

**Reidun Ursin**

**Changing concepts on the role of serotonin in the regulation of sleep and waking**

The story of serotonin and sleep has been developing for more than 50 years, from the discovery in the 1950s that it had a role in brain function and in EEG synchronization. In parallel, the areas of sleep research and neurochemistry have seen enormous developments. The concept of serotonin as a sleep neurotransmitter was based on the effects of lesions of the brainstem raphe nuclei and the effects of serotonin depleting drugs in cats. The description of the firing pattern of the dorsal raphe nuclei changed this concept, initially to the entirely opposite view of serotonin as a waking transmitter. More recently, there has emerged a more complex view on the role of serotonin as a modulator of both waking and sleep. The effects of serotonin on sleep and waking may depend on which neurons are firing, their projection site, which postsynaptic receptors are present at this site, and, not the least, on the functional state of the system and the organism at a particular moment.

**Christopher A. Lowry, Andrew K. Evans, Paul J. Gasser, Matthew W. Hale, Daniel R. Staub and Anantha Shekhar**

**Topographic organization and chemoarchitecture of the dorsal raphe nucleus and the median raphe nucleus**

The role of serotonergic systems in regulation of behavioral arousal and sleep-wake cycles is complex and may depend on both the receptor subtype and brain region involved. Increasing evidence points toward the existence of multiple topographically organized subpopulations of serotonergic neurons that receive unique afferent connections, give rise to unique patterns of projections to forebrain systems, and have unique functional properties. A better understanding of the properties of these subpopulations of serotonergic neurons may aid in the understanding of the role of serotonergic systems in regulation of behavioral arousal, sleep-wake cycles and other physiological and behavioral responses attributed to serotonin. In this chapter, we outline evidence for multiple serotonergic systems within the midbrain and pontine raphe complex that can be defined based on cytoarchitectonic and hodological properties. In addition, we describe how these topographically organized groups of serotonergic neurons correspond to the six major ascending serotonergic tracts innervating the forebrain.

**Robert P. Vertes and Stephanie B. Linley**

**Efferent and afferent connections of the dorsal and median raphe nuclei in the rat**

It is well established that the brainstem contains discrete groups of serotonin-containing neurons with extensive axonal processes that distribute throughout the neuroaxis. Serotonergic neurons have been implicated in a range of functions prominently including the modulation of various events and states of sleep. We describe the efferent and afferent projections of the dorsal raphe (DR) and the median raphe (MR) nuclei. DR fibers distribute widely throughout the forebrain to dopamine-containing nuclei of the ventral midbrain, the lateral hypothalamus, the midline thalamus, amygdala, the dorsal and ventral striatum and adjoining regions of the basal forebrain, and most of the cortex. In contrast to the DR, the MR is a midline/paramidline system of projections. Specifically, MR fibers mainly distribute to forebrain structures lying on or close to the midline including the medial mammillary and supramammillary nuclei, posterior and perifornical nuclei of hypothalamus, midline and intralaminar nuclei of thalamus, lateral habenula, medial zona incerta, diagonal band nuclei,

septum and hippocampus. Overall, MR projections to the cortex are light. With few exceptions, DR and MR project to separate, non-overlapping regions of the forebrain – or, in effect, DR and MR share the serotonergic innervation of the forebrain. Although their outputs are distinct, DR and MR receive common sets of afferent projections from ‘limbic’ cortices, the medial and lateral preoptic areas, lateral habenula, the perifornical, lateral and dorsomedial nuclei of hypothalamus, and several brainstem nuclei prominently including the midbrain and pontine central gray, locus coeruleus, laterodorsal tegmental nucleus and caudal raphe groups. In addition to common afferents, DR receives significant projections from bed nucleus of stria terminalis, lateral septum, diagonal band nuclei, substantia nigra and the tuberomammillary nucleus, while MR receives distinct projections from the medial septum, mammillary nuclei and the interpeduncular nucleus. There are few projections from the amygdala to either DR or MR. In effect, the DR and MR are positioned to integrate of vast array of information from the brainstem and limbic forebrain and through their extensive axonal network influence virtually all parts of the neuroaxis.

**Samüel Deurveilher and Kazue Semba**

**Reciprocal connections between the suprachiasmatic nucleus and the midbrain raphe nuclei: A putative role in the circadian control of behavioral states**

The primary circadian pacemaker resides within the suprachiasmatic nucleus (SCN) in the hypothalamus, and controls the circadian rhythms of virtually all mammalian behaviors and physiological processes, including sleep and wakefulness. Serotonergic neurons in the midbrain dorsal (DRN) and median (MRN) raphe nuclei have been suggested to play an important role in behavioral state control. These neurons also show circadian rhythmicity in their activity, and may be an important target of the SCN circadian signal for organizing circadian sleep-wake rhythms. There are, however, no direct efferent projections from the SCN to the DRN or the MRN, suggesting that most of the SCN neuronal output may be conveyed indirectly. In this review, we first provide an overview of the anatomical evidence for the indirect neuronal pathways from the SCN to the DRN and MRN *via* several hypothalamic nuclei, namely, the medial preoptic area, subparaventricular zone, and dorsomedial hypothalamic nucleus. We discuss functional evidence to suggest that the SCN may influence the regulation of sleep-wake states by sending its circadian signal through these indirect pathways to the raphe nuclei. We then briefly consider the feedback projections from the DRN and MRN to the SCN, and discuss functional evidence to suggest that these projections carry some feedback information to the SCN regarding the vigilance state of the animal. We hypothesize that the reciprocal interactions between the circadian and sleep-wake regulatory systems may ensure a stable yet adaptive rhythmicity of daily sleep-wake cycles.

**Noemí Santana, Julián de Almeida, Guadalupe Mengod and Francesc Artigas**

**Localization of 5-HT receptors in the mammalian cortex**

Serotonergic neurons located in the dorsal and median raphe nuclei innervated the whole neuraxis and are critically involved in a large number of physiological functions, including sleep. Derangements of the serotonergic system are suspected in several psychiatric disorders, including mood and anxiety disorders. A large body of data supports a prominent role of dopamine in cortical function. However, much less is known on the role of serotonin (5-HT) in the neocortex, despite a very dense serotonergic innervation of some areas, such as the frontal lobe. Among other 5-HT receptors, this area contains a high density of 5-HT<sub>1A</sub> and 5-HT<sub>2A</sub> receptors in the rodent, primate and human brains. Using double in situ hybridization,

we reported on the presence of both receptor subtypes in a high proportion of pyramidal neurons and a smaller, yet significant proportion of GABAergic neurons. These data indicate that 5-HT can modulate the activity of cortical networks in a number of ways, including the activation of receptors on projection pyramidal neurons and on local inhibitory interneurons.

**Jason Hannon and Daniel Hoyer**  
**Molecular biology of 5-HT receptors**

Serotonin (5-hydroxytryptamine, 5-HT) is probably unique among the monoamines in that its effects are mediated by as many as 13 distinct G protein-coupled receptors and several ligand-gated ion channels (5-HT<sub>3</sub>). These receptors are divided into seven distinct classes (5-HT<sub>1</sub> to 5-HT<sub>7</sub>) largely on the basis of their structural, transductional and operational characteristics. While this degree of physical diversity clearly underscores the physiological importance of serotonin, evidence for an even greater degree of operational diversity continues to emerge.

**Barry L. Jacobs and Casimir A. Fornal**  
**Brain serotonergic neuronal activity in behaving cats**

A series of studies was conducted on the electrophysiological activity of brain serotonergic neurons in behaving cats. The studies explored a wide variety of behavioral and physiological conditions. In general, neuronal activity of both rostral (mesencephalic and pontine) and caudal (medullary) groups of serotonergic neurons was strongly related to spontaneous changes in behavioral state (highest in active waking and lowest during REM sleep). Across a wide variety of behavioral and physiological conditions (including stressors), the activity of these neurons was relatively unperturbed. However, one condition, motor activity, strongly affected neuronal activity. A general relationship exists between level of tonic motor activity and serotonergic neuronal activity across all groups of serotonergic neurons. Superimposed upon this in some neurons is an additional relationship in which a further, often dramatic, activation is seen in association with repetitive, central motor program (CPG)-mediated behaviors (e.g., feeding, licking, respiration, and locomotion). The exact nature of this relationship varies both with the serotonergic neuronal group (e.g., locomotor-related medullary neuronal activity *versus* grooming-related mesencephalic neuronal activity) and within a particular group (e.g., respiratory-related and feeding-related medullary neuronal activity). We hypothesize that the primary function of this increased serotonergic neuronal activity in association with tonic and repetitive motor activity is to facilitate behavioral output by coordinating autonomic and neuroendocrine function in association with the existing motor demand, and by concomitantly suppressing activity in most sensory information processing channels.

**Kazuya Sakai**  
**Electrophysiological studies on serotonergic neurons and sleep**

When their activity is recorded extracellularly in freely moving animals, all brainstem serotonergic neurons are characterized by a typical bi- or triphasic action potential of long duration, a slow discharge activity, a decrease in spontaneous discharge rate during paradoxical sleep (PS) compared to slow-wave sleep (SWS), a depressant response to serotonergic autoreceptor agonists, and a slow conduction velocity. These unit-recording studies further demonstrate a marked heterogeneity of brainstem serotonergic neurons in

general and of serotonergic dorsal raphe (DRN) neurons in particular. Serotonergic DRN neurons can be subdivided into two “typical” (types I-A and I-B) and four “atypical” (types I-C, II-A, II-B, and II-C) populations, whereas serotonergic medullary neurons can be divided into “complete” and “incomplete” types on the basis of their firing pattern during PS. “Typical” DRN neurons are evenly distributed in the DRN, and their activity is related to the level of behavioral arousal, as they discharge regularly at a high rate during waking (W) and at progressively slower rates during SWS, and cease firing during SWS with ponto-geniculo-occipital waves and PS (type I-A) or only during PS (type I-B). Serotonergic neurons in the nucleus raphe centralis superior (NCS) appear to be very much like type I-A and I-B DRN neurons in their activity across the sleep-waking cycle. In contrast, “atypical” neurons are unevenly distributed in the DRN and exhibit firing patterns distinct from those of “typical neurons”, such as a high rate of tonic activity related to motor activity (type II-A), a highest rate of tonic discharge during SWS with suppression of discharge during both W and PS (type II-B), or a sustained high or low level of tonic activity during PS (type I-C or II-C, respectively). Type II-A neurons are located in the middle portion of the DRN, type II-B in the most rostral and dorsal portion of the DRN, and types I-C and II-C in the ventral portion of the DRN near, or between, the medial longitudinal bundles. The “complete” type of serotonergic medullary neurons is evenly distributed in the medullary 5-HT cell groups, whereas the “incomplete” type is mainly located in the nucleus raphe pallidus. The suppression of discharge of type II-B DRN neurons seen during W appears to result from the activation of serotonergic autoreceptors, whereas the reduction or suppression of discharge during sleep of both typical and atypical serotonergic DRN neurons is caused by the withdrawal of excitatory drives (disfacilitation) resulting from cessation of discharge of norepinephrine, histamine and, to a lesser extent, orexin (hypocretin) (Orx/Hcrt) neurons during sleep. A similar disfacilitation mechanism appears to operate for caudal raphe neurons. These data suggest that different roles are played by brainstem serotonergic neurons in behavioral state control, functional topographic organization, and adrenergic, histaminergic, and/or orexinergic tonic control of serotonergic neurons during wake-sleep states.

**Pierre-Hervé Luppi, Damien Gervasoni, Christelle Peyron, Lucienne Leger, Denise Salvert and Patrice Fort**

#### **Role and origin of the GABAergic innervation of dorsal raphe serotonergic neurons**

Extracellular electrophysiological recordings in freely moving cats have shown that serotonergic neurons from the dorsal raphe nucleus are tonically active during waking, decrease their activity during slow-wave sleep, and are nearly quiescent during paradoxical sleep. However, the mechanisms at the origin of the modulation of activity of these neurons were not identified. To fill this gap, we developed a method allowing extracellular single-unit recordings of neurons, combined with iontophoresis of agonists and antagonists in the head-restrained rat. Using this method, we were able to show that GABA is responsible for the decrease of activity of the dorsal raphe serotonergic cells both during slow-wave sleep and paradoxical sleep. In addition, combining retrograde tracing with cholera toxin B subunit and GAD immunohistochemistry, we showed that the GABAergic innervation of the dorsal raphe nucleus arises from multiple distant sources and not only from local interneurons as classically accepted. Among these afferents, we propose that GABAergic neurons located in the lateral and ventrolateral preoptic area and the pontine ventral periaqueductal gray are responsible for the reduction of activity of the serotonergic neurons of the dorsal raphe nucleus during slow-wave sleep and paradoxical sleep, respectively.

**Sidney B. Auerbach****Regulation of serotonin release by inhibitory and excitatory amino acids**

Serotonergic neurons are spontaneously active with slow regular discharge during alert behavior and decreased activity during sleep. Sleep-related inhibition of serotonergic neurons is mediated by GABAergic inputs that originate in hypothalamic and brainstem sleep centers. During alert behavior, tonic release of GABA contributes to feedback and feedforward inhibitory circuits that, together with serotonin autoreceptors, prevent excess stimulation of serotonergic neurons. Glutamatergic inputs to the raphe originate in the brainstem, several hypothalamic nuclei and cerebral cortex. Although glutamate receptor agonists strongly stimulate serotonergic neuronal discharge, the physiological significance of glutamatergic inputs is not well-established. In the dorsal raphe nucleus (DRN), more glutamatergic fibers terminate on GABAergic than serotonergic neurons. Moreover, GABA normally restrains the excitatory influence of glutamatergic inputs to serotonergic neurons in the DRN. Peptidergic neurons modulate the activity of GABAergic and glutamatergic interneurons that synapse with serotonergic neurons in the DRN. These neuropeptides, for example CRF, endogenous opioids and Substance P, are implicated in responses to environmental challenges. Thus, stress can indirectly influence the activity of serotonergic neurons. In the raphe, GABAergic and glutamatergic interneurons may serve as a final common pathway for integrating information about environmental challenges and inputs from hypothalamic and brainstem centers that control the usual sleep-related inhibition of serotonergic neurons. Neuropeptides might thereby promote alert behavior in order to appropriately cope with stress. However, persistent peptidergic-induced changes in the strength of GABAergic and glutamatergic inputs to serotonergic neurons could contribute to insomnia, anxiety and major psychiatric disorders such as depression and schizophrenia.

**Edward F. Pace-Schott****Serotonin and dreaming**

Many clinical anecdotes and an experimental study have reported intensification of dreaming by the selective serotonin-reuptake inhibitors (SSRIs). However, no published neurochemical dream model invokes serotonin as a dream-promoting neuromodulator or accounts for serotonergic dream enhancement. An experimental study of normal volunteers showed that, although SSRI treatment decreased dream recall frequency, several subject-rated dream-intensity measures were greater during steady-state drug administration compared with pre-drug baseline and early drug treatment. Additionally, such subject-rated dream intensity as well as dream report length and judge-rated bizarreness were greater during acute discontinuation than during pre-drug baseline and drug administration periods. Nightcap ambulatory monitor data showed increased REM latency during treatment and increased REM density during acute discontinuation, indicative of SSRI-induced REM suppression and REM rebound following drug discontinuation, respectively. The bulk of pharmacological evidence suggests that drugs that enhance serotonergic neurotransmission lighten sleep. Sleep-disruptive effects of SSRIs are accompanied by electroencephalographic and electromyographic signs of brain activation, abnormally prominent eye movements in NREM sleep, and REM rebound following drug discontinuation. Explanations of SSRI-induced dream intensification suggested by these findings include, respectively, generalized brain activation during sleep, enhanced NREM dreaming, and within-night REM rebound. Additional clues as to potential causes of serotonergic dream enhancement are provided by: (i) the cellular pharmacology of hallucinogens that act on 5-HT<sub>2A</sub> receptors, (ii) the

phenomenological and functional neuroimaging effects of serotonergic hallucinogens, and (iii) putative neurophysiological mechanisms of lesion-related complex hallucinosis.

**Chiara M. Portas and Janne Grønli**

**Involvement of the 5-HT<sub>1A</sub> and the 5-HT<sub>1B</sub> receptor in the regulation of sleep and waking**

The involvement of the 5-HT<sub>1A</sub> and the 5-HT<sub>1B</sub> receptor in the regulation of sleep and waking is complex due to a multitude of pre-synaptic and/or post-synaptic actions also involving other neurotransmitter systems. Both receptors produce an important inhibitory feed back to the serotonergic raphe neurons. Overall, most studies support the possibility that stimulation of post-synaptic 5-HT<sub>1A</sub> receptors e.g. via systemic administration of a high dose of agonists increases wakefulness and decreases sleep. Local administration of agonists in DRN mainly produces a response similar to the 'low dose' systemic administration decreasing wakefulness and increasing REM sleep via disinhibition of mesopontine REM sleep promoting neurons. Systemic administration of 5-HT<sub>1B</sub> receptors agonists consistently increases wakefulness and decreases REM sleep as do the 5-HT<sub>1A</sub> agonists. The mechanism by which 5-HT<sub>1B</sub> receptors affect state modulation remain elusive. The general arousing effects of 5-HT<sub>1A</sub> and 5-HT<sub>1B</sub> agonists should also be considered in relation to the multiple, largely redundant, neurotransmitter systems which maintain arousal. Finally, 5-HT<sub>1A</sub> and 5-HT<sub>1B</sub> receptor are important modulators of the circadian rhythm largely by affecting the response of the SCN to light and the secretion of melatonin from the pineal gland. The development of more selective ligands seems crucial to further explore the role of these receptors in state modulation.

**Jaime M. Monti and Héctor Jantos**

**Mechanisms involved in the inhibition of REM sleep by serotonin**

Based on electrophysiological, neurochemical, and neuropharmacological approaches, it is currently accepted that serotonin (5-HT) functions to promote waking (W) and to inhibit (permissive role) REM sleep (REMS). Serotonergic neurons of the dorsal raphe nucleus (DRN) fire at a steady rate during W, decrease their firing during slow wave sleep (SWS), and virtually cease activity during REMS. Serotonin released during W activates 5-HT<sub>1A</sub> somatodendritic receptors and 5-HT<sub>2A/2C</sub> receptors expressed by GABAergic interneurons, and induces a decrease of the firing rate of 5-HT cells characteristic of SWS. In addition to local inhibitory circuits, GABAergic neurons of the ventrolateral preoptic nucleus play a role in the deactivation of the 5-HT and all other arousal systems which results in the occurrence of REMS.

Studies on the effects of direct administration of selective 5-HT<sub>1A</sub> (8-OH-DPAT, flesinoxan), and 5-HT<sub>2A/2C</sub> (DOI) receptor agonists into the DRN on REMS tend to indicate that quite different mechanisms are involved in their effects. Direct infusion of 8-OH-DPAT or flesinoxan into the DRN significantly enhances REMS and this effect is prevented by local infusion of the selective 5-HT<sub>1A</sub> receptor antagonist WAY 100635. In agreement with the reciprocal interaction hypothesis of REMS generation, inhibition of DRN serotonergic neurons following somatodendritic 5-HT<sub>1A</sub> receptor stimulation suppressed 5-HT inhibition of mesopontine cholinergic neurons and increased REMS. Infusion of DOI into the DRN induces a significant reduction of REMS in the rat. Pretreatment with selective 5-HT<sub>2A</sub> and 5-HT<sub>2C</sub> receptor antagonists prevents the DOI-induced suppression of REMS. Serotonin-containing neurons of the DRN do not express 5-HT<sub>2A</sub> or 5-HT<sub>2C</sub> receptors. The 5-HT<sub>2A</sub> and 5-HT<sub>2C</sub> receptor-containing neurons are predominantly GABAergic interneurons and

projection neurons. Since DOI inhibits the firing of serotonergic neurons in the DRN and reduces the extracellular concentration of 5-HT, it can be proposed that the DOI-activation of long-projection GABAergic neurons that express 5-HT<sub>2A/2C</sub> receptors would be responsible for the inhibition of cholinergic cells in the laterodorsal tegmental and pedunculopontine tegmental nuclei (LDT/PPT) and the suppression of REMS.

Microinjection of 8-OH-DPAT or flesinoxan into the LDT/PPT induces an inhibitory response on target neurons and the suppression of REMS. Moreover, infusion of DOI into the LDT/PPT selectively decreases REMS. In this respect, activation of 5-HT<sub>2A/2C</sub> receptors expressed by GABAergic interneurons in the LDT/PPT would produce the local release of GABA and the reduction of the behavioral state.

### **Tamas Kitka and Gyorgy Bagdy**

#### **Effect of 5-HT<sub>2A/2B/2C</sub> receptor agonists and antagonists on sleep and waking in laboratory animals and humans**

Several lines of evidence including human and rodent sleep studies with receptor ligands, data from genetically modified mice, and the localization of receptors in key brain structures suggest the important role of 5-HT<sub>2</sub> receptors in the regulation of vigilance. There are three members of the 5-HT<sub>2</sub> receptor family: the 5-HT<sub>2A</sub>, 5-HT<sub>2B</sub> and 5-HT<sub>2C</sub> receptors. Their distribution in the brain as well as their functions in sleep regulation show considerable differences. In summary, activation of 5-HT<sub>2A</sub> receptors results in an increase, and activation of 5-HT<sub>2B</sub> receptors causes a decrease in waking. Tonic activation of 5-HT<sub>2A</sub> receptors by endogenous 5-HT effectively inhibits slow wave sleep. Subtype-selective 5-HT<sub>2C</sub> receptor agonists cause an increase in waking, while the 5-HT<sub>2C</sub> receptor antagonists have little effect. In the case of none subtype-selective compounds, the 5-HT<sub>2A</sub> receptor-mediated effects usually dominate the outcome on sleep-wake stages and thus, inhibition of non-REM (and also REM) sleep could be expected after administration of SSRI antidepressants, while activation of slow-wave sleep could be observed after 5-HT<sub>2</sub> receptor antagonist antidepressants and atypical antipsychotic compounds, although high affinity to other, e.g., cholinergic or adrenergic receptors may mask the outcome in certain cases. Compounds with high affinity to 5-HT<sub>2</sub> receptors (either agonists or antagonists) reduce REM sleep in general.

### **David R. Thomas**

#### **5-HT<sub>7</sub> receptor modulation of sleep patterns**

The 5-HT<sub>7</sub> receptor class is one of seven major subtypes of 5-HT receptor (5-HT<sub>1-7</sub>) exhibiting a distinct profile in terms of structural properties, functional coupling and pharmacology. The receptor is widely localized in the brain and is expressed neuronally, in both terminal and cell body regions, in a number of brain areas relevant to sleep including, pyramidal neurons of the hippocampus, the suprachiasmatic nucleus (SCN) of the hypothalamus and the dorsal raphe nucleus (DRN). Brain functional studies utilizing 5-HT<sub>7</sub> receptor-selective antagonists suggest the 5-HT<sub>7</sub> receptor plays a role in modulating 5-HT neuronal activity in the DRN, a brain area implicated in the control of sleep. Thus, alteration in 5-HT<sub>7</sub> receptor function might indirectly modulate sleep architecture. Consistent with this possibility, systemic administration to rats of selective 5-HT<sub>7</sub> receptor antagonists such as SB-269970, increases the latency to onset of REM sleep and reduces the density of REM sleep, without significant effects on other sleep parameters. A qualitatively similar profile has been reported in 5-HT<sub>7</sub><sup>-/-</sup> knockout mice, which spend less time in REM sleep without alteration in wakefulness or slow-wave sleep. Microinjection of SB-269970 into the DRN in rats produces

effects on REM sleep consistent with those observed following systemic administration. These findings support a role for 5-HT<sub>7</sub> receptors in the DRN in the mechanisms underlying REM sleep formation. To date, no clinical studies have been carried out that investigate the therapeutic potential of selective 5-HT<sub>7</sub> receptor ligands. However, based on the pre-clinical findings, it is tempting to speculate that such ligands might exhibit utility in disorders where disrupted REM sleep is a feature.

### **Joëlle Adrien**

#### **Sleep and waking in mutant mice that do not express various proteins involved in serotonergic neurotransmission such as the serotonergic transporter, monoamine oxydase A, and 5-HT<sub>1A</sub>, 5-HT<sub>1B</sub>, 5-HT<sub>2A</sub>, 5-HT<sub>2C</sub> and 5-HT<sub>7</sub> receptors**

Sleep studies in knock-out mice have investigated the effects on sleep and wakefulness of targeted disruption of genes controlling various proteins involved in serotonergic neurotransmission. It has been the case notably of proteins that regulate serotonin concentration in the extracellular space: the serotonin transporter (5-HTT) and catabolytic enzyme, monoamine oxydase A (MAOA), as well as of serotonergic receptors such as the 5-HT<sub>1A</sub>, 5-HT<sub>1B</sub>, 5-HT<sub>2A</sub>, 5-HT<sub>2C</sub> and 5-HT<sub>7</sub> sub-types.

Mutant mice that do not express the 5-HTT, 5-HT<sub>1A</sub> or 5-HT<sub>1B</sub> receptors exhibit spontaneously larger amounts of Rapid Eye Movement (REM) sleep than their wild-type counterparts. In the case of 5-HT<sub>1A</sub><sup>-/-</sup> and 5-HT<sub>1B</sub><sup>-/-</sup> mice, the sleep phenotype is mimicked by pharmacological blockade of 5-HT<sub>1A</sub> and 5-HT<sub>1B</sub> receptors, respectively. This indicates that no major compensatory mechanisms have developed in these mutants, and that REM sleep is under tonic inhibitory control of serotonin via these receptors, and particularly the 5-HT<sub>1A</sub> sub-type. In contrast, pharmacological blockade of the 5-HTT in wild-type mice has effects on REM sleep opposite to those of the transporter gene deletion. In the same manner, ablation of the monoamine oxydase A gene results in no major impairment of sleep, whereas pharmacological inhibition of MAOA induces dramatic REM sleep decrease. These opposite effects might be related to the desensitization of 5-HT<sub>1B</sub> receptors in 5-HTT<sup>-/-</sup>, and of 5-HT<sub>1A</sub> receptors in MAOA<sup>-/-</sup> mutants, but it seems essentially accounted for by the lack of clearance of serotonin from the extracellular space during early life. Indeed, protection of the brain from this serotonin overload during early life (by treatment with an inhibitor of serotonin synthesis or with a 5-HT<sub>1A</sub><sup>-/-</sup> receptor antagonist) rescues a lasting wild-type phenotype in 5-HTT<sup>-/-</sup> mice. In contrast to the previous mutants, 5-HT<sub>7</sub><sup>-/-</sup> mice exhibit reduced amounts of REM sleep, a profile identical to that obtained in rats after pharmacological blockade of 5-HT<sub>7</sub> receptors. This indicates that the latter receptor type mediate a serotonergic facilitation of REM sleep. Finally, non-REM (NREM) sleep is affected after mutations involving 5-HT<sub>2</sub> receptors. Both 5-HT<sub>2A</sub><sup>-/-</sup> and 5-HT<sub>2C</sub><sup>-/-</sup> mutants exhibit reduced NREM sleep amounts compared to wild-type mice, and no change of REM sleep. However, pharmacological inactivation of each receptor type induces an effect opposite to the genetic invalidation, i.e., enhancement or no change of NREM sleep, and pronounced inhibition of REM sleep.

Investigations of the response to sleep deprivation, total or REM-selective, and to immobilization stress indicate that mutants have lost their homeostatic sleep properties, except for 5-HT<sub>2C</sub><sup>-/-</sup> mice that exhibit enhanced rebound of cortical Slow Wave Activity after sleep deprivation.

In all constitutive mutants examined with pharmacological tools, sleep regulations reflect adaptations at serotonergic proteins other than the one involved in the mutation. These adaptive processes might participate in the sleep phenotype in addition to the mutation itself.



Finally, in order to dissect more precisely the role of serotonin components in sleep regulations, the data obtained from constitutive mutant mice will have to be completed by using the new molecular tools such as inducible knock-out and lentiviral technology. Altogether, the studies performed to date have enlightened the complex role of serotonin in sleep-wakefulness regulations, particularly when taking into account the developmental components. In that sense, constitutive mutants might be interesting to define “critical” developmental periods related to sleep disorders vulnerability, that probably parallel emotional impairments.

**Daniel P. Cardinali, Seithikurippu R. Pandi-Perumal and Venkataramanujan Srinivasan**

#### **Circadian control by serotonin and melatonin receptors: clinical relevance**

This chapter reviews analyze the role of the different serotonin receptors located in the circadian apparatus and their possible clinical implications for the activity of antidepressants. The central oscillator located in the hypothalamic suprachiasmatic nuclei (SCN) received three major inputs: (a) the retinohypothalamic tract, which is extending from the retina and releases glutamate and pituitary adenylate cyclase-activating polypeptide at its nerve endings; (b) the geniculohypothalamic tract, which originates in the retino-recipient area of the intergeniculate leaflet (IGL) and releases neuropeptide Y and  $\gamma$ -aminobutyric acid as transmitters; (c) a dense serotonergic innervation arising from ascending projections of serotonin neurons in midbrain raphe nuclei. Serotonergic projections come directly from the median raphe nucleus and indirectly from the dorsal raphe nucleus via the IGL. Destruction of serotonergic afferents to the SCN modifies circadian behavioral responses to light. At the SCN several serotonin receptors are localized (i.e., 5HT1A, 5HT1B, 5HT2A, 5HT2C, 5HT5A and 5HT7 receptor subtypes). Basic and clinical data on the efficacy of agomelatine, a novel antidepressant that combines a potent agonist activity of melatonin MT1 and MT2 receptors and an antagonist activity of 5-HT2C receptors, are reviewed.

**Larry D. Sanford, Richard J. Ross and Adrian R. Morrison**  
**Serotonergic mechanisms contributing to arousal and alerting**

Serotonin (5-HT) is implicated in the regulation of both behavioral arousal and a brainstem alerting system that operates in wakefulness and in rapid eye movement sleep (REM). Activation of the brainstem alerting system is marked by the presence of ponto-geniculo-occipital (PGO) waves that occur in association with orienting in wakefulness and spontaneously in REM. Local application of serotonergic agents into REM and PGO wave regulatory regions can alter REM, but there is conflicting evidence as to whether 5-HT in the brainstem can independently influence PGO wave generation. A potential site of action of 5-HT outside the brainstem is the amygdala, which can influence arousal as well as neurobiological responses to novel and significant stimuli. The amygdala also modulates the occurrence and amplitude of PGO waves. We discuss the linkages between arousal and alerting systems and the role 5-HT may play in their regulation at brainstem and amygdalar sites.

**Gordon F. Buchanan, Matthew R. Hodges and George B. Richerson**

**Contribution of chemosensitive serotonergic neurons to interactions between the sleep-wake cycle and respiratory control**

Serotonergic neurons in the midbrain and medulla are sensitive to changes in serum CO<sub>2</sub> concentrations. Medullary serotonergic neurons project to brainstem respiratory control centers and stimulate breathing. Midbrain serotonergic neurons project to thalamocortical circuitry responsible for sleep-wake modulation. There is state-dependent modulation of these medullary serotonergic neurons that may be responsible for state-dependent changes in respiratory rate and breathing regularity. Thus, with projections to both respiratory control centers and thalamocortical arousal circuits, chemosensitive serotonergic neurons are poised to induce both arousal and increased ventilation in response to potentially life-threatening increases in PCO<sub>2</sub>. This may have important implications for such clinical conditions as sudden infant death syndrome (SIDS), obstructive sleep apnea (OSA), and panic disorder, disorders in which serotonin is thought to contribute to the underlying pathophysiology.

**Sigrid Carlen Veasey**

**Obstructive sleep apnea: The potential for serotonergic pharmacotherapies**

Obstructive sleep apnea syndrome is a highly prevalent disorder, associated with numerous cardiovascular and neurobehavioral morbidities. A unique feature of this syndrome is the sleep-state dependency of upper airway collapse and obstruction. Indeed, individuals with obstructive sleep apnea have normal breathing while awake and only manifest airway obstruction in sleep. This sleep-state dependency for obstruction strongly supports the concept that drugs targeting the neurochemical events that underlie the state-dependent obstruction should effectively treat obstructive sleep apnea. Tremendous progress has been made in understanding the neurochemical mechanisms involved in state-dependent control of breathing. It is apparent from this work that there are many potential avenues for pharmacotherapies, including several promising directions for serotonergic therapies. This chapter provides an update on the involvement of serotonin in breathing and in apneas, and then summarizes trials of serotonergic agonists and antagonists in animal models and humans with obstructive sleep apnea. Future directions are suggested for successful development of safe and effective serotonergic pharmacotherapies for obstructive sleep apnea.

**Spilios V. Argyropoulos, Sue J. Wilson and David J. Nutt**

**The effects of antidepressant drugs and 5HT<sub>1A</sub> agonists on human sleep**

Antidepressants, in general, affect sleep. The most consistent effect is suppression of the rapid eye movement (REM) sleep, and this is observed both in healthy volunteers and depressed patients. REM is affected most by drugs that block the reuptake of serotonin, like the selective serotonin reuptake inhibitors (SSRIs) and the serotonin noradrenaline reuptake inhibitors (SNRIs). Further, these drugs often disrupt sleep continuity. The 5HT<sub>1A</sub> agonist anxiolytics (azapirones) like buspirone show an REM suppressant effect but they do not affect sleep continuity. We discuss this difference in terms of likely explanatory 5HT mechanisms for the above effects. With chronic treatment, there is gradual diminution of the sleep effects of the SSRIs. Finally, we discuss the subjective sleep effects of these drugs, which are often different from the polysomnographic ones.

**Andrew Winokur and Jayesh Kamath**

**The effect of typical and atypical antipsychotic drugs on sleep of schizophrenic patients**

Patients with schizophrenia commonly report problems related to disturbed sleep, most often presenting as difficulties with sleep onset latency (SOL) or with sleep maintenance, whereas others have identified disrupted circadian sleep control such as phase delay and even free running cycles. Several studies have evaluated objective measures of sleep abnormalities in patients with schizophrenia by means of polysomnographic techniques. While the majority of published reports are associated with substantial methodological problems that confound interpretation of the data presented, some consistent patterns of findings can be observed across a number of studies examining sleep physiology in schizophrenic patients. The most consistently reported findings have included prolonged SOL and increased wake time after sleep onset (WASO). Additionally, some studies reported diminished slow-wave sleep (SWS) time in patients with schizophrenia as compared to healthy controls, and other studies noted short REM latency (REM-L). However, the latter two findings were not as consistently replicated across studies. Considerable progress has been made in recent years in delineating neurobiological mechanisms involved in the regulation of sleep and wake states as well the coordination of transitions within sleep from one state to another (e.g., regulating transitions from non-REM to REM sleep stages). In addition to the major excitatory and inhibitory neurotransmitter systems glutamate and GABA, monoamine neurotransmitter systems, including dopamine (DA), norepinephrine (NE), serotonin (5-HT), acetylcholine (ACh) and histamine have all been demonstrated to play important roles in the sleep state regulation process. Antipsychotic drugs, including both the typical and atypical antipsychotic classes, have all been shown to produce prominent inhibitory effects at DA D2 receptor sites. Additionally, to varying degrees, different antipsychotic drugs produce modulatory effects on 5-HT, NE, ACh and histaminergic receptors, thereby providing a strong rationale for anticipating prominent effects of antipsychotic drugs on sleep physiology when administered to patients with schizophrenia, other psychiatric disorders as well as to healthy control subjects. A number of studies have reported data on effects of antipsychotic drugs on sleep measures in patients with psychiatric disorders and in healthy control groups. Many of the published reports are noted to have significant methodological limitations, making interpretation of the findings tenuous in some cases. Nevertheless, some consistent patterns of findings have been reported to date. In general, administration of typical antipsychotic drugs has been noted to result in improved sleep continuity, as characterized by increased total sleep time, increased sleep efficiency and decreased SOL and WASO. In these studies, SWS was generally not altered but REM-L was occasionally increased. In studies examining effects of the atypical antipsychotic drugs, generally similar patterns of improvement of sleep continuity measures have been reported across the various agents in this class, but some differences have been noted in terms of effects on SWS and on parameters of REM activity. Differences in effects of various atypical antipsychotic drugs on sleep may be explained by their differing pharmacological mechanisms of action. Substantial limitations are noted in terms of the studies of effects of atypical antipsychotic drugs on sleep. For several of the drugs in this class, no published studies have characterized their effects in the clinical populations in which they are used most extensively. Thus, there is a need for much further work in this area of great clinical importance.

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