Recurrent Inhibitory Interneurons of the Rabbit’s Lateral Posterior-Pulvinar Complex

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Zhu, J. Julius and Fu-Sun Lo. Recurrent inhibitory interneurons of the rabbit’s lateral posterior-pulvinar complex. J. Neurophysiol. 78: 3117–3124, 1997. We recorded from 118 neurons in the visual sector of the thalamic reticular nucleus (TRN) in anesthetized rabbits. Cells were identified by their location and characteristic burst responses to stimulation of the primary visual cortex (Cx) and optic chiasm (OX) and were classified into two groups. Type I cells had relatively short latencies from both OX and Cx stimulation, and the latency from OX was always longer than from Cx. In contrast, type II cells had much longer latencies after OX and Cx stimulation, and the latency from OX was always shorter than from Cx. Type I cells were located in the dorsal part of TRN, whereas type II cells were located in the ventral part of TRN. The physiological properties and location of type I TRN cells indicate that they are recurrent inhibitory interneurons of the dorsal lateral geniculate nucleus (LGN). Type II TRN cells most likely function as recurrent inhibitory interneurons of the lateral posterior nucleus-pulvinar complex (LP) because they could be activated antidromically by LP stimulation and orthodromically activated via axonal collaterals of LP cells. Type II TRN cells exhibited a prolonged depression after Cx or OX stimulation. Intracellular recordings showed that a prolonged inhibitory postsynaptic potential was evoked by Cx or OX stimulation. Therefore, these recurrent interneurons of LP, type II cells form mutual inhibitory connections just like those recurrent interneurons of LGN, type I cells. Our data suggest that the geniculocortical and extrageniculocortical visual pathways have similar recurrent inhibitory circuits.

INTRODUCTION
At least two visual ascending pathways exist in mammals. One is the retino-geniculocortical pathway, the primary visual pathway; the other is an extrageniculocortical pathway, which involves the retina, superior colliculus, lateral posterior nucleus-pulvinar complex (LP), and visual cortex. Recently, the importance of the second visual pathway has been emphasized because several lines of evidence suggest that this visual pathway is involved in vision-related orientation and attention (Robinson and Petersen 1992). In humans, positron emission tomography scanning studies reveal that the activity in LP is enhanced selectively during attention-demanding tasks (Corbetta et al. 1991; Grafton et al. 1992; LaBerge and Buchsbaum 1990). This also has been confirmed with single-unit recording in monkeys (Petersen et al. 1985, 1987). Lesion studies provide a similar picture and show that LP is necessary for humans and monkeys to shift attention (Bender and Butter 1987; Rafal and Posner 1987). Taken together these results suggest a direct involvement of LP in attention. Recently, a computer modeling study (Olshausen et al. 1993) predicted that the functional role of this ascending pathway is to provide an attentional “window” that is essential for the primary visual cortex to select and route important visual information to the higher cortical areas.

The neuronal circuitry along the primary visual pathway has been studied well. For example, the recurrent inhibitory circuit of the dorsal lateral geniculate nucleus (LGN) has been characterized particularly well (Ahlsen et al. 1984, 1985; Bal et al. 1995; Dubin and Cleland 1977; Hale et al. 1982; Lindström 1982; Lo and Sherman 1994; Lo and Xie 1987a; Shosaku et al. 1984). Interneurons in this recurrent circuit are located in the thalamic reticular nucleus (TRN) or perigeniculate nucleus, a nucleus containing a pure population of GABAergic cells (Jones 1975, 1985). These inhibitory interneurons receive excitatory inputs from axonal collaterals of both geniculocortical and corticogeniculate fibers and project their axons back to LGN. Synaptic connections and intrinsic properties of these TRN cells play a crucial role in the modulation of ascending transmission through LGN and also promote thalamocortical oscillatory rhythms (Bal and McCormick 1993; Bal et al. 1995; Sherman and Guillery 1996; Sherman and Koch 1986; Steriade and Llinás 1988; Steriade et al. 1993). Whether a similar recurrent inhibitory circuit exists in the second ascending visual pathway is still unknown, although several anatomic studies (Conley and Diamond 1990; Crabtree and Killackey 1989; Fitzgibbon et al. 1995; Rodrigo-Angulo and Reinoso-Suárez 1988) show that LP and the visual sector of TRN are mutually connected. In addition, a previous physiological study in the rat (Sumitomo et al. 1988) has demonstrated that some neurons in the visual sector of TRN can be activated antidromically by LP stimulation.

Recently, we have identified two types of cells in the visual sector of the rabbit’s TRN (Zhu and Lo 1996a). Type I cells respond to optic chiasm (OX) and primary visual cortex (Cx) stimulation with relatively short latencies. Their physiological properties and location within TRN indicate that they are recurrent inhibitory interneurons of LGN as identified by Lo and Xie (1987a). Type II cells respond to OX and Cx stimulation, but with much longer latencies. These type II cells may serve as recurrent inhibitory interneurons of LP. To test this idea, one needs to determine whether these cells project to LP and receive excitatory inputs via axonal collaterals of relay cells in LP. The present study is intended to answer these questions.

METHODS
Male New Zealand rabbits weighing 2.3–3.0 kg were used in this study. During surgery, the rabbits were anesthetized by an
intravenous injection of pentobarbital sodium (Nembutal, 40 mg/kg). Supplemental doses (10 mg/kg) of Nembutal were given as needed to keep animals free from pain reflex and in a state of slow-wave sleep, as determined by monitoring the cortical electroencephalogram (EEG). All pressure points and incisions were infiltrated with lidocaine. During recording, the rabbits also were immobilized by intermittent administration of gallamine triethiodide (Flaxedil) and were respired artificially. End-tidal CO₂ of animals was kept in a range of 3.8±4.5%. Body temperature was maintained within normal range (37.3±0.3°C; mean±SD).

Four silver electrodes were fixed on the surface of the Cx so that we could record EEG and evoked potentials from stimulation of the OX. Several insulated stainless steel electrodes, with an exposed tip of 60 μm in diameter, were inserted stereotaxically into the ipsilateral LP (coordinates: P4.5, L4, D7), LGN (coordinates: P5, L6.8, D8), and OX (coordinates: A3, L1, D14) according to the atlas of Sawyer et al. (1954). The final electrode tip position for OX stimulation was ascertained by finding the position where the threshold of evoked cortical potentials was <50 μA. The positions of the LP- and LGN-stimulating electrode tips were confirmed by Prussian blue reaction at the end of the experiments. Stimulating electrode tracks were reconstructed after conventional histological processing. In some experiments, the LP stimulating electrode was moved up and down to plot threshold-depth curves. The test pulse was composed of a negative pulse (200 μs, 5–250 μA) passing through stimulating electrodes.

Extracellular and intracellular recordings were made in the visual sector of the ipsilateral TRN (coordinates: P3.5, L6, D8.5) with micropipettes filled with 3 M NaCl, 3 M K-acetate or 2% Pontamine Sky Blue in 0.5 M K-acetate solution. The resistance of micropipettes ranged from 20 to 35 MΩ. Pontamine Sky Blue was injected by passing negative current (5–10 μA, 5–10 min) to localize positions of recorded neurons. Recording electrode tracks were reconstructed after conventional histological processing. Electrical signals were displayed on an oscilloscope and recorded photographically. Each photographic record typically contained five superimposed sweeps.

RESULTS

We recorded the extracellular responses of 118 neurons and intracellular responses of 16 neurons in the visual sector of TRN. These cells were identified by their stereotaxic positions and characteristic physiological properties, such as bursting responses to OX and Cx stimulation (Lo 1994; Lo and Xie 1987a; Zhu and Lo 1996a). They were classified further as type I or type II groups based on their response latency to OX and Cx stimulation, location in TRN, and different projection targets. Examples of a type I and type II cell are shown in Fig. 1.

Response latency to OX and Cx stimulation

In the visual sector of TRN, two groups of cells could be distinguished based on their response latencies to OX and Cx stimulation (Zhu and Lo 1996a). Type I cells responded to OX and Cx stimulation with relatively short latencies. For each cell we tested, the response latency to Cx stimulation was always shorter than to OX stimulation (see Fig. 1, top right). Type II cells on the other hand, exhibited much longer latencies to OX and Cx stimulation, and the latency to Cx stimulation was always longer than to OX stimulation (see Fig. 1, bottom right).

Of the 118 extracellularly recorded TRN cells, 61 cells belonged to type I. Figure 2 shows latency distributions of these cells to OX and Cx stimulation. The mean latency to OX stimulation was 3.43±0.52 (SD) ms, whereas that to Cx stimulation was 2.56±0.49 ms. The latencies from OX stimulation ranged from 2.27 to 4.70 ms, whereas those from Cx stimulation ranged from 1.70 to 3.77 ms. The latencies from OX were significantly longer than those from Cx (paired t-test, P<0.001).

The remaining 57 TRN cells were taken as type II cells. The latency distributions of these cells to OX and Cx stimulation also are shown in Fig. 2. The mean latency to OX stimulation was 4.99±0.90 ms, whereas that to Cx stimulation was 7.26±1.69 ms. The latencies from OX ranged...
from 3.49 to 7.24 ms, whereas those from Cx ranged from 3.97 to 10.86 ms. The latencies from OX were significantly shorter than those from Cx \( (P < 0.001) \).

There was a linear correlation between the latencies from OX and Cx in each group of TRN cells (Fig. 2). For both type I \( (r = 0.80, P < 0.001) \) and type II cells \( (r = 0.69, P < 0.001) \), the linear correlations were significant. For type I cells, the regression formula was \( y = 0.84x + 1.27 \), whereas for type II cells, it was \( y = 0.37x + 2.31 \). These results suggest that if a TRN cell receives visual inputs from the retina via fast pathways, it also will be connected to the cortex via fast conducting fibers and vice versa.

Intracellular recordings showed that the latency of excitatory postsynaptic potentials (EPSPs) exhibited similar patterns in these two types of cells (Fig. 3). We successfully impaled seven type I TRN cells and nine type II TRN cells. The resting membrane potential of type I cells \((-66.0 \pm 5.1 \text{ mV})\) was similar to that of type II cells \((-66.2 \pm 4.1 \text{ mV})\), however, there was a remarkable difference in EPSP latencies from either OX or Cx stimulation between the two cell types (Fig. 3). For type I cells, the latency of the EPSP from OX ranged from 1.92 to 3.49 ms with a mean of 2.53 \pm 0.59 ms, whereas the EPSP latency from Cx ranged from 1.37 to 2.69 ms with a mean of 2.01 \pm 0.47 ms. The former was significantly longer than the latter \( (P < 0.001) \). In contrast, for type II cells, the mean EPSP latency from OX \( (4.66 \pm 1.00 \text{ ms}) \) was shorter than that from Cx \( (5.98 \pm 1.60 \text{ ms}, P < 0.001) \). The range of OX latencies for type II cells was from 3.07 to 6.11 ms, which was clearly different from that for type I cells. The range of Cx latency for type II cells was from 3.51 to 8.73 ms and far beyond the range for type I cells.

**Location of type I and type II cells in TRN**

Eight recording electrode tracks were reconstructed to determine the locations of the recorded cells (Fig. 4A). As shown in Fig. 4B, type I cells \( (n = 51) \) were located predominantly in the dorsal part of TRN, whereas type II cells \( (n = 52) \) were located mainly in the ventral part of TRN. In none of the eight penetrations, did the two types of cells comingle. These results indicate that the two types of physiologically distinct cells are segregated in TRN with type I located in dorsal portion and type II, ventral portion.

**Antidromic activation of type II TRN cells from LP stimulation**

Stimulation of LP gave rise to an antidromic spike with a fixed latency in type II cells \( (n = 27) \) as exemplified in Fig. 5A. An IS-SD notch was seen clearly in most cases, suggesting an antidromic invasion of action potential from the axon to cell body. This was further confirmed by a collision test. As shown in Fig. 5B, if LP stimulation was preceded by Cx stimulation at a proper interval, the antidromic spike was blocked. A small increase in the Cx-LP interval resulted in a partial recovery of the antidromic spike (Fig. 5C), whereas further increase in the Cx-LP interval resulted in a complete recovery (Fig. 5D). None of type I cells could be activated antidromically from LP stimulation.

For 11 type II cells, we conducted threshold-depth curves. We moved the LP-stimulating electrode up and down in steps of 250 \( \mu \text{m} \). This allowed us to measure the antidromic activation threshold for each step so that a threshold-depth curve could be plotted (Fig. 6A). The most effective sites (low-threshold points) for antidromic activation of type II
cells were all located in LP, as judged from the depth of the reconstructed electrode tracks. The lowest threshold currents for antidromic spikes were ~10 μA, suggesting that axons of type II TRN cells are indeed present within LP. Sometimes, the threshold-depth curve showed two or more low-threshold points (n = 3; Fig. 6B). This phenomenon has been observed previously and has been used as an index of axonal arborization (Ahlsen 1984; Ahlsen and Lo 1982). Thus these results show that type II cells project to and terminate in LP.

We also examined whether these cell types project to LGN. Of 17 type II cells tested that could be activated antidromically by LP stimulation, none responded antidromically to LGN stimulation. In contrast, the same LGN stimulation antidromically activated type I TRN cells (n = 12). Therefore, type II TRN cells project only to LP and not LGN, whereas type I does not project to LP but instead to LGN.

The antidromic latency of type II cells (n = 27) to LP stimulation is summarized in Fig. 5E. It varied from 0.77 to 1.72 ms and had a peak of 1.00 ms. This latency exhibited a linear correlation with the orthodromic latency from OX (Fig. 7A) or with that from Cx (Fig. 7B). For the former correlation, the regression formula was $y = 4.36x + 0.26$ ($r = 0.68, P < 0.001$), whereas for the latter the regression formula was $y = 4.87x + 1.93$ ($r = 0.53, P < 0.001$). These imply that if a type II TRN cell has a fast conducting axon, it also will receive visual inputs from either OX or Cx via fast conducting pathways and vice versa.

Sometimes, LP stimulation induced an orthodromic response in type II TRN cells with or without the preceding antidromic spike (n = 16; not shown). Presumably, the orthodromic response was mediated monosynaptically by axons of LP cells. The latency of this response ranged from 0.91 to 3.75 ms with a mean of $2.63 \pm 0.65$ ms. The mean orthodromic latency was 1.6 ms longer than the antidromic latency, implying that the conduction velocity of axons of LP cells must be slower than that of type II cells’ axons.
Orthodromic activation of type II TRN cells from axonal collaterals of LP cells

We used a modified collision test, developed previously in our lab (Lo and Xie 1987a), to test if type II cells receive excitatory inputs from axonal collaterals of relay cells in LP. Figure 8A is a schematic diagram of our postulated recurrent inhibitory circuit. If correct, the response from Cx stimulation will collide with preceding spikes evoked by Ox stimulation at some place along axons of LP cells.

Figure 8, B–F, shows the results of a collision test on a type II cell. This cell was activated orthodromically by both Ox and Cx stimulation, and the latency from Cx stimulation was longer than that from Ox stimulation (Fig. 8, B and C). The cortical stimulation intensity was so adjusted as to induce only a single spike in the cell. When the Cx stimulation was preceded by an Ox stimulation at a proper interval, the response from Cx stimulation disappeared (Fig. 8D). Then it partially or totally recovered when the Ox-Cx stimulation interval was increased (Fig. 8, E and F). Similar results were obtained from collision tests using six other type II cells.

Mutual inhibition of type II TRN cells

The existence of mutual inhibition between recurrent inhibitory interneurons of LGN was first proposed based on the observation of a long-lasting depression of TRN cells after stimulation of Cx (Ahlsén and Lindström 1982; Ahlsén et al. 1985; Lo 1985). This idea then was confirmed by intracellular recording studies (Ahlsén et al. 1985; Ulrich and Huguenard 1996; Zhu and Lo 1996b). It is believed that the mutual inhibitory synaptic connections between these TRN cells underlie the inhibition (Cox et al. 1996; Spreafico et al. 1988; Ulrich and Huguenard 1996; Yen et al. 1985). We would like to know whether type II TRN cells also form mutual inhibitory connections.

We first tested whether there was a depression after Cx stimulation in type II TRN cells (Fig. 9B). The probability of discharges from these cells in response to stimulation of Cx was taken as an index. Without any conditioning stimulation, the probability of discharges evoked by a test Cx stimulation was set at 100%. After a subthreshold conditioning stimulation of Cx, the probability of discharges decreased remarkably at certain conditioning-test intervals. The time course of the depression could be obtained by plotting the probabilities of responses against conditioning-test intervals. The time course of the depression could be obtained by plotting the probabilities of responses against conditioning-test intervals. The time course of the depression could be obtained by plotting the probabilities of responses against conditioning-test intervals. Using this method, we revealed that there was a prolonged depression after the Cx stimulation (n = 8). It lasted for ~150 ms and peaked at ~60–80 ms. Stimulation of Ox also resulted in a depression of similar time course (n = 8; Fig. 9A). The results thus suggest that a depression is present in type II cells after the initial excitation.

We then used intracellular recording technique to examine whether the inhibitory postsynaptic potential (IPSP) is responsible for the depression (Fig. 10, C and D). We found...
arborization in the dorsal thalamic nuclei, whereas the other is a widespread, diffuse projection. As judged from the threshold-depth curves of the present study, some type II cells had only one low-threshold point for antidromic invasion, whereas others had multiple low-threshold points, which spread in a large area of LP. However, this does not necessarily mean that type II TRN cells have different axonal arborization patterns, as we did not stimulate the whole LP to determine the exact size of the axonal arborization.

Input of type II TRN cells

Collision tests showed that type II TRN cells receive excitatory inputs via axonal collaterals of relay cells in LP. This is in good agreement with anatomic findings that LP cells project to TRN (Fitzgibbon et al. 1995; Rodrigo-Angulo and Reinoso-Suárez 1988). Because type II TRN cells, presumably GABAergic cells, form reciprocal connections with LP cells, they meet the criteria for recurrent inhibitory interneurons of LP.

Compared with type I cells, type II cells exhibited longer latencies with more variation (jitter) in response to OX stimulation. This might result from the fact that these cells receive retinal inputs via a polysynaptic pathway relayed by the superficial superior colliculus and LP (Abramson and Chalupa 1988; Benevento and Standage 1983; Berson and Graybiel 1991). Previously, we showed that LP-projecting cells in the superficial superior colliculus responded to OX stimulation with a shortest latency of 1.78 ms and a shortest axonal conduction time of 0.59 ms (Zhu and Lo 1994). In this study, we found the shortest latency for type II TRN cells responding to OX stimulation to be 3.49 ms; this was 1.12 ms longer than the shortest time for impulses traveling from OX via superficial superior colliculus to reach LP (1.78 + 0.59 = 2.37 ms). The shortest orthodromic latency of type II cells to LP stimulation was 0.91 ms (see RESULTS), which represents the time needed for impulses from LP to be relayed to TRN. The remaining 0.21 ms (1.12 − 0.91 = 0.21 ms) just allowed one synaptic transmission between

DISCUSSION

In the present study, we found there were two distinct groups of neurons present in the visual sector of TRN of the rabbit. They could be distinguished from one another on the basis of their location and response latencies to OX and Cx stimulation. Type I cells are recurrent inhibitory interneurons of LGN, as previously identified in our lab (Lo and Xie 1987a), whereas type II cells most likely function as recurrent inhibitory interneurons of LP. Both type I and type II cells form mutual inhibitory connections. Therefore the primary visual pathway and extrageniculate visual pathway in the rabbit have similar recurrent inhibitory circuits (Fig. 11) (also see Zhu and Lo 1995).

Output of type II TRN cells

Because type II cells could be activated antidromically by LP stimulation but not by LGN stimulation, LP is the only target for type II cell projections. This observation is consistent with anatomic findings showing that individual cells in the visual TRN project either to LGN or LP (Conley and Diamond 1990; Pinault et al. 1995). Interestingly, the antidromic latency of type II cells is similar to that of type I cells (Lo and Xie 1987a), so the conduction velocities of axons for both types are about the same.

A recent anatomic study revealed that TRN cells project to the dorsal thalamic nuclei with two axonal ramifications patterns (Cox et al. 1996). One pattern is a compact, focal arborization in the dorsal thalamic nuclei, whereas the other is a widespread, diffuse projection. As judged from the threshold-depth curves of the present study, some type II cells had only one low-threshold point for antidromic invasion, whereas others had multiple low-threshold points, which spread in a large area of LP. However, this does not necessarily mean that type II TRN cells have different axonal arborization patterns, as we did not stimulate the whole LP to determine the exact size of the axonal arborization.
the superior colliculus and LP. The above latency analysis further supports that the retinal inputs to type II cells is mediated by above-mentioned polysynaptic pathway.

In the rabbit, type II TRN cells also had much longer latencies to Cx stimulation than did type I cells. Because we showed that the response to Cx stimulation was mediated by axonal collaterals of relay cells in LP, the long latency suggests that the conduction velocity of the axons of LP cells is much slower than that of LGN cells. In addition, the long orthordamic response latency from LP to TRN also suggests that the conduction velocity of the axonal collaterals of LP cells is slower than that of the axons of the type II cells.

Functional significance of recurrent inhibitory circuit in LP

Although LP receives significant visual input from the retina via the superficial layers of the superior colliculus (Abramson and Chalupa 1988; Benevento and Standage 1983; Berson and Graybiel 1991; Liu and Lo 1996; Zhu and Lo 1994), the visual response properties in LP differ substantially from those in the superior colliculus (Chalupa et al. 1983). Properties such as orientation, direction, and color appear to be lacking in LP (Bender 1982; Petersen et al. 1985). It has been proposed that suppressing these properties helps to generate visual salience in LP, and those behavior-related extraretinal afferents to LP may play a major role in modulating the visual responsiveness (Robinson and Petersen 1992; Robinson et al. 1991). All these may be mediated by the recurrent inhibitory interneurons in TRN, because it is well known that the recurrent inhibition of the dorsal thalamic nuclei modulates the ascending sensory information dramatically (Norton and Godwin 1992; Soltesz and Cruelli 1992).

The recurrent inhibitory interneurons of LGN are involved in saccadic suppression of LGN (Lo 1988; Lo and Xie 1987b; Zhu and Lo 1995, 1996b). When the predorsal bundle-projecting neurons in the deep superior colliculus send out a signal to initiate eye movements, they also feed an excitation, via the central lateral nucleus in the thalamus, to the recurrent inhibitory interneurons of LGN. Activation of these interneurons in TRN will suppress the LGN response during saccadic eye movements by generating an IPSP in LGN cells. Because the visual responses of LP cells are also suppressed during saccadic eye movements (Robinson et al. 1991), it will be interesting to examine whether the suppression in LP is also mediated by the recurrent inhibitory circuit to LP.

Oscillatory neuronal activity at alpha (1–13 Hz) and beta/gamma (30–60 Hz) rhythm were found in LP (Molotchnikoff and Shumikhina 1996; Núñez et al. 1992). These rhythms are believed to be related to attention and information processing in the brain (Jefferys et al. 1996; Pare and Llinás 1995; Ray and Cole 1985; Tiitinen et al. 1993). Physiological studies have demonstrated that TRN cells can generate these rhythms intrinsically, and they may function as the pacemaker of the related oscillations in the thalamocortical system (Avanzini et al. 1989; Bal and McCormick 1993; Pinault and Deschênes 1992; Steriade et al. 1987). Therefore the recurrent inhibitory interneurons of LP may also be involved in promoting these oscillatory rhythms in LP during the attention-related behaviors.

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