3 subunits. AMPA receptors consisting of heteromeric combinations of NR1 and NR2B or NR2C subunits (Laurie and Seeburg 1994; Monyer et al. 1994). Currents evoked by activation of NMDA receptors consisting of heteromeric combinations of NR1 and NR2D are far longer in duration than currents evoked by activation of NMDA receptors consisting of NR1 and NR2B or NR2C (Monyer et al. 1994), which could result in greater entry of Ca
+ through NMDA channels at the corticothalamic synapse and facilitate long-term changes of synaptic efficacy. α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor expression also has been examined in the developing rat ventrobasal thalamus. Immunoreactivity for GluR1 protein is not found. Immunoreactivity for GluR2–3 proteins is moderate at all ages, whereas that for GluR4 protein is found from P0 to P9 and declines at more mature ages (Spearefo et al. 1994). The non-NMDA corticothalamic currents, which become pronounced after the first 2 wk of development in the present study are therefore likely to be evoked by the activation of GluR2 and/or GluR3 subunits. AMPA receptors consisting of heteromeric combinations of GluR2 and GluR3 show linear current voltage relationships (Boulter et al. 1990), which is in agreement with what we observed for corticothalamic non-NMDA currents, and are relatively impermeable to Ca
+ (Boulter 1990; Nakanishi et al. 1990; Sakimura et al. 1990). Although little is known about the physiological properties of the GluR4 subunit, the decline in GluR4 expression levels after P9 coincides with the increase in the non-NMDA response in our study.

Kainate receptor gene expression is also regulated developmentally in the thalamus. GluR5 mRNA expression peaks at birth in the thalamus, declines during the first two postnatal weeks, and is absent from the adult dorsal thalamus (Bahn et al. 1994). GluR6, GluR7, and KA2 mRNAs are expressed at lower levels, and these levels do not change significantly during development. GluR7 is the only kainate receptor expressed in the adult thalamus but is restricted to the reticular nucleus (Bahn et al. 1994). These expression patterns suggest that the corticothalamic non-NMDA currents observed toward the end of the second postnatal week probably do not include a significant kainate receptor component.

Voltage dependence and decay kinetics of the corticothalamic NMDA current

It has been hypothesized that lower levels of voltage-dependent block by Mg
+ of NMDA currents in younger neurons, in conjunction with a longer decay time constant, facilitate the induction of long-term changes in synaptic efficacy and synaptic plasticity during development by causing increased and more prolonged activation of NMDA receptors (Ben Ari et al. 1988; Hestrin 1992). Lower levels of voltage dependence of NMDA-receptor–mediated events have been demonstrated in developing hippocampal (Ben Ari et al. 1988; Kleckner and Dingledine 1991) and visual cortical neurons (Kato and Yoshimura 1993), and larger time constants of decay of NMDA currents have been demonstrated in the developing superior colliculus (Hestrin 1992), visual cortex (Carmignoto and Vicini 1992), and lateral geniculate nucleus (Ramoa and McCormick 1994). The present study found no significant developmental differences in the voltage dependence and a moderate decrease in the decay time constant of the pharmacologically isolated, corticothalamic-evoked NMDA currents. NMDA currents showed strong voltage dependence at all ages studied. A lack of developmental differences in the voltage dependence of retinogeniculate NMDA-mediated currents also was demonstrated in the ferret (Ramoa and McCormick 1994), suggesting that NMDA mediated EPSCs at both lemniscal/optic tract and corticothalamic synapses on thalamocortical neurons might not be subject to developmental modulation of voltage dependence.

Linear regression analysis of corticothalamic NMDA-receptor–mediated currents revealed a significant though modest change in the time constant of decay of the current. These changes were less dramatic than previously reported in other CNS structures (Carmignoto and Vicini 1992; Hestrin 1992; Ramoa and McCormick 1994). Because corticothalamic synapses are concentrated on the distal dendrites of thalamocortical neurons (Jones and Powell 1969; Liu et al. 1995; Robson 1984; Wilson et al. 1984), the time course of synaptic currents recorded at presumed somatic locations may have been distorted by electrotonic decay and space clamp difficulties. Because the dendritic arborization of VP neurons changes dramatically during the first two postnatal weeks (Warren and Jones 1997), the degree of distortion would not be uniform across the developmental period studied. The fact that the reversal potentials of the NMDA-receptor–mediated currents tended to be more positive in older neurons (although this relation was not statistically significant) also supports the notion that corticothalamic currents recorded in older neurons were subject to increased space clamp distortion in comparison with currents recorded in
younger neurons. The input resistance of the VP neurons has been shown to decrease with development (Warren and Jones 1997), and this decrease also may distort changes in decay kinetics of NMDA currents.

Slow corticothalamic EPSC(P)s can be elicited after the first postnatal week

High-frequency stimulation of corticothalamic fibers (trains of 10 stimuli at 100 Hz) could evoke slow mGluR-mediated EPSPs or EPSCs lasting up to 30 s in VP neurons from P8. Slow mGluR EPSCs were inward at all holding potentials between −120 and −35 mV and appeared to increase in amplitude with hyperpolarization of the membrane, suggesting that the slow mGluR EPSC was not the result of K⁺ channel closure but probably resulted from activation of a nonselective cation current.

Slow mGluR-dependent EPSPs evoked by stimulation of corticothalamic fibers have been demonstrated in slices of adult guinea pig lateral geniculate nucleus in vitro and in the rat ventrobasal complex in vivo (Eaton and Salt 1996; McCormick and von Krosigk 1992) but could not be recorded in the rat thalamiccortical slice, in vitro (Kao and Coulter 1997). The slow mGluR EPSP in guinea pig lateral geniculate nucleus in vitro was associated with a decrease in input conductance, suggesting that activation of mGluRs at corticothalamic synapses depolarized the thalamic cell by closing K⁺ channels (McCormick and von Krosigk 1992).

The present study extends the previous studies by demonstrating that slow mGluR-mediated corticothalamic EPSPs only can be evoked from the beginning of the second postnatal week. The present study also suggests that activation of a nonselective cation conductance (discussed later) and not closure of K⁺ channels might underlie the mGluR-mediated EPSPs in immature animals.

Typically, high-frequency stimulation of corticothalamic fibers was necessary to evoke slow mGluR EPSP(C)s; similar stimulation paradigms were used to evoke mGluR responses in in vitro studies of the adult guinea pig corticothalamic projection (McCormick and von Krosigk 1992) and in other brain regions (Charpak and Gahwiler 1990). High-frequency stimulation probably is needed because mGluRs are located perisynaptically rather than immediately at the corticothalamic synapse (Vidnyanszky et al. 1996) as at other CNS synapses (Baude et al. 1993; Lujan et al. 1996; Nusser et al. 1994). Large amounts of glutamate released by repetitive stimulation are therefore necessary to reach and activate these receptors. In contrast, a corticothalamic EPSP with an mGluR component can be evoked by a single cortical stimulus in vivo; the lower threshold for activation of mGluR-mediated EPSPs in vivo probably results from the fact that higher numbers of corticothalamic fibers are intact in the in vivo preparation, leading to larger amounts of total glutamate being released. We could not evoke mGluR responses before P8, although we showed that functional postsynaptic mGluRs were present on relay neurons from P0. It is possible that not enough glutamate is released before P8 in vitro to activate the mGluR receptor but that sufficient levels could be released in vivo during this time.

Postsynaptic effects of mGluR activation traditionally are thought to be mediated through activation of mGluR1 or mGluR5 receptor subunits. mGluR1 mRNA is detectable in thalamic relay nuclei during the first postnatal week, and expression levels increase throughout postnatal development (Catania et al. 1994; Shigemoto et al. 1992). mGluR5 protein is present at the day of birth in the thalamus/midbrain area, peaks at P7, and both it and its mRNA dramatically decrease after this time period (Romano et al. 1996). These studies suggest that both mGluR1 and mGluR5 are present in thalamic relay nuclei during early development and that both may be activated by corticothalamic stimuli. At more mature stages, however when corticothalamic stimulation continues to elicit the mGluR-based EPSP, mostly mGluR1 appears to be present.

t-ACPD depolarizes VP neurons from P0 and activates a nonselective cation conductance

Although stimulation-evoked mGluR postsynaptic responses could not be elicited before P8, the mGluR-specific agonist, t-ACPD, when locally applied to developing VP neurons, evoked a large, slow depolarization (10–20 mV for 20–60 s) from the day of birth. This indicates that functional mGluRs, not detected by activation of corticothalamic synapses in vitro, are present on VP neurons during the first postnatal week. The t-ACPD–evoked depolarization was blocked reversibly by the mGluR antagonist, MCPG, associated with an increase in input conductance in a subset of cells, and was never associated with a decrease in input conductance before P13. We reason that the mGluR-activated current in our preparation is a nonselective cation current because it is associated with an increase in input conductance, it reverses near the cation reversal potential, and its reversal potential significantly shifts in the negative direction when external [Na⁺] is reduced, confirming that Na⁺ is a charge carrier in this current. The nominal exclusion of Ca²⁺ did not affect the reversal potential, indicating that Ca²⁺ is not a charge carrier in the current.

This appears to be the first demonstration that a nonselective cation current can underlie the postsynaptic effects of mGluR activation in thalamic neurons. mGluR-activated nonselective cation conductances have been demonstrated in CA1 (Bianchi and Wong 1995; Crepel et al. 1994) and CA3 (Guérineau et al. 1995; Pozzo Miller et al. 1995) hippocampal neurons, and in cerebellar Purkinje cells (Linden et al. 1994; Staub et al. 1992). Currents mediating the slow depolarization of thalamic neurons in response to local application of t-ACPD are direct effects of mGluR activation because these currents were still present in extracellular solutions containing TTX. The characteristics of the t-ACPD current recorded before P13 were very different from mGluR-activated currents described in the adult guinea pig lateral geniculate nucleus, where t-ACPD evoked a current associated with a decrease in input conductance that reversed at −80 to −100 mV, near the K⁺ equilibrium potential (McCormick and von Krosigk 1992). It is possible that activation of a nonselective cation conductance predominates in younger cells and inhibition of K⁺ currents is more dominant in neurons older than those included in our study. Because there is extensive dialysis of intracellular contents with the conventional whole cell recording technique, it is also possible that signal transduction intermediates that link
mGluRs to the closure of K\(^+\) channels were selectively dia-
lized, unmasking currents mediated by activation of nonse-
lective cation conductances. In hippocampal neurons loaded with GTP\(\gamma\)S, GTP\(\gamma\)S, or GTP, which abolish or irreversibly activate G proteins, the cationic current was unaltered, but reduction in \(I_K\) was abolished or irreversibly activated (Guerineau et al. 1995). Hence it is possible that different signal pathways link mGluRs to several different ion chan-
nels and that the whole cell recording configuration might favor detection of one current over the other.

In a small subset of neurons, slow depolarizations evoked by local application of \(t\)-ACPD were preceded by a low-
amplitude, faster hyperpolarization. In one neuron, where this current could be resolved in voltage clamp, it was seen to reverse from outward to inward at \(-90\) mV, near the \(K^+\) equilibrium potential. mGluR-mediated hyperpolarizations were never recorded from cells impaled with \(Cs^+\)-containing electrodes or in external solutions containing TTX. Because the slow hyperpolarization was never observed in the presence of TTX (\(n = 26\) ), it is likely that this current does not arise from direct postsynaptic activation of mGluR receptors but from polysynaptic activation of GABA\(_A\) receptors through excitation of RTN neurons by spiking VP neurons. This current also could result from direct induction of a \(K^+\) current by mGluR activation. mGluR receptors have been shown to be coupled to G-protein-linked, inwardly rectifying \(K^+\) channels in oocytes (Saugstad et al. 1996), and activa-
tion of mGluRs can hyperpolarize neurons of the basolateral amygdala by activation of a TEA sensitive, \(Ca^{2+}\) dependent, \(K^+\) conductance (Rainnie et al. 1994).

Extracellular exposure to \(Cs^+\), \(Ba^{2+}\), and \(Cd^{2+}\) did not significantly reduce the \(t\)-ACPD–induced current. Hence, the nonselective cation current activated by \(t\)-ACPD is dist-
ct from the hyperpolarization-induced cation current (\(I_H\) that is readily antagonized by extracellular exposure to \(Cs^+\) and distinct from the mGluR-activated nonselective cation current in CA3 hippocampal neurons, which is antagonized effectively by \(Cd^{2+}\) and \(Ba^{2+}\) (Guerineau et al. 1995). The mGluR-activated nonselective cation conductance in our preparation resembles the analogous conductance in cerebel-
lar Purkinje neurons and CA1 hippocampal neurons, where the \(t\)-ACPD current is not blocked by \(2\) mM \(Ba^{2+}\) and \(200\) \(\mu\)M \(Cd^{2+}\), respectively (Crepel 1994; Linden et al. 1994).

We thank Drs. Diane O’Dowd, Ivan Soltesz, and Alberto Munoz and G. Hollrigel for practical and theoretical advice during the experiments. We also thank V. Nguyen, P. Nguyen, and H. Truong for technical assistance.

This study was supported by National Institute of Neurological Disorders and Stroke Grants NS-21377 and NS-30109. P. Golshani is a MD/Ph.D student and is supported by American Heart Association Grant 960502Q.

R. A. Warren is supported by the Fonds de la Recherche en Santé du Québec.

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Received 3 October 1997; accepted in final form 27 March 1998.

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