Functional Development of Intrinsic Properties in Ganglion Cells of the Mammalian Retina

GUO-YONG WANG, 1 G.-M. RATTO, 2 SILVIA BISTI, 2 AND LEO M. CHALUPA 1

1Section of Neurobiology, Physiology, and Behavior and the Center for Neuroscience, University of California, Davis, California 95616; and 2Istituto di Neurofisiologia, Consiglio Nazionale delle Ricerche, 56127 Pisa, Italy

Wang, Guo-Yong, G.-M. Ratto, Silvia Bisti, and Leo M. Chalupa. Functional development of intrinsic properties in ganglion cells of the mammalian retina. J. Neurophysiol. 78: 2895–2903, 1997. Sensory neurons manifest pronounced changes in excitability during maturation, but the factors contributing to this ubiquitous developmental phenomenon are not well understood. To assess the contribution of intrinsic membrane properties to such changes in excitability, in the present study whole cell patch-clamp recordings were made from developing ganglion cells in the intact retina of postnatal rats. During a relatively brief developmental period (postnatal days P7-P27) ganglion cells exhibited pronounced changes in the discharge patterns generated by depolarizing current injections. The youngest cells (P7-P17) typically responded to a single spike or a rapidly adapting discharge pattern. In contrast, the predominant response mode of more mature cells (P21-P27) was a series of repetitive discharges that lasted for the duration of the depolarization period, and by P25 all cells responded in this manner. These functional changes characterized all three morphologically defined cell classes identified by intracellular labeling with Lucifer yellow.

To determine if expression of the potassium current (Ia) and the kinetics of the Na-channel related to the increased excitability of developing ganglion cells described above, current- and voltage-clamp recordings were made from individual neurons. The different firing patterns manifested by developing retinal ganglion cells did not reflect the presence or absence of the Ia conductance, although cells expressing Ia tended to generate spikes of shorter duration. With maturation the speed of recovery from inactivation of the Na current increased markedly and this related to the increased excitability of developing ganglion cells. Neurons yielding only a single spike to maintained depolarization were characterized by the slowest speed of recovery; cells with rapidly adapting discharges showed a faster recovery and those capable of repetitive firing recovered fastest from Na-channel inactivation. It is suggested that these changes in intrinsic membrane properties may relate to the different functional roles subserved by ganglion cells during development.

INTRODUCTION

The visual responses of developing cells have often been characterized as sluggish and rapidly adapting in comparison with the brisk and repetitive discharges exhibited by mature neurons (Freidlander and Tootle 1990; Sherman and Spear 1982). Such maturational changes could reflect peripheral as well as central events, including the differentiation of voltage-sensitive channels in the neuronal membrane. To gain a better understanding of the contribution of intrinsic membrane properties to developmentally related increases in neuronal excitability, we have made whole cell patch-clamp recordings from developing ganglion cells.

Retinal ganglion cells offer an attractive model for such investigations because a great deal is known about the ontogenesis of these neurons as well as their patterns of connection (Chalupa 1995). Recent studies have related maturational changes in ganglion cell discharge properties to the period when activity-mediated refinements occur in developing retinofugal pathways (Galli and Maffei 1988; Maffei and Galli-Resta 1990; Meister et al. 1991; Skaliora et al. 1993; Wong et al. 1993; Wong and Oakley 1996). Information is also available about age-related modifications in sodium, potassium, and calcium currents (Huang and Robinson 1997; Rorig and Grantyn 1994; Rothe and Grantyn 1994; Schmid and Guenther 1996; Skaliora et al. 1995). As yet, however, it is uncertain which currents contribute to the maturation of discharge patterns exhibited by developing ganglion cells.

In the present study, voltage- and current-clamp recordings were made from ganglion cells in the intact rat retina obtained from postnatal animals ranging in age from postnatal days P7 through P27. This period spans the time during which rats open their eyes and begin to acquire the ability to respond to visual stimuli. Cells were filled with Lucifer yellow to assess the relation between the different firing patterns exhibited by developing ganglion cells and morphologically defined classes. In addition, we sought to relate the expression of the potassium current (Ia) as well as recovery from Na-channel inactivation to the discharge patterns manifested by developing ganglion cells. Previous studies have reported that the Ia contributes to the repetitive firing of neurons (Aghajanian 1985; Connor and Stevens 1971). However, it has also been suggested that the Ia has little influence on firing rates, but instead shapes spike waveforms (Belluzzi et al. 1985; Storm 1987). In terms of the speed of recovery from Na-channel inactivation, this attribute of sodium channel kinetics has been reported to differ among ganglion cell classes isolated from the adult cat retina (Kaneda and Kaneko 1991). However, spike discharge patterns have not been related previously to recovery from Na-channel inactivation in either developing or mature neurons.

METHODS

Retina preparation

Retinas were obtained from hooded rats ranging in age from P7 to P27, with the day of birth denoted as P0. The animals were purchased from a commercial breeder (Simensen’s Laboratory, Gilroy, CA) or a campus breeding colony. To facilitate the separa-
tion of the retina from the pigment epithelium the animals were dark-adapted. After induction of deep anesthesia with either choral hydrate or pentobarbital sodium, the eyes were enucleated under a dim red light and placed in ice-cold saline solution. The animals were then administered a lethal dose of the anesthetic. Each retina was exposed by a single cut along the ora serrata and the eyecup was dissected into two halves by making an additional cut through the optic disk. The tissue was placed for several minutes in iced saline before peeling the retina from the pigment epithelium. The isolated retina was divided into quarters and stored for at least 1 h in oxygenated Eagle’s minimal essential medium (Sigma) at room temperature before recordings were initiated. Subsequently, a single retinal quarter was placed in a recording chamber, ganglion cell layer up, and gently flattened by repeated stroking with a soft camel-hair brush. The retina was secured in place on the bottom of the recording chamber by covering the tissue with a grid made of fine nylon filaments strung tightly across a steel frame. This method does not allow precise identification of the eccentricity of the labeled cells, but all of our recordings were made from a region above midway between the geometric center and the peripheral rim of the retina.

During recordings the retina was perfused continuously with buffered and oxygenated solution (1.5 ml/min). The solution contained the following (in mM): 140 NaCl, 3.6 KCl, 2.4 MgCl₂, 5.0 N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid (HEPES), 12.0 glucose, 1.2 CaCl₂, and 1.0 pyruvate, pH of 7.4 with tetramethylammonium hydroxide (TMOH). The recording chamber was placed on the stage of a fixed-stage Zeiss microscope equipped with appropriate filters to visualize rhodamine (filter 15, excitation BP 546/12, emission long-pass 590) and Lucifer yellow (filter 05, excitation 395–440, emission long-pass 470) fluorescence. In initial experiments the retinas were maintained at room temperature; in later experiments, which provided most of the data, the retinas were maintained at 34°C for 1–2 h. Recordings sessions typically lasted from 8–12 h and during this period the retina remained viable as indicated by stable resting potentials, the presence of spontaneous discharges, and the ability to evoke action potentials with depolarizing currents.

Cells were filled with Lucifer yellow during the recording, which usually lasted 30–50 min. Subsequently the morphological features of these neurons were reconstructed with a computerized morphometric system (Bio Rad CoMOS, version 7.0) attached to a confocal microscope (Bio Rad MRC-600) equipped with an argon laser mounted on an Olympus microscope. Optical sections were collected sequentially in depth throughout the labeled field in steps of 3–5 μm to obtain a z-series. Between 16 and 30 images, depending on cell size, were compiled by the morphometric system to generate a view depicting the entire perikaryon. Because withdrawal of the patch-clamp electrode sometimes distorted the shape of the soma, such reconstructions could not be made for all recorded cells.

To differentiate ganglion cells from displaced amacrine cells, we used three criteria: 1) somal size, presence of axon, and other aspects of cell morphology visualized after filling with Lucifer yellow; 2) the amplitude of the inward currents; and 3) retrograde labeling after injection of rhodamine latex microspheres into the superior colliculus. The somas of displaced amacrine cells in the rat are <10 μm diam (Perry 1981). Consequently, recordings from cells smaller than 10 μm have been excluded from the reported sample. Amacrine cells in the rat have also been shown to be characterized by relatively small inward (i.e., sodium) currents, as well as small amplitude action potentials (Boos et al. 1993). For these reasons, we discarded data from neurons with such electrophysiological properties. In some experiments recordings were made from retrogradely labeled cells. In these cases, animals were anesthetized by hypothermia and two pressure injections (0.5 μl each) of rhodamine latex beads (Molecular Probes) were made into each superior colliculus through a glass micropipette. After an appropriate survival period (usually 2 days), the retinas were removed as described above. Recordings from retrogradely labeled cells confirmed the validity of the other criteria used for identification of ganglion cells.

Electrophysiological recordings

Recordings were made by using the whole cell configuration of the patch-clamp technique in both current- and voltage-clamp modes (Hamill et al. 1981) with an Axopatch 1-D or an EPC-7 patch-clamp amplifier (List, Darmstadt, Germany). To attain whole cell access, the vitreous and outer limiting membrane overlying the recording area were removed by gently brushing the retinal surface with the tip of a glass pipette. Data were low-pass filtered at rates between 1 and 2 kHz and digitized at rates between 5 and 8 kHz. Electrodes with a tip resistance ranging from 5 to 9 MΩ in saline were prepared from glass pipettes (Blau Brand) by using either a BB-CH puller (Mecanex, Geneva) or a Sutter puller (P87). In some instances, the pipettes were covered near the tip with Sylgard (Dow Corning, Midland, MI) to reduce capacitance artifacts. The electrode solution contained (in mM) 10 NaCl, 130 KCl, 1.0 CaCl₂, 2.0 MgCl₂, 10 HEPES, 2.0 ATP, and 1.1 ethylene glycol-bis-(β-aminoethyl ether) N,N,N’,N’-tetraacetic acid (EGTA), as well as 0.2-mg/ml Lucifer yellow CH (Dilithium salt, Sigma). To minimize the possibility of dialysis, in some experiments Nystatin (250 μg/ml) was added to the electrode solution. The series resistance was 9–13 MΩ in whole cell recordings and 12–19 MΩ in Nystatin patch recordings. Membrane and electrode capacitors were compensated electrically. There were no differences in the results obtained with and without Nystatin, although the use of this antibiotic permitted stable recordings for longer time periods (see Robinson and Chalupa 1997a).

Recordings from cells with a seal resistance of <1 GΩ as well as those with inadequate space-clamp in the voltage-clamp experiments were discarded. The resting potential was measured after the whole cell recording configuration was established and rechecked periodically throughout the recording period. Input resistance was calculated by using Ohm’s law from the voltage change obtained to hyperpolarizing current pulses (−10 to −20 pA) from a holding potential of −60 mV. The mean value of the input resistance was 470 ± 180 (SD) MΩ (n = 11) and variations in this measure did not reflect age differences of the recorded cells. For each cell, 10 steps of different current magnitudes were employed, beginning near threshold to values several times threshold. The range of stimulus intensities tested varied from cell to cell (overall values: 10–350 pA), but this was not related to cell type or the age of the animal. A preprogrammed data acquisition paradigm was implemented with either a National Instrument PC-MIO16 board computer (program designed by GMR) or an Axopatch PCclamp 6 program.

RESULTS

Ontogenetic changes in spiking patterns

The overall sample of retinal ganglion cells from which recordings were made in the current-clamp mode (n = 81, obtained from 24 animals) showed three different spiking patterns in response to maintained depolarizing currents. The resting potential of these neurons was ~56.2 ± 7.8 mV and did not vary as a function of age. One group of ganglion cells yielded only a single spike, generated shortly after the onset of the stimulus (Fig. 1A). In a second group, rapidly adapting spike discharges were observed, consisting of a variable number of low-frequency action potentials that sub-
which recordings were made (P7 to P27) there was a clear age-related shift in the distribution of the different response patterns. In the youngest age group, the predominant response mode to injection of maintained depolarizing current was only a single spike (11/15 cells at P7–P8). By contrast, in more mature retinas (P21 and older) none of the cells responded with only a single spike to such stimulation. During this period there was also an increase in percentage of cells capable of repetitive firing. Although a few such neurons were encountered in the youngest retinas, at older ages repetitively firing cells predominated and at P25–P27 all cells manifested such a response pattern. A developmental change was also evident in the incidence of rapidly adapting cells. These neurons constituted nearly 40% of the population ~2 wk after birth, but in the oldest age group (P25–P27) such cells were no longer encountered. These results demonstrate a pronounced increase in excitability with development and they also imply that at maturity rat ganglion cells are capable of responding to maintained depolarization with repetitive firing.

Figure 3 shows confocal reconstructions of the three morphologically distinct ganglion cell classes from which recordings were made. These three cells types appear equivalent respectively, to the type 1, 2, and 3 ganglion cells described in the Golgi study of Perry (1979). Of the 31 cells that were sufficiently well-filled in the present study to permit unequivocal morphological classification, 5 were type 1, 21 were type 2, and 5 were type 3. Peichl (1989) has shown that the type 1 cells can be further subdivided on the basis of their dendritic branching patterns into $\alpha$- and $\delta$-cells. In our sample, the type 1 cells were all $\alpha$-cells, which constitute 2–4% of the rat ganglion cell population. The preponderance of type 2 cells in our sample most likely reflects the fact that these neurons are characterized by larger
somata than type 3 cells and are much more common than the type 1 cells.

All three cell classes were evident in younger as well as older retinas. Moreover, there was no indication that a given morphological cell class was associated with a specific response pattern. For instance, the three morphologically distinct cells shown in Fig. 3 fired in a repetitive manner during the period of maintained depolarization. Table 1 shows the distribution, in terms of age and firing patterns, of the total sample of the 31 cells classified morphologically as types 1, 2, or 3. Taken all together, these observations indicate that the different spiking patterns manifested by developing ganglion cells to maintained depolarizing currents reflect the maturational state of these neurons rather than morphologically distinct cell classes.

Voltage-clamp recordings

The results of the current-clamp recordings, described above, revealed that the incidence of neurons with three distinct response patterns to maintained depolarization (single spike, rapidly adapting and repetitive firing) shifted markedly during development, reflecting an overall increase in ganglion cell excitability. By voltage-clamp recordings we sought to determine whether the expression of specific membrane conductances related to the different firing patterns observed in developing ganglion cells.

Initially, we focused on the rapidly inactivating $I_A$ because this conductance has been reported to contribute to repetitive firing (Aghajanian 1985; Connor and Stevens 1971). To isolate this conductance, retinal ganglion cell ionic currents were activated by stepping the membrane potential to various maintained test potentials from a holding potential of $-70$ mV. This resulted in a fast inward current followed by a slower, longer lasting, outward current. Two groups of ganglion cells could be differentiated on the basis of their outward currents. In the majority of cases (60.7%, $n = 37/61$) the outward current had two clear components: a rapidly inactivating and a slowly inactivating current (Fig. 4A). In the second group of cells (39.3%, $n = 24/61$) only the slowly inactivating component was present (Fig. 4B). On the basis of their activation and inactivation properties (described below) the rapidly inactivating and slowly inactivating outward currents appeared to correspond to $I_A$ and $I_K$, respectively.

In terms of the activation properties, Fig. 5A shows the responses of two cells with and without the $I_A$ current to step depolarizations of $-20$ mV from a holding potential of $-70$ mV. In both cases the responses have been normalized to peak amplitudes attained with a test potential of $30$ mV. As may be seen, the transient potassium component (thick traces) reached a peak value at $4.5 \pm 2.5$ ms and then declined to baseline in $<25$ ms. At this depolarization value, the other cell showed no appreciable transient outward current (thin traces). Figure 5B depicts the relative amplitudes of $I_A$ and $I_K$ at various test potentials, normalized to the amplitudes attained with a test pulse of $30$ mV, starting from a $-70$ mV holding potential. Note that the $I_A$ began to activate at less than $-40$ mV, whereas the $I_K$ was activated at around $-30$ mV. As shown in Fig. 5C, there was also a clear difference between the two outward components in terms of their inactivation properties.

These characteristics of the $I_A$ and $I_K$ are virtually identical to the attributes of these potassium conductances we documented previously in dissociated cat retinal ganglion cells (Skaliöra et al. 1995). In that study pharmacological agents (tetraethylammonium (TEA) and 4-aminopyridine (4-AP) were used to validate the kinetics and voltage-dependence of activation and inactivation of these potassium currents.

Relation of $I_A$ and $I_K$ to spiking properties

As indicated above, it has been suggested that $I_A$ contributes to the repetitive firing of neurons (Aghajanian 1985; Connor and Stevens 1971). Other investigators have suggested that this conductance plays a role in shaping spike waveform, with little effect on firing rate (Belluzzi et al. 1985; Storm 1987). To assess the relationship between $I_A$ expression and spike discharge patterns and/or spike duration, both current- and voltage-clamp recordings were made from 39 retinal ganglion cells. This revealed that $I_A$ was expressed in neurons characterized by each of the three firing patterns described above. Figure 6 illustrates this point by showing the presence of $I_A$ in two different cells—one that yielded a single spike (Fig. 6A); one that discharged in a repetitive fashion (Fig. 6B). In the overall sample, 11 cells

TABLE 1. RGCs firing patterns in relation to age and cell types

<table>
<thead>
<tr>
<th>Age</th>
<th>Type 1</th>
<th>Type 2</th>
<th>Type 3</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>P7–P9</td>
<td>1-R</td>
<td>4-S</td>
<td>1-A</td>
<td>6</td>
</tr>
<tr>
<td>P10–P12</td>
<td>1-S, 1-R</td>
<td>1-S, 1-A, 1-R</td>
<td>2-S</td>
<td>7</td>
</tr>
<tr>
<td>P13–P17</td>
<td>2-S, 1-A</td>
<td>2-A, 1-R</td>
<td>1-R</td>
<td>3</td>
</tr>
<tr>
<td>P21–P24</td>
<td>1-A, 1-R</td>
<td>2-A, 4-R</td>
<td>5-R</td>
<td>9</td>
</tr>
<tr>
<td>P25–P27</td>
<td>5-R</td>
<td>1-R</td>
<td>6</td>
<td></td>
</tr>
</tbody>
</table>

Number of type 1 cells was 5, type 2 was 21, and type 3 was 5. Total number of cells was 31. S, single spike; A, rapidly adapting; R, repetitive.
responded with a single spike (7 with $I_a$), 9 were rapidly adapting (5 with $I_a$), and 19 fired repetitively (13 with $I_a$). Thus the distinct firing patterns manifested by developing retinal ganglion cells to maintained depolarization did not reflect the presence or absence of the $I_a$ conductance.

The expression of $I_a$ was also examined in relation to the duration of the action potential. Figure 7 shows the distribution of spike durations (half-width at 50% amplitude) as a function of age. Cells lacking $I_a$ are denoted separately from those with this conductance. Note that irrespective of age, cells without $I_a$ tended to have spikes of longer duration. However, there were no age-related differences in the incidence of cells expressing $I_a$ during the developmental period under consideration. Nevertheless, in the overall sample of cells there was a clear trend for spike duration to decrease with maturation. Thus between the ages of P10-P17 the average half-width at half-height of the spikes recorded was $1.77 \pm 0.53$ ms ($n = 19$), whereas in the older cells it was $1.36 \pm 0.39$ ms ($n = 27$). This difference was statistically significant ($P < 0.05$, two-tailed t-test).

Recovery from inactivation of sodium channel

In an earlier study of developing cat retinal ganglion cells (Skaliora et al. 1993), significant changes in sodium current properties were noted, including an increase in Na-current density, as well as a shift in the voltage dependence of both activation and steady-state inactivation. More recently, Schmid and Guenther (1996) have reported that Na-current density in rat ganglion cells attains adult levels by P9, the youngest age group from which recordings were made in the present study. An attribute of the sodium channel kinetics not considered by previous developmental studies is the speed of recovery from sodium channel inactivation. If the time to recovery of sodium channel inactivation was longer in younger cells, this would provide a reasonable explanation for the age related changes in excitability observed in the present study. Hence, we sought to determine whether or not the speed of sodium channel recovery from inactivation related to the ganglion cell firing patterns that differentiated younger from older cells.

The methods for measuring sodium channel recovery from inactivation were essentially the same as those described for cat retinal ganglion cells (Kaneda and Kaneko 1991). Briefly, the firing pattern of the recorded cells to maintained depolarization was identified by current-clamp recording, without any ion-channel blockers. Once this was established, voltage-clamp recordings were made from the same cell to examine recovery from inactivation. A control recording was made by depolarizing the membrane holding potential from $-85$ to $-5$ mV for 50 ms. To assess the speed of recovery, the protocol shown at the bottom of Fig. 8 was used. As may be seen, a 10-s depolarizing step from a holding potential of $-85$ to $-5$ mV was followed by a hyperpolarizing pulse to $-85$ mV of variable duration. At the end of the variable duration hyperpolarizing pulse, the magnitude of the recovered inward currents was assessed by stepping to $-5$ mV for 50 ms. The control and recovery protocols were first performed without tetrodotoxin (TTX) and both protocols were then repeated with TTX (300 nM) in the bath solution. This provided a means for obtaining a measure of sodium currents by subtracting the relevant recordings with TTX from those obtained without this sodium channel blocker.

Figure 8 shows such sodium currents for a cell that fired repetitively (Fig. 8A) and a neuron that generated only a single spike (Fig. 8B). As may be seen, the recovery from $I_{Na}$ inactivation was substantially faster in the repetitively firing neuron. For instance, whereas the amplitude of $I_{Na}$ after 1.1-s hyperpolarization was 64.1% of control, in the repetitive firing cell it was only 23.8% of control in the single spike cell.

The time course of recovery from inactivation could be fitted by a double exponential function. During recovery from inactivation the number of Na channels ready to be opened by depolarization is expressed by the following equation:

**FIG. 5.** A: responses to stepping membrane potential to $-20$ mV from a holding potential of $-70$ mV for 2 cells with and without $I_a$ (thin and thick lines, respectively, depolarization delivered at $t = 0$). Currents are normalized to peak amplitude attained in response to a depolarization to 30 mV to compensate for differences in overall conductance of each cell and to allow for an easier comparison between different kinetics of outward currents. B: dependency of outward current peak amplitude (normalized as above) on step potential from $-70$ mV holding potential. Values for cells without $I_a$ were measured as maximum current reached within 300 ms from onset of depolarization. ■, cells with $I_a$ ($n = 3$); ●, cells without $I_a$ ($n = 3$). C: dependency of peak amplitude of $I_a$ and $I_h$ on holding potential. A cell displaying $I_a$ was clamped from plotted holding potential to a step potential of 10 mV. ●, peak current measured within 20 ms from voltage step onset. △, current measured at 300 ms, well after transient component extinguished. $I_a$ is more rapidly inactivated by depolarizing the holding potential than $I_h$. Age range of the 7 cells that provided data in this figure was P12–P22.
FIG. 6. $I_a$ expressed in a P14 cell that generated only a single spike (in A) as well as in a P24 cell with a repetitive discharge pattern (in B). Top: voltage-clamp recordings, with voltage-clamp protocol shown above. $+$, peak of the $I_a$ current. Middle and bottom: current-clamp recordings. Amplitude of injected current is indicated above each trace. Recordings were obtained at body temperature.

The foregoing observations indicate that the predominant response pattern characterizing immature ganglion cells (i.e., single spike discharge) is closely associated with the slowest speeds of recovery, whereas the discharge pattern manifested by mature cells (i.e., repetitive firing) is related to the fastest speeds of recovery. Moreover, the speed of recovery from ganglion cells were statistically significant ($P < 0.05$, two-tailed tests).

The following equation was used to calculate the time constants of the fast and slow components of the $I_a$ current.

$$N = \frac{N_0}{I_f + I_s} \left[ I_f (1 - e^{-t/f}) + I_s (1 - e^{-t/s}) \right]$$

where $N_0$ represents the total number of Na channels of a cell (which had been inactivated by the preceding depolarization), $f$ and $s$ represent the time constants of the fast and slow components respectively, $I_f$ represents the weight of contribution of the fast component, and $I_s$ that of the slow component (Kaneda and Kaneko 1991). With this equation we calculated the time that sodium channels needed to recover to 50% of control by using a time step of 1 ms. This permitted a comparison of the speed of recovery of Na-channel inactivation among cells (Fig. 9). As may be seen, speed of recovery was fastest in repetitive firing cells ($80 \pm 28$ ms, $n = 8$), intermediate in rapidly adapting cells ($339 \pm 207$ ms, $n = 12$), and slowest in single spike cells ($2,909 \pm 1,294$ ms, $n = 8$). These differences among the three groups of ganglion cells were statistically significant ($P < 0.05$, two-tailed tests).

![Figure 7](https://example.com/fig7.png)

**FIG. 7.** Age-related changes in distribution of half-width of spikes, measured at 50% peak amplitude. ○, cells with $I_a$; ▲, cells without $I_a$; ■, cells for which voltage-clamp recording were not made because of space-clamp problems. These recordings were at body temperature. Height of the spikes varied from 35 to 55 mV, but variation was not age-related.

![Figure 8](https://example.com/fig8.png)

**FIG. 8.** Recovery of $I_{Na}$ from inactivation. These data were obtained by subtracting the recordings with tetrodotoxin (TTX) from those without TTX (see RESULTS). Sodium channels were fully inactivated by depolarizing membrane potential from $-85$ to $-5$ mV for 10 s. $I_{Na}$ was measured by depolarizing membrane potential to $-5$ mV after hyperpolarizing membrane potential from $-5$ to $-85$ mV for various durations ($\Delta t$). Numbers shown above each trace indicate the duration of hyperpolarization ($\Delta t$). A: a P12 ganglion cell that exhibited a repetitive firing pattern in response to maintained depolarization. B: a P11 cell that yielded only a single spike in response to maintained depolarization. Recovery of $I_{Na}$ from inactivation in this cell is much slower than in repetitively firing neuron.
Relation to morphologically defined cell classes

To our knowledge, the present study describes the first attempt to examine developmental changes in intrinsic membrane properties of morphologically identified retinal ganglion cells. Injections of Lucifer yellow permitted the morphological classification of the cells from which recordings were made and three different cell classes could be distinguished, corresponding to the type 1, 2, and 3 cells in the Golgi study of Perry (1979). The functional changes described here during postnatal development were not limited to any particular ganglion cell class. However, our sample was heavily biased for type 2 cells (for the reasons discussed previously), so we cannot rule out the possibility of subtle developmental differences among ganglion cells classes.

In previous work on dissociated fetal and postnatal cat retinal ganglion cells, we also found an ontogenetic increase in the number of cells capable of sustained discharge patterns (Skaliora et al. 1993). However, because the dissociation procedure causes a loss of dendritic processes, cell classes could not be distinguished. In the postnatal cat retina, a proportion of cells with rapidly adapting discharges were still evident at P20. In the present study, we show that in the rat by P25 all cells from which recordings were made responded to depolarizing current injections with repetitive firings. This implies that recordings from older cat cells would yield only repetitive discharges and this has been found to be the case in recent studies in this laboratory (Robinson and Chalupa 1997b). Thus the available evidence indicates that at maturity all (or nearly all) retinal ganglion cells are capable of repetitive discharges to maintained depolarizations in both cat and rat. At the same time, extracellular recordings from retinal ganglion cells of both species have shown that these neurons can respond to light in either a sustained or transient manner (rat, Brown and Rojas 1965; cat, Cleland et al. 1973). This implies that the different response patterns evoked by visual stimuli at maturity (sustained or transient) reflect differences in retinal circuitry, as has been proposed by others (e.g., Werblin 1977).

Current properties in relation to spike discharges

Several recent studies have documented developmental changes in the expression and kinetics of voltage-activated ionic currents (Baraban and Lothman 1994; Bayliss et al. 1994; Gorter et al. 1995; Kandler and Friauf 1995; Keen et al. 1994; Rossi et al. 1994; Schmid and Guenther 1996; Skaliora et al. 1993, 1995), but with few exceptions (e.g., Gruol et al. 1992) previous investigators have not made voltage- and current-clamp recordings from individual neurons. The current- and voltage-clamp recordings from individual retinal ganglion cells in the present study showed that spike duration and the discharge patterns manifested by these neurons can be accounted for by distinct current properties.

In terms of their expression of potassium conductances, retinal ganglion cells could be subdivided into two groups: some cells were characterized by both the $I_K$ and $I_\text{Na}$, whereas only an $I_K$ current was expressed in other cells. This has also been found to be the case in dissociated cat retinal ganglion cells (Skaliora et al. 1995). In the present study, the differential expression of $I_K$ was found to correlate with the duration...
of the action potential because cells expressing this conductance generated action potentials of significantly shorter duration. Moreover, this was the case for both older and younger cells. At the same time there was no obvious relationship between the expression of $I_{Na}$ and the ability of cells to generate repetitive firing as has been reported in other systems (Aghajanian 1985; Connor and Stevens 1971). It is important to emphasize, however, that our findings apply to developing ganglion cells, whereas previous investigators have been concerned with the relation of this conductance to the diverse firing patterns exhibited by mature cells.

The current- and voltage-clamp recordings obtained from individual neurons also revealed that the increase in excitability exhibited by developing ganglion cells relates to changes in the time of recovery from sodium channel inactivation. Cells capable of generating only a single spike to maintained depolarization had the slowest speed of recovery. Rapidly adapting cells recovered at a significantly faster rate and the neurons that fired repetitively were characterized by the fastest recovery. A parsimonious explanation of these observations is that during development there is a progressive change in the membrane properties underlying the speed of recovery from Na-channel inactivation. Moreover, such a change in the kinetics of the Na-channel appears to occur in all morphologically defined cell classes. The procedure we employed to assess the speed of recovery from Na-channel inactivation was essentially identical to that used by Kaneda and Kaneko (1991). These investigators reported that isolated $\alpha$- and $\gamma$-cells obtained from adult cat retinas exhibited significant differences in Na-conductance kinetics, but the relation of speed of recovery from sodium channel inactivation to presumed differences in discharge patterns in these neurons remains to be established.

Recent studies have demonstrated a large diversity of ion channels in retinal ganglion cells. Indeed, in a cogent review of this literature, Ishida (1995) concluded that the available evidence indicates that more ion channels are expressed in ganglion cells than in any other type of retinal neuron. The role(s) of these diverse channels in shaping firing patterns are just beginning to be assessed. In particular, it has been shown that Ca-activated potassium currents in ferret retinal ganglion cells act to modulate discharge patterns in these neurons (Wang et al. 1995). It is reasonable to think that other conductances also contribute to the spiking patterns of retinal ganglion cells, but this remains to be established in future work.

**Concluding remarks**

The developmental period examined here spans the time (P7–P27) when rat pups normally open their eyes, at P11–P14. Interestingly, this corresponds rather closely to the period when rat photoreceptors have been shown to develop their sensitivity to light (Ratto et al. 1991). The time before eye opening also encompasses the developmental period when retraction of initially widespread retinofugal projections has been documented (Land and Lund 1979) and such refinement of connections has been shown to be based on activity-mediated events (Constantine-Paton 1990; Dubin et al. 1986; Hahn et al. 1991; Shatz 1990; Stryker and Harris 1986). Thus at the time that retinal activity is thought vital for activity-mediated refinements, ganglion cells appear predominantly limited to firing single or brief bursts of spikes. By contrast, later in development, when these neurons convey signals from the retina to the visual centers of the brain, they become capable of generating sustained trains of action potentials. It is intriguing to speculate that the membrane properties characterizing immature ganglion cells (e.g., slow recovery of sodium channel inactivation) may have some functional utility for the generation and/or propagation of spontaneous bursts of activity in the developing retina (Galli and Maffei 1988; Masland 1977; Meister et al. 1991; Tootle 1993; Wong et al. 1993). If this were the case, the changes in intrinsic properties documented here might reflect the different roles subserved by the discharge patterns of ganglion cells before and after these neurons are capable of processing visual information.

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Address reprint requests to Leo M. Chalupa, Center for Neuroscience, University of California, Davis, CA 95616.

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**REFERENCES**


Strecker, M. P. and Harris, W. A. Binocular impulse blockade prevents the formation of ocular dominance columns in cat visual cortex. J. Neurosci. 6: 2117–2133, 1986.


