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Functional Properties of Neocortical Neurons

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Abstract. Differences in intrinsic membrane properties, the receptor subtypes available for activation by transmitters and modulators and their coupling to a variety of ionic conductances, and alterations in neuronal membranes and synaptic events during ontogenesis are all major factors determining the behavior of individual neocortical neurons in even the simplest cortical circuit. Interneurons and pyramidal cells in neocortex have quite different input-output behaviors related to differences in membrane currents which allow the interneuron to follow inputs faithfully and the pyramidal cell to have a more integrative role. Modulatory agents can produce profound changes in the excitability of cortical pyramidal cells through interactions with voltage-dependent and Ca^{++}–activated membrane currents. Different classes of cortical neurons can possess different receptor subtypes and can produce quite different responses to a given transmitter. A complex sequence of changes in the electrical properties of neuronal membranes and in synaptic activities occur during ontogenesis, including marked increases in the density of ion channels and delayed development of inhibitory electrogenesis. These variables probably account for the rich repertoire of responsiveness possible in mature and immature cortical circuits during different behavioral states.

INTRODUCTION

The anatomic circuitry and synaptic activities within the neocortex play a critical role in determining the functional capacities and integrative properties of neuronal networks. However, it has become increasingly clear that the electrical properties of single cells can also contribute significantly to cortical circuit function. A large number of variables will influence the output of a given neuron, including: (a) differences in the type and topographic distribution of voltage-dependent membrane conductances among different anatomic classes of cells; (b) the variety of receptor subtypes present, and their coupling to particular ionic conductances and/or intracellular

biochemical events; (c) alterations produced in transmitter and voltage-activated conductances by modulatory substances; and (d) changes in neuronal properties and synaptic events occurring during ontogenesis. We have here selected a number of specific examples for discussion which show how some of these factors influence the properties of neocortical neurons and provide a rich repertory of possible behaviors perhaps not predictable from the skeleton of a circuit diagram.

COMPARATIVE ELECTROPHYSIOLOGY OF SUBCLASSES OF CORTICAL NEURONS

A Variety of Membrane Currents Control Cell Behavior

Although the properties of complex cortical circuits leading to sensory and motor activities have been investigated in elegant detail, the electrophysiological properties of individual cortical neurons of various subclasses (Peters and Jones 1984) are not completely understood. In order to fully describe the operation of cortical circuits and alterations occurring in them during a variety of normal and pathological states, it becomes important to understand the membrane properties of individual elements which can profoundly influence the cortical output. Electrophysiological studies in other structures, such as the cerebellum (Llinás and Sugimori 1980a,b), hippocampus (Wong et al. 1979), and thalamus (Deschenes et al. 1982, Jahnsen and Llinás 1984), have emphasized that, in addition to the classical Na^+–K^+ currents underlying the action potential in squid axons, mammalian neurons are endowed with a large variety of ionic channels whose activation can be affected by voltage, transmitters and modulators, and intracellular ions to generate a rich spectrum of membrane behavior.

Many of these ionic current channels or the associated membrane conductances have been characterized in vertebrate cortical neurons (Table I). It is the interaction of several inward and outward currents activated in the subthreshold voltage range between resting potential and the spike threshold which determines the membrane potential trajectory during prolonged depolarizations and the rate and pattern with which action potentials are triggered (if at all). A few examples particularly important for the control of neuronal discharges are mentioned here:

1) A slow persistent Na^+ current, present in some neocortical neurons (Connors et al. 1982; Stafstrom et al. 1985) and at other sites (Llinás and Sugimori 1980b), is activated rapidly by small depolarizations. It can thus produce a relatively maintained intrinsic "booster" depolarization in response to an excitatory postsynaptic potential (EPSP). This current would tend to counteract the effects of K^+ currents activated in the subthreshold voltage range, and promote repetitive action potential generation.

TABLE 1 Voltage-dependent ionic conductances in cortical neurons

Current	Preparation	Relevant References	Possible Function
Na Currents			
Fast Na	neocortex	Connors et al. 1982	spike current
Slow Na	neocortex	Connors et al. 1982; Stafstrom et al. 1985	maintained depolarization
K currents			
A current	hippocampal cultures	Segal et al. 1984	slow repetitive firing
	hippocampal slice	Zbicz and Weight 1985	
m current	neocortex	Halliwell 1986; McCormick and Prince 1985	accommodation
q current	neocortex	Halliwell 1986	inhibits hyperpolarization
anom. rectifier	olfactory cortex	Constanti and Galvan 1983	
c current	hippocampus	Lancaster and Adams 1986	repolarize spike
AHP current	neocortex	Connors et al. 1982	accommodation
Ca currents			
Transient	olfactory cortex	Constanti et al. 1985	burst firing
(low threshold)	neocortex	Galvan et al. 1986	
Persistent	olfactory cortex	Constanti et al. 1985	calcium entry
(high threshold)	neocortex	Galvan et al. 1986	

2) A rapidly activating but also *inactivating* K^+ current, known as the "A" current (Connor and Stevens 1971), is also present in neocortical (O.P. Hamill, J.R. Huguenard and D.A. Prince, unpublished) and hippocampal (Zbicz and Weight 1985) pyramidal neurons. This current is activated by depolarizations from hyperpolarized membrane potentials and may contribute to spike repolarization. It is a transient current which would promote firing at relatively slow rates by retarding the rate of depolarization between spikes. In neurons of the dorsal raphe the A current is suppressed by activation of α_1 adrenergic receptors (Aghajanian 1985).

3) Another K^+ current, the "M"-current (Adams et al. 1982), has been identified in hippocampal (Halliwell and Adams 1982) and neocortical (Halliwell 1986) pyramidal cells. It is activated slowly by depolarizations in the subthreshold range and functions to counteract prolonged depolarizations and promote spike frequency accommodation. It is reduced by muscarinic agonists (see below).

4) Spike discharges in cortical neurons are associated with Ca^{++} entry, which in turn activates a slow afterhyperpolarization (AHP) mediated by a K^+ current (Hotson and Prince 1980; Lancaster and Adams 1986). This slow Ca^{++}-activated K^+ current (the "AHP" current) promotes spike frequency accommodation of a longer time course than the M current and markedly reduces excitability in neurons after periods of intense activity. Some transmitters have important modulatory effects on the AHP current (see below).

5) Still other K^+ currents (Table I) are activated when the membrane is hyperpolarized from rest. These conductance mechanisms tend to insure that the resting membrane potential is not allowed to remain at highly hyperpolarized levels.

Different Classes of Neurons Possess Different Functional Properties

Anatomic studies have defined a number of different structural and immunocytochemical classes of neuron in the cerebral cortex (Emson and Hunt 1981; Peters and Jones 1984), making it only reasonable to consider whether these structural and biochemical specializations are accompanied by important variations in membrane properties and cell behavior. For example, fast and slow conducting cat pyramidal tract neurons are specialized so that they operate in a phasic and more tonic manner, respectively (Takahashi 1965); differences in structure of these neuronal subclasses have recently been described (Deschenes et al. 1979). Other experiments done using the *in vitro* cortical slice preparation show that in the hippocampus (Masukawa et al. 1982) and neocortex (Connors et al. 1982; McCormick et al. 1985), subsets of neurons may have quite different membrane properties. Given the variables affecting neuronal function mentioned above, we would

speculate that increasing evidence for functional differentiation will be discovered among diverse and even similar appearing anatomic groups of cortical cells. Some examples of this variability and its implication for the function of circuits are discussed here.

Interneurons. One of the key issues in the operation of the cortex is the function of its large variety of local circuit neurons. Because of their relatively small size, not much information about the membrane characteristics of interneurons is available. In recent experiments, however, we have been able to define a number of these properties in identified bitufted and multipolar sparsely spiny or aspiny stellate cells which are probably GABAergic (McCormick et al. 1985). One unambiguous electrophysiological "marker" for these cortical interneurons, previously noted in extracellular recordings (Mountcastle et al. 1969), is the short duration of their action potentials in relation to those of pyramidal neurons (Fig. 1). Compared to pyramidal cells, interneurons tend to have a higher input resistance, a more linear current–voltage relationship, a steep and linear relation between the frequency of generation of action potentials and the amplitude of somatic depolarizing current pulses, and the ability to fire at high frequencies (Fig. 2A). Even when firing at high rates, these neurons show very little if any spike frequency adaptation (Fig. 2B), a feature thought to be related to the absence of appreciable slow outward currents such as the Ca^{++}-activated K^+ current and M-current described above. Some of these properties are similar to those of identified interneurons in hippocampus (Schwartzkroin and Mathers 1978), olfactory cortex (Satou et al. 1983), and visual cortex in turtles (Connors and Kriegstein 1986), suggesting that they may be common to some classes of interneurons and conserved during evolution of vertebrates.

Biophysical properties of interneurons and pyramidal cells. In order to obtain more quantatative and detailed data relevant to the differences in membrane properties of interneurons versus pyramidal cells, and to elucidate the mechanisms underlying the different spike morphologies and spike firing patterns, we have recently applied patch clamp methods (Hamill et al. 1981) to acutely isolated neurons (Kay and Wong 1986) from the sensorimotor area of rat neocortex. Pyramidal cells and interneurons were identified using morphological criteria in conjuction with immunocytochemical staining for glutamic acid decarboxylase: GAD (Oertel et al. 1981). A single type of current was isolated in each experiment and compared in the two cell types of interest.

Na^+ Current—The fast Na^+ current underlying the action potential was essentially the same for the two cell types (Fig. 3A) in terms of its TTX sensitivity (10–100 nM range) and Hodgkin–Huxley type voltage sensitivity

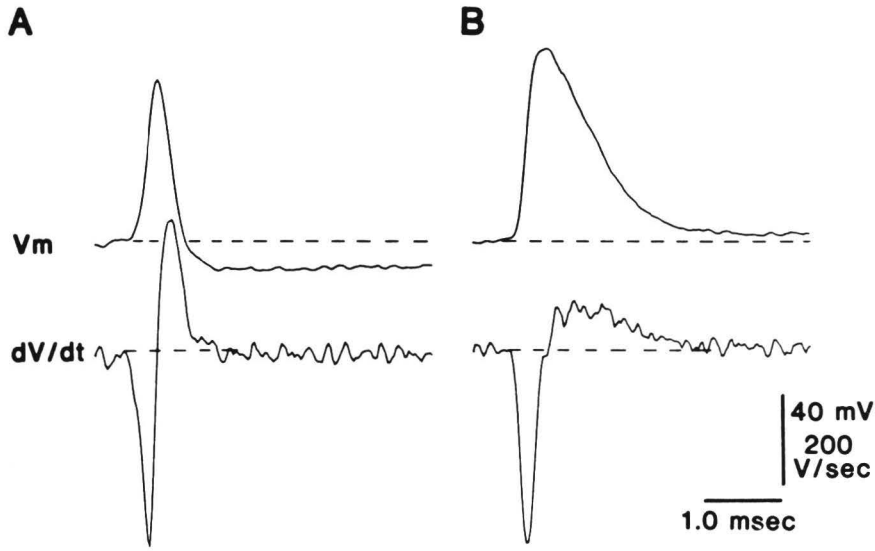

Fig. 1—Characteristics of action potentials (APs) of interneurons and pyramidal cells. **A:** AP of typical fast-spiking interneuron. Top: Deviation in V_m during action potential. Bottom: Voltage trace differentiated with respect to time. Positive slope is downward. **B:** AP of typical regular spiking pyramidal neuron. Note the slower rate of repolarization compared to the fast spiking cell (A). From McCormick et al. 1985.

of activation ($V_{1/2}$ –30 to –35 mV), steady-state inactivation ($V_{1/2}$ –65 to –60 mV), and recovery from inactivation. The estimated density of Na^+ channels in the soma membrane was slightly higher for pyramidal neurons (Fig. 4A).

K^+ Currents—In initial studies we have characterized a delayed rectifier current and a transient rapidly activated K^+ current (I_A) that are very similar to those described in other neurons (Connor and Stevens 1971, Zbicz and Weight 1985). There are marked differences in both the type and density of these two K^+ channels between the two neuron classes (Fig. 3B).

The "A" current was found mainly in pyramidal neurons (cf. Fig. 3B1 and Fig. 3B2), while the delayed rectifier current was present in both neuronal types. The lack of I_A in interneurons may help explain the high spike firing rates as well as the linear f/I functions of these cells (see above).

The kinetics and voltage sensitivity of the delayed rectifier current are qualitatively similar in both neuron classes, in that it undergoes little steady state inactivation, and is half activated at 0 to +10 mV. However, in nonpyramidal neurons, the membrane density of delayed rectifier channels is about 150% of that in pyramidal neurons (Fig. 4B). To the extent that the delayed rectifier current contributes to spike repolarization, this difference

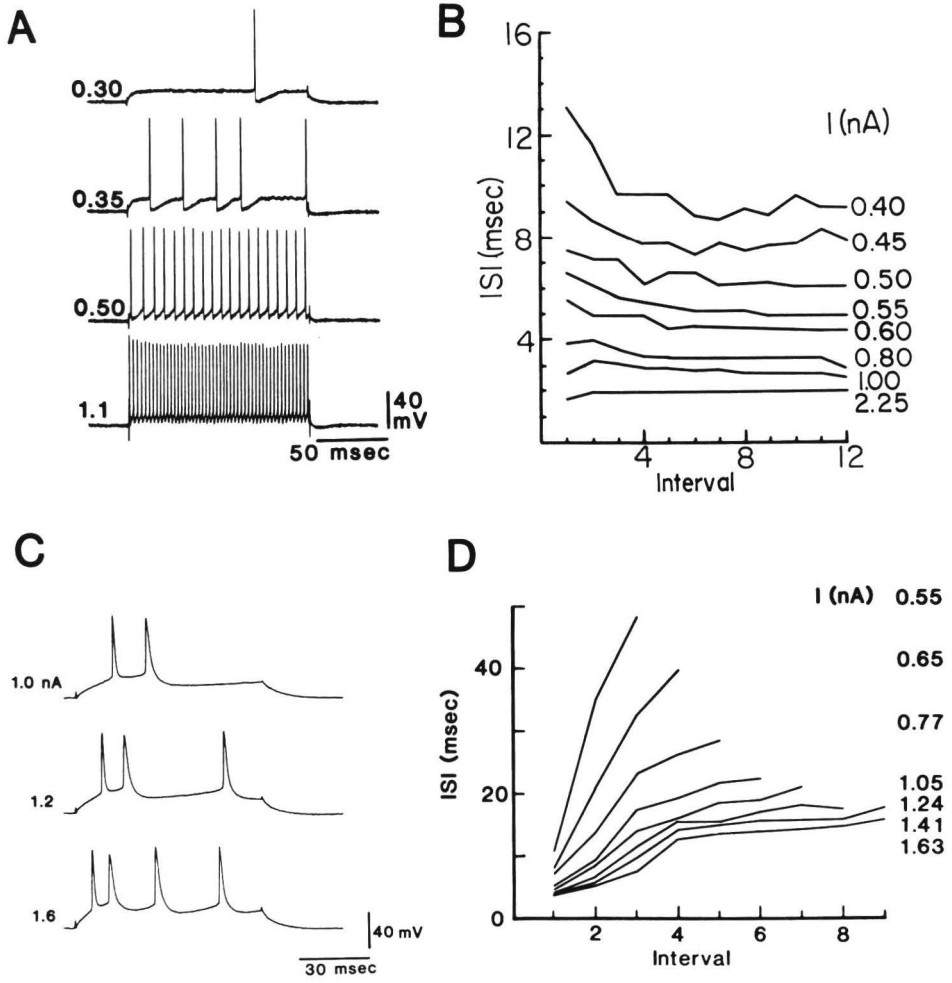

Fig. 2—Response characteristics of interneuron (A–B) and pyramidal cell (C–D). **A:** Responses showing steep relationship between injected depolarizing current pulses and firing frequency, as well as absence of spike frequency adaptation. Magnitude of current (in nA) is indicated to the left of each trace. **B:** Interspike interval (ISI) versus interval number for interneuron illustrated in A. Magnitude of injected current is indicated to right of graph. Note the initial increase in spike frequency during lower-intensity currents, followed by periods of sustained high-frequency firing without adaptation. **C:** Square, suprathreshold current pulses of varying amplitudes cause repetitive spiking that shows marked adaptation in pyramidal neuron. **D:** Graph of interspike interval versus interval number for a pyramidal neuron. Regular spiking cells showed marked adaptation at all levels of current (compare to **B**). From McCormick et al. 1985.

(along with a small contribution from the difference in Na^+ channel density) could contribute to the shorter spike duration of interneurons versus pyramidal cells. The higher K^+ current density in nonpyramidal neurons would result in a more rapid rate of repolarization of the action potential and would therefore result in an action potential of shorter duration (see also dV/dt traces of Fig. 1).

Current clamp recordings (McCormick and Prince 1985; McCormick et al. 1985) suggest that Ca^{++}-activated K^+ current and M-current may be

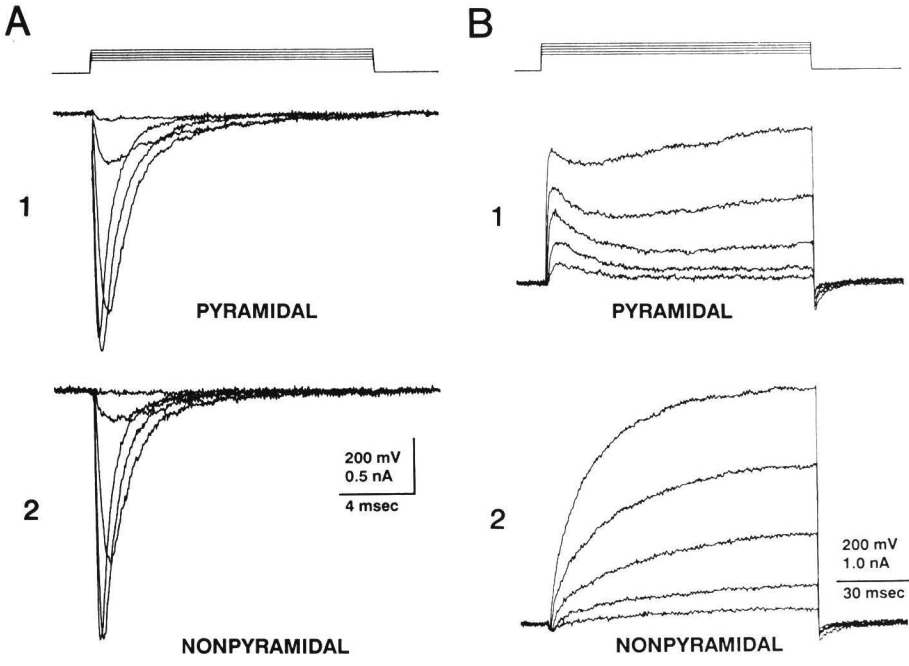

Fig. 3—Ionic currents recorded from whole cell clamps in different neocortical cell classes. **A:** Na currents recorded from pyramidal (**A1**) and nonpyramidal (A2) neurons of similar membrane capacity at developmental stage P2. K and Ca currents were blocked by including CsF and TEA in the intracellular solution and $CdCl_2$ in the extracellular solution. Holding potential: − 100 mV; command steps: − 50 to − 10 mV. Voltage sensitivity of the Na current is similar in both cell types. **B:** K currents recorded from pyramidal and nonpyramidal neurons of similar size at developmental stage P17. Na currents were blocked with 10^{-7} M TTX. Holding potential: −100 mV: command steps: −40 to 0 mV. The pyramidal neuron (B1) generates a rapidly activated, transient outward current with the smallest depolarizations, and with larger depolarizations, a more delayed outward current which becomes larger than the transient current. Nonpyramidal neurons (**B2**), for the most part exhibit only a slowly activating outward current. Capacitative and leak currents have been subtracted in **A** and **B**. From Huguenard, Hamill and Prince, unpublished.

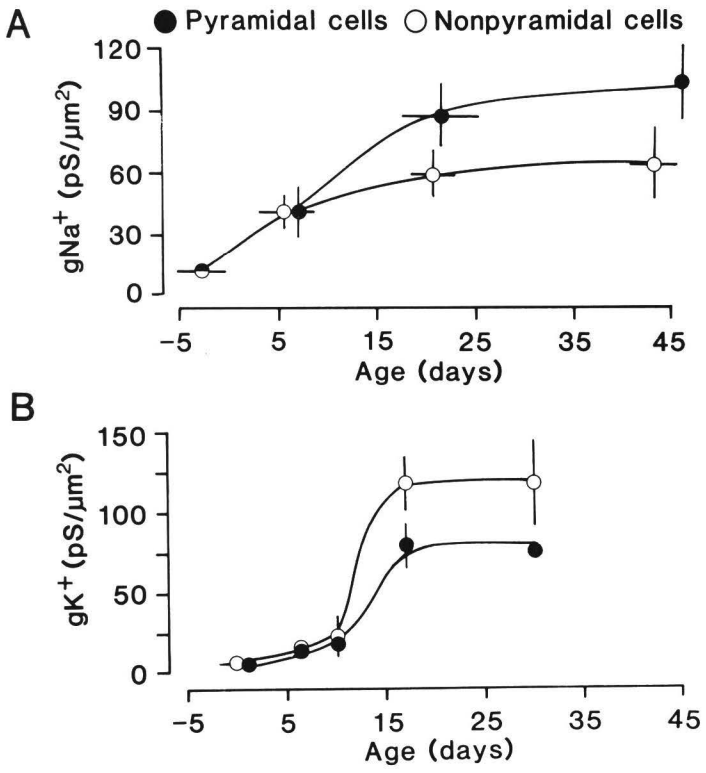

Fig. 4—Comparison of the density of Na and K conductances in membranes of the two different cell classes at different developmental stages. **A:** The peak Na conductance was obtained from the maximum slope conductance of I–V relationships. Pyramidal neurons demonstrated a slightly higher Na current density especially apparent after day P5. **B:** The maximum late (100 ms latency) K conductance was determined analogously to peak Na conductance. Nonpyramidal neurons demonstrated higher late K conductance at developmental stages after P10. From Huguenard, Hamill and Prince, unpublished.

much less prominent in interneurons than pyramidal cells. This remains to be resolved using voltage clamp techniques. Since axonal terminal regions of cortical neurons cannot be studied with standard recording techniques, it is not known whether the differences in the patterns of firing and spike morphologies detectable in somata of different classes of neuron are also present at terminals where they would significantly affect transmitter release. Also, these physiological observations are very limited—there are no comparable data regarding the properties of other inhibitory or excitatory interneurons.

Implications for interneuronal function. The consequences of these properties make interneurons prone to fire repetitively following depolarizations (e.g., Fig. 5), and presumably distribute an inhibitory output to principal cells which is a faithful transform of the excitatory input. Presumed inhibitory interneurons do have a "noisy" baseline in intracellular recordings, probably due to impingement of excitatory synaptic inputs. Orthodromic stimulation shows that inhibitory control of these cells (evoked IPSPs) is less prominent than for pyramidal-type neurons (cf. Fig. 5 with Fig. 13 of Connors et al. 1982). Perhaps one of the consequences of the above properties is a high level of resting discharge in some inhibitory interneurons, as evidenced by a continuous barrage of small inhibitory events which can be recorded from pyramidal neurons of hippocampal (Alger and Nicoll 1980) and neocortical (Galvan et al. 1985) slices under resting baseline conditions. These spontaneous IPSPs, best seen when the neuron is loaded with Cl^- or nitrate, are generated at a frequency of 10–45 Hz and often occur in bursts (B. Wong and D.A. Prince, unpublished data; Fig. 6), perhaps reflecting the tendency of interneurons to fire repetitively. We would speculate that such a continuing inhibitory background, if it occurred *in vivo*, would keep the membrane further away from firing threshold and shunt small excitatory events so that larger, more significant depolarizing inputs will achieve threshold without baseline "noise" in the system.

Studies of the responses of GABAergic and other interneurons to transmitters will be important in understanding the control of inhibitory

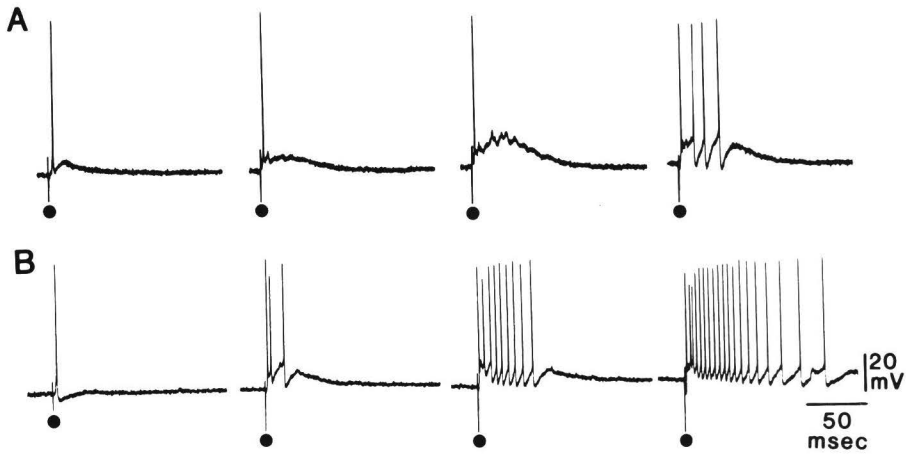

Fig. 5—Orthodromic activation of two fast spiking interneurons leads to repetitive firing. Single shocks (dots) were applied to deep cortical layers at increasing intensity from left to right. No obvious hyperpolarization or inhibition was seen. Resting potential was −76 mV in A and −71 mV in B. From McCormick et al., 1985.

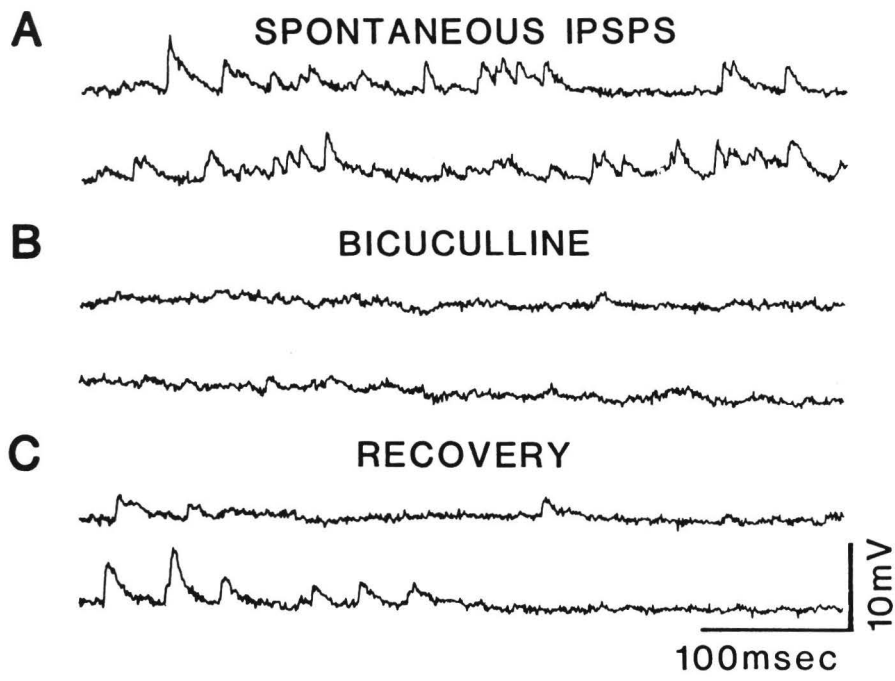

Fig. 6—Positive-going IPSPs recorded from a pyramidal neuron of guinea pig neocortical slice using K-nitrate-filled micropipette. Frequent spontaneous IPSPs were observed immediately after impalement **(A)**, which were blocked by topical application of bicuculline **(B)** and recovered during washout **(C).** Note clusters of IPSPs in A. From B. Wong and D.A. Prince, unpublished.

(and excitatory) circuitry in the cortex. For example, some evidence already indicates that GABAergic interneurons have different kinds of muscarinic receptors and cholinergic responses than cortical pyramidal cells (McCormick and Prince 1985; see below for discussion). If this is a general principle of the organization of ascending transmitter systems, the possibility of selective pharmacological manipulation of subsets of cortical principal cells and interneurons becomes an exciting new research direction and potential future clinical tool.

Pyramidal type neurons. In contrast to the above properties of interneurons, the vast majority of pyramidal neurons in layers II–VI of the sensorimotor cortex are "regular spiking" cells which possess a variety of voltage-activated currents (Table I), including a low threshold noninactivating Na^+ current. At least two of the currents described above, the M-current and the AHP current, significantly modulate the firing of cortical pyramidal neurons and allow them to discharge at low frequencies (Madison and Nicoll 1984). Some

of the neuronal behavioral consequences of these membrane properties contrast sharply with those of interneurons, including significant spike frequency adaptation during depolarization (Fig. 2C and D), IV relationships that are markedly nonlinear in both the depolarizing and hyperpolarizing directions, and a tendency to generate slow spike afterhyperpolarizations.

Another variety of cortical pyramidal neuron has the capacity to generate bursts of three or more spikes when depolarized directly or with an EPSP (Connors et al. 1982; McCormick et al. 1985). These cells are located largely in layer IV and upper layer V and, when labelled intracellularly, are grossly indistinguishable from other nonbursting medium-sized spiny pyramidal-shaped neurons. Burst generating neurons form only a small percentage of those impaled[1] in the sensorimotor cortex, although such behavior is common in other cortical areas (Masukawa et al. 1982). The membrane mechanisms underlying burst discharge in neocortical cells are not known. However, some burst features suggest that activation of a low threshold Ca^{++} spike similar to that in thalamic relay cells (Jahnsen and Llinás 1984) may be involved. The functional significance of burst-generating neocortical pyramidal type neurons is unclear. We have speculated (McCormick et al. 1985) that these neurons may receive strong thalamic excitation which might evoke their burst behavior *in vivo*. If the action potentials recorded in the soma propagate to the terminal axonal arborization where they release transmitter, burst generation would provide one means for establishing a very secure influence on the target neuron, since significantly more release of transmitter would be expected (e.g., Miles and Wong 1986). Such cells could also be involved in some forms of cortical rhythm generation. The axonal arborizations have not been traced; however, from studies in the disinhibited cortex (Connors 1984), it has been concluded that these cells may have widespread intracortical connections so that they serve as pacemakers and synchronizing elements for epileptiform discharge.

Another major unkown with respect to the physiology of cortical pyramidal neurons is the functional properties of their dendritic membranes. Prominent Ca^{++} spikes, such as those found in dendrites of Purkinje cells (Llinás and Sugimori 1980a) and hippocampal pyramidal cells (Wong et al. 1979), are not detectable in somatic recordings from most cortical neurons under normal circumstances, although they can be evoked after K^{+} conductances are reduced (Connors et al. 1982). The suggestion that cortical dendrites may generate short duration (presumed Na) spikes (Purpura et al. 1965), as do hippocampal dendrites (Benardo et al. 1982), remains to be explored. These and other voltage-dependent events could profoundly affect dendritic responsiveness to synaptic inputs and therefore to cell output.

[1] Relative proportions of cell types recorded with microelectrodes could be subject to a selection bias.

ALTERATION OF FUNCTIONAL PROPERTIES OF CORTICAL NEURONS BY MODULATORY AGENTS

Over the past decade, traditional views of the action of neurotransmitters on cortical and other neurons have been substantially altered. One major change has been the discovery that transmitters may serve as "modulatory" substances which interact with neuronal receptors to produce long-term alterations in responsiveness of neurons to other inputs (Kupfermann 1978). The functions of voltage-dependent ion channels may be regulated or modulated by transmitters. Receptors for a number of transmitters, including acetylcholine (M1 and M2), serotonin (type 2), noradrenaline (α_1, α_2 and β_1) and GABA(B), can activate intracellular second messenger systems, either through phosphotidylinositide hydrolysis or G proteins and cAMP, to produce channel protein phosphorylation and long-term changes in excitability in some cases (Greengard 1978; Levitan 1985).

Consequences of Modulatory Actions

Three important examples of modulatory effects of transmitters on intrinsic membrane properties and neuronal behavior are provided by studies of the actions of acetylcholine and norepinephrine on cortical and thalamic neurons:

1) In hippocampal (Benardo and Prince 1982; Halliwell and Adams 1982) and cortical pyramidal neurons (McCormick and Prince 1986), the M current is reduced by activation of muscarinic receptors which are probably of the M1 subtype (McCormick and Prince 1985). Normally this outward K^+ current increases as the pyramidal neuron is depolarized, with a resulting return of membrane potential toward resting levels. This action of ACh is thus voltage-dependent, so that it produces larger depolarizations when applied to depolarized neurons where the M-current is active. An agonist-induced decrease in the M-current would increase the effectiveness of any depolarizing input. (ACh also has a non-voltage-dependent action on a K^+ conductance in the hippocampus (Benardo and Prince 1982)).

2) A second action of ACh on cortical and hippocampal pyramidal neurons is to decrease a slow Ca^{++}-activated K^+ current (AHP current) (Benardo and Prince 1982; Cole and Nicoll 1984; McCormick and Prince 1986b). This current underlies portions of the large and prolonged afterhyperpolarizations which follow repetitive spike activity in principal neurons. Together the AHP current and the M-current are important for the development of spike frequency adaptation during repetitive firing. Applications of ACh to hippocampal (Madison and Nicoll 1984) and cortical (McCormick and Prince 1986b) pyramidal neurons therefore cause a marked decrease in spike frequency adaptation and make the input–output relationships of these cells much steeper and more linear during prolonged depolarizations (Madison and Nicoll 1984). Activation of β_1 adrenergic receptors has a similar action

in the hippocampus through blockade of the I_{AHP} (Madison and Nicoll 1986). One major question with respect to transmitter studies employing local applications or bath perfusions of agonists is whether normal activation of circuits will evoke similar responses. This may be the case for some ACh actions in hippocampus (Cole and Nicoll 1984), but remains to be tested for most of the effects described above and in the following section.

3) The results of recent studies on the effects of acetylcholine and norepinephrine on thalamic relay neurons are relevant to the function of cortical circuitry during activation of afferents from the periphery. Thalamic principal neurons possess a low threshold Ca^{++} spike mechanism which is normally inactive at resting membrane potential. However, when neurons are hyperpolarized into the range from -65 to -80 mV, depolarizing pulses or synaptic potentials can evoke the low threshold Ca^{++} spike and a consequent burst of Na^{+}-mediated action potentials (Jahnsen and Llinás 1984). Transmitter actions which move the membrane into this voltage range would cause thalamic neurons to function in an oscillatory mode, whereas they function in a single spike mode at more depolarized potentials. The changes in information transfer by a relay cell in the guinea pig lateral geniculate nucleus at different membrane potentials following focal applications of a muscarinic agonist are shown in Fig. 7.

Depending upon the species and the region of thalamus studied, ACh may produce one or more of several responses (McCormick and Prince 1987a, c), including a short-lived nicotinic depolarization (prominent in cat), a slow hyperpolarization mediated by an increase in g_K, presumably through activation of an M2 receptor (prominent in guinea pig: Fig. 7), followed by a slow depolarization produced by a voltage-independent decrease in g_K (all species studied: Fig. 7). The latter action is similar to that produced by norepinephrine on the same neurons (McCormick and Prince 1986c). Thus, some of the effects of ACh and NE on membrane potential of thalamic relay cells may be to switch their output between the burst generating mode, which limits information transfer (e.g., during sleep), and the single spike mode, which supplies coherent information relevant to the periphery to cortical circuits (e.g., during arousal) (Livingston and Hubel 1981; Steriade and Deschenes 1984).

Functional Implications of Multiple Receptor Subtypes

Ligand binding studies show that there is a rich assortment of binding sites for transmitters in the cortex and thalamus (see Wamsley 1984 for review). With development of more and more specific ligands, more subtypes of presumed receptors will undoubtedly be described. A major experimental question is whether these multiple binding sites have functional meaning.

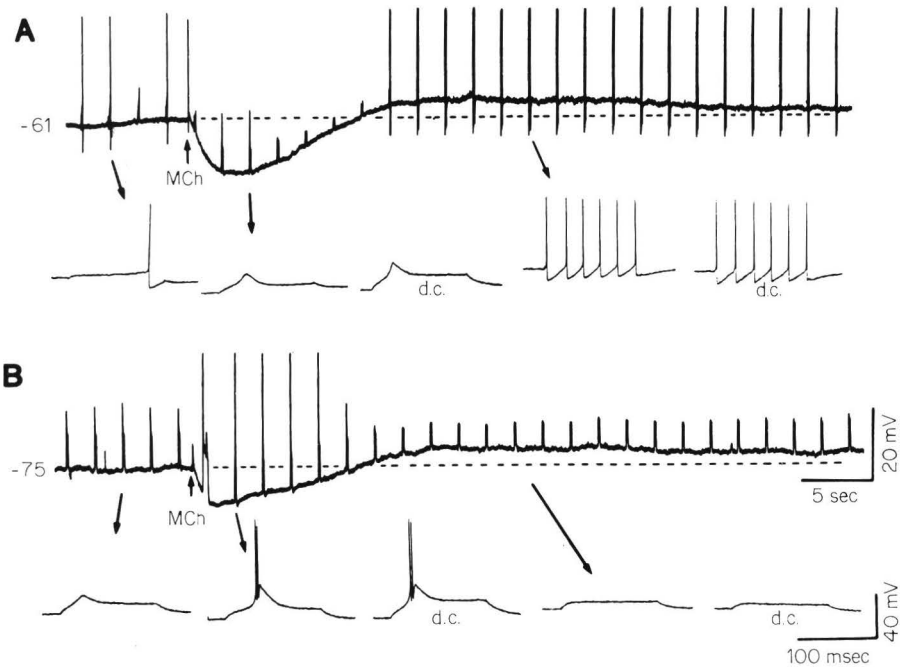

Fig. 7—Effects of methylcholine (MCh) applications to a guinea pig lateral geniculate neuron held at membrane potentials of −61 **(A)** and −75 mV **(B)**. MCh elicits a biphasic hyperpolarizing-depolarizing response in both trials. Responses of neuron to depolarizing current pulses are changed during both phases of the MCh response. Downward arrows show expanded sweeps of individual responses. In **A**, control response of a single spike (1st arrow), is changed to a small low threshold Ca^{++} spike during hyperpolarization (2nd arrow) and to a train of regular spikes during depolarization (3rd arrow), similar to those reponses produced at other times by manipulating the membrane potential with d.c. current alone (d.c.). In **B**, control response shows a small low threshold Ca^{++} spike (1st arrow) which becomes larger and evokes a burst of Na^{+} action potentials during hyperpolarization (2nd arrow), but is blocked during depolarization (3rd arrow). From McCormick and Prince, unpublished.

Do they represent innervated receptors versus nonjunctional receptors which may have functions aside from those of synaptic transmission and synaptically induced modulation? Potential segregation of these receptor subtypes on different classes of neurons and innervation by subgroups of neurons in ascending pathways could have important functional consequences. For example, studies in sensorimotor cortex show that following ACh application, pyramidal neurons develop an initial hyperpolarization associated with increased conductance, followed by a voltage-dependent slow depolarization associated with decreased conductance. Analysis of these responses has

shown that the initial inhibition is due to cholinergic muscarinic excitation of GABAergic interneurons mediated through an M2 receptor-coupled increase in cation conductance, whereas the later slow excitation is a direct action of ACh caused by a M1-mediated decrease in K^+ conductance (M-current) and AHP current. Thus, one transmitter can produce two different postsynaptic effects in cortex via two different receptors segregated on two different subclasses of neurons. (It is also of interest to note that pharmacologically similar M2 receptors on GABAergic neurons in n. reticularis are coupled to a different ionophore than those on cortical GABAergic cells (McCormick and Prince 1986a), so that ACh evokes a hyperpolarization due to an increase in K^+ conductance). It is not known whether single cholinergic neurons in nucleus basalis make contacts onto both cortical GABAergic interneurons and pyramidal cells, or whether separate cholinergic subgroups are involved. In the latter case, activation of the cholinergic system *in vivo* might produce either of these responses in isolation.

From these results, two general kinds of transmitter-affected functional alterations in cortical circuitry could be envisioned. One would occur through the activation of local circuit neurons or recurrent synapses of pyramidal cells and might control local cortical activities operating over relatively short times. Another, mediated by serotonergic, noradrenergic, cholinergic, and perhaps under some circumstances glutamatergic (Thomson 1986) transmitter systems originating in subcortical structures, might have the capacity to produce longer duration, modulatory, "state"-related changes in cortical circuits involving alterations in their gain or integrative function during such behavioral alterations as sleep, arousal, and seizure states.

CHANGES IN CORTICAL FUNCTION DURING DEVELOPMENT

Marked alterations in cortical responsiveness to sensory stimuli occur during development. However, the underlying changes in neuronal properties have not been completely examined. Results of recent experiments using *in vitro* neocortical slices (Kriegstein et al. 1987; McCormick and Prince 1987b) and acutely dissociated cortical neurons (Hamill et al. 1986; Huguenard et al. 1986) have documented an important sequence of changes in intrinsic membrane properties, synaptic events, and some transmitter responses which occur during the course of postnatal ontogenesis in rat sensorimotor cortex.

Ontogenesis of Some Membrane Properties

Although layer V pyramidal type neurons in the immature (postnatal day 1 to 10; P1–10) neocortical slice are similar to their adult counterparts

(>P21) in terms of the qualitative aspects of underlying voltage-dependent conductances, there appears to be a quantitative change in the density of at least some of the associated ion channels during development. In current clamp recordings, neurons of slices from newborn rats tend to have longer duration and smaller amplitude action potentials, much higher input resistances, longer time constants, and more linear relationships between applied current and voltage than do mature cells. At the earliest time of recording (P1), these cells can generate Ca^{++} spikes only when portions of the K^+ conductances are blocked, as is the case in adult cells (Connors et al. 1982). However, the rate of rise of Ca^{++} action potentials increases over time. The rate of both the rise and fall of Na^+-mediated spikes is slower in young animals. These findings indicate that changes in the action potential conductances occur during development.

To determine the underlying basis for some of these observations, we have conducted studies of membrane currents in acutely dissociated rat neocortical neurons (Hamill et al. 1986; Huguenard et al. 1986). The development of active conductance mechanisms (i.e., insertion of ion channels into membranes) occurs at different rates for different ion channels and in different neuronal classes. Na^+ current density in both interneurons and pyramidal cells exhibits a tenfold increase during the first 2–3 weeks postnatal but as noted above, the final density in pyramidal neurons is about 20% larger than in nonpyramidal neurons (Fig. 4A). Delayed rectifier current also undergoes an approximately tenfold increase from P5 to P21 (Fig. 4B). In this case, nonpyramidal neurons end up with a higher density than pyramidal neurons. In both types of neurons these changes in Na^+ and K^+ channel density should contribute to the decrease in spike duration during ontogenesis. There is a striking delay of about five days after birth before channel density increases (not seen in Fig. 4A because of absence of data points between P0 and P5, but see Fig. 4B). A similar time course of changes in spike dV/dt is seen in current clamp recordings of neurons in slices (McCormick and Prince 1987b). Other changes, e.g., those in membrane time constant and input resistance, have a different developmental time course, suggesting that they are regulated by separate mechanisms.

The functional significance of these changes in membrane excitability is unclear. Variations in action potential parameters could certainly affect transmitter release (see below) and the responsiveness of cortex to afferent drives. Subtle differences in input-output relationships exist between mature and immature neurons, such as more rapid spike frequency adaptation and the capacity to fire at lower frequencies in the former (McCormick and Prince 1987b). Such functional differences (which would be a consequence of developmental changes in membrane properties) may have important influences on circuit behavior.

Ontogenesis of Synaptic Potentials

Postsynaptic potentials (PSPs) recorded in immature neurons are longer in duration and more fragile during repetitive activation than in their mature couterparts (Fig. 8). Stimuli may evoke stable PSPs only at very low frequencies (<0.1 Hz) in animals less than P7. When higher frequencies such as 0.5 Hz–1 Hz are used, there is a progressive fall- off in the PSP peak amplitude until, in some instances, very little PSP is obvious after 5 or 6 stimuli. The high input resistance and long time constant of immature neurons would tend to make responses to synaptic currents larger and might in some ways serve to compensate for some of the vagaries of synaptic transmission. For example, small amplitude distal EPSPs would be more likely to evoke spikes in such cells.

A second remarkable feature of synaptic activation is the absence of any evoked IPSPs, either depolarizing or hyperpolarizing, in animals less than about P7. These findings are consistent with those previously reported in neonatal kitten cortex (Oka et al. 1985) and developing rabbit hippocampus (Schwartzkroin 1982), and with biochemical (Coyle and Enna 1976) and anatomical (Blue and Parnavelas 1983; Miller and Peters 1981; Wolff et al. 1984) evidence suggesting that the GABAergic inhibitory system in cortex is not mature during the early postnatal period. Another indication of the immaturity of cortical inhibitory circuitry comes from recent data on the actions of ACh on cortical pyramidal neurons during development (McCormick and Prince, in progress). The indirect inhibitory action of ACh on these cells, mediated via GABAergic interneurons in mature animals, is absent at P3 (Fig. 9A), although the direct slow depolarization is present.

Fig. 8—Long duration PSPs evoked by a subcortical stimulus (dot) in a neuron from a P8 neocortical slice. Stimuli with constant parameters delivered at 0.25 Hz evoke PSPs of progressively smaller amplitudes.

Biphasic responses are seen in neurons from slices of older animals (e.g., P16 of Fig. 9A). Although inhibition cannot be evoked on pyramidal neurons through existing circuitry or indirectly by cholinergic excitation of GABAergic cells at ages <P7–10, direct applications of GABA do activate inhibitory conductances on postsynaptic neurons even in very young animals (Fig. 9B), suggesting that receptors are present but not activated during stimuli. The sequence of development of various subclasses of receptors mediating extracortical influences, and those related to intrinsic cortical connectivity, will have an important influence on the functional capacity of the immature cortical networks.

The implications of this slow development of inhibitory electrogenesis are significant in terms of cortical function. For example, the diffuse responses to light and slow development of more specific visual receptive fields in

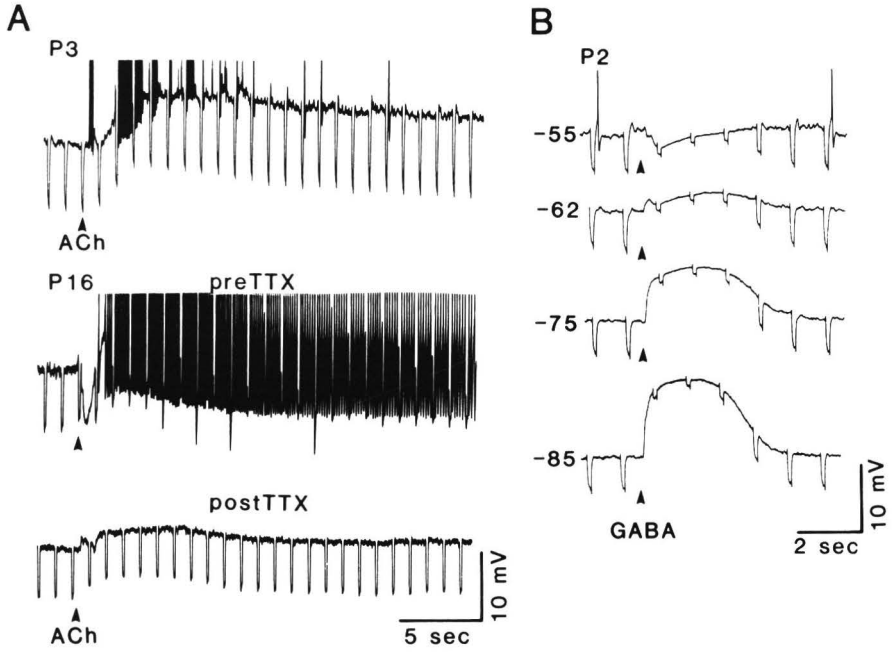

Fig. 9—Responses of neurons of immature neocortical slices to ACh and GABA. **A:** Top: Focally applied ACh evokes only a slow depolarizing response in P3 neuron, whereas at P16 an initial hyperpolarization precedes the later depolarization (middle line). Hyperpolarizing response in same P16 neuron is blocked by TTX (bottom line). **B:** Responses of P2 neuron to focal applications of GABA at membrane potentials indicated on the left of each line. GABA evokes a hyperpolarizing response with a large conductance increase at −55 mV which inverts at more hyperpolarized membrane potentials. From McCormick and Prince, unpublished.

immature cortex might be partially due to immaturity of cortical GABAergic inhibitory interneurons (Grobstein et al. 1973). Similar effects occur in mature cortex treated with anti-GABA agents (Sillito 1984). The mechanisms underlying falloff of repetitively elicited synaptic events are unknown but might relate to the paucity of synaptic vesicles in immature presynaptic terminals (Blue and Parnavelas 1983), to unusual biophysical properties of terminal membranes, such as a reduced complement of Na^+–Ca^{++} channels as has been demonstrated in pyramidal somata (Hamill et al. 1986), or to postsynaptic mechanisms such as altered transmitter reuptake and receptor desensitization.

CONCLUSION

Although data with respect to the properties of various types of cortical neurons are very incomplete, they do suggest that the major differences among cell types found in other parts of the mammalian brain are also characteristic of structurally different groups of cortical neurons. This is certainly true for interneurons versus pyramidal-type cells; and at least two subgroups of the latter, bursting and nonbursting, are apparent. When possible variations in receptor subtypes for neurotransmitters and their coupling to different ionophores are taken into account, together with the modulatory actions of agents on membrane currents that shape neuronal input-output relationships, a great potential variability in the function of even a "hard-wired" cortical circuit becomes possible. Progress in understanding cortical function will depend on more detailed information about cortical circuitry, the electrophysiological properties of particular types of neurons, and the influence of ascending modulatory systems on these cells. Studies of ontogenesis may lead to a better understanding of the control mechanisms underlying regulation of membrane excitability.

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