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PHYSIOLOGICAL REVIEW

Serotonin and sleep

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Summary For 50 years, serotonin has been in the centre of the search for the mechanisms and control of sleep. Serotonergic neurotransmission is related to the behavioural state of the animal and plays an important role in modulation of the behavioural state, by interacting with other brain areas modulating circadian rhythm, sleep and waking. Serotonergic activity may be accompanied by waking or sleep depending on the brain area and receptor type involved in the response, on the current behavioural state and on the concomitant agonism/antagonism of other neurotransmitter systems.

INTRODUCTION

The neurotransmitter serotonin has played a crucial role in the last 50 years of search for the mechanisms and control of sleep. Indeed, for a short period in the 1960s serotonin was by many considered *the* neurotransmitter of sleep, until it turned around, for a while a full 180°, with the recording of raphe neurones in naturally sleeping animals, to serotonin being a waking neurotransmitter. The truth probably lies somewhere in between or, rather, both may be true. From the earliest studies of serotonin and sleep, both types of responses, and often biphasic responses (arousal followed by synchrony and sleep), have been recorded following serotonergic manipulations. As a neurotransmitter, acting on many different receptors, serotonin may be involved in many processes relevant for the control of both sleep and waking, depending on the localization in the brain, the type of receptor it acts on and the current state of the individual. The last decades of research have shown that sleep is a complex process, and the idea that it is governed by a single brain structure or by a single neurophysiologically or neurobiochemically activated mechanism is no longer feasible.

In this paper will be reviewed some of the data indicating the versatile role of serotonin in sleep and waking, including some of the old work which the hypothesis on the sleep-inducing effect of serotonin was based on. Much of the early data seem to have been forgotten in light of the more recent data on raphe nucleus activity and serotonin release in the different sleep and waking stages, which emphasize the role of serotonin as a modulator of waking activities. The early experiments were often done with crude methods and "dirty" drugs, which for each experiment seen in isolation might explain why the results were obtained. However, together they tell a story which is not to be ignored.

Since the description of the cellular activity of the raphe neurones was such a milestone in serotonin–sleep research, this review is in two parts, the early data and the last 25 years of research – with a few exceptions – being grouped separately. During recent decades, a lot of new data on sleep mechanisms and brain areas potentially involved in the control of sleep and waking have appeared. In

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the last part of the paper some of these findings are briefly reviewed, with particular reference to the relations to serotonergic activity.

Several recent reviews emphasize specific aspects of the serotonin, sleep and waking story and should be consulted for more detailed information [1–4].

THE EARLY DATA

Administration of serotonin into the brain

After serotonin had been found in the central nervous system [5], an effect on synchronization and/or sleep was described by several researchers. The biphasic effects of serotonin administration were noted in some of the very first studies on serotonin and sleep. Bradley (cited by Koella [6]) injected low doses of 5-hydroxytryptamine (5-HT) into the lateral ventricle in cats. After an initial period of arousal, signs of drowsiness and sleep occurred. Similar effects was observed following intravenous injection: initial arousal followed by prolonged hypersynchrony was observed following administration of low doses (5–30 μg/kg) in curarized, unanaesthetized cats. In "cerveau isolé" cats (cats with a brain stem transection at the level of the mesencephalic colliculi), injection of serotonin lead to immediate arousal, with subsequent signs of electroencephalogram (EEG) synchrony [7].

Koella and coworkers did several studies aimed at establishing the effects of serotonin on sleep and at clarifying the site and mechanisms of action. They studied recruiting responses, which are thalamic synchronization responses produced by medial thalamic slow frequency (10/s) stimulation. Small doses of serotonin administered into the carotid artery led to an initial attenuation or complete suppression of the recruiting responses. This was followed by a prolonged period of increased recruiting lasting for up to 20 min. Similar biphasic effects were seen in the EEG, i.e. arousal followed by hypersynchrony, and the effects were accompanied by an increase followed by a decrease in pupillary size, suggestive of arousal followed by true sleep. Following a brain stem transection at the pretrigeminal level, only arousal signs were seen [7]. In lightly anaesthetized cats, injection of serotonin into the third and fourth ventricles induced EEG and ocular signs of sleep. In freely moving cats with implanted EEG recording electrodes, injection of serotonin into the fourth ventricle via an implanted cannula induced a typical sleep picture with exaggerated high voltage slow waves within 1–5 min [6]. Thus, in Koella's work, the arousal response seems to be a thalamic phenomenon, while the synchronization–sleep effect was proposed to stem from stuctures in the floor of the fourth ventricle: area postrema and the nucleus tractus solitarius. Nucleus tractus solitarius is a site for integration of visceral information and its modulation by afferent neural systems, among others afferents from the raphe nuclei, serotonin mainly having a depressant action possibly via 5-HT_{1B} receptors [8]. It is also rich in opioid receptors, the stimulation of which produces a hypnotic effect by enhancing slow wave sleep (SWS) [9].

Lesions of the raphe nuclei

The description of serotonergic cells in the raphe nuclei of the brain stem and their projection [10, 11] made it possible to lesion serotonergic neurones selectively. The rostral group, the dorsal and medial raphe nuclei, projecting to the forebrain (thalamus, hypothalamus, striatum, hippocampus, and frontal cortex) was of main interest in this context.

Jouvet and coworkers (see reviews by Jouvet [12, 13]) performed subtotal lesions of the raphe nuclei in cats and found a reduction of sleep parallelled by a reduction of brain serotonin. Together with the *para*-chlorophenylalanine (pCPA) data this was the main evidence for the serotonin–sleep hypothesis, postulating the ascending serotonergic system as essential for SWS [12]. In rats, however, raphe lesions tended to induce hyperactivity but not loss of sleep [14]. Also, it appeared that a temporary raphe lesion by cooling induced sleep when it was performed during waking and induced waking when it was performed during sleep, the opposite of what would be expected [15]

Blocking serotonin formation by *para*-chlorophenylalanine

pCPA is a compound which blocks tryptophan hydroxylase, the enzyme catalysing the transformation of serotonin from its precursor, the amino acid l-tryptophan. Several studies have demonstrated that sleep is blocked or heavily reduced in a period following administration of pCPA, in both cats [12, 16] and rats. In cats, a moderate dose primarily reduced the deep SWS with delta

activity [17]; the same was the case in rats [18]. The insomnia is reversed by 5-hydroxytryptophan (5-HTP), serotonin's immediate precursor [16, 19], although after a delay of 30–60 min [19]. After a moderate pCPA dose, presumably leaving some of the enzyme still active, the pCPA-induced insomnia in rats may be reversed by l-tryptophan [18] or by a selective serotonin re-uptake inhibitor [20].

The pCPA data imply that, without serotonin in the brain, there is no sleep. However, with chronic administration of pCPA, sleep eventually reappears, while brain serotonin is still very low [21]. Following pCPA administration, there is a "release" of the ponto-geniculo-occipital (PGO) activity, which normally accompanies rapid eye movement (REM) sleep in cats. It was hypothesized that this activity initially activated the animals, until habituation occurred [21]. However, close observation indicated that the insomnia starts much earlier than the increase in PGO activity [22].

Increasing brain serotonin via precursor administration

Serotonin is formed within serotonergic neurones from the amino acid precursor l-tryptophan, by the enzyme tryptophan hydroxylase, which is the rate-limiting factor. This enzyme is found only within serotonergic neurones. The first step is the immediate serotonin precursor 5-HTP. The reaction from 5-HTP to 5-HT, or serotonin, is catalysed by 5-HTP decarboxylase. This conversion can take place also outside serotonergic neurones (see [23] for references).

As mentioned above, the immediate precursor 5-HTP reverses the insomnia induced by pCPA and makes the animals go back to sleep. Given alone, however, 5-HTP tends to induce hypersynchrony [19] or drowsiness, which in cats is characterized by a 6–8 c/sec synchronous EEG activity. It is possible that these effects are due to serotonin formation outside serotonergic neurones [23].

Hartmann *et al.* [24] found a reduction of sleep latency following a low dose (1 mg) of l-tryptophan. With a larger load of l-tryptophan, there was no further improvement of sleep latency, but an increase of SWS and a reduction of REM sleep. The 1 mg dose is within the range of normal dietary intake, although competition for serotonin uptake in the brain with other amino acids may reduce the effect of dietary l-tryptophan. However, carbohydrate ingestion alters the brain uptake in favour of l-tryptophan [25]. In a study of l-tryptophan effects in humans in the daytime, we found reduced sleep latency compared with placebo as well as increased EEG alpha activity during waking, suggesting a deactivation effect [26]. Plasma melatonin is increased following L-tryptophan infusions [27], and thus the effect may not solely be a result of increased serotonin. For a while, l-tryptophan was being used as a hypnotic, until such use was halted by the report of several cases of eosinophilia–myalgia syndrome, some leading to death, following intake of the amino acid [28]. Most likely, however, this was due to a contamination of one particular product [25]. In cats, l-tryptophan increases drowsiness [23]. In rats, the results are variable and seem to depend on dose, route of administration and also time of day [29].

In sum, the serotonin precursors seem primarily to induce drowsiness, and the reduced sleep latency and sleep effects may be a consequence of this sedation. The hypersynchrony seen especially after 5-HTP but also following l-tryptophan is consistent with the earlier findings on serotonin application to the brain discussed above and with some more recent pharmacological data discussed later.

THE LAST 25 YEARS OF RESEARCH ON SEROTONIN AND SLEEP

The reports on the unit activity of dorsal raphe neurones [30–32] were the introduction to a new era in serotonin–sleep research. Together with the data on cooling of the raphe nucleus [15], these data were the first to seriously question the serotonin–sleep hypothesis. However, the neurophysiological data were also the result of an important development of techniques, making it possible to collect data from unanaesthetized, spontaneously sleeping animals (e.g. unit recording and microdialysis data). This development was paralleled by developments in pharmacological research on serotonergic neurotransmission, with the introduction of selective serotonin re-uptake inhibitors and the description of a variety of subtypes of serotonergic receptors and increasingly specific agonists and antagonists to these receptors. Recently, developments within molecular biology and genetics have also benefited sleep research.

Dorsal raphe neuronal activity and release of serotonin throughout the sleep–wake cycle

The first study of unit recordings from the dorsal raphe nucleus area, in spontaneously sleeping cats, was published in 1976 [30]. The neurones had their highest activity in waking, and the activity was reduced during SWS to almost complete cessation of firing during REM sleep. These data were soon confirmed [31, 32]. It was also reported that stimulation of the dorsal raphe nucleus induced wakefulness [32]. By means of a fluorescent labelling technique it has been demonstrated that the cells responsible for the dorsal raphe neurone firing pattern are serotonergic [33]. The state dependence has mostly been investigated in the dorsal raphe nucleus. However, the pattern is generally similar in the other raphe nuclei, with some exceptions, one being that some of the caudally projecting nuclei are less related to behavioural state [33].

Recently, the firing pattern of putative serotonergic neurones has also been studied in rats [34]. The neurones exhibit progressive decrease in discharge rate from waking through SWS to REM sleep. The discharge is reduced after systemic administration of a selective 5-HT_{1A} agonist, indicating that it stems from serotonergic neurones.

The findings on extracellular content of serotonin in different brain areas have recently been reviewed [3]. There seems to be a general tendency that serotonin measured in the extracellular fluid, which presumably mirrors the actual release in the area where it is measured, is highest during waking, is reduced during SWS and more reduced during REM sleep. This is the case in the dorsal raphe nucleus in both cats [35] and rats [36], in the brain stem pedunculopontine tegmental (PPT) nucleus in cats [37] and in hippocampus in rats [38]. The pattern is largely consistent with data on the firing activity of the dorsal raphe nucleus in cats [30–32] and rats [34], with the exception that the values measured during REM sleep are comparatively less reduced than the firing decrement in this stage. This may be an effect of the methodology [37] or it may indicate release without firing. Dendritic release of serotonin in the dorsal raphe nucleus during sleep has been suggested as being important for the reduction of dorsal raphe neuronal activity in SWS and REM sleep, via somatodendritic autoreceptors [39].

The data on raphe neurone activity, as well as the microdialysis data, suggest a modulating activity of the raphe neurones, in phase with the state of the animal. Two major questions arise: is the raphe neurone activity instrumental in defining where the animal is on the sleep–wake axis and is the relation to the sleep–wake axis an innate function of these neurones or is it based on afferent information from other parts of the brain?

The presence of GABAergic function in the dorsal raphe neurones has long been recognized. Recently it was demonstrated that extracellular levels of γ-aminobutyric acid (GABA) in the dorsal raphe nucleus are higher in REM sleep than during waking and non-REM (NREM) sleep [40]. Following iontophoretic application of a $GABA_A$ antagonist into the dorsal raphe nucleus, the firing activity of the cells was not suppressed during SWS, suggesting GABAergic modulation as the background for this relationship [41].

There is also evidence for cholinergic, glutamatergic, noradrenergic, dopaminergic and histaminergic input to the the dorsal raphe nucleus [33]. Recently, hypocretin/orexin projections to the raphe nuclei have been described (see [42]). The implications for serotonergic sleep–wake effects of these findings are discussed below.

Selective serotonin re-uptake inhibitor effects on sleep

The antidepressive selective serotonin re-uptake inhibitors (SSRIs) seem to be ideal drugs to investigate serotonergic effects, in that they will, by inhibiting the re-uptake of serotonin from the synaptic cleft, only increase serotonin released at serotonergic terminals, and only when serotonin is released after physiological activity in serotonergic neurones.

Depressive patients generally complain about disturbed sleep, such as sleep onset insomnia, fragmented sleep with frequent awakenings and early morning awakenings. When recorded polygraphically their sleep shows characteristic changes which may suggest defective serotonergic neurotransmission: reduced REM sleep latency, increased phasic REM sleep activity and reduced deep slow wave (delta) sleep. The most consistent effect of the SSRIs, in all species, is a reduction of REM sleep, supporting the hypothesis of a serotonergic inhibition of REM sleep discussed below. It has been suggested that the antidepressant effect is tied to

this effect. However, recently, antidepressant drugs which do not suppress REM sleep have been developed, invalidating the REM sleep hypothesis [43].

Other sleep effects of the SSRIs vary, with the drug and with the species in which they are investigated. In humans, the REM sleep suppressant effect is prominent. There is usually no sedative effect, but the antidepressant effect of the SSRIs tends to improve insomnia complaints in depression [44], although insomnia is a possible side-effect with some SSRIs. In cats and rats, there is a tendency to biphasic effects following acute administration of SSRIs. In cats, waking is increased initially, then after a delay of around 2 h there is an SWS increase, particularly SWS-2 with delta activity [45, 46]. This effect is potentiated with 5-HTP pre-treatment [47]. The findings opened for the idea that serotonergic stimulation yields complex effects on the sleep–waking axis, both sleep-incompatible and sleep-promoting effects [20]. The increase in waking is accompanied by behavioural and motor phenomena and may be secondary to those [46]. In rats, increased waking followed by increased SWS or SWS-2 has been reported from several but not all studies following different SSRIs (see [1] for references). The biphasic effect is potentiated by an adenosine A_1 antagonist, suggesting modulation of serotonin release by adenosine [48].

There are regional differences in effects of different SSRIs. The synchronizing effect is less pronounced in both cats and rats following an SSRI with low potency in the dorsal raphe and the descending raphe projection [20, 46]. Thus the localization of the serotonergic effect may be important for the type of effect on sleep and waking. A synchronizing effect via serotonergic modulation of ascending sensory information from the spinal cord has been suggested [49].

Practice Points

1. Administration of the serotonin precursor amino acid l-tryptophan reduces sleep latency. A protein-rich meal followed by carbohydrate intake 2 h later may have the same effect.
2. Depressive patients may have characteristic changes in their sleep pattern. The changes are reversed by treatment with SSRIs.
3. SSRIs improve sleep complaints in depressive patients as a part of the antidepressive effect. SSRIs are not effective as hypnotics, and may have insomnia as a side-effect.

Is the receptor subtype important?

With the description of multiple serotonergic receptors [50], the question arouse whether it was possible to assign the divergent effects of serotonin on sleep to different receptors. The development of multiple pharmaceutical agents which may stimulate or block the various receptors has been central in this research. Most of the work on this aspect of the serotonin–sleep–waking problem so far has been done on the 5-HT_{1A} and 5-HT_2 receptor and has been reviewed in detail [2].

The 5-HT_2 receptor antagonist ritanserin promotes sleep in both humans [51] and rats [52], suggesting that this receptor was promoting waking activity, although in cats the antagonist reduces sleep [53]. However, ritanserin did not block the SSRI waking effects in rats, just adding to the SWS effect [54], suggesting different mechanisms. Also, power spectral data indicated that the sleep following ritanserin was different from normal sleep [55]. A mechanism for an arousing effect of 5-HT_2 receptors may be found in the thalamic reticular nucleus and the sleep spindle generating network of relay cells, cortical cells and reticular nucleus cells [56]. The 5-HT_2 receptors have been postulated to modulate the activity in the thalamus in the direction of single spike activity, i.e. waking [57]. Thus, serotonin acting on 5-HT_2 receptors in the thalamus may facilitate single spike activity and waking, but this does not seem to be the full answer to why serotonergic stimulation leads to waking.

The 5-HT_{1A} receptors are located postsynaptically on many neurones, as well as somatodendritically on dorsal raphe neurones. Stimulation of the somatodendritic autoreceptors presumably leads to reduction of the neuroneal activity. Extracellular serotonin decreased following microdialysis application of the 5-HT_{1A} agonist 8-OH-DPAT into the dorsal raphe in cats [58], supporting the concept of release control by the somatodendritic 5-HT_{1A} autoreceptors in the raphe neurones. Perfused into the dorsal raphe nucleus, 8-OH-DPAT increase REM sleep but has no effect on other sleep stages [58, 59].

Stimulation of 5-HT_{1A} receptors via systemic administration of agonists has repeatedly been shown to increase waking. Administration of the agonist 8-OH-DPAT increases waking and reduces REM sleep; however, within SWS, there is a delayed increase of highly synchronized sleep (SWS-2) [59]. The 5-HT_{1A} agonist ipsapiron increases slow wave

activity in both humans [60] and rats [61]. *In vitro* stimulation of 5-HT_{1A} receptors on basal forebrain cholinergic neurones increases rhythmic burst activity mediated by low threshold calcium spikes [62]. Thus, possibly, 5-HT_{1A} receptor stimulation in the forebrain may facilitate if not SWS at least synchronization within SWS. In the spinal cord, local infusion of 8-OH-DPAT reduces waking and increases SWS, but does not increase SWS-2 in particular [49]. This suggests a deactivating effect of spinal serotonergic transmission. This is consistent with the finding of differential effects on SWS-2 of different SSRIs discussed above [46].

Thus there are some indications that 5-HT_{1A} receptors may facilitate sleep or sleep characteristics at least in some central nervous system areas. The increase of waking seen following systemic administration of 5-HT_{1A} agonists does not necessarily contradict this picture. As discussed above, this may be due to serotonergic effects incompatible with sleep (e.g. the serotonin syndrome [59]) or sleep modulating serotonergic effects from different locations in the central nervous system. The 5-HT_{1A} autoreceptors in the dorsal raphe neurones complicate the interpretation of some of the data. With systemic administration of 5-HT_{1A} receptor modulating agents, it it often unclear whether the effect stems from post-synaptic receptors or whether the effect is a result of altered serotonin release at the terminals secondary to modulation of the autoreceptors.

The preoptic/basal forebrain area, sleep and serotonin

This area has been in focus as a possible site for sleep–wake regulation for many decades. A group of cholinergic cells which project monosynaptically to the entire limbic telencephalon and neocortex, modulating arousal and attention, is located here (reviewed in [63]). However, the area has a longer history of research on sleep-promoting functions. On the basis of von Economo's findings in an insomniac subgroup of patients suffering from encephalitis lethargica, Nauta [64] did transections of the anterior hypothalamus at the level of the optic chiasm in rats. He found that the rats displayed continuous motor activity until they died after a few days. Many years later this was followed up by Sterman and Clemente [65] who demonstrated that sleep could be induced by stimulation of the basal forebrain-pre-optic area in cats. Also, sleep was reduced after electrolytic preoptic lesions [66]. Sleep active cells are found in the ventrolateral preoptic (VLPO) area in cats [67], and a population of cells which showed c-fos activation after sleep was described in rats [68]. Lesions of the VLPO cluster cause reduction in NREM sleep and delta activity, while lesions in the region containing scattered VLPO neurones medial or dorsal to the cluster are more associated with loss of REM sleep [69].

The GABAergic neurones identified in this area have been implicated in the sleep-promoting effects [63]. Many of the cells also contain galanin [70]. Several recent studies suggest a reciprocal interaction between this area and serotonergic raphe neurones. *In vitro* data indicate that some of the VLPO cells are inhibited by serotonin [71], suggesting serotonergic input, and both afferent input from [72] and efferent projections to [70, 73] the dorsal raphe nucleus have been demonstrated. Thus it is possible that serotonergic raphe neurones may modulate sleep-promoting cells in the VLPO and, vice versa, that GABA- (and/or galanin-) ergic VLPO cells may modulate the activity of serotonergic raphe neurones and contribute to the decrease of activity during NREM sleep and REM sleep. Jouvet [13] has suggested that serotonin released in the preoptic region during waking may participate in a homeostatic sleep factor process. The clock-like action of dorsal raphe neurones may be marking duration and intensity of waking, then herald a cascade of genomic events which will trigger sleep onset. Such a chain of events may involve the modulation by serotonin of preoptic–VLPO area neurotransmitter projections as described above or, possibly, the serotonergic modulation of the sleep-promoting hormones vasoactive intestinal peptide (VIP) and prolactin described below. The sleep-active cells are also thermosensitive, and preoptic warming slows down the activity of neurones in hypothalamus and (presumably serotonergic) cells in dorsal raphe with high activity during waking [34].

The cholinergic basal forebrain cells receive afferent input from both the dorsal and the median raphe nuclei, with both excitatory and inhibitory effects [74]. Although only a minority of these connections are serotonergic, a serotonergic modulation of these neurones, and thereby modulation of cortical activation, is possible. Recently it was demonstrated that sleep deprivation in cats increases basal forebrain adenosine as measured by

microdialysis [75]. A prominent effect of adenosine in the brain is its ability to inhibit the release of neurotransmitters, including serotonin. These effects are probably mediated by pre-synaptic A_1 receptors in most systems and may be due to inhibition of calcium entry into nerve terminals (reviewed in [76]). Thus, one possible mechanism for the sleep effects of adenosine is via adenosinergic modulation of serotonin release. It has been demonstrated that adenosine perfused into the dorsal hippocampus of rats decrease extracellular 5-HT levels [77].

Dorsolateral hypothalamus, hypocretin/orexin, serotonin and sleep

Recently, a new peptide, hypocretin or orexin has been described in neurones of the dorsal and lateral hypothalamus (see overview by Mignot [42]). Two versions of the peptide, hypocretin 1 and 2 or orexin A and B, and corresponding receptors have been described. It appears that narcoleptic dogs have a mutation in the hypocretin 2/orexin B gene. Some but not all narcoleptic patients are deficient of hypocretin in the spinal fluid. Also, orexin knockout mice have episodes of behavioural arrests similar to human narcolepsy [78]. The hypocretin/orexin neurones have wide projections to multiple neuronal systems [79], in particular to many areas of the hypothalamus and amygdala. They project to areas which may be involved in sleep and arousal: the tuberomammillary nucleus, the locus coeruleus, the raphe nulei, the PPT nucleus, the laterodorsal tegmental (LDT) nucleus and the nucleus tractus solitarius. The effect is excitatory, and it has been speculated that the hypocretin/orexin projections may drive the modulatory effects on sleep and waking attributed to these areas [42]. An arousing effect of hypocretin 1/orexin A together with activation of locus coeruleus firing has been demonstrated by intracerebroventricular (i.c.v.) application in rats [80], and local administration into the locus coeruelus increases waking and suppresses SWS and REM sleep [81].

I.c.v. administration of hypocretin 1/orexin also suppress plasma prolactin [80]. Prolactin is secreted during sleep. Data indicate that serotonin, acting directly at the posterior pituitary, stimulates the secretion of prolactin and VIP, probably by releasing an unidentified mediator from melanotropes [82]. The hypnogenic effect of VIP (see [13]) is probably mediated via prolactin. There also prolactin-containing neurones in the hypothalamus and preoptic area. Hypothalamic prolactin is thought to participate in the modulation of neurotransmission involved in REM sleep generation [83]. Apparently the distribution of neurones described as prolactin immunoreactive is similar to the distribution of the hypocretin/orexin neurones, and it has recently been demonstrated that hypocretin/orexin and prolactin coexist in the same neurones [84]. Thus there are possibilities of an interplay between hypocretin/orexin, prolactin and serotonin in sleep modulation and in narcolepsy. The mechanisms of such interactions remain to be elucidated.

Circadian rhythm, the suprachiasmatic nucleus, serotonin and sleep

The suprachiasmatic nucleus (SCN), which is central in the regulation of circadian rhythm, has one of the densest serotonergic terminal plexes in the brain. Also, the intergeniculate leaflet which projects to the SCN receives serotonergic input from the dorsal raphe nucleus, while SCN receives input from the median raphe nucleus [4]. However, it is not clear how serotonin is involved in the modulation of the circadian rhythm. It has been suggested that serotonin modulates the sensitivity of the circadian system to light [4]. Another hypothesis is that serotonin mediates phase-shifting effects of behavioural stimuli on circadian rhythms [85]. Serotonin can advance the pacemaker when applied during the subjective day and delay the pacemaker when applied during the subjective night [86]. *In vitro* data implicate stimulation of 5-HT_7 receptors on pacemaker cells in the phase advance effect of serotonin. A number of neuromodulators may influence the serotonergic phase shifts [86]. There are projections, both exitatory and inhibitory, from the SCN to the preoptic area which may convey information on circadian phase to this sleep-promoting area [87].

Thalamocortical projections, sleep spindles and serotonin

The 7–14 c/sec sleep spindles which identify the onset of sleep in the EEG are the result of a thalamocortical oscillatory process generating rhythmic burst activity by low threshold Ca^{2+} spikes.

GABAergic neurones of the reticular nucleus, thalamocortical relay cells and cortical pyramidal cells participate in the spindle generation [56]. This oscillatory activity may be modulated by neurotransmitters, including serotonin. There is a dense serotonergic innervation of the reticular nucleus, possibly arising from the dorsal raphe nucleus or the nucleus raphe pontis. The receptors involved in this modulation are probably 5-HT_2 receptors (reviewed in [88]). Serotonin has a potent excitatory action on the GABAergic reticular nucleus neurones *in vitro*, associated with the occurrence of single-spike activity. However, within a certain range of the membrane potential, i.e. with moderate depolarization, there is an increased probability of rhythmic oscillations. This is consistent with a model for control of reticular thalamic oscillations [89]. In this model it is proposed that the oscillating properties of the reticular nucleus depends on the level of the membrane potential, which may be modulated by serotonin and noradrenalin by the blocking of a potassium current ("leak K^+"). A moderate serotonergic input may slightly depolarize the reticular nucleus cells and generate oscillatory activity and sleep spindles. With no such input there is resting hyperpolarization and no oscillations, while strong serotonergic input lead to depolarization and a switch to single-spike activity as in waking. This excitation of the reticular nucleus neurones probably occurs through activation of 5-HT_2 receptors. In behaving cats, there is a decrease of dorsal raphe unit activity before and during, but not after, the occurence of a sleep spindle [31].

During later stages of sleep, the spindle oscillations are progressively reduced and replaced by thalamocortical oscillations with slower frequencies, delta waves [1–4 Hz] and slow oscillations. Hyperpolarization of the cells is essential for the generation of delta waves, which, in contrast to the sleep spindle generation, may take place in single cells (basal forebrain cells [62], thalamic sensory relay cells and cortical cells [56]). The delta wave activity in cortical cells is synchronized via local mechanisms or via thalamic interconnections [56]. Serotonin can modulate the excitability of cortical cells in a number of different manners, including inhibition, excitation and voltage-dependent facilitation. Also, a mix of the different actions of serotonin in different types of cells may result in even more complex modulation [88].

Serotonin and REM sleep

One characteristic aspect of dorsal raphe neurone activity is the reduction of firing to almost nothing during REM sleep. This phenomenon is not unique for the serotonergic neurones; it also applies to the neuroneal activity of the noradrenergic locus coeruleus neurones. A model for the alternation between SWS and REM sleep postulates either the locus coeruleus or the raphe nucleus as the REM sleep inhibitory area [90, 91].

Pharmacological studies indicate that increased synaptic serotonin reduces REM sleep. Increasing brain serotonergic activity by precursor loading with 5-HTP and l-tryptophan [19, 23], as well as with SSRI administration [20, 46], reduces REM sleep.

REM sleep is generated in the pontine region of the brainstem. A thorough overview of the research leading to this conclusion is given by Siegel [92]. Different, but adjacent, structures in this region have been implicated in REM sleep generation. The role of mesopontine cholinergic neurones in the LDT nucleus and PPT nucleus in the modulation of REM sleep has been reviewed by McCarley *et al.* [91]. The serotonergic modulation of REM sleep is postulated to be due to stimulation of 5-HT_{1A} receptors in the LDT and PPT nuclei. There is evidence that LDT and PPT nuclei receive serotonergic fibres [93], and serotonin is released in the PPT nucleus in a state-related manner, highest during waking and lowest during REM sleep [37]. Some of the neurones in these nuclei show preferential activity during REM sleep ("REM-on cells") [91]. Lesions in this area decrease REM sleep, while electrical stimulation of the LDT nucleus increase REM sleep [94]. When these neurones are perfused via microdialysis with the 5-HT_{1A} agonist 8-OH-DPAT, the discharge is almost completely suppressed [95], supposedly as a result of inhibition of the REM-on cells [91]. Consistent with this, microinjection of serotonin into the LDT nucleus suppresses REM sleep [96]. Also, infusion of a 5-HT_{1A} agonist in the dorsal raphe nucleus via microdialysis increases REM sleep in both cats [58] and rats [59], presumbaly via stimulation of dorsal raphe autoreceptors, reduced serotonin release and reduced serotonergic inhibition of the REM sleep generating neurones. Systemic administration of a 5-HT_{1A} antagonist reduces REM sleep, presumably via antagonism of the autoreceptors and increased serotonin release and REM sleep inhibition [97].

Another area which has been implicated in the generation of REM sleep is an area adjacent to the locus coeruleus, peri-locus coeruleus alpha in the medio-dorsal pontine tegmentum (see [92]). Neurones in this area display an increase in discharge rate just prior to and during REM sleep [98], and lesions suppress REM sleep [99]. Application by microdialysis of noradrenaline and adrenaline, but not serotonin, to this area suppresses REM sleep; in the rostral area noradrenaline and adrenaline induce REM sleep without atonia [100]. The different areas implicated in REM sleep may be important for the different physiological aspects of this sleep stage (atonia, PGO activity, EEG desynchronization); these areas may interact to generate REM sleep [92].

Recent research indicate that nitric oxide (NO) is involved in the regulation of REM sleep (see Burlet *et al.* [101] for an overview). NO acts as a brain intracellular messenger and modulator of neurotransmitter release. It is co-localized with serotonergic dorsal raphe neurones in rats and with cholinergic LDT–PPT neurones. NO synthesis is catalysed by nitric oxide synthase enzymes. Administration of NO synthase inhibitors into the dorsal raphe nucleus in rats reduces REM sleep, hypothesized as being a result of reduced serotonin release within the dorsal raphe [101, 102]. Administration of NO synthesis inhibitors into the mesopontine reticular formation of cats also decreases REM sleep, interpreted as an effect via modulation of acetylcholine release [103]. Some studies also find an effect on SWS of NO modulation in the PPT nucleus.

CONCLUSION

The central characteristic of most of the serotonergic raphe neurones is their activity in relation to the sleep–wake cycle. According to Jacobs and Azmitia [33] serotonin acts to modulate the function of target organs with respect to the sleep–wake axis. A common way of interpreting the raphe activity data is, however, much more simple: the dorsal raphe and serotonergic neurotransmission is seen as an arousal system [42, 70], being turned more or less off during sleep. Alternatively, as suggested by Jouvet [13], the dorsal raphe activity initiates a process which eventually results in sleep. One may also raise the more subtle question of whether the raphe neurone activity, and target organ serotonin release, is instrumental in defining where the animal is on the sleep–wake axis. If so, is it the amount of activity of raphe neurones, and serotonin release, which determines the state, or has the frequency, or possibly pattern, of raphe neurone firing any information value in itself? An example of a possible frequency dependence is the "window" for sleep spindle activity in the thalamus, where a moderate serotonergic input may slightly depolarize the reticular nucleus and generate oscillatory activity, whereas no serotonergic input results in no oscillations and stronger input in single spike activity.

Another question that one may raise concerns the background of the state dependence of the raphe neurones. It is clear that this activity may be modulated by many other neurotransmitters; however, there is also an increasing amount of data indicating that serotonergic activity may modulate other structures involved in sleep and waking, like the thalamic reticular nucleus, the VLPO and the SCN. Most likely there is an interplay between the raphe nucleus and other areas, involving many other neurotransmitters and neurotransmitter systems than the serotonergic. What actually starts sleep may vary from one situation to the next.

There are also indications of differential effects with regard to sleep and waking dependent on the type of receptors receiving the serotonergic input and where they are located. In the thalamus, 5-HT_2 receptors are probably responsible for the excitation of the reticular nucleus leading to single-spike activity and waking. This could be an important mechanism for the arousing effects of serotonin. It could also be a mechanism by which serotonin modulates the oscillatory activity of the reticular nucleus and thereby the state of the individual. Serotonin may also modulate cortical activation, via modulation of the cholinergic basal forebrain cells, or modulation of cortical cells. More directly, 5-HT_{1A} receptor stimulation seems to facilitate deep SWS or at least slow wave activity within sleep. The mechanism for this effect is not clear. Several data sets point towards the descending raphe nuclei for mediating such effects; also, the basal forebrain has been implicated via (*in vitro*) data on oscillatory activity via 5-HT_{1A} receptors. Jouvet [13] has suggested that release of serotonin during waking may initiate a cascade of genomic events in neurones in the preoptic area, thus linking serotonin to a homeostatic regulation of SWS.

It is evident that the differential effects of serotonin on sleep and waking cannot occur simultaneously, although, with a massive serotonergic stimulation such as that following SSRI administration, both increased waking and increased slow wave activity are seen within the time period of drug effects. The point is that, as a neurotransmitter and neuromodulator, serotonin influences a multitude of different, sometimes opposite, physiological responses, depending on the receptor type and localization in the brain, on whether the receptor is pre- or postsynaptic, and depending on the current state of the individual.

Research Agenda

1. Investigate interactions between prolactin, hypocretin/orexin, serotonin, sleep and narcolepsy.
2. Research on the sedating–hypersynchrony effects of serotonergic stimulation.
3. Clear up current controversies on REM sleep generating areas, serotonergic inhibition of such areas and the role of nitric oxide.

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