Sleep-Wake Related Discharge Properties of Basal Forebrain Neurons Recorded With Micropipettes in Head-Fixed Rats

Maan Gee Lee, Ian D. Manns, Angel Alonso, and Barbara E. Jones
Department of Neurology and Neurosurgery, McGill University, Montreal Neurological Institute, Montreal, Quebec H3A 2B4, Canada
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Lee, Maan Gee, Ian D. Manns, Angel Alonso, and Barbara E. Jones. Sleep-wake related discharge properties of basal forebrain neurons recorded with micropipettes in head-fixed rats. J Neurophysiol 92: 1182–1198, 2004. First published March 17, 2004; 10.1152/jn.01000.2003. The basal forebrain has been shown to play an important role in cortical activation of wake and paradoxical sleep (PS), yet has also been postulated to play a role in slow wave sleep (SWS). In an effort to determine whether these different roles may be fulfilled by different cell groups, including cholinergic and GABAergic cells, we recorded from 123 units in waking-sleeping, head-fixed rats using micropipettes to allow juxtacellular labeling. Functional sets of intermingled cell groups emerged as units whose discharge was as follows: 1) maximum in active wake (aW) and positively or not correlated with EEG gamma activity, while positively correlated with nuchal EMG activity, and thus potentially facilitatory for waking and behavioral arousal (12%); 2) maximum in SWS or SWS-PS and positively correlated with delta EEG activity, while not or negatively correlated with EMG activity, and thus potentially promotive for sleep with cortical slow activity and/or accompanying behavioral changes (16%); 3) maximum in PS or PS and aW and positively correlated with gamma and theta EEG activity, while negatively or not correlated with EMG activity, and thus potentially promotive for cortical activation during PS or PS and W (62%); and 4) equivalent across all states and thus not involved in state regulation (10%). Units of each group also manifested different firing patterns typified as slow tonic (19.5%), fast tonic (32.5%), or fast phasic (48%), including rhythmic bursting (6%). Through these diverse cell groups, the basal forebrain has the capacity to modulate cortical activity, behavior, and/or related physiological processes across the sleep-waking cycle and thereby regulate the sleep-wake state of the animal.

INTRODUCTION

The basal forebrain may play manifold roles in the modulation of sleep-wake states and cortical activities (Jones 2000, 2003). This diversity has been evident since early studies showing that electrical stimulation in the basal forebrain, shown to serve as the extra-thalamic relay from the brain stem reticular formation to the cerebral cortex, could elicit cortical activation (Starzl et al. 1951) or cortical slow activity and slow wave sleep (SWS) (Sterman and Clemente 1962a,b). Similarly, lesions of the basal forebrain have been associated with loss of cortical activation (Bujska et al. 1988; Stewart et al. 1984), or reciprocally, loss of cortical slow wave activity and SWS (Szymusiak and McGinty 1986a).

Discovery of cholinergic cortically projecting neurons in the basal forebrain indicated that the cholinergic neurons could be responsible for stimulating cortical activation (Mesulam et al. 1983; Shute and Lewis 1967). Indeed, acetylcholine (ACh) release was enhanced in association with evoked and naturally occurring fast cortical activity during waking and paradoxical (or rapid eye movement) sleep (PS or REMS) (Celesia and Jasper 1966; Jasper and Tessier 1971; Marrosu et al. 1995). Through the basal forebrain, however, the cholinergic neurons are co-distributed with noncholinergic neurons, including GABAergic neurons, that could play different roles in cortical modulation and sleep-wake state (Gritti et al. 1993, 1997). Indeed, electrical stimulation of the basal forebrain that elicited ACh release was found to have excitatory effects on cortical unit activity, whereas stimulation at adjacent sites that did not elicit ACh release was found to have inhibitory effects on cortical unit activity (Jimenez-Capdeville et al. 1997). Such results indicate that opposing actions on cortical activity could be mediated by different groups of basal forebrain neurons.

Single unit recording studies in naturally sleeping-waking animals have found units that discharge at higher rates during waking and/or paradoxical sleep in association with cortical activation and other units that discharge at their highest rates during cortical slow wave activity and SWS (Detari et al. 1984; Koyama and Hayaishi 1994; Szymusiak and McGinty 1986b). These results clearly indicated that different cell groups in this region would play different roles in modulating cortical activity and sleep-wake states. However, given the diversity of basal forebrain neurons, which contain multiple different neurotransmitters, including ACh, GABA, and glutamate (Manns et al. 2001), and give rise to different efferent projections, including the cerebral cortex, posterior hypothalamus, and local vicinity (Gritti et al. 1994, 1997; Zaborszky and Duque 2000), precise knowledge of the particular neurons having particular discharge properties is necessary to understand their roles in modulating cortical activity and state.

In an effort that was first begun in vitro, we aimed to determine the specific roles of cholinergic and other noncholinergic basal forebrain cell groups in the modulation of cortical activity (Alonso et al. 1996; Khatib et al. 1992). We were able to extend this investigation in recent years to in vivo studies by employing juxtacellular recording and labeling of neurons in association with EEG activity in anesthetized animals (Manns et al. 2000b). Found to have distinctive properties in vitro, the cholinergic neurons were similarly found in vivo to discharge in rhythmic high-frequency bursts that occurred with cortical activation and rhythmic theta-like EEG activity (Manns et al. 2000b). In contrast to cholinergic cells, identified GABAergic neurons were heterogeneous in their properties, but in the majority, discharged at higher rates in association
with slow wave activity than with cortical activation ("cortical activation-off") (Manns et al. 2000a). Cholinergic and GABAergic neurons, as well as putative glutamatergic or peptide-containing neurons (Duque et al. 2000; Manns et al. 2003), thus possess different properties and profiles of discharge and could accordingly play different and distinctive roles in modulating cortical activity and sleep-wake states.

The aim of this study was to record from basal forebrain neurons in unanesthetized rats to characterize their discharge profile and properties in relation to EEG activity and natural sleep-wake states and accordingly determine the characteristics of the cell population and whether particular cell groups possess properties previously identified in the anesthetized animal as typical of cholinergic or GABAergic neurons. For this purpose, glass micropipettes were employed to facilitate high fidelity recording of single units and moreover to permit juxtacellular labeling of the recorded cells. Such recordings were thus carried out in head-fixed rats habituated to sleep during the day in the stereotaxic frame (Souliere et al. 2000).

Multiple units were recorded across sleep-wake states per animal, although only one or two units could be labeled per animal to insure unambiguous identification of those cells. Dual staining for neurotransmitter phenotype was undertaken in the labeled units for future focused study of specific cell groups. In this study, however, unbiased sampling of units was performed to characterize the basal forebrain cell population and estimate proportions of cell groups and subgroups with particular state-related discharge properties. Units were classified by statistical analysis according to the state-dependence and profile of their discharge rate across active waking: SWS and PS. The relationship of their discharge to gamma, theta, or delta EEG activity and to nuchal EMG activity was also examined by simple correlations and used to aggregate sets of cell groups that may be functionally related by their potential modulation of specific EEG activity or behavior. Based on our previous differentiation of neurons as having slow tonic or fast phasic, burst or cluster, and irregular or rhythmic firing patterns among chemically identified cell groups in anesthetized rats (Manns et al. 2000a,b, 2003), we further sought to characterize and distinguish units within each group according to firing pattern. In the head-fixed, unanesthetized rat, we found a myriad of cell types representing multiple groups according to state-related profile and several subgroups according to properties of discharge that nonetheless form functionally related sets according to their potential influence on EEG activity or behavior across the sleep-waking cycle.

**METH ODS**

**Animals and surgery**

All procedures were approved by the McGill University Animal Care Committee and the Canadian Council on Animal Care. Adult Long-Evans rats (200–250 g, Charles River, St. Constant, Quebec, Canada) were employed. They were maintained throughout the experiments on a 12/12-h, light/dark schedule with lights on during the day and fed ad libitum except during head-fixation. For surgery, they were anesthetized with a cocktail of ketamine, xylazine, and acepromazine (65/5/1 mg/kg, 2 ml/kg, ip) and maintained with lower doses of the same cocktail (1 ml/kg, ip) when necessary during surgery. They were placed in a stereotaxic frame (David Kopf Instruments, Tujunga, CA) for implantation of chronically indwelling electrodes and placement of an aluminum U-shaped frame (2 × 2 cm) that was attached by screws to a sliding carriage adapter (GFG, Lyon, France) for subsequent painless fixation of the head (Souliere et al. 2000). Stainless steel screws were threaded into the skull over the olfactory bulb (OB), prefrontal cortex (PF), retrosplenial cortex (RS), parietal cortex (P), and occipital cortex (O) for EEG recording, and two screws were threaded into the right frontal bone and the interparietal bone as reference and ground electrodes, respectively. Two Teflon-coated silver wires were inserted into the neck muscles, and another two silver wires were inserted into the whisker pad for monitoring EMG. The bone was covered with a thin layer of acrylic cement (Superbond, Sun Medical), except over bregma and the region caudal to it overlying the basal forebrain. The U-shaped frame was fixed by screws to the carriage adapter, which was set such that the frame was centered over bregma and the region caudal to it. The electrodes and their pin connectors were embedded in dental cement while leaving a well inside the U-shaped frame that was filled with sterile bone wax.

**Habituation to head-fixation**

After recovery from surgery (2 days), the rats were habituated to the head-fixation device. When their heads were fixed between the carriage adapter, their bodies were lying comfortably in a Plexiglas box that allowed free movement of body and limbs except for twisting of the body or reaching the top of the head with their forepaws (Parry and McElligott 1993). Over a period of 6–9 days, the rats were allowed to habituate to the Plexiglas box and then to the head-fixation within the box by repetitive sessions, increasing the duration each day as the rat remained calm, and then began to sleep for longer periods within the apparatus (30 min, 1–2 h, and 3–6 h). A cereal treat was given to the rat following each session. At the end of the training period, they remained calm for periods of ~6 h and typically slept the majority of the time, as is natural for rats during the afternoon. Episodes of W, including brief periods of active waking (aw), typically occurred together with longer episodes of SWS and PS during the afternoon recording sessions. Previous studies documented that the percentage of sleep-wake states in the head-restrained rats was not different from that of freely moving rats and that no physiological signs of stress were evident in the animals (Parry and McElligott 1993; Souliere et al. 2000).

**Single-unit and polygraphic recording**

After 6–9 days of habituation and before the first single-unit recording session, rats were once again anesthetized with a cocktail of ketamine, xylazine, and acepromazine (65/5/1 mg/kg, 2 ml/kg, ip), and holes were drilled (~2 mm) in the skull overlying the basal forebrain areas of each side. Leaving the dura mater intact, the well was sealed again with sterile bone wax. After 1 day of recovery, daily recording sessions of ~6 h were performed over a maximum of 5 days. On the first day of recording on each side, the dura mater was cut following application of one drop of lidocaine. On subsequent days, the surface of the brain was cleaned with sterile saline using a cotton swab after local application of lidocaine (which has a plasma half-life of 10 min and terminal half-life through liver of 90 min). Approximately 1 h later, recording was begun and continued for ≤6 h. Following each recording session, the well was re-sealed with sterile bone wax.

Single units were recorded using glass micropipettes (~1-μm tip) that were filled with 0.5 M NaCl, and for the last cell recorded, ~5% Neurobiotin (Nh, Vector Laboratories, Burlingame, CA). Their impedance was commonly around 40 MΩ (20–100 MΩ). The approximate two coordinates of recording sites were 0.5–0.8 mm lateral and 2.0–2.5 mm lateral to Bregma. Micropipettes were lowered vertically to ~7 mm from the surface of the brain using a Kopf High Speed Micropositioner (Model 660, David Kopf Instruments) that allowed
accurate digital readings of vertical descent into the basal forebrain and fine control of step advances (1 or 2 μm) for approaching cells. The precise vertical coordinates at which cells were recorded were read from the Micropositioner and were generally located ~7.5–8.5 mm below the cortical surface (see RESULTS). Recording and juxtacellular labeling were done using an intracellular amplifier (Neurodata IR-283A, Cygnus Technology, Delaware Water Gap, PA). The unit signal was amplified (2000×) and filtered (0.3–3 KHz) using a CyberAmp (380, Axon Instruments, Union City, CA) and acquired at 16 KHz for on-line viewing with Axoscope. At acquisition (100 μA) to verify that the recorded activity was derived from a single unit as determined by amplitude consistency and shape of the spikes. The unit signal was simultaneously acquired and digitized at 8 KHz together with EEG and EMG signals (amplified 5000×, filtered 0.5–60 and 10–100 Hz, respectively, and digitized at 250 Hz by CyberAmp) for recording and analysis using EEG and sleep-wake state dedicated software (Harmonic v5.2, Stellaire, Montreal, Canada). Video recording of behavior was also acquired simultaneously by the same software. On the last day of recording from one side, the last cell recorded during aW, SWS, and PS was labeled with Nb using the juxtacellular method, as described previously (Manns et al. 2000b; Pinault 1996). Depending on the amplitude of the unit spikes, which reflected the distance of the electrode from the cell, currents of 1 nA up to a maximum of 50 nA (commonly ~10 nA) were applied (with 200-ms pulses) through the intracellular amplifier (Neurodata) to modulate the activity of the unit for 3–10 min (commonly ~5 min) for juxtacellular labeling with Nb.

Data analysis

For analysis, units were selected that had been recorded for >5 min and during at least one episode of aW, SWS, and PS. Together with the synchronized video images of behavior, electrophysiological records of EEG and EMG were scored (using Harmonie) by 10-s epochs for sleep-wake state, distinguishing active wake (aW), quiet wake (qW), transition to slow wave sleep (IWS), slow wave sleep (SWS), transition to paradoxical sleep (IPS), and paradoxical sleep (PS). As largely described previously (Maloney et al. 1997), aW was scored according to the presence of high and phasic EMG together with high-frequency and theta (4.5–8 Hz) activity on the RS EEG (and associated video images of movement); qW by the presence of moderate to low tonic EMG with low-voltage fast EEG activity; SWS by the presence of low EMG with slower irregular medium-voltage EEG activity and occasional spindles (12–14 Hz); SWS by the presence of low EMG with slow delta (1–4.5 Hz) high-voltage EEG activity together with occasional spindles; IPS by almost continuous spindle activity (progressively slowing to ~10 Hz); and PS by the presence of minimal EMG, marking neck muscle atonia, and fast EEG activity riding on prominent theta waves on RS cortex. The unit activity was subsequently analyzed per 10-s epoch in each sleep-wake state for average discharge rate (spikes per second), instantaneous firing frequency (f) using the first modal peak of the interspike interval histogram (ISIH), rhythmicity of discharge and its frequency using the autocorrelation histogram (fACH), and cross-correlated EEG activity using the spike-triggered average (STA) as described previously (Manns et al. 2000b). Gamma (30–58 Hz) power, delta (1–4.5 Hz) power, and theta (4.5–8 Hz) activity (measured as the ratio of theta/delta powers) were measured per epoch along with EMG amplitude (30–100 Hz) for correlation with unit spike rate.

The state dependency of average unit discharge rate was tested for each unit by ANOVA followed by paired comparisons (using 1-tailed t-test, P < 0.01) for the three principal states: aW (W), SWS (S), and PS (P) (ignoring quiet wake and transitional sleep stages). By this analysis, 12 different state-dependency profiles emerged that were encoded using a capital letter to indicate the state(s) with the maximal average discharge rate(s), a subscripted letter to indicate the state(s) with the minimal rate(s), and a lowercase letter to indicate the state with an intermediate rate: \( W, W_p, W_s, W_{SP}, WS, WS_p, W_{SP}, wS, wS_p, wS_P, wP, \) and \( W_P \). A 13th state-independent profile emerged that was encoded as wsp. Each unit was classified into 1 of the 13 groups according to its discharge profile. The relationship of spike rate to gamma, delta, and theta EEG activity and to EMG amplitude was examined across epochs and states for each unit by simple correlations. Functional sets of cell groups were inferred by common correlations across groups of unit discharge rate with these activities.

Each unit was further distinguished according to its discharge properties during the maximally active state. First, it was classified as slow or fast using a cutoff of 14.5 Hz for the peak frequency of the principal mode of the ISIH, as corresponding to the distinction of fast EEG activity in the beta range at >14.5 Hz. Among the fast spiking units, their discharge was further distinguished as phasic or tonic by comparing the interval of the average discharge rate to the ISIH distribution. For this comparison, the area under the log-transformed, and thus normalized, ISIH curve of the primary mode was calculated. If the area to the right of the interval corresponding to the average discharge rate (1/average rate) represented <5% of the total area (P < 0.05), the unit was classified as “phasic.” Otherwise, it was classified as “tonic.” Whether phasically firing units discharged predominantly in high-frequency bursts (>80 Hz) or lower frequency spike clusters (<80 Hz) was also assessed by examining the peak frequency of the principal mode of the ISIH. A cutoff of 80 Hz was selected according to previously determined profiles of burst versus cluster discharge of units first in our in vitro (Alonso et al. 1996) and in vivo anesthetized preparations (Manns et al. 2003).

Units in the fast, phasic group were further distinguished as being either “rhythmic” or “nonrhythmic” according to the ACHS. Using a hanning window, the log power spectrum of the right half (1 s) of the ACHS was calculated. If the log power of a frequency was >2.5 SD from the mean of the log powers of all frequency bins (250), the power at that frequency was considered as significantly different from background activity. Accordingly, the unit discharge was classified as “rhythmic.” Given the epoch length sampled, only frequencies of >1 Hz were considered.

The CV was calculated from the SD of the average discharge rates for the three principal states (aW, SWS, and PS) divided by the mean of the average discharge rates of the three states. The modulation ratio (MR) was calculated from the average discharge rate for the maximally active state divided by the average discharge rate for the minimally active state among the three principal states.

All analyses of raw data and plotting were done using Matlab (v5, MathWorks, Natick, MA), and statistical group analysis was done using Systat (v10, SPSS, Chicago, IL). Figures were compiled using Adobe Illustrator 9 (Adobe, San Jose, CA) for electrophysiological data and Adobe Photoshop 6 (Adobe) for photographic data.

Histochemistry

After the recording and labeling of units, the rat was anesthetized with an overdose of sodium pentobarbital (~100 mg/kg, ip) and perfused with 50 ml physiological saline and 300 ml 4% paraformaldehyde solution (pH 7.4). The brain was removed from the skull and incubated in 30% sucrose for 2–3 days until sinking. Brains were frozen in isopentane (to −50°C) and held in a deep-freezer (−80°C) until processing. Coronal frozen sections were cut and collected serially at 25 μm through the basal forebrain. For revelation of Nb, sections were incubated for 2.5 h in Cy2-conjugated streptavidin (1:1,000, Jackson ImmunoResearch Laboratories, West Grove, PA). Sections were then processed subsequently for immunohistochemical staining of neurotransmitter enzymes that will be reported in subsequent publications. Nb-labeled cells were located by epifluorescence using a Leica DMLB microscope. Images of the labeled cells were acquired, and their location was mapped onto a computer resident atlas with the aid of NeuroLucida (v5, MicroBrightField, Williston, VT). Coordinates of other cells that were recorded but not
labeled were utilized to map their estimated location on the same atlas sections. For this purpose, a correction factor was calculated for the vertical (87%) plane according to the difference between the coordinate of the depth of the electrode tip registered by the Micropositioner and that of the Nb-labeled cell on the atlas image.

RESULTS

Cell group classification and localization

In 17 rats, 123 units were recorded across all sleep-wake states including aW, qW, tSWS, SWS, tPS, and PS. Of these, 11 of 15 units submitted to labeling protocol were successfully labeled with Nb (Fig. 1A) and could thus be plotted onto computer-based atlas sections through the basal forebrain (Fig. 1, C–F). All Nb-labeled units were located in the region of the substantia innominata (SI) and magnocellular preoptic nucleus (MCPO), where the cholinergic neurons of the basal forebrain are situated together with GABAergic and other cortically projecting neurons (Gritti et al. 1997). Other units were plotted by the coordinates used in recording (Fig. 1, open symbols; see METHODS). According to these, the majority of cells were situated within the region of the SI and MCPO, and some cells in the immediately adjacent nuclei of the globus pallidus (GP), bed nucleus of the stria terminalis (BST), or lateral preoptic area (LPO), where GABAergic and other noncholinergic projection neurons of the basal forebrain are also located (Gritti et al. 1997).

Examining the average discharge rate of each unit as a function of the three principal states, aW, SWS, and PS, using ANOVA, it was found that the discharge of 111/123 units (90%) varied significantly as a function of state. With post hoc analysis (using paired t-test with Bonferroni correction, P < 0.05), it was determined in which state (or states) the average discharge rate was significantly higher, lower, or intermediate with respect to the others for each unit. For the states in which the rate was significantly higher than in other states, those states were recorded as having the maximal rates (marked by a capital letter, e.g., W); for those in which the rate was significantly lower, they were recorded as having the minimal rates.
(marked by a subscripted letter, e.g., \(^w_p\)), and for those in which the rate was lower than in the maximal and higher than in the minimal, they were recorded as having an intermediate rate (marked by a small letter, e.g., \(\text{W}_p\), to yield e.g., \(\text{W}_p\), for the highest rate in \(\text{aW}\), lowest rate in \(\text{SWS}\), and intermediate rate in \(\text{PS}\)). In the event that the average rates did not differ significantly between two states, those state rates were considered equivalent, as maximal (e.g., \(\text{WS}_p\)), minimal (e.g., \(\text{W}_p\)), or neither for the state-unrelated \((\text{wsp})\) group. According to these comparisons, units could be classified as 1 of 12 logically possible state-related groups or 1 state-unrelated group, as displayed on a clock face in Fig. 2. To reflect the passage from \(\text{W}\) through \(\text{SWS}\) to \(\text{PS}\) of the normal wake-sleep cycle, the groups were numbered to begin with maximally \(\text{W}\)-active (\(\text{W}-\text{max-active}\)) and move clockwise to maximally \(\text{SWS}\)-active (\(\text{S-max-active}\)) and to maximally \(\text{PS}\)-active (\(\text{P-max-active}\)).

The one state-unrelated group was numbered 13, displayed in the middle of the clock face, to yield: 1: \(\text{W}_p\), 2: \(\text{W}_p\), 3: \(\text{W}_p\), 4: \(\text{W}_p\), 5: \(\text{W}_p\), 6: \(\text{W}_p\), 7: \(\text{W}_p\), 8: \(\text{W}_p\), 9: \(\text{W}_p\), 10: \(\text{W}_p\), 11: \(\text{W}_p\), 12: \(\text{W}_p\), and 13: \(\text{wsp}\). In the final analysis, at least one unit belonged to each group, except group 4: \(\text{WS}_p\).

The correlation of spike rate with EEG and EMG activity across states was also examined for individual units of each cell group. As had been established previously (Maloney et al. 1997), gamma power, theta (measured as the ratio of theta/delta power), and delta power along with EMG amplitude varied significantly as a function of state and stage (as confirmed by ANOVA, \(P < 0.05\) for each variable). Each also differed significantly between stages (as determined by Bonferroni-corrected post hoc paired comparisons, \(P < 0.05\)). In this study, gamma and theta were the highest in \(\text{PS}\), delta in \(\text{SWS}\), and \(\text{EMG}\) in \(\text{aW}\) (e.g., see Figs. 4–7). By examining correlations of spike rate with gamma, theta, and delta EEG activity and with EMG amplitude, four major sets of cell groups emerged (Table 2; Fig. 2).

1) In \(\text{W-max-active}\) units (1–3), the average spike rate was most commonly positively correlated with EEG activity (\(P < 0.05\) in 9/15 units, \(r = 0.36\) for overall average across set), while positively or not correlated with gamma EEG activity. 2) In \(\text{S-max-}\) and \(\text{SP-max-active}\) units (5–8), the average spike rate was most commonly positively correlated with delta EEG activity (\(P < 0.05\) in 15/20, \(r = 0.40\)). 3) In \(\text{P-}\) and \(\text{WP-max-active}\) units (9–12), the spike rate was most commonly positively correlated with gamma EEG activity (\(P < 0.05\) in 63/76 units, \(r = 0.44\)) and theta EEG activity (\(P < 0.05\) in 69/76 units, \(r = 0.53\)), while being negatively or not correlated with EMG activity. 4) Finally, in \(\text{wsp}\) state-unrelated units, the average spike rate was not correlated with EEG or EMG activity. Across all groups, 59% of units showed a significant positive correlation with gamma and theta power, and 15% showed a significant positive correlation with delta power, while 14% showed a significant positive correlation with EMG amplitude (and 33% a significant negative correlation).

The proportion of units that were maximally active in the different states differed markedly (Figs. 2 and 3; Table 1). A minority was maximally active during \(\text{aW}\) alone (1–3; \(\text{W-max-active}\), \(~12\%\)). Another minority was maximally active during \(\text{SWS}\) alone (5–7; \(\text{S-max-active}\), \(~10\%\)) or SWS and \(\text{PS}\) (SP-max-active, 8; \(\text{SP-active}\), \(~7\%\)). The majority of the units were maximally active during \(\text{PS}\) alone (9–11; \(\text{P-max-active}\), \(~50\%\)) or \(\text{W}\) and \(\text{PS}\) (WP-max-active, 12; \(\text{WP-active}\), \(~12\%\); Table 1). Finally, a minor proportion discharged at equivalent rates across all states, belonging to the state-unrelated group (13; \(\text{wsp}\), \(~10\%\)). The \(\text{W-max-active}\), \(\text{S-}\) or \(\text{SP-max-active}\), \(\text{P-}\) or \(\text{WP-max-active}\), and \(\text{wsp}\) state-unrelated units were distributed across the basal forebrain with no apparent aggregation of cell types (Fig. 1). There was also no significant difference in anterior-posterior or vertical coordinates of the recorded units across the 13 cell groups (ANOVA, \(P > 0.05\)).

The units of each group (1–13) had variable frequencies and patterns of firing (Fig. 3; Table 1). Units were thus further classified according to their instantaneous firing frequency and pattern during the maximal discharge state. First, units were divided according to whether the peak of the primary mode of the ISIH was in a low (\(~14.5\) Hz) or higher (\(>14.5\) Hz) frequency range (see METHODS). Some units were thus classified as “slow” spiking (20% of all units); most were classified as “fast” spiking (\(~80\%\) of all units; Table 1). Second, by comparing the average discharge rate to the instantaneous firing frequency distribution of the primary mode (see METHODS), fast spiking units were further subdivided according to whether they showed a distinct phasic pattern of discharge or were otherwise classified as tonic. The majority was classified as phasic (48% of all units, Table 1; shown in black in Fig. 3) and a minority as tonic (\(~33\%\) of all units). Of the fast, phasically discharging units, one-half discharged at a high burst-like frequency (\(\text{ISIH} > 80\) Hz) and one-half at a lower cluster-like frequency (\(<80\) Hz; see METHODS). Among these, a certain number (\(~6\%) belonging to particular groups also discharged rhythmically. The average measures for units shar-
ing similar properties and profiles of discharge are accordingly presented for resulting subgroups in Table 3.

The particular discharge properties of the prominent cell groups \( n > 4 \) are detailed below for each functional set of cell groups.

**TABLE 1. Number and proportion of units with common sleep-wake related discharge profile and properties**

<table>
<thead>
<tr>
<th>Group</th>
<th>All</th>
<th>Slow</th>
<th>Fast</th>
<th>Fast</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1: ( W_p )</td>
<td>8</td>
<td>1</td>
<td>7</td>
<td>0</td>
<td>6.5</td>
</tr>
<tr>
<td>2: ( W_{sp} )</td>
<td>6</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4.9</td>
</tr>
<tr>
<td>3: ( W_{sp} )</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0.8</td>
</tr>
<tr>
<td>4: ( W_{sp} )</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>5: ( W_{sp} )</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1.6</td>
</tr>
<tr>
<td>6: ( W_{sp} )</td>
<td>6</td>
<td>3</td>
<td>0</td>
<td>3</td>
<td>4.9</td>
</tr>
<tr>
<td>7: ( W_{sp} )</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>3.3</td>
</tr>
<tr>
<td>8: ( W_{SP} )</td>
<td>8</td>
<td>1</td>
<td>2</td>
<td>5</td>
<td>6.5</td>
</tr>
<tr>
<td>9: ( w_{sp} )</td>
<td>15</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>12.2</td>
</tr>
<tr>
<td>10: ( w_{sp} )</td>
<td>15</td>
<td>2</td>
<td>6</td>
<td>7</td>
<td>12.2</td>
</tr>
<tr>
<td>11: ( w_{sp} )</td>
<td>3</td>
<td>4</td>
<td>9</td>
<td>18</td>
<td>25.2</td>
</tr>
<tr>
<td>12: ( w_{sp} )</td>
<td>15</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>12.2</td>
</tr>
<tr>
<td>13: ( w_{sp} )</td>
<td>12</td>
<td>2</td>
<td>1</td>
<td>9</td>
<td>9.8</td>
</tr>
<tr>
<td>( \Sigma )</td>
<td>123</td>
<td>24</td>
<td>40</td>
<td>59</td>
<td>100.0</td>
</tr>
</tbody>
</table>

*Number and percentage of units classified according to their discharge profile and properties.

**W-max-active cells**

Representing \(~ 12% \) of all units recorded, W-max-active cells were grouped into 1: \( W_p \) \( (n = 8) \), 2: \( W_{sp} \) \( (n = 6) \), and 3: \( W_{sp} \) \( (n = 1) \) (Table 1; Figs. 2 and 3). The discharge of these units was most commonly positively correlated with EMG activity \( (P < 0.05 \) in 9/15, overall average \( r = 0.36 \), maximum 0.75; Table 2; see Fig. 4 as an example). Their discharge rate was also significantly positively correlated with gamma EEG activity in many units \( (7/15) \) but was not significantly or was negatively correlated in others \( (r = 0.10 \) average for set) and rarely significantly positively correlated with theta \( (2/15, r = 0.05) \). The rate was commonly negatively correlated with delta activity \( (13/15, r = 0.41) \). Two W-max-active cells \( (1 W_p \) and 1 \( W_{sp} \) were labeled with Nb and located at different levels in the MCPO (Fig. 1).

Most units \( (7/8) \) in group 1: \( W_p \) had fast instantaneous firing frequencies and were classified as fast, tonic (Tables 1 and 3). These units maintained a relatively high average discharge rate across states \( (\text{means of} 30 \text{ Hz in aW to} 22 \text{ Hz in SWS and} 25 \text{ Hz in PS}) \) (Table 3; Fig. 3). The average discharge rates did not differ significantly from the instantaneous firing frequencies \( (\text{mean,} 47 \text{ Hz}) \) during aW, indicating the predominantly tonic discharge pattern during the max-active state. Despite continued firing across states and a relatively low median CV \( (0.26) \) of rates across states, the rate changes...
FIG. 4. Discharge properties and profile of a representative W-Max active unit from group 2: \$W_{sp}\$ (fast, tonic) unit (u172) that discharges on average at a maximal rate during aW (A), intermediate rate during SWS (B), and minimal rate during PS (C). According to the peak instantaneous firing frequency during aW (~78 Hz, D), the firing is fast, and although it appears to be phasic, is classified as tonic [since the corresponding interval of the average spike rate during waking falls within 95% of the interspike interval histogram (ISIH) distribution]. There is also a lack of evidence for bursting or rhythmicity in the autocorrelation histogram (ACH) during aW (E). Average spike rate (F) decreased progressively from aW (~12 Hz) to SWS (~4 Hz) to PS (2 Hz) and was associated with a moderately high CV (0.9) and modulation ratio (MR; 3.6). It was not significantly correlated with gamma EEG power (G, $r = -0.04$) or theta (H, $r = -0.20$), although it was significantly negatively correlated with delta EEG power (I, $r = -0.36$), while being significantly positively correlated with EMG amplitude (J, $r = 0.58$, $n = 52$, $P < 0.01$). In this and Figs. 4–7, the unit discharge is presented with EEG and EMG activity for 10-s epochs of aW (A), SWS (B), and PS (C). Spike amplitude is cut at 1 mV. The unit discharge is analyzed for instantaneous firing frequency (from the peak of the primary mode of the ISIH, D) and rhythmicity of discharge (from the ACH, E). Average spike rate (per second displayed on a linear scale, F), gamma EEG power (30–58 Hz, G), ratio of theta (4.5–8 Hz)/delta (1–4 Hz) EEG power (H), delta EEG power (1–4.5 Hz, I), and EMG amplitude (30–100 Hz, J) are displayed per state together with the SE. aW, active awake; qW, quiet wake; tSWS, transition to SWS; SWS, slow wave sleep; tPS, transition to PS; PS, paradoxical sleep.
between aW (max) and SWS (min) for these units nonetheless corresponded to a modulation ratio median of 1.7 (Table 3).

Most units (5/6) in group 2: W sp were fast spiking and either tonic or phasic (Tables 1 and 3). Their average spike rate decreased from aW to equivalently minimal rates during SWS and PS (Fig. 3) and was often positively correlated with EMG activity and not with EEG gamma or theta (see Fig. 4). Their discharge in aW was comprised of irregular trains, clusters, or bursts of spikes with mean ISIH frequencies of >70 Hz (Table 3). As evident for a unit shown in Fig. 4, their discharge was

---

**FIG. 5.** Discharge properties and profile of a representative SWS-max unit from group 6: \(S_p\) (fast, phasic) unit (c16u02) that discharges on average at a minimal rate during aW (A), a maximal rate during SWS (B), and equivalent minimal rate during PS (C). As evident in the recording (see expanded trace of 500 ms period of unit activity in B: a), the ISIH (D), and ACH (E), the unit discharges in a distinctly phasic manner with high-frequency bursts (114-Hz peak frequency of the principal mode of the ISIH). This bursting occurs maximally during SWS, although it is also evident (by central peak in ACHs) during other states with a much lower incidence. The average spike rate (F) increased from aW (1.6 Hz) in the eSWS to be highest during SWS (3.8 Hz) and decreased in tPS to be equivalently low in PS (1.8 Hz) as in aW. Spike rate was not significantly correlated with gamma (G, \(r = 0.13\)), was significantly negatively correlated with theta (H, \(r = -0.30\)), and was significantly positively correlated with delta EEG power (I, \(r = 0.53\), \(n = 180\) observations, \(P < 0.001\)), while being significantly negatively correlated with EMG amplitude (J, \(r = -0.38\)). See Fig. 4 for general details.
predominantly tonic or irregular if phasic, as evident by a dispersed ISIH lacking a clear secondary mode and a flat ACH for the aW state. The median CVs for this group are relatively high and the median modulation ratios very high (~3.5–12), reflecting a relatively strong reduction in average discharge rate during sleep, relative to waking (Table 3).

**S- and SP-max-active cells**

Representing ~10% of all units recorded, S-max-active cells comprised groups 5: wS_p (n = 2), 6: wS_p (n = 6), and 7: SP (n = 4), and representing ~7% of the total, SP-max-active units comprised group 8: wSP (n = 8) (Table 1; Figs. 2 and 3).
The discharge rate of these groups was most commonly significantly positively correlated with delta EEG activity (P < 0.05, in 15/20 with an average for the set of r = 0.40, maximum 0.84; Table 2; see Fig. 5 for an example). The discharge of some units was also negatively correlated with EMG activity (P < 0.05 in 6/20 particularly in group 8, with
an average for that group of \( r = -0.20 \) (maximum \(-0.40\)). Two S-max-active (1 \( wS_p \) and 1 \( wSP \)) cells and one \( wSP \) cell were labeled with Nb and located in the SI or MCPO (Fig. 1).

Units in group 6: \( wSP \) all discharged at a slow average rate, but one-half of them (\( n = 3 \)) with high-frequency spike bursts in a distinctly phasic manner during the maximally active state of SWS (Tables 1 and 3; Fig. 3). Their discharge rate was strongly positively correlated with delta (see Fig. 5). The fast, phasic units typically discharged in bursts of spikes, with high instantaneous firing frequencies (mean primary mode peak, 208 Hz) and low average discharge rates (mean, 2.7 Hz) during SWS (Fig. 5; Table 3). As seen in the ISIH and ACH (Fig. 5), the bursting was also evident in other states. Bursting occurred with the highest incidence and regularity during SWS, often in association with slow waves (<4 Hz). The units tended to increase their average discharge rate in the transition states, increasing from aW passing into qW and tSWS leading up to SWS and then decreasing in tPS and PS (as evident by ANOVA for rate across 6 stages and 2nd-order polynomial contrast, \( P < 0.05 \)). The median CV and modulation ratio of these \( wSP \) fast, phasic units were moderately high (0.87 and 4.7, Table 3), indicative of their higher average discharge rate during SWS (mean, 2.7 Hz) relative to that during W and PS (\(-0.6-0.7 \) Hz).

Of the units in group 8: \( wSP \) units, most were fast spiking (7/8) and discharged in a distinctly phasic manner (5/7; Tables 1 and 3; Fig. 3). Their discharge was often negatively correlated with EMG activity. Most fired in high-frequency bursts (mean fISIH, 105 Hz) with a relatively high incidence during both SWS and PS (mean average discharge rates of \(-3.5 \) Hz) and lower incidence during W (mean, \(-1.8 \) Hz, Table 3). As evident from the ACH, none of these units discharged rhythmically. They had a CV median of 0.6 and modulation ratio median of 3.8 (Table 3).

### P- and WP-max-active cells

Representing \(-50\%\) of all cells, P-max-active units comprised groups 9: \( wSP \) (\( n = 15 \)), 10: \( wWP \) (\( n = 15 \)), and 11: \( wWP \) (\( n = 31 \)), and representing 12\% of all units, WP-max-active cells formed group 12 (\( n = 15 \); Table 1; Figs. 2 and 3). As a set (groups 9–12), the discharge rate of these units was most commonly positively correlated with gamma EEG activity (\( P < 0.05 \) in 63/76 units with overall average for set: \( r = 0.44 \), maximum \( 0.87 \)) and theta EEG activity (69/76 units with overall average \( r = 0.53 \), maximum 0.92; Table 2, see Figs. 6 and 7 as examples). It was also significantly negatively correlated with delta EEG activity (59/76 with overall average \( r = -0.39 \), maximum \( 0.85 \)). The discharge of many units was significantly negatively correlated with EMG amplitude (34/76, see Fig. 6 as an example), although not significantly correlated in most (overall average \( r = -0.16 \)). Six P-max-active cells (2 \( wSP \) and 4 \( wWP \)) were labeled with Nb and located within the region of the MCPO or SI (Fig. 1).

Units in group 9: \( wSP \) were composed of slow spiking (\( n = 5 \)) as well as fast spiking (\( n = 10 \)) units (Tables 1 and 3; Fig. 3). Of the fast spiking, one-half (\( n = 5 \)) displayed a distinctly phasic pattern of discharge. Discharging in an irregular manner, the slow spiking units (mean fISIH, 9 Hz) progressively increased their low rate of discharge from a mean of 0.6 Hz in aW to 1.4 Hz in SWS to reach a maximum of 2.4 Hz in PS (Table 3). Also discharging in an irregular manner, the fast, tonic units progressively increased their relatively higher average rate of discharge from a mean of 10.5 Hz in aW to 13.4 Hz in SWS to reach a maximum of 21.8 Hz in PS (Table 3). The fast phasic units discharged predominantly in clusters of spikes during PS with a mean peak instantaneous firing frequency of 63 Hz and mean average discharge rate of \(-6 \) Hz (Table 3). Judging from the lack of a clear second mode in the ISIH and of evidence for rhythmicity in the ACH, they did not discharge rhythmically during PS. They increased their average rate of discharge progressively from aW (mean, \(-1.5 \) Hz) to qW (\(-1.8 \) Hz), tSWS (3.3 Hz), SWS (3.4 Hz), IPS (5.2 Hz), and PS (7.4 Hz; ANOVA, \( P < .05 \) for repeated measures test of rate across 6 stages and post hoc hypothesis test for 1st order polynomial, linear trend). They had a moderately high CV (median 0.83) and modulation ratio (7.3) of average discharge rate across the principal states (Table 3).

Units in group 10: \( wWP \) were composed of a small number of slow spiking cells (\( n = 2 \)) together with a majority of fast spiking cells (\( n = 13 \)) of which most had a distinct phasic pattern of discharge (\( n = 7 \); Tables 1 and 3; Fig. 3). Their discharge was often negatively correlated with EMG amplitude, while also being positively correlated with gamma and theta (see Fig. 6). The fast, phasic units discharged at an equivalently minimal average rate during aW (mean, 1.5 Hz) and SWS (1.5 Hz) and at a maximal rate during PS (6 Hz; Table 3). As shown by an example in Fig. 6, they could fire in a very selective manner during PS. In fact, their increase in discharge began during the transition into PS as confirmed by the increase in rate in tPS over SWS (post hoc hypothesis, \( P < 0.05 \), following ANOVA of rate across 6 stages, \( P < 0.05 \)). During PS, they tended to discharge in clusters of spikes with a mean instantaneous firing frequency of \(-46 \) Hz (Table 3). In one of these cells, the clustered spike discharge (fISIH of 44

### Table 2. Correlation coefficients of spike rate with EEG activities and EMG amplitude*

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Ga</th>
<th>Th/De</th>
<th>De</th>
<th>EMG</th>
</tr>
</thead>
<tbody>
<tr>
<td>W-Max</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1: W_p</td>
<td>8</td>
<td>0.31</td>
<td>0.25</td>
<td>-0.55</td>
<td>0.35</td>
</tr>
<tr>
<td>2: W_s</td>
<td>6</td>
<td>-0.07</td>
<td>-0.11</td>
<td>-0.34</td>
<td>0.33</td>
</tr>
<tr>
<td>3: W_sp</td>
<td>1</td>
<td>-0.58</td>
<td>-0.59</td>
<td>0.31</td>
<td>0.53</td>
</tr>
<tr>
<td>4: WS_p</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average</td>
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<td>0.10</td>
<td>0.05</td>
<td>-0.41</td>
<td>0.36</td>
</tr>
<tr>
<td>S- and SP-Max</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5: wSP</td>
<td>2</td>
<td>-0.14</td>
<td>-0.53</td>
<td>0.38</td>
<td>0.17</td>
</tr>
<tr>
<td>6: wSp</td>
<td>6</td>
<td>-0.32</td>
<td>-0.40</td>
<td>0.60</td>
<td>0.06</td>
</tr>
<tr>
<td>7: wSP</td>
<td>4</td>
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<td>-0.19</td>
<td>0.43</td>
<td>-0.04</td>
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<tr>
<td>8: wSP</td>
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<td>0.01</td>
<td>0.09</td>
<td>0.25</td>
<td>-0.20</td>
</tr>
<tr>
<td>Average</td>
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<td>-0.14</td>
<td>-0.18</td>
<td>0.40</td>
<td>-0.05</td>
</tr>
<tr>
<td>P- and WP-Max</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9: wSP</td>
<td>15</td>
<td>0.35</td>
<td>0.50</td>
<td>-0.08</td>
<td>-0.22</td>
</tr>
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<td>0.42</td>
<td>0.50</td>
<td>-0.32</td>
<td>-0.26</td>
</tr>
<tr>
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<td>0.62</td>
<td>-0.53</td>
<td>-0.20</td>
</tr>
<tr>
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</tr>
<tr>
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<td>0.53</td>
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<td>-0.16</td>
</tr>
<tr>
<td>13: wsp</td>
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<td>0.03</td>
<td>-0.02</td>
<td>-0.01</td>
<td>0.01</td>
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*Average correlation coefficients per group based on calculations for individual units across sleep-wake state (10-s epochs) between spike rate and EEG activity (Ga) power (30–58 Hz), the ratio of theta (Th) power (4.5–8 Hz) over delta (De) power (1–4.5 Hz), or De power and EMG amplitude (30–100 Hz).
Clusters or bursts of spikes with a low average rate of discharge (mean $f_{ACH}$, 93.51 Hz) occurred rhythmically at 6 Hz during PS (data not shown). The CV and MR were moderately high in this cell subgroup (medians, 0.9 and 4.8).

Units in group 11: $w$, $P$ were composed of a small number of slow spiking cells ($n = 4$) and a large number of fast spiking cells ($n = 27$) of which the majority showed a distinctly phasic pattern of discharge ($n = 18$; Tables 1 and 3; Fig. 3). Their discharge rate was often negatively correlated with EMG activity or otherwise not correlated with EMG, while being commonly positively correlated with gamma and theta (see Fig. 7). With a mean peak instantaneous firing frequency of 95 Hz, the phasically discharging units appeared to discharge in clusters or bursts of spikes with a low average rate of discharge being intermediate during aW (mean, $\sim$3.5 Hz), minimal during SWS ($\sim$0.8 Hz) and maximal during PS ($\sim$8.0 Hz). The increase in rate from SWS preceded PS during the transitional stage, tPS (post hoc hypothesis, $P < 0.05$, following ANOVA of rate across 6 stages, $P < 0.05$). The CV and MR were moderately high (medians, 0.8 and 5.2). One-third of the fast spiking units ($n = 6$) discharged in a rhythmical manner during PS, as shown for a representative unit in Fig. 7. Typically, these fast, phasic, rhythmic units discharged in bursts or clusters of spikes (mean $f_{ISIH}$, 93.51 ± 13.46 Hz) at a theta frequency (mean $f_{ACH}$, 6.7 ± 0.82 Hz) and in a continuous manner during PS (Fig. 7). They also discharged in the same manner in specific association with theta during aW. For all these rhythmic units, the unit discharge was significantly correlated with theta EEG activity recorded from the retrosplenial cortex (as determined by spike triggered average, STA, data not shown). These rhythmically discharging $w$, $P$ units had high CVs and particularly high modulation ratios (medians, 0.99 and 22).

### Table 3

<table>
<thead>
<tr>
<th>Group</th>
<th>$n$</th>
<th>$f_{ISIH}$</th>
<th>aW</th>
<th>qW</th>
<th>tSWS</th>
<th>SWS</th>
<th>tPS</th>
<th>PS</th>
<th>CV</th>
<th>MR</th>
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<tbody>
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<td>Slow</td>
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<td>4.58</td>
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<td>1.13</td>
<td>1.39</td>
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</tr>
<tr>
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<td>2</td>
<td>7.42</td>
<td>4.17</td>
<td>0.33</td>
<td>0.46</td>
<td>0.07</td>
<td>0.00</td>
<td>0.00</td>
<td>1.69</td>
<td>4710.20</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>7.20</td>
<td>7.06</td>
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<td>0.60</td>
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<td></td>
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<td>0.66</td>
<td>0.51</td>
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<td>0.62</td>
<td>0.75</td>
<td>2.39</td>
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<td>0.51</td>
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<td>23.36</td>
<td>21.92</td>
<td>26.40</td>
<td>25.19</td>
</tr>
</tbody>
</table>

Values are mean ± SE for firing frequency or discharge rate per cell group. *Instantaneous firing frequency representing that of the first modal peak of the ISIH ($f_{ISIH}$). †Average discharge rate (spikes per second) in different states (aW, active wake; qW, quiet wake; tSWS, transition to SWS; SWS, slow wave sleep; tPS, transition to PS; PS, paradoxical sleep) CV of average discharge rates during the principal states (aW, SWS, and PS). §Modulation ratio (MR) calculated as average discharge rate of maximally active state divided by average discharge rate of minimally active state among the principal states. Median values are presented for CV and MR per cell group. Bolded values indicate those during the maximally active state or during two similarly maximally active states.
Units in group 12: W,P units were comprised of slow spiking (n = 4) as well as fast spiking (n = 11) units, of which the majority discharged phasically (n = 6; Tables 1 and 3; Fig. 3). Their discharge rate was positively correlated with gamma and theta and mostly not correlated with EMG activity. In the latter units, the fast, phasic discharge occurred in clusters or bursts of spikes (with a mean fISIH of ~70 Hz) and a low average discharge rate in aW (mean, 8.8 Hz) and PS (7.3 Hz) that was higher than that in SWS (4.9 Hz). The clusters of spikes were not rhythmic. The median CV and MR for these units were 0.35 and 2.13 (Table 3).

State-unrelated cells

Representing ~10% of all units, group 13: wsp (n = 12) did not show significant variation of average discharge rate as a function of sleep-wake state (Tables 1 and 3). Similarly, their discharge rate was not significantly correlated with EEG or EMG activity (Table 2).

The wsp units were comprised of slow spiking (n = 2) and fast spiking neurons (n = 10) of which almost all displayed a distinct phasic pattern of discharge (n = 9, Table 1). These latter units discharged in bursts of spikes (fISIH mean = 127 Hz) associated with a very low average discharge rate across states (~1 Hz; Table 3). All discharged in an irregular manner.

DISCUSSION

These results reveal that in the vast majority, basal forebrain neurons manifest systematic changes in discharge rate as a function of sleep-wake state and comprise diverse groups with distinct state-related discharge profiles such as to have the capacity to regulate collectively the three principal states of the sleep-waking cycle. Their discharge is correlated with EEG and/or EMG such that they may influence cortical activity and/or behavior across this cycle. The firing patterns are also varied with the majority of units being fast spiking and firing in a phasic manner, some in rhythmic bursts or clusters of spikes. By these various profiles and patterns of discharge, distinct groups of neurons have the capacity to stimulate cortical activation and promote wake and/or PS or to dampen cortical activation and promote SWS.

Proportions and locations of cell groups

Classification in this study by statistical analysis of state dependence and difference of average spike rate across aW, SWS, and PS, revealed that discharge rate varies significantly as a function of state in 90% of basal forebrain units. This proportion is higher than that estimated in earlier studies (70%), in which a MR > 2 was considered to reflect state dependence of discharge (Szymusiak and McGinty 1986b). Here, we actually found that the MR (calculated as maximal over minimal mean state rates) and also CV (calculated as variation between mean state rates) were not reliable in differentiating state-dependent from state-independent rate changes due to the unaccounted variance in the mean rate values per state. In any event, small, as well as large, but consistent differences in average discharge rate may be functionally significant in state regulation.

In the large proportion of units represented by P-max-active cells (49%), the discharge rate was significantly higher in PS compared with aW in this study. Similarly, in another study in head-fixed rats, most neurons in the medial septum-diagonal band of Broca nuclei (MS-DBB) were found to fire at higher average rates during PS than during waking, including waking with theta activity (Sweeney et al. 1992), as also considered to indicate active waking in this study. In previous studies in freely moving cats, 46% of basal forebrain units were characterized as W-active because they discharged greater two times faster during W than non-REM sleep and described as having a similar discharge rate during REM sleep as during W (Szymusiak and McGinty 1986b). More recently in freely moving rats, 57% of basal forebrain units were characterized as wake related because they discharged at a >1.25 times higher rate during W than during non-REM sleep but were not examined during REM sleep (Alam et al. 1999). However, in another early study in freely moving cats (Detari et al. 1984), 56% was found to discharge at higher rates in PS compared with W, similar to the proportion estimated here. Since the absolute rates in waking depend on the situation and behavior of the animal, such distinctions could be arbitrary for those units involved in stimulating cortical activation during both PS and W. In this study, it is possible that lower discharge rates in some units during “active” waking were due to the relative immobility of the animal, even though the rat was able to move his body and limbs relatively freely as well as his jaw, nose, whiskers, and eyes. Between aW and PS, the levels of gamma and theta were both significantly higher on average in PS than in aW in this study, whereas theta but not gamma was significantly higher in PS in freely moving rats previously studied in this laboratory (Maloney et al. 1997), thus suggesting on average a slightly lower arousal in the aW state of the head-fixed rat. To better determine the relationship of the unit discharge to cortical versus behavioral arousal, we examined the correlation of unit discharge rate with EEG gamma and theta or delta activity and with EMG activity. We were accordingly able to distinguish and aggregate cell groups according to the relationship of their discharge to these activities. The discharge of the W-max-active units was most commonly positively correlated with EMG activity and thus primarily related to behavioral arousal, whereas the discharge of P- or WP-max active units was most commonly positively correlated with EEG gamma and theta activity and thus primarily related to cortical activation. It can be concluded that the majority of basal forebrain units (62%) discharges in association with cortical activation during PS or PS and W as P- or WP-maxactive cells and do so at consistently high rates during PS when gamma and theta are consistently high. Only a minority of units (12%) discharges in association with behavioral arousal during W as W-max-active cells. Another minority (16%) discharges in association with delta activity and slow wave sleep as S or SP-max active cells.

As evident from the location of Nb-labeled cells and from the recording coordinates of unlabeled cells, the W-max-active, S- or SP-max-active, and P- or WP-max-active cells were co-distributed and intermingled through the SI-MCPO and adjacent areas. The units were thus located in the region of cholinergic neurons but could be cholinergic, GABAergic, or putative glutamatergic cells, which are similarly co-distributed and intermingled in the rat basal forebrain (Manns et al. 2001). They might also contain neuropeptides, including somatostatin, neuropeptide Y, or galanin found in basal forebrain neu-
rons (Duque et al. 2000; Zaborszky et al. 1991). Their neurotransmitter identity will be examined in the future by a focused study of particular cell groups, which are most likely to contain particular neurotransmitters. The recorded units probably comprise magnocellular cortically projecting neurons but may also comprise co-distributed smaller, caudally, hypothalamic or brain stem, or locally projecting neurons (Gritti et al. 1994, 1997, 2003; Grove 1988; Zaborszky and Duque 2000). In the cat, wake (REM)-active units were also co-distributed with SWS-active cells and both were found to comprise cortically and brain stem-projecting neurons when examined by antidromic activation (Szymusiak and McGinty 1986b, 1989). Accordingly, the intermingling of functionally differentiated cell groups corresponds to the intermingling of chemically and hodologically differentiated cell groups that may provide the substrate for integral though differentiated modulation of cortical activity and/or other processes including motor activity, temperature, and autonomic functions that vary in a correlative manner across the sleep-waking cycle.

Discharge properties and patterns

Across cell groups, the units manifested diverse discharge properties in the unanesthetized, head-fixed rats that were comparable to properties previously observed in the in vitro brain slices and the in vivo anesthetized rats for cholinergic, GABAergic, or putative glutamatergic neurons (Alonso et al. 1996; Khat et al. 1992; Manns et al. 2000a,b, 2003). Across the population, the majority of units (~80%) were fast spiking (fISIH > 14.5 Hz), and almost one-half (48%) were phasic in their discharge pattern in head-fixed rats, similar to the respective proportions found in the anesthetized rats (~80 and 48%). High-frequency burst-like discharge (>80 Hz intra-burst frequency) and lower frequency cluster-like discharge (<80 Hz intra-cluster frequency), which were originally distinguished in vitro (Alonso et al. 1996; Khat et al. 1992) and observed in the in vivo anesthetized preparation (Manns et al. 2000a, b, 2003), were both evident here in different units belonging to various state-related groups in head-fixed rats. A similar prominence of phasic or “bursty” discharge was previously noted for basal forebrain neurons in the naturally sleep-waking cat (Detari et al. 1984). Szymusiak et al. (2000) also found units in the cat that discharged in high-frequency bursts during SWS, but never in the rat and never in association with cortical activation of waking or REM sleep in rat or cat. Our results clearly differ from theirs, since we found bursting units in the rat basal forebrain that burst during SWS and others that fired in bursts or clusters during PS and W. In this study, however, glass micropipettes were employed and permitted the clear isolation and recording of single units with high-amplitude spikes. Since these results are perfectly consistent with our previous results of both in vivo and in vitro recordings, they would appear to be reliable in documenting phasic discharge in bursts or clusters of spikes as a common mode of discharge among basal forebrain units.

Among the phasically discharging units recorded here, a subgroup also discharged rhythmically in association with theta activity recorded on the limbic cortex during aW and PS. Such rhythmically bursting units resemble those in the MS-DBB (Petsche et al. 1962). Rhythmically discharging units were first identified in the basal forebrain in the brain slice (Alonso et al. 1996; Khat et al. 1992) and in the anesthetized rat in association with rhythmic cortical activity (Manns et al. 2000a, b, 2003). In contrast to the results here in the unanesthetized rat, in which a small minority (~6%) of the basal forebrain neurons manifest rhythmic firing during their max-active state, almost one-half (45%) manifest rhythmic firing during cortical activation in the anesthetized rats. Similarly in the MS-DBB, a smaller proportion of units (~30%) discharged rhythmically in awake, freely moving rats (King et al. 1998; Sweeney et al. 1992) but that (~40–80%) in urethane-anesthetized rats (Dutar et al. 1986; Stewart and Fox 1989). Although rhythmic bursting could possibly be diminished during waking due to head-fixation here in the unanesthetized rat, it would not be affected by that condition during PS. Indeed, the vast majority (94%) of neurons in the MS-DBB were reported to fire in rhythmic bursts during PS in head-fixed rats (Sweeney et al. 1992). It must therefore be assumed that, in the basal forebrain, synaptic activity during natural waking and PS is much more irregular and has the capacity to thus render the phasic discharge less regular than present in the anesthetized rat or brain slice. It is nonetheless significant that a proportion, albeit minor, of basal forebrain neurons discharge rhythmically and may thus influence the cerebral cortex in a rhythmic manner.

Functionally related sets of cell groups

Individual units with state-related activity were classified by statistical analysis into 12 logically possible groups, of which 11 had at least one cell as member. Cell groups were then grouped into sets according to the relationship of their discharge to EEG and/or EMG activities. These sets are discussed according to their potential influence on cortical activity or behavioral and other processes of the sleep-waking cycle.

Only a small minority of units discharged maximally in W alone as W-max-active cells (groups 1–3: W_p, W_sp, and W_sp, ~12%). The discharge of these units was most commonly positively correlated with nuchal EMG activity and less consistently positively correlated with gamma EEG activity, suggesting that they may influence behavioral arousal or cortical activation contingent on behavioral arousal. They could stimulate movement or increased muscle tone and thermogenesis or other ortho-sympathetic processes associated with arousal and cortical activation, possibly by local or descending projections to the posterior hypothalamus and brain stem. Indeed, Szymusiak et al. (2000) assessed that unit discharge in the basal forebrain of freely moving rats was most highly correlated with head and limb movements. They also found cold-sensitive units in the DDB that discharge maximally during wake when they may stimulate thermogenesis (Alam et al. 1997). W-max-active units could thus be involved in diverse yet parallel or integrated processes of cortical and behavioral arousal.

Only a small minority of units were maximally active during SWS and minimally active during W and/or PS (groups 5–7: wS_p, wS_p, and wS_p, ~10%). Another group was equivalently active during SWS and PS (group 8: wSP, 7%). The activity of these S- and SP-max-active units was most commonly positively correlated with delta activity. Many of these could correspond to units that discharged maximally in association with slow wave activity in anesthetized animals, in which they were called “cortical activation-off” units (Manns et al. 2000a).
In those studies, the majority (>65%) of such cells were identified as GABAergic neurons. Whether SWS-active units are GABAergic will be the subject of future focused studies by juxtacellular labeling and immunohistochemical staining of such units in the head-fixed rat. In the cat, SWS-active neurons were found to give rise to either ascending cortical projections or descending brain stem projections (Szymusiak and McGinty 1989). Neurons with direct projections to the cerebral cortex that are maximally active during SWS could have a direct influence on the cortex and accordingly dampen cortical activation or promote slow wave activity. Here, we found a certain number of neurons (particularly in group 6: wS2) that discharged in high-frequency bursts of spikes (average ~200 Hz) in association with slow wave activity, similarly to specific GABAergic neurons that we had identified in anesthetized animals as cortical activation-off and cortically projecting neurons (Manns et al. 2000a). Such GABAergic units could directly modulate slow wave activity. Indeed, in early studies, electrical stimulation of the basal forebrain was shown to elicit cortical slow wave activity followed by behavioral SWS (Sterman and Clemente 1962a). We found other slow spiking, nonbursting units, that resembled SWS-active units found in the freely moving cat (Szymusiak and McGinty 1986b) and cortical activation-off cells found in the anesthetized rat (Manns et al. 2000a). Given our failure to antidromically activate similar units in the anesthetized rat, we thought it likely that such units could project to local or caudal sites in the posterior hypothalamus or brain stem. They could exert a local or descending inhibitory influence on other W or W-PS-active cells and thus indirectly dampen cortical activation, or (particularly in group 8) they could indirectly influence motor or autonomic processes, including respiration, blood pressure, and heart rate that are, respectively, inhibited or decreased by stimulation of basal forebrain and adjacent preoptic area (Hess 1957; Sterman and Clemente 1962b). They could also be warm-sensitive, SWS-active neurons involved in stimulating heat loss while promoting sleep like those found in the DBB and adjacent preoptic area (Alam et al. 1996, 1997). Accordingly, SWS or SWS-PS active basal forebrain units may have a direct or indirect dampening influence on cortical activation and/or behavioral arousal and its associated physiological correlates for the promotion of sleep. That such units represent a small minority (~16%) of cells would allow for the majority of cells to rest during SWS, the state of reduced energy consumption for the brain (Maquet et al. 1990) as well as the body.

Units that were maximally active during PS (groups 9: wS2P, 10: wS2P, 11: wS2P, ~49%) or PS and W (12: W2P, ~12%) comprised the majority of cells (62%). Their discharge was most commonly positively correlated with gamma and theta EEG activity, which was maximal during PS. The discharge of many cells was also negatively correlated with EMG activity, suggesting that some that are relatively selectively active during PS (particularly in group 10: wS2P) could be involved in active processes of motor inhibition producing the muscle atonia specific to PS. Both electrical and chemical stimulation of the basal forebrain have been shown to produce sensory-motor inhibition, “adynamia,” and even cataplexy (Hess 1954; Lineberry and Siegel 1971; Nishino et al. 1995; Siegel et al. 1986). Such units could exert a local or descending inhibitory influence on the posterior hypothalamus and/or brain stem reticular formation. On the other hand, some PS-active units could selectively stimulate cortical activation during PS (particularly of group 10), whereas other W-PS-active units would do so during both PS and waking (particularly of groups 11 and 12). Some of these W-PS-active units are likely to be cortically projecting neurons and cholinergic, which were shown to increase their rate of discharge in association with cortical activation in anesthetized rats (Manns et al. 2000b). Indeed, the discharge rate profile of many of the W-PS active units correlates well with ACh release in cortex and hippocampus that have been shown to be lowest in SWS and equivalently high or higher during PS compared with W (Marrosu et al. 1995). However, W-PS active units could also include certain cortically projecting GABAergic or putative glutamatergic cells (Gritti et al. 1997, 2003; Manns et al. 2001), which were also shown to increase their discharge rate in association with cortical activation in anesthetized rats (Manns et al. 2000a, 2003). Collectively, W-PS active units would form the extrathalamic relay to the cerebral cortex from the brain stem reticular formation and thereby transmit impulses for the widespread cortical activation that accompanies the states of both W and PS. Indeed, specific chemical stimulation of basal forebrain neurons, including cholinergic, evokes high-frequency gamma activity and theta activity in association with waking and PS (Cape and Jones 1998, 2000; Cape et al. 2000). Conversely, neurotoxic lesions or pharmacological inactivation of basal forebrain neurons diminishes cortical activation or gamma and theta along with the states of waking and PS (Berntson et al. 2002; Cape and Jones 2000; Stewart et al. 1984), thus clearly demonstrating the importance of these W-PS-active units in stimulating cortical activation along with these states. As first shown in anesthetized rats, the stimulation of cortical theta activity may also occur by direct modulation from rhythmically discharging basal forebrain units (Manns et al. 2003). Indeed, such rhythmic discharge that was cross-correlated with theta activity was observed in specific W-PS active units (particularly of group 11: w2P, ~5%) here in the head-fixed rats. Given their discharge properties, some of these units could correspond to cholinergic neurons, which discharge rhythmically in high-frequency bursts (Manns et al. 2000b); however, others could correspond to GABAergic or glutamatergic neurons, which also discharge rhythmically in lower frequency clusters of spikes (Manns et al. 2000a, 2003). Their precise chemical identity will be examined in future focused studies. As rhythmic pacemaker neurons, they may allow the basal forebrain to function in an autochthonous manner together with the MS-DBB to stimulate theta activity and perhaps initiate states of attentive, active waking or PS.

Role of basal forebrain in sleep-wake state and cortical modulation

This study reveals highly diverse basal forebrain neurons that collectively have the capacity to modulate cortical activity, behavior, and/or other processes across the sleep-waking cycle. The diversity of function of the basal forebrain is not surprising given the diversity of structure, whereby the population comprises neurons containing ACh, GABA, or glutamate as neurotransmitters along with somatostatin, neuropeptide Y, or galanin as neuropeptides (Duque et al. 2000; Zaborszky et al. 1991), and also comprises neurons projecting to different targets including the cerebral cortex, the posterior hypothala-
mus and brain stem, and local neurons (Gritti et al. 1997, 1999, 2003; Grove 1988; Zaborszky and Duque 2000). From the current physiological data together with the immunohistochemical and neuroanatomical data, the functionally and chemo-neuroanatomically different cell subgroups appear to be intermingled through the basal forebrain. They include W-active neurons that may be primarily involved in facilitating motor activity or physiological processes underlying behavioral arousal. They include neurons that are most active with cortical slow wave activity and thus potentially promoting SWS. They include a majority of neurons that are active during PS, some perhaps involved in decreasing or inhibiting muscle tone, but all potentially involved in facilitating cortical activation. W-PS-active units would have the capacity to stimulate high-frequency gamma activity and also rhythmic theta activity in the cerebral cortex during PS and wake. The major role attributed to basal forebrain cholinergic and other neurons in cortical plasticity and memory (Damasio et al. 1985; Kilgard and Merzenich 1998; McLin et al. 2002; Wenk 1997) could thus be fulfilled while stimulating cortical activation during waking but perhaps more consistently during PS, when the large majority of units here discharged at their highest rate. Indeed, recent evidence suggests that memory consolidation may be enhanced during the state of REM sleep (Stuckgold et al. 2001). This diverse population of neurons thus has the capacity to stimulate waking and arousal, to promote sleep and rest, and to generate that extraordinary paradoxical state of sleep when the body rests while the brain works, perhaps to consolidate memories while fashioning images known as dreams.

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Present addresses: M. G. Lee, Department of Pharmacology, School of Medicine, Kyungpook National University, Dong-In 2-101, Daegu 700-422, Korea; I. Manns, Max-Planck-Institut für Medizinische Forschung, Abteilung Zellphysiologie, Jahnstrasse 29, 69120 Heidelberg, Germany.

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