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Membrane and synaptic properties of developing lateral geniculate nucleus neurons during retinogeniculate axon segregation

(ferret/slices/intracellular recording)

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ABSTRACT **During the first postnatal month in the ferret (*Mustela putorius furo*), the projections from the retina to the lateral geniculate nucleus (LGN) become segregated into eye-specific layers and ON and OFF sublayers, a process that is thought to depend in part on neuronal activity. Remarkably, virtually nothing is known about the physiological features of LGN neurons during this period. We have recorded intracellularly from 46 A-layer neurons in slices of the ferret LGN between the ages of postnatal days 7 and 33. The passive membrane properties and current–voltage relationships of the developing neurons were similar in many, though not all, respects to those of adult LGN neurons. Action potentials in younger animals were smaller in amplitude and longer in duration than in older animals, but cells at all ages were capable of producing spike trains whose latency and spike number varied with stimulus intensity. In addition, cells at all ages responded with low-threshold potentials upon release from hyperpolarization. Slightly more than half of the LGN neurons responded to optic tract stimulation with excitatory postsynaptic potentials (EPSPs), inhibitory postsynaptic potentials (IPSPs), or EPSP–IPSP pairs, beginning with the youngest ages. Thus, as early as the second postnatal week, and much before the onset of pattern vision, LGN neurons have many of the membrane and synaptic properties of adult thalamic neurons. These data are consistent with LGN cells playing a significant role in activity-dependent reshaping of the retinogeniculate pathway.**

Neuronal activity has been shown to play an important role in development of connections within the mammalian visual system. For instance, in the retinogeniculate pathway of the fetal cat, blockade of sodium-dependent action potentials by infusion of tetrodotoxin prevents the normal segregation of retinal ganglion cell axons into eye-specific layers (1). Whether this is due to interruption of presynaptic activity, postsynaptic activity, or both is unknown. Earlier studies have shown that retinal ganglion cells are electrophysiologically active at a young age (2–4), but, remarkably, virtually nothing is known about the physiological properties of neurons in the lateral geniculate nucleus (LGN) during the period of retinogeniculate afferent segregation. Indeed, the functional state of immature thalamic neurons in general has been little explored to date. Using intracellular recordings from thalamic slices, we have examined the membrane and synaptic properties of developing LGN neurons in the ferret (*Mustela putorius furo*) during the first postnatal month, when retinogeniculate afferents in that species sort into eye-specific layers and into ON and OFF sublayers (5, 6). While the adult thalamus has been extensively studied, we are not aware of any previous intracellular examination of LGN neurons during the period of afferent reorganization. A brief version of the results has been published in abstract form (7).

MATERIALS AND METHODS

Animals. Twenty-one ferret kits ranging in age from postnatal day 7 (P7) to P33 were obtained from litters born in our colony to timed-pregnant mothers (Marshall Farms, North Rose, NY). The number of animals used at each age is given in parentheses: P7 (1), P9 (4), P10 (1), P15 (1), P16 (1), P17 (2), P18 (2), P22 (1), P23 (3), P29 (2), P30 (1), P33 (2).

Preparation and Maintenance of Slices. Kits were deeply anesthetized with sodium pentobarbital (35 mg/kg i.p.) and Metofane, and perfused intracardially with chilled (4–6°C) oxygenated artificial cerebral spinal fluid (ACSF). The ACSF contained (in mM) 126 NaCl, 20 $NaHCO_3$, 3 KCl, 1.2 $MgSO_4$, 1.25 NaH_2PO_4, 2.5 $CaCl_2$, and 10 dextrose and was pH 7.4 when saturated with 95% O_2/5% CO_2 (vol/vol). The brain was quickly removed from the cranium, and the cerebral cortices and cerebellum were dissected away. The remaining brain was glued to a cutting dish, covered with chilled ACSF, and sliced in the horizontal plane at 350–400 μm on a Vibratome (Technical Products, St. Louis, MO). The LGN was dissected out from each section and transferred to the recording chamber (Fine Science Tools, Belmont, CA). The LGN slices rested at the interface of continually perfusing ACSF warmed to 35°C and humidified atmosphere of 95% O_2/5% CO_2. Recordings began 2 hr after the transfer.

Recording and Stimulation. The health of the LGN slices was assessed by recording extracellularly with a tungsten electrode. Areas in which vigorous extracellular activity could be detected on the oscilloscope and audio monitor were selected for subsequent intracellular recording. Extracellular and intracellular recordings were restricted to the A layers, located in the medial half of the LGN. Intracellular recording electrodes (40–60 MΩ) were made from thin wall capillary tubing (WPI, Sarasota, FL) on a Flaming–Brown puller (Sutter Instruments, San Rafael, CA) and filled with 4 M potassium acetate. Intracellular current injection and voltage recordings were carried out in the bridge mode with an Axoclamp 2A amplifier (Axon Instruments, Foster City, CA). For stimulation of the optic tract, a bipolar stimulating electrode made from Teflon-coated platinum–iridium wire was placed on the optic tract at the lateral edge of the slice. Current pulses of 100–800 μA, duration 50–80 μs, were delivered at 0.3–0.5 Hz to stimulate the optic tract.

Data Analysis. Digitized electrical signals were collected on-line as well as stored on videotape for off-line analysis. The pClamp software (Axon Instruments) was used to analyze responses to current injection and synaptic stimulation.

Input resistance and membrane time constant for each cell were determined during small hyperpolarizing current steps,

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Abbreviations: LGN, lateral geniculate nucleus; P*n*, postnatal day *n*; PSP, postsynaptic potential; EPSP, excitatory PSP; IPSP, inhibitory PSP.

typically between 0.1 and 0.3 nA, and represent an average of three measurements. Time constants were measured during the charging phase of the voltage response, since in some neurons low-threshold potentials were evoked during the discharging phase (see below). The time constant was estimated by using a least-squares procedure to fit the voltage response to a single-term exponential. The resting membrane potential was adjusted for the tip potential recorded at the end of each impalement.

RESULTS

A total of 46 cells in the A layers of the LGN met the criteria for inclusion in the study. These were cells with stable resting membrane potentials of −50 mV or deeper for which recordings lasted long enough for time constants and input resistances to be measured. An analysis of the 46 cells showed that there was no correlation (Pearson $r = 0.069$, $P > 0.05$, test of significance of r) between resting membrane potential and input resistance, indicating that leakage of current at the recording electrode was not a significant problem (8).

Passive Membrane Properties. Fig. 1 shows the resting membrane potential, input resistance, and membrane time constant for each cell in the study. As can be seen, these properties varied considerably at each age. Resting membrane potential and time constant showed no significant change over the period studied ($r < 0.15$, $P > 0.05$ for each membrane property). However, there was a very slight though statistically significant trend toward declining input resistance with age ($r = -0.29$, $P < 0.05$). For the 46 cells pooled, mean resting membrane potential (±SD) was −62.4 ± 7.2 mV, mean input resistance was 58.9 ± 28.0 MΩ, and mean time constant was 7.1 ± 3.1 ms. The mean resting potential and input resistance were similar to those of adult LGN neurons (e.g., see ref. 9), but the time constant was considerably shorter than in the adult (adult mean time constant = 16.4 ± 13.4 ms; ref. 9).

Current–Voltage Relationships. Adult thalamic neurons are characterized by numerous voltage-dependent currents (10), one of which produces prominent outward rectification during depolarization. To determine whether similar conductances were present in the immature LGN, current–voltage (I–V) relationships were studied by injecting a series of square current pulses of varying amplitude (−0.8 to +0.7 nA) for 120–200 ms and recording voltage responses. A representative example of such recordings and the resulting I–V plot are shown in Fig. 2. As in the adult (e.g., see ref. 11), LGN neurons at all ages responded in a linear manner to weak hyperpolarizing pulses of less than 0.4 nA. In addition, they displayed outward rectification when depolarizing current was injected. Occasionally, as in the adult (12), nonlinearities were observed with strong hyperpolarizing current, occurring toward the end of the pulse as an upward deflection in potential. Thus, at least some of the channels contributing to the complex intrinsic membrane properties of the adult thalamus are present in the immature LGN (see also *Discussion*).

Action Potentials and Low-Threshold Potentials. Neurons generated fast spikes during initial penetration by the electrode. Once impaled, the cells ceased spiking and did not fire unless depolarized by current injection. Action potentials could be evoked by depolarizing current pulses beginning at the youngest ages. The action potentials would typically be followed by an afterhyperpolarization of 8–20 ms. One difference between young and older animals was seen in the amplitude and duration of action potentials, measured in a subset of cells ($n = 27$) between P9 and P33. Spike amplitude (range 36.3–98.0 mV) increased with age ($r = 0.418$, $P < 0.05$), while spike duration (range 6.3–1.0 ms) decreased significantly with age ($r = -0.709$, $P < 0.01$).

Spike trains could be evoked at all ages by long depolarizing pulses and reached frequencies of 120–140 Hz with the highest current injection (0.7 nA). An example is shown in Fig. 3*A*. Several properties of the traces in Fig. 3*A* were typical of the sample and similar to the properties of the adult LGN. First, the number and frequency of spikes in a train increased with the amplitude of current injection. Second, the initial spike in each train arrived sooner as current amplitude was increased. Third, the frequency of spikes declined during a given spike train. In some cells, the spike amplitude also became attenuated during the course of a spike train.

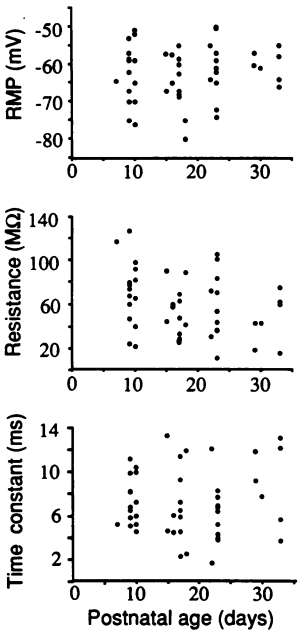

FIG. 1. Scatter plots showing the resting membrane potential (RMP), input resistance, and membrane time constant for each cell in the study.

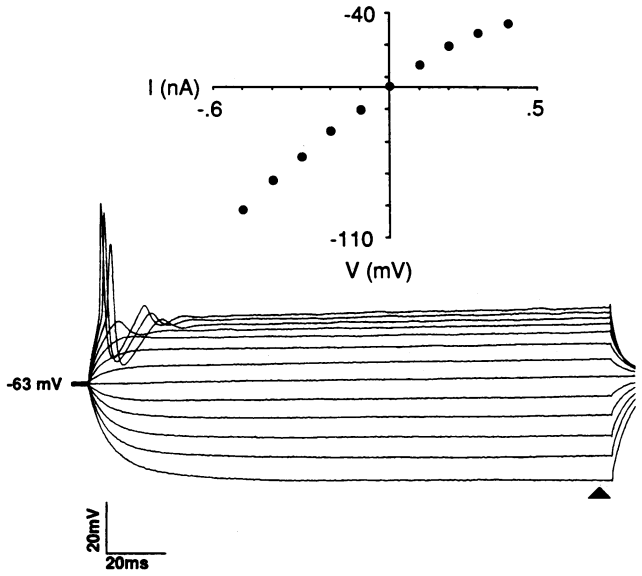

FIG. 2. Current–voltage relationships of an LGN neuron at P22. Voltage traces, averaged from three series of square-wave current injections, are shown on bottom. Arrowhead indicates where membrane potential measurements were taken for the current–voltage (I–V) plot shown on the top. The three traces in which action potentials occurred are not included in the I–V plot.

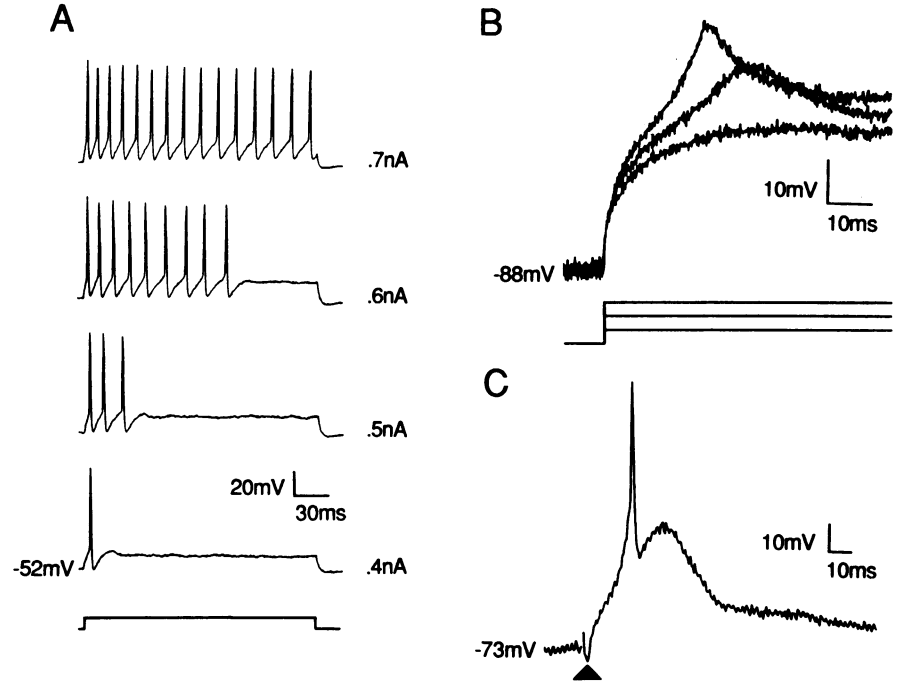

FIG. 3. Action potentials and low-threshold potentials evoked by current ejection and synaptic activation. (*A*) Spike trains evoked in a P15 cell by varying levels of square-wave depolarizing current. The amplitude of the current pulses is shown to the right. The trace at the bottom indicates the pattern of current injection. The membrane potential of the cell was depolarized to −52 mV from rest by constant current injection. (*B* and *C*) Low-threshold potentials evoked at different ages and by different means in LGN neurons. (*B*) A cell from a P9 ferret is hyperpolarized with steady current and depolarized by square-wave pulses of current of varying amplitude. Traces at the bottom of *B* represent pattern of current injection. (*C*) A P18 cell hyperpolarized by current injection is depolarized by synaptic input (arrowhead) after optic tract stimulation, resulting in a low-threshold potential and action potential.

Low-threshold potentials were observed in 28 of the 46 recorded cells, beginning with the youngest ages. As in adult cells (13), they were produced by depolarizing the cell after it had been hyperpolarized. Two means of evoking low-threshold potentials are shown in Fig. 3. In Fig. 3*B*, a cell that had been hyperpolarized to −88 mV by steady current injection responded with graded low-threshold potentials to pulses of depolarizing current of varying amplitude. In Fig. 3*C* a cell that had been hyperpolarized to −73 mV responded with a low-threshold potential when it was depolarized by synaptic input. Low-threshold potentials were commonly seen when a cell was released from current-imposed hyperpolarization. The potentials were similar to those mediated by low-threshold voltage-gated calcium channels (13), and unpublished experiments (C.A.W.) indicate that they remain after voltage-gated sodium channels have been blocked by tetrodotoxin.

Synaptic Responses. Postsynaptic potentials (PSPs) were studied at stimulus intensities just past the threshold for eliciting a response. Synaptic responses were distinguishable from stimulus artifact by their delay in response and the sensitivity of their amplitude to membrane potential. In addition, the stimulus artifact, but not the PSP, would reverse direction as polarity of the stimulating electrode was changed.

PSPs could be elicited in 26 of the 46 LGN neurons by stimulating the optic tract. The PSPs were initiated with short latencies after optic tract shock (0.8–4.5 ms); most appeared to be excitatory responses (n = 19), although purely hyperpolarizing responses (n = 4) and excitatory-inhibitory PSP (EPSP-IPSP) pairs (n = 3) were also observed, as they are in adult LGN slices (9, 14–16). Synaptic responses of all three types were present from the earliest ages.

Fig. 4 shows EPSPs and IPSPs as well as plots of the PSP amplitude vs. membrane potential. The EPSPs (Fig. 4*A*), while not recorded with inhibition blocked, appeared to be purely depolarizing. They peaked at a short latency, declined in amplitude with depolarization, and had an extrapolated reversal potential close to 0 mV, consistent with glutamatergic synapses. The IPSPs (Fig. 4*B*) had slower latencies to peak and reversed at −62 mV, consistent with γ-aminobutyric acid type A ($GABA_A$) receptor-mediated inhibitory synapses.

DISCUSSION

We have examined membrane and synaptic properties of neurons in slices of the ferret LGN between P7 and P33. During this period, retinogeniculate afferents complete segregation into eye-specific layers and ON and OFF sublayers (5, 6). Although neuronal activity has been postulated to play a key role in retinal afferent segregation (e.g., see ref. 1), very little has been known about the functional properties of developing LGN neurons and therefore how they might contribute to activity-dependent remodeling of the retinogeniculate pathway.

Using intracellular recordings, we have found that LGN cells in the postnatal ferret are remarkably similar to cells of the adult thalamus in many of their membrane and synaptic properties (9–16). For instance, both excitatory and inhibitory synapses are present as early as the second postnatal week. In addition, young LGN neurons are capable of generating low-threshold potentials and action potentials. The physiological maturity of developing LGN neurons contrasts with the significant morphological change in soma size (5) and dendritic complexity (17, 18) that takes place in the LGN during the first postnatal month. Before discussing the significance of the results, we will consider some of the technical issues in interpretation of the data.

Technical Considerations. One possible explanation for such adultlike functional properties in developing LGN neurons is that our recordings were biased toward mature cells.

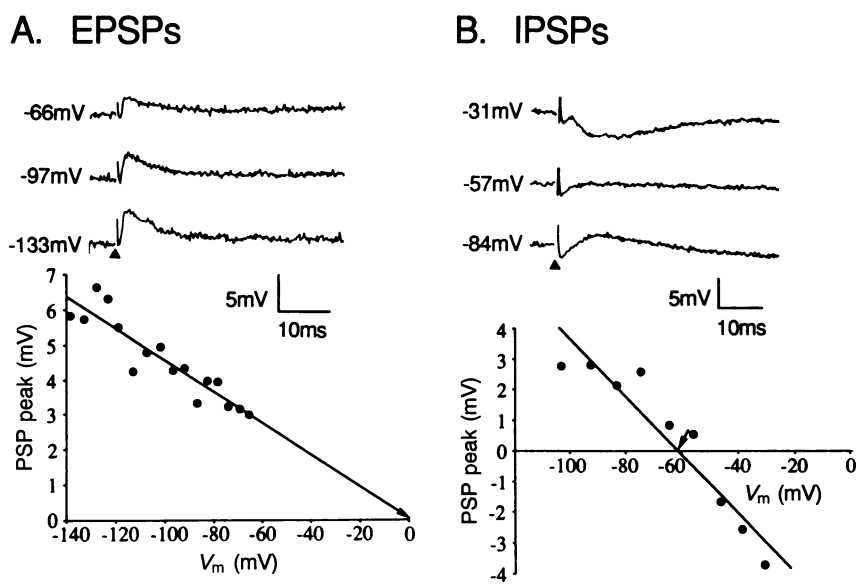

FIG. 4. Single EPSPs (*A*) and IPSPs (*B*) elicited by stimulation of the optic tract in two LGN neurons at P9. The voltage traces recorded during optic tract stimulation at different membrane potentials are shown on the top. Arrowheads indicate optic tract stimulation. In the top trace of *B*, the slight upward deflection prior to onset of the IPSP is due to optic tract shock artifact. The artifact has been attenuated in *A* and *B*. Plots of PSP peak vs. membrane potential (V_m) are shown on the bottom. A regression line is drawn through the points to estimate the reversal potential. The expected reversal potentials are indicated by the arrows.

We cannot rule out the possibility that immature cells were missed because of difficulties in impaling or maintaining them. However, intracellular labeling of cells in slices of ferret LGN at comparable ages (18) indicates that cells of diverse soma sizes and dendritic morphologies can be impaled with these procedures. At the least, we conclude that many neurons in the LGN have strikingly adultlike functional characteristics as early as P7.

Another potential problem is that current spread from stimulation of the optic tract may have activated axons of nonretinal origin within the slice, generating the PSPs we observed. However, in many cases (20 of 46 cells), no PSPs were recorded, even at very high stimulation intensities. This suggests that PSPs generated by nonspecific activation of axons were rare or nonexistent (see also ref. 9).

The failure to elicit PSPs in all neurons raises the question of whether some cells lacked functional retinal afferents. This might be expected, since retinal afferents were becoming reorganized during the period studied. However, technical difficulties such as disconnection of retinal afferents from their target neurons by a suboptimal plane of section could also play a role. In slices of adult LGN as well, there is a substantial failure rate for eliciting retinogeniculate PSPs (e.g., see ref. 9), although all A-layer LGN neurons are thought to be retinally innervated.

LGN Neurons and Retinogeniculate Development. Previous extracellular recording studies in the neonatal rabbit (19) and fetal and neonatal cat (20), as well as electron microscope studies in the hamster (21) and cat (22), have shown that retinogeniculate synapses are present early in development, even before the onset of light-driven activity. Our experiments demonstrate that functional excitatory and inhibitory synapses exist by P7–9 in the ferret LGN, an age that corresponds in gestational and developmental stage to embryonic days 49–51 in cats (5, 23). Inhibition in the ferret LGN was seen 10 days before its reported onset in the prenatal cat (20). Our preliminary results (24) indicate that synaptic transmission in the developing LGN, as in the adult (e.g., refs. 9 and 16), is likely to be mediated by excitatory amino acid receptors, including the *N*-methyl D-aspartate subtype of receptor, and by type A γ-aminobutyric acid receptors.

Adult thalamic neurons possess a rich diversity of membrane currents (see ref. 10 for review). These include a fast and a persistent sodium current, low-threshold and high-threshold calcium currents (13, 25), a hyperpolarization-activated cation current (12), and at least four distinct potassium currents, including a fast A current, a slow A current, an inactivating delayed rectifier current, and a calcium-dependent potassium current (26–28). While we have not examined explicitly the ionic bases of the membrane currents in developing LGN cells, we find significant similarities between developing and adult neurons. These include, importantly, the fact that developing LGN cells are capable (*i*) of firing fast action potentials, including trains of spikes whose latency and number are related to stimulus strength, and (*ii*) of generating low-threshold potentials at hyperpolarized membrane voltages, a feature fundamental to state-dependent gating of thalamic neurons (13). Low-threshold T-type calcium currents have been observed in developing kittens (29); our description of these potentials by the second postnatal week in ferrets pushes their appearance into significantly earlier developmental stages.

The fact that LGN cells possess an impressive range of physiological features by the second postnatal week argues strongly that the functional state of the postsynaptic cell is an important cofactor in any activity-dependent reshaping of retinogeniculate afferents. Indeed, specific physiological features we have encountered could be critical if synapse stabilization involves temporal coactivation of pre- and postsynaptic cells and the favoring of strong (temporally correlated) inputs over weak (temporally uncorrelated) inputs (30). For instance, many, if not all, developing LGN neurons are synaptically connected to retinal afferents during the period of retinogeniculate reorganization. In addition, LGN cells are able to detect differences in stimulus intensity and to modulate their output accordingly; thus, stronger synaptic inputs would produce stronger postsynaptic responses. At the same time, however, the wide range of membrane currents in thalamic cells suggests that retinogeniculate interactions during early development will be complex, depending on both the nature of the synaptic input and the functional state of the postsynaptic cell.

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