WhystaypanosomesCausesleeping
sickness

African trypanosomiasis or sleeping sickness is hallmarked by sleep and wakefulness disturbances. In contrast to other infections, there is no hypersomnia, but the sleep pattern is fragmented. This overview discusses that the causative agents, the parasites Trypanosoma brucei, target circumventricular organs in the brain, causing inflammatory responses in hypothalamic structures that may lead to dysfunctions in the circadian-timing and sleep-regulatory systems.

When microbes meet the brain, diseases characterized by disturbances in sleep may evolve. Two remarkable examples of this are African trypanosomiasis or sleeping sickness, which is caused by subspecies of the parasite Trypanosoma brucei (Tb) (FIGURE 1A), and encephalitis lethargica (von Economo encephalitis or “sleepy sickness”). Although the infectious agent that caused encephalitis lethargica was never isolated, the lesions in the brain described by von Economo in 1930 (70) predicted contemporary knowledge about how certain diencephalic regions are involved in sleep-wakefulness regulation (61). On the other hand, African trypanosomiasis has a well-known etiology, Tb, but the mechanisms underlying the sleep disruption remain elusive.

Tb spreads to humans by the biting of tsetse flies (FIGURE 1B), which belong to the genus Glossina and are intermediate hosts and vectors of the parasites. The sleep disruption caused by Tb in humans is characterized by episodes of nocturnal insomnia and daytime sleep and not by hypersomnia. In fact, the patients may sleep even less than normally. Thus the sleep disturbances contrast those usually seen during infections, when the individual spends increased time in sleep as a generalized host response to the invading microbe. In this overview, we will highlight and discuss the origin, cause, and unique characteristics of the sleep-wake disturbances that are a hallmark of sleeping sickness.

Human African Trypanosomiasis or Sleeping Sickness

Sleeping sickness occurs in ~250 foci in 36 sub-Saharan countries (FIGURE 2) and is an example of a reemerging zoonosis. At the beginning of the 20th century, an outbreak on the northern shore of Lake Victoria killed two-thirds of the population of Uganda. During this period, Aldo Castellani found the parasites in the cerebrospinal fluid (CSF) of patients with sleeping sickness (7). Programs to control tsetse flies were then instituted, but due to warfare, political instability, and civil unrest, the prevalence now approaches that of the 1920s in certain areas (most notably in Angola, Congo, Uganda, and southern Sudan). About 60 million people live in endemic areas, but less than 4 million of them are under surveillance.

Human African trypanosomiasis (HAT) is found in two clinically distinguishable forms in separate geographical areas (FIGURE 2), although there is an overlap between the two forms in many foci of central Africa. Tb gambiense, occurring mainly...
west of the Rift valley, causes the Gambian or West African form of the disease, whereas *Tb rhodesiense*, east of the valley, causes the Rhodesian or East African form of sleeping sickness. The Gambian form of sleeping sickness is characterized by a slow progression of the disease lasting for months or even years, whereas the Rhodesian form is characterized by a more rapid progression (weeks to a few months). If untreated, both infections are lethal, and the pathological changes in both forms of the disease are similar. The Rhodesian form may thus be regarded as a compressed Gambian form. Another, nonhuman infectious strain, *Tb brucei*, has been widely used in experimental rodent infections.

For practical therapeutic purposes, the disease has been divided into two stages. The first is a hemolymphatic systemic stage; the second, corresponding to neuroinvasion of the parasites, is defined by the presence of *Tb* and/or an increased number of white blood cells in the CSF. During this encephalitic stage, a complex neuropsychiatric disorder evolves, leading to sensory, motor, and psychiatric disturbances, with alterations of sleep representing the most characteristic sign. For treatment of the second stage of the disease, arsenic preparations that pass the blood-brain barrier (BBB) are still the drugs of choice, although they are associated with a high degree of toxicity, which can even be lethal. It is, however, not clear how and when during the infection the blood-borne parasites penetrate the BBB. In experimental rodents infected with *Tb brucei*, it has been determined that the parasites do not penetrate the BBB in the early encephalitic stage, when they are localized to the choroid plexus and other circumventricular organs, as well as to peripheral nerve root ganglia; at these sites there is also an infiltration of lymphocytes (36). Thus from such regions products from the trypanosomes and inflammatory cytokines may diffuse into the surrounding tissue and be released into the CSF.

**Disturbances in Sleep and Sleep-Wake Rhythms During Trypanosome Infection**

Sleep is divided into two distinct phases: sleep with rapid eye movements (REM) and sleep without such movements (non-REM). In humans, four stages of non-REM sleep have been characterized by EEG; these include low-frequency slow wave sleep (SWS) stages with EEG synchronization. Individuals usually fall asleep by entering non-REM sleep, which is followed by shorter episodes of REM sleep. Alternations between sleep and wakefulness follow an endogenous circadian rhythm of ~24 h, and it has been proposed that REM sleep is mainly coupled to circadian mechanisms, whereas SWS depends primarily on homeostatic processes (15) (see below).

The sleep disturbances characteristic of HAT typically imply diurnal somnolence (which gave the disease its name) and in many cases nocturnal insomnia. In patients with the Gambian form of HAT, continuous 24-h polysomnographic recordings, including EEG, showed that the diurnal rhythm of sleep-wakefulness disappears progressively during the disease (10). In particular, REM sleep was affected and occurred throughout the entire sleep-wake cycle at late stages of HAT. The latency to SWS and REM sleep was decreased, and sleep-onset REM sleep occurred, often without any intermediary non-REM sleep (10). Therefore, as mentioned above, in contrast to what the name indicates, sleeping sickness does not involve hypersomnia but leads instead to a disorganization and fragmentation of the 24-h rhythm of the sleep-wake pattern, resulting in sleep episodes during the day and wakefulness periods during the night.

In experimental models of *Tb brucei* infections, fragmented sleep episodes were recorded in acute infections in rats (49), and both length and amount of SWS were reduced in infected rabbits (69). We have used a rat model with a chronic course of disease, reaching a terminal stage 55–60 days after infection (but still shorter than the human forms of disease). In this model, a marked fragmentation of the sleep structure was observed, with frequent awakenings during SWS episodes, a reduction of the average length of SWS episodes, and a reduced REM sleep latency (22). Interestingly, the onset of sleep changes was predictive of the terminal stage.
Sleeping Sickness: A Circadian Rhythm Disorder?

Besides fragmentation of the sleep-wake cycle in HAT, other data call attention to a disruption of endogenous rhythms during the disease. Alterations in the circadian fluctuations of cortisol, prolactin, and growth hormone secretion, as well as of plasma renin activity, have been described in patients (57). However, the rhythm of the secretion of the pineal hormone melatonin, which is a parameter commonly used as a marker of internal circadian control, is preserved in HAT (14). The perturbation of circadian rhythm parameters in HAT and in trypanosome-infected experimental animals (62) raises the question of whether sleeping sickness is a circadian rhythm disease that reflects disturbances in brain regions involved in the control of the sleep-wake cycle.

In mammals, the control of the daily sleep-wake cycle, as well as of endogenous rhythms in behav-
ioral, hormonal, and immune functions, is mainly regulated by bilateral nuclei in the anterior hypothalamus, the suprachiasmatic nuclei (SCN), which thus function as a master “biological clock.” We will here discuss whether and how the input pathway, the oscillator, and/or the output pathway of the mammalian circadian system (58) are affected by trypanosome infections.

Input pathways

Light signals synchronize, or entrain, the phase of the oscillator (the SCN) rhythm to the external light-dark (LD) cycle via the retinohypothalamic tract, the main input pathway (FIGURE 3), in which glutamate is considered to be the main neurotransmitter (47). A subpopulation of melanopsin-containing retinal neurons, which project to the SCN, has been implicated in the circadian phototransduction cascade (27). Retinal fibers reach the “core” region of the SCN (38). Photic stimuli cause a rapid induction of the immediate-early gene c-fos (38), which encodes for the nuclear phosphoprotein Fos, in the SCN.

In rats infected with Tb brucei, Fos could not be detected in the SCN during the light period of the LD cycle and Fos induction in response to light pulses was markedly decreased (54). Tracing studies indicated that the retinohypothalamic tract was not disrupted in infected rats, whereas glutamate receptor subunits showed reduced expression in the retinorecipient part of the SCN (41). This reduction in glutamate receptor subunits may underlie the blunted Fos protein response to light stimuli observed in trypanosome-infected rats.

Oscillator

Although the SCN is not the only circadian oscillator (1, 26), these nuclei contain a master pacemaker in mammals, coordinating endogenous biological rhythms with the external environment (26, 58). In normal rodents, SCN neurons exhibit a circadian oscillation in spontaneous firing activity, in vivo and in vitro, which in both nocturnal and diurnal animals is highest during the subjective light phase of the day and low during the dark hours (46). The circadian oscillations in the SCN activity are maintained by cyclic products of sets of “clock genes,” which provide negative and positive feedback loops between the cytoplasm and the nucleus of SCN neurons (26, 38, 58).

Intrinsic SCN electrical activity has been studied in acute horizontal slices from trypanosome-infected rats, in which the neuronal firing was altered, with a decrease in spontaneous postsynaptic excitatory activity (42). Although these data suggest that synaptic communication between the SCN neurons is affected during Tb infection, preliminary studies using a luciferase (luc) reporter of the clock gene Period (Per) 1 in transgenic rats did not reveal alterations in the cyclic expression of the Per-luc construct in SCN slices (44). Together the data suggest that this parasitic infection affects synaptic activities of the SCN network but may not target one key component of the molecular clock machinery in the pacemaker neurons.

Output pathways

Through monosynaptic and polysynaptic pathways, information processed in the SCN is conveyed to brain regions, primarily various hypothalamic nuclei (FIGURE 3) and some nuclei in the thalamus and brain stem, as well as to the pineal gland. Thereby the SCN regulate neuroendocrine signals controlling diurnal rhythms in secretion of hormones (such as cortisol, prolactin, growth hormone, renin, and melatonin) as well as rhythms in body temperature and locomotor activity (32, 58).

In our rat model of chronic trypanosome infection, the diurnal rhythm of melatonin secretion in
the urine was preserved when rats were kept on a 12:12-h LD cycle but the amplitude of the nocturnal peak was decreased (35). The fragmented sleep pattern could be restored by administration of melatonin (23), the hormone that has the ability to reset the SCN. In infected rats maintained in LD cycles, the amplitude of locomotor activity rhythm was markedly blunted, whereas the amplitude in body temperature rhythm was preserved but with a phase advance of 3 h of its nocturnal peak (22). Recent results obtained from infected rats kept in constant darkness also demonstrated that locomotor activity and body core temperature rhythms are preserved but that the amplitude of locomotor activity and of wheel-running activity is significantly reduced (44).

In view of the wealth of recent data indicating that the SCN can influence sleep and arousal by a number of neural pathways, in the following section we will briefly discuss how alterations in the oscillator function may affect the regulation of sleep and the alternation of sleep and wakefulness. We will also point out data showing that this communication is reciprocal, enabling sleep centers to influence the biological clock.

**Do Trypanosomes Interfere with Interactions Between the Circadian-Timing and Sleep-Regulating Systems?**

**Homeostatic and circadian-timing processes**

The now generally recognized model of sleep regulation (15) is based on the integration of a homeostatic process (implying increase of sleep pressure with duration of wakefulness, a process that controls the onset and duration of sleep with a greater propensity for sleep after a longer wake time) and a circadian-timing process (controlling the sleep-wake cycle in relation to specific phases of the LD cycle). The propensity to initiate sleep, the length and incidence of sleep episodes, and the intensity of sleep would be determined by this two-process system. Recent knowledge has unraveled a cross-talk between the circadian-timing and sleep-related systems. However, it is still unclear to what

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**FIGURE 3. Neural pathways interconnecting the circadian-timing and sleep-regulatory centers; these neural pathways and centers, and in particular the hypothalamic ones, could be potentially dysregulated in *Tb* infections**

Not all of the connections between these centers are indicated; the major input and output pathways of the suprachiasmatic nucleus (SCN) are indicated by thick arrows. From the environment, photic information is conveyed through the retinohypothalamic tract to the SCN, which is the master circadian pacemaker in the mammalian brain. The main output of the SCN is to other hypothalamic cell groups: the subparaventricular zone (SPZ), dorsomedial nucleus (DMH), and paraventricular hypothalamic nucleus (PVH). Via these centers, as well as through direct, less-dense connections, information can be conveyed from the SCN to regions implicated in sleep regulation: the sleep-promoting ventrolateral preoptic nucleus (VLPO) of the anterior hypothalamus, aminergic cell groups implicated in arousal such as the histamine-containing cells of the tuberomammillary nucleus (TM) of the posterior hypothalamus and noradrenergic cells of the locus ceruleus (LC) in the brain stem, and the neurons of the dorsolateral and posterior hypothalamus that contain the peptide orexin/hypocretin (O/H neurons). The latter neurons are also implicated in arousal and exert an excitatory effect on TM neurons. In addition, direct connections or polysynaptic pathways link the SCN with the cholinergic mesopontine tegmentum [the pedunculopontine (PPT) and laterodorsal tegmental (LDT) nuclei] and the serotonergic cells of the raphe nuclei in the brain stem, which are also key centers of sleep-regulatory circuits. Sitting in circumventricular organs, including the posterior pituitary (connected with the PVH), the trypanosomes are in a strategic position to influence hypothalamic centers, directly or through the cerebrospinal fluid, by means of molecules released by the parasite or cytokines released by the host as a defense mechanism.
degree the circadian and homeostatic processes are interrelated at the cellular and system levels.

SCN-lesioned rats and mice sleep during both subjective day and night, but the total amount of sleep is unaffected (19, 30), indicating that the homeostatic process is independent of the biological clock. SCN lesions in squirrel monkeys result instead not only in a loss of the circadian rhythms in sleep and wakefulness but also in a reduction of the total waking time (20). Thus the latter data from the monkey, as well as findings based on genetic alterations of the circadian clock system in the mouse (50), point out an interaction between the normal circadian clock function and the homeostatic control of sleep. Furthermore, it has been demonstrated that the SCN may be actively involved in the initiation of sleep (17).

Interactions between the SCN and regions involved in sleep and arousal

A number of neural pathways have been recently identified and implicated in the interaction between the circadian-timing and sleep-regulatory centers through hypothalamic structures near the SCN (FIGURE 3). These include the subparaventricular zone (to which the SCN projects densely) and the paraventricular and dorsomedial hypothalamic nuclei. Through these regions, information is transferred from the SCN to the noradrenergic locus ceruleus in a circuit that could be implicated in arousal (4). Direct connections of the SCN with the orexin/hypocretin-containing neurons have also been described (2). These neurons, located in the dorsolateral and posterior hypothalamic areas, are implicated in arousal (65). In addition, SCN information reaches another cell group that plays a role in arousal, the histamine-containing neurons of the tuberomammillary nucleus (TM) of the posterior hypothalamus, which are modulated by influences of the amineergic brain stem cell groups (24). The SCN is connected to the TM directly, as well as via the sleep-promoting ventrolateral preoptic nucleus of the anterior hypothalamus and via the dorsal subparaventricular zone (13, 40). The orexin/hypocretin-containing neurons exert an excitatory effect on TM cells directly and by disinhibition (21). Orexin receptor-1 is expressed in subsets of hypothalamic cells, including SCN and TM neurons (5, 29).

Circadian information can thus be transmitted to arousal and sleep-promoting centers (FIGURE 3), i.e., neural centers that subserve the transition of sleep stages during sleep as well as sleep-wake transitions. These regions have been proposed to be involved in a bistable “flip-flop” model of how the brain switches from sleep to wakefulness by reciprocal inhibitory circuits (61). A decreased resistance in this circuit would weaken the stability of the switch and could therefore result in disruption of the sleep-wakefulness pattern (61).

Sites of action of the trypanosomes

Reduced functions of the SCN, as observed in **Tb brucei**-infected rats, could hypothetically weaken the stability of the sleep switch. In particular, trypanosomes could affect regions close to the CSF. Hypothalamic centers could be especially vulnerable, because, as mentioned above, circumventricular organs, including the median eminence, as well as the posterior pituitary, which is directly connected with the paraventricular hypothalamic nucleus (FIGURE 3), are sites for the earliest localization of the parasites (FIGURES 3 AND 4). In support of this assumption, a marked induction of proinflammatory cytokines (FIGURE 5A) (56) and activation of microglia (12) were detected in periventricular regions, with prevalence in the hypothalamus, in trypanosome-infected rats (FIGURE 5, B AND C). This microglia activation paralleled the onset and progressive alterations in sleep parameters (12). It should be noted that, despite the longstanding and marked inflammatory reaction, there are few signs of neurodegeneration in trypanosome-infected brains (36).

On the other hand, a feedback loop from sleep centers to the SCN has recently been demonstrated (17), showing that specific sleep states correlate with changes in the electrical activity of the SCN. Thus by affecting periventricularly located sleep centers and sleep-regulation pathways, trypanosomes could also affect the feedback to the SCN. Furthermore, SCN transplants can sustain circadian activity rhythms by means of a diffusible signal, which may reach its neural targets through the CSF or the extracellular space (63). As described above, trypanosomes would also be in a strategic position to interfere with such diffusible signals. Together, the above data indicate that, because of their targeting in the brain, trypanosomes have the...
propensity to interfere with the coupling between the SCN circadian clock and sleep-regulatory mechanisms.

Do Inflammatory Cytokines Play a Key Role in Sleep Disturbances During Trypanosome Infections?

Since Tb are extracellular parasites, the effects on sleep pattern and on the sleep-wake cycle should be related to molecules released from the trypanosomes and/or to molecules released during the host response to the infection. The latter include a plethora of inflammatory molecules released into the blood and in the brain, and these could potentially affect brain function. In our studies, we focused on the effect of the proinflammatory cytokine interferon (IFN)-γ on the SCN, because this molecule plays a crucial and paradoxical role in the infection.

There is a positive correlation between plasma levels of IFN-γ, and of this cytokine in particular, and the severity of the disease in patients with HAT (57). We have recently observed that, although IFN-γ can control the growth of the parasite, this cytokine has the paradoxical effect to promote its neuroinvasion through the BBB at late stages (45). In the rat brain, the expression of the receptor for IFN-γ is particularly high in the retinorecipient part of the SCN (FIGURE 6) and in areas such as the piriform and enthorinal cortex, midline thalamus, medial hypothalamus, and TM (60). In rats entrained in a 12:12-h LD cycle, the IFN-γ receptor expression in the SCN was found to be low during the subjective day and increased during the early subjective night, with the highest expression at zeitgeber time (ZT; time in hours after light goes on) 15 (43) (FIGURE 6).

Other data also point out an influence of IFN-γ on the circadian-timing system. For example, IFN-γ administered subcutaneously in mice at ZT 12, but not at ZT 0, caused a marked reduction of clock gene expression, i.e., Per mRNA levels, in the SCN (52). Intracerebroventricular injections of IFN-γ alter locomotor activity in hamsters (9). IFN-γ operates in synergy with tumor necrosis factor (TNF)-α (53), which in fact was first isolated from rabbits infected with trypanosomes under the name of cachectin (36). IFN-γ and TNF-α in combination with the bacterial endotoxin lipopolysaccharide blunted the spontaneous firing activity and reduced spontaneous excitatory postsynaptic activity in slices of the SCN (42). These studies indicate that a proinflammatory cytokine such as IFN-γ can alter SCN functions both in vitro and in vivo. In addition, the expression of IFN-γ receptors in the TM also provides a possibility for the cytokine to affect the arousal system directly.

Do Trypanosomes Use Sleep Disturbances as a Strategy to Fool the Host?

Sleeping sickness embraces a number of paradoxes. One of these is, as mentioned above, the lack of hypersomnia, i.e., a component of the “acute-phase response or sickness behavior.” Hypersomnia is elicited by proinflammatory cytokines, most notably interleukin (IL)-1β and TNF-α, which are highly induced also during trypanosome infections in humans (59) and experimental rodents (56) (FIGURE 5A).
somnogenic IL-1β and TNF-α increase non-REM sleep (37), and this effect is possibly mediated through prostaglandin D2 and E2 (28) and/or the free radical nitric oxide (33). How then can trypanosomes cause changes in the sleep pattern and not hypersomnia?

During microbe-host interactions, the host may show behavioral disturbances that could favor either the survival of the host or the microbe (6). Sickness behavior with hypersomnia is advantageous for the host because it conserves energy to be used in generating fever to combat infections caused by temperature-sensitive microbes. For the trypanosome, on the other hand, hypersomnia of the host may present a disadvantage. It could disrupt the life cycle of the parasite by preventing spread back to the tsetse fly, which preferentially bites moving objects during daytime. Trypanosomes may therefore have an evolutionary advantage by preventing hypersomnia during the day. Speculatively, this may have been obtained by selection of trypanosome strains, inducing an enhanced proportion of anti-inflammatory cytokines that inhibit non-REM sleep (37) or promote synthesis of antibodies that neutralize proinflammatory cytokines, or by selection of trypanosome strains secreting molecules that directly counteract the somnogenic effects of cytokines (68). It is also noteworthy that responses to cytokines, at least in the immune system, can be inhibited by so-called suppressors of cytokine signaling (3). However, it has not yet been examined whether such suppressor molecules are expressed in brain regions during trypanosome infection.

Common Features of Sleep Alterations in African Trypanosomiasis, Narcolepsy, and Sleep Disturbances During Aging

The sleep disturbances in sleeping sickness patients have certain aspects in common with those of narcoleptics. Narcolepsy is a sleep disorder characterized by abnormal transitions from wakefulness to REM sleep, with “sleep attacks” during wakefulness, episodes of sudden muscle weakness (cataplexy), and disturbed nocturnal sleep. A close link between narcolepsy and disruption of the excitatory orexin/hypocretin system was found in dogs (39), mice (11, 25), and rats (8), as well as humans (51, 55, 67). Such disruption is associated with hypocretin ligand and receptor mutations or loss of orexin-producing neurons. Like in sleeping sickness, the total sleep time in narcoleptics is not changed, but REM sleep latency is reduced; the circadian rhythm in body temperature is preserved (16). The orexin/hypocretin peptides and their receptors have not been analyzed in sleeping sickness so far.

Sleep-wake disturbances in narcoleptic dogs (31) show certain similarities to those in old dogs (66). Aging dogs display fragmented sleep-wake cycles with excessive daytime sleep episodes. Like in sleeping sickness, aging is associated in rodents with reduced Fos activation in the SCN following photic stimuli, with a reduced nocturnal peak in melatonin secretion in the urine and alterations in latency of REM sleep in rodents and humans (48, 64, 72). The circadian cycling of Perl expression in the SCN of old rats remains robust but with a slightly shortened free-running period (71).
However, it has been recently reported that reduction of Clock and alterations in Bmal1 gene expression are linked to aging (34). As mentioned previously, although the clock gene Per1 is a crucial component of the pacemaker machinery, it may not be sufficient alone for maintaining a robust biological clock function (34). Thus in contrast to a total lesion of the SCN, when, as mentioned above, circadian rhythms are abolished, it can be envisaged that dysfunctions in the biological clock can have different effects on the various output systems. This may explain why certain parameters of the circadian rhythm system are affected and others are preserved in conditions such as sleeping sickness, narcolepsy, and aging.

Concluding Remarks

The disturbances of the sleep-wake regulation in sleeping sickness are unique and not typical for infectious diseases. We speculate that this type of dysfunction could in fact be a result of an evolutionary survival strategy for the trypanosome. However, the main question remains: how do the parasite and the nervous system interact to impact specifically the sleep-wake regulatory circuits in the brain? The answer is not yet clear, but the information available so far suggests that circadian control of the sleep-wake cycle and sleep regulation may be influenced during trypanosome infection, leading to disturbed coupling between the sleep-related and circadian-timing systems. Investigating the similarities to sleep-wake alterations in other conditions, for instance in experimental models of narcolepsy and during aging, is intriguing and can stimulate the design of further studies in this field. By exploring the pathogenesis of sleeping sickness, insights may be obtained for a better understanding of sleep disturbances associated with other neuropsychiatric disorders and aging.

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References


