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INTRACELLULAR RECORDINGS FROM BINOCULARLY ACTIVATED CELLS IN THE CAT'S DORSAL LATERAL GENICULATE NUCLEUS

Sivert LINDSTRÖM and Andrzej WRÓBEL

Department of Physiology, University of Göteborg, Box 33031, S-400 33 Göteborg, Sweden and
Department of Neurophysiology,
Nencki Institute of Experimental Biology, 3 Pasteur St., 02-093 Warsaw, Poland

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Abstract. Five binocularly activated cells near the interlaminar layers of the dorsal lateral geniculate nucleus have been studied with intracellular recording techniques. Four neurons were relay cells and antidromically activated from the visual cortex. They received monosynaptic excitation and disynaptic inhibition from Y type retinal gang-lion cells in both eyes and disynaptic recurrent inhibition. The fifth cell was similar to perigeniculate neurons. It received disynaptic excitation from retinal ganglion cells in both eyes and monosynaptic excitation from antidromically activated relay cell axons. It was also inhibited from all these sources after an additional synaptic delay. The cell had a large receptive field, about twice the center size of neighboring relay cells, and gave on-off responses from the entire response area. Such displaced perigeniculate like cells may explain why relay cells issue occasional axon collaterals within the dorsal lateral geniculate flucleus.

INTRODUCTION

In adult cats ganglion cells from the two eyes terminate in separate layers of the dorsal lateral geniculate nucleus (dLGN), as is the case for most larger mammals. The postsynaptic cells, both relay cells and local

inhibitory interneurons, receive a strictly monocular excitation from either the left or the right eye. Occasional cells with binocular excitation have been observed, however, mainly within or near the interlaminar layers (6, 8). In a large sample of dLGN neurons recorded by Sanderson (19) only 0.60/c were binocularly activated. Because of their rare occurrence these cells have never been properly characterized.

Our interest in these neurons arose from two unexplained observations. Relay cells of dLGN issue axon collaterals in the perigeniculate nucleus (4, 9, 12), where recurrent inhibitory interneurons are located (2, 7). A few relay cells give off one or two additional collateral branches within the dLGN, some near the interlaminar layers. There is no evidence for direct connexions between such collaterals and relay cells or intrageniculate interneurons (15, 16). So what are the target cells of these branches?

The other unexplained observation concerns brain stem neurons with presumed inhibitory action on recurrent inhibitory cells in the perigeniculate nucleus (1, 5). The axons of these cells avoid the main layers of the dLGN but have some termination in the interlaminar zones – again with an unknown target. Were some interlaminar cells displaced perigeniculate neurons, both findings would be easily explained. Note that most perigeniculate cells are binocularly activated (7, 19, 22). Here we describe the properties of a few intracellularly recorded dLGN cells with binocular excitation.

METHODS

Observations were obtained from cats, anesthetized with pentobarbital sodium (Nembutal, Abbot), initial dose 25-35 mg/kg, supplemented as needed to maintain the animal in a state of slow wave sleep. The animals were paralyzed with gallamine triethiodide (Flaxedil, May and Baker Ltd), 5-7 mg/kg/h and artificially ventilated. End-expiratory CO_2 was kept at 3.50%, body temperature at 38°C and blood pressure above 110 mmHg. For visual stimulation the pupills were dilated, accommodation paralyzed and the eyes fitted with contact lenses and focused an a tangent screen in front of the animal. Unipolar stimulation electrodes were placed on the optic nerves behind the eye bulbs, in the optic tract and in the visual cortex. Control recordings from the two optic nerves excluded cross-activation due to current spread. Glass micropipettes filled with 3 M potassium acetate were used for intra- and extracellular recordings of dLGN cells on the right side. Cells were classified on the basis of receptive field properties, antidromic activation from the visual cortex and synaptic input from retinal ganglion cells and relay cell

axons. Their locations were judged from microelectrode depth readings, using transitions between dLGN layers with contra and ipsilateral visual inputs as reference points and from reconstructions of electrode tracts in Nissl stained serial sections.

RESULTS

Intracellular recordings have been obtained from five binocularly activated dLGN neurons. Four of these cells were sampled among more than 400 penetrated dLGN neurons in experiments devoted to other problems. They were all located near or within the interlaminar layers between lamina A, Al and C. One cell was found in experiments especially devoted to a search for binocular dLGN cells. Fourteen penetrations were made through the dLGN in these experiments. The regions around the interlaminar layers between A and Al and between Al and C were carefully explored for cells with binocular excitation. In addition to 40 perigeniculate cells above lamina A, 143 dLGN neurons were isolated with extra- and/or intracellular recordings. Besides the cell included in our sample only one more unit with binocular input was observed within the dLGN proper. The latter cell was only recorded extracellularly. It had a rather small spike and we could not be absolutely certain that the recordings originated from a single cell. These explorations convinced us that binocularly activated cells are indeed rare within the dLGN.

With intracellular recordings there can be no doubt that the responses are from the same cell. Four of five binocularly activated cells in our sample were relay cells as demonstrated by their antidromic activation from the visual cortex. The cell shown in Fig. 1 A-D was found in the transition zone between lamina A and Al. It had an antidromic latency of 0.6 ins. It received EPSPs from the contralateral (C) and ipsilateral (D) optic nerves with comparable latencies (1.3 and 1.2 ins). Optic tract stimulation evoked a summed EPSP with a spike (truncated by the high recording gain, B).

The local synaptic delay of the EPSPs were estimated by an extrapolation procedure. PSP latencies from optic nerves and optic tract stimulation were plotted against the respective conduction distances (as illustrated for another cells in Fig. 2). The intercept of the extrapolation plot gives a good measure of synaptic linkage (10). This delay (which includes a true synaptic delay, spike initiation time at the stimulation site and decrease in impulse velocity in terminal branches) is below 1 ins for a monosynaptic and between 1 and 2 ins for a disynaptic pathway. The delay was 0.7 ms for the EPSPS from both eyes, demonstrating

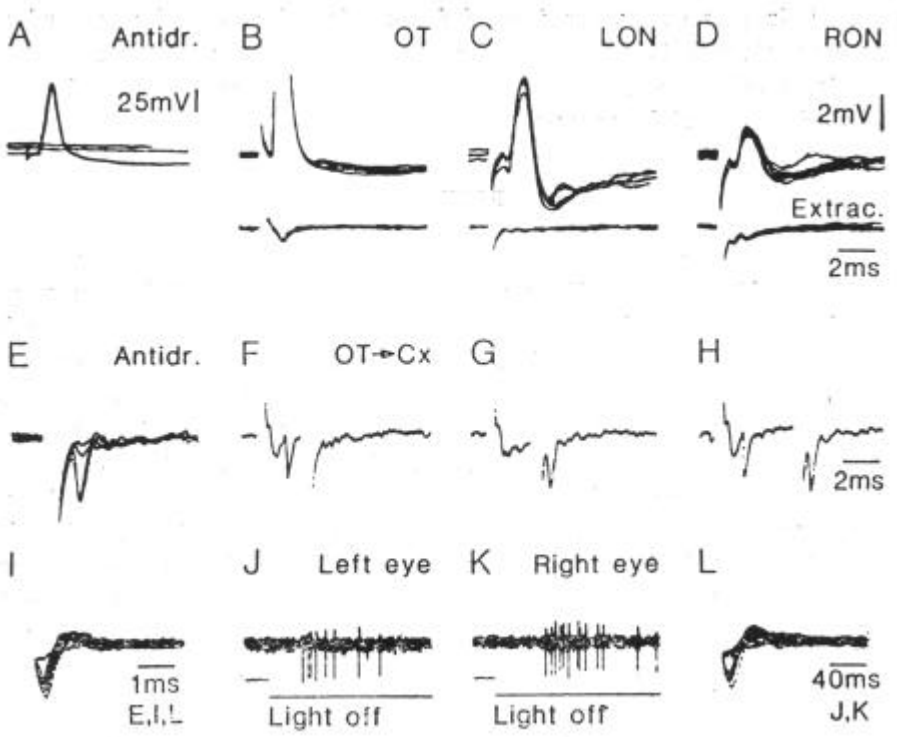

Fig. 1. Binocularly activated relay cells in the dLGN. Intracellular recordings in *A-D* and extracellular recordings in the *E-L* are from two different neurons. *A,* antidromic spike evoked by stimulation of the visual cortex (Cx) at threshold intensity; *B,* Monosynaptic EPSP from the optic tract (OT); *C-D,* monosynaptic EPSPs from the contralateral left (LON) and ipsilateral right (RON) optic nerves. The decay phase of all EPSPs is distorted by concomitant disynaptic IPSPs. Lower traces in *B-D* show the extracellular responses. Time calibration in *D* is for *A-D,* voltage calibration for *B-D; E,* antidromic spike at threshold; *F-H,* collision test. In *F* the antidromic spike is blocked by a preceding orthodromic spike evoked at monosynaptic latency by optic tract stimulation. The antidromic spike invaded the cell body when the orthodromic spike failed (G) or at longer intervals (H). Lower traces show the cell's off-response to a light spot centered in the receptive field of the left (J) and right (K) eyes. Flanking traces (I, L) show the superimposed spikes of the same discharges at higher sweep speeds.

that both effects were mediated by monosynaptic connections from retinal ganglion cells.

The cell also received IPSPs of feed-forward type from both eyes. The extrapolated delay of these IPSPs were 1.3 and 1.4 ins, i.e. within the disynaptic range. Both EPSPs and [PSPs were mediated by fast conducting optic tract fibers of Y types as calculated from the slope of the lines in the extrapolation diagram. The conduction velocities of the

responsible axons in the left optic nerve were 66 and 51 *m/s*, respectively. As• for typical relay dells a large disynaptic recurrent IPSP was evoked in the cell by antidromic activation of relay cell axons in the cortex (not illustrated).

The binocular cell found in the special search experiment was also a relay cell. It was encountered in the interlaminar zone between layers Al and C and is illustrated with extracellular recordings in Fig. 1 E-L. The upper row shows the antidromic spike with a latency of 0.8 ms (E) and a collision test (F-H). At short intervals (1.7 ins) the antidromic spike was blocked by a preceding monosynaptic spike evoked by optic tract stimulation (F). It waS conducted to the cell body when the Orthodromic spike failed (G) or when the interval between the two stimuli was prolonged (H). The cell had an off-center receptive field of Y type as tested through either eye. The receptive fields were located about 350 lateral to the area centralis and their centers were 20 in diameter.

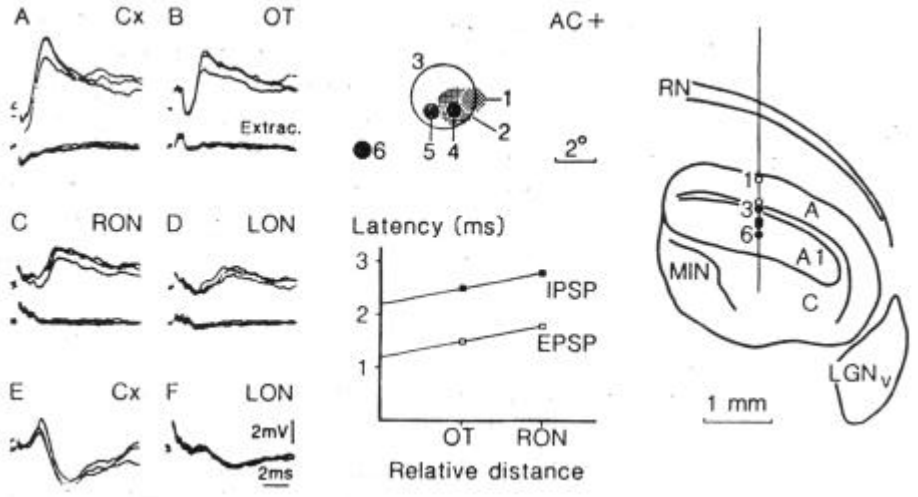

Fig. 2. Intracellular recordings from a perigeniculate like interlaminar cell. *A.* mcnosynaptic EPSP evoked by cortex stimulation; *B-D,* disynaptic EPSPs evoked from stimulation of the optic tract and the right and left optic nerves. Lower traces in each pair are the corresponding extracellular responses. The same stimuli also evoked IPSPs with an extra synaptic delay. The records in E-F were obtained with the cell depolarized and they show the disynaptic and trisynaptic IPSPs elicited by cortex and left optic nerve stimulation. Calibrations in *F* are for all records. The lower middle diagram shows the extrapolation procedure used to determine the synaptic linkage of EPSPs and IPSPs from the right optic nerve. The upper diagram shows the large on-off receptive field of the cell (3) together with the field centers of neighboring principal cells in lamina *A* (1-2) and Al (4-6) after alignment of the two eyes; *AC,* area centralis. The position of the different cells within the dLGN is shown by the reconstructed track to the right.

The lower *traces* show the transient off-responses to light spots flashed in the receptive field centers, tested separately for each eye. The evoked spikes are superimposed with higher sweep speeds in the flanking records to illustrate that one and the same cell was responding. The findings were confirmed by intracellular recordings after penetration of the cell.

The remaining two binocular relay cells were also influenced by Y axons with monosynaptic excitation and disynaptic inhibition from both optic nerves. They were found close to the A/Al and Al/C interlaminar zones. The latter cell was peculiar in having an on-center receptive field in the ipsilateral and an off-center field in the contralateral eye.

The fifth interlaminar cell was similar to perigeniculate neurons in all respects. The location of the cell between lamina A and Al is shown by the reconstructed track in Fig. 2. The cell had large receptive fields in both eyes and gave very transient on-off responses over the entire response areas. The ipsilateral right eye was most effective. The size and position of the receptive field in that eye is shown in Fig. 2 (large open circle) together with the field centers of neighboring relay cells. The cell was lost before we could map its field in the contralateral eye with any accuracy, but it did at least partly overlap with the right eye field. Thus, both fields seemed to be in register with those of surrounding relay cells. Note that the binocular cell had a receptive field which was about twice the center size of relay cells 1 and 2 in lamina A. These two cells belonged t~ the Y system, as did the binocular cell (cf. below).

The records to the left (Fig. 2 A-D) show EPSPs evoked by stimulation of the visual cortex, optic tract and right and left optic nerves. The EPSP from the cortex (A) had a latency of 1.0 ms and was clearly mediated through a monosynaptic linkage by antidromically activated relay cell axons. The total latency, including a synaptic delay, is much shorter then the conduction time for the fastest cortico-geniculate neurons (10). The latency is in fact too short even for axons of X type relay cells (14).

The extrapolation procedure was used to estimate the synaptic linkage of the EPSPs from the optic nerves. The intercept was 1.2 ms for the EPSPs from both nerves (Fig. 2) implying that the excitation was mediated through disynaptic pathways. A disynaptic linkage would of course be expected with an excitatory input from relay cell axon collaterals. Like perigeniculate cells (3) the neuron also received IPSPs from the same sources as the excitation. Two examples are shown by the lowermost records, obtained with the cell depolarized. The ‘latency was about a millisecond longer for the IPSPs than for the corresponding

EPSPs, suggesting that the pathway was disynaptic from the cortex and trisynaptic from the optic nerve. Similar IPSPs. are found in perigeniculate neurons and caused by mutual inhibitory connections between these cells (3). Both the EPSP and the IPSP after optic nerve stimulation were mediated by fast conducting axons of Y type (conduction velocity 67 m/s).

DISCUSSION

The small population of binocularly activated neurons within or near the interlaminar layers of the dLGN appears to be functionally heterogeneous. Some cells are undoubtedly relay cells as demonstrated by their antidromic activation from the visual cortex while others resemble perigeniculate neurons.

The binocular relay cells received monosynaptic excitation from Y type retinal ganglion cells. From experiments with intracellular tracer injections (4, 12) it is known that such afferents contact a specific cell type within the dLGN similar to the class I cell of Guillery (13). The dendrites of these cells do not respect lamina borders – cells located near the interlaminar zone may have dendrites that penetrate more than 100 rtm into another LGN lamina. It may be tempting to relate the binocular excitation of some relay cells of Y type to such morphological findings. Crossing dendrites can not be the sole explanation, however, since such dendrites are far more common than binocular excitation. In fact all relay cells with crossing dendrites observed by Ahlsen et al. (4) and by Friedlander et al. (12) were monocularly activated. Thus, the synaptic connexions of the dLGN are more specific than suggested by the dendritic distribution of relay cells.

May be binocular relay cells simply result from occasional errors of development? In elegant in vitro experiments, Shatz and Kirkwood (20) found that most geniculate neurons are binocularly activated during early stages of development. They gradually loose their binocularity when the nucleus differentiates into layers with separate innervation from the left or right eye. With such a process it is not entirely surprising that some neurons at the lamina borders may maintain a binocular input into adulthood. It is hard to believe that these binocular neurons should have any specific function since they constitute less than one per cent of the adult dLGN cell population (19). The suggestion that binocular relay cells come from error of development is supported by the unusual convergence of excitation from on-center and off-center ganglion cells found in one of our neurons.

The cell with disynaptic excitation from optic nerve fibers presuma-

bly represents displaced perigeniculate neurons. Apart from its position, its synaptic connections and receptive field properties were indistinguishable from typical perigeniculate neurons. Such displaced cells must be very few in number. Among more than 400 penetrated dLGN neurons only one was found. None was encountered in specific search experiments although 40 perigeniculate cells were recorded above the dLGN. Similar binocular cells with large on-off receptive fields have been observed sporadically by others near the interlaminar layers (21, 22). To be certain that these cells are indeed displaced perigeniculate neurons it would be necessary to demonstrate that they form inhibitory connections with relay cells. This is not easy. to do with physiological means but some clues might be obtained from immunohistochemistry. The interlaminar layers do contain some. GABAergic cells, as would be expected (11, 18). It is not yet known, however, if these cells have morphological features in common with perigenicu.late cells. Until such evidence is obtained we have to be content with the finding that some. interlaminar cells have physiological properties indistinguishable from those of perigeniculate neurons.

The monosynaptic excitation of these cells following antidromic activation of geniculo-cortical axons undoubtedly originates from occasional intrageniculate axon collaterals of relay cells (4, 9, 12). Most likely these cells also form the targets of brain stem neurons with selective axonal projections to the perigeniculate and interlaminar regions (1). Thus the present finding can account for some hitherto unexplained discrepancies between the physiology and morphology of the LGN.

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Note *added in proof.* After this paper was submitted for publication a report by Montero (17) appeared describing GABAergic neurons, with ultrastructural features of perigeniculate cells, in the interlaminar zone of the cat's dLGN.

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