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Synaptic Responses of Cortical Pyramidal Neurons to Light Stimulation in the Isolated Turtle Visual System

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The resistance of the turtle brain to hypoxic injury permits a unique *in vitro* preparation in which the organization and function of visual cortex can be explored. Intracellular recordings from cortical pyramidal neurons revealed biphasic responses to flashes of light, consisting of an early phase (50–100 msec) of concurrent inhibitory and excitatory activation, followed by a longer, inhibitory phase (250–600 msec) composed of summated Cl^--dependent postsynaptic potentials mediated by GABA. This response sequence results from the coactivation of pyramidal and GABAergic non-pyramidal cells, followed by feed-forward and possibly feedback pyramidal cell inhibition, and is partly dependent on differences in the membrane properties of pyramidal and non-pyramidal neurons.

An understanding of cortical function can be approached by an analysis of the properties of individual cellular elements and the way they respond to physiological activation along specific pathways. For example, elegant studies based on recordings of unit activity in mammalian visual cortex during patterned light stimulation have revealed a complex array of receptive-field properties (Hubel and Wiesel, 1977). Local cortical inhibition mediated by GABA appears to determine some of these response properties (Sillito, 1984). A more detailed analysis of the synaptic events underlying the integration of visually evoked cortical activity will require intracellular recording from identified cell types, including subclasses of pyramidal neurons and inhibitory interneurons. This is difficult to achieve in the mammalian brain, but the unique metabolism of the turtle brain (Belkin, 1963; Sick et al., 1982) renders the turtle visual system an ideal system for cellular physiological studies (Waldow et al., 1981; Connors and Kriegstein, 1986; Kriegstein and Connors, 1986).

The intracortical structure of the turtle is much simpler than that of the mammal. The turtle cortex has only 3 layers, containing 2 general classes of neurons: pyramidal cells, which form the main output of the cortex, and aspiny or sparsely spiny non-pyramidal neurons (Ramon y Cajal, 1911, 1917). Despite its relative simplicity, turtle visual cortex bears a phylogenetic relationship to mammalian neocortex (Diamond and Hall, 1969; Northcutt, 1981) and appears to have some similar structural and physiological features. In the present study, intracellular cortical responses to visual stimuli were obtained in a preparation in which the intact turtle visual system was isolated *in vitro* (Kriegstein, 1985), as shown in Figure 1, *A, B*. This approach has allowed the testing of specific aspects of cortical circuitry that may have relevance to the organization of telencephalic cortex in general.

Materials and Methods

Young specimens (3–4 cm shells) of red-eared turtles (*Pseudemys scripta*) were cooled to 0°C, decapitated, and the brains surgically removed with eyes and optic nerves attached and maintained at 22°C at the liquid–gas interface in a recording chamber perfused with aerated (95% O_2, 5% CO_2) turtle saline containing (in mM) NaCl, 96.5; KCl, 2.6; $CaCl_2$, 4.0; $MgCl_2$–$6H_2O$, 2.0; $NaHCO_3$, 31.5; and dextrose, 10, at pH 7.4. Access to the visual cortex was obtained by making a U-shaped incision in the medial cerebral hemisphere, which preserved the laterally coursing optic radiations (see Fig. 1*B*). Light flashes were provided by a Grass model PS2 photostimulator, which projected a 3-mm-diameter light beam onto the contralateral eye via a fiber-optic conduit that terminated 1.5 cm from the cornea (see Fig. 1, *A, B*). Long-duration flashes were provided by a Dolan-Jenner Fiber-Lite illuminator (model 170-D) connected to the same fiber-optic conduit. Responses to visual stimuli were observed for up to 6 hr *in vitro* (Kriegstein, 1985).

Intracellular electrodes were fashioned from capillary tubing (Fredrick Haer) filled with 4 M K-acetate (pH 7.0) and beveled to resistances of 50–100 MΩ. An active bridge circuit was used to pass current through the intracellular recording electrode, and the signal was amplified and displayed on an oscilloscope screen and recorded on magnetic tape. Data were digitized and stored on floppy disks for analysis, using a MINC-23 computer (Digital Equipment Co.).

Focal drug application was accomplished by means of compressed N_2 gas (2–3.5 kg/cm^2) that was pulsed to the back of a micropipette broken to a tip diameter of 2–4 μm. Quantity was controlled by varying the pulse duration from 2 to 1000 msec.

Results

Flash-evoked intracellular responses were recorded from 26 pyramidal neurons. Recordings made with dye-filled microelectrodes indicated that pyramidal cells can be distinguished from non-pyramidal neurons by their distinctive action potential (AP) characteristics, including multiple AP amplitudes, relatively long AP duration, and rapid spike-frequency accommodation to prolonged depolarizing current pulses (Connors and Kriegstein, 1986). A characteristic pyramidal cell response to a brief (10 μsec) flash of light was demonstrated by the neuron illustrated in Fig. 1*C*, 1. The response was biphasic, consisting of early excitation (50–100 msec) composed of EPSPs that often triggered one or more APs, and a subsequent inhibitory phase (250–600 msec) composed of discrete IPSPs associated with net hyperpolarization and a decrease in input resistance (R_N). There were frequent spontaneous EPSPs and IPSPs in these cells, but

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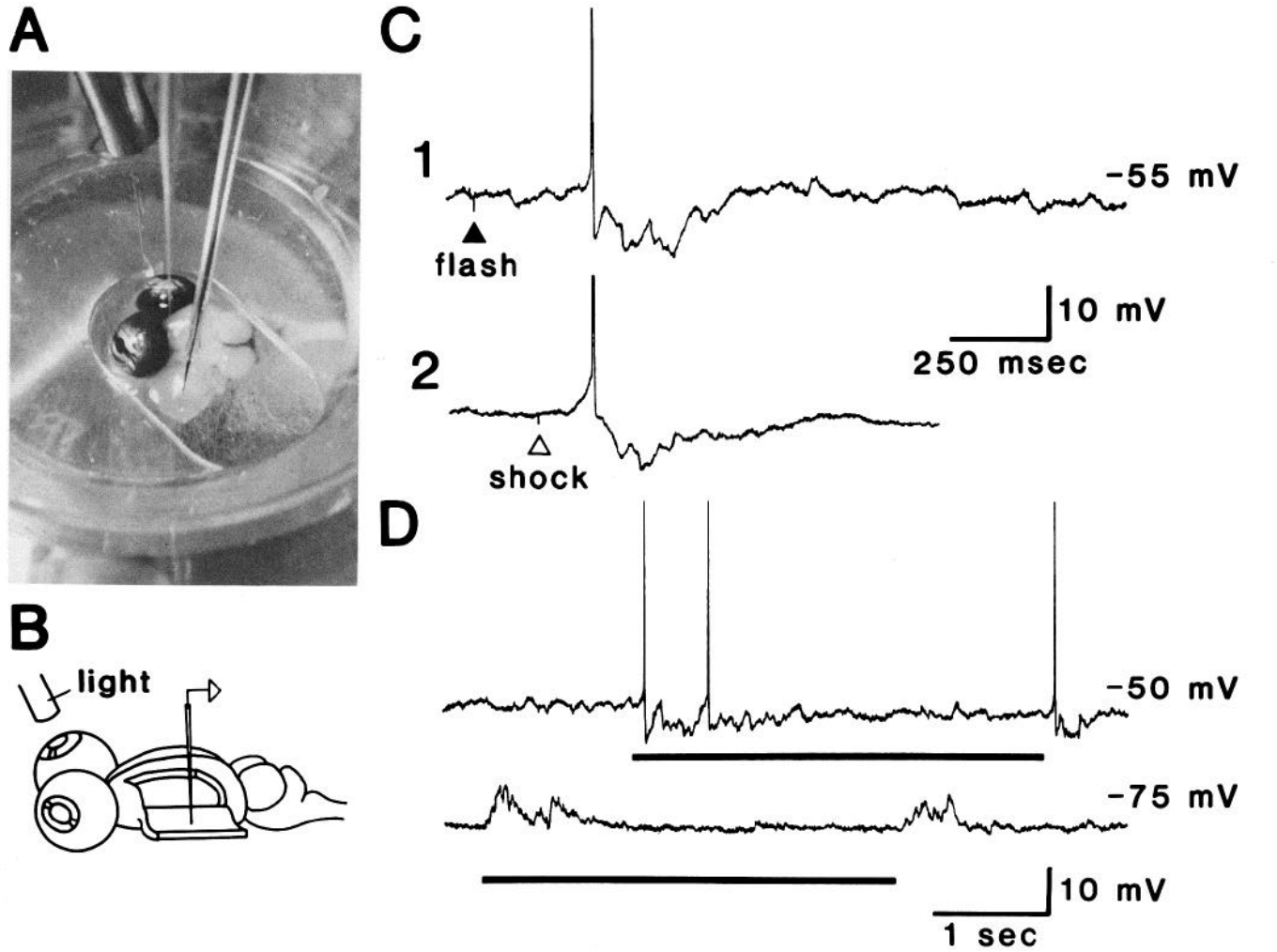

Figure 1. *A*, Photograph of a living *in vitro* preparation. *B*, Access to the visual cortex was obtained by a cortical flap that preserved the laterally coursing optic radiations. *C*, (*1*) Pyramidal cell response to a brief (10 μsec) flash of light; (*2*) the cortical pyramidal cell response to an electrical shock applied to the optic nerve via a suction electrode mimicked the intracellular response recorded to a flash of light (*1*) but with a shorter latency. *D*, Long light flashes produced similar ON and OFF responses in pyramidal cells (*top trace*). Synaptic events were inverted by hyperpolarizing the cell membrane to −75 mV (*bottom trace*). AP amplitudes are truncated in these figures. Solid bar indicates duration of light flash.

almost no spontaneous APs when unstimulated in a darkened room.

Long flashes of light (3–4 sec) produced pyramidal cell responses that typically consisted of both ON and OFF components, each of which resembled the response to a brief light flash (Fig. 1*D*, top trace). The flash-evoked inhibitory synaptic events could be inverted by injecting negative current to hyperpolarize the cell membrane (Fig. 1*D*, bottom trace). The mean reversal level of the inhibitory response measured for 6 neurons at the latency-to-peak inhibition (large arrow in Fig. 2*A*, 1) was −69.6 mV (±3.3 mV SD; Fig. 2*A*, 2). The extrapolated reversal potential of the early response was highly variable, but was approximately −50 mV for the example shown in Figure 2*A* (small arrow in Fig. 2*A*, 1). This suggested that the excitatory response may be shunted by concurrent inhibition, since the reversal potential for this phase of the response was lower than one would expect from unopposed EPSPs, and that the hyperpolarizing response may result from an increase in Cl^- conductance (gCl^-).

The ionic dependence of the long-lasting hyperpolarizing phase of the light-evoked response was tested by recording from 8 cortical neurons with KCl^--filled microelectrodes. In all cells, intracellular Cl^- injection resulted in gradual inversion of the IPSPs to depolarizing events lasting 250–1000 msec (Fig. 2*B*, 1 and 2). This result confirmed that a component of the hyperpolarizing phase of the flash response consisted of prolonged activation of Cl^--dependent IPSPs. When bursts of APs occurred in pyramidal neurons, a post-discharge hyperpolarization developed (Figs. 2*B*, 2; 3*A*) that was not present in the absence of spike discharge. This might have resulted from an increase in gK^+ activated by Ca^{2+} entry during the AP burst, or from the recruitment of a new burst-dependent IPSP.

Most Cl^--dependent cortical IPSPs are mediated by the transmitter GABA. The GABA dependence of Cl^--mediated IPSPs in pyramidal neurons was tested by applying the GABA antagonists picrotoxin (500 μM; $n = 3$) or bicuculline methiodide (100 μM; $n = 4$) focally to the cortex. A large, early flash-evoked EPSP developed, which lasted 200 msec and triggered a pyramidal cell burst discharge (Fig. 3*A*). The enhancement of the early flash-evoked response by GABA blocking agents supports the idea that there is coactivation of excitatory and inhibitory elements during this phase of the response.

The IPSPs recorded in pyramidal cells are presumed to arise

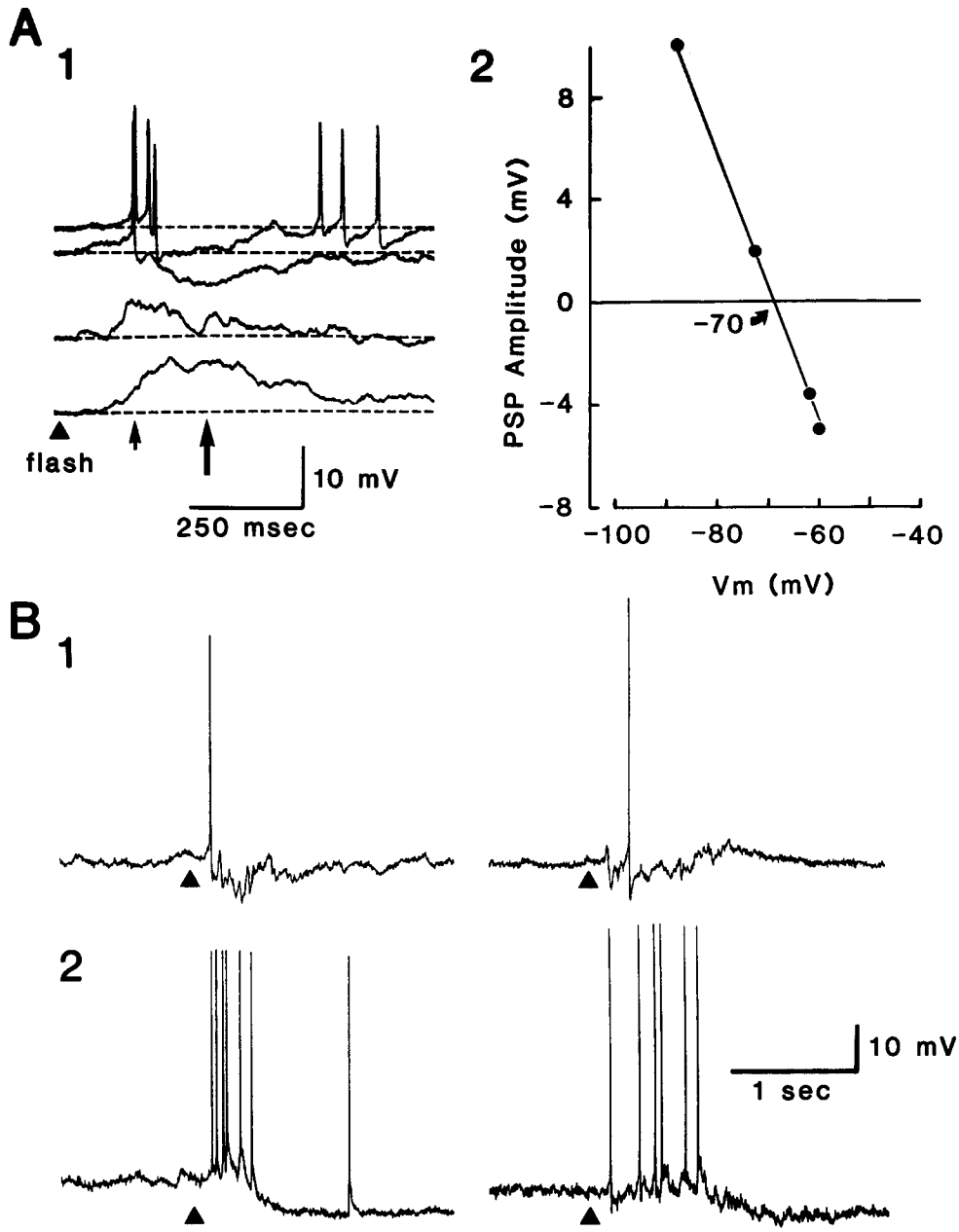

Figure 2. *A*, (*1*) Averages of 3 flash-evoked responses obtained for a pyramidal cell held at each of a series of 4 potentials between −50 and −90 mV. Spikes are truncated; (*2*) plot of PSP amplitude calculated at the latency-to-peak inhibition indicated by the *large arrow in 1*. The reversal potential for this response is −70 mV. *B*, (*1*) *Right and left panels* show flash-evoked responses for 2 different cells following initial impalement with KCl^--filled microelectrodes; (*2*) following 15 min of intracellular Cl^--injection, the light-evoked responses inverted to depolarizing events that lasted 250–1000 msec and fired APs. A post-discharge hyperpolarization also developed.

from the activation of local circuit non-pyramidal cells that stain positively for a variety of GABAergic markers (Blanton et al., 1987). Non-pyramidal cells are encountered infrequently in microelectrode penetrations through turtle cortex, in part because they are sparse (Smith et al., 1980). However, it is possible to record unit activity from presumed non-pyramidal cells by placing an extracellular microelectrode into the molecular layer of turtle cortex, which contains only non-pyramidal cell somata. In 3 experiments, either sequential or simultaneous recordings were obtained from an intracellular electrode placed in a pyramidal cell and an extracellular electrode recording the activity of neurons in the molecular layer. The response to a flash of light is shown in Figure 3*B*. The intracellular electrode recorded the typical pyramidal cell response, dominated by a series of IPSPs (Fig. 3*B*, bottom trace), while the extracellular electrode recorded units firing in response to the flash (top trace). Unit firing in the molecular layer extended through the time period of intracellularly recorded IPSPs in the nearby pyramidal neuron. The flash-activated units were presumably GABAergic non-pyramidal cells in the turtle visual cortex that mediate the Cl^--dependent IPSPs seen in neighboring pyramidal neurons.

Discussion

This intracellular study of the cortical response to sensory stimulation has been able to test a variety of predictions of cellular behavior derived from anatomical, biochemical, and physiological studies of turtle cortex. Retrograde labeling and degeneration studies have shown that geniculocortical afferents occupy the superficial molecular layer and form 6 times more excitatory terminations onto non-pyramidal cell dendrites than onto pyramidal cell dendrites (Smith et al., 1980). Immunohistochemical studies using GABA and GAD antibodies have shown that molecular layer non-pyramidal cells are GABAergic (Blanton et al., 1987), and ultrastructural evidence suggests that

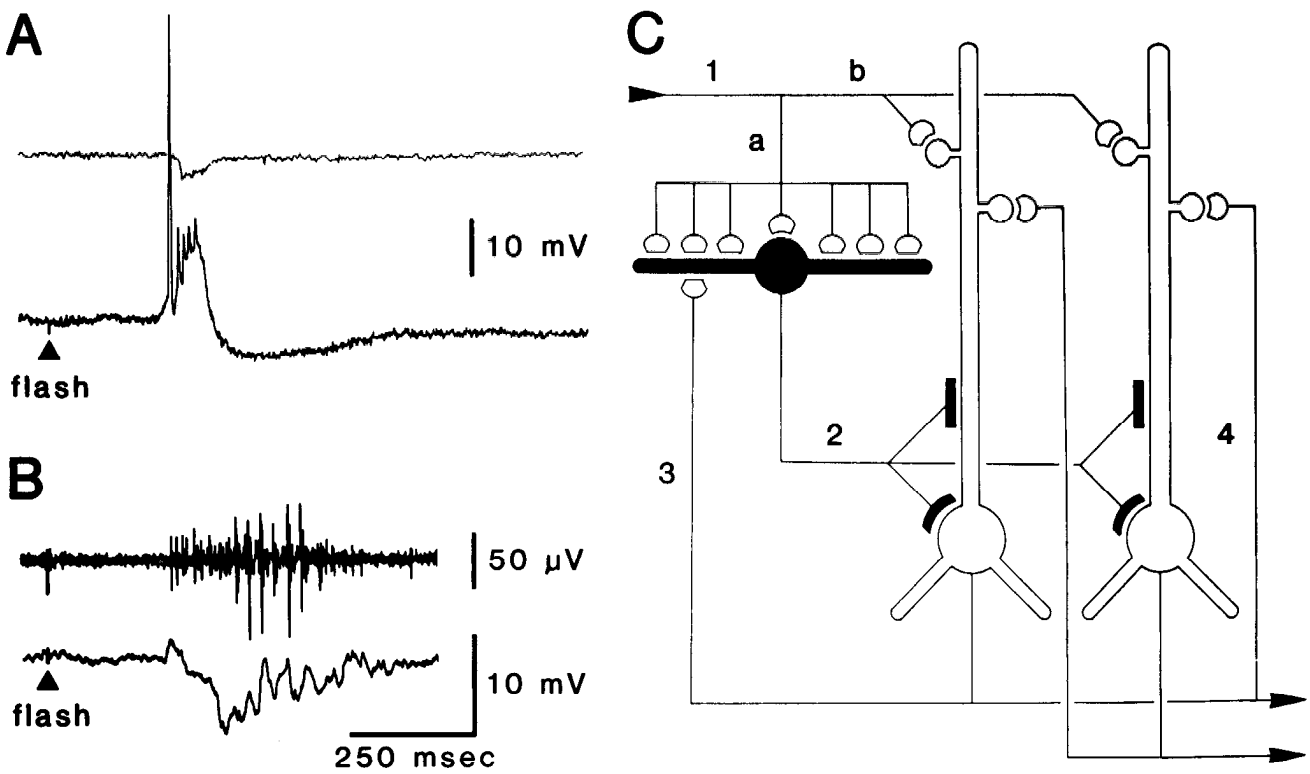

Figure 3. *A,* The GABA dependence of Cl^--mediated IPSPs in turtle cortex was tested by applying picrotoxin (500 μM) to the cortex. A large early EPSP lasting 200 msec was observed, leading to a pyramidal cell burst discharge in response to a light flash. Note the post-burst hyperpolarization. *Top trace* is a simultaneous field-potential tracing showing a synchronized population discharge. *B,* An extracellular recording of unit activity in the molecular layer of the cortex (*top trace*) reveals neurons discharging in response to a flash of light. The latency of unit firing corresponds to the latency of IPSPs recorded intracellularly in a nearby cortical pyramidal neuron (*lower trace*). *C,* Schematic of the cortical circuitry described in the text. Geniculocortical afferents contact both GABAergic non-pyramidal cells (*1a*) that provide feed-forward inhibition of pyramidal cells (*2*) and pyramidal cell apical dendrites (*1b*). There are also pathways for recurrent inhibition (*3*), and reciprocal excitation (*4*).

they form inhibitory terminals onto the somata and proximal dendrites of pyramidal neurons (Ebner and Colonnier, 1975, 1978; Smith et al., 1980). The principal cortical pathways discussed above are illustrated schematically in Figure 3*C*. Therefore, as hypothesized by Smith et al. (1980), an afferent volley along geniculocortical pathways would powerfully excite inhibitory non-pyramidal cells and coactivate pyramidal cell dendrites. The non-pyramidal cells would presumably mediate GABAergic feed-forward inhibition of pyramidal neurons. Recurrent inhibitory pathways (Kriegstein and Connors, 1986) may also contribute to the long latency of the inhibitory response. Moreover, differences between pyramidal and non-pyramidal cell membrane properties, particularly the characteristic tendency of pyramidal cell APs to show accommodation while non-pyramidal cell APs are nonaccommodating (Connors and Kriegstein, 1986), lead to the prediction that, in the summated response, inhibition will outlast excitation. As reported here, the pyramidal cell response to visual stimulation of the eye consisted of early, phasic excitation superimposed upon long-lasting tonic inhibition.

Previous studies of visual responses in turtle cortex (for a review, see Belekhova, 1979) did not address the issue of intracortical inhibition because the extracellular recording methods generally applied to *in vivo* experiments were not adequate for analyzing IPSPs. The present *in vitro* study has allowed a significantly more detailed cellular analysis of the cortical events engaged by activity along the visual pathway, emphasizing the role played by physiologically distinct cell types in determining the cortical responses to an afferent volley and revealing the key role played by GABA-mediated intracortical inhibition.

It is interesting to note that pyramidal cell inhibition induced by visual stimulation appears to consist mostly of Cl^--dependent IPSPs. In contrast, when the afferent layer of turtle cortex is stimulated directly, an additional long-latency K^+-dependent IPSP is evoked (Kriegstein and Connors, 1986). This IPSP may result from coactivation of one or more of the nonthalamic afferent pathways, which include cholinergic, catecholaminergic, and peptidergic fibers (Parent and Poitras, 1974; Reiner et al., 1984; Ouimet et al., 1985). The *in vitro* turtle visual system, therefore, is a promising model for the study of the cortical effects of selectively activated subcortical pathways and of certain aspects of functional organization that may be common to the higher vertebrate cortex.

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