P-Cells and I-Cells in in Vitro Slices of the Lateral Geniculate Nucleus of the Cat

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OGAWA, T., ITO, S. and KATO, H. *P-Cells and I-Cells in in Vitro Slices of the Lateral Geniculate Nucleus of the Cat.* Tohoku J. exp. Med., 1980, **130** (4), 359-368 — Extra- and intracellular recordings were performed from neurons in in vitro slices of the lateral geniculate nucleus (LGN) prepared from cats anesthetized with Nembutal. Based on responses to stimulation of the optic tract (OT), neurons were classified into three types, i.e., P-cells (principal relay cells), I-cells (intrinsic cells) and unclassified cells. P-cells were characterized by an IPSP following an EPSP or an action spike which appeared at a short latency in response to OT stimulation, while I-cells were characterized by a long lasting EPSP on which a burst of spikes were superimposed. These observations were comparable with those reported in situ. The incidence of P- and I-cells was investigated for a total of 118 neurons recorded intracellularly from in vitro slices of LGN of cats at various ages; 73.0% and 5.4% for P- and I-cells respectively in 1-day-old kittens, 71.4 and 9.5% in 3-day-old, 81.8 and 9.1% in 1-week-old, 80.8 and 7.7% in 6-week-old kittens, and 82.6 and 13.0% in adult cats. From these findings it was concluded that there exist physiologically differentiated P- and I-cells in the LGN even at birth and that I-cells are responsible for intrageniculate inhibition. ——— in vitro slices; lateral geniculate nucleus; principal and intrinsic cells; cat

It is well documented that the dorsal lateral geniculate nucleus (LGN) of the cat consists of at least two major types of cells: principal cells (P-cells) which relay retinal inputs to the visual cortex (VC), and intrinsic cells (I-cells) whose axons ramify within the LGN (Bishop et al. 1953; Guillery 1966; Tömböl 1966/7; Sakakura 1968; Famiglietti and Peters 1972; Ono and Noell 1973; Cleland et al. 1976; Dubin and Cleland 1977). Recently Kato (1974), using in vitro slices of the cat LGN, has revealed that similar categorization of P- and I-cells was possible on the basis of the same physiological criteria as in situ; P-cells are characterized by a single spike followed by a prolonged inhibition in response to a single shock given to the optic tract (OT) and also to a single shock given to the ipsilateral visual cortex for antidromic activation of the LGN neurons, while physiological features of I-cells are a burst of spikes at a high rate in response to a single shock to the OT and to the VC (Sakakura 1968; Ono and Noell 1973; Dubin and Cleland 1977).

The present experiments were designed to extend the previous study (Kato

1974) using the techniques of extracellular and intracellular recordings from in vitro slice preparations of the cat's LGN. A preliminary report has been published elsewhere (Kato et al. 1978).

METHODS

Slices of the LGN were cut from 7 kittens ranging from 1 day to 6 weeks of age and from 2 adult cats more than one year of age. After fixing the head of a cat anesthetized with Nembutal (30–40 mg/kg, i.p.) on a headholder, the cranium and the dura were widely opened. The whole brain was isolated quickly by cutting the cranial nerves and by transectioning the brain stem with a sharp-edged spatula. The isolated brain was immediately transferred en masse to a chopping board where it was cut into two hemispheres as shown in A1 of Fig. 1. Then the temporal and occipital lobes were taken off massively with forceps (A2 and 3), so that the LGN could be easily identified with reference to the OT, which appeared as a small protrusion close to the medial geniculate nucleus and the superior colliculus as shown in A2. The LGN with the OT connected was excised from the brain with small scissors after making two incisions (arrows in A3) close to and in parallel with the rostro-caudal axis of the LGN with a sharp razor blade. The excised LGN+OT was immediately transferred onto a piece of blotting paper soaked with a medium equilibrated with a gas of 5% CO₂ in O₂, so that slices about 1 mm thick were sectioned at right angles to the long axis of the LGN with a razor blade as shown in Fig. 1B. Sectioning was

Fig. 1. A and B: Schematic diagrams for preparation of slices from lateral geniculate nucleus of cats anesthetized with Nembutal. LGN, lateral geniculate nucleus; OT, optic tract; MGN, medial geniculate nucleus; SC, superior colliculus; IC, inferior colliculus. In B it is shown that isolated LGN is being sliced with a razor blade which has been attached to vibrating element of an electric shaver. C: Arrangement of recording and stimulating electrodes on a slice immersed in medium on the bottom of experimental chamber. A pair of acupuncture needle electrodes (S) was inserted into OT for electrical stimulation and glass microelectrode (E₁) was advanced into slice to record intracellular responses from a neuron in A or A₁ layer of LGN. Another acupuncture needle electrode (E₂) was inserted into slice for recording field potentials to OT stimulation to check conditions of slice. This electrode was also useful to fix slice in place together with a silver wire (W) placed somewhere on slice.

facilitated by making the razor blade vibrate parallel to its sharp edge by attaching to a vibrating element of an electric shaver while it was pressed down gently perpendicularly to the LGN surface.

Three or four slices were obtained from one LGN and they were placed quickly in an incubation chamber. The time required from opening the dura till placement of slices in the incubation chamber was 7 min at the longest, usually within 5 min. Slices thus obtained were incubated for more than one hr in the chamber filled with a circulating medium before recordings were commenced. The composition of the medium was as follows (mM): NaCl 124, KCl 5.00, NaH_2PO_4 1.25, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 2.00, $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ 2.00, NaHCO_3 26.0 and glucose 10.0 (Schwartzkroin and Altschuler 1977). At a recording session one of the slices was transferred onto the bottom of an experimental chamber which was perfused with the medium. The temperature of the medium was kept at $37 \pm 1^\circ\text{C}$ throughout the experiments.

Arrangement of recording and stimulating electrodes for a slice maintained in the experimental chamber are schematically shown in Fig. 1C. A bipolar electrode consisting of a pair of acupuncture needles (S) insulated with varnish except for 0.5 mm from the tip, was placed in the OT for electrical stimulation. Stimulating pulses of 0.05–0.2 msec in duration were fed by means of an isolating unit. Another acupuncture needle electrode (E_2) was inserted into the slice so that it could lead off field potentials evoked by OT stimulation and also fix the slice in place. A small silver wire (W) was also placed on the other end of the slice for ensuring fixation.

For extra- and intracellular recording of single neuron activities, a glass micro-electrode (E_1) filled with 3 M KCl was inserted into A or A_1 layer of the sliced LGN under visual control. Recording equipments consisted of a bridge circuit for passing current through an intracellular microelectrode, a preamplifier, a dual beam oscilloscope in which one beam was used for DC recordings and the other for high gain AC recordings, a loud speaker, and a camera for photographic recording. A reference electrode of a chlorinated silver wire was placed in the medium.

RESULTS

When a microelectrode was introduced into the LGN, unitary spike discharges were most frequently encountered at depths from 50 to 250 μm from the cut surface of the preparations. After scrutinizing the properties of extracellular responses to OT stimulation, the microelectrode was further advanced slowly using a hydraulic driving system. A square-shaped current pulse (10 msec in duration 70 V in strength, tip positive) was fed to the microelectrode through the bridge circuit to facilitate the impalement of a cell. Penetration of a cell was recognized by a sudden appearance of a deep negative membrane potential with excitatory and inhibitory postsynaptic potentials (EPSPs and IPSPs) in response to OT stimulation. But in most cases especially in young kittens action potentials were small in amplitude (less than 40 mV) or abortive and PSPs were small as well even when the resting membrane potential of the cells was deep such as -60 to -90 mV. A total of 118 neurons were recorded intracellularly on which the present report was based.

P- and I-cells

Fig. 2 shows examples of two different types of responses to OT stimulation. They were all extracellularly recorded. Neurons shown in the upper row (A, B, C) discharged a spike with a short latency which was sometimes followed by a

Fig. 2. Two types of responses recorded extracellularly from slices of different cats. Upper three neurons (A, B, C) were classified as P-cells and lower ones (D, E, F) as I-cells. P-cells responded with a spike at short latency while I-cells fired a burst of spikes in response to OT stimulation. Neurons A and B were recorded from 6-week-old kitten, C and D from 1-week-old, E from 1-day-old and F from adult cat.

second spike after a spike-free interval of 20–100 msec as can be seen in Figs. 4A1 and 5B. Neurons shown in the lower row (D, E, F) were characterized by repetitive spike discharges evoked by single OT stimulation. Latencies of the first spikes of the burst responses were variable depending upon the strength of stimulus, the stronger the stimulus the shorter the latency. The response burst consisted of 3–10 spikes at a mean rate of 130/sec (45–350/sec) with a duration of 20–80 msec provided the intensity of stimulus was so strong as to usually evoke the former type of response. The amplitude of spikes within the burst was in some cases so small as to be just above the base lines as shown in E and F, and their width was usually narrower than that of the former type of cell.

These two types of response pattern, i.e., single and burst discharges, correspond to those of P- and I-cells respectively which have been described for *in situ* preparations (Sefton and Burke 1966; Burke and Sefton 1966a; Sakakura 1968; Ono and Noell 1973; Sumitomo et al. 1976; Dubin and Cleland 1977). Thus it may be admissible to adopt the same dichotomy for LGN neurons of *in vitro* slices responsive to OT stimulation.

With P-cells there is good reason to believe that the discharge free phase following the first spike is associated with an inhibitory process (Burke and Sefton 1966b, c; Ono and Noell 1973; Sumitomo and Iwama 1977). One of the tests for this point is to show that the test responses are suppressed during this phase when paired shock experiments are performed at varying intershock intervals as shown in Fig. 3A. The strength of stimulus in each pair was fixed at such a level that each stimulus could produce a spike at a short latency. Responses to conditioning and test stimuli at increasing intershock intervals are shown in 2 through 4, and responses to test shocks alone are given at the bottom (5). When test stimuli were applied within 35 msec following conditioning stimuli (2, 3, and 4), spikes to be elicited by the former were fractionated, leaving prepotentials alone. Such suppressive effects on a spike generating mechanism lasted for about 60 msec in

this cell. In contrast to P-cells, no suppression could be disclosed in I-cells. Instead, summation effects could be observed in I-cells. An example is given in Fig. 3B. When a strong stimulus was applied to the OT, an I-cell discharged a burst of 4–5 spikes at a latency of 4.2 msec to the first spike. During the paired shock experiment the intensity of stimulus was reduced to such a level that

Fig. 3. A: Responses recorded from P-cell to paired shocks given to OT at various intershock intervals. Conditioning stimulus elicited always a spike with short latency (1). When test stimulus with the same strength was applied close to conditioning stimulus (2–4), test responses were suppressed leaving only prepotentials. At 5 control test response is shown. B: Paired shock experiment with I-cell. Intensity of conditioning and test stimuli was adjusted to such a level as to elicit only single spike with latency of 13 msec (1 and 5). When test stimuli were applied at close intervals after conditioning stimulus, increased numbers of spikes were evoked by test stimuli (summation effect).

only one spike with a latency of about 13 msec was elicited by conditioning and test stimuli alone (1 and 5). When test stimuli were applied close to a conditioning stimulus (2, 3 and 4), responses evoked by test stimuli were a burst of 3–5 spikes with a shorter latency than when test stimuli were applied in isolation. Such summation effect was found to continue for as long as 70 msec. The phase of suppression following the initiation of the first spike in P-cells and the phase of summation in I-cells thus revealed were found to correspond to a prolonged IPSP and EPSP respectively as illustrated in the upper (1) and lower (2) traces of Fig. 4A.

A P-cell (A1) identified by aforementioned criteria gave rise to only a hyperpolarizing potential in response to an OT shock which was adjusted so strong as to be just below threshold for the first spike discharge. This hyperpolarizing potential had the same time course and latency as the hyperpolarizing potential following the first spike elicited by strong stimuli. This indicates that

Fig. 4. A: Intracellular recordings from P- (1) and I-cells (2) in response to OT stimulation recorded from 6-week-old kitten. IPSP was produced in P-cell and EPSP with spike discharges superimposed in I-cell. At 1, low gain DC and high gain AC recordings were simultaneously displayed.

B: Spike discharges and IPSPs induced by intracellular application of depolarizing current in P-cell. Arrows indicate IPSPs occurring just after a burst of spikes during current application.

the hyperpolarizing potential following the first spike is not an after-hyperpolarization associated with a spike, but an IPSP. Furthermore, that this potential could be inverted in polarity by passing a hyperpolarizing current through an impaled electrode supports the above view. The latency of the IPSP was about 5 msec, 1 msec longer than that of the first spike and the duration was about 40 msec.

The intracellular records of I-cell responses, as shown in A2 were also obtained on OT stimulation. When the intensity of stimulus was weak, only a small EPSP was obtained without accompanying any spikes. At a moderate intensity, an OT shock elicited an EPSP of 20 msec duration having a slowly rising phase on which one or two spikes were superimposed. A strong stimulus produced a long-lasting EPSP of about 55 msec duration on which a burst of several spikes was superimposed (A2).

Since the P- and I-cells illustrated in Fig. 4A were located close to each other in the same slice preparation and the time course of the IPSP observed in the P-cell was very much like that of spike discharges observed in the I-cell, it may well be reasoned that the I-cell is responsible for the generation of the IPSP in the P-cell (Kato 1974).

There have been two concepts concerning the neuronal circuitry involving I-cells (Dubin and Cleland 1977). One is that I-cells are activated directly by retinal afferents (feedforward connection) and the other that I-cells are activated by axon collaterals of P-cells (feedback connection). The most convincing solution of this problem will be given by intracellular stimulation of a P-cell and recordings therefrom. If excitation of a P-cell is responsible for activation of I-cells which in turn exert an inhibitory action upon the P-cell, i.e., if the feedback connection hypothesis is valid, it should be possible to record an IPSP in the impaled P-cell

after action spikes are induced by intracellular stimulation of the cell (Kato et al. 1979). Records of responses from a P-cell as shown in Fig. 4B strongly suggest this possibility. Passing a depolarizing current of 0.15 nA through an intracellular electrode caused a discharge of spikes in the P-cell. Spikes were not uniformly distributed for a period of passage of the depolarizing current, but some groupings of discharges followed by slowly developing hyperpolarizing potentials were observed. These hyperpolarizations were not always fixed in time to the last spike of the preceding group of spikes, sometimes occurring with some delay following the preceding spikes and other times without a detectable delay (Fig. 4B). These findings suggest that the hyperpolarization following a burst of discharges produced by intracellularly applied current is nothing but an IPSP which is mediated by I-cells which are activated by recurrent axon collaterals of the impaled P-cell.

P- and I-cells in the kitten LGN

In this series of experiments our interest was also directed toward the development of inhibitory action of I-cells in the kitten's LGN. In vitro slices of LGN were prepared from kittens ranging from 1 day to 6 weeks in age, where two types of neuronal responses were searched for.

Examples of two types of neurons are illustrated in Fig. 5, P-cells being in

Fig. 5. Responses recorded from P- (left column) and I-cells (right column) of in vitro slices obtained from kittens of various ages. Recordings of neurons in A and E were made from 6-week-old kittens, B and F from 1-week-old, C and G from 3-day-old, and D and H from 1-day-old kittens. Low gain DC and high gain AC recordings are simultaneously displayed except at A and E where only DC recordings were shown. Calibrations for AC recordings are given in parentheses.

the left column (A-D) and I-cells in the right column (E-H), sampled from cats of various ages. Cells recorded from 6-week-, 1-week-, 3-day- and 1-day-old kittens are shown in this order from top to bottom. Except the top records (A and E), the records are displayed in two fashions, DC (upper) and high gain AC (lower).

Neuron A responded either with a spike followed by an IPSP or with an IPSP in isolation to OT stimulation. Neuron B responded either with or without an EPSP followed by an IPSP, at the end of which a spike was generated. Comparing traces with an EPSP and without an EPSP discloses an onset latency of the IPSPs to be 1 msec longer than that of the EPSP. Neurons C and D recorded from very young kittens gave rise to only IPSPs whose latencies were 5.1 and 2.7 msec respectively.

All cells in the right column in Fig. 5 gave rise to long lasting EPSPs of 40–80 msec duration on which a burst of spikes were superimposed, although spikes of cells F, G and H were abortive which might be due to some damage of cells caused by the impalement. These cells should be regarded as I-cells according to the criteria for identification.

The frequency of occurrence of each cell type in sliced LGN from cats of various ages is summarized in Table 1. Of a total of 118 neurons examined, 91 neurons belonged to P-cells (77.1%), 10 to I-cells (8.5%). The remaining 17 neurons (14.4%) could not be classified as either type and were put together into an unclassified cell group at the right column in Table 1. From these data it might

TABLE 1. *Frequency of occurrence of P- and I-cells in slices obtained from kittens of various ages and adult cats*

Age	Number of animals	Number of cells	P-cells Number (%)	I-cells Number (%)	Unclassified cells Number (%)
1 day	2	37	27 (73.0)	2 (5.4)	8 (21.6)
3 days	1	21	15 (71.4)	2 (9.5)	4 (19.0)
1 week	1	11	9 (81.8)	1 (9.1)	1 (9.1)
6 weeks	3	26	21 (80.8)	2 (7.7)	3 (11.5)
Adult	2	23	19 (82.6)	3 (13.0)	1 (4.3)

be stated that P- and I-cells are already differentiated within the LGN even in kittens 1 and 3 days old. Furthermore, the incidence of P- and I-cells seems to increase progressively with the growth of an animal at the expense of unclassified cells, although there is no statistically significant difference (χ^2 -test) in observed frequencies of three types of cells among differently aged cats ($\chi^2=4.99$; $df=8$; $p>0.50$).

DISCUSSION

The present results have revealed that the dichotomy of P- and I-cells is feasible in *in vitro* slices of cat's LGN and that there exists a feedback inhibitory mechanism in such preparations by showing that a burst of spikes produced by intracellular injection of a depolarizing current pulse was accompanied by a prolonged IPSP.

Dubin and Cleland (1977) have suggested that there are two possible inhibitory circuits in the LGN, i.e., a feedforward inhibition involving intrageniculate interneurons which receive direct inputs from retinal afferents and a feedback inhibition involving perigeniculate interneurons which receive axon collaterals of principal cells in the LGN (Ahlsén et al. 1978). In our preparations it was histologically demonstrated that there remained some structures surrounding the LGN including the perigeniculate nucleus. Hence the feedback inhibition as suggested in the present experiments might be considered to be mediated by the remaining perigeniculate neurons. The crucial test for this possibility will be to show that the IPSP which is generated in a P-cell fired by intracellular current injection is eliminated by complete resection of the perigeniculate nucleus from LGN slices. No experiments have been done yet along this line.

Another important point to be discussed here is that intrageniculate inhibition could be demonstrated even in a one-day-old kitten. According to Daniels, Pettigrew and Norman (1978) surround inhibition was not detected in the LGN neurons of neonatal kittens, but in kittens older than 3 weeks. Histological studies have shown that intrinsic neurons are already distinguishable in the LGN of newborn kittens and rats (Tömböl 1966/7; Morest 1969; Parnavelas et al. 1977) and that the density of synapses in the LGN observed in newborn kittens is about 25% of the adult value (Cragg 1975). These physiological and histological data may well be interpreted as showing that intrageniculate inhibition is already functioning to some extent at birth although it is not organized in such a fashion as to support surround inhibition which is detected only by light stimulation. Our results will favor this interpretation.

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