Neurotransmitters and Sleep

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Sleep is an active process, not just a default state when there is less incoming sensory information. It can be understood best by considering fluctuating levels of a series of neurotransmitters including the biogenic amines and acetylcholine. The effects of these neurotransmitters are not unique to sleep, but also subserve a wide range of other functions, including affect, sexual behavior, and appetite. The mechanism by which the most common hypnotics work is by binding to the benzodiazepine recognition site of the γ-aminobutyric acid$_A$–benzodiazepine receptor complex, which mediates action of the most widely distributed inhibitory neurotransmitter in the nervous system. It is possible that some endogenous sleep factors indirectly alter the properties of this receptor complex.

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In this article, we will review the major neurotransmitters that have a role in sleep as seen in the historical context of our growing understanding of sleep regulation. We begin our saga with the general view of sleep in the first half of the 20th century. Until the 1940s or so, the predominant view in neuroscience was that sleep was a passive resting state, often thought of in terms of electrical analogies. Putting this in a cultural context, the electrification of America was an event of recent memory, and the Freudian view of the mind was largely comprised of images of changing levels of energy in various hypothetical structures. It is not surprising, then, that many viewed sleep as something which occurred when the batteries were depleted, or as a kind of resting state that appeared when there was not enough stimulation. This view reached its culmination with the work of Moruzzi and Magoun[1] in the 1940s, who expressed in modern terms, and provided anatomical confirmation for, a notion that had been considered since the 18th century, i.e., that there was a structure deep in the brain which, when stimulated, brought about wakefulness.[2] This was, of course, the ascending reticular activating system. They described a polymorphous network rising from the upper brain stem reticular core that, after passing through intralaminar and other thalamic nuclei, impinges diffusely in the cortex, where it has an excitatory function.[2] It can be thought of as having 3 columns moving from medially to more laterally—the raphe nuclei, the medial zone (including the gigantocellular reticular nucleus), and the lateral zone.[3] The major neurotransmitters involved appear to be acetylcholine and glutamate, as well as certain neuropeptides such as substance P and neurotensin. It was thought that sensory input, in addition to providing specific information about the world around us, also sends nonspecific stimulation to this ascending system, which in turn activates the cortex. A second, more anterior pathway is thought to go to the basal forebrain and the anterior hypothalamus. It is also important to understand that the ascending reticular activating system is a physiologic description of function; it is not necessarily the same as the anatomical reticular formation, but rather comprises a subsection of it.[3]

The view that the reticular formation is the dominant mode by which an organism becomes alert was consistent with findings from physiologic preparations from the 1930s, such as the *cerveau isolé* cat, which appeared to be asleep and manifested high-amplitude slow electroencephalogram (EEG) waves after separation of the cerebrum from the brain stem by transection at the intercollicular level below the third cranial nerve nucleus[4]; a lower transection, at the level of the first cervical segment of the spinal cord (*encéphale isolé*) allowed an animal to remain awake.

On the other hand, a growing body of evidence was also beginning to suggest that this passive view of sleep was insufficient.[5] Studies from the 1930s indicated that electrical stimulation of parts of the nervous system could induce sleep.[6] These studies were sometimes difficult to interpret, however, because in some cases only certain stimulating frequencies were effective, and formal statistical analyses were not always available. A second line of evidence that appeared was that lesions of some areas in the mid-pons enhanced wakefulness,[7] suggesting that there might be areas that actively inhibited the reticular

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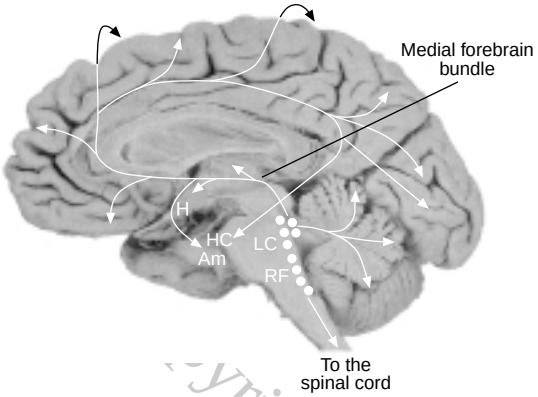
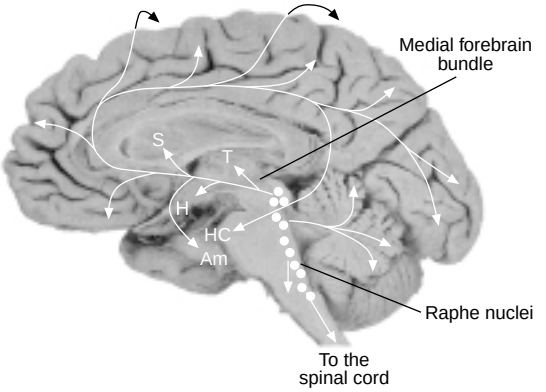
Figure 1. Schematic Indication of Central Nervous System Projections of Noradrenergic Neurons[a]



[a]Adapted from Nolte,[3] with permission. Abbreviations: Am = amygdala, H = hypothalamus, HC = hippocampus, LC = locus ceruleus, RF = reticular formation.

Figure 2. Schematic Indication of Central Nervous System Projections of Serotonergic Neurons[a]



[a]Adapted from Nolte,[3] with permission. Abbreviations: Am = amygdala, H = hypothalamus, HC = hippocampus, S = septal nuclei, T = thalamus.

activating system. Perhaps less troubling, but still a consideration, was the large disparity between the time durations involved in the electrical processes involved in neuronal action potentials and the phenomena of sleep. It was difficult to picture, for instance, a system in which firing rates measured in milliseconds could systematically regulate sleep phenomena that occurred with regularity over hours or even days. It seemed easier to picture these much slower processes as being regulated by the rise and fall of concentrations of chemical substances.

The final blow to the passive view of sleep regulation came with the discovery of rapid eye movement (REM) sleep in 1953.[8,9] Once it became clear that sleep was not a unitary process, but rather was comprised of 2 very different, alternating states (non-REM and REM sleep), it was very difficult to describe sleep as a resting condition toward which the nervous system drifts in the absence of stimulation. It seemed much more likely that it is an actively regulated process.

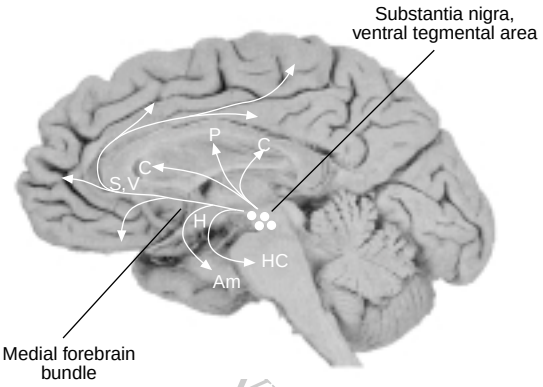
As long as sleep was considered a passive process, traditional "dry" neurophysiology—the study of electrical processes—seemed adequate; the active view, however, demanded a more complex formulation. Fortunately, in these same years, technical advances made possible what came to be known as "wet" neurophysiology, the study of neurotransmitters. The finding primarily responsible for this was the discovery by Falck et al.[10] that monoamines, when exposed to formaldehyde vapor, would fluoresce, thus introducing histofluorescence techniques capable of tracking neurotransmitter pathways. The diffuse, polymorphous qualities of the reticular activating system had made it difficult to understand anatomically; now, important neurotransmitter pathways could be histologically examined with precision. In the rest of this article, we look at the results of studying the pathways of the major neurotransmitters involved in sleep and then conclude with some comments about how hypnotics may act on these pathways to induce sleep.

MAJOR NEUROTRANSMITTERS IN SLEEP

The major biogenic amines, serotonin and norepinephrine, have cell bodies largely located in the brain stem, with major ascending pathways rising through the reticular formation to the thalamus and going anteriorly and bathing the basal forebrain and the anterior hypothalamus. Noradrenergic neurons are concentrated in the pons and medulla in the locus ceruleus near the floor of the fourth ventricle, as well as in the lateral medullary reticular formation.[3] As seen in Figure 1, ascending projections, primarily through the central tegmental tract, pass through the hypothalamus and thalamus, as well as limbic areas, ultimately innervating virtually all of the cerebral cortex. Serotonergic neurons, found at all levels of the brain stem, originate primarily in the raphe nuclei; ascending fibers through the medial forebrain bundle pass through the forebrain and ultimately innervate virtually all areas of the cortex, particularly sensory and limbic areas[3] (Figure 2). Firing rates of the biogenic amines are highest in waking, decline in non-REM sleep, and are virtually silent in REM sleep.[11] Of course, it should be recalled that these same neurotransmitters subserve a huge range of physiologic functions—not just sleep, but also affect, appetite, reproductive behavior, and so on—which suggests why the same processes in depression, for instance, that cause an alteration in affect and reproductive interest also affect sleep.

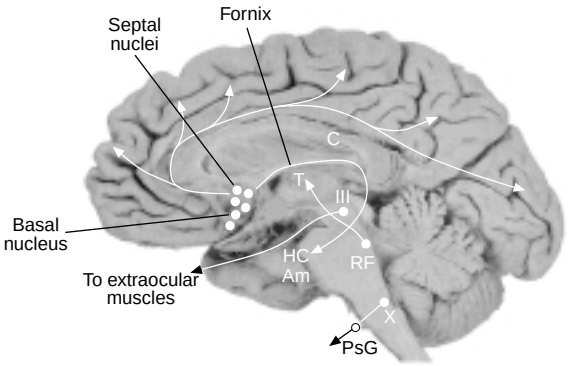
Dopamine has a similar pathway, except that it is largely confined in origin to the midbrain in the dorsal substantia nigra and the nearby ventral tegmental area (Figure 3). There are 3 major ascending pathways to the

Figure 3. Schematic Indication of Central Nervous System Projections of Dopaminergic Neurons[a]



[a]Adapted from Nolte,[3] with permission. Abbreviations: Am = amygdala, C = caudate nucleus, H = hypothalamus, HC = hippocampus, P = putamen, S = septal nuclei, V = ventral striatum.

Figure 4. Schematic Indication of Central Nervous System Projections of Cholinergic Neurons[a]



[a]Adapted from Nolte,[3] with permission. Abbreviations: III = oculomotor nucleus (representing motor neurons in general), X = dorsal motor nucleus of the vagus (representing preganglionic autonomic neurons in general), Am = amygdala, HC = hippocampus, PsG = parasympathetic ganglion cell, RF = reticular formation, T = thalamus.

caudate nucleus and putamen, limbic structures such as the amygdala, and the cortex.[3] In general, dopamine leads to alertness, which explains, for instance, why compounds such as bupropion, which has significant dopaminergic properties, have such major side effects in causing awakenings and disturbed sleep.

The biogenic amine histamine is concentrated in the posterior hypothalamus,[12] primarily in the tuberomammillary nucleus (TMN). It receives input from the reticular formation, and projects to the cortex,[13,14] where it tends to depolarize cells and promote waking. That is why antihistamines are used so widely as sedatives, because they inhibit that area. The TMN may also play an inhibitory role in some forms of behavioral reinforcement and learning,[15] and lesions of its E2 region have an anxiolytic-like effect.[16] Inhibitory galininergic and γ-aminobutyric acid (GABA)-ergic fibers from sleep-active neurons in the ventrolateral preoptic area (VLPO) impinge on the TMN, as well as on the serotonergic dorsal raphe nuclei and noradrenergic locus ceruleus; it has been hypothesized that this mechanism quiets these arousal systems during sleep.[17] It is also possible that this is one of the mechanisms by which GABAergic hypnotic medications may promote sleep.

Acetylcholine is found in neurons in the basal forebrain (particularly the nucleus basalis), but also in parts of the reticular formation, as well as the caudate nucleus and putamen[3] (Figure 4). The cells of the basal forebrain project widely to the cortex, and in effect become the ventral, extrahypothalamic extension of the reticular formation to the cortex.[18] Lesions and pharmacologic inhibition of this area appear to diminish fast EEG activity and arousal. Cholinergic neurons of the brain stem and basal forebrain are inhibited by adenosine,[18,19] which may be the mechanism by which adenosine and its agonists increase slow wave sleep[20] and by which caffeine, which blocks adenosine receptors, may enhance wakefulness.[21] Pharmacologic manipulation of brain stem cholinoceptive cells of the pontine gigantocellular tegmental (FTG) fields suggests that acetylcholine plays an important role in REM sleep initiation.

A family of neurotransmitters which has received much interest recently is the orexins (hypocretins). These neuropeptides, which appear to be involved in regulation of both eating behavior and wakefulness,[22] are localized in neurons in the perifornicular nucleus of the posterior hypothalamus, from which projections go to sleep-regulatory areas including the preoptic area.[23] Direct injection of orexin-A into the preoptic area of rats results in increased wakefulness and decreased REM sleep.[24] Studies[25] from an animal model suggest that genetic alterations of the orexin-2 receptor may be involved in the genesis of narcolepsy.

The final neurotransmitter we will consider, and the one that may be most relevant to the consideration of hypnotic medications, is GABA, which is the dominant inhibitory neurotransmitter in the nervous system. In 1977, two groups independently described what, in those years, was called the "Valium receptor" and is now called the $GABA_A$-benzodiazepine receptor complex. It is one of the members of a ligand-gated ion channel superfamily, which includes nicotinic acetylcholine, glycine, and serotonin-3 receptors.[26] It is comprised of 3 distinct but interacting functional units: a benzodiazepine recognition site, a $GABA_A$ recognition site, and a chloride ionophore. When a GABA agonist binds to the GABA recognition site, the ionophore is opened; negative chloride ions enter the cell, making the neuron relatively more negative on the inside and stabilizing it at a level below that needed for spike generation. When benzodiazepines, or the newer nonbenzodiazepine

hypnotic compounds such as zaleplon and zolpidem, bind to the benzodiazepine recognition site, they cause a complex interaction with the GABA recognition site, the end result of which is to increase chloride ion flux by increasing the frequency of channel opening.

Structurally, the receptor complex is comprised of at least 5 subunits, each of which has multiple isoforms. Each of these subunits is made up of 4 membrane-spanning sections, which include consensus phosphorylation sites.[27] It is interesting that one of the possible endogenous sleep factors—the unsaturated fatty acid amide oleamide—can activate protein kinases[28] and hence might alter the phosphorylation state of drug receptors. So it is very possible that one way that some endogenous sleep factors influence sleeping and waking is by altering the properties of the $GABA_A$-benzodiazepine receptor by a mechanism of changing the phosphorylation state.

As mentioned earlier, this complex receptor structure is made up of multiple subunits, and each of the alpha, beta, and gamma sites themselves have multiple isoforms. Clinically, the one of particular interest is the alpha subunit, which has at least 6 isoforms, leading to a large range of possible types of receptors. One major categorization is into type I and type II receptors, which are determined by the type of alpha subunit. The original benzodiazepines were nondiscriminatory—they bound equally to type I and type II—whereas the newer, nonbenzodiazepine compounds such as zaleplon and zolpidem are much more specific for type I. It has been speculated by some investigators that this binding property may lead to their greater selectivity for sleep-related effects.

Drug names: bupropion (Wellbutrin), diazepam (Valium and others), zaleplon (Sonata), zolpidem (Ambien)

Disclosure of off-label usage: The author has determined that, to the best of his knowledge, no investigational information about pharmaceutical agents has been presented in this article that is outside U.S. Food and Drug Administration–approved labeling.

REFERENCES

1. Moruzzi G, Magoun HW. Brain stem reticular formation and activation of the EEG. Electroencephal Clin Neurophysiol 1949;1:455–473
2. Steriade M. Arousal: revisiting the reticular activating system. Science 1996;272:225–226
3. Nolte J. The Human Brain: Introduction to Functional Anatomy. St. Louis, Mo: Mosby; 1999
4. Bremer F. Preoptic hypnogenic area and the reticular activating system. Arch Ital Biol 1973;111:85–111
5. Jouvet M. Biogenic amines and the states of sleep. Science 1969;163: 32–41
6. Hess WR. Hirnreizversuche uber den mechanismum des schlafes. Arch Psychiatr Nervenkr 1929;86:287–292
7. Batini C, Magni F, Paelstini M, et al. Neural mechanisms underlying the enduring EEG and behavioral activation in the midpontine pretrigeminal cat. Arch Ital Biol 1959;97:13–25
8. Aserinsky E. Memories of famous neuropsychologists: the discovery of REM sleep. J Histories Neurosci 1996;5:213–227
9. Aserinsky E, Kleitman N. Regularly occurring periods of eye motility and concomitant phenomena during sleep. Science 1953;118:273–274
10. Falck B, Hillarp NA, Thieme G, et al. Fluorescence of catecholamine and related compounds condensed with formaldehyde. J Histochem Cytochem 1962;10:348–354
11. Hobson JA, McCarley RW, Wyzinski PW. Sleep cycle oscillations: reciprocal discharge by two brainstem neuronal groups. Science 1975;18:55–58
12. Steinbusch HWM, Mulder AH. Immunohistochemical localization of histamine in neurons and mast cells in the rat brain, vol 3: classical transmitters in the CNS, pt 2. In: Bjorklund A, Hokfelt T, Kuhar MJ, eds. Handbook of Chemical Neuroanatomy. Amsterdam, the Netherlands: Elsevier; 1984
13. Sakai K. Neurons responsible for paradoxical sleep. In: Wauquier A, Gaillard JM, Monti JM, eds. Sleep: Neurotransmitters and Neuromodulators. New York, NY: Raven Press; 1985
14. Lin J-S, Sakai K, Vannj>Mercier G, et al. A critical role of the posterior hypothalamus in the mechanisms of wakefulness determined by microinjection of muscimol in freely moving cats. Brain Res 1989;479:225–240
15. Huston JP, Wagner U, Hasenohrl RU. The tuberomammillary nucleus projections in the control of learning, memory and reinforcement processes: evidence for an inhibitory role. Behav Brain Res 1997;83:97–105
16. Frisch C, Hasenohrl RU, Krauth J, et al. Anxiolytic-like behavior after lesion of the tuberomammillary nucleus E2-region. Exp Brain Res 1998;119: 260–264
17. Sherin JE, Elmquist JK, Torrealba F, et al. Innervation of histaminergic tuberomammillary neurons by GABAergic and galaninergic neurons in the ventrolateral preoptic nucleus of the rat. J Neurosci 1998;18:4705–4712
18. Jones BE. Basic mechanisms of sleep-wake states. In: Kryger M, Roth T, Dement WC, eds. Principles and Practice of Sleep Medicine. Philadelphia, Pa: WB Saunders Co; 2000:134–154
19. Rainnie DG, Grunze HCR, McCarley RW. Adenosine inhibition of mesopontine cholinergic neurons. Science 1994;263:689–692
20. Benington JH, Kodali SK, Heller HC. Stimulation of A1 adenosine receptors mimics the electroencephalographic effects of sleep deprivation. Brain Res 1995;692:79–85
21. Radulovacki M, Virus RM, Djuricic-Nedelson M. Adenosine and adenosine analogs: effects on sleep in rats. In: McGinty DJ, Morrison A, Drucker-Colin R, eds. Brain Mechanisms of Sleep. New York, NY: Raven Press; 1985
22. Hagan JJ, Leslie RA, Patel S. Orexin A activates locus coeruleus cell firing and increases arousal in the rat. Proc Natl Acad Sci U S A 1999;96: 10911–10916
23. Peyron C, Tighe DK, Van Den Pol AN, et al. Neurons containing hypocretin (orexin) project to multiple neuronal systems. J Neurosci Res 1998;18:9996–10015
24. Methippara MM, Alam MN, Szymusiak R, et al. Effects of microinjection of orexin-A into the preoptic area on sleep-wakefulness in rats. Sleep 2000;23(suppl 2):A93–A94
25. Lin L, Faraco J, Li R, et al. The sleep disorder canine narcolepsy is caused by a mutation in the hypocretin (orexin) receptor 2 gene. Cell 1999;98: 365–376
26. Ortells MO, Lunt GG. Evolutionary history of the ligand-gated ion-channel superfamily of receptors. Trends Neurosci 1995;18:121–127
27. Leidenheimer NJ, Browning MD, Harris RA. $GABA_A$ receptor phosphorylation: multiple sites, actions and artifacts. Trends Pharmacol Sci 1991;12: 84–87
28. Murakami K, Routtenberg A. Direct activation of purified protein kinase C by unsaturated fatty acids (oleate and arachidonate) in the absence of phospholipids and Ca^{2+}. FEBS Lett 1985;192:189–193