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SLEEP-WAKE MECHANISMS AND BASAL FOREBRAIN CIRCUITRY

Laszlo Zaborszky [1] and Alvaro Duque [2]

[1] Center for Molecular and Behavioral Neuroscience, Rutgers, The State University of New Jersey, Newark, NJ, [2] Department of Neurobiology, Yale University, School of Medicine, New Haven, CT

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1. ABSTRACT

The seminal studies by von Economo in humans (1) and by Nauta (2) in rats implicated specific basal forebrain areas at the preoptic level as important in sleep regulation. In the last two decades, studies employing recording of single neurons and monitoring of sleep parameters with subsequent chemical and electron microscopic identification of the synaptic input-output relations of these recorded neurons, provided an increasingly detailed understanding of the function of specific neurotransmitters and corresponding chemically specific neuronal circuits in the forebrain in relation to sleep-wake states. In this review, first the electrophysiology of cholinergic and parvalbumin-containing GABAergic basalo-cortical projection neurons is described, followed by an examination of possible functional interconnections between basal forebrain neuropeptide Y- (NPY) and somatostatin-containing putative interneurons and cholinergic projection neurons. A survey of various inputs to basal forebrain neurons that show state-related changes is then discussed in relation to their possible effects via basal forebrain circuitry on cortical activity. This treatise suggests that cholinergic and GABAergic projection neurons of the basal forebrain are anatomically in a unique position to enable the channeling of specific cellular and homeostatic states from different subcortical systems to the cortical mantle to modulate behavioral adaptation and cognitive functions.

2. INTRODUCTION

In 1975 Hobson and his colleagues (3), proposed a model to explain the alternating rapid eye movement (REM) and non-REM (NREM or slow wave sleep=SWS) sleep states by suggesting that the underlying mechanism is a reciprocal interaction between REM-on (cholinergic) and REM-off (serotoninergic and noradrenergic) cells in the brainstem. Over the subsequent years many details of the sleep-wake control systems have been disclosed as summarized in two recent reviews by Allan Hobson and his coworker (4-5). Sleep is a complex phenomenon: it is characterized by specific cortical electroencephalographic (EEG) waveforms and synchronized electrical activity (oscillations) in large scale networks, particularly in the corticothalamic system (Steriade, this volume). It is

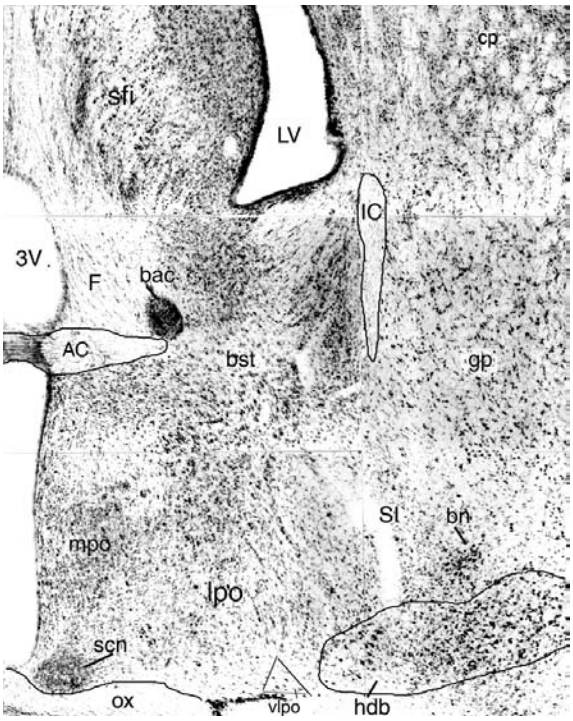

Figure 1. Photomontage of a Nissl-stained coronal section at the level of the caudal crossing fibers of the anterior commissure. Triangular box delineates neurons in the caudal portion of the ventrolateral preoptic nucleus (vlpo). Abbreviations: AC= anterior commissure; bn= basal nucleus of Meynert (see text for explanation); bac=bed nucleus of the anterior commissure; bst= bed nucleus of the stria terminalis; cp= caudate putamen; F= fornix; gp=globus pallidus; hdb= horizontal limb of the diagonal band; IC= internal capsule; LV= lateral ventricle; mpo= medial preoptic nucleus; lpo= lateral preoptic area; ox=optic chiasm; scn= suprachiasmatic nucleus; sfi= septofimbrial nucleus; SI substantia innominata; 3V= third ventricle.

assumed that sleep-wake transitions are accomplished by coordinated interactions between neural circuits of the hypothalamic circadian, the mesopontine ultradian REM-NREM oscillators, and GABAergic neurons in the preoptic-anterior hypothalamic region. Changing levels of adenosine, cytokines, growth factors and other substances, acting via specific receptors in these circuits, mediate the homeostatic sleep pressure (see corresponding chapters by Krueger, Obal, Porkka-Heiskanen and Borbely, this volume). This intermittent sleep pressure gives rise to sleep-wake cycles which are modulated by activity of the brainstem and forebrain arousal systems that use noradrenaline, serotonin, histamine, acetylcholine and orexin/hypocretin among others as their transmitters.

Recent interest in basal forebrain (BF) research was prompted by discoveries in the late seventies and early eighties showing that a specific population of neurons in this region, namely those that use acetylcholine (ACh) as their transmitter and project to the cerebral cortex, are seriously compromised in Alzheimer's disease (6-9). In addition, studies using single unit recordings in the BF and adjacent preoptic-anterior hypothalamic regions in combination with EEG monitoring indicated that neocortical activation critically depends on corticopetal inputs from these regions (10-15). *In vivo* recordings of single neurons, in combination with EEG and transmitter identification of the recorded neurons opened the way to determine the function of specific cell types in modulating cortical activity (16-19). These studies have provided a preliminary view of the dynamic interplay between BF and cortical circuits as well as possible interactions among specific cell types within the BF. This paper will review the effect of various inputs on BF circuitry and the possible consequence on cortical activity.

3. BASAL FOREBRAIN: PRESENCE OF HETEROGENEOUS CELL POPULATIONS

Figure 1 shows a Nissl-stained coronal section in the forebrain of the rat at the level of the caudal crossing fibers of the anterior commissure. At this level several structures that are important in sleep regulation can be identified including the medial and lateral preoptic areas, ventrolateral preoptic nucleus, that contain sleep-activating neurons (3, 4) and the suprachiasmatic nucleus, the circadian pacemaker. As subsequent figures will disclose, this general area is populated by cholinergic and GABAergic corticopetal neurons and various interneurons that interdigitate in a complex fashion. Cholinergic and non-cholinergic neurons, however, are distributed in extended forebrain territories across several different cytoarchitectonic areas, including the medial septum, ventral pallidum, diagonal band nuclei, substantia innominata (SI), and peripallidal regions (Figs. 2 and 3). In this chapter, the term BF is applied to those territories of the forebrain that contain cholinergic projection neurons. According to this definition, cholinergic cells in the medial septum/vertical diagonal band (*ms/vdb*) complex, that project primarily to the hippocampus, are part of the BF. In reference to the clinical literature, cholinergic neurons in the horizontal limb of the diagonal band (*hdb*), SI and peripallidal areas projecting to neocortical areas are often equaled with the term basal nucleus of Meynert. It is beyond the scope of this paper to dwell on nomenclature issues and the reader is referred to two reviews (20, 21). As discussed elsewhere (22, 23), cholinergic neurons in rodents establish a more or less continuous band extending from the rostral pole of the septum to caudal peripallidal areas, where any demarcation would be artificial. Within this space, however, cholinergic neurons show higher and lower densities and it is possible that the high density clusters in rodents correspond to aggregates of cholinergic neurons in primates (23). Some of these high density locations are labeled in Figures 2 and 3 as *bn*. In the same cytoarchitectonically defined territories in addition to cholinergic neurons, a heterogeneous population of neurons is present. Figure 3 schematically shows in four coronal

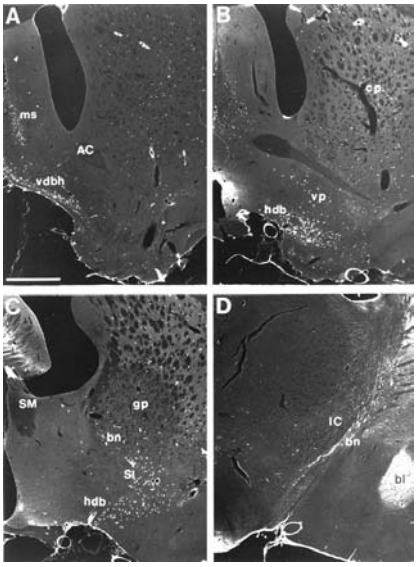

Figure 2. Dark-field photomicrographs of rostro-caudal (A-D) series of coronal sections stained for choline acetyltransferase to show the distribution of cholinergic neurons. Abbreviations: bl= basolateral nucleus of the amygdala; ms= medial septal nucleus; vdbh= horizontal part of the vertical limb of the diagonal band; vp= ventral pallidum; SM= stria medullaris. Reprinted from (55).

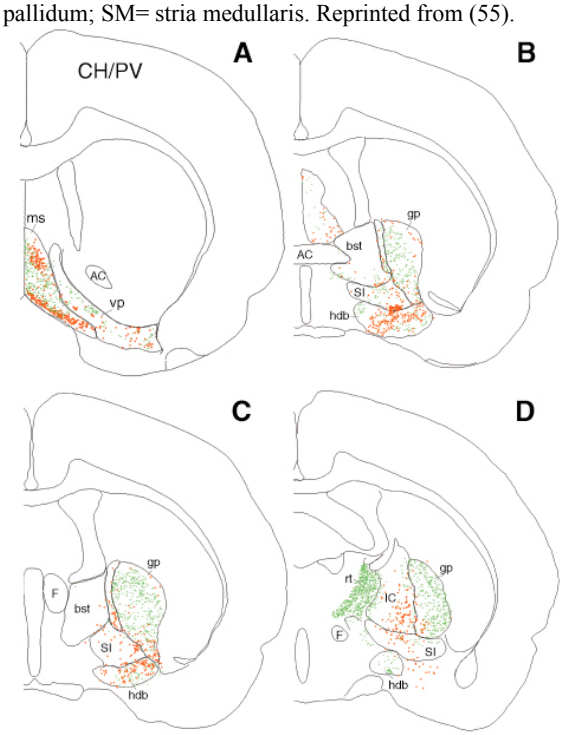

Figure 3. Distribution of cholinergic (red), and parvalbumin-containing (green) neurons at four rostro-caudal levels in the forebrain. The Nissl-stained section shown in Figure 1, was prepared from the same brain and fits between levels B and C. These maps were generated by superimposing the original Neurolucida® maps plotted from sets of sections alternately stained for choline acetyltransferase and parvalbumin, using landmarks and alignment techniques. rt= reticular thalamic nucleus.

sections the distribution of parvalbumin (PV) neurons (green) that are used to label a subpopulation of GABAergic neurons. According to tracing studies many of these PV-neurons project to the cerebral cortex and hippocampus (23, 24). In addition to PV, two additional calcium-binding proteins: calbindin and calretinin are used to label non-cholinergic neurons in the BF. Both calbindin and calretinin-containing neurons project to the cerebral cortex albeit in fewer number than cholinergic or PV-containing neurons (23, 24). At present it is unclear what is the transmitter in these two neuronal populations. Although two-dimensional sections do not show a clear organization of the various cell populations, computational studies (Figure 4) that apply spatial and numerical constraints on 3-D mapped cells suggest that individual BF neuronal populations form partially segregated bands that are twisted around each other (22). Figure 4E proposes a scheme showing that the BF is composed of several, partially segregated projection bands that can be further subdivided into chemically specific mini-bands. The functional consequence of this new aspect of organization has not been explored but should be dealt with in future electrophysiological mapping studies. Cortically projecting cholinergic and GABAergic neurons are intermingled with different types of interneurons, including those which contain the peptides somatostatin and neuropeptide Y (NPY), as displayed in Figures 5 and 6. For delineation of the BF as defined above, in Figures 3 and 5, cholinergic neurons are also depicted in red. Despite the systematic arrangement of cholinergic and PV cells from rostral to the more caudal areas, there are important physiological and anatomical differences in the BF rostro-caudal axis. For example, NPY and somatostatin interneurons are less numerous in the *ms/vdb* complex than in more caudal areas. Also, the intrinsic electroresponsiveness of cholinergic neurons in the septal area is different from those that are located in more caudal BF regions (25). In our deliberations we will focus primarily on this caudal, cortically projecting component of the BF.

4. DIVERSE ELECTROPHYSIOLOGY OF BASAL FOREBRAIN NEURONS

Data correlating cortical EEG with discharge profiles of BF neurons (10, 11, 26, 27) together with electrophysiological evidence that ACh acts as a slow excitatory neurotransmitter in the neocortex (28) have been taken as support for the hypothesis that BF neurons would relay the activating influence from the brainstem reticular formation to the neocortex (29-33). Discrepancies between the effect of selective and non-selective lesions of the BF cholinergic system (34), as well as the complex effect of stimulations in this region on cortical multiunit activity (35) suggested that in addition to the cholinergic neurons, other neural components from this region must participate in the regulation of cortical activity. Due to the anatomical complexity of this region, establishment of unequivocal electrophysiological signatures of the different BF neurons was not possible until efforts were made that allowed the identification of their chemical characteristics.

4.1. Unanesthetized animals

Szymusiak and McGinty (26), in freely moving cats, divided neurons in the *hdb*, lateral preoptic area, SI and olfactory tubercle into three groups: waking-active,

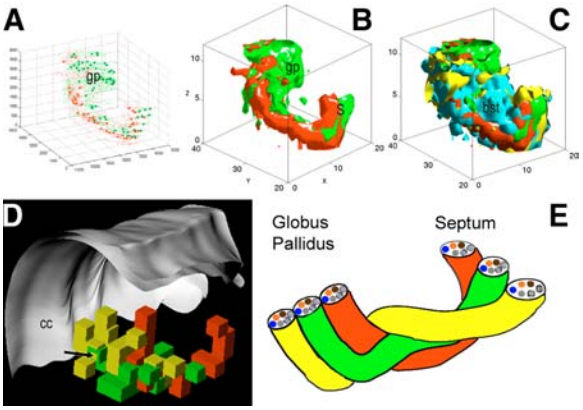

Figure 4. Computational models of the basal forebrain cell populations. **A**: Differential density scatter plot to show the spatial distribution of cholinergic (red dots) and parvalbumin (green dots) containing neurons in the basal forebrain. Filled circles mark the high-density locations where the density of cholinergic or parvalbumin cells is higher than 15 in the unit space (250 x 250 x 50 µm). Data from this and subsequent panels (B and C) are derived from a brain alternately stained for choline acetyltransferase, parvalbumin, calretinin and calbindin. Cells were plotted using a computerized microscope and the Neurolucida® software. Axis scaling is in µm corresponding to the *x*, *y* and *z* coordinates according to the Neurolucida® database. **B**: Combined iso-density surface rendering of cholinergic (red) and parvalbumin (green) neurons. Iso-color surfaces are rendered around volumes that contain ≥ 5 cells per unit space (250 x 250 x 50 µm). Note that the bulk of parvalbumin-containing cells are located medially from the cholinergic cells in the septum (S) but laterally in the globus pallidus (gp). The numbers in the box plots represent voxel (unit space) indices along the *x* and *y* axis. Numbers along the *z* axis mark the set of four sections that were combined into composite 'layers'. *x*-axis: medio-lateral, *y*-axis: antero-posterior, *z*-axis: rostro-caudal. **C**: Combined iso-density surface rendering of cholinergic (red), parvalbumin (green), calretinin (yellow), and calbindin (blue) neurons. Looking from a three-dimensional perspective, these four cell types seem to occupy longitudinal, obliquely (lateromedially) oriented 'U'-shaped lamellar or band-like subspaces. The different cell sheets seem to be twisted and attached to each other in a complicated fashion, however, closer observation suggests that the four cell sheets display a pattern of association in the entire basal forebrain. **D**: The differently colored boxes (500 x 500 x 50 µm) in the basal forebrain are derived from three individual animals that received retrograde tracer injections along specific longitudinal stripes in the parietal/frontal cortex. Red: medial strip of the cortex along the M1/M2 region; green: the S1 barrel cortex; yellow: around the rhinal sulcus. The boxes represent spaces where non-cholinergic projection neurons are represented by at least 6 neurons. As can be seen only one box (arrow) is shared among the three animals, suggesting that specific medio-lateral stripes of the neocortex receive segregated input from the basal forebrain. **E**: Schematic illustration to show the relationship of the three projection bands from panel (D) and the notion that each of these bands may contain several transmitter-specific sub-bands. Panel A, D, E from Zaborszky (81). cc=corpus callosum.

(i.e., waking discharge rates were > 2 times NREM sleep rates); state-indifferent and sleep-active. In this latter group the discharge rates of cells during alert waking were low, and maximal discharge rates occurred during NREM sleep. The increased discharge of sleep-active cells anticipated the onset of NREM sleep. Although there was a considerable overlap in the location where these three cell populations were found, it was noted that sleep-active neurons tended to be restricted to more ventral areas in each of the investigated BF regions. Since atropine (1.5 mg/kg, i.p.) resulted in an increase of firing rates during NREM of sleep-active neurons and assuming that cholinergic neurons are modulated by inhibitory autoreceptors, it was suggested that the group of sleep-active neurons might use ACh as their transmitter (26). In another study, Detari *et al.* (36) similarly, in freely moving cats, recorded neurons primarily at the borders between putamen/globus pallidus and globus pallidus/ internal capsule. These neurons were divided into five groups on the basis of the modifications of firing rates and patterns during the entire sleep-wake cycle. Two of these groups (#3, #4) were characterized by a strong increase of firing rates in transition from SWS to quiet wakefulness (W) or REM sleep. In one of these groups (#3, n=27), that was characterized by regular firing in W and REM and by bursty pattern in SWS, 66% of the neurons showed an increase of firing at the SWS→REM transition that preceded the cortical activation by an average latency of 26 sec. In this study, only a small percentage of neurons were found that increased their firing during SWS. Neurons with the latter characteristics were found by these investigators (37) also in the olfactory tubercle in an earlier study. Although, no attempts were made to identify the transmitter character of the neurons in either study, Detari *et al.* (36) suggested that the wake-active neurons (group #3) belong to cholinergic projection neurons.

The first study in unanesthetized rats was done by Buzsaki *et al.* (11). In this study, recordings were performed only during wakefulness and drowsiness. It was found that neuronal firing increased more than twofold in the majority of BF cells during transitions from standing still to drinking and running. More recently, in freely moving rats, wake-active and sleep-active neurons were recorded in relation to their thermosensitivity in BF areas (38). Furthermore, Szymusiak and colleagues (33) also investigated the action of adenosine and GABA receptor antagonists on the state-dependent discharge pattern of BF neurons; however, the transmitter character of the recorded neurons was not identified.

4.2. Anesthetized animals

Neurons in anesthetized rats with faster firing rates during fast cortical EEG activity were termed F-cells, to distinguish them from their counterparts, the S-cells, whose firing rates increase during high amplitude slow cortical EEG activity (32). It was noted that there is a great diversity among F- and S-cells in terms of conduction velocity, spontaneous and evoked neuronal activity, and timing of changes in unit activity in relation to EEG activity, indicating that F- or S-cells are far from being homogeneous cell populations. In addition, there are also

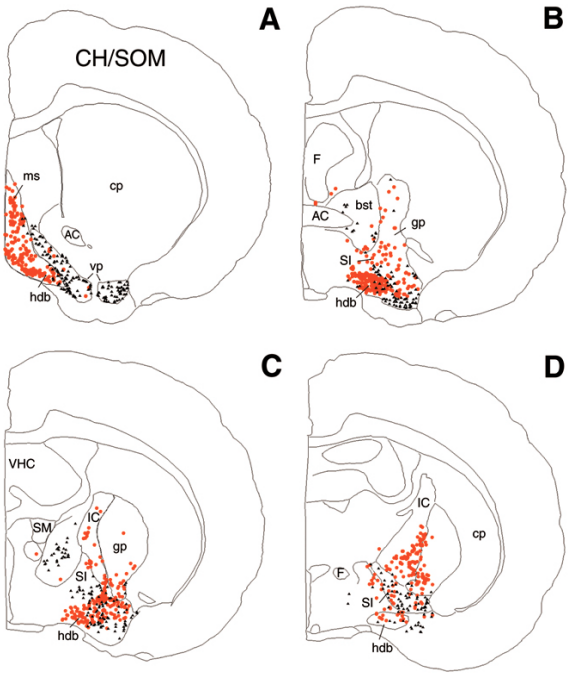

Figure 5. Distribution of cholinergic (red) and somatostatin (black) containing neurons at four rostro-caudal (A-C) coronal levels plotted from a rat brain that was double-stained for choline acetyltransferase and somatostatin. VHC= ventral hippocampal commissure.

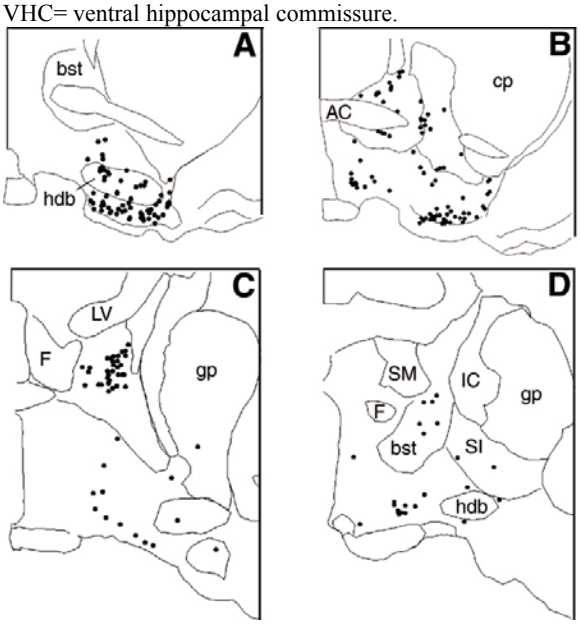

Figure 6. Distribution of neuropeptide Y-containing neurons at four rostro-caudal levels in the rat forebrain. Each dot represents one cell body.

neurons whose activities remain unchanged despite EEG cortical changes (15).

After our initial study of the electrophysiology and morphology of BF non-cholinergic neurons using the juxtacellular labeling technique (39, 40), we and others (16-18) combined this technique with EEG monitoring in anesthetized rats with subsequent immunohistochemistry in order to identify the transmitter of the recorded neurons. Cholinergic neurons were found to correspond to some of the F-type neurons reported earlier in that they show increased action potential firing during cortical EEG activation. In addition to characterizing cholinergic neurons, our study (16) identified for the first time the EEG correlation of PV-containing, putative GABAergic neurons. PV neurons, similar to cholinergic neurons, had a strong positive correlation with EEG activation, indicating that there are also PV cells among the F-cells. Since GABAergic basalo-cortical axons were found to terminate exclusively on cortical GABAergic interneurons (41), our finding is compatible with the notion that at least a subpopulation of PV-containing basalo-cortical neurons promotes functional activation in the cerebral cortex by disinhibition (35).

Manns and coworkers (17, 18) emphasized the rhythmicity of cholinergic neurons and their possible participation to promote cortical theta and gamma rhythmicity. We (16) noted that cholinergic neurons did not show robust bursting during spontaneous or stimulation induced EEG activation. In fact burst firing was not characteristic for any cell type identified in our study. Furthermore the stimulation-induced rhythmic burst firing of a PV neuron in our study was remarkably similar to the cholinergic neurons described by Manns *et al.* (17). These findings indicate that a more extensive database of identified cells is needed before electrophysiological criteria can be used to reliably distinguish different BF cell types. Also, a preliminary study (42) noted that theta and beta/gamma local field potentials in the BF occurred often independently of such rhythms in the hippocampus and olfactory bulb. Thus, it remains to be determined to what extent BF network activity contributes to various rhythmicities in cortical areas across the sleep-wake cycle. In this respect it is relevant to mention that selective lesions of BF cholinergic neurons with 192-IgG-saporin did not change the total amount spent in wakefulness, but caused a transient (7-8 days) impairment of the circadian distribution of sleep-wakefulness, indicating that BF cholinergic neurons are not necessary for the maintenance of wakefulness but play an enabling role (43-45). On the other hand, the power of EEG was suppressed in each frequency band after 192-IgG-saporin, suggesting that cholinergic BF neurons contribute to the generation of normal EEG independent of sleep regulation (45).

A major unresolved issue is whether ascending basalo-cortical neurons also contain glutamate as their transmitter. Although various attempts were made to label putative BF glutamatergic neurons, including immunolabeling with phosphate activated glutaminase (46, 47), only the recently cloned vesicular glutamate transporters can reliably label neurons that use glutamate as transmitter (48). We, and others (49-51), have recently described neurons in the BF that express a specific vesicular glutamate transporter, Vglut2. Although a large number of cells in BF areas express Vglut2 mRNA, whether or not these glutamatergic neurons project to the cortex and what their function is in relationship to the sleep-wake cycle remains to be determined.

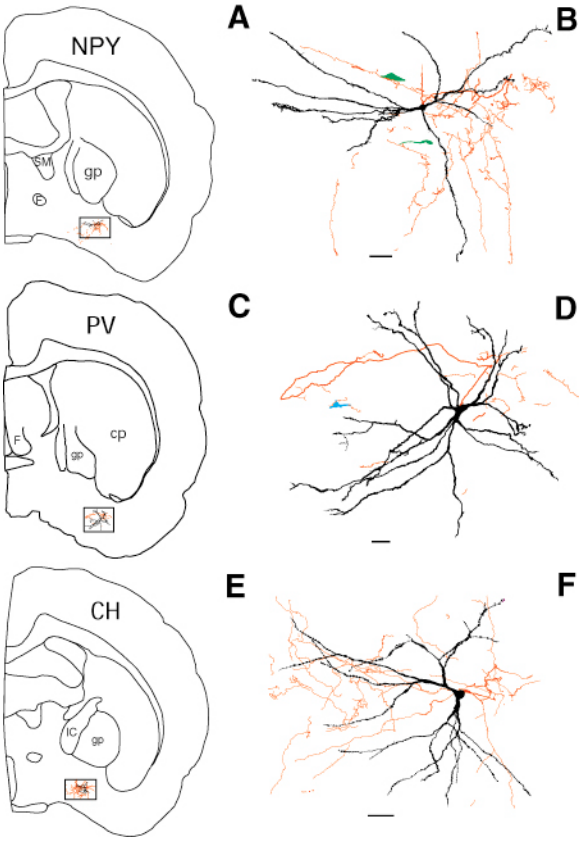

Figure 7. Partial reconstruction of juxtacellularly filled neuropeptide Y (NPY), A-B; parvalbumin (PV), C-D and cholinergic neuron (CH), E-F. Cell body and dendrites are black, axon collaterals in red. Scale bar: 50 µm. The boxed area in each map shows the approximate location of the respective neuron. In (B) green symbols represent two cholinergic cell bodies that are approached by axon-collaterals from the NPY neuron. In the case of the lower cholinergic cell, electron microscopy revealed that the NPY axon established synaptic contact with the cholinergic dendrite. A cholinergic cell body is indicated by blue symbol in (D) that is approached by a PV-containing axon-collateral.

5. LOCAL PROCESSING IN THE BASAL FOREBRAIN

5.1. GABAergic neurons

Using combined microdialysis/unit recording methodology, Alam *et al.* (52) have shown in freely moving rats that neurons in the BF are under various degree of GABAergic inhibition across the sleep-wake cycle. Sources of GABAergic input include axon-collaterals of basalo-cortical PV-GABAergic neurons (Figure 7D), GABAergic neurons of the nucleus accumbens (53) and other local GABAergic neurons, including those located in the preoptic-anterior hypothalamic regions (54). Increased activity of preoptic neurons during sleep onset has been hypothesized to mediate, in part, the sleep-related suppression of BF wake-active neural discharge (33).

5.2. Somatostatin

Somatostatin a 14- or 28-amino acid-containing neuropeptide has been identified in synapses on cholinergic projection neurons (55). A portion of these somatostatin-containing terminals may originate from local neurons distributed mainly in the ventral pallidum, SI and around the *hdb* as shown in Figure 5. Systemic or intracerebroventricular injections of octreotide, a somatostatin analog, suppress SWS for 1 hr followed by slight increases of SWS 2-3 hrs postinjection (56, 57). It is suggested that the octreotide-induced SWS inhibition is mediated by the inhibition of growth hormone-releasing hormone (GHRH) neurons in the hypothalamus or in the preoptic area, where hypothalamic GHRH axons terminate and where microinjection of GHRH promote sleep (58, 59). Systemic octreotide treatment also enhances REM sleep (56) and an antiserum against somatostatin administered in the brainstem solitary nucleus blocks the REM-promoting activity of locally applied carbachol (60), indicating that the somatostatin effect on REM is mediated via intracerebral action. Using *in vitro* patch clamp techniques, our preliminary studies suggest that somatostatin inhibits GABAergic miniature postsynaptic currents in BF cholinergic neurons (Momiyama and Zaborszky, in preparation). These data are compatible with the idea that the effect of intraventricularly applied octreotide is mediated in part via inhibition of the GABAergic/cholinergic (61) interaction in the BF. It would be interesting to know if BF somatostatin neurons also show sleep-related activity and how they influence the firing rate of cholinergic neurons.

5.3. Neuropeptide Y neurons

In addition to neurons that increase their firing during cortical activation, several studies described the presence of BF cells that reduced their firing rate during cortical EEG activation in anesthetized rats (18, 32). These so-called S-cells were suggested to be local GABAergic interneurons, as they could not be activated antidromically from the cortex (32). We identified several functionally S-type cells (n=9), some of which (n=4) were stained positively for NPY (Figure 7A). Although in immunostained material the number of NPY neurons is relatively low (Figure 6), their function might be significant because they possess abundant axon collaterals, some of which enter into synaptic contacts with cholinergic profiles (Figure 7B). Although NPY axons have been described innervating cholinergic neurons in bulk immunolabeled material (19, 62), these studies could not ascertain that the source of NPY-containing synapses on cholinergic neurons indeed originate from local neurons. In the lack of *in vitro* studies, it is unclear how NPY neurons affect the firing properties of cholinergic neurons. However, considering their opposite firing relation to EEG activation as compared to that of cholinergic neurons, the possibility that they contain GABA (63), and the conditions required for release of neuropeptides (64), it is likely that burst firing of NPY neurons could result in a pronounced modulation of GABAergic-cholinergic transmission at least in the ventral pallidum where cholinergic cell bodies are richly innervated by GABAergic terminals (61). It is unknown how many cholinergic neurons are contacted by a single

NPY neuron or what are the other postsynaptic targets of the local NPY axon-collaterals in addition to cholinergic neurons since in the axonal arborization space of a single NPY neuron, large numbers of non-cholinergic neurons exist. These neurons include PV and other calcium-binding protein-containing neurons, some of which may contain glutamate (see Figure 9 in ref. 22).

5.4. Cholinergic local interaction

Several studies in brain slices containing the septum and the diagonal band have documented that cholinergic neurons are capable of releasing ACh locally in an impulse-dependent manner (65-69). We have recently shown that cholinergic projection neurons indeed possess extensive local collaterals (16, 19 and Figures 7E-F), whose postsynaptic targets remain to be established. Because of the paucity of cholinergic synapses on cholinergic neurons (70), it is expected that cholinergic-cholinergic interactions in BF areas are limited. Carbachol (a cholinergic agonist) injections in the lateral preoptic area and SI, in dogs and cats, significantly reduced SWS and increased wakefulness (71, 72). Also, it was found that microinjections of carbachol into the BF significantly reduced the ability of simultaneously injected carbachol into the pons to elicit REM sleep (72). Since cholinergic neurons are hyperpolarized by muscarine (73) and muscarinic receptor agonists strongly excite non-cholinergic, presumably GABAergic neurons in BF slices (74), it is unlikely that corticopetal cholinergic neurons were activated in the carbachol microinjection experiments. From these studies it is uncertain whether endogenous ACh shows state-dependent efflux in the BF and whether carbachol acted on preoptic GABAergic or other non-cholinergic BF neurons with descending projections to the mesopontine tegmentum (75, 76). A preliminary study in rats (77) suggested that lateral preoptic sites, from where carbachol injections induced wakefulness, receive cholinergic projections only from the mesopontine tegmentum, thus it remains to be elucidated whether ACh release from local collaterals in the BF affects the sleep-wake cycle.

6. CORTICAL INPUT TO THE BASAL FOREBRAIN

In urethane anesthetized rats, a continuous spectrum of EEG patterns appears. For the sake of simplicity, two patterns with rhythmically recurring periods of cortical activation were differentiated to provide a simple way to correlate BF unit activity (16). Using transcortical EEG electrodes, under light anesthesia, periods of low voltage fast activity (LVFA) alternate with epochs of SWS at a frequency of 0.2-0.3 Hz (pattern I). At deeper anesthesia, deep-positive inactive periods and short activations riding on deep-negative deflections alternate at a rate ≤1 Hz (pattern II). According to our study (16), in pattern I EEG, firing changes started in BF neurons earlier than seen in pattern II. At present, it is unknown where such generalized patterns are initiated. However, the data is compatible with the hypothesis that pattern I is generated by BF cells and/or transmitted from the brainstem via the BF to the cortex. On the other hand, our finding that all BF units recorded under EEG pattern II showed delayed increased firing in relation to cortical activation suggests that under these circumstances, the state of the cortex reflected in the EEG may be transmitted via descending corticofugal fibers to BF neurons. This suggestion is in line with the observations of Dringenberg and Vanderwolf (78), that stimulation of the lateral and ventrolateral orbitofrontal cortex induced bilateral LVFA and suppression of large irregular slow activity in the EEG recorded from the sensory-motor cortex. Administration of atropine, a centrally acting cholinergic antagonist, produced a partial block of this stimulation-induced LVFA.

We recently described the termination of cortical axons in the BF using correlated light- electron microscopic methods (79). This study confirmed an earlier suggestion (80) that the cortical feedback to the BF originates only from a restricted portion of the cortex, including prefrontal, insular, and piriform cortices. Furthermore, we found that prefrontal axons terminate exclusively on non-cholinergic cells, including PV-containing GABAergic neurons. In addition to PV neurons, we noted at least another unidentified cell population that received prefrontal input. It would be important to investigate whether NPY or somatostatin neurons are among the neuronal populations that receive input from the prefrontal cortex and may mediate the prefrontal stimulation induced EEG effects described above, at least in part via cortically projecting cholinergic or GABAergic neurons. It is unclear whether this prefrontal feedback to the BF has any role in sleep regulation or if it contributes to selective activation of cortical regions during various cognitive operations as proposed in recent reviews (23, 81).

7. BRAINSTEM INPUT TO THE BASAL FOREBRAIN

7.1. Noradrenergic-adrenergic effects via the basal forebrain on cortical arousal

Noradrenaline (NE) has long been considered an important modulator of cortical EEG and behavioral arousal. Jouvet (82) suggested that NE is necessary for the normal maintenance of tonic EEG activation. During wakefulness, the discharge rates of the noradrenergic locus coeruleus (LC) neurons are closely tied to the state of arousal. During NREM sleep, LC neurons in rats, cats and monkeys show a progressive decrease in firing rate and become nearly silent before the onset of REM sleep (3, 83-86). The noradrenergic innervation of the forebrain originates in the LC, and electrical or chemical stimulation of the LC area is effective in shifting the electrocorticogram (EcoG) or hippocampal EEG in anesthetized rats from low-frequency-high amplitude to high frequency-low amplitude activity. This effect is blocked by the alpha-2 agonist, clonidine (86). On the other hand, bilateral lesions of the LC in cats, with a consequent 85-95% depletion of cortical NE, resulted in control values of EEG activation immediately after recovery from surgery (87), suggesting that the role of LC is largely enabling rather than maintaining the wake pattern as suggested originally by Jouvet.

A scenario that LC-NE acts via BF cholinergic neurons to influence cortical activity is suggested by our

anatomical studies showing dopamine-beta-hydroxylase-containing synapses on cholinergic dendrites that originate in part from the LC (88, 89). Functional studies, as described below, corroborate this notion. For example, high frequency stimulation (100 Hz, 1 ms pulses, 20 μA) of the LC area in urethane-anesthetized rats produced EcoG activation in the neocortex and hippocampus, and these effects are abolished by systemic treatment with the anti-muscarinic drugs scopolamine or atropine (27). Single pulse stimulation of the LC area enhanced discharge of the majority of F cells in the BF and inhibited a significant portion of S cells (23, 78). An amphetamine-induced increased turnover rate of cortical ACh was also abolished by intraseptal administration of the alpha-adrenergic blocker phenoxybenzamine (90, 91). Furthermore, NE microinjections into the BF in freely moving rats produced a dose-dependent increase in gamma activity, a decrease in delta activity and an increase in waking (92). These effects on cortical EEG activity were interpreted by Jones and coworkers as being attributable to the depolarization and excitation of cholinergic BF neurons by NE as had been demonstrated previously in *in vitro* studies (93).

Recently, we have described that adrenaline-containing neurons also innervate BF cholinergic neurons (94). Although an effect of adrenaline on cortical arousal has been suggested ever since the description of the brainstem adrenaline containing cell groups (95), studies are lacking to support this notion. Histochemical evidence for the existence of several types of adrenergic receptors in the BF has been brought forward by autoradiographic receptor-binding, immunocytochemical, and *in situ* hybridization techniques (94). However, no attempt was made to identify the cellular localization of such receptors. In our preliminary studies (96) $alpha_{2A}$-adrenergic receptors were found to be localized in a subpopulation of BF cholinergic as well as in a substantial number of non-cholinergic neurons. Since $alpha_{2A}$-adrenergic receptors are associated with neuronal inhibition, NE (or adrenaline) may disinhibit the GABAergic/cholinergic link, thereby causing a facilitatory action on cholinergic neurons. This indirect excitatory effect of NE/adrenaline may be paralleled by a direct excitatory action through putative $alpha_1$ adrenergic receptors on cholinergic neurons as suggested by Fort *et al.* (93). Thus, considering the presence of different neuronal populations among the electrophysiologically identified F and S types of neurons and the various adrenergic receptors, NE/adrenaline may affect cortical arousal via the BF according to a complicated cellular mechanism that awaits to be determined in future studies.

7.2. Basal forebrain in mediating serotoninergic effect on cortical arousal

The activity of dorsal raphe (DR) neurons, one of the main sources of serotoninergic (5-HT) innervation of the forebrain, shows state-dependent changes across the sleep-wake cycle. The firing of putative 5-HT neurons shows wake-active REM-off discharge characteristics: in both cats and rats the activity of these neurons declines as the animal becomes drowsy and declines even further in SWS. Finally, the activity of putative DR-5HT neurons becomes virtually silent in REM sleep (97-100). There is *in vitro* intracellular evidence to suggest that 5-HT produces cortical activation by enhancing the membrane excitability of neocortical pyramidal neurons (101, 102). Finally, 5-HT-dependent cortical activation is not affected by cholinergic, dopaminergic, noradrenergic, (103) or histaminergic blockade (104), suggesting that the activating effect of 5-HT release is exerted by its direct action in the cortex. Recent studies in unanesthetized rats suggest that DR neurons have enabling effects on cortical arousal, since altering discharge rates of DR neurons did not result in changes in the vigilance state (99, 100).

An indirect effect of the raphe-serotonin neurons on the cortex via BF neurons was also suggested by both electrophysiological and anatomical studies. Microinjection of serotonin into the BF induces a decrease in the gamma component of cortical EEG in unanesthetized rats (92). This EEG effect was attributed to the hyperpolarization and inhibition of cholinergic neurons by 5-HT, as shown earlier in *in vitro* guinea pig slices (105). *In vivo* experiments in which the DR was stimulated showed a more complex scenario. Single pulse stimulation of the DR produced excitatory or inhibitory responses with latencies ranging from 3-26 ms in about 20% of cortically projecting BF neurons tested in barbiturate-anesthetized rats (106). In a more recent study in which F and S cells in the BF of urethane–anesthetized rats were recorded, Detari *et al.* (107) found that single pulse DR stimulation elicited a short-latency, short-duration excitation in the majority of both F and S cells, although in a few F and S cells the initial reaction was inhibitory. Interestingly, tail pinch induced excitation in most of F cells and in half of the S-cells while the other half of the S-cells were inhibited. LVFA induced by noxious stimulation, such as tail pinches, is abolished by 5-HT antagonists (108), suggesting that neocortical activation due to noxious stimulation depends on the DR/5-HT system. The interpretation of the effects of stimulation is complicated by the presence of different cell types in the DR: only about one third of the neurons contain 5-HT, and they are mixed with dopaminergic, GABAergic and peptidergic neurons (109-112). Thus, these neuronal populations may have been co-activated by electrical stimulation.

Previous preliminary anatomical (113) and biochemical (114) studies from this laboratory provided evidence that the BF might be a relay station conveying neural information from 5-HT cells toward cortical areas. However, there is no agreement about the potential postsynaptic sites. Light microscopic studies (113, 115) suggested that cholinergic cells may be among the BF targets, but an electron microscopic study in the rat septum failed to identify serotoninergic synapses on cholinergic neurons (116). On the other hand, 5-HT fibers formed synapses with PV-containing- GABAergic neurons in the septum (116). Additionally, it was found in a preliminary study that 5-HT axons synapse on calretinin-containing neurons in the septum and in more caudal BF areas, including the SI (117). Calretinin neurons in the septum are supposed to represent a subpopulation of GABAergic neurons, although in the supramammillary nucleus calretinin has been colocalized with glutamate (118).

According to the reciprocal interaction model for REM sleep regulation (3), 5-HT exerts an inhibitory influence on mesopontine REM-on neurons through postsynaptic 5-HT_{1A} receptors and also inhibits the firing of DR/5-HT neurons acting on somatodendritic 5-HT_{1A} autoreceptors (119-121). This same 5-HT receptor subtype is also expressed in a subpopulation of *ms/vdb* cholinergic neurons (122). Data suggests that disruption of hippocampal theta by the action of 5-HT is mediated in part via the septum (123). In the absence of 5-HT synapses on BF cholinergic neurons, however, it is unclear how a reduced 5-HT efflux through altered activation of $5HT_{1A}$ postsynaptic receptors could contribute to increased firing in cholinergic BF neurons during REM sleep.

7.3. Input to the basal forebrain from the mesopontine tegmentum

Since the seminal studies of Moruzzi and Magoun (124) and Jouvet (125) it is accepted that the pontomesencephalic reticular formation is critical for the initiation and maintenance of cortical activation during wakefulness and REM sleep. In line with these early studies, unit recordings in unanesthetized cats revealed that neurons in the pontomesencephalic tegmentum increase their firing rate before transitions from NREM to REM sleep occur (126).

The concurrent increase in cortical ACh release following stimulation of the pedunculopontine tegmental nucleus (PPT) (127) strongly suggests that BF cholinergic neurons are activated by PPT stimulation. The projection from the reticular formation to the forebrain was originally presumed to be cholinergic since the most effective sites for EEG activation were in the vicinity of cholinergic cell bodies in the PPT (126). Using microdialysis to deliver receptor antagonists to the BF, however, it was found that ACh release and cortical EEG activation evoked by PPT stimulation were not blocked by either muscarinic or nicotinic antagonists, but were greatly reduced by a non-specific glutamate antagonists, kynureic acid (127). Also, ACh inhibits BF cholinergic neurons *in vitro* (73). These findings suggest that the excitatory input to BF neurons from the PPT area may be via glutamatergic axons (128). Electrical stimulation in the mesopontine tegmentum was also used to assess their effect on the activity of BF neurons. Stimulation of the midbrain central tegmental field (ventral and lateral to the central gray matter and dorsal to the red nucleus) in freely moving cats elicited short latency orthodromic excitatory or inhibitory responses in wake-active and sleep-active neurons in BF areas, including the *hdb*, ventral globus pallidus and rostral portions of the SI, rich in cholinergic and GABAergic corticopetal neurons (129, 130). Single pulse stimulation of the PPT area produced excitation in the majority of F and S cells in the BF (107).

Cholinergic, putative glutamatergic and GABAergic neurons are codistributed within the mesopontine tegmentum, including the PPT and the laterodorsal tegmental (LDT) nuclei (131, 132). Some GABAergic cells in the PPT/LDT showed increased expression of the immediate early gene *c-fos* after REM sleep deprivation (133) and thus could correspond in part to the electrophysiologically identified REM-on (134) and/or Wake/REM-on cells (119). Retrograde tracer studies in rats indicated ascending projections from the PPT/LDT nuclei and the central tegmental field to BF areas, however, the majority of retrogradely labeled cells did not colocalize with choline acetyltransferase, the definitive marker for cholinergic neurons (135).

The nucleus reticularis pontis oralis (RPO) has been identified as an important site for generating various signs of cortical activation, including REM and hippocampal theta (123). The transmitter of these tonic movement-REM neurons apparently has not been identified. On the other hand, GABAergic neurons in the RPO showed decreased c-fos expression after paradoxical sleep deprivation and are thought to be SWS-on or REM-off cells (136). In preliminary studies, we have shown that axons arising from the RPO synapse with PV-containing neurons in the BF, however the transmitter identity of the ascending axons was not determined (23). Some of the pontomesencephalic GABAergic neurons project to the posterior lateral hypothalamus (132), or represent local interneurons and innervate neighboring monoaminergic neurons (137). In the lack of a systematic investigation at the electron microscopic level, it is unclear if putative glutamatergic, GABAergic or cholinergic projections from the mesopontine tegmentum synapse with specific neuronal types or indiscriminately contact neurons in the BF to mediate cortical arousal.

7.4. Ventral tegmental area input to basal forebrain in mediating arousal

The ventral tegmental area (VTA) is the source of dopamine (DA) containing neurons that project to the prefrontal cortex, ventral striatum, BF and hypothalamus. Neural interactions among these areas have been implicated in mediating motivated behavior (138-140). We have recently described that VTA and substantia nigra pars compacta (SNc) neurons synapse on cholinergic and PV-containing neurons in the BF and could thus transmit reward-related information to these neurons (141, 142). Although the firing rate or pattern of DA neurons in the VTA and SNc are not significantly modulated by the sleep-wake cycle or anesthetics (143, 144), the administration of DA D1 receptor agonists produces EEG desynchronization and behavioral arousal (145). Low doses of dopaminomimetic agents acting on D2-like inhibitory autoreceptors on the cell bodies or terminals of VTA neurons induce sleep (146). Also, mice with deleted dopamine transporter show increased wakefulness and decreased NREM sleep (147). Furthermore, sleep disturbances in Parkinson's disease and related disorders and their influence with dopaminergic medication suggest involvement of the dopaminergic system in sleep-wake regulation (148-150). Clearly, the effects of midbrain-dopamine on sleep mechanisms await further investigations.

Its has been recently discovered that VTA non-dopaminergic, putative GABAergic, neurons increase firing rates during active wakefulness and REM sleep, relative to

quiet wakefulness. During deprived SWS, there was a direct correlation between decreased VTA-GABA neuron firing rates and increased delta wave power (151). It remains to be definitely established whether VTA-GABAergic neuronal activity contributes to, or only reflects, cortical activity. However, it is interesting to note that neurons in the VTA project to somatostatin-containing, putative GABAergic neurons in the BF (152). Furthermore, a preliminary study indicated that VTA neurons that project to the SI and adjacent BF areas contain GABA (54). Since somatostatin-containing neurons in the BF innervate cholinergic corticopetal neurons (55), a VTA-GABAergic/BF-GABA-ACh link might represent another extrathalamic route for cortical activation.

8. AMYGDALA INPUT TO THE BASAL FOREBRAIN

It has been long known that high frequency unilateral stimulation in the amygdaloid body activates bilaterally the ECoG and induces LVFA in anesthetized cats (153, 154). This activation may be mediated by the BF since it can be blocked by lidocaine injections in the SI or attenuated by systemic administration of centrally acting muscarinic receptor antagonists (155, 156). The involvement of the BF is also supported by anatomical evidence showing that projections from the amygdala terminate in the SI (157-161). Although a pharmacological study (162) and an electron microscopic study (163) suggested the existence of glutamatergic inputs from the amygdala to the BF, another morphological study indicated the presence of inhibitory (164) inputs from the amygdala to the BF. Similarly, even though amygdalofugal axons were shown to synapse on cholinergic neurons in the ventral pallidum (163) and SI (165), the majority of amygdala efferents to the BF terminated on non-cholinergic neurons of unknown transmitter identity. In line with these seemingly contradictory results, single-pulse stimulation of the amygdala excited the majority of F cells in the BF, while inhibiting the majority of S cells (156). Interestingly, in an early study Kreindler and Steriade had shown that within the basolateral amygdala there are two antagonistic systems, one that desynchronizes and another one that synchronizes the background activity of the neocortex. Both reactions persisted following a complete midbrain transection (166). Another route by which the amygdala stimulation may participate in EEG and sleep modulation is via its projections to the lateral pontine region, as suggested by Calvo and Simon-Arceo (167).

9. HYPOTHALAMUS AND BASAL FOREBRAIN CIRCUITS INVOLVED IN SLEEP-WAKE MECHANISMS

A large body of evidence accumulated since the classical study of the Viennesa epidemic of encephalitis lethargica by von Economo (1) showing that inflammatory lesions in the posterior wall of the third ventricle caused somnolence, while lesions in more rostrally situated parts of the hypothalamus were paralleled with insomnia. Later Walle Nauta, in his elegant study in rats (2) using a series of knife cuts, suggested that neurons in the lateral preoptic/hypothalamic regions, about the level of the suprachiasmatic nucleus, have specific importance for sleep regulation ("sleep centre"). On the other hand, neurons in the posterior hypothalamus, at the mammillary region that give rise to ascending pathways to be relayed at successive rostral hypothalamic levels, are important in the maintenance of the waking state ("waking centre").

9.1. Preoptic/anterior hypothalamic area

Subsequent lesion or stimulation studies in various species (168-173) confirmed that the preoptic/anterior hypothalamic (POAH) and adjacent BF areas have a hypnogenic function. In addition, studies in cats and rats in which axon-sparing neurotoxins such as kainic or ibotenic acids were deposited into POAH and neighboring BF structures, revealed that the insomnia could be attributed to the destruction of neuronal cell bodies and not axonal fibers of passage. Both the duration and number of SWS and REM sleep episodes were reduced following these lesions, although in the chronic course SWS bouts did consolidate. A substantial cell population in the median preoptic nucleus (MnPN), medial and lateral preoptic areas and more laterally in the cholinergic rich regions of the BF in cats and rats show sleep-related discharge patterns (129, 130, 174-178). The sleep-related neurons included heterogeneous populations: SWS/REM-related neurons exhibiting activation during both phases of sleep compared to W; SWS and REM neurons exhibiting selective activation during either NREM or REM sleep, respectively. While sleep-related neurons are intermingled with wake-active neurons in extended POAH-BF areas, sleep-related neurons are concentrated in two specific regions: in the MnPN and within the ventralmost aspect of the lateral preoptic area (VLPO). Similarly to neurons in the MnPN, the firing of VLPO neurons anticipated sleep onset (176-178). Some of the sleep-active neurons that showed elevated discharge in deep SWS in the POAH may be projection neurons as they gave antidromic responses evoked from the external capsule, cingulate bundle or mesencephalic reticular formation (129). Since POAH neurotoxic lesions also impaired EEG spindling (172), it is likely that these lesions involved cholinergic or GABAergic neurons projecting to the thalamic reticular nucleus (179-181), the locus of the spindle-genesis (Steriade, this volume).

Recently, it has been shown that neurons in the VLPO (182-185) and in the MnPN (186) show c-fos activation proportional to the amount of time spent in sleep. In the VLPO the number Fos-immunoreactive neurons was proportional to SWS sleep (182, 183), while the number of Fos-immunoreactive neurons in the area extending dorsally and medially from the VLPO ('extended VLPO') was highly correlated with REM but not SWS sleep (185). Sixty percent of sleep-active neurons in the extended VLPO and 90% of sleep-active cells in the VLPO-cluster contain galanin, an inhibitory peptide, and many of these galanin-positive neurons also express GABA (184, 185). The VLPO has been shown to project to the histaminergic tuberomammillary nucleus in the posterior lateral hypothalamus (187-189). It is suggested that this particular projection would promote SWS (190). On the other hand,

GABAergic projections from the VLPO and extended VLPO to the LC and DR cell groups might promote REM sleep by inhibiting the discharge of brainstem aminergic nuclei (190). Neurons in the MnPN project to the DR-serotoninergic and LC-noradrenergic neurons (191) and to the VLPO (192). MnPN may be part of a POAH sleep-promoting network, although MnPN neurons are also involved in thermoregulation, while VLPO neurons apparently are not (186). Local warming of the POAH area resulted in inhibition in a substantial population of wake-active cells in the cat cholinergic BF areas (130) suggesting that sleep-active neurons may inhibit wake-active neurons. Our observations that symmetric, inhibitory type, synapses originating from the medial preoptic area were found on cholinergic neurons in the SI (193) are compatible with this notion. Interestingly, axons from the VLPO were also traced to the cholinergic BF area, but they seemed to avoid cholinergic cell bodies (187). It remains to be assessed in electron microscopic studies whether VLPO GABA/galaninergic axons innervate specific BF non-cholinergic neurons. Other studies, using *in vitro* slice preparation have shown that a subpopulation of VLPO-GABAergic neurons are inhibited by ACh (194), and the origin of this ACh was suggested to be the mesopontine tegmentum (77). Finally, it is suggested that GHRH axons projecting from the paraventricular/periventromedial region (195) to the POAH region could mediate the SWS promoting action of GHRH (58, 196), since microinjection of GHRH directly into the medial preoptic area elicits enhanced SWS and GHRH antagonists in the same area suppress spontaneous SWS (59).

9.2 The hypocretin/orexin system of the lateral hypothalamus

Two new neuropeptides, hypocretin-1 and hypocretin-2 (also called orexin-A and orexin-B) were identified in about 1,200 neurons in the perifornical-dorsolateral hypothalamus of rats (197, 198). These neurons project in addition to the neocortex to such diverse regions, as the BF, VLPO, tuberomammillary nucleus, DR, LC, pontine reticular formation and the PPT/LDT tegmental nuclei that are all involved in behavioral state control (199, 200). Hypocretins operate through Hcrt-1 and Hcrt-2 receptors that show differential distribution. For example, in the BF, septum and the pontine reticular formation, neurons express mostly Hcrt-2 (201, 202), while in the LC the predominant receptor is Hcrt-1 (203).

Fos expression in orexin neurons correlates positively with the amount of wakefulness and negatively with the amounts of SWS and REM sleep (204). Hcrt-1 efflux was significantly higher during active waking than during SWS in the hypothalamus and higher during REM sleep than during SWS in both the hypothalamus and BF in freely moving cats (205). This is compatible with the data of Alam *et al.* (206) that many neurons in the rat perifornical area show elevated discharge in active waking and REM sleep as compared to SWS. Since in this electrophysiological study no attempt was made to identify the transmitter character of the recorded neurons, and in the perifornical area hypocretin cells are intermingled with other cell types, including melanin-concentrating hormone expressing cells (207), the unequivocal electrophysiological signature of hypocretin/orexin cells awaits further studies. For example, they may correspond to wake-related neurons that exhibited reduced discharge during both SWS and REM sleep when compared to that during waking (206) neurons (202, 217).

These findings, together with studies showing that intraventricular (208, 209) or BF (210) microinjections of hypocretins produce an increase in wakefulness, suggest that the activation of hypothalamic hypocretin neurons may promote or contribute to the maintenance of wakefulness. The excitatory effects of hypocretins on noradrenergic neurons of the LC, serotoninergic neurons of the DR, histaminergic neurons of the tuberomammillary nucleus, cholinergic neurons of the LDT and cholinergic and PV neurons of the BF have been described (211-217). Hypocretin-containing axons establish asymmetric, excitatory type synapses on septal cholinergic neurons and Hcrt-2 receptors have been found on PV-containing, septal GABAergic neurons (202, 217). Orexin/hypocretin-containing terminals were also found in the VLPO area (192) and microinjections of hypocretins into the lateral preoptic area increased wakefulness and suppressed all sleep stages, especially deep SWS and REM sleep (218).

It has been shown recently that adrenaline-containing axons synapse on orexin/hypocretin containing neurons in the perifornical area (219): a transmitter interaction that could mediate the sedative action of the alpha$_2$-adrenoreceptor-mediated anesthetic agent, dexmedetomidine (220). Interestingly, adrenaline-containing axons synapse with asymmetric synapses on orexin-containing dendrites, but show symmetric synapses with orexin-positive cell bodies, an observation that was noted also with adrenaline synapses on cholinergic neurons of the BF (94). The functional significance of this phenomenon is unclear.

9.3. Posterior lateral hypothalamus

Lesions in the posterior lateral hypothalamus, including the area of the histaminergic tuberomammillary nucleus produce somnolence and hypersomnia, whereas stimulation promotes wakefulness (221-226). The existence of interactions between preoptic hypnogenic and posterior hypothalamic wake-promoting neurons was already suggested by Nauta (2) and is supported by the demonstration that muscimol injection in the ventrolateral part of the posterior hypothalamus restores sleep in cats previously rendered insomniac by the injection of the cell-specific neurotoxin ibotenic acid into the VLPO area (173). Neurons in the posterior lateral hypothalamus, in rats and cats, using chronically implanted electrodes, were classified as waking-related (W) W/REM-related and REM-related (227, 228). W-related neurons decreased their discharge in SWS sleep, and remained firing at low rates during REM sleep. A subpopulation of these neurons discharge very little during REM sleep, and qualified as REM-off neurons. It is suggested that these latter units may correspond to histaminergic neurons (225, 227), although neither their cortical projections nor their transmitter were identified. Histaminergic neurons in the ventrolateral part of the

posterior hypothalamus innervate the entire forebrain as well as brainstem regions (229, 230) and may affect multiple neuronal sites involved in behavioral-state control. For example, histamine microinjections into the VLPO area produce dose-dependent increases in wake, and blockade of histamine synthesis in the POAH increases sleep and decrease wakefulness (226). The VLPO area is richly innervated by histaminergic fibers (192), however in an *in vitro* study (194) VLPO-GABAergic neurons were unaffected by histamine. Since muscimol injection in the same site of the preoptic area evoked severe insomnia (225), and all histamine-immunoreactive neurons in the tuberomammillary nucleus also contain GABA (231), further studies are necessary to understand the mechanism by which histamine and GABA elicit arousal in the VLPO. In addition, histaminergic fibers innervate neurons lateral to the VLPO, i.e. *hdb* and SI, that contain cholinergic and GABAergic corticopetal neurons. Indeed, an excitatory action of histamine on BF cholinergic neurons has been shown *in vitro* (232) and a projection from the tuberomammillary/posterior hypothalamic area to cholinergic neurons has been described (193), although no evidence exists to indicate whether histaminergic axons indeed synapse with cholinergic or other non-cholinergic neurons of the BF.

9.4. The supramammillary nucleus

The supramammillary nucleus is a relay station within the ascending pathway from the nucleus reticularis pontis to the septo-hippocampal complex and thought to play a modulatory role in reticularly elicited hippocampal theta (123). Supramammillary fibers may regulate the electrical activity of the hippocampus by both a direct pathway as well as via an indirect pathway through the septal complex. Supramammillary, presumably glutamatergic, fibers terminate both on cholinergic and PV-containing GABAergic neurons in the *ms/vdb* complex (233, 234). It has not been investigated whether supramammillary fibers also contact cholinergic and/or GABAergic cells in more caudal areas of the BF where neurons with predominant projection to the cortical mantle instead of the hippocampus are found.

9.5. The homeostatic and circadian control of sleep–wake cycles

Adenosine is an endogenous somnogen; its accumulation in specific brain regions during prolonged wakefulness might constitute the physiological basis of homeostatic sleep need (sleep pressure). During prolonged wakefulness accumulating adenosine produces immediate ionic effects reflected in decreased neuronal discharge in BF wake-active, putative cholinergic neurons. Adenosine may indirectly activate sleep-promoting neurons in the VLPO via an inhibition of presynaptic GABA release onto these neurons, resulting in disinhibition (235-238). At present, the origin of this GABA input to the VLPO-GABAergic neurons is unclear.

According to the two-process model of sleep regulation (239), the homeostatic sleep pressure with duration of wakefulness must be integrated with circadian propensity to initiate sleep. In the absence of the suprachiasmatic nucleus (SCN), the circadian pacemaker, the total amount of sleep is unchanged, but there is no light-induced variation in sleep onset (240). The VLPO receives inputs from the retina and the SCN. In addition, circadian influence can reach the VLPO indirectly through the median, medial preoptic area and the dorsomedial hypothalamic nucleus, all of which receive dense projections from the SCN and project to the VLPO (192, 241-244). Thus, the VLPO is anatomically well positioned to integrate homeostatic and circadian drives and to influence forebrain and brainstem arousal systems. It is unclear if BF neurons receive circadian information directly from the SCN or via preoptic or dorsomedial hypothalamic projections to cholinergic neurons whose existence has been established by electron microscopy (193).

10. CONCLUDING REMARKS

Figure 8 is an attempt at summarizing the synaptic input and interconnections of specific BF neurons in rat with their EEG-correlated firing properties in anesthetized animals whenever appropriate data are available. Unfortunately, at present the sleep-waking related discharge characteristics of neurochemically identified BF neurons in awake behaving animals have not been established. It would be interesting to know if application of similar criteria, used to classify sleep-wake related cell groups in other brain areas, would lead to segregation of BF neurons along different transmitter types. Alternatively, it is possible that neurons with the same transmitter show temporarily distinct activation/inhibition patterns depending upon the prevailing state of afferent control. Fifteen years ago, Cliff Saper suggested in an influential review (245) that neurons comprising the diffuse corticopetal systems may give off collaterals at multiple levels along their ascending path towards the cerebral cortex and affect the function of rostrally located cortical projection neurons. Study of the local axonal arborization of electrophysiologically and chemically identified neurons and the application of computational methods that show the specific spatial associations between the various cell populations, suggest that the BF is not a diffuse structure and its neural elements might be capable of distinct operations. The increasing use of combined rather than correlated functional anatomical studies (246) helps to understand how the firing properties of projection (cholinergic, PV-containing GABAergic) and local interneurons (NPY) relate to cortical EEG. The establishment of the input/output relationships, receptor-makeup and synaptology of electrophysiologically and neurochemically identified neurons will likely reveal that the various ‘diffuse’ ascending systems affect cortical arousal via the BF according to a very complex fine tuned cellular mechanism. Of course, if we treat the BF as a diffuse structure then sleep-related information may reach any of its cell population from all important brainstem and diencephalic arousal systems. However, even though all BF cell types are innervated by noradrenergic/adrenergic axons, the data suggest that the end-effect of NE on the different BF neurons would be different due to the presence of various adrenergic receptors and the different intrinsic

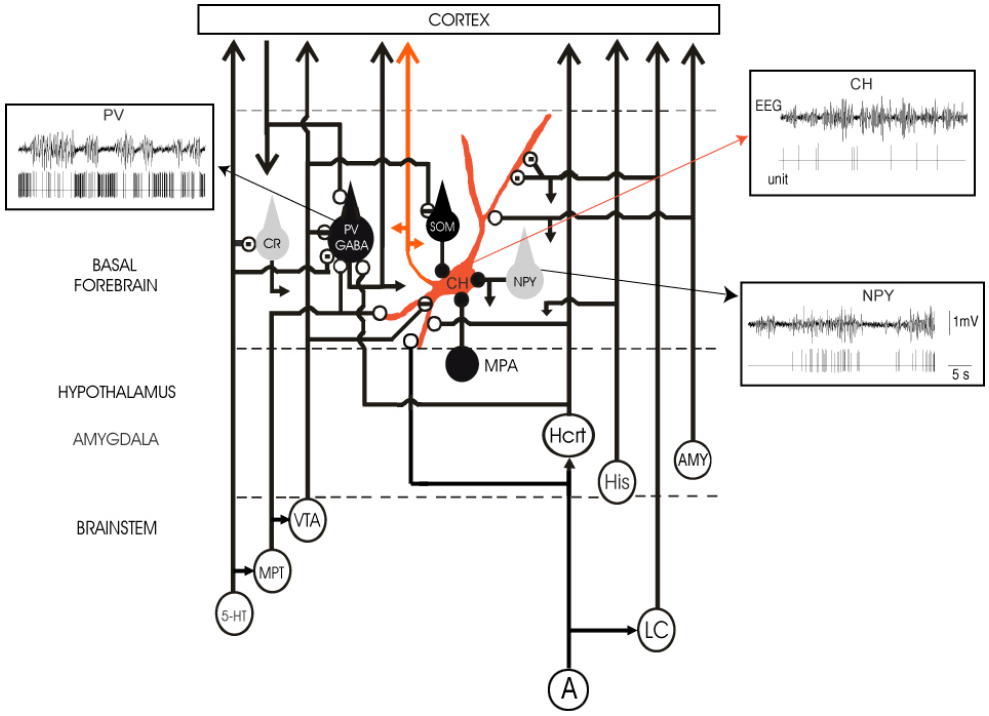

Figure 8. Schematic diagram (modified from [19]) illustrating the mode of termination of different ascending afferents to cholinergic (CH) parvalbumin (PV), somatostatin (SOM), neuropeptide Y (NPY) and calretinin (CR) containing neurons in the basal forebrain. Some of the interconnections between ascending aminergic cell groups are also indicated. Black filling in cell bodies indicates GABA, while gray tone in CR and NPY cell bodies indicates that data are equivocal as to whether the neuron is GABAergic or glutamatergic. Inhibitory synapses are drawn in full black, excitatory synapses with open symbols. Within the open symbols a minus sign represent dopaminergic synapses, a dot marks a noradrenergic or serotoninergic synapses. Axon collaterals with free endings indicate that no firm data is available about the postsynaptic target. Only those neurons and connections are included here that have been cross-correlated on the basis of fine structural, tracing and/or immunocytochemical identification of the same elements. Note that afferents to cholinergic neurons selectively innervate different portions of the neuron. Amygdala (AMY) axons from the basolateral amygdala (163), central nucleus of the amygdala (165) noradrenergic axons from the locus coeruleus (LC) (88) terminate on distal dendrites. Somatostatin, NPY (19, 70), GABAergic (61), medial preoptic (MPA) (193) and dopaminergic (141) axons innervate predominantly the cell bodies and proximal dendrites of cholinergic neurons. Corticofugal axons originating in the prefrontal cortex terminate on dendritic shafts of parvalbumin-containing neurons (79). Parvalbumin-containing neurons also receive on their soma input from the ventral tegmental area-substantia nigra (VTA-SN) (142). These synapses were GABA-negative (142), however, GABAergic axons from the VTA synapse in prefrontal cortex (247). Synapses originating in the VTA on BF-somatostatin neurons were not tested for the presence of GABA (152). Parvalbumin (23), and cholinergic (Kallo and Zaborszky, in preparation) neurons also receive input from the mesopontine tegmentum (MPT). Inputs from serotoninergic (5-HT) neurons to CR (117) and PV (116) neurons in the BF have been described. Synapses on midbrain dopaminergic neurons originating from the mesopontine cholinergic (131), glutamatergic (250) and serotoninergic (251) axons have also been indicated. Serotonin inputs to the mesopontine neurons are described in references (248, 249). Adrenergic (A) input to the LC (252), to orexin/hypocretin (Hcrt) neurons (217) and to cholinergic neurons in the basal forebrain (94) have been described. Hcrt-axons synapse with cholinergic (217) and PV-containing neurons (202) in the BF. Histamine (His) synapses on BF neurons are apparently not described. Data are based on studies of the rodent basalocortical neurons; the presence of catecholaminergic and GABAergic synapses on cholinergic neurons of the nucleus basalis in primates has been recently confirmed (253). The VLPO is omitted due to the lack of electron microscopic data on synaptic connections. EEG-correlated unit firing of CH, PV and NPY neurons are from (16).

properties of transmitter-specific neuronal populations. Further specialization may be achieved if ascending monoaminergic axons do not contact indiscriminately all cell types. For example, the available electron microscopic data suggest that 5-HT axons selectively contact non-cholinergic BF neurons. Also the subtle differences in the synaptology of adrenergic synapses on cholinergic or orexin-containing neurons may presage differences in synaptic transmission and transduction mechanisms. Nothwithstandingly, corticopetal cholinergic and GABAergic neurons are uniquely positioned to integrate the constant flow of cellular and homeostatic states derived from the ascending subcortical systems and to channel this momentarily changing neural pattern to the cortical mantle to modulate alertness. As we argued elsewhere (23, 81), against the relatively 'diffuse' termination of the ascending

brainstem and hypothalamic axons, the restricted input from the prefrontal cortex to PV-containing BF neurons (79) might be instrumental in communicating state-related changes from BF neurons to specific posterior sensory areas to modulate selective cognitive processes. It is unclear, however, if the same BF neurons that receive sleep-related brainstem or diencephalic input are the ones that also mediate specific functions, like selective attention and sensory plasticity.

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Send correspondence to: Laszlo Zaborszky, M.D., D.Sc., Center for Molecular and Behavioral Neuroscience, Rutgers, The State University of New Jersey, 197 University Avenue, Newark, NJ 07102, USA, Tel: 973-353-1080, Fax: 973-353-1844, E-mail: zaborszky@axon.rutgers.edu