Cellular Bases of Neocortical Activation: Modulation of Neural Oscillations by the Nucleus Basalis and Endogenous Acetylcholine

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In the mammalian neocortex, the EEG reflects the state of behavioral arousal. The EEG undergoes a transformation, known as activation, during the transition from sleep to waking. Abundant evidence indicates the involvement of the neurotransmitter acetylcholine (ACh) in EEG activation; however, the cellular basis of this involvement remains unclear. We have used electrophysiological techniques with *in vivo* and *in vitro* preparations to demonstrate actions of endogenous ACh on neurons in auditory neocortex. *In vivo* stimulation of the nucleus basalis (NB), a primary source of neocortical ACh, (1) elicited EEG activation via cortical muscarinic receptors, (2) depolarized cortical neurons, and (3) produced a change in subthreshold membrane potential fluctuations from large-amplitude, slow (1–5 Hz) oscillations to low-amplitude, fast (20–40 Hz) oscillations. The NB-mediated change in pattern of membrane potential fluctuations resulted in a shift of spike discharge pattern from phasic to tonic. Stimulation of afferents in the *in vitro* neocortex elicited cholinergic actions on putative layer 5 pyramidal neurons. Acting via muscarinic receptors, endogenous ACh (1) reduced slow, rhythmic burst discharge and facilitated higher-frequency, single-spike discharge in burst-generating neurons, and (2) facilitated the appearance and magnitude of intrinsic membrane potential oscillations. These *in vivo* and *in vitro* observations suggest that neocortical activation results from muscarinic modulation of intrinsic neural oscillations and firing modes. Rhythmic-bursting pyramidal neurons in layer 5 may act as cortical pacemakers; if so, then modifying their discharge characteristics could alter local cortical networks. Larger, intercortical networks could also be modified, due to the widespread projections of NB neurons. Thus, NB cholinergic neurons may play a critical role in producing different states of neocortical function.

Early electrophysiological recordings of neocortical activity revealed the EEG activation (desynchronization) that signifies behavioral arousal (Jasper and Carmichael, 1935). Since then, attempts to identify brain mechanisms of arousal have focused on brainstem neurons and the cholinergic system, since either behavioral arousal or stimulation of the midbrain reticular formation results in cortical release of ACh and EEG activation

(Moruzzi and Magoun, 1949; Kanai and Szerb, 1965; Jasper and Tessier, 1971). Recent studies have identified the nucleus basalis (NB) of Meynert of the basal forebrain as the primary source of cortical ACh (Johnston et al., 1981; Rye et al., 1984). Given that brainstem cholinergic neurons do not, in general, project to neocortex, but that ascending projections from the reticular formation do terminate in the NB region (Wainer and Mesulam, 1990), the NB may form an important link in ACh-mediated cortical activation (Shute and Lewis, 1967; Detari and Vanderwolf, 1987; Steriade and Buzsaki, 1990).

Several lines of evidence indicate a relationship between NB activity, ACh, and arousal. The discharge rates of NB neurons vary with the degree of EEG activation, greater NB activity being associated with an activated cortex (Detari and Vanderwolf, 1987; Buzsaki et al., 1988). Also, excitotoxic NB lesions or systemic administration of muscarinic cholinergic receptor antagonists renders the cortical EEG resistant to desynchronization in awake animals (Stewart et al., 1984; Buzsaki et al., 1988). Finally, stimulation of the NB results in cortical ACh release and EEG activation (Belardetti et al., 1977; Casamenti et al., 1986; Metherate and Ashe, 1991). These findings indicate a role for NB cholinergic neurons in cortical activation.

NB cholinergic activity is also implicated in higher cognitive functions. In humans, Alzheimer's disease is associated with degeneration of NB neurons and corresponding losses of cortical cholinergic markers, which parallel the degree of cognitive dysfunction (Davies and Maloney, 1976; Perry et al., 1978; Whitehouse et al., 1982; Sims et al., 1983). Similarly, NB and cortical ACh deficits in experimental animals impair performance in specific behavioral tasks (Hagan and Morris, 1988). Electrophysiological studies indicate that the discharge rates of NB neurons increase transiently during the acquisition of learned behaviors (Rigdon and Pirch, 1986; Richardson and DeLong, 1990) and are closely tied to the expectation of reward (Richardson and DeLong, 1990; Wilson and Rolls, 1990).

While much correlative evidence points to a critical role for NB cholinergic neurons in arousal and cognition, the cellular bases of such actions at the cortex are unclear. As an initial step in understanding these processes, we have used *in vivo* and *in vitro* preparations of neocortex to determine the cellular consequences of cholinergic involvement in neocortical activation.

Materials and Methods

In vivo experiments. Male Sprague–Dawley rats (250–350 gm) were anesthetized with urethane (ethyl carbamate, 1.5 gm/kg, i.p.), supplemented as needed (0.2 gm/kg) so that noxious stimulation (e.g., tail pinch) did not elicit either reflex movement or changes in EEG activity. A catheter inserted into the external jugular vein allowed for subsequent drug administration. The rat was placed in a stereotaxic apparatus, on

a heating pad maintained at 37°C. A glass microelectrode (3 M NaCl) inserted into auditory cortex was used to record extracellular activity (bandpass DC–10 kHz), which was filtered off line for preparation of illustrations (bandpass for EEG, 1–100 Hz; unit activity, 0.1–10 kHz, positive polarity upward). Analysis of the frequency distribution of power (power spectrum analysis) in the EEG over the 1–50 Hz range was performed, using computer software (AXOGRAPH, Axon Instruments), by digitally sampling the signal off line at 200 Hz. For simultaneous extracellular and intracellular recordings, an insulated stainless steel electrode (50 μm diameter) was used to record the EEG at a 1.0 mm depth, while a microelectrode (4 M K+-acetate, 40–80 MΩ) was used for intracellular recordings, with current injection by way of an active bridge circuit. Following placement of electrodes, the cortex was covered with warmed agar (4% in saline). Additional procedures to minimize pulsations for intracellular recordings included tracheal cannulation and lumbar suspension.

Bipolar NB stimulation (tip separation, about 0.5 mm, generally in the mediolateral plane) consisted of 0.1-msec-duration stimulus pulses, in trains of 20–100 pulses, 100–200/sec, 100–500 μA. After the optimally effective NB stimulation sites had been determined (see Results), the stereotaxic coordinates that were routinely used for placement of the stimulating electrodes were (in mm, relative to bregma) AP −1.4, ML 2.0, and 6.4 below the pial surface (coordinates for the medial electrode; cf. Paxinos and Watson, 1986). For chemical stimulation, glutamate (30–180 nmol in saline) was infused using a syringe pump over 15 sec to 5 min via a 30 gauge cannula attached to the stimulating electrodes. Cortical administration of atropine sulfate was by iontophoresis (20–60 nA; multibarrel iontophoresis micropipette glued to the recording electrode) or direct application to the cortical surface (50–320 μM in saturated cotton gauze). Experiments were terminated by barbiturate overdose, followed by transcardial perfusion with formalin and saline for histology.

In vitro *experiments.* Methods for *in vitro* experiments have been detailed elsewhere (Cox et al., 1992). Briefly, coronal slices from rat auditory cortex were maintained in an interface chamber at 32°C in physiological solution (in mM): NaCl, 125; KCl, 2.5; MgSO₄, 1.2; CaCl, 2.5; KH₂PO₄, 1.25; NaHCO₃, 25; glucose, 10; gassed with 95% O₂ and 5% CO₂ for pH 7.4. Neurons were impaled with microelectrodes (4 M K+-acetate, 60–100 MΩ), and current clamp recordings were made using an amplifier with an active bridge circuit. Tetanic stimulation consisted of 8–40 pulses at 20/sec, 20–100 μA, delivered via bipolar electrodes to the deep gray matter, usually several hundred microns ventromedial to the recording site. This site of stimulation lay along the presumed path of NB cholinergic afferents, which enter the lateral neocortex ventrally and then turn dorsally and course in the deep layers of a given coronal plane (Kristt et al., 1985; Saper, 1987). In some cells, 6,7-dinitroquinoxaline-2,3-dione (DNQX), D-2-amino-5-phosphonovalerate (APV), and picrotoxin were used to attenuate activity resulting from amino acid transmission. Throughout the Results, all averages are ± SEM.

Results

Results from in vivo experiments

In 41 experiments on urethane-anesthetized rats, large-amplitude, 1–5 Hz fluctuations dominated the local field potential (EEG) recorded in the middle and lower layers (range, 500–1000 μm) of auditory cortex (Figs. 1–3). Tetanic stimulation of the NB region of the basal forebrain elicited EEG activation (Fig. 1A; n = 36), resulting in the appearance of higher-frequency EEG components and decreased power in the 1–5 Hz range (Fig. 1B). The desynchronization endured for approximately 1–20 sec, and more intense or longer-duration stimulation desynchronized the EEG for longer periods of time (Fig. 1A). Tetani repeated more frequently than about 0.1/sec also resulted in longer periods of desynchronization. Recordings from superficial cortical layers revealed EEG fluctuations that were similar in frequency but reduced in amplitude from those in middle and deep cortical layers. In these superficial recordings, NB stimulation generally produced weak or no observable desynchronization of the local EEG. However, continuing the electrode penetration to the middle and deep cortical layers revealed

clear NB-mediated desynchronization. These data suggest that the sources of superficial and deep field potentials under the present conditions may not be identical, and that the site of NB-mediated action lies within the deeper layers. However, it is possible that the effects of NB stimulation are only less *noticeable* superficially, if the sources of superficial field potentials are more widely distributed and/or less synchronized than those deeper within the cortex.

Atropine antagonized the NB-induced EEG desynchronization (Fig. 1A,B) when administered iontophoretically within the cortex (20–60 nA; n = 5), directly to the cortical surface (50–320 μM; n = 4), or systemically (3.2–15 mg/kg; n = 2). In each case, atropine not only blocked EEG desynchronization, but also increased the amplitude and degree of synchronization, implying a tonic action of endogenous ACh. Increasing the intensity of NB stimulation could partly overcome the atropine block. These effects of atropine suggest that NB-mediated EEG activation involves muscarinic cholinergic receptors located within the neocortex.

The effectiveness of NB stimulation in eliciting cortical activation varied with the anesthetic used. In urethane-anesthetized animals with clear NB-induced EEG activation, systemic administration of subanesthetic doses of pentobarbital (5.0–8.4 mg/kg) effectively blocked EEG desynchronization without increasing EEG synchrony (n = 3; not shown). The pentobarbital effect endured for 30–60 min.

Evidence that the tetanus-induced EEG activation primarily involved the subset of NB neurons that project to auditory cortex was obtained in a series of 20 consecutive experiments. In these experiments, the NB stimulating electrode was positioned in a single penetration, to preclude tissue damage from multiple penetrations, and to facilitate accurate determination of stimulation sites from histological sections. To facilitate reliable grouping of data across experiments, the rostrocaudal distance of each site from the anterior commissure was determined from histological material. Sites of stimulation that effectively elicited EEG activation lay in the middle and ventral aspects of the globus pallidus and substantia innominata, at an average rostrocaudal distance of 0.77 mm (range, 0.54–1.04 mm) from the anterior commissure (Fig. 2A, upper section). This region contains the magnocellular cholinergic neurons of the NB that project to auditory cortex (Wenk et al., 1980; Saper, 1984; Moriizumi and Hattori, 1992). In contrast, stimulation sites located more rostrally in the NB were less effective in eliciting EEG activation in auditory cortex (Fig. 2A, lower section). This finding is consistent with data indicating that the rostral NB projects to other cortical regions (Wenk et al., 1980). Thus, forebrain stimulation that effectively elicited EEG activation in auditory cortex likely involves the NB neurons that project to auditory cortex. The further importance of these data is twofold. First, they suggest that the topographic nature of NB projections to neocortex (McKinney et al., 1983; Rye et al., 1984) could produce localized EEG activation. Second, they indicate that axon fibers passing rostrocaudally through the NB are not responsible for changes in the cortical EEG following tetanic stimulation.

Further evidence that NB neurons, and not fibers of passage, mediated EEG activation was provided by chemical stimulation of the NB region with the excitatory amino acid glutamate. Glutamate infusion (30–180 nmol) via a cannula glued to the stimulating electrodes desynchronized the cortical EEG (Fig. 2B; n = 8). Saline infusion of equal volume produced no effect. In general, glutamate produced weaker cortical activation than did
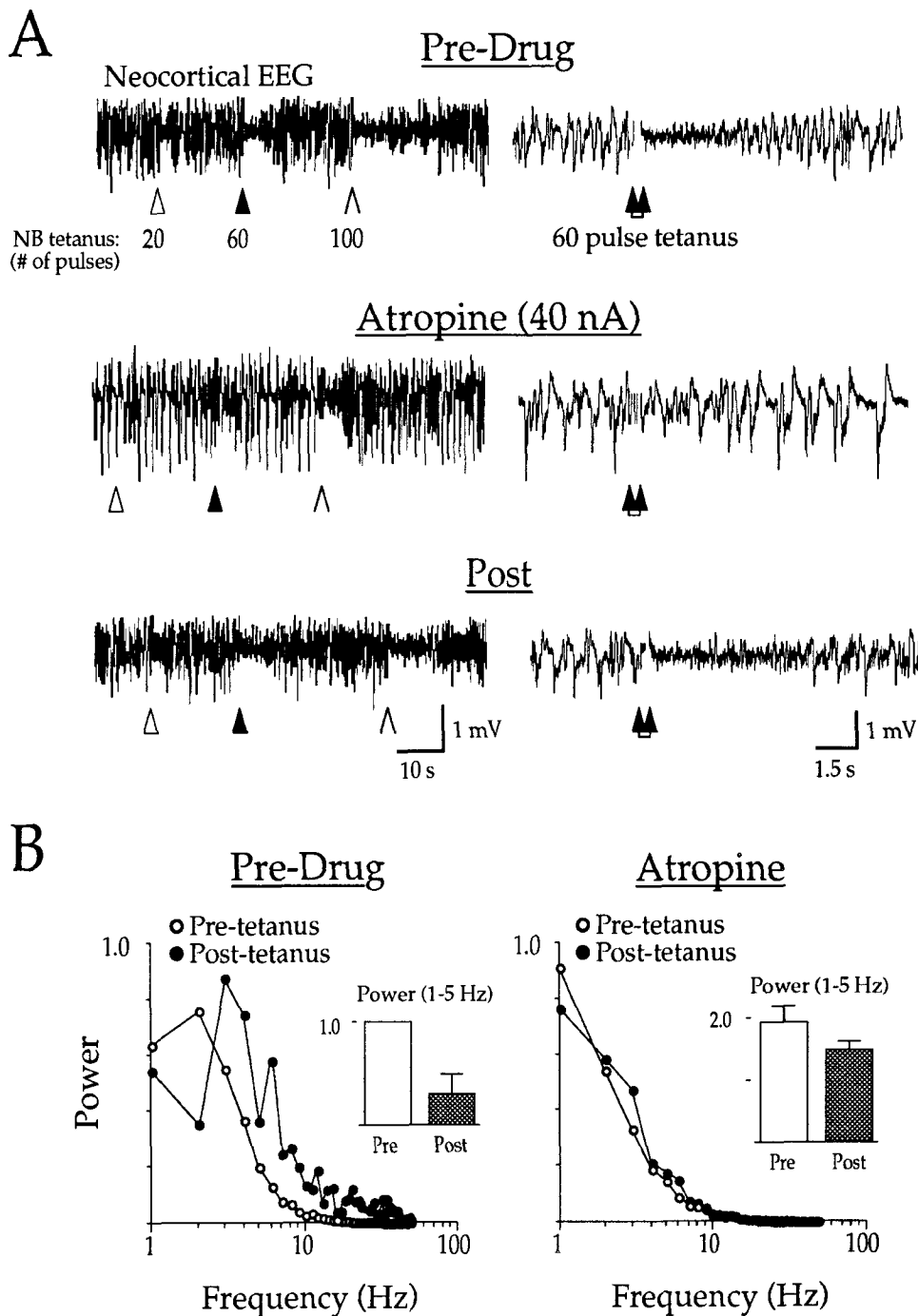
Figure 1. NB stimulation *in vivo* elicited EEG activation mediated by cortical muscarinic receptors. *A*, NB tetani (200/sec, 500 μA) of increasing number of pulses (*arrowheads*) elicited correspondingly longer durations of EEG desynchronization. Effect of 60 pulse tetanus is shown at faster speed to the *right* (duration of tetanus indicated by *arrows*; artifacts are blanked). Atropine, administered iontophoretically (40 nA) within auditory cortex, enhanced synchronous oscillations and antagonized the effect of NB stimulation. Increasing stimulus intensity (e.g., 100 pulse stimulus) partly overcame the atropine blockade. Recovery was seen at about 1 hr (*Post*). *B*, Averaged ($n = 5$) frequency power spectra show that NB stimulation resulted in increased high-frequency components (*Pre-drug* spectra; individual functions contributing to each average were normalized to the peak power under that condition, i.e., pre- or posttetanus). NB stimulation also produced decreased power in the 1–5 Hz range (histogram in *inset*; power normalized to pretetanus value for each experiment). Atropine antagonized the effect of NB stimulation on EEG frequency components and power in the 1–5 Hz range. Note that the increased EEG amplitude and synchrony under atropine (seen in *A*) resulted in increased power in the 1–5 Hz range relative to control (note change in histogram scale). Data are from five experiments in which (1) atropine was administered within the cortex (either iontophoretically or directly to the cortical surface), and (2) NB-mediated EEG desynchronization endured for longer than 2 sec (to allow for adequate digital sampling).

electrical stimulation, a finding that may reflect the difficulty in effectively stimulating cortically projecting NB neurons that are relatively few in number yet distributed over a wide area (Bigl et al., 1982; Saper, 1984). However, the data do suggest that neurons whose somas lie in the NB region mediate activation of the cortical EEG.

Simultaneous extracellular unit and EEG recordings in the cortex showed that at middle and deep (>500 μm) recording sites, single- or multiple-unit activity invariably coincided with the negative phase of EEG fluctuations, resulting in a phasic pattern of discharge (Fig. 3*A*). Tetanic NB stimulation produced a tonic pattern of unit discharge during EEG activation ($n = 16$). A return to the phasic discharge pattern occurred as the EEG resynchronized. Thus, NB stimulation elicited EEG activation, increased single-unit discharge, and a shift from a phasic to a tonic discharge pattern.

Intracellular recordings *in vivo* revealed that prior to NB stimulation, abrupt, large-amplitude membrane depolarizations appeared to coincide with EEG negativities (Fig. 3B_1; shown at higher resolution in the lower part of the figure; $n = 17$). NB stimulation produced membrane depolarization of 5.2 ± 1.9 mV (range, 1–10 mV) (Fig. 3B_2) that lasted for the duration of EEG activation. Small-amplitude, fast (approximately 20–40 Hz) fluctuations replaced the abrupt, large-amplitude depolarizations, and were superimposed on the maintained depolarization. After several (1–20) seconds of maintained depolariza-
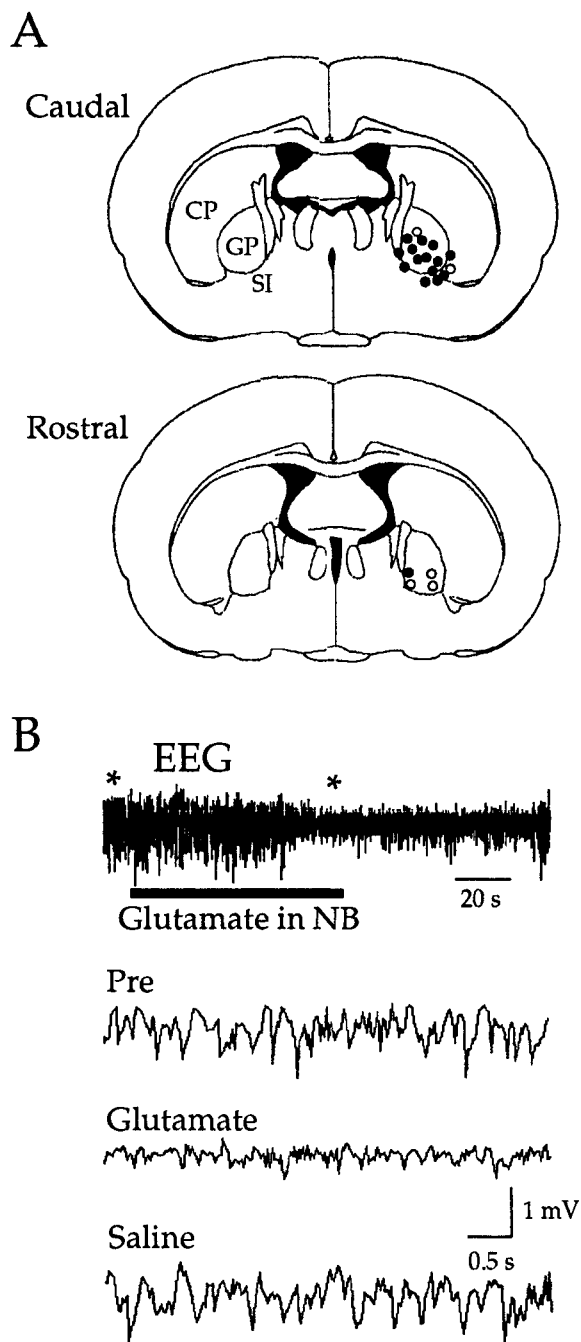
A

B

Figure 2. *A*, Effective (*solid circles*) and ineffective (*open circles*) stimulation sites for eliciting EEG activation in 20 consecutive experiments. Caudal stimulation sites (mean rostrocaudal distance from the anterior commissure, 0.77 mm; range, 0.54–1.04 mm) were more effective (87.5 %; *n* = 14 of 16), whereas rostral stimulation sites (mean distance, 0.28 mm; range, 0.08–0.46 mm) were less effective (25%; *n* = 1 of 4). Effective stimulation sites lay in middle and ventral aspects of the caudal globus pallidus (GP) and substantia innominata (SI), source of the NB cholinergic projection to auditory cortex (Wenk et al., 1980; Saper, 1984). Atlas sections modified from Paxinos and Watson (1986). CP, caudate putamen. *B*, Glutamate (180 nmol in saline), delivered via a 30 gauge cannula attached to the stimulating electrode, desynchronized the EEG for >1 min (recovery can be seen at *end of trace*). Portions of the EEG before and during glutamate-induced desynchronization are marked by *asterisks* and shown below at higher resolution. Subsequent saline infusion of equal volume was without effect.

tion, the membrane potential returned to its original level, and the EEG and intracellular fluctuations simultaneously reverted to their original patterns. Subsequently, steady current injection through the intracellular recording electrode showed that membrane depolarization, per se, did not alter the pattern or frequency of membrane potential fluctuations, but only increased the probability of cell discharge, which occurred in a phasic pattern (Fig. 3B_3). Tetanic NB stimulation from this depolarized level did reveal the excitatory nature of NB actions, which produced increased, tonic discharge (Fig. 3B_4).

Neurons that did not discharge spontaneously at rest, or discharged infrequently, allowed the modification of subthreshold membrane potential fluctuations to be clearly observed (Fig. 3B; *n* = 8). These neurons had resting membrane potentials of −68 ± 2.7 mV and spike amplitudes (measured from firing threshold) of 68 ± 1.7 mV. In spontaneously firing neurons (resting membrane potential, −40 to −60 mV; *n* = 9), NB stimulation appeared to modify membrane potential activity similarly, resulting in depolarization and increased firing frequency. Note that in neurons that discharged either spontaneously or during steady current injection, the changes in firing patterns before (Fig. 3B_3) and after (Fig. 3B_4) NB stimulation resembled the phasic to tonic changes in extracellularly recorded firing patterns (Fig. 3*A*).

Taken together, the data obtained *in vivo* indicate that NB-induced EEG activation appears to involve a process mediated by cortical muscarinic receptors that results in modification of membrane potential fluctuations in cortical neurons. The modification of membrane potential fluctuations alters the pattern of spike discharge, from phasic to tonic firing. This process may be neuromodulatory, for while it is usually accompanied by membrane depolarization, it appears to act independently of depolarization, and results in relatively enduring changes in neuronal activity. To study more closely the modification of neocortical neurons by endogenous ACh, we turned to the *in vitro* neocortical slice preparation.

Results from in vitro experiments

Evidence for neocortical actions of endogenous ACh was obtained from 50 neurons in the auditory cortical slice. These neurons had a mean resting potential of −75 ± 1.2 mV, spike amplitude of 72 ± 1.3 mV (measured from firing threshold of −58 ± 1.5 mV), and apparent input resistance of 37 ± 3.0 MΩ. Neurons never discharged spontaneously, nor did they display prominent membrane potential fluctuations *at the resting membrane potential*. Based on electrophysiological criteria (Connors et al., 1982; McCormick et al., 1985; Chagnac-Amitai et al., 1990; Mason and Larkman, 1990) and location in the slice (range, 600–1300 μm from the pial surface), these neurons were likely to be pyramidal neurons from layers 5 and 6.

At the resting membrane potential, tetanic stimulation of the deep cortical gray matter elicited rapid membrane depolarization and spiking followed by an afterhyperpolarization and, occasionally, a small-amplitude slow depolarization. When tetanic stimulation was presented during steady membrane depolarization to near threshold by intracellular current injection, the slow membrane depolarization was more readily apparent (cf. Fig. 5B; peak amplitude, 2.9 ± 0.4 mV, range 1–9 mV; duration, 174 ± 24 sec, range 35 sec to 5 min; *n* = 17). In some cells, a clear depolarization only occurred in the presence of the anticholinesterase eserine (1.0 μM; *n* = 2), whereas in other cells eserine increased the magnitude of the slow depolarization (to
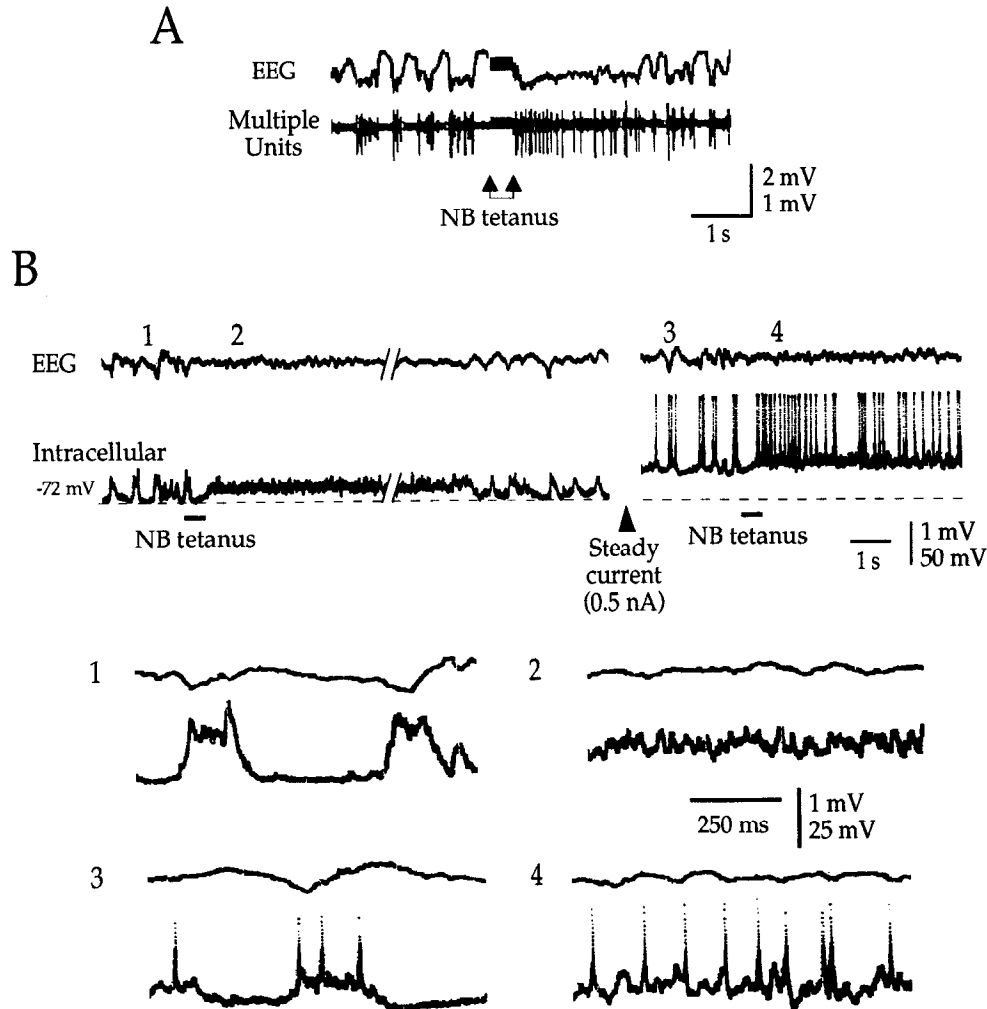
Figure 3. NB stimulation *in vivo* simultaneously activated the cortical EEG and altered the frequency and pattern of spike discharge and subthreshold neural activity. *A*, Extracellularly recorded unit activity (depth, 987 μm from the pia) coincided with the negative phase of EEG fluctuations, resulting in a phasic discharge pattern (prior to the tetanus). NB tetanus (artifact blanked) elicited EEG activation and tonic unit discharge. Note negative shift of EEG during desynchronization (EEG is DC-coupled in this trace only). Unit and EEG activity reverted to their original patterns within seconds. *B*, Simultaneous EEG and intracellular recording (depth, 571 μm) prior to tetanus revealed abrupt membrane depolarizations from the resting membrane potential (−72 mV) that coincided with negative EEG fluctuations (*top left panel*; EEG on *top*, transmembrane potential on *bottom*; shown below in *1* at higher resolution). NB tetanus elicited EEG activation, a 10 mV depolarization, and fast, small-amplitude membrane potential fluctuations (*2*). EEG and intracellular activity recovered to original patterns of fluctuations within about 10 sec (*gap in record* indicates a 4 sec interval). Subsequent steady injection of intracellular current (0.5 nA; *top right panel*) produced membrane depolarization of about 10 mV *greater* than that produced by the NB tetanus; however, this did not alter the pattern or frequency of oscillations (*3*). Steady current injection did result in spontaneous membrane depolarizations eliciting spikes in a phasic discharge pattern, and revealed the excitatory nature of NB actions; that is, the NB-mediated increased frequency of membrane fluctuations gave rise to increased spike discharge in a tonic firing pattern (*4*). Epochs of EEG and intracellular activity (labeled *1–4*) are shown below with greater resolution; spikes are truncated in *3* and *4*.

241 ± 66% of control; *n* = 3), suggesting the involvement of endogenous ACh. The muscarinic receptor antagonists atropine (1.0 μM; *n* = 3) or pirenzepine (0.1–1.0 μM; *n* = 8) attenuated the slow depolarization (by 85 ± 6%), indicating cholinergic action via muscarinic receptors. The voltage dependence and pharmacological characteristics of the tetanus-induced slow depolarization resembled the characteristics of depolarizations induced by muscarinic agonists acting on cortical neurons (Cole and Nicoll, 1984b; McCormick and Prince, 1986), as well as the characteristics of muscarinic synaptic activity in diverse regions of the CNS and PNS (Ashe and Yarosh, 1984; Cole and Nicoll, 1984a; Misgeld, 1989; Benardo, 1991). Together, these data indicate the presence in auditory neocortex of a slow EPSP mediated by ACh acting at muscarinic receptors.

Two actions of the tetanus-induced slow depolarization are particularly relevant to the present discussion on control of neocortical neural oscillations. First, the slow depolarization modified the characteristics of neurons that generated either single or repetitive burst discharges. At membrane potentials close to rest (about −75 mV), such neurons responded to depolarizing current pulses either with a *single burst* of action potentials (two or three spikes riding on a depolarizing envelope), or with *rhythmic, repetitive bursts* at 4.7 ± 0.9 Hz (Fig. *4A*; *n* = 26) (see also McCormick et al., 1985; Agmon and Connors, 1989; Chagnac-Amitai et al., 1990; Silva et al., 1991). During steady membrane depolarization by intracellular current injection, current pulses elicited only single-spike discharge (i.e., no bursts), which occurred at a higher frequency (10–20 Hz)
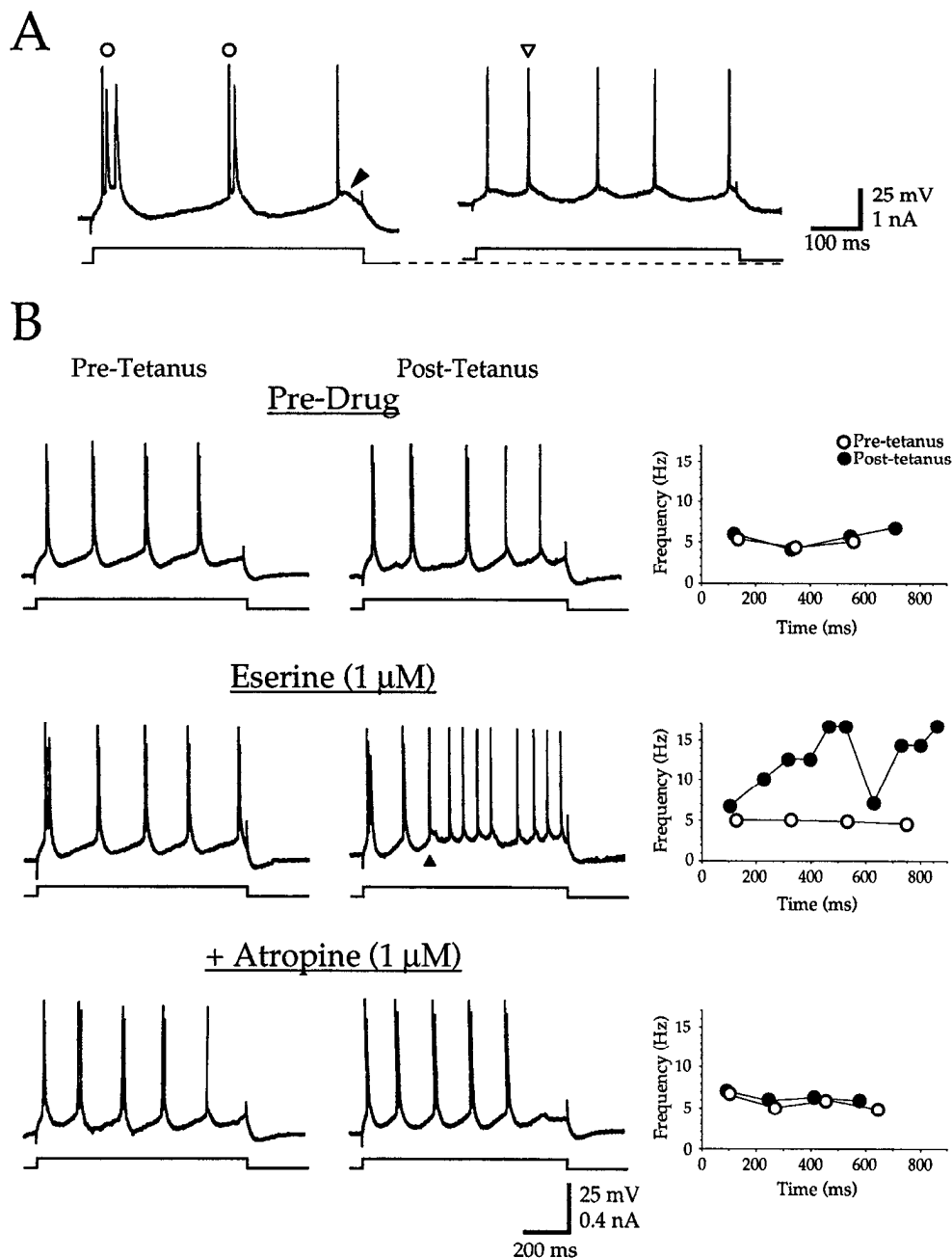
Figure 4. Muscarinic action *in vitro* resulted in switch from low-frequency bursting to higher-frequency single-spike discharge in rhythmic-bursting neurons. *A*, Depolarizing current pulse from rest (−77 mV) elicited rhythmic, repetitive bursts of two or three spikes (*open circles*), with an afterdepolarization (*solid arrowhead*) following a solitary spike. During steady depolarization, a current pulse elicited only single spikes (*open triangle*) without prominent afterdepolarizations. *B*, In the same cell, a longer (900 msec) current pulse elicited rhythmic, repetitive bursting at about 5 Hz. Tetanic stimulation (40 pulses, 20/sec, 100 μA) appeared to produce a reduced tendency to burst since the current pulse elicited two single spikes and one fewer burst. The discharge frequency of the neuron is quantified in the *graph to the right of the traces*, which indicates firing frequency throughout the 900 msec current pulse determined from each interburst (for bursts) or interspike (for single spikes) interval. In the predrug condition, the tetanus did not appreciably change the frequency of discharge, which remained at about 5 Hz despite the appearance of single spike activity. Eserine clearly enhanced the effect of the tetanus, resulting in the current pulse eliciting many single spikes and fewer bursts. Note the abrupt shift from bursting to single-spike firing modes (*arrowhead*), and the relative absence of both afterdepolarizations and large afterhyperpolarizations during single spike firing. As indicated by the graph, the tetanus changed the discharge frequency from about 5 Hz to up to 17 Hz during single-spike activity. Correction for the slight (2 mV) depolarization elicited by the tetanus did not change the response to current pulses (not shown). Atropine completely blocked the effects of the tetanus and eserine.

than the rhythmic bursts (Fig. 4*A*). Thus, these neurons can exhibit one of two modes of firing, either bursting or single spike, depending on the membrane potential. However, following te-tanic stimulation at membrane potentials close to rest, current pulses elicited more single spikes and a reduced tendency to

burst, despite little change (e.g., 0–2 mV) in the baseline mem-brane potential (Fig. 4*B*; *n* = 12). Eserine (1 μM; *n* = 3) clearly enhanced this effect, indicating action of endogenous ACh. Mus-carinic antagonists (1 μM atropine or 0.1–1.0 μM pirenzepine; *n* = 3) blocked the effect, indicating the involvement of muscarinic
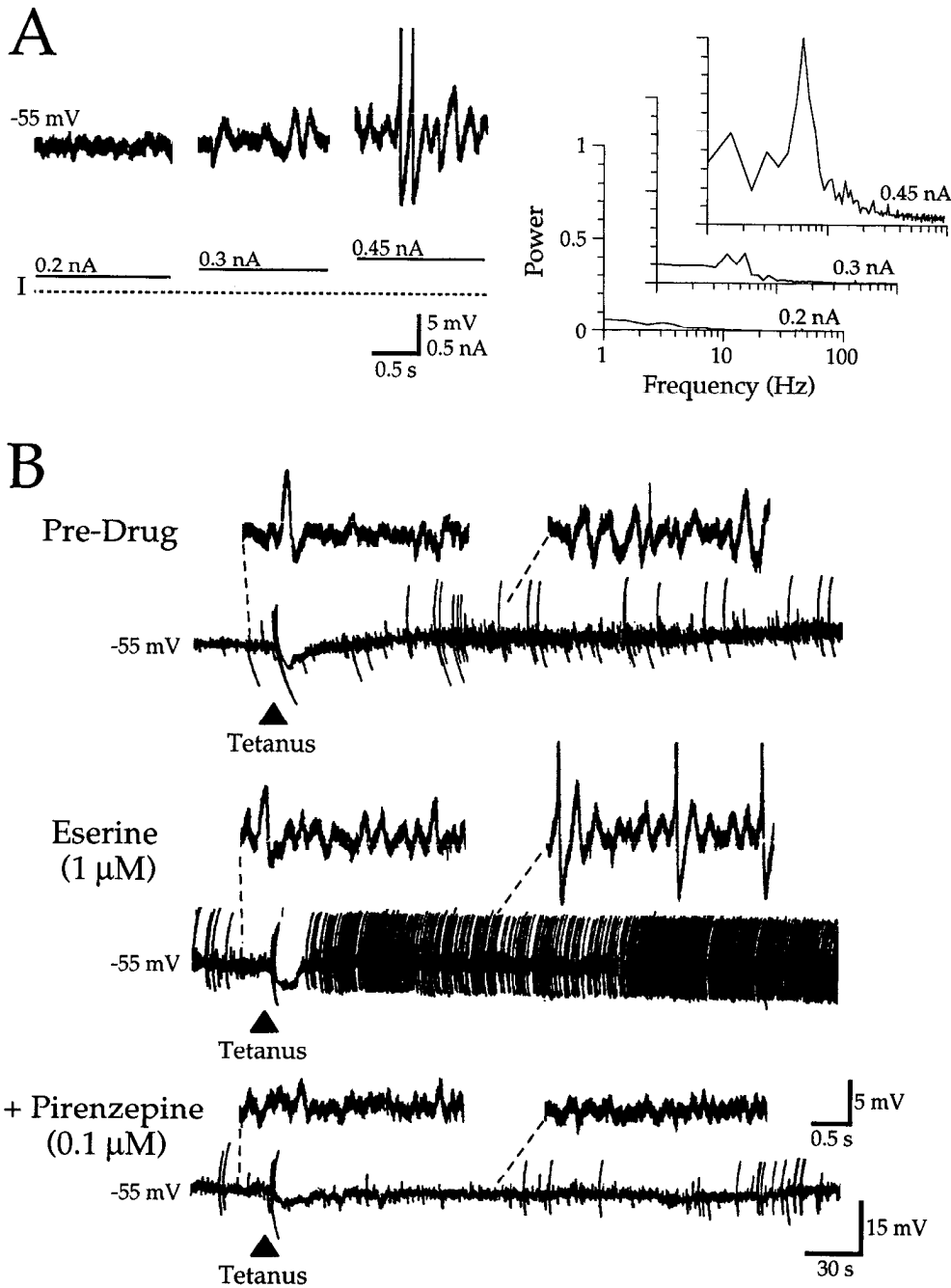
A

B

Figure 5. Muscarinic facilitation of intrinsic membrane potential oscillations *in vitro. A,* Membrane depolarization through passage of current (*I*; *broken line* indicates zero current) through the recording electrode resulted in the appearance of membrane potential oscillations that increased in frequency and amplitude, and could elicit spikes (truncated in figure), with increasing depolarization. Power spectra derived from records at each level of injected current show the emergence of oscillations that peak at about 6 Hz (spectra are derived from 15–20 sec epochs and are normalized to the peak value of the function for 0.45 nA). *B,* In the same cell, at a membrane potential where oscillations were not prominent, tetanic stimulation elicited a slow hyperpolarization followed by a small-amplitude, long-lasting depolarization and the appearance of membrane potential oscillations (shown at higher resolution in *inset*). Following recovery, bath application of eserine by itself resulted in the appearance of membrane oscillations (i.e., oscillations can be seen prior to tetanus). Subsequently, the tetanus produced sustained spiking elicited by oscillations. Pirenzepine blocked the effects of both eserine and the tetanus.

receptors. Thus, endogenous muscarinic actions can shift the mode of cell firing, from low-frequency bursting to higher-frequency, single-spike discharge.

The effectiveness with which tetanic stimulation reduced burst generation differed between rhythmically bursting and single burst neurons. Suppression of burst generation by tetanic stimulation occurred invariably in rhythmically bursting neurons (100%; *n* = 7 of 7). These neurons were located primarily in a narrow region of cortex 800–1000 μm from the pial surface. Tetanus-induced burst suppression occurred less frequently in single burst neurons (27%; *n* = 3 of 11); such neurons were not preferentially distributed in the cortex. These data suggest that the modification of rhythmic burst generation is an important feature of endogenous cholinergic actions, and one that is pref-

erentially directed at one class of neuron, the rhythmically bursting pyramidal neuron found in cortical layer 5 (Agmon and Connors, 1989; Chagnac-Amitai et al., 1990; Mason and Larkman, 1990).

A second action of the tetanus-induced slow depolarization was to modify intrinsic membrane potential oscillations. In many neurons, depolarization to near firing threshold elicited oscillations (5–10 Hz) that increased in frequency and amplitude with increasing depolarization (Fig. 5*A; n* = 42) (cf. Leung and Yim, 1991; Llinas et al., 1991; Silva et al., 1991). The oscillations persisted in the presence of agents that attenuate amino acid–mediated synaptic transmission (10 μM DNQX, 100 μM APV, 10 μM picrotoxin; *n* = 9), but were blocked by tetrodotoxin (1 μM; *n* = 2). Since fast synaptic transmission in auditory cortex

is mediated by amino acids (Cox et al., 1992), these findings support the intrinsic nature of the oscillations, and implicate voltage-dependent Na^+ currents in their generation.

Tetanic stimulation facilitated the frequency and amplitude of intrinsic oscillations, sometimes resulting in spike discharge (Fig. 5*B*; $n = 26$). The facilitation of oscillations could be partly attributed to tetanus-induced slow depolarization; however, in the presence of eserine, oscillations could appear prior to the tetanus at membrane potentials where they were not previously seen (Fig. 5*B*). This suggests that potentiation of ongoing cholinergic activity resulted in a decreased threshold for oscillations. Subsequent tetanic stimulation in eserine resulted in further facilitation of the oscillations, which sometimes led to sustained repetitive discharge ($n = 9$). Atropine or pirenzepine did not attenuate oscillations, but did reduce the effects of eserine and tetanic stimulation ($n = 9$). Thus, muscarinic synaptic actions could facilitate the appearance and magnitude of intrinsic membrane potential oscillations.

Discussion

The present results indicate that NB stimulation *in vivo* (1) elicits EEG activation via cortical muscarinic receptors, (2) depolarizes cortical neurons, and (3) produces a change in membrane potential fluctuations from large-amplitude, slow oscillations to low-amplitude, fast oscillations. The change in membrane potential oscillations results in a shift in firing pattern, from phasic to tonic discharge. In the *in vitro* neocortex, actions of endogenous ACh at muscarinic receptors (1) reduce slow, rhythmic burst discharge and facilitate higher-frequency, single-spike discharge in burst-generating neurons, and (2) facilitate the appearance and magnitude of intrinsic membrane potential oscillations. These data suggest that NB activity elicits a cortical cholinergic synaptic potential that induces EEG activation via, at least partly, muscarinic modulation of intrinsic neocortical oscillations and firing modes.

The effect of forebrain stimulation appears to involve NB neurons that project to auditory cortex. Anatomical studies that combine retrograde labeling with identification of cholinergic neurons indicate that the NB cholinergic projection to rat auditory cortex arises from neurons in the medial and ventral portions of the caudal globus pallidus, and adjacent portions of the substantia innominata and internal capsule (Bigl et al., 1982; Saper, 1984; Moriizumi and Hattori, 1992). Electrolytic lesions of this region significantly reduce cholinergic markers in auditory cortex (Wenk et al., 1980). In the present study, electrical or chemical stimulation of this NB region effectively activated the EEG in auditory cortex, using tetanus parameters or glutamate doses that elicit cortical ACh release (Kurosawa et al., 1989). In contrast, stimulation of the rostral NB was relatively ineffective in activating auditory cortex, a finding consistent with rostral NB projections to visual, but not auditory, cortex (Wenk et al., 1980). These data, plus those indicating that cortical administration of atropine blocked the activating effect of NB stimulation, implicate the NB cholinergic projection to auditory cortex in mediating EEG activation. Further, the effectiveness of caudal, but not rostral, NB stimulation suggests that NB neurons could exert local, as well as global, control over neocortical activity.

The present findings support the hypothesis that the NB forms the cortical link of a cholinergic ascending reticular activating system (Shute and Lewis, 1967; Detari and Vanderwolf, 1987; Steriade and Buzsaki, 1990). While stimulation of the brainstem

reticular formation results in cortical activation and ACh release (Moruzzi and Magoun, 1949; Kanai and Szerb, 1965; Celesia and Jasper, 1966), there are few direct cholinergic projections from the brainstem to cortex (Wainer and Mesulam, 1990). Rather, the major cholinergic projection to cortex arises from the NB, which in turn receives inputs from a wide variety of structures, including the reticular formation (Wainer and Mesulam, 1990). Thus, direct cholinergic control of cortical activation is probably mediated via the NB (Detari and Vanderwolf, 1987; Steriade and Buzsaki, 1990; present results).

While the NB likely plays an important role in cortical activation, several neural systems that ascend through the neuraxis in parallel may contribute to activation under different circumstances. Stimulation of mesopontine cholinergic nuclei in the brainstem results in cortical activation, an effect that persists even after excitotoxic lesions of the NB (Steriade et al., 1991). Since mesopontine cholinergic nuclei primarily innervate the thalamus, this suggests that thalamocortical projections, which may utilize glutamate as a neurotransmitter (Fox and Armstrong-James, 1986; Tsumoto et al., 1986; Hicks et al., 1991), contribute to processes of activation. Non-cholinergic brainstem nuclei such as the locus coeruleus and raphe nuclei that project diffusely to neocortex have also been implicated in activation (Vanderwolf, 1988; Berridge and Foote, 1991). How and when these neural systems contribute to processes of activation are unclear; however, the putative neurotransmitters released by these systems may converge at the cortex onto common cellular mechanisms. That is, norepinephrine, serotonin, and metabotropic actions of glutamate all can exert some neuromodulatory effects similar to those mediated by ACh acting at muscarinic receptors (Nicoll, 1988; McCormick, 1989; Baskys, 1992). Thus, final control over cortical excitability and rhythms may depend on the interplay of several neural systems, of which NB neurons form the primary source of *direct* ACh-mediated cortical actions.

Known muscarinic actions of exogenous or endogenous ACh on cortical neurons include voltage-dependent membrane depolarization and increased input resistance, probably due to decreased K^+ conductance (Krnjevic et al., 1971; Halliwell and Adams, 1982; Cole and Nicoll, 1984a,b; McCormick and Prince, 1986). Thus, while cholinergic actions and spontaneous membrane potential oscillations are reduced at the large resting potentials found *in vitro*, both occur and can interact upon membrane depolarization *in vitro*, or at the lesser membrane potentials found *in vivo*. In light of the voltage dependence of ACh actions, the blockade of NB-induced EEG activation by pentobarbital administration may be due to the hyperpolarizing effect of barbiturates (Nicoll and Madison, 1982), which could move the membrane potential out of the voltage range for effective ACh action.

An important finding of the present study, obtained both *in vivo* and *in vitro*, is that apparent neuromodulatory actions of endogenous ACh can modify the discharge mode of cortical neurons. Bursting and single-spike firing modes are characteristic of some neurons in the neocortex and thalamus (Connors et al., 1982; Jahnsen and Llinas, 1984; McCormick et al., 1985). In such neurons, membrane depolarization results in the transition from bursting to single-spike firing modes. The present study indicates that similar changes can also be produced by ACh, independently of changes in the resting membrane potential, but perhaps dependent on the presence of other depolarizing inputs that move the membrane potential within the range of

voltage-dependent cholinergic actions. These data provide evidence for neuromodulatory actions of ACh acting via muscarinic cholinergic receptors. It is probable that such changes are integral to the functional relevance of cortical activation. For example, the transition from sleep to waking is associated with a change in firing pattern of cortical neurons from bursting to single spike, and a concurrent increase in responsiveness to afferent stimuli (Livingstone and Hubel, 1981). Notably, NB actions that elicit neocortical activation also facilitate cortical responsiveness to thalamic inputs (Metherate and Ashe, 1991).

Since low-frequency EEG activity under urethane anesthesia shares physiological and pharmacological characteristics with slow delta waves that occur during sleep (Fox and Armstrong-James, 1986; Armstrong-James and Fox, 1988), data from the present study can be used to hypothesize a cellular basis and functional significance of EEG activation. For example, during slow waves, cortical neurons may discharge to excitatory inputs only during depolarizing phases of membrane potential fluctuations, and therefore display poor responsiveness to afferent stimuli (Steriade and McCarley, 1990). Muscarinic neuromodulation, generated by NB activity, could reduce slow oscillations and burst generation, and facilitate higher-frequency (~ 10 Hz) intrinsic oscillations and single-spike discharge. These actions would make neurons more responsive to afferent stimuli, and contribute to higher-frequency EEG oscillations (e.g., in the alpha, or 8–12 Hz, range) of waking states.

Of particular interest is the consistent modification by endogenous ACh of rhythmic-bursting neurons. Correlative anatomical and physiological studies indicate that such neurons are invariably pyramidal neurons located in cortical layer 5 (Agmon and Connors, 1989; Chagnac-Amitai et al., 1990; Mason and Larkman, 1990). Of the cortical laminae, this region appears to have an intrinsic capacity to generate rhythmic field potential oscillations (Connors, 1984; Steriade et al., 1990; Silva et al., 1991), a finding that has led to a hypothesized pacemaker function for layer 5 bursting neurons (Connors, 1984; Silva et al., 1991). These neurons also have axonal arborizations that largely remain within layer 5 (Chagnac-Amitai et al., 1990), and may therefore mediate synchronization of intralaminar neural activity. Notably, when intracortical inhibitory mechanisms are slightly depressed *in vitro*, these neurons are excited in phase with rhythmic field potential oscillations, whereas other putative pyramidal neurons are generally inhibited, suggesting that layer 5 bursting neurons may mediate synchronized bursts (Chagnac-Amitai and Connors, 1989). Since rhythmic-bursting neurons discharge in bursts at a frequency of about 5 Hz, within the range of slow EEG waves, and discharge in the single-spike mode at higher frequencies (10–20 Hz) corresponding to those of the activated EEG, neocortical rhythms may reflect, at least in part, the firing mode of burst neurons. Release of ACh from the terminals of NB neurons could shift the firing mode from bursting to single spike, reducing synchronous activity in layer 5. Thus, cortical activation could reflect cholinergic modification of an intrinsic cortical pacemaker.

Finally, this study reveals several clues as to the nature of the cholinergic involvement in higher cognitive functions. It is well established that a significant NB and cholinergic deficit parallels the cognitive dysfunction in Alzheimer's disease (Perry et al., 1978; Whitehouse et al., 1982; Sims et al., 1983). Since the NB likely mediates cortical activation, loss of NB neurons could underlie the increased delta (1–5 Hz) power and decreased alpha (8–12 Hz) power observed in the EEG of awake Alzheimer's

patients (Mody et al., 1991). However, recent findings have placed greater emphasis for cognitive function on cortical oscillations near the 20–40 Hz frequency range. These higher-frequency cortical oscillations occur in attentive humans and animals, and during sensory stimulation can be transiently synchronized *among* cortical regions (Freeman and Skarda, 1985; Eckhorn et al., 1988; Gray and Singer, 1989; Engel et al., 1991; Pantev et al., 1991; Ribary et al., 1991). These fast oscillations are hypothesized to contribute to the perception of sensory stimuli by functionally "binding" cortical regions that subserve complementary sensory functions (Eckhorn et al., 1988; Crick and Koch, 1990; Engel et al., 1991). Notably, it appears that cortical 40 Hz oscillations are also reduced in Alzheimer's disease (Ribary et al., 1991).

In light of the possible significance of fast cortical oscillations, the present demonstration of NB-elicited 20–40 Hz membrane potential fluctuations is of particular interest. The NB appears to be optimally situated to evaluate sensory stimuli for significance, and to regulate cortical responsiveness accordingly. While NB neurons do not normally respond to sensory stimuli (Lamour et al., 1986), they may do so when the stimuli are behaviorally meaningful (Rigdon and Pirch, 1986; Richardson and DeLong, 1990). Activation of the NB by *significant* stimuli could therefore result in high-frequency oscillations that would optimize cortical responsiveness. Due to its widespread projections, the NB could coordinate cortical oscillations among diverse cortical regions, binding these regions into larger, functionally significant networks (Freeman and Skarda, 1985; Eckhorn et al., 1988; Gray and Singer, 1989; Weinberger et al., 1990; Engel et al., 1991).

In summary, the findings of this study suggest cellular bases for the involvement of the NB cholinergic system in arousal as well as in higher cognitive functions. It is possible that subsequent investigations may reveal that seemingly different NB functions actually reflect a continuum of actions. In any case, understanding the cellular basis of NB function, as well as the basis of NB dysfunction, will be a priority for future research.

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