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RESEARCH NOTE

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Feedback inhibition in the cat's lateral geniculate nucleus

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Abstract Feedback inhibition is generally believed to be a ubiquitous feature of brain circuitry, but few specific instances have been documented. An example in cats is the supposed feedback circuit involving relay cells of the lateral geniculate nucleus and cells of the perigeniculate nucleus (a part of the thalamic reticular nucleus): geniculate relay cells innervate the perigeniculate nucleus, which, in turn, provides an inhibitory, GABAergic projection back to the lateral geniculate nucleus. However, feedback inhibition at the single-cell level requires that a given perigeniculate cell project back onto the same geniculate relay cell that innervates it. We probed for this in an in vitro slice preparation of the cat's lateral geniculate nucleus. We evoked a single action potential in a geniculate cell via a brief, depolarizing pulse delivered through an intracellular recording electrode and looked for any evoked hyperpolarizations. For 6 of the 36 geniculate cells tested, we observed a long-lasting hyperpolarization after the action potential, and much of this was eliminated by application of bicuculline, suggesting synaptically activated inhibitory postsynaptic potentials. We interpreted this to be clear evidence that a given neuron may inhibit itself via circuitry mediating feedback inhibition in the cat's lateral geniculate nucleus.

Key words Perigeniculate nucleus
Thalamic reticular nucleus · IPSP · Vision

Introduction

The thalamus acts as a sort of dynamic filter to regulate the flow of information to cortex. This has been most intensely studied and is best understood for the cat's lateral geniculate nucleus, which controls the flow of retinal information reaching cortex (Singer 1977; Burke and Cole 1978; Sherman and Koch 1986, 1990; Sherman 1993). Retinogeniculate transmission is largely controlled by nonretinal inputs, which comprise 80–90% of all synaptic drive onto relay cells. Important among the nonretinal inputs are local, inhibitory cells that use γ-aminobutyric acid (GABA) as a neurotransmitter; these include interneurons and cells of the perigeniculate nucleus, which is part of the thalamic reticular nucleus. Their activity helps to regulate the excitability of relay cells and thus the gain of retinogeniculate transmission. The circuit involving the perigeniculate nucleus forms the focus of this investigation.

As Fig. 1 illustrates, the precise functional organization of this circuit is unclear. For instance, perigeniculate cells are said to be involved in "feedback" inhibition (Lindström 1982). This is because perigeniculate cells receive input from relay cells and project back into the lateral geniculate nucleus (see Fig. 1). Thus massive, synchronized activation of the optic tract or radiations leads in relay cells to multisynaptic inhibitory postsynaptic potentials (IPSPs) that are attributed to feedback inhibition via perigeniculate cells (Burke and Sefton 1966; Lindström 1982). However, whether or not this represents feedback inhibition at the single-cell level depends critically on the connectivity patterns of individual cells. Two extreme examples are shown in Fig. 1, although a combination of these patterns is also plausible (not illustrated). In either example, synchronous activation of geniculate cells via optic tract stimulation will lead to feedback IPSPs in these cells. The critical difference between the two examples depends on whether a perigeniculate cell innervates the same relay cell from which it receives input (Fig. 1 A) or whether it innervates only other relay cells (Fig. 1 B). Only the former (Fig. 1 A) represents true feedback inhibition at the level of single cells. As a result, activity in any one of the three cells of Fig. 1 A would lead to that cell's feedback inhibition. Figure 1 B represents lateral inhibition rather than

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feedback inhibition at the single cell level. Furthermore, Fig. 1 B leads to feedback disinhibition, which is the opposite of feedback inhibition. Consider activation in cell 2 in Fig. 1 B: this leads to activation of perigeniculate cells **a** and c, which inhibits geniculate cells 1 and 3; this, in turn, reduces activation of perigeniculate cell b, which finally disinhibits cells 2. Until this circuitry involving the lateral geniculate and perigeniculate nuclei is understood at the single-cell level, the actual function of this circuit, be it inhibition, disinhibition, or a combination, cannot be determined.

Note that available evidence for inhibitory circuitry usually involves massive activation of afferent pathways (Burke and Sefton 1966; Lindström 1982), and such activation of the optic tract cannot distinguish between the circuits of Fig. 1. Clearly a more precise approach is needed to address this issue. We sought to do this for the perigeniculate "feedback" circuit illustrated in Fig. 1 by intracellularly activating only a single relay cell and determining whether or not this ever produces an IPSP in that cell, which, by definition, would represent feedback inhibition at the single-cell level.

Materials and methods

Our experiments were performed in vitro on slices taken through the lateral geniculate nucleus from adult cats. Our general methods for this procedure have been reported previously (Scharfman et al. 1990), and we shall briefly outline them here. We anesthetized each cat with pentobarbital (30–35 mg/kg i.v.) and performed bilateral craniotomies above the lateral geniculate nuclei. The thalamus on each side was removed quickly after transcardial perfusion with ice-cold artificial cerebrospinal fluid (ACSF), modified as described by Aghajanian and Rasmussen (1989) to increase the viability of the slices. The ACSF consisted of 2.5 mM KCl, 2.0 mM $CaCl_2$, 1.0 mM $MgSO_4$, 26 mM $NaHCO_3$, 1.25 mM NaH_2PO_4, 10 mM *d*-glucose, and 252 mM sucrose. It differs from normal ACSF in that 252 mM sucrose is used in the modified version instead of 124 mM NaCl in the normal solution. We sectioned the lateral geniculate nucleus parasagittally or coronally at 500 μm and placed the slices at room temperature in modified ACSF for 1 h followed by normal ACSF for at least 30 min. We then transferred the slice to an interface chamber, where it was perfused with oxygenated, normal ACSF at 33° C. We used glass micropipettes (filled with 4 M (KAc) with resistances of 55–70 MΩ) to record intracellularly from geniculate neurons in such slices, and we visualized recording sites as lying within the A-laminae. We delivered brief depolarizing pulses through these intracellular electrodes to evoke single action potentials in the recorded cells.

Our chief test that an evoked hyperpolarization was an IPSP was to determine its sensitivity to the $GABA_A$ antagonist bicuculline. To do this, bicuculline methiodide (Sigma) was dissolved in normal ACSF at various concentrations and a droplet (roughly 2 μl) of this bicuculline solution was focally applied to the surface of the slice near the recorded cell. As a control, we applied a droplet of ACSF without bicuculline. Our intracellular recordings were too brief to record a return to baseline following bicuculline application, the action of which often lasted for more than 1 h.

Results

By using suitably brief and low-amplitude depolarizing pulses delivered to the geniculate cell through the recording electrodes at an appropriate membrane potential (V_m; typically 1 nA for 1 ms at -50 mV to -60 mV), we evoked single spikes. Any resultant postsynaptic potential is, by definition, feedback at the single-cell level. We averaged at least 20 trials to detect weaker signals. V_m was adjusted as needed to ensure that it did not vary between trials. We successfully accomplished this experiment for 36 geniculate cells recorded in vitro.

Action potentials evoked a period of hyperpolarization in all cells. We observed that these hyperpolarizations became smaller as the cells were hyperpolarized from -55 mV to -65 mV, but we were unable to demonstrate reversal of these hyperpolarizations, because low-threshold Ca^{2+} spikes intruded at more hyperpolarized membrane levels (Jahnsen and Llinás 1984a, b; Steriade and Llinás 1988). In any case, we relied mainly on effects of the $GABA_A$ blocker bicuculline to demonstrate that at least some of these hyperpolarizations were in part synaptically evoked (i.e., IPSPs). We found evidence for such feedback IPSPs in 6 of

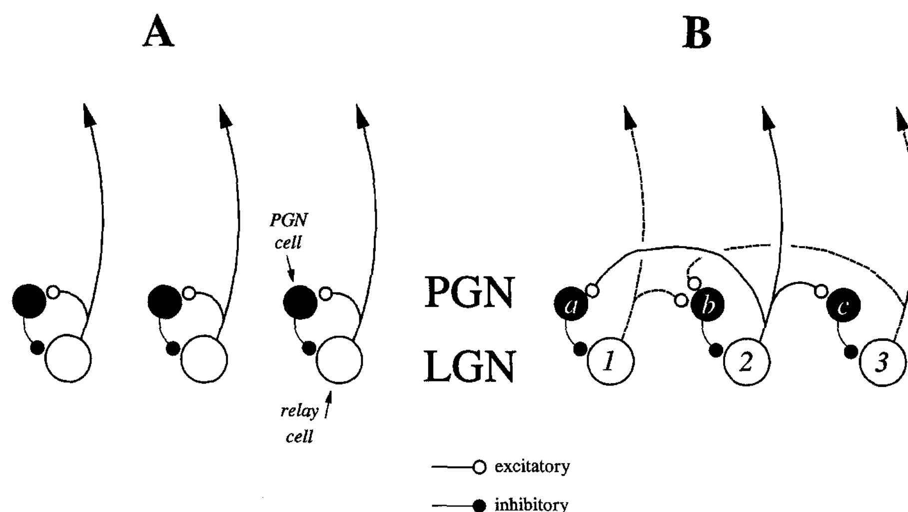

Fig. 1 A, B Schematic view of two different functional circuits involving the lateral geniculate (*LGN*) and perigeniculate nuclei (*PGN*). **A** Circuit showing feedback inhibition at the single-cell level, since a relay cell innervates a perigeniculate cell that, in turn, innervates the same relay cell. **B** Circuit without feedback inhibition at the single-cell level, since the perigeniculate cell innervated by a relay cell does not, in turn, innervate that relay cell. The numbers (*1, 2, 3*) and letters (*a, b, c*) are used to identify specific cells in the text

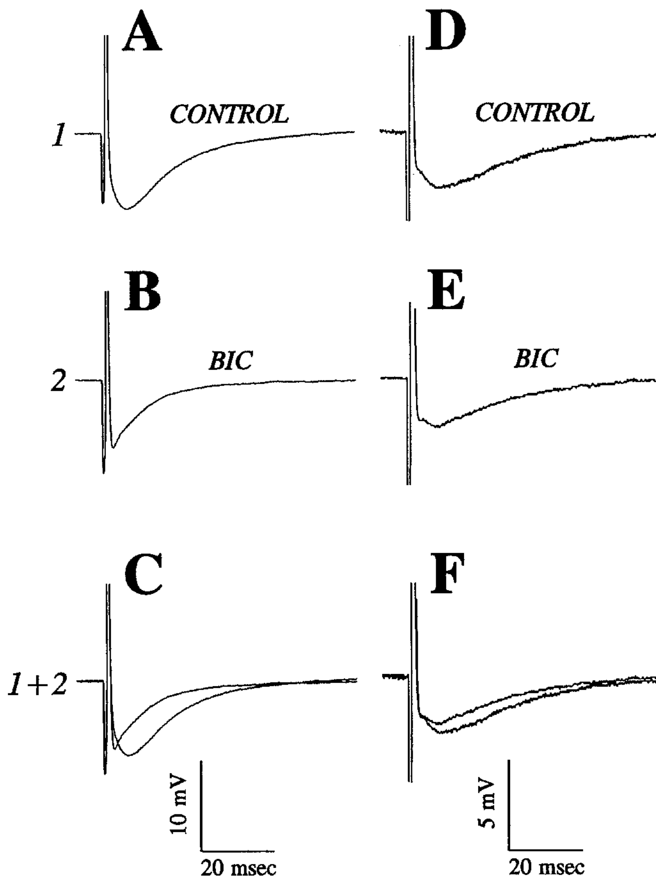

Fig. 2A–F Hyperpolarization in a geniculate relay cell following an action potential evoked from a brief, depolarizing pulse delivered intracellularly. Each trace shown results from averaging 20 individual traces. **A–C** one relay cell; **D–F** a different relay cell. These hyperpolarizations were relatively long-lasting. **A, D** Control traces (1), showing hyperpolarization after the action potential. **B, E** Traces showing effects of bicuculline (*BIC*) application (2), which reduces the hyperpolarization. **C, F** Comparison of control and bicuculline application (1 + 2), more clearly showing that at least part of the evoked hyperpolarization is sensitive to bicuculline

the 36 cells tested. Figure 2 shows two examples. The action potential elicited a substantial hyperpolarization (Fig. 2A, D) that was reduced in amplitude and modified in time course by bicuculline (Fig. 2B, E); Fig. 2C, F directly compares the control condition with that during bicuculline application.

It should be noted that we found no evidence of detectable shifts in either input resistance or V_m due to bicuculline application, suggesting little or no background level of GABA release to activate the $GABA_A$ receptors. In any case, we interpret this to mean that the reduction in the evoked hyperpolarization induced by bicuculline is not an artifact of input resistance or V_m.

We thought that many cells might not show feedback inhibition in this experiment, because a single action potential might be insufficient to elicit it. We thus used bursts of activation varying in frequency and duration to evoke trains of action potentials in the geniculate cells. However, if no hyperpolarization sensitive to bicuculline was seen after a single action potential, none was ever evoked by a burst of action potentials (not illustrated).

In each of the six cells displaying apparent feedback inhibition, a brief hyperpolarization persisted during bicuculline application (Fig. 2B, E), and a similar hyperpolarization occurred in the remaining 30 cells. We have not thoroughly investigated these responses, but their short latencies and time courses suggest that each reflects a single component afterhyperpolarization. Another possible source of evoked hyperpolarization could be activation of $GABA_B$ receptors (Bloomfield et al. 1988; Crunelli et al. 1988; Soltesz et al. 1989; Crunelli and Leresche 1991). However, $GABA_B$ responses in the lateral geniculate nucleus tend to be quite long-lasting (typically 200–300 ms), but, in our experiments, the evoked hyperpolarizations sensitive to bicuculline lasted for less than 100 ms, and the bicuculline-insensitive hyperpolarizations were even briefer (see Fig. 2). We thus feel that we did not evoke $GABA_B$ responses in our experiments. More data are needed to resolve this issue of contributions to the evoked hyperpolarizations other than $GABA_A$ responses.

Discussion

Our main conclusion is that some geniculate neurons are clearly under the partial control of inhibitory feedback circuits at the single-cell level. We emphasize the preliminary nature of this study. Much more needs to be done to determine more fully the pharmacological action of the feedback circuit, and, as noted below, other methods must be employed to characterize the types and numbers of geniculate cells exhibiting such feedback inhibition. Our only purpose here is to offer evidence that feedback inhibition at the single-cell level does exist for at least some geniculate cells.

To demonstrate feedback inhibition at the single-cell level, it is necessary to ensure that any inhibition seen can be clearly attributed to activity initiated in the cell under investigation and cannot be attributed to other activity. Obviously, IPSPs seen in a geniculate neuron after activation of the optic tract can be completely explained on the basis of activity initiated by cells other than the one under investigations (see Fig. 1). This is not feedback inhibition at the single-cell level in the sense that we use the term. Even IPSPs associated with spontaneously occurring action potentials in the cell under investigation are not proof of feedback inhibition at the single-cell level, because this "spontanous" action potential might be due to an input that is common to and concurrently activates other geniculate cells, and activity in these other geniculate cells could initiate the inhibition seen. It thus seems necessary to initiate the action potential in the cell under study and only in this cell in order to demonstrate whether or not it leads to feedback inhibition at the single cell level. We believe we

have achieved this via the technique of intracellular activation of the geniculate cell.

We emphasize that, although only a minority of geniculate cells (6 of 36) displayed clear inhibitory feedback in our study, there are at least three reasons why this might be a gross underestimate. First, a positive result in our study requires that action potentials from only the recorded geniculate cell are sufficient to activate the feedback inhibitory circuit. It is interesting that a single action potential is indeed sufficient in at least some cases, but perigeniculate cells might typically require convergent input from several coactivated sources to fire. Second, for inhibitory feedback to be seen, it is necessary for the requisite circuitry to survive the in vitro preparation, and, in many cases, the axons of either the relay cells or the inhibitory cells may have been interrupted by the slicing. Indeed, morphological studies from this laboratory (e.g., Friedlander et al. 1981; Uhlrich et al. 1991) suggest that, even though we did our best to minimize this problem when we made the slices for recording, we would inevitably interrupt this circuit for most cells from which we recorded. Third, the bicuculline may have failed to reach or affect postsynaptic receptors on many cells, perhaps because of inadequate penetration or other factors.

Because of the reasons to expect false negatives in these in vitro experiments, we do not place much faith in our observation that a majority of geniculate cells failed to demonstrate feedback inhibition. Perhaps all relay cells in more physiological preparations display this phenomenon. In any case, given that feedback inhibition at the single-cell level is present for at least some geniculate cells, what is the specific circuitry over which it functions? The most likely candidate is that illustrated by Fig. 1A. However there is another circuit that cannot yet be ruled out: a fraction of relay cells exhibit intrageniculate collaterals (Friedlander et al. 1981), and these may innervate interneurons, which, in turn, feed back inhibition to their afferent relay cells. Clearly more work needs to be done with approaches other than the in vitro methodology used here to determine both the actual percentage and types of relay cell that display feedback inhibition as well as the specific pathways involved.

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