Early Development of X-Cells in Kitten Lateral Geniculate Nucleus

Abstract. *Lateral geniculate nucleus cells of the kitten were classified as X-cells or Y-cells with a contrast reversal test and their latencies to optic chiasm shock were measured. X-cells with mature latencies were found as early as 21 days. Y-cells did not have adult latencies at 40 days. The early development of some X-cells may be due to differential rates of fiber myelination and synaptic maturation within the lateral geniculate nucleus.*

The X/Y distinction has been used to classify neurons in the mammalian retina and lateral geniculate nucleus (LGN) (*1*, *2*). The original X/Y classification was based on responses of neurons to contrast reversal of patterns in their receptive fields (*3*). For X-cells a place in the receptive field could be found at which light and dark areas of striped gratings could be reversed without causing a response from the cell. For Y-cells no such place could be found. The X/Y terminology has also been used to denote a cell's conduction velocity or latency to an electrical stimulus. Hoffmann *et al.* (*2*) called moderate-conducting cells (19 to 24 m/sec) X-cells and fast-conducting cells (36 to 44 m/sec) Y-cells, and Stone and Fukuda proposed (*4*) that these cell groups were equivalent to the original X/Y cell groups identified with contrast reversal tests. Neuroanatomists have suggested that large cells in the retina and LGN are Y-cells (*5*). Partly because of the anatomical correlations that can be made, X/Y is probably a more fundamental classification than "on" compared to "off" cells.

Along with classification schemes for cells, visual physiologists have been interested in the development of cells. For example, single units in both the visual cortex (*6*) and superior colliculus (*7*) have been studied in kittens. With regard to the development of the visual cortex, in fact, there is a controversy in the literature about the age of appearance of orientation selective responses and the effects of experience on those responses (*8*).

Since it has been suggested that the X- and Y-cells of the LGN project directly to different kinds of cortical neurons (*9*), we thought that a study of the development of X and Y responses in the LGN, besides being of interest in itself, could help us understand more about the development of visual cortex neurons. The data we present here suggest that some LGN X-cells mature before Y-cells and other X-cells, and that synaptic delay in the LGN may be an important factor in this phenomenon.

Table 1 shows our data base. We studied 237 single LGN units which responded to optic chiasm (OX) stimulation in 17 kittens ranging in age from 6 days to 40 days. These ages correspond roughly to a time just before eye-opening to a time near the middle of the critical period (*10*). The kittens were prepared for single-unit recordings with methods similar to

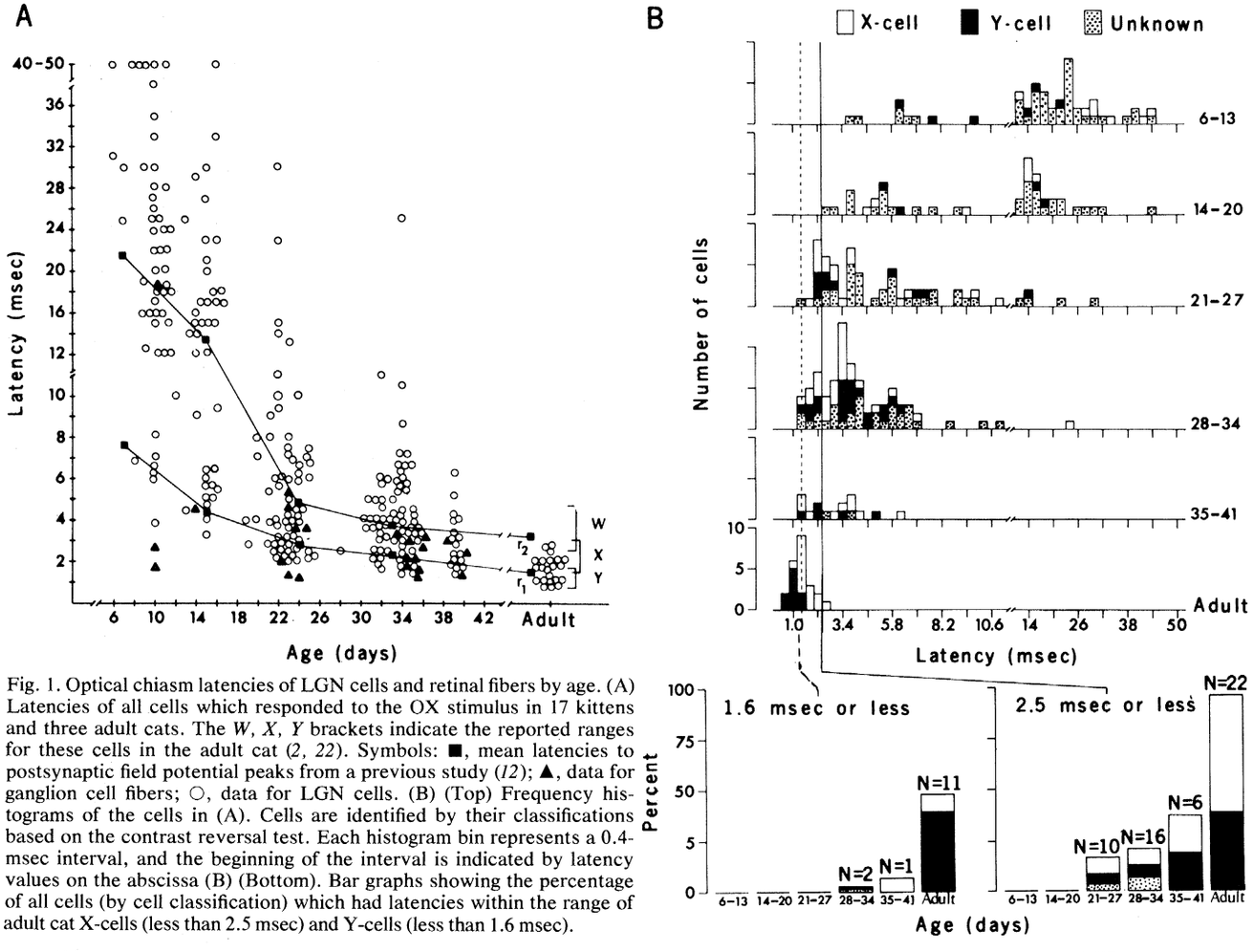

Fig. 1. Optical chiasm latencies of LGN cells and retinal fibers by age. (A) Latencies of all cells which responded to the OX stimulus in 17 kittens and three adult cats. The *W*, *X*, *Y* brackets indicate the reported ranges for these cells in the adult cat (*2*, *22*). Symbols: ■, mean latencies to postsynaptic field potential peaks from a previous study (*12*); ▲, data for ganglion cell fibers; ○, data for LGN cells. (B) (Top) Frequency histograms of the cells in (A). Cells are identified by their classifications based on the contrast reversal test. Each histogram bin represents a 0.4-msec interval, and the beginning of the interval is indicated by latency values on the abscissa (B) (Bottom). Bar graphs showing the percentage of all cells (by cell classification) which had latencies within the range of adult cat X-cells (less than 2.5 msec) and Y-cells (less than 1.6 msec).

those used for adult cats (*11*) except that plastic head braces replaced the earbars. Bipolar stimulating electrodes were placed in the chiasm which was located by recording "swish" from the optic fibers in response to a strobe flash. Electrode placements in the chiasm and LGN were confirmed with histology. A WPI Anapulse stimulator and isolator were used to shock the chiasm. We located the LGN with a previously worked out coordinate system (*12*), isolated single units with tungsten-in-glass microelectrodes (*13*), and recorded each cell's response to OX stimulation on Polaroid film. Later, the cells' latencies were determined from these photographs (*14*) without knowledge of receptive field characteristics.

For our X/Y testing, images at luminances from 200 to 500 cd/m^2 were rear-projected onto a tangent screen (6 to 10 cd/m^2) with a Prinz 150-W projector. These luminance values are higher than those used in most studies of adult cats, but it must be remembered that for kittens less than about 4 weeks of age, the optical pathway to the retina is clouded (*15*). We tested each cell for its X/Y property with a contrast reversal test (*16*), and recorded a variety of other receptive field characteristics (*17*).

Before the time of optic-nerve myelination, near the start of the third week (*18*), we could classify with certainty only about one-third of the cells encountered as X or Y (*19*). Also, during this time cells were difficult to drive electrically (see Table 1). In most cases, dual instead of single shock pulses and high (30 to 50 hertz) rates of shock were necessary to evoke reliable responses. Even then, there was considerable variability in the spike latencies for a single cell (*20*). The latencies of all cells we encountered are plotted by age in Fig. 1A. Most cells of kittens younger than 20 days had OX latencies greater than 12 msec and greater than nearly all cells of older kittens.

Figure 1B, top, shows the frequency distributions for cell latencies by cell classification: X, Y, or unknown. Prior to the third week very few of the short-latency cells are X-cells, but after this time both X- and Y-cells are found at all latencies. The adult pattern of short-latency X-cell populations begins emerging in only the oldest kitten, 38 to 40 days of age. The means for X- and Y-cells of kittens 21 to 35 days of age did not differ significantly (*21*).

X-cells of the adult cat LGN have OX latencies ranging from about 1.6 to 2.5 msec (mean 1.8 msec), whereas Y-cells have latencies ranging from about 0.8 to 1.6 msec (mean 1.2 msec) (*2, 22*). Beginning at 21 days, one-fourth of all of the X-cells found in kittens have OX latencies within the adult X-cell latency range. In contrast, we found only one Y-cell with an OX latency within the adult Y-cell range in all of the kittens studied (see Fig. 1B, bottom). The mean latency for the fastest X-cells (less than 2.5 msec) in the oldest kitten was about 1.88 msec [standard deviation (S.D) 0.2], about equal to the adult value. The mean latency for the fastest Y-cells (less than 2.5 msec) in the oldest kitten was about 1.7 msec (S.D. 0.18), 0.5 msec from the adult value. The one Y-cell in the adult range had a latency of 1.6 msec. This suggests to us that at least some of the X-cells in kitten LGN have matured very early in terms of OX response latency.

The appearance of adultlike OX latencies as early as 21 days is surprising in view of the fact that the distance between the optic chiasm and the LGN doubles after this time because of growth (*12*). It is likely, however, that the continuing myelination of optic fibers (*18*) provides a velocity increase which offsets the increase in conduction distance.

More complete early myelination of these X-cell afferent fibers may be responsible for their fast maturation. Also, we have evidence that suggests that synaptic mechanisms within the LGN may be important. Eight times in our experiments we recorded OX-evoked responses from an LGN cell and a retinal fiber simultaneously, and in three of these dual recordings we were able to isolate both units and ascertain that they had the same receptive field characteristics. While this does not provide conclusive evidence for a functional relationship, the inference has been made by others (*23*) and our data indicate that it

Table 1. Subjects and cells sampled.

Age (days)	Kittens (*N*)	LNG cells (*N*)		Retinal fibers in LGN (*N*)*
		OX driven	Not driven	
6 to 13	5	51	25	3
14 to 20	5	41	10	1
21 to 27	3	61	5	7
28 to 34	3	70	2	9
35 to 41	1	14	0	3
Total	17	237	42	23

* These were units within the LGN which had all the characteristics of optic tract fibers including all-or-none responses, perfect following of a 100-hertz OX stimulus, and typical fiber spike waveform.

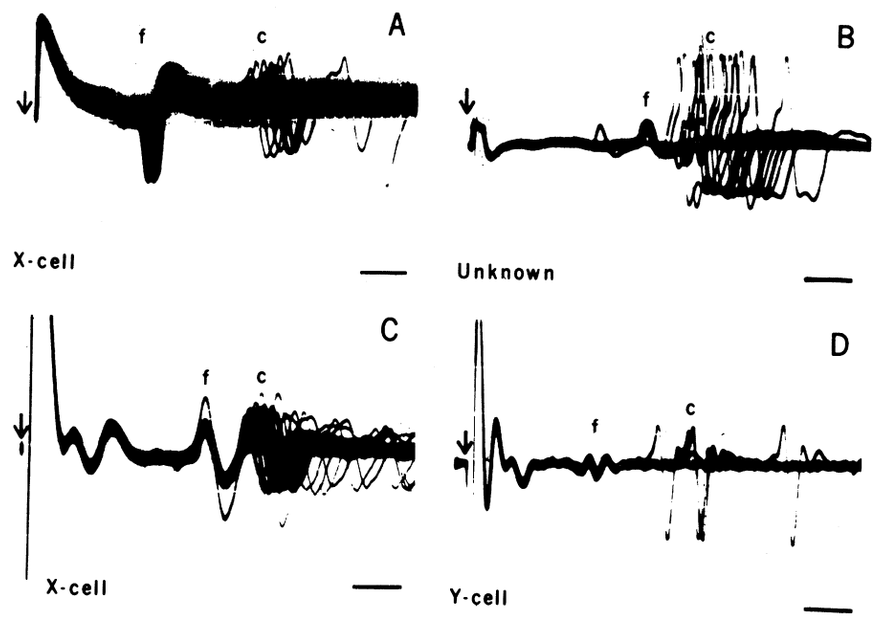

Fig. 2. Responses of four retinal fiber (*f*)–LGN cell (*c*) pairs to 100-hertz OX stimulation. Stimulus onset is indicated with an arrow. The time marker represents 0.5 msec in (A) and (C) and 1.0 msec in (B) and (D). In each case the retinal fiber shows perfect following while the LGN cell is irregular and variable in latency. (A) X-cell pair from K29 (21 days). (B) Unclassified cell pair from K29 (21 days). (C) X-cell pair from K30 (33 days). (D) Y-cell pair from K30 (33 days).

might be so (*24*). Examples of four fiber-cell pairs are shown in Fig. 2. In three of the four pairs both units were isolated and found to have similar field characteristics. The delays between the retinal fiber spike and the LGN cell spike for the two X-cells are about 1.2 msec (1.0 to 2.0) (21-day-old kitten) and 0.5 msec (0.5 to 0.8) (33-day-old kitten). The Y-cell pair of the 33-day-old kitten had an interspike delay of 2.0 msec (1.4 to 3.8), and the unclassified pair (21-day-old kitten) had a delay of 1.6 msec (0.5 to 2.8). This suggests the possibility that developmental events within the LGN may contribute to the observed latency difference between cells.

In adult cats the LGN cell responses are time-locked to the maintained activity of one to three retinal ganglion cells (*25*); however, this relationship has not been demonstrated in the kitten. It has been our finding that LGN cells of kittens younger than about 4 weeks of age have zero to very low (< 1 per second) maintained rates of firing, while retinal fibers have higher rates (*26*). It is likely that temporal and spatial summation are a requirement for driving immature LGN cells, in which case any single fiber-cell pair might not be related as in the adult cat. Certainly this should be investigated. Our finding, however, is that when the visual pathway is stimulated electrically, activating large numbers, if not all afferents to the LGN, cell pairs have different fiber-cell response intervals. And, in addition, the data we have indicate the possibility of longer intervals for immature Y-cells. The different fiber-cell response intervals, as well as the variability in intervals observed within a single pair of units, might be an indication that there is summation between synapses of different retinal afferents at single LGN cells in kittens. Loss of this convergent input, as well as synaptic maturation, might then account for synaptic delay changes. Furthermore, these processes may have different time courses for X- and Y-cells.

In addition to early OX latency maturation, we also find that some X-cells develop surround responses, surround inhibition, adult receptive field sizes, and mature responses to moving targets by 21 to 34 days and prior to nearly all Y-cells (*26*).

Our data show that before myelination of optic-nerve fibers, all LGN cells have long OX latencies and immature receptive field properties. After myelination onset it appears that some of the X-cells mature to their adult response character quickly. If some cells in the visual cortex receive primarily Y-cell input from the LGN (*9*), then some of those cells may mature more quickly than their counterparts receiving Y-cell input. This may account for the fact that some mature cells have been observed in young kitten visual cortex (*8*). Also, a protracted developmental period for Y-cells might help to account for some of their modifiability with visual deprivation (*27*).

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11. Surgical procedures were performed under Fluothane anesthesia (1 to 2 percent) delivered in a mixture of nitrous oxide and oxygen (2 : 1 by volume). A local anesthetic was applied to wound edges and the subsequent procedures were continued with N_2O, O_2, and CO_2 (70, 27, and 3 to 4 percent, respectively), paralysis being maintained with a constant infusion of Flaxedil (10 mg per kilogram per hour) in 5 percent dextrose. Fluids were infused at a rate of 0.84 ml/hour, and kittens were respired at a rate of 50 cycles per minute with a gas volume of 10 to 15 cm^3 per stroke. Temperature was maintained at 36° to 38°C, and vital signs were monitored.
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16. Two abutting hemicircles (contrast 0.9 to 0.96) were projected through perpendicular pieces of polarized glass onto the screen to form a pattern over the receptive field center and surround region. The pattern could be adjusted in size from 2° to 12°; cells with larger fields could not be tested. A large Polaroid sheet was rotated between the projector and the screen to darken and brighten the two hemicircles symmetrically, leaving the total luminance in the field constant. The border between the two hemicircles could be moved around on the screen to search for a contrast reversal null position.
17. A Nova II minicomputer was used to present flashing and moving spots of various sizes and to collect spike data. Based on this data, we classified cells as sustained or transient and also noted their field sizes, presence of surround responses, strength of surround inhibition, and responses to different velocities of stimulus movement.
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19. Many cells in young kittens had responses that were not definitely typical of X- or Y-cells in that a position could be found in the receptive field at which the response to contrast reversal was weaker but not totally abolished. These cells were not classified. Cells were classified as X only if there was a clear null position, and as Y only if the response remained as strong at reversal as at unmasking of the bright hemicircle. Many immature cells failed to respond to the unmasking of the lighted half of the pattern and thus could not be tested.
20. The mean range of spike latencies for all cells of kittens 6 to 20 days of age was 6.5 msec (S.D. 3.8). At 21 to 27 days the mean range dropped to 1.5 msec (S.D. 0.5), and it was adultlike (0.5 msec, S.D. 0.2) at 35 to 41 days.
21. The means for all X-cells/Y-cells at 21 to 27 days and 28 to 34 days were 4.6 msec (S.D. 3.1)/5.1 msec (S.D. 2.6) and 3.4 msec (S.D. 1.2)/3.8 msec (S.D. 1.3), respectively.
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24. According to our data: (i) in the eight unit pairs the mean interspike latency difference decreased with age; (ii) the range of interspike latency differences (0.6 to 2.7 msec) was small compared to the possible range given random occurrence of any fiber-cell pair (see Fig. 1); (iii) in all eight cases the cell spike followed the fiber spike, regardless of the fiber spike latency (note in Fig. 1 that many fibers encountered had long latencies); and (iv) our electrode tips were small (5 to 10 μm in length and 2 to 4 μm in diameter).
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28. This research was supported in part by grants from the Spencer Foundation and by NIH grants 1F32NS05038-01 and 1F32EY05069-01 to J.L.N. and J.D.D., and by PHS grant MH25852 to J.D.P. We thank L. Crowder and G. Blasdel for technical assistance and the Lederle Laboratories for providing Flaxedil.
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Hair Element Content in Learning Disabled Children

Abstract. *Hair samples from 31 learning disabled and 22 normal children were analyzed for content of 14 elements. Significant group differences were determined and a discriminant function was completed which separated the groups with 98 percent accuracy. Elevated lead and cadmium content in the learning disabled group is viewed as being of particular importance.*

The influence of heavy and trace elements on human physical functioning has been well studied (*1*). Much less is known about the effects of these elements on behavior. The finding reported here, of a significant relation between the content of these elements in the hair and learning disabilities, represents an initial