Developmental Changes in Calcium Channel Types Mediating Central Synaptic Transmission

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Multiple types of high-voltage-activated Ca^{2+} channels trigger neurotransmitter release at the mammalian central synapse. Among them, the ω-conotoxin GVIA-sensitive N-type channels and the ω-Aga-IVA-sensitive P/Q-type channels mediate fast synaptic transmission. However, at most central synapses, it is not known whether the contributions of different Ca^{2+} channel types to synaptic transmission remain stable throughout postnatal development. We have addressed this question by testing type-specific Ca^{2+} channel blockers at developing central synapses. Our results indicate that N-type channels contribute to thalamic and cerebellar IPSCs only transiently during early postnatal period and P/Q-type channels predominantly mediate mature synaptic transmission, as we reported previously at the brainstem auditory synapse formed by the calyx of Held. In fact, Ca^{2+} currents directly recorded from the auditory calyceal presynaptic terminal were identified as N-, P/Q-, and R-types at postnatal day 7 (P7) to P10 but became predominantly P/Q-type at P13. In contrast to thalamic and cerebellar IPSCs and brainstem auditory EPSCs, N-type Ca^{2+} channels persistently contribute to cerebral cortical EPSCs and spinal IPSCs throughout postnatal months. Thus, in adult animals, synaptic transmission is predominantly mediated by P/Q-type channels at a subset of synapses and mediated synergistically by multiple types of Ca^{2+} channels at other synapses.

Key words: N-type calcium channels; P/Q-type calcium channels; postnatal development; transmitter release; central synapse; slice

Neurotransmitter release is triggered by Ca^{2+} entry through presynaptic voltage-dependent Ca^{2+} channels (Katz, 1969). In the mammalian CNS, fast synaptic transmission is mediated synergistically by multiple types of high-voltage-activated Ca^{2+} channels, including N-type, P/Q-type, and R-type Ca^{2+} channels (Luebke et al., 1993; Takahashi and Momiyama, 1993; Regehr and Mintz, 1994; Umemiya and Berger, 1994; Wheeler et al., 1994; Wu et al., 1998). Recently, however, the contribution of N-type Ca^{2+} channels to rat auditory brainstem synaptic transmission was found to be restricted to the early postnatal period (Iwasaki and Takahashi, 1998). A similar transient contribution of N-type channels to neuromuscular transmission was found in neonatal rats (Rosato Siri and Uchitel, 1999). These findings raise the possibility that the contribution of N-type Ca^{2+} channels to synaptic transmission might be developmentally regulated at other CNS synapses. We have examined this possibility at cerebellar, thalamic, cerebral, and spinal cord synapses in rats of various postnatal ages. Although it is clear that N-type Ca^{2+} channels contribute to synaptic transmission at many developing synapses, our results suggest that, at a subset of CNS synapses, there is a developmental switch to P/Q-type Ca^{2+} channels.

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MATERIALS AND METHODS

Preparation and solutions. Sagittal slices of cerebellum and thalamus, coronal slices of occipital neocortex, and transverse slices of brainstem (150- to 200-μm-thick) were prepared from 5- to 40-d-old Wistar rats killed by decapitation under halothane anesthesia. Transverse slices (250-μm-thick) were prepared from lumbar spinal cord of 21- to 54-d-old Wistar rats dissected after laminectomy under urethane anesthesia (2.4 gm/kg, i.p.). Each slice was perfused with artificial CSF (aCSF) containing (in mM): 125 NaCl, 2.5 KCl, 26 $NaHCO_3$, 10 glucose, 1.25 NaH_2PO_4, 2 $CaCl_2$, and 1 $MgCl_2$, pH 7.4, with 95% O_2 and 5% CO_2. Neurons in slices were visually identified with a 40 or 60× water immersion objective attached to an upright microscope (Axioskop, Zeiss, Oberkochen, Germany; or BX50WI, Olympus Opticals, Tokyo, Japan). For recording IPSCs, patch pipettes were filled with an internal solution containing 140 mM CsCl, 9 mM NaCl, 1 mM EGTA, 10 mM HEPES, and 2 mM MgATP, pH 7.3 adjusted with CsOH, and 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) (10 μM; Tocris Cookson, Bristol, UK) was added to the aCSF. To isolate GABAergic IPSCs from glycinergic IPSCs, strychnine (0.5 μM; Sigma, St. Louis, MO) was added to the aCSF. To isolate glycinergic IPSCs from GABAergic IPSCs, bicuculline (10 μM; Sigma) was added to the aCSF. For recording EPSCs, pipettes were filled with an internal solution containing 35 mM CsF, 100 mM CsCl, 1 mM $MgCl_2$, 10 mM EGTA, and 10 mM HEPES, pH 7.3 adjusted with CsOH, and bicuculline (10 μM) and strychnine (0.5 μM) were added to the aCSF. To isolate non-NMDA-EPSCs, D-2-amino-5-phosphonopentanoic acid (D-AP-5) (Tocris Cookson) was included in the aCSF. For recording calcium currents from the calyx of Held, tetraethylammonium chloride (TEA-Cl) (10 mM; Nakarai, Kyoto, Japan) and tetrodotoxin (TTX) (1 μM; Wako, Osaka, Japan) were added to the aCSF. The presynaptic patch pipettes were filled with (in mM): 110 CsCl, 40 HEPES, 0.5 EGTA, 1 $MgCl_2$, 12 Na_2 phosphocreatine, 10 TEA-Cl, 2 ATP-Mg, and 0.5 GTP.

Recording, drug application, and data analysis. Whole-cell voltage-clamp recordings of synaptic currents were made from visually identified neurons at the holding potential of −70 mV (unless otherwise noted) using a patch-clamp amplifier (Axopatch 200B). Postsynaptic and presynaptic electrodes had resistances of 2–4 and 5–7 MΩ, respectively. The

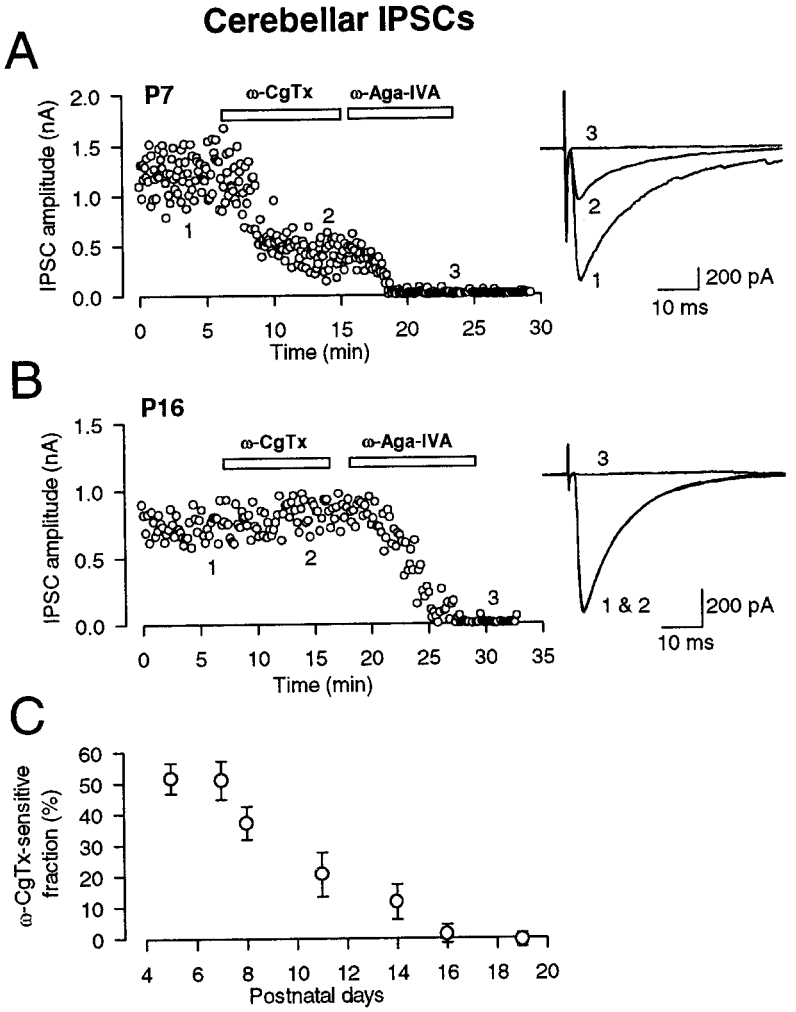

Figure 1. Developmental decline in the ω-CgTx sensitivity of GABAergic IPSCs in deep cerebellar nuclear cells. IPSCs were recorded in the presence of CNQX (10 μM) and strychnine (0.5 μM) and were blocked by bicuculline (10 μM; data not shown). *A*, At P7, ω-CgTx (3 μM) reduced the amplitude of IPSCs by 68%. Subsequent application of ω-Aga-IVA (200 nM) blocked the remaining IPSCs. *B*, At P16, ω-CgTx no longer affected IPSCs, whereas ω-Aga-IVA blocked IPSCs. Superimposed sample records (*A*, *B*) are averages of 10 consecutive IPSCs at a holding potential of −70 mV before ω-CgTx application (*1*), after ω-CgTx application (*2*), and after ω-Aga-IVA application (*3*). In this and following figures (Figs. 2, 4), each data point represents the amplitude of an individual synaptic current. *C*, The fraction of IPSCs blocked by ω-CgTx application at different postnatal days. *Symbols* and error bars are mean ± SEMs amplitudes derived from five to eight cells at each age.

access resistance for postsynaptic recording was 6–12 MΩ. The access resistance for presynaptic recording was 12–20 MΩ and compensated by 70%. Stimulation of synaptic input was made with a glass pipette filled with 1 M NaCl. The pipette was positioned in the vicinity of Purkinje cell axons to evoke GABAergic IPSCs in deep cerebellar nuclear cells (Takahashi and Momiyama, 1993), in the reticular nucleus thalami (RNT) to evoke GABAergic IPSCs in thalamic relay cells, in the vicinity of neighboring interneurons to evoke glycinergic IPSCs in spinal dorsal horn neurons, and in the layer VI border of the white matter to evoke non-NMDA-EPSCs in layer IV pyramidal cells in visual cortex. Synaptic currents were evoked at 0.1–0.2 Hz. Presynaptic Ca^{2+} currents were evoked by a 10 msec depolarizing pulse from −80 mV holding potential to −10 mV under voltage clamp at 0.1 Hz. Synthetic ω-Aga-IVA (200 nM; Peptide Institute, Osaka, Japan) and ω-conotoxin GVIA (ω-CgTx) (3 μM; Peptide Institute) were dissolved in oxygenated aCSF containing cytochrome c (1 mg/ml; Sigma) just before bath application. Records were low-pass filtered at 2–5 kHz and digitized at 10 kHz by a LM-12 interface (Dagan Instruments, Minneapolis, MN) or Digidata 1200 (Axon Instruments, Foster City, CA). Leak subtraction of Ca^{2+} currents was made by a P/N protocol (Takahashi et al., 1998). Values in the text and figures are given as means ± SEMs, and unless otherwise stated, differences between groups were evaluated by Steel's multiple comparison test, with $p < 0.05$ taken as the level of significance. All experiments were performed at room temperature (23–27°C).

RESULTS

Developmental decline of ω-conotoxin-sensitivity in GABAergic IPSCs

IPSCs were evoked in deep cerebellar nuclear cells by stimulating putative Purkinje cell axons extracellularly in the presence of CNQX (10 μM) and strychnine (0.5 μM). The IPSCs were blocked by bicuculline (10 μM), indicating that they are mediated by $GABA_A$ receptors (data not shown). At postnatal day 7 (P7), the N-type Ca^{2+} channel blocker ω-CgTx at a saturating concentration (3 μM) partially and irreversibly (data not shown) blocked the amplitude of IPSCs (Fig. 1*A*). The remaining fraction of IPSCs after ω-CgTx application (49.1 ± 6.2%; $n = 5$) was almost completely abolished by the P/Q-type Ca^{2+} channel blocker ω-Aga-IVA (200 nM). These results confirm our previous report (Takahashi and Momiyama, 1993), indicating that multiple Ca^{2+} channels are involved in synaptic transmission at this synapse at P6–P8. However, in older animals, the blocking effect of ω-CgTx became progressively less (Fig. 1*C*) until it was eventually lost at P16 (Fig. 1*B*), with the ω-CgTx-sensitive fraction being <2% ($n = 5$) (Fig. 1*C*). In contrast, ω-Aga-IVA nearly abolished IPSCs in rats older than P16 (Fig. 1*B*), suggesting that GABAergic inhibitory transmission from Purkinje cells to deep nuclear cells is exclusively mediated by the P/Q-type Ca^{2+} channels in mature animals.

GABAergic neurons in RNT provide a major inhibitory innervation onto thalamic relay cells, thereby contributing to thalamocortical rhythm generation (Steriade and Llinas, 1988). Bicuculline-sensitive GABAergic IPSCs were evoked in thalamocortical relay neurons in the laterodorsal (LD) thalamic nucleus by stimulating the RNT in the presence of CNQX (10 μM),

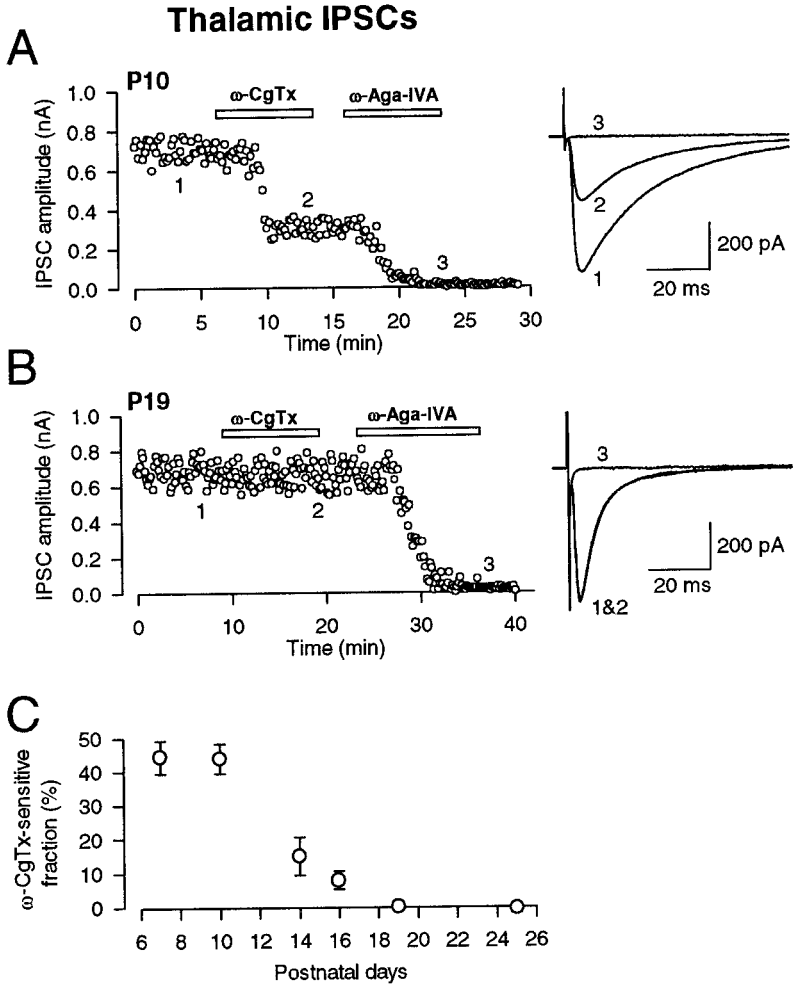

Figure 2. Developmental decline in the ω-CgTx sensitivity of GABAergic IPSCs in the LD thalamic nucleus. IPSCs were recorded in the presence of CNQX (10 μM), D-AP-5 (50 μM), and strychnine (0.5 μM) and could be blocked by bicuculline (10 μM; data not shown). *A*, At P10, ω-CgTx (3 μM) reduced the amplitude of IPSCs by 53%. Subsequent application of ω-Aga-IVA (200 nM) blocked the remaining IPSCs. Sample records of 10 IPSCs before ω-CgTx application (*1*), after ω-CgTx application (*2*), and after ω-Aga-IVA application (*3*) were averaged and superimposed (*A*, *B*). The holding potential was −70 mV. *B*, At P19, ω-CgTx no longer affected IPSCs, whereas ω-Aga-IVA almost completely blocked IPSCs. *C*, The fraction of IPSCs blocked by ω-CgTx application at different postnatal ages. *Symbols* and error bars as above (n = 5–6).

strychnine (0.5 μM), and D-AP-5 (50 μM). At P7–P10, ω-CgTx attenuated thalamic IPSCs (Fig. 2*A*) by 55.8 ± 3.1% (n = 11) (Fig. 2*C*). The fraction remaining after ω-CgTx application was abolished by ω-Aga-IVA (Fig. 2*A*). Similar to cerebellar IPSCs, the ω-CgTx-sensitive fraction decreased as animals matured (Fig. 2*C*). At P19, IPSCs were no longer attenuated by ω-CgTx but were completely abolished by ω-Aga-IVA (Fig. 2*B*). These results, and those at the brainstem auditory EPSCs (Iwasaki and Takahashi, 1998), suggest that an N-type to-P/Q-type switch of presynaptic Ca^{2+} channel type may be common among many central synapses.

If transmitter release increases with development, postsynaptic receptors may become saturated by transmitters. Also, as reported at the calyx of Held (Chuhma and Ohmori, 1998), the relationship between Ca^{2+} influx and transmitter release may shift developmentally and become saturated with Ca^{2+} influx in normal external [Ca^{2+}]. These might cause an apparent decline of ω-CgTx sensitivity. To exclude these possibilities, we have reduced IPSCs by reducing external [Ca^{2+}] to 1 mM and increasing [Mg^{2+}] to 2 mM. Although this treatment reduced cerebellar and thalamic IPSCs down to 31.2 ± 2.2% (n = 5) and 26.2 ± 2.9% (n = 5), respectively, ω-CgTx still had no effect on IPSCs (99.1 ± 1.7% remaining at P17 cerebellum; 102.9 ± 2.9% at P20 thalamus; n = 5 each). During postnatal development, thalamic IPSCs showed a clear kinetic speeding at the decay time, possibly because of the developmental switch of $GABA_A$ receptor α subunits (Onodera and Takahashi, 1996). No such kinetic change was observed for the GABAergic IPSCs between cerebellar Purkinje cell and deep cerebellar nuclear cell (Fig. 1), as reported for the basket/stellate cell–Purkinje cell IPSCs (Pouzat and Hestrin, 1997).

Developmental elimination of multiple calcium channel types at the calyx of Held

Developmental decline of ω-CgTx sensitivity in synaptic currents may be caused by the disappearance of N-type Ca^{2+} channels from presynaptic terminals or a decoupling of presynaptic Ca^{2+} channels from the exocytotic machinery. To determine which of these changes takes place, we recorded Ca^{2+} currents directly from the giant presynaptic terminal, the calyx of Held, in the brainstem slices (Borst et al., 1995; Takahashi et al., 1996, 1998; Forsythe et al., 1998; Wu et al., 1998). At P7, Ca^{2+} currents were partially blocked by ω-CgTx (3 μM) and also by ω-Aga-IVA (200 nM), with the magnitude of suppression being 28.4 ± 2.4 and 55.3 ± 2.4%, respectively (n = 5) (Fig. 3*A*). The substantial fraction (16.4 ± 2.8%; n = 5) remaining after application of both toxins was completely blocked by Cd^{2+}. These results confirm those reported by Wu et al. (1998, 1999), suggesting that N-, P/Q-, and R-type channels coexist at the presynaptic terminal and contribute to synaptic transmission at this age. At P10, all three types of Ca^{2+} channels were still present at the presynaptic

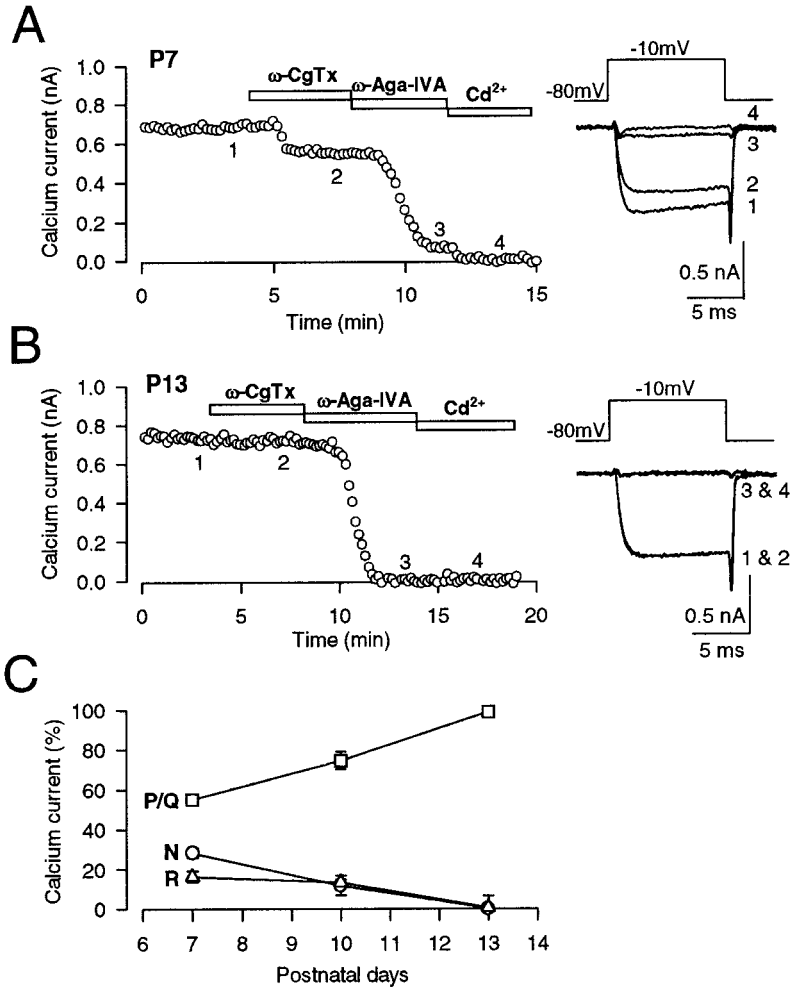

Figure 3. Developmental decline of N- and R-type Ca^{2+} channels in the giant presynaptic terminal, the calyx of Held. Presynaptic Ca^{2+} currents (Ip_{Ca}) were evoked by a 10 msec depolarizing pulse from −80 mV holding potential to −10 mV under voltage clamp every 10 sec in the presence of TTX (0.1 μM) and TEA-Cl (10 mM). *A*, At P7, ω-CgTx (3 μM) reduced the amplitude of Ip_{Ca} by 23% (*2*), whereas ω-Aga-IVA (200 nM) by 66% (*3*). The fraction remaining after application of both toxins (11%) was abolished by Cd^{2+} (100 μM; *4*). *B*, At P13, ω-CgTx had no effect on Ip_{Ca}, whereas ω-Aga-IVA almost completely abolished Ip_{Ca} (*3*) with no appreciable remaining Cd^{2+}-sensitive component (*4*). *C*, The fraction of Ip_{Ca} blocked by ω-CgTx (*N*, ○), ω-Aga-IVA (*P/Q*, □), and that insensitive to the toxins but blocked by Cd^{2+} (*R*, △) at three different postnatal ages. *Symbols* and error bars derived from five to eight cells at each age.

terminal, but N-type channels were significantly reduced, and P/Q type channels increased (Fig. 3*C*). At P13, ω-CgTx no longer affected presynaptic Ca^{2+} currents, whereas ω-Aga-IVA completely abolished them (Fig. 3*B*) (Takahashi et al., 1996). After application of ω-Aga-IVA, little Cd^{2+}-sensitive component remained. These results suggest that N-type and R-type Ca^{2+} channels are lost from the calyceal presynaptic terminals, being replaced by P/Q type Ca^{2+} channels during postnatal development.

Persistent ω-CgTx sensitivity of cerebral cortical EPSCs and spinal cord IPSCs through postnatal development

Although developmental loss in the contribution of N-type Ca^{2+} channels was observed at various central synapses, this was found not to be a general rule. Non-NMDA-EPSCs were evoked in layer IV pyramidal cells of visual cortical slices by stimulating at the borders between the white matter and layer VI in the presence of D-AP-5 (50 μM), strychnine (0.5 μM), and bicuculline (10 μM). These EPSCs are likely to derive from excitatory afferents containing geniculo-cortical projections, which represent the main component of the excitatory input to layer IV neurons from subcortical structures, as well as from cortical connections (Katz and Callaway, 1992; Carmignoto and Vicini, 1992). At P40, non-NMDA-EPSCs had fast kinetics in rise and decay times relative to those at P10 (Fig. 4), suggesting that transmitter release may become more synchronous with development at this synapse. However, we observed no change in the relative contribution of different Ca^{2+} channel types over this period. At P10, ω-CgTx blocked non-NMDA-EPSCs (Fig. 4*A*) by 42.0 ± 4.8% ($n = 6$). The blocking effect of ω-CgTx remained similar, at least until P40 (Fig. 4*B*). The remaining fraction of EPSCs after ω-CgTx was almost completely blocked by ω-Aga-IVA. These results suggest that both N-type and P/Q-type Ca^{2+} channels contribute to synaptic transmission throughout the postnatal developmental period at this synapse.

Another example of persistent ω-CgTx sensitivity during development was observed for glycinergic IPSCs in dorsal horn neurons of the spinal cord, evoked by stimulating neighboring interneurons. These IPSCs evoked in the presence of CNQX (10 μM), bicuculline (10 μM) and D-AP-5 (25 μM) were blocked by strychnine (0.5 μM; data not shown), suggesting that they were mediated by glycine receptors. At P21–P27, ω-CgTx (3 μM) blocked glycinergic IPSCs (Fig. 5*B*) by 49.9 ± 7.1% ($n = 7$), which is similar in magnitude to that reported previously for these synapses at P4–P8 (51 ± 9%) (Takahashi and Momiyama, 1993). At P44–P54, ω-CgTx similarly blocked glycinergic IPSCs (by 36.9 ± 8.9%; $n = 8$; not significantly different from P4–P8 or P21–P27) (Fig. 5*A*,*B*). At all ages, ω-Aga-IVA abolished EPSCs remaining after the ω-CgTx application (Fig. 5*A*). Thus, these results are similar to those for cerebral cortical EPSCs but clearly contrast with those for cerebellar and thalamic IPSCs and brainstem auditory EPSCs (Iwasaki and Takahashi, 1998).

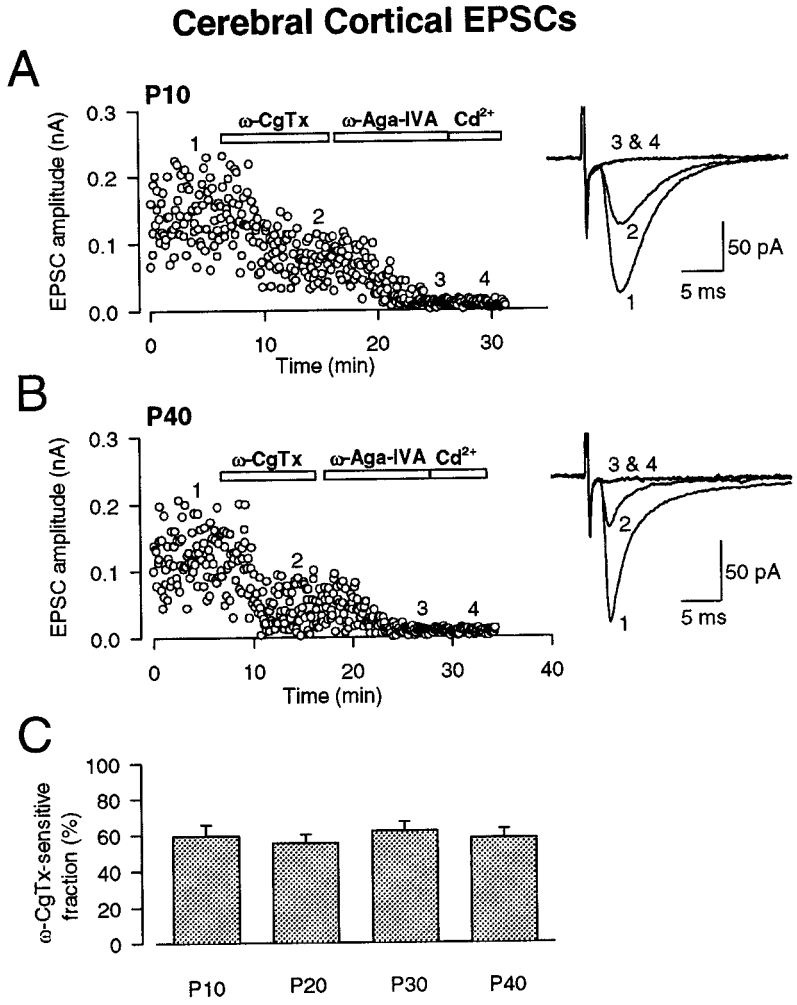

Figure 4. Persistent ω-CgTx sensitivity of non-NMDA-EPSCs in pyramidal neurons of visual cortex. EPSCs were recorded in the presence of D-AP-5 (50 μM), bicuculline (20 μM), and strychnine (0.5 μM) and blocked by CNQX (10 μM; data not shown). *A*, At P10, ω-CgTx (3 μM) reduced the amplitude of EPSCs by 43%. Subsequent application of ω-Aga-IVA (200 nM) almost completely blocked the remaining EPSCs. *B*, At P40, ω-CgTx reduced the amplitude of EPSCs by 64% in this cell, and subsequent application of ω-Aga-IVA (200 nM) almost completely blocked the remaining EPSCs. Sample records of 20 EPSCs before ω-CgTx application (*1*), after ω-CgTx application (*2*), after ω-Aga-IVA application (*3*), and after Cd^{2+} application (*4*) were averaged and superimposed (*A*, *B*). The holding potential was −70 mV. *C*, The ω-CgTx-sensitive fraction at different postnatal ages. The mean ± SEMs derived from five to six cells are shown in bar graphs.

DISCUSSION

Using type-specific Ca^{2+} channel blocker toxins, we have demonstrated that the contributions of N-type Ca^{2+} channels to cerebellar and thalamic inhibitory synaptic transmission are lost during postnatal development. These results are consistent with those at the rat auditory brainstem excitatory synapse (Iwasaki and Takahashi, 1998) and neuromuscular junction (Rosato Siri and Uchitel, 1999), suggesting that Ca^{2+} channels involved in transmitter release switch developmentally from N-type to P/Q-type at various mammalian fast synapses. Direct recordings of presynaptic Ca^{2+} currents from the auditory brainstem presynaptic terminals indicated that both N-type and R-type Ca^{2+} channels disappear with postnatal development. As illustrated in Figure 6, the disappearance of N-type Ca^{2+} channels at the cerebellar and thalamic inhibitory synapses occurred several days later than those at the brainstem auditory synapse (Iwasaki and Takahashi, 1998) or neuromuscular junction (Rosato Siri and Uchitel, 1999).

What is the mechanism underlying the developmental switch of Ca^{2+} channel types? One possibility would be the type-specific regulation of *de novo* synthesis of Ca^{2+} channels during development. Another possibility would be the Ca^{2+} channel type-specific sorting, which is developmentally regulated. Within a given type of neuron, Ca^{2+} channel subtypes are differentially sorted between soma and neurites (Christie et al., 1995; Mouginot et al., 1997; Doughty et al., 1998; Plant et al., 1998). In fact, at the early postnatal period, N-type Ca^{2+} channels are involved in synaptic transmission at the nerve terminal of cerebellar Purkinje cells (Takahashi and Momiyama, 1993), whereas these channels are not expressed at the soma (Mintz et al., 1992). Similarly, in facial motoneurons of neonatal rats, P/Q-type Ca^{2+} channels are involved in synaptic transmission (M. D. Rosato Siri and O. D. Uchitel, unpublished observation) but not expressed in the soma (Plant et al., 1998). At the nerve terminals of anteroventral cochlear neurons, the calyx of Held, we have shown that N- and R-type Ca^{2+} channels are replaced by P/Q-type Ca^{2+} channels with development. In contrast, multiple types of Ca^{2+} channels at the soma of these neurons do not exhibit developmental changes (Doughty et al., 1998). All of these results suggest that channel type-specific sorting mechanisms rather than the regulation of *de novo* synthesis may underlie the developmental switch of presynaptic Ca^{2+} channels.

What is the functional outcome of the N-type to-P/Q-type Ca^{2+} channel switch? At the calyx of Held of immature animals, for example, Ca^{2+} channel subtypes are located differentially, with N- and R-type Ca^{2+} channels being more distant from release site than P/Q-type Ca^{2+} channels (Wu et al., 1999). Our previous (Iwasaki and Takahashi, 1998) and present results indicate that these remote Ca^{2+} channels disappear with postnatal development. This will change the spatiotemporal profile of presynaptic Ca^{2+} channel domain (Augustine et al., 1991) toward more synchronous transmitter release (Chuhma and Ohmori,

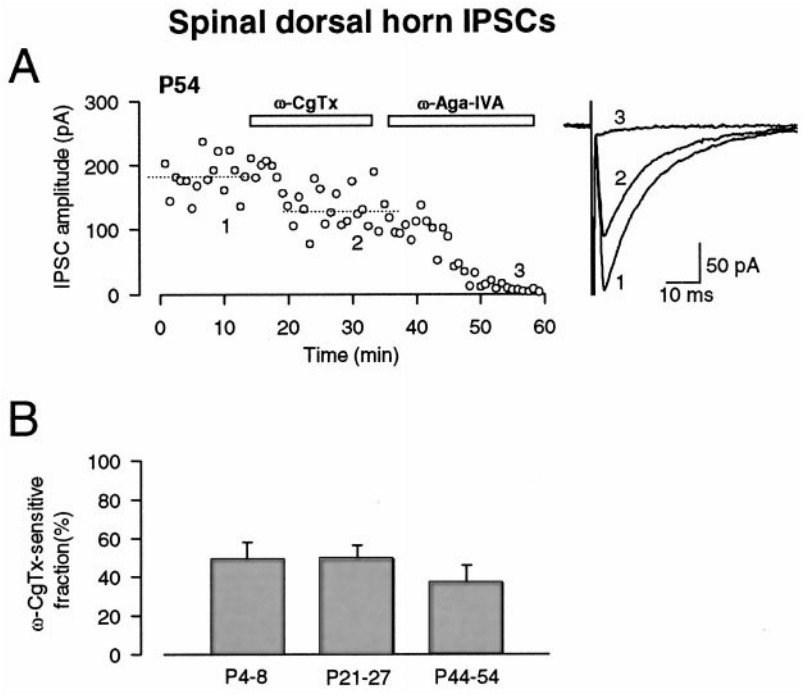

Figure 5. Persistent ω-CgTx sensitivity in glycinergic IPSCs in dorsal horn neurons of spinal cord. IPSCs were recorded in the presence of CNQX (10 μM), D-AP-5 (25 μM), and bicuculline (20 μM) and were blocked by strychnine (0.5 μM; data not shown). *A*, At P54, ω-CgTx (3 μM) reduced the amplitude of IPSCs by 28%. Subsequent application of ω-Aga-IVA (200 nM) blocked the remaining IPSCs. Each *symbol* represents the mean amplitude of 10 consecutive IPSCs. Sample records of 20 IPSCs before ω-CgTx application (*1*), after ω-CgTx application (*2*), and after ω-Aga-IVA application (*3*) were averaged and superimposed. Holding potential was −40 mV. *B*, The ω-CgTx-sensitive fraction at different postnatal periods. The mean ± SEMs derived from seven to eight cells (holding potentials between −40 and −70 mV) are shown in bar graphs. Data at P4–P8 are taken from Takahashi and Momiyama (1993). No significant difference between P21–P27 and P44–P54 ($p = 0.281$).

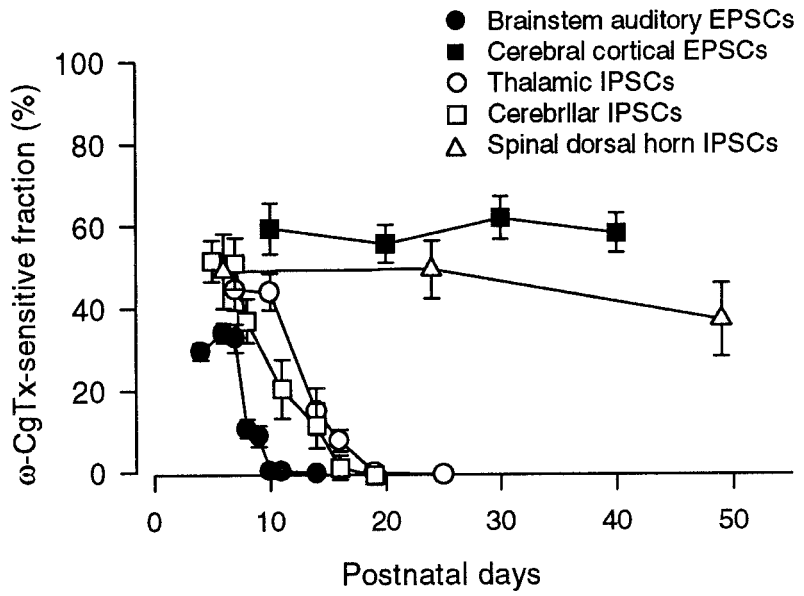

Figure 6. Age-dependent changes in the ω-CgTx-sensitive fraction at different central synapses. Data for brainstem auditory EPSCs are taken from Iwasaki and Takahashi (1998). EPSCs and IPSCs are indicated by *filled* and *open symbols*, respectively. Brainstem (●) and cerebral (■) EPSCs, and cerebellar (□), thalamic (○), and spinal (△) IPSCs. *Symbols* and error bars indicate mean ± SEMs.

1998). It has been reported that the G-protein-coupled receptors, such as adenosine receptors (Mogul et al., 1993; Umemiya and Berger, 1994) or metabotropic glutamate receptors (Stefani et al., 1998), are differentially linked to N- or P/Q-type Ca^{2+} channels in the presynaptic terminals. Such a differential linkage might also arise, at least in part, from differential localization of Ca^{2+} channel subtypes relative to the functional domain of G-protein-coupled receptors (Takahashi et al., 1998). In this respect, developmental redistribution of Ca^{2+} channels in combination with developmental changes in the presynaptic receptor expression (Baskys and Malenka, 1991; Elezgarai et al., 1999) may contribute to remodeling of presynaptic modulation.

In contrast to cerebellar and thalamic IPSCs and brainstem auditory EPSCs, cerebral cortical EPSCs and spinal cord dorsal horn IPSCs remained similarly sensitive to ω-CgTx throughout postnatal development (Fig. 6). In fact, N-type channel α_{1B} subunit immunoreactivity has been detected at the nerve terminals of dorsal cerebral cortex (Westenbroek et al., 1992) and spinal cord (Westenbroek et al., 1998) of adult rats. In adult animals, hippocampal synaptic transmission is mediated in part by N-type Ca^{2+} channels (Luebke et al., 1993; Wheeler et al., 1994). However, in hippocampal neurons in culture, the relative contribution of N-type Ca^{2+} channels to synaptic transmission has been reported to decline with days in culture (Scholz and Miller, 1995). It is possible that a similar developmental decline of N-type Ca^{2+} channels occurs at hippocampal synapses *in situ* as well.

Besides neurotransmission, N-type Ca^{2+} channels are thought to be involved also in cell migration (Komuro and Rakic, 1993) and synaptogenesis (Vigers and Pfenninger 1991) during the early development. The contribution of N-type Ca^{2+} channels to syn-

aptic transmission seems general among synapses in developing animals, but it remains only in a subset of synapses in mature animals. It has been reported that N-type Ca^{2+} channels are specifically involved in nociceptive transmission (Chaplan et al., 1994; Omote et al., 1996; Westenbroek et al., 1998); therefore, ω-CgTx can be a potential analgesic agent for chronic pain treatment (Miljanich and Ramachandran, 1995). Thus, it would be important to clarify what other functional roles bear N-type Ca^{2+} channels remaining at mature CNS synapses.

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