Nature of Inhibitory Postsynaptic Activity in Developing Relay Cells of the Lateral Geniculate Nucleus

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INTRODUCTION

Inhibitory activity in the lateral geniculate nucleus (LGN) plays a critical role in determining the gain and efficacy of retinogeniculate signal transmission (Sherman and Guillery 1996). However, compared with our knowledge about the development of excitatory activity in LGN, there is limited information about the ontogeny of inhibitory activity during the period of retinogeniculate axon segregation. In some mammalian species (e.g., ferret and cat), inhibitory activity seems to initially appear during the late phases of retinogeniculate axon segregation (Pirchio et al. 1997; Ramoa and McCormick 1994; Shatz and Kirkwood 1984; but see White and Sur 1992) and becomes more prevalent thereafter as LGN cells take on adult-like receptive-field properties (Tavazoie and Reid 2000; Tootle and Friedlander 1986). In the cat, the emergence of inhibitory activity at or near birth also corresponds to the appearance of glutamic acid decarboxylase (GAD) staining in somata and terminals of A-layer LGN cells (Shotwell et al. 1986). In the rat, the expression of GABAergic elements in LGN occurs at earlier prenatal times (Bentivoglio et al. 1991; Lauder et al. 1986; Laurie et al. 1992). This suggests that in rodents inhibitory activity emerges earlier in development, perhaps preceding or at least coinciding with the period of retinogeniculate axon segregation (Guido et al. 2003; Guido and Žiburkus 2001; Jeffery 1984).

A number of other issues regarding the nature and origin of inhibitory activity in the developing LGN remain unresolved. In the adult LGN, optic tract stimulation evokes a monosynaptic excitatory postsynaptic potential (EPSP) that is followed by two inhibitory postsynaptic potentials (IPSPs), an early fast one mediated by a $\text{GABA}_A$ $\text{Cl}^-$ conductance and a slow one mediated by a $\text{GABA}_B$ $\text{K}^+$ conductance (Crunelli et al. 1988). It is not clear whether these inhibitory events develop at different rates, or when they do emerge, if they are part of a feed-forward circuit involving LGN interneurons or a recurrent one involving neurons of the thalamic reticular nucleus. Moreover, inhibitory activity in the LGN has been implicated in both monocular and binocular signaling (e.g., Guido et al. 1989; Xue et al. 1987). The circuitry underlying binocular inhibitory interactions has received limited attention (Lindström 1982), and its emergence during development has yet to be investigated. To examine these issues, we studied the synaptic responses of developing LGN cells in an in vitro isolated brain stem preparation (Fig. 1) (Lo et al. 2002; see also Shatz and Kirkwood 1984 for a similar preparation in the developing kitten). This preparation is especially suited for the study of synaptic transmission because large segments of each optic nerve as well as the intrinsic circuitry of the LGN remain intact.

METHODS

Long Evans rat pups ranging in age from P1 to P21 were anesthetized with halothane and killed by decapitation. The brain was excised and placed in solution of artificial cerebrospinal fluid [ACSF (in mM): 124 NaCl, 2.5 KCl, 1.25 NaH$_2$PO$_4$, 1.0 MgSO$_4$, 26 NaHCO$_3$, 10 dextrose, 2 CaCl$_2$, saturated with 95% O$_2$-5% CO$_2$, pH = 7.4].

Figure 1 depicts the isolated brain stem and our experimental approach. The excised 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. The lateral surface of the thalamus and midbrain were exposed by removing the forebrain. The isolated brain stem was submerged and perfused continuously (4–5 ml/min) with warmed (31–33°C) ACSF. In experiments that required electrical stimulation of the optic nerves, the isolated brain stem preparation was warmed (31–33°C) ACSF. In experiments that required electrical stimulation of the optic nerves, the isolated brain stem preparation was controlled recording chamber. The lateral surface of the thalamus and midbrain were exposed by removing the forebrain. The isolated brain stem was submerged and perfused continuously (4–5 ml/min) with warmed (31–33°C) ACSF. In experiments that required electrical stimulation of the optic nerves, the isolated brain stem preparation was

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modified to include large segments of each optic nerve (Fig. 1). The nerves were cut just behind the optic disk and dissected from the base of the skull, thus sparing at least 4–5 mm of each optic nerve. Using a ventral approach, the brain was cut along the midline axis while leaving the optic chiasm intact. Recordings began 1–3 h after incubation and were done at a depth of 50–250 μm below the pial surface of the LGN.

Whole cell recordings were obtained with pipettes made of borosilicate glass filled with a solution containing (in mM) 140 K gluconate, 10 HEPES, 1.1 EGTA-Na, 0.1 CaCl₂, 2 MgCl₂, 2 ATP-Mg, 0.2 GTP-Na (pH = 7.2) and pulled to a final tip resistance 5–7 MΩ. In some cases, we used sharp-tipped electrodes filled with either 4 M potassium acetate (KAC) or a 2% solution of biocytin dissolved in 2 M KAC. Sharp-tipped electrodes were pulled to a final tip resistance of 70–90 MΩ. Intracellular recordings were done in current clamp mode with an Axoclamp 2B amplifier using techniques described elsewhere (Guido et al. 1997, 1998; Lo et al. 2002). All neuronal activity was displayed on a storage oscilloscope, digitized at 10 kHz, and stored directly on computer.

During intracellular recording, some LGN cells (n = 18) were filled with biocytin by passing alternating positive and negative current pulses (±1 nA, 30 ms, 100–300 pulses) through the recording electrode. Tissue containing biocytin-filled cells was placed in a fixative solution of 4% paraformaldehyde dissolved in 0.1 M phosphate buffer for 72 h. The LGN was sectioned (400 μm) in the coronal plane and processed using the ABC method (Guido et al. 1997, 1998). Labeled cells were photographed and drawn using a camera lucida attached to a microscope.

To evoke synaptic activity in LGN, single square-wave pulses (0.1–0.3 ms, 0.1–5.0 mA) were delivered at a rate of 0.20–1.0 Hz through a pair of low-impedance (0.2–0.3 MΩ, thin-gauged Ir wires) stimulating electrodes are positioned in each optic nerve and a recording electrode is inserted into the LGN. Electrical stimulation of the optic nerves (or optic tract) activates retinal axons and evokes synaptic activity in LGN.

We then adjusted current intensity in 1, 2, 5 or 10% increments from this initial threshold value until an EPSP of maximal amplitude was reached. Various ligand-gated antagonists were bath applied to ascertain the pharmacology underlying EPSP [N-methyl-D-aspartate (NMDA): D(-)-2-amino-5-phosphono-pentanoic acid (APV, 100 μM) and IPSP (GABA_A: bicuculline, 10–25 μM, and GABA_B: 2-hydroxy-saclofen, 100 μM) activity.

**RESULTS**

We studied the synaptic responses evoked by optic tract or optic nerve stimulation of 397 LGN cells in rats that were 1–21 days old. All cells exhibited a resting membrane level of −55 to −68 mV, action potentials that exceeded 60 mV, and near-threshold synaptic responses >3 mV. All recordings were restricted to regions in LGN that in the adult receive retinal input from the contralateral eye (Reese 1988).

Representative voltage responses to intracellular current injection and a synaptic response (at postnatal day 11) evoked by optic tract stimulation are shown in Fig. 2. These responses are typical of rodent relay cells (Crunelli et al. 1987; Williams et al. 1996). The voltage responses to intracellular current injection reflect a number of active membrane properties. These include low-threshold Ca²⁺ spikes (Fig. 2A, T) and burst firing (Fig. 2A, B), a mixed cation conductance (Fig. 2A, H) that prevents membrane hyperpolarization, and a transient K⁺ conductance (Fig. 2A, A) that delays spike firing during membrane hyperpolarization. Sustained membrane depolarization also evoked a train of action potentials that showed frequency adaptation, due largely to the activation of an after-hyperpolarizing (AHP, Fig. 2A) response that follows each action potential. Electrical stimulation of retinal afferents also evoked.
postsynaptic responses typical of rodent relay cells (Crunelli et al. 1988). After postnatal day 11, the synaptic responses were comprised of EPSP/IPSP pairs (Fig. 2B, see following text for details). Figure 2, C and D, provides some examples of LGN cells filled with biocytin during intracellular recording. Labeled cells (n = 18) had relatively large somata and multipolar dendritic arbors consistent with those of class A thalamocortical cells (Grossman et al. 1973; Parnavelas et al. 1977; Webster and Rowe 1984) In some instances, we could also identify an axon exiting the LGN. Taken together, these results indicate that our recordings were likely restricted to relay cells.

Representative responses in Fig. 3 underscore the dynamic changes occurring in synaptic transmission during early postnatal life. Between P1 and P2, 69% (22 of 32 cells) of the synaptic responses evoked by optic tract stimulation were purely excitatory. All other excitatory responses (31%) were coupled with some form of inhibitory activity. As shown in Fig. 3A, pure EPSPs had long decay times that were decreased by membrane hyperpolarization or by application of the NMDA receptor antagonist APV (n = 12). By P5, all retinaevoked EPSPs were followed by IPSPs. When inhibitory activity was evoked by optic tract stimulation, it was always coupled with a preceding EPSP. It is also important to note that for a given cell, IPSP activity was evoked at the same stimulus intensity as the accompanying EPSP.

Examples of EPSP/IPSP pairs are shown in Fig. 3, B–D. During the first postnatal week, IPSPs were mediated largely by GABA<sub>A</sub> receptors (Fig. 3B). They were of short-duration (20–50 ms), reversed at membrane levels more negative than −80 mV, and blocked by the application of bicuculline (n = 11). Note also that the GABA<sub>A</sub>-mediated response greatly curtailed the duration of the preceding EPSP (Crunelli et al. 1988; Lo et al. 2002; Ramoa and McCormick 1994). Additional GABA<sub>B</sub>-mediated IPSPs emerged at P3–4 but were not prevalent until after the first postnatal week (Fig. 3, C and D). GABA<sub>B</sub>-mediated IPSPs followed GABA<sub>A</sub> IPSPs, were longer in duration (200–300 ms), reversed at membrane levels more negative than −95 mV, and were blocked by the application of hydroxysaclofen (n = 10). We used the preceding measures as a means to distinguish different inhibitory responses (Crunelli et al. 1988; Lo et al. 2002; Ramoa and McCormick 1994; Zhu and Lo 1999).

Figure 4 plots the incidence of GABA<sub>A</sub>- and GABA<sub>B</sub>-mediated inhibition as a function of age for 130 LGN cells. GABA<sub>A</sub> responses were present as early as P1–2 (31%) and showed a rapid increase thereafter so that by P5 nearly every cell (98%) expressed GABA<sub>A</sub>-mediated IPSPs. GABA<sub>B</sub> responses developed more slowly. At P1–2, these responses were rare (6%) but showed a gradual increase with age so by P9–10 all cells possessed GABA<sub>B</sub> activity.

We were also able to evoke synaptic responses by stimulating the optic nerves in preparations where large segments of each optic nerve were preserved (Fig. 1). This approach has proven useful for determining whether developing LGN cells are binocularly responsive. Developing LGN cells receive excitatory retinal input from both eyes (Guido and Ziburkus 2001; Guido et al. 2003). Moreover, binocular excitatory responses are transient and show an age-related decrease that coincides with the recession of uncrossed retinal projections (ipsilateral eye) terminating in LGN (see Table 1) (Guido and

**Fig. 2.** Electrophysiological properties and synaptic responses of biocytin-filled relay cells. A: examples of voltage responses evoked by current pulse injection. Depolarization produces an outward rectification (A) that delays spike firing. Note the afterhyperpolarization (AHP) that follows each action potential that leads to spike frequency adaptation. Membrane hyperpolarization produces a strong inward rectification (H) while the passive repolarization from a hyperpolarized state activates a rebound low-threshold Ca<sup>2+</sup> spike and burst discharge. B: example of postsynaptic activity evoked by electrical stimulation of optic tract at P18. The excitatory postsynaptic potential (EPSP, E) is followed by a pair of inhibitory postsynaptic potentials (IPSPs, I): a short one that occurs in conjunction with an EPSP and a longer slower one that follows the early IPSP (see Fig. 3 for details). C: photomicrograph of 2 relay cells at P6 filled with biocytin during intracellular recording. D: camera lucida reconstruction of biocytin-filled relay cell filled at P18. Labeled cells have dendritic morphology consistent with those of class A thalamocortical cells.
By P19, LGN cells receive excitatory monocular input from the retina. Examples of the binocular responses evoked by optic nerve (ON) stimulation at P11–P14 are shown in Figs. 5 and 6, A and B. Separate stimulation of the contralateral or ipsilateral ON evoked an EPSP/IPSP pair. In fact, between P11 and P14, a time when inhibitory responses have fully matured, 58.9% of (79/134) LGN cells tested exhibited a binocularly mediated EPSP/IPSP pair.

It is important to note that binocular responses cannot be attributed to inadvertent current spread. We preserved large segments of each ON (Fig. 1) and could evoke binocular responses at stimulus intensity levels that were at threshold for evoking postsynaptic responses (0.1–0.5 mA). Moreover, when concurrent shocks were delivered to each optic nerve (shocks are timed so that responses are evoked at the same time), the excitatory component of the synaptic response reflected a summation of the response evoked by each nerve separately (n = 4). An example is shown in Fig. 5. The amplitude of the EPSP evoked by contralateral ON stimulation was 12 mV, to ipsilateral ON stimulation was 8 mV, and to concurrent stimulation 19 was mV. If the stimulating current was indeed spreading from one electrode across both optic nerves, then stimulation of both nerves should lead to occlusion and a reduced response (Shatz and Kirkwood 1984).

During the time when binocular excitatory responses were waning, we encountered a subset of LGN cells in which contralateral ON stimulation evoked an EPSP/IPSP pair and
ipsilateral ON stimulation evoked only IPSP activity. Although these responses were somewhat rare (5%, 12/244), they first appeared at P12, and as shown in Table 1 became somewhat more prevalent during the third postnatal week of life (11.7%, 8/68). Examples of these binocularly mediated inhibitory responses are shown in Fig. 6, C–F. These binocularly mediated IPSPs were evoked at stimulus intensity levels that were at threshold for evoking a postsynaptic response (0.1–0.5 mA) or at levels that were 10 times greater than those used to evoke a threshold response (Fig. 6, D and E). The absence of an accompanying EPSP (preceding the IPSP) at high levels of stimulation suggests some retinal afferents from the ipsilateral eye synapse exclusively onto LGN interneurons (Ahlström, 1985; Lindström 1982). In a normal ACSF solution, IPSPs evoked by ipsilateral optic nerve stimulation had both a short and long-lasting hyperpolarizing component (Fig. 6, C and D), suggesting that both GABA_A and GABA_B receptors subtypes were involved (n = 3). Robust IPSP activity was also evident during the bath application of bicuculline (n = 9; Fig. 6, E and F). These IPSPs were of long duration and reversed at membrane levels more negative than −90 mV (Fig. 6F), indicating they were likely mediated by GABA_B receptor activation.

**DISCUSSION**

Our results indicate that inhibitory synaptic responses in the rat’s LGN were present during the first week of postnatal life and that GABA_A and GABA_B responses developed at different rates. GABA_A responses emerge as early as P1–2, and by P5 almost all cells expressed IPSPs that utilized this receptor subtype. GABA_B-mediated activity was rare during the first few postnatal days, but the incidence increased gradually with age so by P10 all cells exhibited activity through this receptor subtype.

It could be that the delayed onset of GABA_B activity observed in the present experiment is related to the response firing of interneurons evoked by optic tract stimulation. There is some evidence to suggest that GABA_B-mediated postsynaptic responses favor repetitive burst-like firing of presynaptic afferents (Bal et al. 1995; Huguenard and Prince 1994; Kim et al. 1997). Burst-induced GABA_B responses in thalamic relay cells involves a recurrent feedback circuit with interneurons of the thalamic reticular nucleus. With regard to the present experiments, perhaps the absence of GABA_B activity at the earliest postnatal ages is related to an inability of immature interneurons to fire burst discharges in response to optic tract stimulation. Although such activity might be necessary to evoke GABA_B activity via the recurrent feedback circuit, it does not seem required for the activation of GABA_B responses.

**TABLE 1.** Incidence of binocular excitatory and inhibitory responses

<table>
<thead>
<tr>
<th>Postnatal Age</th>
<th>n</th>
<th>Binocular Excitatory Response, %</th>
<th>Binocular Inhibitory Response, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>P0–7</td>
<td>22</td>
<td>63.6</td>
<td>0</td>
</tr>
<tr>
<td>P8–P14</td>
<td>177</td>
<td>64.4</td>
<td>2.2</td>
</tr>
<tr>
<td>P14–P21</td>
<td>68</td>
<td>29.4</td>
<td>11.7</td>
</tr>
</tbody>
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Recordings were restricted to regions in the lateral geniculate nucleus that in the adult receive retinal input from the contralateral eye.
FIG. 6. Nature of postsynaptic activity evoked by contralateral (left) and ipsilateral (right) ON stimulation. A: example of binocularly innervated LGN cell. Separate stimulation of the contralateral and ipsilateral ON evokes an EPSP/IPSP pair. The IPSP is sufficient to activate a rebound low-threshold (LT) Ca^{2+} spike. B: example of another binocularly innervated cell recorded at low (0.2 mA) and high (1.0 mA) stimulus intensities. C: example of a cell in which contralateral ON stimulation evokes an EPSP/IPSP pair and ipsilateral ON stimulation produces IPSP activity. In both instances, the inhibitory activity is composed of a short and long component. The latter was sufficient to activate LT Ca^{2+} spikes and burst discharges. D and E: examples of 2 different cells in which ipsilateral ON stimulation evokes IPSP activity at both low (0.2 mA) and high (2.0 mA) stimulus levels. F: example of a cell in which IPSP activity evoked by ipsilateral ON stimulation is recorded at 3 different membrane levels. At −100 mV, the IPSP reverses. Records in A and B and E and F were obtained during bath application of bicuculline. The action potentials in A have been truncated for clarity. Except where indicated, all responses were recorded at −60 mV.
evoked by a feed-forward inhibitory pathway. Recordings from LGN interneurons indicate that a single shock of optic tract evokes a unitary EPSP with a single spike riding its peak (Lindström 1983; Williams et al. 1996; Zhu and Lo 1999), and this form of activation can reliably evoke robust GABA B activity in relay cells (Crunelli et al. 1987; Lo et al. 2002; Soltez et al. 1989). Moreover, we have also shown that at early postnatal ages repetitive activation of the optic tract is no more capable of evoking GABA B responses than single shocks (Lo et al. 2002) (Fig. 10). Typically, repetitive activation (20 and 50 Hz) of the optic tract evokes a large plateau-like depolarization with no evidence of inhibition. Finally, we have tested frequencies as high as 100 Hz and have seen no sign of inhibition (n = 3, data not shown).

Although GABA A and GABA B inhibition develop at different rates, overall the relatively early maturation of inhibitory responses in the rat differs from that reported in other mammalian species. For example, in the ferret, optic tract-evoked IPSP activity appears during postnatal week 3–4 (Ramoa and McCormick 1994; but see White and Sur 1992), a time when retinogeniculate axon segregation is nearly complete (Linden et al. 1981). A similar timing was found in the developing kitten. Using an isolated diencephalon preparation and extracellular recording techniques, Shatz and Kirkwood (1984) were able to detect inhibitory activity near the late stage of gestation and early postnatal life, a time when retinogeniculate axon segregation is adult-like (Shatz 1983). By contrast, the maturation of inhibitory responses in the rat seems to occur during the early phases retinal axon segregation (Guido and Ziburkus 2001; Guido et al. 2003; Jeffery 1984). Based on the anterograde labeling pattern of retinofugal projections with the subunit B cholera toxin, we estimated that during the first week of life the uncrossed retinal projections occupy between 50 and 90% of LGN (Guido and Ziburkus 2001; Guido et al. 2003). The fact that inhibition in the LGN develops during early (rat) and late phases (ferret and cat) of retinogeniculate axon segregation suggests that such activity may not play a major role in the activity-dependent consolidation of adult-like patterns of retinogeniculate connectivity.

The present results add a new dimension to the development of synaptic circuitry in LGN. During early development, retinal fibers from crossed (contralateral eye) and uncrossed (ipsilateral eye) pathways share common terminal space in LGN and form functional excitatory and inhibitory connections. At later ages, starting at the time of eye opening (P14), synapses between uncrossed retinal axons and relay cells recede, and there is a loss of binocular responsiveness. That is, ipsilateral ON stimulation can no longer evoke EPSP/IPSP pairs. However, in some cells ipsilateral ON stimulation still evokes IPSP activity. In these instances, synapses between uncrossed retinal axons and inhibitory LGN interneurons seem to be maintained.

Binocular inhibitory interactions in the adult LGN have been a subject of intense inquiry, especially in the cat (e.g., Guido et al. 1989; Xue et al. 1987) but also in the monkey (Schroeder et al. 1990). Typically, these studies involved the use of in vivo preparations and showed, through extracellular averaging techniques, that visual stimulation of one (or nondonnant) eye had a relatively weak and varied influence on visual responses activated by the other (or dominant) eye. Because of this, the actual incidence of binocular inhibitory interactions has been difficult to ascertain with estimates ranging from 29% (Guido et al. 1989) to 75% (Xue et al. 1987). The circuitry underlying binocular interactions has also been difficult to delineate. Perhaps the best and most direct evidence for an inhibitory binocular circuit was detailed by Lindström and colleagues. Using intracellular recordings in an in vivo cat preparation, a disynaptic, feed-forward inhibitory circuit was identified (Lindström 1982). These feed-forward binocularly mediated IPSPs were shown to arise from separate interneurons and not from a single one that received convergent retinal input (Ahlsén et al. 1985). In the rat, interneurons are dispersed throughout the LGN (Gabbot et al. 1986) and have extensive branching patterns that span large regions (Webster and Rowe 1984; Zhu and Lo 1999). Thus it is conceivable that the binocular inhibitory interactions reported here are consistent with a feed-forward circuit involving interneurons that receive separate input from the two eyes. Finally their presence in the rodent also suggests binocular inhibitory circuits in LGN are highly conserved across mammalian species.

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