SUBCORTICAL MODULATORY SYSTEMS

(Honors Class, March 11, 2004)

Over the past 50 years, the basic mechanisms of sleep-wake states have been studied with an interdisciplinary approach embracing neurophysiology, neuroanatomy and neurochemistry. Early studies employed lesions and stimulation of the brain to identify regions and delineate neural systems that are involved in the generation and maintenance of wakefulness and sleep. Such experimental studies were also important in identifying the neuroanatomical substrates of coma and the extreme sleep perturbations that occur in association with brain lesions in humans. Neurophysiological research has employed recording of single neurons in the brain to discriminate putative wake- and sleep-generating neurons and to understand the cellular mechanisms of sleep-wake state generation. Over the past 25 years, research has focused on the involvement of specific neurotransmitters and corresponding chemically specific neuronal circuits in the generation of sleep and wakefulness.

Brain Electrical Activity During Waking and Sleep States

The states of wakefulness and sleep are characterized by a set of three cardinal physiological correlates: brain wave activity (electroencephalogram, or EEG), eye movements, and muscle tone.

The background electrical activity of the brain in unanesthetized animals was described in the 19th century, but it was first analyzed in a systematic fashion by Hans Berger in the late twenties in the last century, who introduced the term electroencephalogram (EEG) to denote the record of the variations in potential recorded from the brain. The EEG can be recorded with scalp electrodes through the unopened skull or with electrodes on or in the brain. The term electrocorticogram (ECoG) is sometimes used to refer to the record obtained with electrodes on the pial surface of the cortex.

In an adult human at rest with mind wandering and eyes closed, the most prominent component of the EEG is a fairly regular pattern of waves at a frequency of 8-12/s and an amplitude of about 50 μV when recorded from the scalp. This pattern is the alpha rhythm (alpha spindles). It is most marked in the parieto-occipital area, although it is sometimes observed in other locations. A similar rhythm has been observed in a wide variety of mammalian species. Alfa spindles also appear during the transitional period between wake and sleep. Large slow waves with a frequency of 1-4/s is called delta waves. Theta: 4-8 Hz. Beta waves has a frequency of 14-20 Hz; gamma:frequency 20-60Hz. When the eyes are opened, the alpha rhythm is replaced by fast, irregular low-voltage activity with no dominant frequency. A breakup of the alpha pattern is also produced by any form of sensory stimulation or mental concentration such as solving arithmetic problems. A common term for this replacement of the regular alpha rhythm with irregular low-voltage activity is desynchronization*, because it represents a

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* Desynchronization is an improper term to characterize active state since cognitive operations are associated with fast frequency (gamma) synchronized oscillations in large scale networks.

breaking up of the synchronized activity of neuronal elements responsible for the wave pattern. Because desynchronization is produced by sensory stimulation and is correlated with the aroused, alert state, it is also called the arousal or alerting response.

Sleep Patterns. There are two different kinds of sleep: rapid eye movement (REM) sleep and non-REM or slow-wave sleep. Non-REM sleep can be divided into several stages. A person falling asleep first enters stage 1, which is characterized by slight slowing of the EEG. Stage 2 is marked by the appearance of sleep spindles (12-14Hz) and high voltage biphasic waves called K complexes, which occur episodically against a background of continuing low voltage EEG activity. As sleep deepens, waves with slower frequencies (0.1-4 Hz, mainly delta) and higher amplitude appear on the EEG. The characteristic of deep sleep is a pattern of rhythmic slow waves, indicating synchronization.

REM/Paradox Sleep. The high-amplitude slow waves seen in the EEG during sleep are sometimes replaced by rapid, low voltage, irregular EEG activity, which resembles that seen in alert animals and humans (Figs. 2,4). However, sleep is not interrupted: indeed, the threshold for arousal by sensory stimuli and by stimulation of the reticular formation (RF) is elevated. The condition has been called paradoxical sleep. There are rapid, roving eye movements during paradoxical sleep, and for that reason is also called REM sleep. There are no such movements in slow-wave sleep, and consequently it is often called non-REM sleep. Another characteristic of REM sleep is the occurrence of large phasic potentials, occurring in groups of 3-5, that originate in the pons and pass rapidly to the lateral geniculate body and thence to the occipital cortex. For this reason, they are called ponto-geniculo-occipital (PGO) spikes. There is a marked reduction in skeletal muscle tone during REM sleep despite the rapid eye movements and PGO spikes. The hypotonia is due to increased activity of the reticular inhibiting area in the medulla, which brings about decreases in stretch and polysynaptic reflexes by way of both pre- and postsynaptic inhibition. REM sleep is also characterized by dreaming episodes.

Mechanisms of Arousal. Initial Studies (1935-1980)

Bremer discovered in 1935 that when the neuraxis of a cat is transected at Cl (encephale isole), with artificial respiration and precaution for maintenance of blood pressure, the animal shows the EEG and pupillary signs of normal sleep-wakefulness cycles. In contrast, when the transection is made at the mesencephalic level, just caudal to the motor nuclei of the third cranial nerve (cerveau isole), there ensured a permanent condition resembling sleep.

Bremer's discovery led to the concept of sleep as a passive process, as a deactivation phenomenon, while, wakefulness is an active state maintained by afferent input to the brain and sleep ensues when that input is removed, as in the cerveau isole cat, or falls below a certain critical level, as in normal sleeping. In the cervau isole preparation, olfactory input to the brain remains, but strong olfactory stimuli produce only a transient activation that does not outlast the stimulus. Visual pathways from the retina to the cortex are also intact, but visual stimuli do not evoke widespread activation of the EEG in the cervau isole animal, as they do in intact animals. Although Bremer

tentatively concluded that deafferentation per se is sufficient to induce sleep, this last observation concerning visual stimuli indicates that some neural mechanism in addition to the direct sensory pathways is required for the maintenance of wakefulness.

In 1949 Moruzzi and Magoun discovered that rapid stimulation (50-200/sec) of the brainstem produced activation of the EEG (low voltage fast electrical activity, or LFA), an effect evoked by stimulation of the central core of the brainstem in a region extending upward from the bulbar RF to the mesodiencephalic junction, the dorsal hypothalamus, and the ventral thalamus. In many features the activation produced by RF stimulation resembles the arousal produced by natural stimulation. When the RF is stimulated via implanted electrodes in sleeping animals, behavioral awakening and EEG desynchronization result. This is also true in animals after section of the long ascending sensory systems in the mesencephalon but does not occur after lesions of the mesencephalic RF. Indeed, after extensive lesions of the mesencephalic RF, animals may be comatose for many days and unresponsive to any stimuli (Lindsey et al., 1949; French and Magoun, l952). If they survive, they may show good recovery of sensory and motor functions but display various and sometimes prolonged periods of somnolence, with marked refractoriness for arousal, which when evokable, may not outlast the arousing stimuli. In contrast, animals surviving transection of the long ascending and descending tracts of the midbrain, but with no RF lesion, show no alterations of the sleep-wakefulness cycle, are readily aroused and then show activated EEGs, although they are profoundly deficient in the sensory spheres .

Subsequently by neuroanatomic techniques it was determined that the neurons of the RF receive collateral input from visceral, somatic, and special sensory systems and send long ascending projections into the forebrain via a dorsal pathway to thalamic nuclei and a ventral pathway to and through the hypothalamus, subthalamus and ventral thalamus and hence primarily through the intralaminar thalamic nuclei to the cortex (Jones and Yang, 1985).The ascending reticular system was thus identified located in the brainstem core and giving rise to long ascending forebrain projections, that was necessary and sufficient for the tonic maintenance of the cortical activation and behavioral arousal of wakefulness. The possibility was considered that a background of maintained activity within this ascending brain stem activating system may account for wakefulness, while reduction of its activity either naturally, by barbiturates or by experimental injury and disease, may respectively precipitate normal sleep, contribute to anesthesia or produce pathological somnolence.

Later, Szerb, Jasper and their coworkers showed (1965) that parallel to EEG desynchronization during arousal or paradoxical sleep there is an increased release of acetylcholine (ACh) over the whole cortex . The correlation of the different EEG epochs with the amount of ACh released in the neocortex and hippocampus was confirmed recently using the more sophisticated technique of in vivo dialysis (Marrosu et al., 1995). (Fig.).

In the 1920s, von Econonomo concluded that a “sleep regulating center” was present within the midbrain and diencephalon. Subsequent clinical studies (ref.: Plum, 1980) confirmed that lesions of the oral pontine and midbrain tegmentum or posterior hypothalamus and subthalamus are associated with somnolence, stupor and coma, while lesions in the preoptic/anterior hypothalamic area led to prolonged insomnia.

Further investigations in the 1960s and 1970s indicated that in the chronic course,

the brainstem reticular formation was not absolutely necessary for wakefulness, because cortical activation could eventually recover, given sufficient time after lesions or transections. Although ablation of the thalamus does lead to a temporary loss of cortical activation; however, in the chronic course, cortical activation does return. Furthermore, cortical desynchronization can still be elicited by stimulation of the midbrain reticular formation immediately after thalamic ablation, which indicates that another, alternate extrathalamic route and relay to the cortex must exist. With the development of increasingly sensitive biochemical, histochemical and immunocytochemical techniques in combination with tracing studies confirmed the presence of several extrathalamic corticopetal pathways that may participate in regulating state related behavioral changes.

Figure . summarizes brain regions and regulatory circuits involved in sleep. The sleep-wake cycle is a complex phenomenon: it is characterized by specific cortical EEG waveforms and synchronized electrical activity (oscillations) in large scale networks, in particular in the corticothalamic system. It is assumed that sleep-wake transitions are accomplished by coordinated interactions between wake-promoting and sleep promoting cell groups. Changing levels of adenosine and other substances, acting via specific receptors in these circuits mediate the homeostatic sleep pressure. The sleep-wake cycle is modulated by activity of hypothalamic circadian system. Wake-promoting neurons use noradrenaline, serotonin, histamin, acetylcholine and orexin/hypocretin as their transmitters, while sleep-promoting cells contain GABA and galanin.

'DIFFUSE' ASCENDING SYSTEMS

Lorente de No (1938) noticed that two types of fibers enter the cerebral cortx: one terminate primarily in layers III and IV of a restricted area of the cortex, the second give off multiple radially oriented collaterals that innervate primarily LI and VI over wide areas in the cortex (Fig.). He called the first type of fibers 'specific', while the second 'non-specific'. He thought that specific fibers originate in the specific sensory thalamic nuclei mediating visual, auditory and somatosensory information. On the other hand, he thought that non-specific fibers originate in the so-called non-specific (intralaminar, medial and midline) thalamic nuclei. Anatomical studies in subsequent years established that the non-specific afferents to the cortex originate in addition to the intralaminar thalamic nuclei, in several brainstem and forebrain regions and together represent the diffuse extrathalamic corticopetal systems that will be described in detail below (Fig.).

1. The Noradrenergic- Locus Coeruleus-Cortical Projection (Figs)

Anatomy. Considerable evidence indicates that the locus coeruleus (LC) noradrenergic (NE) projection to the cerebral cortex is highly collateralized, both within the cortex and between it and other structures. There may also be a crude medial-to-lateral topographical ordering to the coeruleocortical projection, but the distributions of cells projecting to different cortical sites largely overlap. Immunohistochemical studies, using an antibody against dopamine-beta-hydroxylase (the enzyme that synthesizes noradrenaline) suggest that noradrenergic axons establish conventional synapses in the cortex.

Physiology. Coeruleocortical neurons in rats and monkeys show long-duration action potential and slow conduction velocities. LC neurons tend to fire synchronously, often in bursts in response to peripheral sensory stimuli; this is usually followed by a quiescent period, which is thought to represent autoinhibition.

Studies on the effects of NE on neurons in sensory cortical areas suggest that the net result of NE release is an improvement in the signal-noise ratio. During wakefulness, the discharge rates of LC neurons are closely tied to the state of arousal, as measured electroencephalographically. During sleep, LC neurons in rats, cats and monkeys show a progressive decrease in firing rate as slow-wave sleep deepens, then become nearly silent before the onset of rapid eye movement or desynchronized sleep. Neurons in the cerebral cortex, thalamic reticular nucleus and thalamic relay nuclei change their activities in vivo from periodic and rhythmic spike bursts during natural, slow wave sleep to tonic firing of trains of single spikes during waking and REM sleep in behaving cats with chronic implants. Similar changes in firing pattern occur in vitro neurons in the cerebral cortex, thalamic reticular nucleus and thalamic relay nuclei in response to NE. The slow depolarization results from the reduction of K+ conductances and the enhancement of I_h. Peri-LC bethanechol infusion results in an increase firing of LC neurons that is followed consistently, within 5-30 sec, by a shift from low-frequncy, high amplitude to high frequency, low amplitude activity in the neocortical EEG. The infusion-induced changes in EEG are blocked by pretreatment (icv) with the alpha-2 agonsit clonidine or beta-antagonist propanolol. Injection of clonidine bilaterally immediately adjacent to LC induced a shift in neocortical EEG. These observations indicate that the level of LC activity are not only correlated with, but causally related to EEG measures of forebrain activation (Fig.).

In addition to changes in LC discharge preceeding corresponding changes in the EEG, LC discharge rates also covary with orienting behavior. LC discharge associated with orienting behavior is phasically most intense when automatic, tonic behaviors (sleep, grooming or consumption) are suddenly disrupted and the animal orients toward the external stimuli. Evidence also indicates that moderate LC activation accompanies optimal information processing, whereas high discharge rates accompany, and perhaps, produce a hyperarousal that may lead to poor performance in circumstances requiring focused, sustained attention.

2. Raphe-Cortical Projection (Fig. 17)

Anatomy. The cortical serotoninergic innervation arises in the dorsal (DR= dorsal raphe) and superior central raphe nuclei, cell groups located ventral to the cerebral aqueduct along the midline of the brainstem. Ascending fibers travel primarily in a paramedian position trough the midbrain reticular formation and ventral tegmental area (VTA) to the diencephalon, where they enter the *medial forebrain bundle*. From this point, their course is similar to the other diffuse cortical projection systems: a lateral systems of fibers turns laterally and runs through the substantia innominata to external capsule, while a medial pathway continues rostrally through the septum, dividing into a branch that runs back through the fornix to the hippocampal formation and another branch that runs over the

genu of the corpus callosum and into the frontal cortex and cingulate bundle. The median raphe nucleus contributes primarily to the medial pathway, whereas the dorsal raphe fibers contribute to both projections.

Physiology. The electrophysiological characteristics of serotoninergic neurons in the dorsal and median raphe nuclei are in many ways similar to those of noradrenergic neurons. Specifically, raphe neurons discharge at a relatively slow, regular rate, have long-duration action potentials (3-4/ms), posses slowly conducting axons and show evidence of inhibitory autoreceptors. Intracellular recording studies shows that the slow, regular firing rates of dorsal raphe neurons is related to "pacemaker" potential in these neurons. The activity of 5HT neurons in the dorsal and median raphe nuclei in the unanesthetized cat relates closely to the wake-sleep cycle. During active wakefulness the discharge rate averages 3.5 impulses/s. With the onset of drowsiness, the rate begins to fall, and about 2-10 s before the onset of REM sleep, the raphe neurons fall silent (Fig.). Iontophoretic application of 5HT to cortical neurons suggest that, like NE, the effect of 5HT on cortical neurons may depend on the ongoing state of activity of the target neuron. Electrical stimulation of the raphe is very effective in inducing neocortical activation, this effect can be blocked by serotoninergic receptor anatgonits such as ketanserin. Similarly, cortical activation induced by noxious stimulation such as tail pinching, an effect that involves the 5HT systems, is blocked by serotoninergic depletion (Dringenberg and Vanderwolf, 1998).

3. The Midbrain Dopaminergic System (Fig.)

DA neurons are concentrated in several cell groups in the brainstem. DA neurons have homeostatic and regulatory roles that they allow the forebrain and cortical neuronal systems to function normally. A lesion of the midbrain dopaminergic neurons disturbs many of the brain integrative functions not directly related to sensory, motor processes or arousal. Lesion of the ventral tegmental area (A10 or VTA) results in hypoactivity, a complete blockade of the locomotor stimulating effect of amphetamine, aphagia, adipsia, deficit in initiation and incentive to respond in an avoidance task, frontal neglect syndrome, attentional impairments. DA neurons are activated when the animal is presented with a behaviorally relevant stimulus requiring a response. However, the DA system appears to be primarily involved during the acquisition phase of this event, with little or no activation when the animal is overtrained on the task (Schultz). According to Schultz the DA neurons generate an error signal in the prediction of reward.

The firing rate or pattern of DA neurons in the VTA and SNc is not significantly modulated by the sleep-wake cycle or anesthetics. However, mice with deleted dopamine transporter show increased wakefulness and decreased NREM sleep. Furthermore, sleep disturbances in Parkonson's disease and their alleviation with dopaminergic medication suggest involvement of the dopaminergic system in sleep-wake regulation (Aldrich, 2000).

4. Hypothalamocortical Projection

Posterior hypothalamic lesions cause profound and prolonged coma, which in monkeys or humans may last for years. These observations suggest that the destruction of

hypothalamic neurons that innervate the cerebral cortex causes an irreversible deficit in cortical function.

Four distinct hypothalamic cell groups that project to the cerebral cortex have been distinguished.

1) In the tuberal lateral hypothalamus, cortical projection neurons are located in clusters in the zona incerta, the perifornical area, and along the medial edge of the internal capsule. These neurons innervate the entire cortical mantle, predominantly on the ipsilateral side. Many of the neurons in the perifornical region contain orexin/hypocretin.

Histaminergic (H) neurons (fig.). Neurons in the tuberomammillary nucleus (TMN) on each side of the brain innervate the entire cerebral cortex bilaterally, many of these neurons synhetize histamin. The histaminergic system innervates the entire forebrain as well as brainstem regions that are involved in behavioural-state control. A number of recent report suggest that histaminergic projections from the tuberomammillary nucleus of the hypothalamus may act to modulate EcoG actibvity and sleep-waking states. Intracerebral or intraventricular administration of H or histaminergic agonists appears to produce neocortical activation.

Neurons in this region in rats and cats, using chronically implanted electrodes, were classified as waking-related (W), W/REM-related and REM-related. W-related neurons decreased their discharge in NREM sleep, and remained at low rates during REM sleep. A subpopulation of these neurons discharge very little during REM sleep, and qualified as REM-off neurons (Fig.). It is suggested that these latter units may correspond to histaminergic neurons. Thus the histaminergic neurons fire in relation to the EEG with a pattern similar to that of the noradrenergic and serotonergic neurons of the lower brainstem. This is compatible with a action of histamine on cortical neurons as reducing the accommodation of firing (Fig.).

Orexin/hypocretin (Fig.). Orexin cells are localized exclusively in the tuberal region of the hypothalamus ventral to the zona incerta and extend 1 mm rostrocaudally (in rat) behind the paraventricular nucleus. In addition to food intake regulation, this system has been implicated in neuroendocrine, cardiovascular, gastrointestinal control, water balance. Mutation in the hypocretin receptor or the absence of ORX (hypocretin null mutant mice) cause in mice periods of behavioral arrest that strongly resembled the cataplectic attacks and sleep-onset REM periods characteristic of narcolepsy in dogs and humans. The release of orexin/hypocretin shows state-related changes: it is smaller in SWS than quiet wake and REM sleep (Fig.) and ICV injections of ORX into rats at light onset (the major sleep period) increases arousal and locomotor activity and decreases REM sleep without affecting non-REM sleep (Fig.). The effect of orexin in addition to its direct cortical projections is mediated via the widespread projection of ORX cells (Kilduff and Peyron, 2000). These neurons project in addition to the neocortex to such diverse regions, as the basal forebrain, preoptic area, TMN, DR, LC, mesopontine tegmentum, nuclei that are all involved in behavioral state control. Hypocretins operate through Hcrt1 and Hcrt2 receptors that show differential distribution. For example, in the basal forebrain, septum and the pontine reticular formation, neurons express mostly, while in the LC, the predominant receptor is Hcrt1.

Fos expression in orexin neurons correlates positively with the amount of wakefulness and negatively with the amounts of non-REM and REM sleep. This finding,

together with studies that intraventricular or basal forebrain injections of hypocretins produced increase in wakefulness, suggest that the activation of hypothalamic hypocretin neurons may promote or contribute to the maintenance of wakefulness.

5. Basal Forebrain Corticopetal System (Figs.)

The magnocellular neurons in the basal forebrain (termed in human Basal nucleus of Meynert) consists of a series of clusters of large, darkly staining cortical projection neurons running through several structures in the basal forebrain, including the medial septal and diagonal band nuclei, the substantia innominata and peripallidal areas. In rat cholinergic cells make up only about half of the neurons projecting to the prefrontal and somatosensory areas, the rest is GABAergic or peptidergic. GABAergic cells are often visualized using the presence of parvalbumin, a calcium-binding protein in these neurons (Fig.). The projection is topopgraphic and individual axons seem to innervate only restricted cortical areas.

Basal forebrain corticopetal neurons show rhythmic, spontaneous firing pattern (at an average rate of approximately 20 impulses/sec) and the discharge rate of these neurons is tightly coupled with cortical electrical activity: increased discharge frequency of basal forebrain neurons during waking and REM sleep is consistently associated with EEG desynchronization, while lower firing of BFC neurons is paralleled with EEG synchronization. Electrical stimulation in the basal forebrain results in short-latency excitation of neocortical neurons in the frontal cortex, long lasting EEG desynchronization and release of ACh in the cortex (Figs.). Recently, using the juxtacellular recording and filling technique, two types of cortically projecting neurons (cholinergic, parvalbumin-containing GABAergic) and a neuropeptide Y containing local neuronal type have been identified in anesthesia while monitoring the EEG (Figs.). Since the firing properties of cholinergic and NPY-containing neurons show opposite pattern to the same EEG epoch, a possible functional circuit within the BF can be envisaged (Fig.).

In rats, the highest frequency activity of BF neurons was observed during running, followed by drinking and immobility. The decrease in the firing rate correlated with the increase of the power of slow activity in the neocortex. A further decrease occurred in several BF neurons at the onset of high voltage neocortical spindles, occasionally present during immobility in the rat. The permissive action of BF neurons on spindle occurrence is also suggested by increased incidence of spindling after damage to the BF and in aged rats with shrunken cholinergic cells. These actions can also be explained by putative inhibitory influences of basal forebrain cholinergic (BFC) neurons upon the spindle pacemaker, on the reticular thalamic (RE) nucleus.

The mechanism, how in BFC neurons low firing in slow wave sleep changes to more active state (in arousal or REM sleep) is less well understood. It is likely that ascending noradrenergic fibers from the locus coeruleus may play an active role in alert state, while in REM sleep, when the locus coeruleus and the raphe cells are silent, perhaps ascending glutamatergic axons from the mesopontine tegmentum could stimulate BFC neurons. Indeed, LC axons synapse on BF cholinergic neurons and BF injection of NE affect specific cortical rhythm (Fig.). Also, kainic acid injection into the substantia innominata of the basal forebrain rapidly blocks the effect of reticular stimulation onto cortical evoked responses (Levandowski and Singer, 1993). In urethane-anesthetized rats

stimulation of the LC area produces EcoG activation in the neocortex and hippocampus and these effects are abolished by systemic treatment with antimuscarinic drugs scopolamine or atropine. These observation suggest that the release of ACh and possibly the cholinergic input from the basal forebrain (see below) to the cortex, play critical role in the EcoG activation induced by the LC (Dringenberg and Vanderwolf, 1998).

During arousal the BFC not only inhibit reticular thalamic bursting activity, but through their projections in the neocortex, the released ACh in extensive areas in the cortex provides a steady background of neocortical activity that may enhance the effect of other afferents (for example those transmitting specific sensory inputs) to the neocortex.

Summary of the diffuse ascending modulatory systems

Although the original concept of Lorente de No about specific and nonspecific thalamocortical systems has not stood the test of time, nevertheless the diffuse cortical projection systems share, to a greater or lesser extent, certain anatomical and physiological features that make it useful to consider them as a whole. For example, in the rat all of the diffuse cortical projections tend to most heavily to innervate superficial layers (LI-II) and deep layers (LV-VI), avoiding the middle layers (III-IV) that in most areas receive the bulk of the specific thalamo-cortical projections.

Experiments involving iontophoresis of monoamines or acetylcholine onto cortical neurons make it clear that these substances primarily act, by means of complex effects on membrane channels, to modulate the ongoing activity of the neuron. Instead of serving strictly excitatory or inhibitory roles, these substances can either enhance or impair discharge of the neuron to other inputs, and the total effect depends on the physiological state of the target neuron.

Another emerging finding that supports this unitary view is the similarity of unit activity patterns in the various cortical projection cell groups. These observations suggest that the brainstem and basal forebrain projections to the cerebral cortex are primarily concerned with modulating the general level of cortical arousal as well as attention and motivation. The diffuse nature of this innervation, which includes the entire cortical mantle, and the prominent collateralization of these projections are also consistent with a role in regulation of the overall level of cortical activity and mental state. On the other hand, the remarkable topographic specificity of the hypothalamic and basal forebrain projections to the cerebral cortex suggests that these diffuse cortical projections could selectively modify specific sensory, emotional or behavioral functions.

The Mesopontine Cholinergic Cells (fig.). REM Sleep

Figure . shows that most of the thalamic nuclei receive cholinergic input from two nuclei in the mesopontine tegmentum, the pedunculopontine (PPT) and laterodorsal tegmental (LDT) nuclei. The PPT and LDT in rat, monkey and human contain cholinergic and glutamatergic neurons whose axons project forward into the forebrain, particularly into the thalamic nuclei but also into the lateral hypothalamus and basal forebrain. A few cholinergic axon terminate in the prefrontal cortex. Lesions of the midbrain reticular

formation, which diminish or eliminate cortical activation, as the early physiologists demonstrated, would destroy the cholinergic neurons located in the mesopontine tegmentum. However, more discrete neurotoxic lesions of the majority of the mesopontine cholinergic neurons did not produce any notable deficit in cortical activation (See Jones, 1994).

Neurons in the brainstem PPT area shows increased firing in advance of EEG desynchronization during REM sleep (Fig.). That the brainstem reticular formation has a role in the blockade of synchronized EEG rhythms is known from early experiments showing that periodic spindle sequences appear on the EEG after transections at the collicular level (Bremer's cervau isole preparation) and that both spindles and slow waves are readily erased by high-frequency electrical stimulation of the upper brainstem reticular core (Moruzzi and Magoun, 1949). Because passing fibers could be activated by electrical stimulation, the use of microinjections of axon sparing neurotoxins within the rostral brainstem reticular core helped to demonstrate that perikarya in the rostral and caudal parts of the midbrain reticular formation were indeed responsible for the EEG desynchronizing reaction and behavioral arousal Since many components of these brainstem-thalamic influences are antagonized by acetylcholine blockers, the cholinergic projection from the mesopontine PPT and LDT nuclei were soon confirmed as important element in the desynchronization process. Figs. show the location of cell groups involved in controlling the various events in REM sleep.

REM Sleep

Paradoxical sleep (PS) was the term originally applied by Jouvet and his colleagues in 1959 to periods of behavioral sleep during which the eyes moved rapidly and the cerebral cortex showed a pattern of activity similar to that of the waking brain in the cat. This unusual association of parameters had been identified and described in humans several years earlier. This type of sleep has according to its principal characteristics, been called PS, REM (rapid eye movement) sleep, desynchronized sleep, active sleep, and dream sleep. The principal and distinguishing characteristics of PS are low voltage fast activity on the EEG, REMs recorded from the electrooculogram and muscle atonia recorded from the neck electromyogram (EMG). During PS, the REMs are accompanied by phasic activity within the visual system (PGO spikes). The manifestation of this same phasic activity occurs peripherally as REMs and also as twiches of facial, hypoglossal and distal limb muscles. At the same time that this phasic activity is being internally generated, somatic reflexes are inhibited, reflecting an inhibition of both sensory input and motor output. Sensory transmission is inhibited by both presynaptic inhibition of the primary afferent fibers and postsynaptic inhibition of sensory relay neurons. Somatic motoneurons of the spinal cord and brainstem are tonically inhibited as evident by hypewrpolarization of the membrane of these cells. Within the autonomic nervous system, reflexes are also attenuated, as manifest by marked alteration of cardiovascular, respiratory and temperature regulation during this state. Fig. 4 summarizes the location of cell groups involved in controlling the major events in REM sleep.

PS occurs in a cyclic manner following a given period of SWS which corresponds in the human approximately 45-85 min (progressing from longer to shorter periods through the night). PS endures on the average 5min in the cat and 5-65 min in the human. The average length of the sleep cycle beginning with SWS and ending with REM sleep is approximately 90 min in man and corresponds to a basic rest-activity cycle. This ultradian rhythm is normally correlated with an ultradian tempertaure cycle of approximately 90 min. Over the course of this cycle during sleep, body and brain temperature decrease during SWS relative to waking and increase during PS relative to SWS. In correlation with the cyclic temperature changes, CBF and metabolism also change during the sleep cycle. Glucose metabolism is also reduced during SWS and is increased to its highest levels through PS. Thus the sleep cycle corresponds to a basic rest-activity cycle of the brain.

PS has been identified in most mammals and in birds. Across mammals, the duration of PS is a function of the sleep cycle length, that increases with the size of the body and brain across species. PS has been consistently found to occur in its greatest amounts in the fetus or immature newborn animal. This would suggest that PS may be crucial to the development of functional circuits, such as those for co-ordinated eye-head movements, locomotion or complex species-specific behaviors. Absolute and prolonged deprivation of PS, like that of total sleep, leads to the death of the animal associated with weight loss, hypothermia in a period of two to eight weeks in adult rat. Thus PS can be viewed as an important function both during development and in adulthood, important perhaps for sensorimotor programming in development and information processing though life and also more fundamentally vital for physiological and metabolic functions of the brain not yet fully understood but as part of a basic rest-activitry cycle.

The results of the transsections studies indicated the importance of the pontine tegmentum in the generation of the phasic and tonic activation as well as the inhibitory processes of PS. Transmission of phasic activation evident as PGO spikes from the pons to the lateral geniculate, occurs along a pathway ascending from and though the dorsolateral pontomesencephalic tegmentum. Tonic cortical activation, associated with the state of PS depend upon multiple systems that relay activation from the branistem reticular formation to the cerebral cortex and which in addition to the thalamocortical relay, include a ventral, extrathalamic pathway and relay through the hypothalamus and basal forebrain. Transections studies also suggested that the medullary ventral reticular formation serves s the relay and final common pathway in producing the inhibition within the spinal cord.

Following neurotoxic lesions of the pontomesencephalic area, including the cholinergic neurons, PS was eliminated 2-3 weeks. Incipient PS episodes reappered following 3 weeks and were characterized by low voltage fast EEG activity in association with minimal PGO spike-like activity and minimal REM and in association with abnormal persistence of neck muscle tone. These results suggests that cholinergic neurons of the dorsolateral pontomes. tegmentum may be critically invoplved in the initiation and maintenace of the state of PS and the associated phasic PGO spikes. Pontomesencephalic cholinergic neurons have been found to give rise long projections into the forebrain, predominantllty to the thalamus and could thus mediate a cholinergic influence upon EEG and PGO. Although cholinergic neurons of the PPT/LDT area send descending projections through the tegmentoreticular tract to the medullary reticular formation (RF), pharmacological studies do not support a direct cholinergic role in the motor inhibition of PS. Specifically, it seems that pontine tegmental neurons that receive a cholinergic innervation may in turn via projections to the medullary reticular formation transmit signals involved in the motor inhibition that naturally occcurs during PS. The non-cholinergic neurons of the tegmentoreticular system may utilize glutamate as transmitter, since injection of Glu into the medullary RF produce muscle atonia. Neurons of the medial medullary RF may either relay or contribute to the reticulospinal influence that results in motor inhibition (Fig.).

Since cholinergic (REM-on) neurons are active during PS while LC noradrenergic and serotoninergic raphe (REM-off neurons) cells are silent there is a reason to believe that a direct or indirect interaction between the cholinergic and monoaminergic system may underlie the fundamental properties and generations of this state, as suggested by McCarley and Hobson in the late seventies. In narcoleptic* dogs, biochemical studies have revealed higher concentration of muscarinic agonists and the symptoms can be reduced by muscarinic antagonists. Reciprocally, evidence indicates that both catecholamines and 5-HT metabolism may be deficient and that drugs which enhances synaptic concentration of monoamines can reduce the cataleptic or narcoleptic attacks in dogs and humans. Figure summarizes the updated version of the reciproc-interaction model to explain the REM-nonREM alternation. As this scheme shows in addition to cholinergic cells, REM-on neurons contain gluatamate and local GABAergic neurons. Additionally, descending GABAergic projections from the preoptic area, ventral periaqueductal region contribute to the increased GABA release (Figs.) during REM sleep in the noradrenergic and serotoninergic nuclei.

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* Narcolepsy is irresistable attacks of sleep associated with cataplexy, paralysis and/or hallucinations. These attacks represent a sudden onset of REM sleep, motor inhibition and dream activity.

Thalamocortical Oscillations in the Sleeping and Aroused Brain

Since 1980, major progress has been made in investigating the mechanisms of generating rhythmic activity in thalamocortical systems. Studies, using simultaneous intra and extracellular recordings in multiple sites of thalamic and neocortical areas both in vivo and in vitro as well as computer simulations have revealed the ionic conductances that contribute to the intrinsic oscillatory properties of neurons and also demonstrated how these oscillations of isolated neurons can be transformed by interactions with other neurons into rhythmic patterns (Steriade, McCormick, Sejnowski).

Fig. shows the synaptic organization in the thalamus. Different areas of the cerebral cortex receive inputs from various thalamic nuclei. In turn, cortical neurons of layer 6 innervate topographically appropriate regions of both the dorsal thalamus and reticular nucleus (RE). The RE cells receive excitatory inputs from axon collaterals of thalamic neurons that project to the cortex and of cortical neurons that project to the thalamus; RE cells project to specific relay neurons and also innervate other cells of the RE. All neurons in the RE are GABAergic.

The majority of neurons in the mammalian brain have two basic modes of operation: tonic (steady) firing during EEG-desynchronized behavioral states and burst discharges during EEG synchronized sleep. The burst discharge mode appears to be an intrinsic features of several neuronal types. An extreme example of the complex interplay of sequentially linked ionic conductances is the oscillatory mode.

Figure summarizes the different types of NREM sleep oscillations in the thalamo-cortical networks. Sleep spindles (with a frequency of 7-14 Hz=alpha waves) are the epitome of EEG synchronization during light sleep. Slow waves or delta waves (1-4 Hz), and slow rhythm (0.1-Hz) prevail during the deep stage of non-REM sleep. Figure . shows that cortical spindle sequences occur nearly simulatenously during natural sleep in humans and cats and decortication disrupt the widespread coherence of thalamic spindles. Three factors account for the appearance of spindle and delta rhythms. Two of them consist of intrinsic properties and ionic conductances that allow thalamic cells to oscillate and synchronizing synaptic networks that include the reticular thalamic nucleus. The other factor is the dampened activity in ascending cholinergic brainstem reticular projections that normally act to prevent the occurrence of, or to block ongoing spindle and delta oscillations.

Spindle oscillations consist of waxing-and-waning field potentials of 7-14 Hz, grouped in sequences that last for 1-3 s and recur once every 3 to 10 sec (Fig.). The EEG spindles are the electrographic landmarks for the transition from waking to sleep that is associated with loss of perceptual awareness. These oscillations are generated in the thalamus as the result of synaptic interactions in a network in which the main players are the inhibitory neurons of the reticular thalamic (RE) neurons, thalamocortical relay cells and cortical pyramidal neurons. Through their connections, the RE is uniquely positioned to influence of the flow of information between the thalamus and cerebral cortex.

Intracellularly (Fig.), spindles are characterized in RE neurons by a slowing, growing and decaying depolarizing envelope with superimposed spike barrages, whereas in thalamocortical neurons spindles are associated with cyclic long-lasting hyperpolarizations that eventually lead to rebound bursts transferred to the cortical pyramidal neurons. The synchronization of this oscillation between neighbouring cells in either the RE or relay nuclei results from a large overlap in the afferent and efferent connections. That the RE

nucleus is the pacemaker of spindle rhytmicity is demonstrated by abolition of spindle waves in RE deprived thalamocortical neurons and preservation of spindle rhythms in RE neurons disconnected from their thalamic and cortical inputs.

Delta waves. High-amplitude, slow delta waves (1-4Hz) are most frequently observed during stage 4 sleep in the normal brain. The rhythmicity of the cortical delta waves is explained by the triggering effect of the periodic quasi synchronous thalamocortical inputs. The thalamus can maintain a rhythmic oscillation in the delta range due to hyperpolarization-dependent intrinsic property of thalamocortical neurons and their network connectivity with the GABAergic reticular nucleus. The depth profile of the slow delta waves and the observed field-unit relationship are compatible with the hypothesis that the extracellularly recorded delta waves reflect inhibition of pyramidal cells mediated by GABAergic interneurons. However, interneurons decrease their firing rates during the deep-positive slow waves. An alternative explanations is that the delta waves reflect the summation of long-lasting AHPs of layer V pyramidal neurons.

Although the intrinsic properties of thalamic neurons are fundamental in allowing them to oscillate, in intact brain, these properties are subject to controlling influences from modulatory ascending systems (cholinergic, noradrenergic, serotoninergic, histaminergic) that change the functional mode of single neurons as well as to the influence of a pacemaker (the reticular thalamic nucleus), which, by virtue of its connections to all thalamic nuclei, synchronizes the activity of thalamic neurons. The ascending modulatory axons collectively innervate the entire expanse of the cerebral cortex and the thalamus (both the relay and reticular nuclei). Through specific receptors, these transmitters induce changes in the membrane properties of the thalamic and cortical neurons promoting more tonic activity and inhibiting those ionic conductances which are responsible for the oscillatory mode.

EEG desynchronization is characterized by the disruption of spindle oscillations in the thalamocortical systems during both waking and REM sleep and upon midbrain reticular stimulation (Fig.). The effects of the putative neurotransmitters released by ascending activating systems, as revealed by in vivo and in vitro experiments, confirm that all these neurotransmitters help maintain the waking state and for ACh, also the dreaming state (Fig.). The changes in firing between sleep and arousal are accomplished by depolarization of the membrane potential in the thalamocortical neurons by 5-20 mV, which inactivates the low-threshold Ca^{2+} current and therefore inhibit burst firing. Brainstem peribrachial stimulation blocks an ongoing spindle sequence in RE neurons by producing a large hyperpolarization (Fig.) associated with an increase in membrane conductance. Electrical stimulation in the region of brainstem cholinergic and noradrenergic neurons, or direct application of ACh or NE, results in prolonged depolarization of thalamocortical cells. In thalamocortical cells, these transmitter-induced depolarizations results from muscarinic ACh and alpha1 adrenergic receptors. The peribrachial-evoked hyperpolarization in RE neurons is a muscarinic effect, as it is blocked by scopolamine. The firing rates of neurons in brainstem PPT neurons increase in anticipation of awakening or before REM sleep (Fig.) in further support of the origin of desynchronization.

During wakefulness, enhanced synaptic excitability of thalamocortical systems is accompanied by an increased efficacy of the fine inhibitory sculpturing of afferent information. It has been already mentioned that brainstem modulatory systems, particularly the cholinergic one inhibits the spindles at their site of genesis, the reticular

thalamic nucleus, ACh, however, also induces an enhancement of the stimulus-specific inhibition by excitation of local circuit neurons in the thalamic relay cells

The switch-off: sleep active neurons in the ventrolateral preoptic nucleus (VLPO)

A large body of evidence suggest that neurons in the preoptic/hypothalamic area, adjacent to the basal forebrain, play an important role in triggering sleep, especially NREM sleep. For example, lesions involving this region in humans, cats and rats induce long-lasting insomnia, whereas its stimulation can be sleep-promoting in animals Furthermore, several groups described cells in the preoptic/anterior hypothalamic areas of cats and rats that increased their discharge in anticipation of non-REM sleep onset More recently, it has been shown that a dense cell cluster in the ventrolateral preoptic area (VLPO) shows c-fos activation proportional to the amount of time spent in sleep but not circadian time. Moreover, the majority of these VLPO cells show elevated discharge rates in both SWS and REM sleep as compared to waking. These neurons express GABA/galanin and project to the hypothalamic tuberomammilllary nucleus. Furthermore, a projection from the VLPO and the surrounding preoptic cells to the locus coeruleus, dorsal raphe and PPT-LDT cell groups has been described. It is suggested that this descending GABAergic pathway might promote REM sleep by inhibiting the discharge of brainstem aminergic and cholinergic nuclei. Figs. show the location and projections from the VLPO.

Homeostatic and Circadian Regulation of Sleep

Recent studies suggest that mesopontine and BF cholinergic neurons are under the tonic inhibitory control of endogeneous adenosine, a neuromodulator released during brain metabolism. Increased metabolic activity during waking may cause an increase in both intra and extracellular adenosine. Consequently, cholinergic neurons are under increasing inhibitory influence through adenosine receptors. During the reduced metabolic activity of sleep, on the other hand cholinergic neurons are slowly released from the adenosine inhibition due to their low level of production. These suggestive data would constitute a long sought coupling mechanism that links neuronal control of EEG arousal to the effect of prior wakefulness (Strecker et al., 2000; Fig.).

Binding of adenosine to A1 receptors in a subpopulation of cholinergic neurons in the ventrolateral basal forebrain may preferentially activate the PLC pathway to mobilize internal calcium that activate PKC. Activated PKC then increases the DNA binding activity of the transcription factor, nuclear factor κB (NF-κB) which is known to alter the expression of several behavioral state regulatory factors, including interleukin-1Beta, tumor necrosis factor-Alpha, nitric oxide synthase, cyclooxygenase-2 and even A1 adenosine receptor mRNA. These changes may contribute to the long-term effects of sleep deprivation (for review see Basheer et al., 2002).

According to the two-process model of sleep regulation (Borbely, 2001), 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 day/light variation in sleep timing. The VLPO receives input from the SCN and retina

and receive input from adenosine receptor rich neurons of the diagonal band. Thus the VLPO is anatomically well-positioned to integrate homeostatic and ciracadian drives and to influence forebrain and brainstem arousal systems. Circadian influence can reach the VLPO also through the medial preoptic area and the dorsomedial hypothalamic nucleui that receive dense projections from the SCN and projects to the VLPO .

Summary of Sleep-Wake Cycle and Sensory Processing

1) Single unit recordings showed that the discharge rate of thalamo-cortical and corticofugal neurons is generally higher in REM sleep than in the waking state. In addition, the otho- and/or antidromic excitability of these cells was the same or higher in REM than in awake animals.

2)At the cortical level, evoked potential studies of thalamic and cortical regions in different sensory modalities suggests that their synaptic excitability diminishes from waking to SWS but surpasses waking values in REM sleep. Finally, in contrast to wakefulness, REM sleep was accompanied by a reduction of inhibitory activity in cortical neurons.

3) Studies in humans found that the percentage of awakenings evoked by sensory stimuli decreased from stage I to stage IV with REM sleep displaying intermediary values.

These studies draw our attention to the central paradox of REM sleep. Namely, that stimuli which are perceived in the waking state do not awaken subjects in REM sleep, even though the amplitude of the primary evoked cortical responses is generally similar to or higher than, in the waking state. In other words, although the thalamo-cortical network appears to be at least as excitable during REM sleep as in waking state, the input is mostly ignored. The lack of behavioral response to suprathreshold sensory stimuli reflect a difference in the way the brain processes sensory input. REM sleep can be considered as a modified attentive state in which attention is tuned away from the sensory input toward memories.

The synaptic transmission of sensory information through the thalamus and the cerebral cortex is enhanced during the states of waking and REM sleep, compared with EEG-synchronized sleep. The obliteration of synaptic transmission occurs in the thalamus at the first EEG signs of drowsiness, before overt behavioral manifestation of sleep and despite the unchanged magnitude of the incoming (prethalamic) volley. The amplitude of the monosynaptically evoked wave of thalamic and cortical field response is greatly increased both during EEG-desynchronized behavior states (waking and REM sleep) in chronic experiments and on brainstem reticular stimulation in acutely prepared animals. These changes are observed in all sensory and motor thalamocortical systems. The synaptically relayed component progressively diminishes in amplitude from the very onset of EEG synchronization during drowsiness and is completely obliterated during EEG-synchronized sleep, in spite of the unchanged amplitude of the presynaptic component. The blockade of synaptic transmission through the thalamus prevents the cerebral cortex from elaborating a response and is a necessary deafferentation prelude for falling asleep. Neocortical delta waves indicates that the principal neurons of the cortex are engaged in a collective burst mode of operation (synchronous hyperpolarization, synchronization), and the EEG waves themselves reflect the long-lasting AHPs that follow such bursts. This ‘closed loop’ state is therefore, characterized by delta waves and long-refractoriness of cortical neurons, precluding high fidelity information processing and transfer. Cellular refractoriness explains why the cortex cannot process incoming information, wheras the ionic basis of the same refractoriness (AHP) explains the current sources of delta waves. However, population bursting and associated calcium flux into the cells is a prerequsite for the expression of early genes and for the induction of long-term changing underlying memory formation.

Besides a parallel increase in spontaneous and evoked discharges during EEG-desynchronized states, the signal-to-noise ratio increases in cortical neurons. These results are explained in the light of the data on the action of various modulatory systems. The locus coeruleus acts as an enabling device by suppressing weak inputs and enhancing strong inputs, thus increasing the efficiency of feature extraction from sensory information and switching emphasis from one set of inputs to another. Arousal is invariably coupled to

increased discharge of BF and brainstem cholinergic, noradrenergic locus and serotoninergic raphe neurons. A common property of these diffuse activating systems is that they block the calcium-mediated potassium conductance (AHP) and attenuate accommodation of the action potentials. This mechanism, in turn, prevent burst firing of the cells, help switching neurons from the bursting state to the single spike mode and blocks slow waves. In addition, these subcortical neurotransmitters induce a gamma frequency oscillation (desynchronized pattern) by activating networks of inhibitory interneurons. Synchronous gamma activity (40Hz) has been hypothesized that binding and segmentation in perception are dynamically encoded in the temporal relationship between coactivated neurons. It has been suggested that gamma oscillation in the EEG represent summation of fast IPSPs of principal cells as a result of coherent, phase-locked activity of interneurons. From this perspective, the term desynchronization is misleading. What seems to happen during arousal is a switch from slow to fast oscillatory pattern. In the 'activated' state of the cortex fast firing Na+ spikes allow for a high-fidelity transmission of neuronal information.

In addition of the effect of general arousal, Mountcastle, Wurtz, Hubel and Livingston et al. described another type of modulation, called selective attention. In experiments in monkeys with fixation on a target, it has been shown that the enhanced responsiveness does not merely occur with changes in general arousal but is more specifically related to the directed attention to the target light. Other studies on the somatosensory cortex of behaving primates have also shown that the response of single neurons increases when the monkey "attends" to the part of the body that is to receive the stimulus (Mountcastle). In the visual cortex, Livingston and Hubel's experiments (Fig. 64) explained the effect of arousal on sensory processing.

In summary, while not all aspects of arousal can be explained, it is assumed that arousal includes a series of interrelated events in the thalamocortical, basalocortical and brainstem-thalamic networks, namely 1) an enhanced responsiveness to sensory stimuli in the thalamocortical relay neurons which allow a faithful transfer of sensory information to the neocortex, a 2) blockade of the bursting activity of the thalamic reticular neurons, which during sleep states inhibit globally the transfer of information from the sensory afferents to the thalamocortical relay neurons. 3) Arousal is also characterized by an enhanced activity in BFC neurons, that through their widespread projection to cortical areas modulate through ACh release the responsiveness of postsynaptic neurons. 4) Increased activity in ascending brainstem modulatory systems, primarily by the cholinergic nuclei of the brainstem and the ascending monoaminergic pathways, respectively. 5) Finally, arousal is also characterized by increased efficacy of inhibitory sculpturing in local circuit neurons. For normal behavioral arousal it is a prerequisite the simultaneous activation of several neural circuits. Activation of either system alone may be sufficient to exert an activating effect on their respective target (i.e. thalamus or neocortex), but it is not sufficient for maintaining a normal interaction between the brain and environment.

Figure is a recent model of Saper suggesting the flip-flop switch mechanism of forebrain circuits and their stabilization by the orexin/hypocretin cells of the lateral hypothalamus.

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