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Synaptic Mechanisms Regulating the Activation of a Ca\textsuperscript{2+}-Mediated Plateau Potential in Developing Relay Cells of the LGN

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INTRODUCTION

Many aspects of neuronal development depend on the sequestration of intracellular Ca\textsuperscript{2+} (Ghosh and Greenberg 1995). A major source is acquired via the activation of ligand-gated N-methyl-D-aspartate (NMDA) channels that allow for the influx of Ca\textsuperscript{2+} in an activity-dependent manner (Constantine-Paton et al. 1990). However, NMDA receptors need not be the sole source of Ca\textsuperscript{2+}. For example, in sensory thalamic nuclei like the lateral geniculate nucleus (LGN), relay neurons possess a variety of voltage-gated Ca\textsuperscript{2+} channels. Most notable in the LGN are the low-threshold (T-type) and high-threshold (L- and N-type) Ca\textsuperscript{2+} currents (Coulter et al. 1989; Hernandez and Pape 1989; Huguenard 1996). The low-threshold Ca\textsuperscript{2+} current that underlies burst firing has been the subject of extensive investigation and found to play a major role in the state-dependent modulation of retinogeniculate transmission (McCormick and Bal 1997; Steriade et al. 1993). By comparison, high-threshold Ca\textsuperscript{2+} channels have received far less attention. While these have been well characterized in dissociated cell preparations (Budde et al. 1998; Kammermeier and Jones 1997), their interplay with synaptic responses has yet to be explored. The paucity of such information may in part be due to technical reasons. Such study requires a preparation that allows for intracellular access and intact synaptic circuitry, conditions not found in either dissociated cell or conventional slice preparations. Therefore we used a unique isolated brain stem preparation (Hu 1993) to examine the nature of synaptic transmission in the developing LGN. This preparation is especially suited for the study of retinogeniculate transmission. Unlike a conventional thalamic slice preparation, in the isolated brain stem preparation, a large segment of the optic tract (OT) as well as the intrinsic circuitry of the LGN remain intact. The isolated brain stem is readily maintained in vitro and has been used successfully to examine the synaptic activity in a number of CNS structures including thalamus (Hu 1993; Xia and Lo 1996), hypothalamus (Hu and Bourque 1992), and superior colliculus (Lo and Mize 2000). Our results indicate the synaptic responses of developing LGN cells exhibit a large and sustained synaptically evoked Ca\textsuperscript{2+}-mediated depolarization. This event provides LGN cells with a rich source of Ca\textsuperscript{2+} during strong postsynaptic depolarization.

METHODS

Sprague-Dawley rat pups ranging in age from postnatal day 1 (P1) to P24 were anesthetized with Halothane and killed by decapitation. The brain was excised and placed in a bubbled (95% O\textsubscript{2}-5% CO\textsubscript{2}) solution of artificial cerebrospinal fluid (ACSF, see following text). The brain was cut in half along the midline axis, glued to a silver plate, and placed into a well of a temperature-controlled recording chamber. Separate recordings were done on each hemisphere. With the aid of a dissecting microscope positioned above the chamber, the lateral surface of the thalamus and midbrain were exposed by gently

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removing the forebrain. The isolated brain stem was then submerged and perfused continuously (4–5 ml/min) with warmed (28–33°C) ACSF [which contained (in mM) 124 NaCl, 2.5 KCl, 1.25 NaH₂PO₄, 1.0 MgSO₄, 26 NaHCO₃, 10 dextrose, and 2 CaCl₂, saturated with 95% O₂-5% CO₂, pH = 7.4]. Recordings began 1–3 h after incubation and were done at a depth of 50–200 μm below the pial surface.

For whole cell intracellular recording, patch electrodes were pulled horizontally in two stages from borosilicate glass and filled with a solution containing (in mM) 140 K gluconate, 10 HEPES, 1.1 EGTA-Na, 0.1 CaCl₂, 2 MgCl₂, 2 ATP-Mg, and 0.2 GTP-Na, pH = 7.2 to a final tip resistance of 5–7 MΩ. Whole cell patch recordings were done in current-clamp mode with an Axoclamp 2B amplifier using the techniques described by Blanton et al. (1989). Briefly, the formation of the whole cell configuration was indicated by a sudden drop in seal resistance and a DC drop of ∼60 mV or more. After breaking in, the series resistance was completely compensated with bridge balance of the Axoclamp-2B amplifier. The junction potential was left uncorrected. Only those cells that exhibited a resting membrane level more negative than −55 mV, an input resistance >300 MΩ, and overshooting action potentials were included in the study. In some instances (n = 8), we switched the recording mode from current clamp to single continuous voltage clamp (SEVC) to record postsynaptic currents at a holding potential of −60 mV. We also conducted extracellular single-unit recordings (n = 20). For these we used patch electrodes filled with 1 M NaCl (1–3 MΩ). All neuronal activity was displayed on a storage oscilloscope, digitized at 10–20 kHz, and stored directly on computer.

To evoke synaptic activity in LGN, single square-wave pulses (0.1–0.5 ms, 0.1–1.0 mA) were delivered at a rate of 0.20–1.0 Hz through a pair of thin gauged Ir wires (WPI) placed on the surface of the OT. To examine the extent to which EPSPs summate spatially we adjusted current intensity in 1, 2, 5, or 10% increments (12–15 steps) above a stimulus level that elicited a threshold response. We then constructed amplitude by stimulus intensity plots (Figs. 7 and 8), and these were used to estimate the number of retinal inputs converging onto a single LGN cell (Chen and Regehr 2000). To examine the extent to which EPSPs summate temporally, optic tract was stimulated repetitively at 20 Hz for 0.1 s (Fig. 10, A and B) or at 50 Hz for 1 s (Fig. 10C).

Various ligand-gated antagonists were bath applied to ascertain the pharmacology underlying EPSP [NMDA: D(-)-2-amino-5-phosphonopentanoic acid (APV), 100 μM] and IPSP (GABAergic: bicuculline 10 μM and GABA_ref: 2-hydro-saclofen 100 μM) activity. The dihydropyridine, nitrendipine, was also used (10–20 μM) to determine whether L-type Ca²⁺ channels contributed to synaptically evoked depolarizations. Unless otherwise stated, a single preparation was used for each drug application.

RESULTS

We studied the synaptic responses evoked by OT stimulation of 311 LGN cells in rats that were 1–24 days old. Of these, 20 responses were recorded extracellularly and 291 using intracellular techniques. For the latter, cells exhibited a resting membrane level of −60 to −65 mV, an input resistance of 300–500 MΩ, action potentials >60 mV, and near-threshold synaptic responses >3 mV.

Single-unit extracellular recordings indicated that developing LGN cells exhibit three distinct firing modes in response to OT stimulation. Examples of these modes are shown in Fig. 1A. At relatively low stimulus intensities (0.1–0.3 mA), OT stimulation evoked a single action potential. This is the typical response observed in the adult (Burke and Sefton 1966; Lo and Sherman 1991) and one that we consistently observed between P10 and 24 (n = 8), even when higher stimulus intensities were

![FIG. 1. Synaptic transmission in developing lateral geniculate nucleus (LGN) cells. A: extracellular recordings from a single LGN cell at P2. Electrical stimulation of optic tract (OT) evokes 3 different response patterns. Left: at stimulus threshold, OT stimulation evokes a single spike. Middle: moderate intensity levels (2–5 times greater than value in left trace) elicits a short train of spikes. Right: at strong stimulus levels, OT activation elicits a high-amplitude (>30 mV), long-lasting (>300 ms) depolarization (plateau potential) that contains a long train of spikes.](jn.physiology.org)
employed. However, at ages earlier than P10 (n = 12), higher levels of stimulation elicited two additional firing patterns. A moderate increase in stimulus intensity (2–5 times above threshold), gave rise to a short train of spikes that had a 100–300 ms in duration. Higher levels of stimulation (5–10 times above threshold) produced a sustained discharge (300–1,000 ms) composed of spikes with variable amplitude. Intracellular whole cell recordings shown in Fig. 1B depict the underlying synaptic events that correspond to each of these response modes. Note the striking similarity in the extracellular responses of Fig. 1A with the intracellular ones of Fig. 1B. Weak levels of stimulation produced a conventional EPSP that had a single spike riding its peak. Moderate levels of stimulation produced a somewhat longer depolarization that gave way to a short train of spikes. Higher levels of stimulation elicited a long-lasting (300–1,300 ms), slow decaying (<0.01 mV/ms) depolarization (25–40 mV). Riding the crest of this response was a train of spikes of variable amplitude. We refer to this response profile as the plateau potential. In close to half of all cells tested between P1 and 24 (139/291, 48%), suprathreshold levels of OT stimulation evoked a plateau potential. Representative examples of plateau potentials are shown in Figs. 3–7 and 10. However, as shown in Fig. 2, the incidence of the plateau potential declined with age. At P1–2, 90.6% of the encountered cells (29 of 32 cells) exhibited a synchronically evoked plateau potential. By P13–14, the incidence dropped to 43.5% (20 of 46 cells) and by P20 to <1% (1 of 12 cells). Although the incidence declined with age, we did not detect any systematic changes with age in either the duration or amplitude of the plateau potential. These parameters varied considerably within and between cells and were influenced by several factors including, membrane level (Fig. 3), the nature of EPSP and IPSP activity (Figs. 4 and 5), stimulus intensity (Figs. 6–8), and the degree of spatial and temporal summation present (Figs. 6–8 and 10). These factors and the manner in which they influenced the plateau potential are described in detail in the following text.

Pharmacological experiments indicate the plateau potential was mediated by the activation of high-threshold L-type Ca\(^{2+}\) channels. In each of 13 cells tested, the L-type Ca\(^{2+}\) channel antagonist, nitrendipine blocked the expression of the plateau potential (Figs. 3, 4A, and 7B). Cells treated with nitrendipine still maintained robust EPSP activity. For example, at membrane levels of −60 to −65 mV and at stimulus intensities strong enough to evoke a plateau potential, EPSPs had peak amplitudes between 12 and 23 mV. Figure 3 depicts a cell in which nitrendipine blocked a plateau potential. At a resting level of −65 mV, strong stimulation of OT evoked a large plateau potential. When the membrane level was hyperpolarized to −93 mV (Fig. 3A), the underlying EPSP, while quite large (40 mV), was unable to trigger a plateau potential. Figure 3B shows the application of nitrendipine abolished the plateau potential but left the underlying EPSP/IPSP event intact. At −65 mV, this EPSP has a peak amplitude of 20 mV but a relatively short duration (20 ms). The duration of the EPSP was curtailed by the presence of IPSP activity (see also Fig. 5A). It was also possible to evoke a large EPSP at a hyperpolarized membrane potential of −105 mV (Fig. 3B) during nitrendipine application. This EPSP was comparable in amplitude and duration to those evoked under control conditions (Fig. 3A). These results indicate the plateau potential is mediated by L-type Ca\(^{2+}\) channels and that EPSP-induced depolarizations are needed to activate this channel activity.

The example in Fig. 3A also underscores the voltage dependency of plateau potential activation. At a resting membrane level −65 mV, strong stimulation of OT evoked a large plateau potential. However, when the membrane was hyperpolarized to −93 mV, the same level of stimulation was not sufficient to evoke a plateau potential. Instead, OT stimulation evoked a conventional EPSP. Typically, we found at resting levels of −60 to −65 mV, EPSPs >20–25 mV (thereby driving the membrane potential above −40 mV) was sufficient to activate plateau potentials.

Another critical factor in determining the activation of the plateau potential was EPSP duration. As shown in Fig. 4A, nitrendipine completely abolished the plateau potential while leaving the underlying EPSP intact. This EPSP, like those of all

![Figure 2](https://www.jn.org)
thalamic relay cells, contained two overlapping components, an early fast one that relies on non-NMDA receptor activity and a slower, longer voltage-dependent one that is mediated by NMDA receptor activity (Scharfman et al. 1990). We found that EPSPs recorded in the presence of nitrendipine (Fig. 4A), or those evoked at relatively low levels of stimulation (Fig. 4B), contained an APV-sensitive NMDA component. In each of 12 cells tested, when the NMDA antagonist APV was applied to block the late excitatory component, EPSPs were no longer sufficient in duration to activate a plateau potential. A representative example is shown in Fig. 4C. Under control conditions, strong stimulation evoked a large plateau potential (Fig. 4C, trace 1). However, in the presence of APV, the same level of stimulation was not sufficient to evoke a plateau potential (Fig. 4C, trace 2). These results also indicate the remaining non-NMDA component of the underlying EPSP was not sufficient in duration to activate the plateau potential. This was true whether high-intensity, single shocks (Fig. 4C) or repetitive shocks (3 pulses at 20 Hz) were employed (n = 3).

We also examined how inhibitory synaptic activity influenced the plateau potential. Even at early postnatal ages (P0–10), many synaptic responses were composed of EPSP/IPSP pairs (Crunelli et al. 1988; Lo and Sherman 1991). A typical example is shown in Fig. 5A. IPSP activity evoked at low levels of stimulation (i.e., below the level required to activate plateau potentials) often times contained both an early short-duration and a late long-lasting hyperpolarization (Fig. 5A, top). The former is mediated by GABA A and the latter by GABA B receptor activation (Crunelli et al. 1988). Both components have reversal potentials at membrane levels more negative than −90 mV (Fig. 5A, bottom). Figure 5B shows the pattern of EPSP/IPSP activity at low, moderate, and strong levels of OT stimulation. Weak OT stimulation (Fig. 5B, trace 1) evoked a response profile similar to that shown in Fig. 5A. At moderate and strong levels of stimulation, these inhibitory

FIG. 3. Pharmacology and voltage dependency of the plateau potential. A: control responses showing postsynaptic activity at 2 different membrane levels. At −65 mV, strong stimulation of OT evoked a plateau potential. At more hyperpolarized level of −93 mV, the same level of stimulation evoked a large postsynaptic potential. B: recordings from the same cell after drop application (10 μM) of nitrendipine. At −65 mV, this L-type Ca 2+ antagonist abolishes the plateau potential while leaving the underlying EPSP/IPSP intact. The EPSP has a short early peak (20 mV) that is followed by 2 IPSPs, an early short-lasting hyperpolarization and a late long-lasting one. At −105 mV, a large postsynaptic potential is present that is comparable in amplitude (45 mV) to the potential recorded under control conditions at −93 mV.

FIG. 4. Excitatory postsynaptic events underlying the activation of the plateau potential. A: synaptic responses recorded in same cell before (1) and after (2) the application of nitrendipine (10 μM). The L-type Ca 2+ channel antagonist abolishes the plateau potential while leaving the underlying EPSP intact. B: synaptic responses evoked at a relatively weak stimulus intensity before (1) and after (2) APV (100 μM) application. APV reduces the overall amplitude and duration of EPSP, blocking the slow-rising late, NMDA component and preventing spike discharge. C: synaptic responses evoked before (1) and after (2) the application of APV. In the control response (1), the EPSP evokes a plateau potential. Application of APV (2) blocks the NMDA component of EPSP that prevents the activation of the plateau potential. The non-NMDA receptor component persists but fails to activate the plateau potential.
responses become more difficult to see (Fig. 5B, traces 2 and 3) because of the activation of a plateau potential. Evidence for an early inhibitory response appears as a notch that precedes the plateau potential (see Fig. 5B, *). However, the late inhibitory component cannot be delineated because it is masked by the slow-decay of the plateau potential. To better understand the manner in which inhibitory activity modulates the expression of the plateau potential, we recorded synaptic responses after the application of selective GABA antagonists (n/H11005/9). Figure 5C shows that GABA A activity affected the stimulus threshold required for the activation of the plateau potential. Weak levels of stimulation evoked an EPSP/IPSP pair (Fig. 5C, trace 1). After

FIG. 5. Inhibitory postsynaptic events underlying the activation of the plateau potential. A: example of EPSP/IPSP pair evoked by weak levels of OT stimulation. Top: at resting levels, IPSP activity is composed of an early fast event that immediately precedes the EPSP and a slower longer event that follows the early IPSP. Bottom: IPSPs reverse at membrane levels more of −110 mV. B: 3 responses at progressively larger stimulus levels depicting the nature of GABA activity in relation to the plateau potential. Low level of stimulation evokes an EPSP/IPSP pair (1). The IPSP contains an early, short (GABA A) and long-lasting (GABA B) hyperpolarization. At higher levels of stimulation, the early IPSP is still apparent (see *), but the late one is masked by the plateau potential. C: synaptic response evoked by weak stimulation, before (1) and after (2) GABA A receptor blockade by bicuculline (10 μM). Weak stimulation (1) evokes an EPSP/IPSP pair. After GABA A blockade with bicuculline (2), the same weak stimulus evokes a plateau potential. D: plateau potentials evoked at high-intensity levels before (1) and after (2) GABA B blockade with 2-hydroxysaclofen (100 μM). Blockade of GABA B increases the duration of the plateau potential.

FIG. 6. Spatial summation of EPSPs evokes the plateau potential. A and B: records from 2 cells showing responses (superimposed) to an increase in stimulus intensity. A: a progressive increase in stimulus intensity (range, 0–1.0 mA, 0.9- to 1.2-mA steps) leads to a step-wise increase in EPSP amplitude. At high stimulus intensities, inputs summate and evoke plateau potentials. Numbered traces correspond to responses evoked at progressively higher levels of stimulation.
GABA_A blockade with bicuculline (Fig. 5C, trace 2), the same stimulus evoked a plateau potential \((n = 5)\). These results suggest that GABA_A activity affects the intensity of OT stimulation needed to evoke the plateau potential, perhaps by truncating the amplitude and/or duration of the underlying EPSP (Crunelli et al. 1988; Ramoa and McCormick 1994) (see also Figs. 5A and 3B). Figure 5D shows that GABA_B activity modulated the duration of plateau potential. Strong stimulation of OT evoked a large but relatively brief plateau potential (Fig. 5D, trace 1). The application of the GABA_B antagonist, 2-hydroxysaclofen, increased the duration of the plateau potential by \(-40\%\) \((n = 4)\), largely by slowing down its rate of decay (Fig. 5D, trace 2).

In summary, the expression of the plateau potential relied on excitatory synaptic responses that result in a strong and sustained membrane depolarization, whereas inhibitory responses influenced the intensity of OT stimulation needed to evoke the plateau potential (GABA_A activity) or the duration of the plateau potential (GABA_B activity).

At young postnatal ages, increased membrane depolarization was readily accomplished by the spatial (Figs. 6 and 7) and temporal summation of EPSPs (Fig. 10). The extent to which EPSPs summate spatially are best illustrated by examining synaptic responses at different stimulus intensities (Allen et al. 1977; Bartlett and Smith 1999; Chen and Regehr 2000; Mariani and Changeux 1981; Mock et al. 1997; O’Brien et al. 1978). In developing LGN cells, a systematic increase in stimulus intensity above threshold led to incremental increases in EPSP amplitude and the subsequent activation of a plateau potential. Representative examples are shown in Fig. 6, A and B. Numbered traces depict a series of responses evoked by progressively larger stimulus intensities (range, \(0-1.0\) mA, \(0.9-1.2\)-mA steps). EPSP amplitude grew incrementally, and when amplitudes in excess of \(30\) mV were reached, plateau potentials often emerged. These discrete increases in EPSP amplitude in response to elevations in stimulus intensity are taken to reflect the successive recruitment of active inputs innervating a single cell (Allen et al. 1977; Bartlett and Smith 1999; Chen and Regehr 2000; Mariani and Changeux 1981; Mock et al. 1997; O’Brien et al. 1978). When EPSP amplitude is measured and plotted as a function of stimulus intensity, it can be used to obtain estimates in the number of retinal inputs converging on a single LGN cell (Chen and Regehr 2000; see also Bartlett and Smith 1999; Mock et al. 1997). Examples of such plots and corresponding responses at different ages are shown in Figs. 7 and 8. At early ages, such as P3 and P8, a systematic elevation in stimulus threshold led to a step-wise increase in EPSP or EPSC amplitude, and as shown in Fig. 7A, the activation of a plateau potential. The graded steps in amplitude are better illustrated when the plateau potential is blocked pharmacologically (Fig. 7B) or prevented by voltage clamping (Fig. 7C). Taken together, the steps evident in the amplitude by stimulus plots of Fig. 7 show these cells received input from 7 to 10 retinal ganglion cells. By contrast, the responses from older animals revealed this feature of retinogeniculate connectivity was transient. Figure 8 shows that at P18 and P19, cells had fewer inputs (1–4). As a result, they showed less spatial summation and lacked plateau potentials.

Estimates of retinal convergence for 72 cells tested at different stimulus intensities (see preceding text) are summarized in the scatterplot of Fig. 9. We found a significant reduction in retinal convergence with age \((r = -0.64, P < 0.001)\). Before P7 we estimated that LGN cells receive input from as many as 7–12 retinal ganglion cells (see also Chen and Regehr 2000). By P14, LGN cells begin to resemble their adult counterparts and receive far fewer (between 1 and 4) inputs. Comparing the scatterplot of Fig. 9 with the plot of Fig. 2 reveals the loss of inputs at older ages is accompanied by a reduced incidence in the plateau potential. These results suggest the high degree of retinal convergence occurring at early ages provides a greater opportunity for spatial summation, thereby increasing the probability that synaptic responses will lead to the activation of the plateau potential.

The repetitive activation of optic tract reveals that the plateau potential could be evoked by the temporal summation of EPSPs. Figure 10A depicts the responses of an LGN cell to a 20-Hz train of three stimulus pulses delivered at a constant stimulus intensity. The response to the initial shock (Fig. 10A, trace 1) evoked a singular EPSP, while those generated by subsequent shocks (Fig. 10A, traces 2 and 3) gave rise to plateau potentials. Figure 10B reveals the plateau potential could also be evoked by a combination of spatial and temporal summation. A 20-Hz train of three stimulus pulses delivered at relatively low stimulus intensities (i.e., levels that were unable to evoke a plateau potential when single shock was employed) readily evoked a plateau potential. As stated in the preceding text, the plateau potential was most prevalent prior to eye opening, at times when spontaneous retinal activity takes the form of periodic high-frequency burst discharges (Galli-Resta and Maffei 1990; Meister et al. 1991; Wong et al. 1993). To determine whether such activity is sufficient for activating the plateau potential, we recorded the synaptic activity evoked by a tetanus protocol (50-Hz train for 1 s) designed to mimic early intrinsic retinal activity \((n = 8)\). Figure 10C (top) shows this form of high-frequency stimulation led to a massive summation of EPSPs and evoked a long-lasting depolarization \((>1\) min). Moreover, this tetanus-induced depolarization was mediated largely by L-type \(\text{Ca}^{2+}\) channel activation. Figure 10D (bottom) shows that nitrendipine greatly reduced the amplitude and duration of this large depolarization \((n = 3)\).

**DISCUSSION**

Thalamic relay cells possess both low (T-type) and high (L- and N-type, and two \(\alpha\)-conotoxin MVIIIC-sensitive ones) threshold \(\text{Ca}^{2+}\) channels (Coulter et al. 1989; Hernandez-Cruz...
and Pape 1989; Huguenard 1996; Kammermeier and Jones 1997). At membrane levels more negative than −65 mV, depolarization activates a T-type current that leads to a large Ca\(^{2+}\)-mediated triangular depolarization and burst firing (Jahnsen and Llinas 1984). The burst firing mode of relay cells has been studied extensively, serving as the primary basis for

**FIG. 8.** Synaptic responses to incremental increases in stimulus intensity at P18 and 19. Responses were obtained during the bath application of bicuculline (10 μM). Cells recorded at older ages exhibited fewer inputs.

**FIG. 9.** Scatterplot showing the number of retinal inputs an LGN cell receives as a function of age. Each point represents a single cell. ○, cells that shared the same number of inputs. —, a significant reduction in inputs with increasing age (r = −0.64, P < 0.001).
oscillatory activity during different behavioral (sleep) or pathological states (absence seizures) (McCormick and Bal 1997; Steriade et al. 1993). Prominent among the family of high-threshold Ca\(^{2+}\) channels for thalamic relay cells is the L-type (Budde et al. 1998; Kammermeier and Jones 1997). These are activated at membrane levels that generally exceed \(-40\) mV and result in a high-amplitude depolarization that shoulders the Na\(^{+}\) spike. L-type Ca\(^{2+}\) channels are believed to regulate cell excitability, perhaps helping to support a tonic firing mode in relay cells (Budde et al. 1998, 2000). However, a more specific role during retinogeniculate signal transmission has not been fully explored. Recent imaging studies suggest they may play an important role in the modulation of synaptic responses. Although high-threshold Ca\(^{2+}\) channels generally reside on the soma (Munsch et al. 1997; Zhou et al. 1997), the L-type channel in particular tends to cluster at the base of dendrites (Budde et al. 1998), thereby putting them in close proximity to retinal terminals (Wilson et al. 1984). This arrangement puts them in an ideal position to affect the gain of retinally mediated activity (Budde et al. 1998). Indeed, we found that EPSP activity evoked by strong stimulation of the optic tract activates L-type Ca\(^{2+}\) channels. Their activation triggers a high-amplitude, slow-decaying, sustained depolarization that rides atop the EPSP. We refer to this event as a plateau potential. This event is virtually identical to the synaptically evoked plateau potential recorded in neurons of the developing rodent superior colliculus (Lo and Mize 2000) and shares some similarities to those reported in developing structures of the brain stem (Rekling and Feldman 1997) and spinal cord (Morisset and Nagy 1999).

In some central synapses, L-type Ca\(^{2+}\) channels facilitate synaptic transmission by regulating transmitter release from presynaptic terminals (e.g., Bonici et al. 1999; Jensen et al. 1999). However, we saw no evidence for such a role in LGN. The blockade of L-type Ca\(^{2+}\) activity with nitrendipine abolished the plateau potential but left underlying postsynaptic events intact. The fact that EPSPs as well as IPSPs were not compromised by nitrendipine indicates L-type Ca\(^{2+}\) channels modulate retinogeniculate transmission at postsynaptic sites rather than at presynaptic ones (Budde et al. 1998, 2000; Munsch et al. 1997; Zhou et al. 1997).

To evoke the plateau potential, EPSPs were required to produce a strong and sustained membrane depolarization. Neither Na\(^{+}\) spikes nor the early, fast AMPA component of EPSPs could trigger the plateau potential. This suggests the synaptic activation of the plateau is both voltage and time dependent. We found at least three ways in which EPSP activity led to heightened membrane depolarization and the activation of the plateau potential. These included NMDA receptor activation, and the spatial and/or temporal summation of EPSPs. Interestingly, these events prevail during early postnatal life, a time when retinal afferents are actively sorting into eye specific domains (Jeffery 1984). The timing of these events may also explain why the plateau potential was developmen-

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**FIG. 10.** Temporal summation of synaptic responses evokes plateau potentials. A: repetitive shocks of a constant stimulus intensity delivered at 20 Hz produce a temporal summation of EPSPs. The response to the 1st shock (1) evokes an EPSP, while those evoked by subsequent shocks (2 and 3) gives rise to a plateau potential. B: a train of 3 stimulus pulses delivered at 20 Hz at progressively larger stimulus levels produces both the spatial and temporal summation of EPSPs. C: high-frequency stimulation (50 Hz, 1 s) evokes a long-lasting (~1 min) plateau potential. D: another example showing that a plateau potential evoked by tetanus is blocked by nitrendipine (20 \(\mu\)M). In C and D, the insets depict expanded traces taken during tetanus (see *) and Na\(^{+}\) spikes are filtered for clarity.
tally regulated, showing a high incidence at P1–7. For example, at early postnatal ages relay cells possess a prominent NMDA current with channel kinetics that favor long open times and slower decay rates (Chen and Regehr 2000; Ramoa and McCormick 1994; Ramoa and Prusky 1997, but see Hohnke et al. 2000). In rodents, these features of excitatory transmission are transient. By the time of natural eye opening (P14–16) excitatory events are faster and shorter, in part because they have a larger AMPA-to-NMDA current ratio as well as faster NMDA decay times (Chen and Regehr 2000). We also found at young ages (<P7), relay cells receive input from several (7–12) retinal ganglion cells. In fact, our estimates seem conservative. Others report that developing LGN cells are innervated by as many as 20 retinal ganglion cells (Chen and Regehr 2000). This contrasts the adult state, where LGN cells are reported to receive input from just one or a few retinal ganglion cells (Chen and Regehr 2000; Cleland et al. 1971; Hamos et al. 1987; Mastronarde 1987; Ursey et al. 1999). Such remodeling happens quickly during development. Between P14 and 24, we found more than a threefold reduction in the number of retinal inputs innervating a single LGN cell. It is conceivable that a change in electrotonic compactness due to an increase in dendritic complexity led to a failure to detect weak synaptic inputs in mature cells. However, our estimates are in accord with other electrophysiological studies showing the receptive field properties of LGN cells are dominated by one or a few retinal ganglion cells (Cleland et al. 1971; Mastronarde 1987; Ursey et al. 1999). Moreover, retinal terminals form synapses on proximal regions of relay cell dendrites (Hamos et al. 1987; Wilson et al. 1984), thereby minimizing potential space clamp problems.

The high degree of retinal convergence coupled with heightened NMDA activity observed at early ages would certainly favor the spatial (and temporal) summation of EPSPs and greatly increase the likelihood that synaptic responses will lead to the activation of the plateau potential. The high degree of summation at early developmental ages may also minimize the influence of GABA-mediated inhibition (Guyon and Leresche 1992), which we found to affect both the threshold and duration of the plateau potential.

The age-related decrease in the incidence of the plateau potential could also be due to nonsynaptic factors such as a developmental difference in the density of L-type Ca	extsuperscript{2+} channels or an inherent difference in channel kinetics between immature and mature LGN cells. However, this possibility seems unlikely. Both Ca	extsuperscript{2+}-imaging and -electrophysiological studies indicate that mature relay cells possess a high density of active L-type Ca	extsuperscript{2+} channels (Budde et al. 1998, 2000; Couler et al. 1989; Hernandez-Cruz and Pape 1989; Huguenard 1996; Munsch et al. 1997; Zhou et al. 1997).

Although in the present study we used electrical stimulation to evoke the plateau potential, it is reasonable to assume that such activation occurs endogenously at early postnatal ages. Prior to eye opening, retinal ganglion cells exhibit a high degree of spontaneous activity in the form of synchronous bursts that traverse across the retina in a wave-like fashion (Galli-Resta and Maffei 1990; Meister et al. 1991; Wong et al. 1993). This patterned retinal activity is also capable of driving LGN cells to fire prolonged bursts of action potentials (Mooney et al. 1996). Such early spontaneous retinal activity also seems capable of activating the plateau potential. Repetitive activation of OT fibers or the use of a tetanus protocol that approximated the intrinsic activity of retinal ganglion cells evoked a large and long-lasting depolarization that was mediated by L-type channel activation. The sustained nature of this Ca	extsuperscript{2+}-mediated depolarization also suggests that early retinal activity can support long-term changes in synaptic efficacy. Indeed, in both LGN (Ziburkus and Guido 1999) and superior colliculus (Lo and Mize 2000), L-type Ca	extsuperscript{2+} activity was necessary for the induction of tetanus induced changes in synaptic strength. Such associative changes are believed to represent the “Hebbian” substrate for synapse stabilization and the activity-dependent remodeling of retinofugal connections (Constantine-Paton et al. 1990; Cramer and Sur 1995).

Taken together, these results underscore the idea that the functional state of the developing retinogeniculate synapse is well suited for the activity-dependent sequestration of Ca	extsuperscript{2+}. Moreover, the activation of NMDA receptors need not be the sole source of Ca	extsuperscript{2+} during synaptic transmission, but a much larger and longer influx can occur via the activation of L-type Ca	extsuperscript{2+} channels. The Ca	extsuperscript{2+} influx associated with the synaptically evoked plateau potential could play an important role in the experience-dependent modification of the visual system, triggering a cascade of signal transduction pathways that lead to the maturation and stabilization of retinogeniculate connections.

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