Rhythmically Discharging Basal Forebrain Units Comprise Cholinergic, GABAergic, and Putative Glutamatergic Cells

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INTRODUCTION

Via widespread cortical projections, the basal forebrain serves as the major extrahalamic relay from the brain stem activating system to the cerebral cortex and plays an important role in arousal, learning, and memory (Damasio et al. 1985; Dunnett et al. 1987; Jones 2000; Wenk 1997). Basal forebrain stimulation elicits high-frequency gamma (30–60 Hz) and theta (4–8 Hz) electroencephalographic (EEG) activity (Cape and Jones 2000; Stewart et al. 1984; Stewart and Vanderwolf 1987) and also produce deficits on multiple learning tasks (Dunnett et al. 1987). Together with gamma, theta rhythm may provide a template for coherent activity and promote synaptic plasticity across distributed cortical networks (Huerta and Lisman 1993; Larson et al. 1986). Theta is controlled by rhythmically discharging neurons in the medial septum-diagonal band of Broca (MS-DBB) projecting to the hippocampus (Bland and Colom 1993) and neurons in the magnocellular preoptic-substantia innominata area (MCPO-SI) projecting to the cortex (Cape and Jones 2000).

Long thought to fulfill the important roles of the basal forebrain (Jones 2000; Wenk 1997), cholinergic neurons, which were recently identified by juxtacellular labeling and recording, have been found to increase their rate of discharge with cortical activation (Duque et al. 2000) and moreover to discharge in high-frequency bursts in association with rhythmic theta-like activity (Cape et al. 2000; Manns et al. 2000b). Yet, the cholinergic neurons may have a more subtle role than originally believed in processes of arousal, learning, and memory (Dunnett et al. 1991; Wenk 1997). Blocking muscarinic receptors does not prevent high-frequency cortical activity elicited by basal forebrain stimulation (Metherate et al. 1992) nor eliminate all theta activity (Vanderwolf 1975). Similarly, selective cholinergic lesions decrease the amplitude of but do not eliminate all theta activity (Bassant et al. 1995; Lee et al. 1994) nor produce the learning and memory deficits (Baxter et al. 1995; Dunnett et al. 1991; Wenk 1997) that the original nonselective lesions had done, suggesting that other basal forebrain neurons contribute to these functions. The noncholinergic neurons thought to play the most important part in theta generation (Lee et al. 1994) were assumed to be GABAergic neurons (Gritti et al. 1997; Kohler et al. 1984). By juxtacellular recording and labeling, we recently discovered a group of rhythmically discharging cells that were GABAergic and could accordingly fulfill this role (Manns et al. 2000a). However, we also found other neurons that discharged rhythmically and did not appear to be either cholinergic or GABAergic.

In chemo-neuroanatomical studies, noncholinergic/non-GABAergic basal forebrain neurons have been found to rep-
resent an important contingent of cortically projecting neurons (Gritti et al. 1997) and most recently to contain phosphate-activated glutaminase (PAG) (Manns et al. 2001), the synthetic enzyme for transmitter glutamate (Bradford et al. 1978; Kaneko and Mizuno 1988). In the present study employing juxtacellular labeling with neurobiotin (Nb) and recording of neurons in association with EEG activity, rhythmically discharging basal forebrain cells were characterized then identified by their neurotransmitter enzymes to determine whether a distinct group of these neurons are noncholinergic/non-GABAergic neurons and, if so, possibly glutamatergic.

METHODS

Animals and surgery

Experiments were performed on adult urethane-anesthetized Long Evans rats (n = 93, 200–250g, Charles River, St. Constant, Quebec) in which 1 to 2 units were recorded and labeled in each. For a total of 121 Nb-labeled neurons, data were drawn from rhythmic units analyzed in studies of choline acetyltransferase-positive (ChAT+) and glutamic acid decarboxylase-positive (GAD+) cells (Manns et al. 2000a,b) and combined with new data from additional rhythmic units (n = 25) to render totals of rhythmic Nb-labeled ChAT+ cells (n = 11), GAD+ cells (n = 8), ChAT-negative/GAD-negative cells (n = 10), and PAG+ cells (n = 13) reported here. According to procedures described in detail in the previous studies, the anesthetized animals were positioned in a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA) for both the surgery and subsequent recording. For the purpose of recording local field potentials and in some cases antidromically activating basal forebrain units, bipolar electrodes were placed (with respect to bregma) in the hippocampus (AP −3.4 mm, L +2.1 mm, and V −2.0 mm), prefrontal cortex (AP +2.0 mm, L +1.0 mm, and V −2.0 mm), olfactory bulb (AP +6.4 mm, L +1.0 mm, and V −2.5 mm), entorhinal cortex (AP −7.4 mm, L 5.3 mm, and V −7.8 mm) and/or piriform cortex (AP −2.2 mm, L 5.5 mm, and V −8.5 mm). In some experiments, enzymocytochemical (EMG) activity was recorded with stainless steel wires from the intercostal muscles to monitor respiration.

Unit recording and labeling

Juxtacellular recording and labeling was done with an intracellular amplifier (IR-283; Neurodata Instruments). Unit recordings were performed with glass microelectrodes, filled with 0.5 M potassium or sodium acetate and ~5.0% neurobiotin (Vector Laboratories, Burlingame, CA). Recorded units were characterized in association with spontaneous irregular slow activity and somatosensory stimulation-induced rhythmic slow activity along with higher frequency gamma activity on the EEG as described previously (Manns et al. 2000a,b). The stimulation consisted of a continuous pinch of the tail for ~50 s or more. Antidromic activation was tested from the prefrontal cortex, olfactory bulb, piriform, and entorhinal cortex. Spike widths were measured from positive inflection to first zero-crossing using >128 averaged spikes.

After the recording and characterization of isolated neurons, they were labeled using the juxtacellular technique as described previously (Manns et al. 2000b; Pinault 1996). The animals then received an overdose of urethan and were transcardially perfused with a 4% paraformaldehyde solution or with a modified Zamboni’s solution, containing 0.3% paraformaldehyde and 75% saturated picric acid in 0.1 M sodium phosphate buffer (pH 7.0) used for immunostaining of PAG (Kaneko and Mizuno 1988; Manns et al. 2001).

Histochemistry

Coronal frozen sections were cut serially at 30 μm through the basal forebrain. In the first step, they were incubated for 2 h in Cy2-conjugated streptavidin (1:800, Jackson ImmunoResearch Laboratories, West Grove, PA) to reveal Nb. After location of an Nb-labeled cell by fluorescence microscopy (in the following text), the section containing the cell was further processed sequentially to establish if it was GAD+, ChAT, or GAD−/ChAT− in the first series or PAG+ in the second series of experiments. For this immunostaining, sections were first incubated overnight in a primary antibody for GAD (rabbit anti-GAD 67 antisemir, 1:3000, Chemicon, Temecula, CA) or PAG [rabbit anti-PAG antisemir, 1:6000, supplied by T. Kaneko (Akiyama et al. 1990)]. They were subsequently co-incubated in Cy3-conjugated donkey anti-rabbit antisemir (1:1000, Jackson ImmunoResearch Laboratories) to reveal GAD- or PAG-immunostaining together with Cy2-conjugated streptavidin to refresh the Nb staining. After examination by fluorescence microscopy, the sections were incubated overnight with a primary antibody for ChAT (rat anti-ChAT monoclonal antibody, 1:2, Boehringer-Mannheim) and subsequently co-incubated with 7-amino-4-methylcoumarin-3-acetic acid (AMCA)-conjugated donkey anti-rabbit antisemir (1:50, Jackson ImmunoResearch Laboratories) to reveal ChAT-immunostaining together with Cy2-conjugated streptavidin to refresh the Nb staining again. Data are presented for Nb+ rhythmic cells that were ChAT+, GAD+, ChAT−/GAD−, or PAG+. In the latter group, cells were also established as PAG+/ChAT− (n = 11). Sections were viewed by fluorescent microscopy using a Leitz Dialux microscope equipped with a Ploemopak-2 reflected light fluorescence illuminator with Leica filter cubes for ultraviolet (A), fluorescein (13), and rhodamine (N2.1).

Data analysis

Analysis of physiological data were performed on stationary periods of recording from prestimulated and stimulated conditions, as previously described in detail (Manns et al. 2000b). For the EEG, spectral analysis was performed to determine the dominant peak frequency. Gamma band activity was computed as the area of the spectral analysis was performed to determine the dominant peak frequency. The spike-triggered average (STA) was used to estimate the extent of cross-correlation between spike trains and EEG activity. To determine whether the actual unit-to-EEG STA was significantly different from random unit-to-EEG patterns, it was compared with an STA computed using a spike train generated from randomly shuffled interspike intervals of the original spike train. The actual unit-to-EEG STA was considered significantly different from the random unit-to-EEG STA with a probability of ≤0.05 using the Wilcoxon test. All analysis of raw data were done with Matlab (5, The MathWorks, Natick, MA).

For statistical comparison of the properties of ChAT+, GAD+, ChAT−/GAD−, and PAG+ cells, both nonparametric and parametric tests were done using Systat (9.0, SPSS, Chicago, IL). Figures were compiled using Adobe Photoshop (6.0 Adobe Systems, San Jose, CA) for photomicrographs and Origin (5.0, Microcal Software, Northampton, MA).
RESULTS

During somatosensory stimulation, ~45% of basal forebrain neurons recorded and labeled with Nb (n = 121) fired rhythmically during slow activity or theta that appeared in association with increased gamma (t = 4.50; df = 34; P < 0.001) on the cortex or hippocampus in urethan-anesthetized rats. Nb-labeled and immunostained rhythmic cells were distributed through the MCPO-SI (n = 42) and included numerous ChAT+ cells (n = 11) and somewhat fewer GAD+ cells (n = 8, Fig. 1). Overlapping in size and distribution, the rhythmically discharging ChAT+ [23.2 ± 1.2 μm] and GAD+ (24.7 ± 2.0 μm) cell groups differed in specific characteristics of their discharge (Fig. 2, A and B). As compared with the ChAT+ cells, the GAD+ cells had a more narrow spike width (t = -2.72, df = 17, P = 0.015); they discharged at a higher average rate during prestimulation (t = 4.536, df = 17, P < 0.001) and decreased, as opposed to increased, their average rate with oscillatory firing during stimulation (Table 1).

A considerable number of neurons that discharged rhythmically during cortical activation proved to be both ChAT- and GAD-negative by triple immunostaining (Fig. 1C, n = 10). They displayed certain physiological properties that differentiated them from both the ChAT+ and GAD+ rhythmically discharging cell groups (Fig. 2C; Table 1). Most prominent was their distinctive pattern of firing characterized by very regular clusters of spikes that appeared with cortical rhythmic slow activity during somatosensory stimulation (Fig. 2C). In subsequent experiments, Nb-labeled neurons with these characteristics were processed for immunostaining of PAG, the synthetic enzyme for transmitter glutamate. All such cells were found to be immuno-positive for PAG (Figs. 1D and 2D, n = 13). Similar to ChAT+ and GAD+ neurons, ChAT−/GAD−, and PAG+ cells were medium-to-large sized neurons (22.6 ± 1.6 and 25.5 ± 2.4 μm) and located in both the MCPO and SI.
### TABLE 1. Firing characteristics of ChAT+, GAD+, ChAT−/GAD−, and PAG+ rhythmically discharging basal forebrain neurons

<table>
<thead>
<tr>
<th>Cell Group</th>
<th>Rate PSH, Hza</th>
<th>Frequency ISH, Hzb</th>
<th>Oscillatory Indexc</th>
<th>Frequency ACH, Hzd</th>
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<tbody>
<tr>
<td></td>
<td>Pre-stimulation</td>
<td>Stimulation</td>
<td>Pre-stimulation</td>
<td>Stimulation</td>
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<tr>
<td>ChAT+</td>
<td>11.0 ± 0.06</td>
<td>5.73 ± 1.42</td>
<td>13.18 ± 2.28*</td>
<td>38.65 ± 13.11</td>
</tr>
<tr>
<td>GAD+</td>
<td>8.50 ± 0.02</td>
<td>16.78 ± 2.10</td>
<td>7.68 ± 2.0*</td>
<td>29.09 ± 5.88</td>
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<tr>
<td>ChAT−/GAD−</td>
<td>10.58 ± 0.03</td>
<td>6.12 ± 1.45</td>
<td>8.24 ± 1.34</td>
<td>16.54 ± 2.69</td>
</tr>
<tr>
<td>PAG+</td>
<td>13.02 ± 0.02</td>
<td>9.26 ± 1.52</td>
<td>9.52 ± 1.27</td>
<td>22.46 ± 4.24</td>
</tr>
<tr>
<td>Average ANOVAf</td>
<td>13.0 ± 0.02 (1)</td>
<td>9.02 ± 0.09</td>
<td>9.83 ± 0.9</td>
<td>26.55 ± 3.97</td>
</tr>
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</table>

Means ± SE values are presented for different physiological measures in the different cell groups. *Peristimulus histogram (PSH) measurements of average discharge rate. †Interspike interval histogram (ISH) measurements of instantaneous firing frequency. ‡Frequency of rhythmic activity during pre- and stimulation periods calculated from the autocorrelation histogram (ACH, fit with a Gabor function) including all units for stimulation and only those discharging rhythmically during pre-stimulation for that condition.  §For spike width, a one-way ANOVA between cell groups was performed. For all other measures, a two-way ANOVA with stimulation and cell group as factors was performed and followed by post hoc tests for condition per cell group and for cell group per condition, if significant interaction or group effects were found. F values along with df for the main effects and interaction along with df error are presented (with bold symbols also marking main stimulation or cell group effects) and significant differences of post-hoc tests indicated (by unbolded symbols for significant differences between pre- and stimulation conditions per group or between cell groups per condition). (Other simple comparisons between groups per condition are presented in text.) One to three symbols indicates P < 0.05, 0.01 or 0.001, respectively.

(1.1) The ChAT−/GAD− and PAG+ rhythmic cells had several properties that distinguished them from the ChAT+ and GAD+ rhythmic cells, including a spike width, which was significantly larger than that of the GAD+ cell group (Table 1; PAG+ vs. GAD+: t = 2.99, df = 18, P = 0.008). Their average discharge rates were significantly lower than that of the GAD+ neurons during the prestimulation period (Table 1; PAG+ vs. GAD+: t = 2.96, df = 19, P = 0.008). Their instantaneous firing frequencies during stimulation, average ~20 Hz, were significantly lower than that of the GAD+ neurons, average ~40 Hz (Table 1; PAG+ vs. GAD+: t = −3.39, df = 19, P = 0.003) and that of the ChAT+ neurons (average: ~75 Hz, PAG+ vs. ChAT+: t = −2.34 df = 22, P = 0.029). The ChAT−/GAD− and PAG+ units had virtually no high-frequency spiking in their discharge (with the proportion of intervals <12.5 ms or >80Hz: 0.8 ± 4.0% and 0.4 ± 3.6% of the ISIH), and were not different from GAD+ cells (with 5.3 ± 4.5%; PAG+ vs. GAD+: t = −1.74, df = 17, P = 0.097) but were distinctly different from ChAT+ cells in this regard (with 17.6 ± 3.9%; PAG+ vs. ChAT+: t = −2.65, df = 22, P = 0.015 with an overall significant difference across cell groups according to ANOVA, F = 4.38, df = 3, 38; P = 0.010).

Across the ChAT+, GAD+, ChAT−/GAD−, and corresponding PAG+ rhythmic cell groups, there was a similar and significant increase in rhythmic firing during somatosensory stimulation-induced cortical activation, as reflected in the oscillatory index (Table 1). For all cell types, the frequency of the rhythmic unit activity during cortical activation was also similar (according to the autocorrelation histogram, Table 1). That frequency (average: 1.70 ± 0.09 Hz) was significantly slower than the frequency of the hippocampal theta activity (3.07 ± 0.11 Hz; t = 13.9, df = 29, P < 0.001) but not than that of the prefrontal cortex activity (1.57 ± 0.55 Hz; t = 1.2, df = 37). The proportion of rhythmic units whose activity cross-correlated significantly with hippocampal activity was relatively low (26%), whereas the proportion whose activity cross-correlated with prefrontal cortex activity was high (74%) and significantly greater (χ2 = 16.1, df = 1.0, P < 0.001). A proportion of the cells could also be antidromically activated from the prefrontal cortex (~10%).

Relation of rhythmic discharge to EEG of different cortical areas

Cholinergic, GABAergic, and unidentified neurons in the MCPO-SI are known to project to the olfactory bulb (Zaborszky et al. 1986). The olfactory bulb cortex displays rhythm...
mic slow activity that is related to respiration and most commonly slower than hippocampal theta, although also equally as fast during particular behaviors (Komisaruk 1970; Macrides et al. 1982; Vanderwolf 1992). To examine the possibility that rhythmic basal forebrain units may discharge in association with rhythmic slow activity on the olfactory bulb, units were recorded along with activity from the bulb and from intercostal muscles, as an indicator of respiration. As illustrated for a PAG+ neuron (Fig. 3), the rhythmic discharge appeared to occur in close association with and at a similar frequency as the olfactory bulb activity during somatosensory induced cortical activation. During the stimulation, the frequency of the olfactory bulb activity increased significantly from $1.25 \pm 0.05$ to $1.46 \pm 0.07$ ($t = 2.5$, df = 18, $P = 0.024$) as did the frequency of all rhythmic unit discharge (Table 1). However, the oscillatory index of the olfactory bulb did not change with stimulation, despite rhythmic basal forebrain units may discharge in association with olfactory bulb activity (Table 1). As illustrated for a PAG+ neuron (Fig. 3), all rhythmic neurons recorded in association with olfactory bulb activity ($n = 23$) were significantly cross-correlated with its activity during stimulation, as well as with that of the intercostal muscles, which was $>90\%$ coherent with the olfactory bulb activity. As for the neuron shown in Fig. 3, a relatively high proportion of rhythmic units could also be antidromically activated from the olfactory bulb ($48\%$ of cells tested).

As it is known that basal forebrain neurons collectively and some individually project to multiple limbic and olfactory-related cortical areas (Luiten et al. 1987; Paolini and McKenzie 1997; Saper 1984), the possibility was examined that the rhythmic basal forebrain units discharged in a correlated manner with many areas including the entorhinal and piriform cortex. As shown for a PAG+ neuron (Fig. 4), the rhythmic discharge during cortical activation was often cross-correlated with rhythmic EEG activity across multiple areas, commonly including the entorhinal cortex and piriform cortex. For all rhythmic units examined, there was a significant correlation with activity of the entorhinal cortex ($8/12$) and piriform cortex ($3/3$) as well as prefrontal cortex ($28/39$), olfactory bulb ($23/23$), and, less frequently, hippocampus ($8/31$), as mentioned in the preceding text. Additionally, instances of antidromic activation of labeled rhythmic units were found from each of these cortical areas (including entorhinal cortex, $n = 2$ and piriform cortex, $n = 1$, in addition to prefrontal cortex, $n = 4$ and olfactory bulb, $n = 11$; and excluding hippocampus, which was not tested) with a couple of instances of activation from more than one cortical area. As shown for a PAG+ cell (Fig. 4), the peak frequency of the unit could correspond to the peak frequency of all cortical leads during slow irregular activity; whereas, during stimulation, it most often corresponded to the dominant peak frequencies of the olfactory bulb, piriform, and prefrontal cortex and not to those of the hippocampal and entorhinal cortex, which were higher than the others. On the other hand, the unit activity did correspond to a secondary lower peak frequency in the entorhinal cortex, accounting for the significant cross-correlation between the two in this case as in other cases for the entorhinal cortex and some cases for the hippocampus (not shown).

**DISCUSSION**

As it becomes increasingly apparent that noncholinergic, in addition to cholinergic, neurons of the basal forebrain play significant parts in modulating cortical activity and behavior, it becomes increasingly important to learn the identity and discharge properties of these neurons. Here for the first time, we identify putative glutamatergic cortically projecting basal forebrain neurons that discharge rhythmically in association with cortical activation. These putative glutamatergic, together with GABAergic and cholinergic, neurons may accordingly participate in modulating cortical activity in a rhythmic manner and thereby promoting coherent activity and plasticity within distributed cortical networks.

**Distinct properties of rhythmic cell groups**

Although indistinguishable according to their anatomical distribution or size, cholinergic, GABAergic, and glutamatergic rhythmically discharging cell groups could be distinguished from one another by their firing properties. The cholinergic cells displayed high-frequency bursts ($>80$ Hz) in vivo (Manns
et al. 2000b) as in vitro (Khatb et al. 1992). GABAergic neurons displayed lower frequency trains of spikes at \(~40\) Hz. The largest number of cells displayed prominent clusters of spikes at \(~20\) Hz in vivo that closely resembled rhythmic clusters of spikes recorded in vitro in noncholinergic neurons (Alonso et al. 1996). This cell group was neither cholinergic nor GABAergic as they contained neither ChAT nor GAD. They did contain PAG, the synthetic enzyme for transmitter glutamate (Bradford et al. 1978; Kaneko and Mizuno 1988) and can thus be considered to be putatively glutamatergic. Utilization of glutamate as a neurotransmitter would depend on vesicular glutamate transporters (VGLUTs) that are evident immunohistochemically in nerve terminals but not cell bodies (Fremeau et al. 2001; Jones, unpublished observations). Given that each cell type could be antidromically activated from cortex, we assume that the rhythmically discharging glutamatergic, together with GABAergic and cholinergic, cells are cortically projecting, like those identified in our chemo-neuroanatomical studies (Manns et al. 2001) and can accordingly modulate cortical activity.

That basalo-cortical projection neurons comprise a glutamatergic in addition to GABAergic and cholinergic contingent had appeared possible in previous studies examining the influence of these afferents on cortical neurons. The predominant role of acetylcholine (ACh) on cortical output neurons is believed to be excitatory due to slow depolarizing muscarinic actions on pyramidal cells (McCormick and Prince 1986) and diverse muscarinic and nicotinic actions on different interneurons (Elaagouby et al. 1991; Porter et al. 1999; Xiang et al. 1998). Projecting only to interneurons (Freund and Meskenaite 1992), GABAergic basal forebrain neurons have been postulated to exert a parallel disinhibitory influence on output neurons. Yet in the olfactory bulb, the cholinergic together with the GABAergic input cannot account for the large field potentials (Nickell and Shipley 1988) or short-latency excitatory postsynaptic potentials (EPSPs) in output neurons (Kunze et al. 1992) that are evoked by stimulation of the basal forebrain, thus implicating another basalo-cortical influence. The possibility revealed here that this basalo-cortical influence could be glutamatergic is substantiated by studies of co-cultured slices or cells showing that the predominant response of hippocampal cells to stimulation of basal forebrain neurons is a fast excitatory postsynaptic current mediated by an excitatory amino acid (Gahwiler and Brown 1985; Laiwand and Brown 1992).

During cortical activation, the cholinergic, GABAergic, and putative glutamatergic cell groups all displayed a slow oscillatory discharge that did not differ in frequency or degree of rhythmicity across the groups but did differ according to the instantaneous firing frequencies within the oscillatory spike trains. Cholinergic neurons discharge with high-frequency bursts that would maximize ACh release to most effectively induce and modulate rhythmic slow activity, while promoting synaptic plasticity (Huerta and Lisman 1993; Konopacki et al.

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**FIG. 4.** I: unit discharge of a rhythmic PAG+ basal forebrain neuron (00051114) recorded in parallel with multiple cortical sites: hippocampus (Hipp), entorhinal cortex (Ent), prefrontal cortex (PF), piriform cortex (Pir), and olfactory bulb (OB). II: as evident in the STAs, the unit activity is cross-correlated with all the cortical signals except the hippocampal one (*, \(P < 0.05\)). Evident in the ACHs, there is a high degree of rhythmicity during the stimulation. III: power spectra of the EEG signals and unit ACHs indicate that the unit has the same dominant frequency as all cortical leads during the prestimulation period. During the stimulation period, the unit has the same dominant frequency as all leads except the hippocampal and entorhinal (Ent 1*), which, however, has a secondary peak (Ent 2*) at the same frequency as that of the unit. Calibration bars for amplitude: 0.1 mV for EEG and 1.0 mV for unit; time scale bar = 1 s for EEG-unit recordings in I and corresponds to the scale (in seconds) for the graphs in II.
GABAergic neurons could have a very precise temporal influence in pacing rhythmic slow and fast activities through their target interneurons in cortex, as they presumably also do in hippocampus (Brazhnik and Fox 1999; Lee et al. 1994). The glutamatergic neurons fire in distinct clusters of spikes at ~20 Hz that through fast EPSPs could promote beta rhythm activity, which occurs in association with sensory sampling (Boeijinga and Lopes da Silva 1989; Kay and Freeman 1998; Wrobel 2000) and permits coherent discharge between pyramidal neurons across long distances in the cortex (Kopell et al. 2000). The slow rhythmic discharge could stimulate rhythmic activity in various target neurons in cortex through fast responses associated with AMPA and kainate receptors or through slower responses associated with N-methyl-D-aspartate and metabotropic receptors, which can also facilitate rhythmic activity (Cobb et al. 2000; Flatman et al. 1983). Slow rhythmic activity stimulated by glutamatergic input is known to enhance synaptic plasticity (Granger and Lynch 1991; Lynch 1998; Malenka and Nicoll 1999). The slow rhythmic activity promoted by the coordinated discharge of the cholinergic and glutamatergic neurons could maximize such plasticity in the cortex (Cobb et al. 2000; Ji et al. 2001). With the precise timing afforded by the GABAergic neurons, the coordinated discharge by the three basal forebrain cell groups could regulate coherent rhythmic slow activity and promote coherent high beta-gamma activity in distributed cortical target areas. Such synchronized activity would facilitate sensory sampling and temporal binding of sensory features across distributed cortical regions (Engel and Singer 2001; Granger and Lynch 1991). It would also promote synaptic plasticity in active cortical networks (Granger and Lynch 1991; Huerta and Lisman 1993; Larson et al. 1986).

**Relationship of rhythmic unit discharge to cortical activity**

The slow rhythmic discharge of the glutamatergic, like GABAergic and cholinergic, basal forebrain neurons commonly occurred at a frequency that was lower than that of limbic or hippocampal theta and similar to that recorded in the medial prefrontal cortex (Manns et al. 2000a,b). Here, it was discovered that the activity of the rhythmic cells was also correlated with activity of the olfactory bulb to which neurons in the MCPO-SI project (Zaborszky et al. 1986) and from which they could often be antidromically activated. The activity of the olfactory bulb and its neurons is synchronized with respiration in part to activation of olfactory receptors during inspiration (Macrides and Chorover 1972). The cross-correlated discharge of the rhythmic basal forebrain neurons with olfactory bulb activity could be due to respiratory-olfactory related afferent input to these neurons (Paolini and McKenzie 1997). However, whereas the olfactory activity, like respiratory activity, was equally highly rhythmic during the prestimulation and stimulation periods, the unit discharge was more highly rhythmic during the stimulation period in association with cortical activation. These results suggest that rhythmic firing by the basal forebrain units could be stimulated by afferents from the brain stem activating system, which would be excited during somatosensory stimulation. Their rhythmic firing could be directly stimulated by neurons of the brain stem reticular formation, which also fire in association with respiration under high drive conditions (Chen et al. 1991; Pagano 1966). Or their rhythmic discharge could emerge from their intrinsic oscillatory properties (Alonso et al. 1996) that would be unmasked by neuromodulatory influences from the brain stem afferents. Evidence has long suggested that the olfactory bulb depends on a centrifugal input for modulation of respiratory related rhythmic activity (Chaput 1983; Ravel et al. 1987). The present results would suggest that the basal forebrain rhythmically discharging neurons could supply that input, which would include glutamatergic in addition to GABAergic and cholinergic afferents. Collectively, they could enhance responses in association with the respiratory rhythm in the olfactory bulb to odors and in other cortical areas to relevant sensory inputs and motor outputs as a function of behavior (Komisaruk 1970; Macrides et al. 1982).

Basal forebrain neurons are also known to project to the entorhinal cortex (Alonso and Kohler 1984; Luiten et al. 1987) and were recently shown by us to comprise a major contingent of glutamatergic, in addition to GABAergic and cholinergic neurons (Manns et al. 2001). Along with the piriform cortex, the entorhinal cortex receives input from the olfactory bulb (Switzer et al. 1985). Here the basal forebrain rhythmic unit discharge was significantly cross-correlated with the activity in the piriform and entorhinal cortex, from where units could also be antidromically activated. The cross-correlated activity occurred with the primary slow peak of the olfactory bulb-piriform cortices and the secondary slow peak of the entorhinal cortex. Although the olfactory bulb-piriform cortex maintain the slower respiratory-olfactory related rhythms during many behaviors, as here in the anesthetized rat, they dynamically shift and couple to the higher hippocampal-entorhinal theta rhythm during olfactory-related behaviors (Boeijinga and Lopes da Silva 1989; Kay and Freeman 1998; Macrides et al. 1982; Vanderwolf 1992). We propose that the rhythmically discharging basal forebrain neurons of the MCPO-SI may be involved in parallel with those of the MS-DBB in coordinating slow rhythmic activity across the olfactory-entorhinal-hippocampal axis and beyond to other cortical areas during coordinated exploratory behaviors, such as sniffing and whisking (Komisaruk 1970). The basal forebrain neurons could accordingly promote coherent rhythmic slow activity across the entire cortical mantle to enable coordinated sampling, learning, and remembering of sensory experiences during waking and reactivation of such experiences during PS (Louie and Wilson 2001).

By differential action on different cortical cells, glutamatergic, GABAergic, and cholinergic basal forebrain neurons could thus generate rhythmic slow activity while stimulating or pacing higher frequency beta or gamma activities. The resulting cortical activation would promote integrated processes and behaviors while enabling their plasticity. The rhythmically discharging glutamatergic together with the GABAergic and cholinergic neurons would thus collectively fulfill the essential role of the basal forebrain in arousal, learning, and memory.
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