AROUSAL SYSTEMS

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1. ABSTRACT

The brain contains autochthonous neural systems that evoke waking from sleep in response to sensory stimuli, prolong or enhance arousal in response to special stimuli, and also generate and maintain wakefulness regardless of sensory stimuli during the active part of the day. Through ascending projections to the cortex, these arousal systems stimulate cortical activation, characterized by high frequency gamma and low frequency rhythmic theta activity, and through descending projections to the spinal cord, they stimulate muscle tonus along with sensory-motor responsiveness and activity. They are comprised of neuronal aggregates within the brainstem reticular formation, thalamus, posterior hypothalamus and basal forebrain, and they utilize multiple different neurotransmitters. Within the brainstem, neurons of the reticular formation, which predominantly utilize glutamate as a neurotransmitter, stimulate cortical activation by exciting the widespread projecting neurons of the nonspecific thalamo-cortical projection system, which similarly utilize glutamate, and neurons of the ventral extra-thalamic relay systems located in the posterior hypothalamus and basal forebrain, many of which also utilize glutamate. In addition, these systems have descending projections by which they can enhance or modulate muscle tonus and activity. Articulating with these are cholinergic neurons of the ponto-mesencephalic tegmentum and basal forebrain that promote cortical activation during waking and also during rapid eye movement sleep (REMS), in association therein with muscle atonia. Dopaminergic ventral mesencephalic neurons stimulate a highly motivated and positively rewarding state during waking and may also do so during REMS. In contrast, noradrenergic locus coeruleus neurons promote an aroused waking state and prevent REMS as well as slow wave sleep (SWS). Serotonergic raphe neurons promote a seemingly quiet or satiated waking state, which though exclusive of REMS, can actually be conducive to SWS. Histaminergic neurons of the posterior hypothalamus act like noradrenergic neurons in enforcing waking and are joined by neurons in the region that contain orexin, a neuropeptide recently shown to maintain waking and in absentia to be responsible for narcolepsy, or the inability to maintain wakefulness. These multiple arousal systems are grossly redundant, since no one system is absolutely necessary for the occurrence of waking; yet they are differentiated, since each plays a special role in waking and sleep. During SWS, they are submitted to an inhibitory influence arising in part at least from particular GABAergic neurons co-distributed with many neurons of the arousal systems and also concentrated within the basal forebrain and adjacent preoptic region.

2. INTRODUCTION

In the early 20th century, many scientists believed that the flow of sensory input into the brain maintained the wake state, whereas the cessation of that input resulted in sleep (1). This view positing a passive occurrence of wake and sleep was overturned in 1949 by Moruzzi, Magoun and their colleagues (2-4), who demonstrated that wake was not altered in a long-lasting manner by stimulating or interrupting sensory pathways but was so modified by
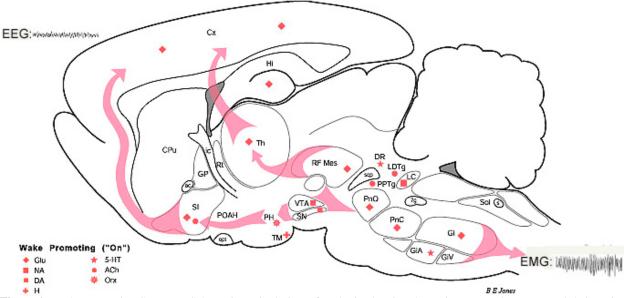
Figure 1. Wake Promoting Systems. Schematic sagittal view of rat brain showing the major neuronal systems and their major excitatory pathways (arrows) involved in promoting the EEG fast activity (upper left) and EMG high muscle tone and activity (lower right) characteristic of the waking state. The major ascending pathways emerge from the brainstem reticular formation (RF, most densely from the mesencephalic (RF Mes) and oral pontine (PnO) fields) to ascend along 1) a dorsal trajectory into the thalamus (Th) where they terminate upon (midline, medial and intralaminar) nuclei of the nonspecific thalamo-cortical projection system, which in turn projects in a widespread manner to the cerebral cortex (Cx) and 2) a ventral trajectory through the lateral hypothalamus up to the basal forebrain where they terminate upon magnocellular basal neurons, shown in the substantia innominata (SI and present in the medial septum-diagonal band complex), which also in turn project in a widespread manner to the cerebral cortex (and hippocampus, Hi) (5). Descending projections collect from multiple levels of the reticular formation (though most densely from the caudal pontine, PnC, and medullary gigantocellular, Gi, fields) to form the reticulo-spinal pathways. The major transmitter systems that promote waking and discharge maximally ("On") during waking contribute to these ascending and descending systems and are represented by symbols where their cell bodies are located. Glutamatergic (Glu) neurons comprise the vast population of neurons of the reticular formation, the diffuse thalamo-cortical projection system and a contingent of the basalo-cortical projection system. Cholinergic neurons, containing acetylcholine (ACh), are located in the laterodorsal and pedunculopontine tegmental (LDTg and PPTg) nuclei in the brainstem from where they project along with other reticular neurons dorsally to the thalamus and ventrally to the posterior hypothalamus and basal forebrain, as well as to the brainstem reticular formation. Noradrenergic (NA) neurons of the locus coeruleus (LC) send axons along the major ascending and descending pathways to project in a diffuse manner to the cortex, the subcortical relay stations, brainstem and spinal cord. Dopaminergic (DA) neurons of the substantia nigra (SN) and ventral tegmental area (VTA) project along the ventral pathway in the nigro-striatal system and meso-limbo-cortical system, respectively. Serotonergic neurons containing 5-hydroxytryptamine (5-HT) of the midbrain (including the dorsal raphe, DR) project to the forebrain, including the cerebral cortex (and hippocampus), as well as the subcortical relay stations, and those of the medulla (in raphe pallidus and obscurus, not shown, as well as pars alpha of the gigantocellular field, GiA) project to the spinal cord. Histaminergic (H) neurons of the tuberomammillary nucleus (TM) project in a diffuse manner to the forebrain and cortex. Orexinergic (Orx) neurons in the (peri-fornical and lateral) mid and posterior hypothalamus (PH) project diffusely through the forebrain, brainstem and spinal cord. Cholinergic and adjacent glutamatergic neurons that project to the cerebral cortex (and to hippocampus) are located in the basal forebrain within the substantia innominata (SI, magnocellular preoptic nucleus and globus pallidus, GP, and also in the medial septum-diagonal band complex). Modified from (159).

manipulating the brainstem reticular formation. Thus for more than half a century now, scientists have recognized that through autochthonous neural structures, the brain actively generates and maintains arousal. The arousal systems comprise ascending networks projecting to the cerebral cortex, which stimulate cortical activation reflected as fast EEG activity, and descending networks projecting to the spinal cord, which stimulate sensory-motor activation reflected as high EMG activity, (Figure 1). They reside within the brainstem, thalamus, hypothalamus and basal forebrain. They utilize diverse chemicals as neurotransmitters or modulators. They are accordingly

multifaceted yet grossly redundant since any one neural system is not necessary and may be sufficient for maintaining wakefulness.

3. BRAINSTEM AROUSAL SYSTEMS

3.1. The reticular formation

The reticular formation is composed of neurons lying amongst passing fibers within the central core of the brainstem that accordingly has a net-like (reticular) appearance. The neurons have long radiating dendrites that extend out through passing fibers and thus have the

capacity to receive input from those fibers and their multiple sources. From the medulla through the pons and into the midbrain, the reticular neurons acquire converging inputs from different sources including peripheral sensory systems relaying somatic or visceral sensory, auditory, vestibular, or visual inputs and the cerebral cortex sending sensory and motor-related outputs. They are thus in a position to respond to peripheral afferents and central efferents. As the first experiments by Moruzzi and Magoun showed (2), electrical stimulation of the reticular formation elicits cortical activation and increased muscle tonus in an anesthetized or sleeping animal. The reticular formation thus has the capacity to awaken, to alert and to render responsive the animal or human to incoming sensory input or outgoing central commands. Moreover, it has the capacity to maintain wakefulness, arousal and responsiveness in absence of those signals. As subsequent experiments by Magoun and his colleagues proved by lesions (4), cortical activation and behavioral wakefulness are maintained despite interruption of sensory pathways in the brain, whereas they are not maintained after destruction of the reticular formation in the presence of intact sensory input. Accordingly, the reticular formation possesses autochthonous mechanisms for sustaining activity and maintaining a waking state of the organism.

Reticular neurons send ascending projections into the forebrain along two major pathways, a dorsal one extending into the thalamus and a ventral, extra-thalamic one extending into the hypothalamus and up to the basal forebrain (Fig. 1; see for review (5). The early physiological studies demonstrated that the activating influence of the reticular formation upon the cerebral cortex is transmitted by relay through these subcortical stations (6). Neurons with ascending projections to these relays discharge at their highest rates during cortical activation (7). Reticular neurons also send descending projections into the spinal cord along dorsal funicular pathways to the dorsal horn and ventral and lateral funicular pathways to the intermediate zone and ventral horn (Fig. 1; see for review (5). Early physiological studies showed that different fields of reticular neurons exert facilitatory or inhibitory influences upon sensory and motor transmission in the cord (8). Such influences would be differentially exerted during states of arousal or sleep. Although the reticular neurons with ascending projections are most concentrated within the mesencephalic and oral pontine reticular formation (RF Mes and PnO) and those with descending projections are most concentrated within the caudal pontine and medullary reticular formation (PnC, Gi, GiA and GiV), they overlap to a considerable degree and some have bifurcating axons with ascending and descending projections (Fig. 1, (9)). The reticular formation can thus function in a differentiated but also integrated manner to influence cortical activation and sensory-motor activity during wakefulness and sleep.

The neurotransmitter utilized by the large population of neurons in the reticular formation with long ascending and/or descending projections is likely the excitatory amino acid, glutamate (Glu, Fig. 1). Immunohistochemical studies employing antibodies for glutamate or its synthetic enzyme, phosphate activated glutaminase, indicate that the vast majority of these neurons can produce glutamate for use as a neurotransmitter (5, 10). *In situ* hybridization for the vesicular glutamate transporter (VGluT) further indicates that reticular neurons have the capacity to package and thus release glutamate as a neurotransmitter (11). Another small number of neurons distributed through the reticular formation synthesize GABA and appear in the majority to project locally onto the larger projection neurons of the reticular core (5, 12-15). The reticular projection neurons are thus under inhibitory control by local GABAergic neurons, a control which could be differentially exerted during different states. Some groups of GABAergic neurons send long ascending projections from the ponto-mesencephalic tegmentum or descending projections from the medullary reticular formation. Glutamatergic and GABAergic long projection systems may function differentially in waking and sleep to respectively promote or dampen cortical activation and sensory-motor activity.

Many anesthetic agents act by blocking glutamatergic transmission (16, 17) and would thus block the action of the ascending reticular activating system and descending reticulo-spinal facilitatory system. Many other anesthetic agents, such as the barbiturates, act by enhancing GABAergic transmission through $GABA_A$ receptors (18) and could thus act by inhibiting projection neurons of the reticular formation.

3.2. The cholinergic ponto-mesencephalic neurons

The cholinergic ponto-mesencephalic neurons utilize acetylcholine (ACh) as a neurotransmitter and are located in the laterodorsal tegmental and pedunculopontine tegmental nuclei (LDTg-PPTg) at the level of the oral pons and caudal mesencephalon (Fig. 1). Like neurons of the reticular formation, they also have long dendrites that extend into the path of traversing fibers, including importantly those of the central reticular fasciculus, the major ascending pathway of the brainstem reticular formation (19). They thus have the capacity to receive input from reticular neurons and also the noradrenergic locus coeruleus neurons, which utilize the same pathway (see below). The cholinergic neurons give rise to ascending projections that parallel those of the reticular formation, extending dorsally into the thalamus and ventrally into the hypothalamus and basal forebrain (12, 20-23). Electrical stimulation of the LDTg-PPTg elicits cortical activation, while exciting thalamic neurons (24). Presumed cholinergic cells, which have been recorded across sleep-wake states, are most active in association with cortical activation of wakefulness or that of REMS (25, 26). Release of ACh is high in the thalamus in association with cortical activation of both states (27). These cholinergic neurons are thus ostensibly important for cortical activation that occurs in both waking and REMS. On the other hand, their influence upon reticulospinal or spinal systems appears to be inhibitory and more specifically associated with REMS, since injections of carbachol, the combined muscarinic and nicotinic cholinergic agonist, into the pontine reticular formation elicits muscle atonia in association with cortical activation, typical of REMS (28-

31). Enhancement of ACh levels by inhibitors of acetylcholinesterase (AChE), the catabolic enzyme, promotes cortical activation and waking, but can also promote REMS under conditions of monoamine depletion (32). Lesions of the cholinergic LDTg-PPTg complex do not produce deficits in cortical activation and waking but lead to a loss of REMS (33). Accordingly, cholinergic ponto-mesencephalic neurons normally stimulate cortical activation, perhaps during behaviorally quiet wake periods and during REMS, when they also promote sensory-motor inhibition and muscle atonia.

3.3. The noradrenergic locus coeruleus neurons

The noradrenergic locus coeruleus neurons utilize noradrenaline (NA) as a neurotransmitter and are clustered in the periventricular gray at the midpons, just behind the cholinergic cells (LC, Fig. 1). Their dendrites extend within the gray and adjacent tegmentum. In contrast to reticular and cholinergic neurons, they give rise to a diffuse innervation of the entire brain that includes the cerebral cortex and the spinal cord (9, 34). En route to the cortex, they also provide an innervation to the subcortical relay stations in the thalamus, hypothalamus and basal forebrain. They thus have the capacity to influence the cortex directly while recruiting the subcortical relays of the brainstem arousal systems. Chemical stimulation of the locus coeruleus elicits cortical activation (35). Pharmacological enhancement of NA release by administration of amphetamine elicits cortical activation in association with behavioral arousal, which depends also on release of dopamine (DA, see below) (36). Across the natural wake-sleep cycle, the noradrenergic neurons discharge during waking, decrease discharge during SWS and cease firing altogether during REMS (37). They are thus specifically involved in cortical activation as well as sensory-motor activity during waking. In fact, their activity appears to prevent the appearance of sleep and REMS in a permissive manner (38). Their maximal discharge during waking is often associated with highly aroused conditions, including stress (39). Pharmacological depletion of NA by inhibition of its synthesis leads to a mild hypersomnia (40). But lesions of the noradrenergic locus coeruleus neurons do not have long lasting effects upon cortical activation or waking (36). These neurons may accordingly correspond to a central sympathetic system that stimulates and enhances cortical activation and arousal, particularly during periods of stress, but is not necessary for the simple occurrence of these during waking.

3.4. The dopaminergic ventral mesencephalic neurons

The dopaminergic ventral mesencephalic neurons utilize dopamine (DA) as a neurotransmitter and are located in the substantia nigra (SN) and ventral tegmental area (VTA, Fig. 1), as well as retrorubral field (not shown). Their dendrites extend out into adjacent regions and those of the medial SN and VTA amongst fibers ascending from the brainstem and descending from the forebrain within the medial forebrain bundle. They give rise to ascending projections particularly along the ventral pathway through the medial forebrain bundle up to the dorsal striatum (comprising the nigro-striatal system) and to the basal forebrain, ventral striatum and cerebral cortex (comprising the meso-limbo-cortical system). Stimulation of the ventral mesencephalon promotes behavioral arousal that is positively rewarding since animals engage in self-stimulation with electrodes in this region (41, 42). Drugs that stimulate release of DA such as amphetamine and cocaine also lead to an aroused and positively rewarding state (43). Surprisingly, recording studies have not found differences in average discharge rate of dopaminergic neurons across sleep-wake states (44, 45). However, the dopaminergic neurons fire in bursts of spikes during aroused and positively rewarding waking conditions (46) and could also do so during REMS (44, 47), when they would be active in series with the cholinergic ponto-mesencephalic neurons (47). DA release is greatest during aroused and rewarding waking situations, including food and drug intake (43, 48). Block of DA receptors antagonizes the rewarding properties of food and drugs (49, 50). Pharmacological depletion of catecholamines also results in a decrease in waking and mild hypersomnia (40). Lesions of the ventral mesencephalic tegmentum result in behavioral akinesia and aphagia (51, 52). There is also evidence to show that such lesions diminish fast cortical activity of attentive arousal (53). While also seemingly active during REMS, dopaminergic neurons play a special role in waking, stimulating arousal and attention that includes locomotor and exploratory behaviors particularly associated with positively rewarding stimuli and situations.

3.5. The serotonergic raphe neurons

The serotonergic raphe neurons utilize serotonin (5-hydroxytryptamine, 5-HT) as a neurotransmitter and are distributed through midline (raphe) nuclei of the brainstem, including the midbrain dorsal raphe nucleus (DR), from where many ascending projections originate to forebrain and cortex, and pontine and medullary raphe nuclei (including raphe pallidus and magnus and pars alpha of the gigantocellular field, GiA), from where descending projections to the spinal cord originate (Fig. 1). Stimulation of midbrain raphe nuclei leads to a behavioral inhibition, arresting movement and eating during waking (54). Substances which increase release or synaptic levels of serotonin produce a quiet waking state seemingly associated with satiety, since they prevent eating and sexual behavior, and are also employed in humans as anxiolytic and anti-depressant drugs (55-61). Reciprocally, pharmacological depletion of serotonin leads to acute insomnia (62) and an aroused waking state marked by increased eating and sexual behavior (63). Lesions of raphe nuclei produce chronic insomnia and agitation (62). Although serotoninergic neurons were accordingly once thought to comprise the SWS-generating system of the brain, they were not found to be active during sleep, but instead to discharge maximally during waking (64, 65). Release of serotonin is also greatest during waking, less during SWS and minimal during REMS (66, 67). Certain serotonergic neurons discharge maximally during waking in association with rhythmic movements such as grooming (68). They may accordingly be part of arousal systems, yet involved in specific waking behaviors or conditions, such as rhythmic motor pattern generation or quiet, satiated conditions that might facilitate the onset of sleep.

Collectively, the brainstem arousal systems serve to generate cortical activation and sensory-motor

responsiveness as multiple parallel systems that are grossly redundant, though differentiated in their specific roles and the waking behaviors or conditions they promote. In the forebrain, they all project upon the subcortical relay stations that also comprise arousal systems of the brain located in the thalamus, hypothalamus and basal forebrain.

4. THALAMO-CORTICAL ACTIVATING SYSTEM

The nonspecific thalamo-cortical projection system comprises multiple thalamic nuclei, including midline (rhomboid and reunions), medial (centromedial and ventromedial) and intralaminar (centrolateral and paracentral) that commonly do not project to one specific cortical region but to multiple regions. All of these nuclei receive ascending input from the reticular, cholinergic, noradrenergic and serotonergic neurons (9). Thalamic neurons are excited by glutamate, ACh and NA, but may be inhibited by serotonin (69, 70). They project in a very widespread manner to the cerebral cortex (Fig. 1, (71, 72)). Moreover, the midline nuclei project in a very diffuse manner to all cortical areas (including hippocampus, (72)). As known from the early physiological studies, stimulation of the nonspecific thalamo-cortical projection system evokes widespread and prolonged cortical activation (73). Containing both glutaminase (74) and VGluT (75, 76), thalamo-cortical neurons appear to be glutamatergic and accordingly to excite cortical neurons by release of glutamate. The nonspecific thalamo-cortical projection nuclei discharge spontaneously in association with cortical activation during waking, as well as REMS (77). Although clinical cases and early experimental studies involving lesions of the nonspecific thalamo-cortical projection system indicated that these neurons play a critical role in maintaining cortical activation (78, 79), more recent studies employing neurotoxins for selective cellular lesions have failed to document a loss of cortical activation following destruction of thalamic neurons (80). Obviously, both nonspecific and specific thalamo-cortical inputs are important for cortical activity and the cognitive content of consciousness, but they may not be essential for maintenance of EEG fast activity, a waking state and crude consciousness of that state. Accordingly the parallel, ventral extra-thalamic pathway from the brainstem into the hypothalamic and basal forebrain relays to the cortex plays a critical role.

GABAergic reticularis thalamic neurons surround the thalamo-cortical relay nuclei in a net-like (thus the name) shell from which they project upon the thalamo-cortical projection neurons. Receiving input from the brainstem arousal systems (above), the reticularis thalamic neurons become disfacilitated and consequently hyperpolarized when the brainstem systems decrease their discharge at the onset of sleep (70). Due to special membrane properties (notably a calcium low threshold spike), the reticularis neurons begin to burst from this hyperpolarized level and through this burst and the consequent release of GABA to inhibit the thalamo-cortical projection neurons with sleep onset and continuation (81). Determined by the frequency of their bursting, they also participate in the propagation of spindles (12 -14 Hz) and

delta (1-3 Hz) or slower (< 1 Hz) waves during SWS within thalamo-cortical circuits (82).

5. HYPOTHALAMIC AROUSAL SYSTEMS

5.1. The posterior hypothalamus

The posterior hypothalamus comprises multiple nuclei and regions, including the lateral hypothalamus, that lie in the path of ascending fibers from the brainstem arousal systems (Fig. 1, PH). Like other similarly positioned neurons through the forebrain, many have radiating dendrites that extend out into passing fibers of the medial forebrain bundle, carrying ascending fibers from the brainstem and also descending fibers from the forebrain (83). They accordingly may be responsive to multiple collateral inputs from brainstem and forebrain arousal systems. Stimulation of the posterior hypothalamus elicits a cascade of arousal responses, including cortical activation and motor activity together with sympathetic responses involving pupillary dilatation, increased respiratory rate, increased heart rate and increased blood pressure (84). Neurons in the lateral posterior hypothalamus project directly to the cerebral cortex (85), and others project into the brainstem and spinal cord, innervating reticular, monoamine and sympathetic neurons (86, 87). They also have an excitatory influence upon the hypothalamo-pituitary-adrenal axis (88). Although neurons in the posterior hypothalamus contain certain peptides (see below), many contain VGluT and would thus appear to utilize glutamate as a primary neurotransmitter (89). In addition, however, both local and cortically projecting neurons appear to synthesize GABA (90, 91). The role of long, including cortically, projecting GABAergic neurons depends upon their specific target neurons and remains to be fully elucidated for the hypothalamus and basal forebrain (see below). In studies of unit discharge by posterior hypothalamic neurons, all cells are reported to fire maximally during waking and decrease their discharge during SWS (92, 93). Many are off during REMS as well, although others discharge at a high rate during REMS. Many also show increased discharge in association with movement during waking. Inhibition of the posterior hypothalamic neurons by local microinjection of the $GABA_A$ agonist, muscimol, leads to diminished waking and hypersomnia (94). In early studies employing electrolytic techniques, lesions of the posterior hypothalamus, particularly of the lateral posterior hypothalamus, produced dramatic decreases in waking along with akinesia and aphagia (4, 95-98). However, in more recent studies employing neurotoxic lesions, destruction of neurons in the posterior hypothalamus did not produce the long lasting deficits in waking (99). Neurons in this area thus represent another component of a grossly redundant yet multifaceted arousal system. Hypothalamic neurons control autonomic and neuroendocrine function and in the posterior region are important for activation of the sympathetic nervous system and also the hypothalamo-pituitary-adrenal axis during arousal.

5.2. The histaminergic tuberomammillary neurons

The histaminergic tuberomammillary neurons utilize histamine (H) as a neurotransmitter and are located

in the ventrolateral posterior hypothalamus lateral to the mammillary nuclei (Fig. 1). They receive multiple inputs from the brainstem and forebrain systems and project in a diffuse manner through the brain, including to the cerebral cortex (100). As part of the posterior hypothalamus, the effects of stimulation and lesion of this region (above) could be in part due to effects upon the histaminergic neurons therein. However, it should be emphasized that the cortically projecting neurons of the posterior hypothalamus include large populations of cells that are not histaminergic and are located dorsal to those cells. Similarly, wake-active neurons recorded in the posterior hypothalamus (above) include large numbers of non-histaminergic neurons (93). On the other hand, histaminergic neurons are thought to be wake-active neurons that like monoaminergic neurons cease their discharge during REMS (92, 93). The role of histamine in the maintenance of waking and cortical activation has been revealed pharmacologically since anti-histaminergic drugs produce somnolence (101, 102). Most recently, it has also been shown that rats lacking histamine due to knock out of its synthetic enzyme show less arousal in response to novel environments although they do not show decreased basal amounts of waking (103).

5.3. The orexinergic peri-fornical neurons

The orexinergic peri-fornical neurons contain the peptide orexin (Orx), also called hypocretin) that they are presumed to utilize as a neuromodulator and are scattered through the posterior to mid hypothalamus surrounding the fornix and within the lateral hypothalamic area (Fig. 1). These orexin/hypocretin producing neurons are believed to be important for maintaining waking since they, the peptide or the receptor are lacking in narcoleptic humans, dogs and knock out mice (104-106). Like other posterior hypothalamic neurons, they give rise to diffuse projections that include the cerebral cortex (107). They also project to and excite other arousal systems, including the locus coeruleus (108, 109), the nonspecific thalamo-cortical projection system (110), the histaminergic neurons (111, 112) and the cholinergic basal forebrain neurons (below, (113). It is possible that they correspond to wake-active and REMS-off cells recorded in the peri-fornical region of the posterior hypothalamus (114) and would thus exert their control only during waking. Orexin, as according to its name, may also promote eating in addition to arousal. Notably, however, it appears to have a role in energy regulation, maintaining a relatively high rate of metabolism in association with activity and arousal in normal animals (115). This role may be fulfilled by influences upon the sympathetic nervous system and the hypothalamo-pituitary-adrenal axis, as well as central arousal systems.

Neurons of the posterior hypothalamus thus include multiple cell types of which many are probably glutamatergic, some GABAergic, histaminergic, orexinergic and others awaiting identification or study. Different cell groups may play subtly different roles in arousal, including stimulation of eating, metabolism, sympathetic and adrenal activation, locomotion and/or cortical activation associated with arousal.

6. BASAL FOREBRAIN INTERLEAVED WAKE AND SLEEP PROMOTING SYSTEMS

6.1. The cholinergic basal forebrain neurons

The cholinergic basal forebrain neurons are distributed from the medial septum (MS)-diagonal band

(DB) nuclei most rostrally to the magnocellular preoptic nucleus (MCPO), substantia innominata (SI) and globus pallidus (GP) caudally (Fig. 1). Like reticular neurons, they have long radiating dendrites that extend out through the passing fibers of the medial forebrain bundle. From these, they receive input from all the brainstem and hypothalamic arousal systems, including glutamatergic reticular neurons, cholinergic ponto-mesencephalic neurons, noradrenergic locus coeruleus neurons, dopaminergic ventral mesencephalic neurons, histaminergic tuberomammillary neurons and orexinergic peri-fornical neurons (20, 100, 107, 116, 117). They are excited by glutamate (118), NA (119), DA (unpublished), histamine (120) and orexin (113). In contrast, they are inhibited by serotonin, an action that may serve in serotonin's role of facilitating sleep (above, (121)). They are also hyperpolarized by ACh through muscarinic receptors, which serve undoubtedly as autoreceptors, yet are excited through nicotinic receptors, which could possibly serve as specific heteroreceptors for inputs from other cholinergic neurons (122). The cholinergic neurons project to the cerebral cortex in a widespread manner, yet with a topographic organization (123-125). MS-DB neurons project densely to the hippocampus, whereas the MCPO-SI-GP magnocellular basal neurons provide the innervation to the neocortical mantle. Similarly to the MS-DB for hippocampal activity (126), chemical stimulation of the basal forebrain suppresses delta activity and SWS, and elicits cortical activation, characterized by increased fast, gamma (30-60 Hz) and rhythmic slow, theta (4-10 Hz) EEG activities (127). Selective activation of cholinergic neurons with neurotensin similarly suppresses delta activity and SWS and elicits cortical activation with gamma and theta, but it not only increases waking, it also increases REMS (128). It thus appears that as for the brainstem cholinergic neurons, the basal forebrain cholinergic neurons stimulate cortical activation but do not exclusively or by themselves alone evoke behavioral arousal. Like the brainstem neurons, they can promote REMS, complete with cortical activation and muscle atonia. Many units recorded in the basal forebrain have been found to discharge at their highest rates during waking and REMS, although none have yet been identified as cholinergic (129-131). In the cerebral cortex, release of ACh is high during both waking and REMS relative to SWS, and in the hippocampus, it is actually highest during REMS (132-134). During waking, very high amounts of ACh release occur during eating, suggesting that also like the brainstem cholinergic neurons, the basal forebrain neurons may be involved together with dopaminergic neurons in positively rewarding activities including food intake (42, 135). Along with positive reinforcement, the cholinergic basal forebrain neurons have been implicated in mechanisms of synaptic plasticity, learning and memory (136, 137), which are of course linked to their role in cortical activation and attention (138).

6.2. The non-cholinergic basal forebrain neurons

The non-cholinergic basal forebrain neurons are co-distributed with the cholinergic cells through the same nuclei (see above) and include glutamatergic as well as GABAergic neurons (Fig. 1). Most recently evidence has been presented that the cortically projecting magnocellular

basal neurons include glutamatergic, GABAergic and cholinergic cells (139-141). In addition, it has been found that many cortically projecting neurons discharge rhythmically in association with rhythmic theta-like EEG activity and these comprise glutamatergic, GABAergic and cholinergic neurons (142, 143). All three cell groups thus discharge in association with cortical activation and moreover may stimulate rhythmic theta-like oscillations in cortex during aroused waking and REMS. By presumably acting upon different target neurons, the three cell groups thus collectively comprise the important basal forebrain relay to the cerebral cortex from the brainstem reticular formation and other arousal systems. Moreover, basal forebrain neurons also receive input from olfactory sensory afferents (144). They may thus be responsible for the cortical activation that can be produced in the cerveau isolé preparation by olfactory stimulation and the recovery of spontaneous cortical activation that occurs in that preparation with time (145). Accordingly the basal forebrain should be considered as a forebrain arousal system that can function in advance and even in absence of the brainstem activating system and the collateral sensory inputs from somatosensory, auditory and visual modalities therein received.

Particular GABAergic basal forebrain neurons may promote SWS. That basal forebrain neurons could play a role in SWS was evident from early experiments showing induction of SWS with stimulation of the basal forebrain (146, 147) and loss of SWS with lesions of the basal forebrain (148). More recently, discrete stimulation of closely spaced sites in basal forebrain was shown to produce inhibition of cortical neurons from some sites and excitation from others (149), and neurotoxic lesions were reported to produce deficits of sleep and spindles in addition to decreases in fast EEG activity (150, 151). These paradoxical findings may reflect the interleaving of different cell types within the basal forebrain. Indeed, many basal forebrain neurons discharge at their highest rates during SWS and can be virtually silent during waking and REMS (129, 131). In anesthetized animals, a majority of GABAergic cells discharge at higher rates in association with irregular slow wave activity than with stimulation-induced cortical activation (152). Accordingly, it appears that SWS-active neurons are co-distributed with wake- and REMS-active neurons within the basal forebrain. They must be differentiated according to their projections, discharge properties, neurotransmitters and receptors. Whereas presumed wake-active cells, such as the cholinergic neurons, are excited by NA, presumed SWS-active cells, notably particular GABAergic neurons, appear to be inhibited by NA (153, 154). They accordingly share properties with neurons in the adjacent preoptic area, including the ventrolateral preoptic area (155, 156), where putative sleep generating GABAergic neurons are also inhibited by NA (157). Such GABAergic neurons may give rise to local projections, inhibiting the cholinergic cells, but also to long projections including to the posterior hypothalamus (158).

In addition to forming the important ventral extra-thalamic relay from the brainstem activating systems to the cerebral cortex, the basal forebrain thus also compromises autochthonous neural systems for the reciprocal regulation of waking/sleeping and associated cortical activation/de-activation in the forebrain.

7. CONCLUSIONS AND PERSPECTIVE

The brain contains multiple autochthonous arousal systems that evoke awakening from sleep in response to sensory stimulation, maintain waking during the active part of the day even in absence of sensory stimulation and prolong or heighten waking in response to particular stimuli or conditions. These systems act upon the cerebral cortex to stimulate high frequency gamma together with theta activity while suppressing slow delta, and they act upon the spinal cord to stimulate sensory-motor responsiveness and activity. They also enlist the peripheral sympathetic system and hypothalamo-pituitary-adrenal axis to enforce, enhance or prolong arousal and activity. Situated within the brainstem, thalamus, posterior hypothalamus and basal forebrain, the arousal systems are comprised of neuronal groups that utilize glutamate, ACh, NA, DA, 5-HT, or H (Fig. 1) and that form interconnected networks, responsive and often excited by their congener's neurotransmitters. Grossly redundant, no one system is essential for the elicitation or maintenance of the waking state. However, each is distinctive in the particular activation or waking condition it evokes. Future challenge lies in understanding the specific involvement and importance of each system, such as the cholinergic in evoking cortical activation during waking with elevated attention, motivation or reward yet also in association with behavioral quiescence and muscle atonia during REMS, the dopaminergic in inducing a highly motivated and positively rewarding condition during waking and also during REMS, the noradrenergic in evoking cortical activation only during waking and often in association with high arousal and stress, or the serotonergic in producing a quiet waking state and apparently relaxed and satiated mood that is actually conducive to the onset of sleep. Neuropeptides, such as the recently identified orexin (Fig. 1) and many others that await discovery, may have the capacity to enlist multiple arousal systems in particular constellations to enforce a waking state. Another challenge lies in understanding how interleaved or adjacent, often GABAergic, SWS-active neurons become active, perhaps through changes in the sensitivity of their unique receptors or through the influence of sleep-inducing neuropeptides, to periodically inhibit the arousal systems and induce sleep.

8. ACKNOWLEDGEMENTS

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