Altered Neuronal Activity Rhythm and Glutamate Receptor Expression in the Suprachiasmatic Nuclei of Trypanosoma brucei-infected Rats

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Abstract. The parasites Trypanosoma brucei cause African trypanosomiasis (sleeping sickness), a severe neuropsychiatric disease with marked disturbances of sleep-wake alternation. The sites of brain lesions are not well characterized. The present experimental investigation is focused on the hypothalamic suprachiasmatic nuclei, which play a role of a biological clock entraining endogenous rhythms in the mammalian brain. The electrophysiological properties of these neurons were analyzed in slice preparations from trypanosome-infected rats. The neuronal spontaneous activity, which shows a circadian oscillation, was markedly altered in the infected animals, displaying a reduced firing rate and phase advance of its circadian peak. The direct retinal fibers, which play a pivotal role in entrainment of the circadian pacemaker, displayed a normal density and distribution in the suprachiasmatic nuclei of infected animals after intracocular tracer injections in vivo. At the postsynaptic level, immunohistochemistry and Western blotting revealed in the suprachiasmatic nuclei of infected rats a selective decrease of the expression of glutamate AMPA GluR2/3 and NMDAR1 receptor subunits that gate retinal afferents. These data disclose an impairment of the neuronal functions in the biological clock in African trypanosomiasis, and may serve to unravel functional and molecular mechanisms behind endogenous rhythm disturbances.

Key Words: Brain slices; Circadian pacemaker; Circadian rhythm; Cytokines; Glutamate receptors; Interferon-γ; Nervous system.

INTRODUCTION

Human African sleeping sickness, caused by subspecies of the extracellular parasite Trypanosoma brucei (T.b.), is a complex syndrome hallmarkled in its advanced stages by a disruption of the sleep-wake cycle (1–3) that parallels the severity of the disease (2). Alterations in the circadian rhythm of cortisol and prolactin hormonal secretion have also been described (4), pointing to a dysregulation of the mechanisms responsible for the generation and synchronization of physiological and endocrine functions during the disease. However, relatively nonspecific neuropathological alterations have been reported in this disease (5), and the question of whether the clinical disturbances in circadian biological rhythms correlate with alterations in neuronal functions in the infected brain has not been addressed.

In the mammalian brain a circadian pacemaker for endogenous rhythms has been identified in the hypothalamic suprachiasmatic nucleus (SCN). Circadian pacemakers generate rhythmicity and must be reset by the environment on a daily basis (6); the SCN is synchronized by light, conveyed to the SCN through direct and indirect retinal afferents (7), and by nonphotic stimuli (8). Administration of exogenous melatonin may also affect the SCN, in which melatonin receptors are concentrated (reviewed in 9, 10).

We have adopted a model of experimental T.b. brucei infection in rats with a relatively slow course of disease (11). In this model of T.b. infection in rats, marked changes in the structure of sleep with a fragmentation of slow wave synchronized sleep, as well as alterations of the body temperature circadian rhythm, have been observed (12, 13). The organization of the sleep pattern can be restored by acute administration of melatonin to the infected animals (14). Gene expression may be dysregulated in the SCN during T.b. infection, since the induction of the protein product of the immediate early gene c-fos in response to light is altered in the SCN of infected rats during the early subjective night (15, 16).

The spontaneous electrical activity of SCN neurons in slice preparations undergoes a circadian rhythm with a characteristic peak of activity at circadian time (CT) 6–8 (17–22) (CT 0 corresponds to the “lights-on” time). Therefore, the study of SCN in slices allows direct assessment of the intrinsic properties of SCN neurons. To test the hypothesis that African sleeping sickness involves functional disturbances in the SCN activity, we recorded in vitro the spontaneous electrical activity of SCN neurons during T.b. infection in rats. As for the input pathway that conveys environmental information to the circadian pacemaker for entrainment, we investigated in vivo whether the organization of presynaptic retinal afferents was modified by trypanosome infection.

Glutamate is the main transmitter released from the retinal afferents to the SCN (22, 23). Several lines of
evidence have indicated that excitatory amino acids mediate the effect of light on this nucleus, and the downstream activation of SCN neurons involves both N-methyl-D-aspartate (NMDA) and \( \alpha \)-amino-3-hydroxy-5-methyl-4-isoxazolpropionate (AMPA)/kainate receptors, and calcium-dependent production of the free radical nitric oxide (see 24 for review). On this basis, we studied in trypanosome-infected animals the immunopositivity to some of the postsynaptic glutamate receptor subunits that have been reported to gate retinal inputs through the SCN.

MATERIALS AND METHODS

Animals and Infection Procedure

All the procedures involving animals were conducted under institutional guidelines and ethical committee approval. Male, adult Sprague-Dawley rats (145–155 g, obtained from B & K Universal AB, Sollentuna, Sweden) were kept on a 12 h light and 12 h dark schedule throughout the course of the experiments. The animals had continuous access to food and water.

The rats were infected by intraperitoneal injection with pleomorphic T. b. brucei (AnTat 1/1, derived from stablate EATRO 1125, Laboratory of Serology, Institute of Tropical Medicine “Prince Leopold,” Antwerp, Belgium). A volume of 0.5 ml, containing 25,000–30,000 trypansomes, was injected in each animal. After 10 days, blood samples were taken from the tip of the tails to verify parasitemia.

Slice Preparation and Electrophysiological Recording

Hypothalamic slices were prepared from a total of 20 rats, which were sacrificed during the light phase of the day by decapitation under terminal halothane anesthesia. The brains were quickly removed from the skulls and placed in ice-cold Ringer solution. A 500-μm-thick hypothalamic slice containing the SCN of both sides was then cut on the horizontal plane on a vibratome and perfused with an oxygenated Ringer solution (35–35.5°C) containing (in mM): NaCl, 126, KCl, 2.5, MgCl₂, 1.3, NaH₂PO₄, 1.2, NaHCO₃, 2.0, CaCl₂, 2.0, glucose and 26 NaHCO₃. This procedure was performed within 5 minutes (min) from the time of decapitation.

The single unit spontaneous activity of SCN neurons was recorded extracellularly (17–19) with a glass microelectrode (resistance 2–4 MΩ) containing 1% NaCl. The signals were amplified and converted to digital signal, and the spikes were stored and analyzed by software Spike2. Only cells with a regular firing rate were recorded, each one for approximately 3–4 min, and the average rate of activity was calculated. The firing rates of each cell were averaged into 2 hour (h) intervals using a 1 h lag in order to determine the time of peak activity.

In the first set of experiments, recordings were made between CT 5 and CT 12 (CT 0 corresponds to the onset of the light phase of the brain slice donor) in slices from 6 control and 6 infected animals, 36–45 days post infection (p.i.). In a second set of experiments, continuous recordings were performed between CT 0 and CT 23 in slices from 4 infected animals, 40–45 days p.i., and between CT 4 and CT 11 in slices from 4 control animals. Significance of the differences in and between groups was determined with the Student’s t-test and one- and two-way analysis of variance (ANOVA), followed by the Duncan’s post-hoc test.

Tract Tracing

Three T. b. brucei-infected rats, 40 days p.i., and 3 control rats were unilaterally injected under anesthesia (chloral hydrate, 400 mg/kg i.p.) with 5 μl of 1% cholera toxin subunit B conjugated with horseradish peroxidase (HRP; List Biological Laboratories Inc., Campbell, Calif, US) into the vitreous body of the eye (25). After 48 h, the rats were again anesthetized and transcardially perfused with saline followed by 1.25% glutaraldehyde and 1% paraformaldehyde in phosphate-buffered saline. After cryoprotection in sucrose, 40-μm-thick sections were cut through the optic nerves and the diencephalon with a freezing microtome and incubated for HRP histochemistry with tetramethylbenzidine, nitroprusside and hydrogen peroxide in acetate buffer. The sections were mounted on gelatin-coated slides, quickly dehydrated and coverslipped, and studied at the microscope under bright-field illumination.

Immunohistochemistry and Histology

Immunopositivity to glutamate receptor subunits was examined in a total of 11 rats (5 control and 6 infected rats, 42 days p.i.). Under deep chloral hydrate anesthesia, the rats were perfused through the heart with saline followed by 4% buffered paraformaldehyde at CT 4 (2 control and 3 infected) or at CT 10 (3 control and 3 infected). A tissue block containing the diencephalon was dissected out, soaked in 20% buffered sucrose for cryoprotection, and cut with a microtome into 30-μm-thick serial sections. Every third section through the SCN was incubated with anti-GluR1 (diluted 1:2,000), GluR2/3 (1:1,000), and NMDAR1 (1:500) polyclonal antibodies raised in rabbits (Chemicon, Temecula, Calif, US). These antibodies have been previously characterized and extensively used for the study of glutamate receptors in the rat brain (26, 27). The material was processed for immunohistochemistry (sections from infected and control animals were processed in the same solutions) using biotinylated goat anti-rabbit IgG and the avidin-biotin-peroxidase protocol (ABC kit, Vector; nickel-intensified 3′diaminobenzidine was used as chromogen in the final step of the procedure.

The GluR1, GluR2/3, and NMDAR1 immunostaining was evaluated in the SCN by means of densitometric analysis. In addition, the immunostaining to these glutamate receptor subunits was studied in the diencephalon by 2 investigators unaware of the experimental group assignment. In the sections processed for NMDAR1 immunohistochemistry, immunoreactive cells in the SCN were counted in all animals in 3 comparable levels regularly spaced through the anteroposterior extent of the nucleus. However, in the study of GluR1 and Glu R2/3 immunoreactivity, the high intensity of the immunostaining in the SCN hampered a clear detection of individual cell bodies, and cell counts were therefore not performed. Neurons immunostained by NMDAR1 were also counted in the supraoptic nucleus in 3 sections from 2 infected and 2 control rats. All the results of the densitometric analysis and cell counts were statistically analyzed with the Student’s t-test.
The slices subjected to the electrophysiological recordings were also examined histologically for glial reaction and presence of trypanosomes. To this purpose, they were briefly fixed in 4% paraformaldehyde and cut with a cryostat into 10 μm thick sections. To examine glial reaction in these sections, immunohistochemistry was performed using the rabbit polyclonal antibody anti-glial fibrillary acidic protein (GFAP, Sigma, St Louis, Mo, US; diluted 1:10,000), and the mouse monoclonal antibody anti-OX 42 (Sera-Lab Ltd, Crawley Down, UK; 1:1000), which recognize the complement C3Bi receptor of the macrophage/microglia lineage. To detect trypanosomes, a rabbit trypanosome-infection antiserum was used (28). Those sections were incubated overnight in primary antibodies, and then for 1 h in biotinylated goat anti-rabbit or rabbit anti-mouse secondary antibodies. They were subsequently processed with the ABC kit, using 3-amino-9-ethylcarbazole as the chromogen.

To evaluate the occurrence of neuronal changes, 3 infected animals at 42 days p.i. and 2 control rats were perfused under deep barbiturate anesthesia with 4% buffered paraformaldehyde. After cryoprotection in sucrose, serial sections were cut through the diencephalon with a cryostat at a 10 μm thickness. These sections were stained with cresyl violet-acetic acid.

**Immunoblotting**

Four infected (46 days p.i.) and 4 uninfected rats were used for immunoblotting analysis. These animals were decapitated under halothane anesthesia. The SCN were rapidly dissected from the brains and immediately placed in lysis buffer (1% SDS, 10 mM Tris-HCl, pH 7.4) and sonicated. An equal volume of sample buffer (1X = 125 mM Tris-HCl, pH 6.8, 2% SDS, 5% glycerol, 0.003% bromophenol blue, and 2% 2-mercaptoethanol) was added and the mixture was boiled for 5 min. To control for equal amount of protein added to the gels, optical densities of the lysates were determined spectrophotometrically at 205 nm and equivalent amounts of material from each sample were resolved by SDS-PAGE. Blots were blocked in 3% BSA (w/v) in PBS, pH 7.4, 0.05% (v/v) Tween 20 (TPBS; blocking buffer), and then probed with (a) anti-GluR1 (0.4 μg/ml), (b) anti-GluR2/3 (0.1 μg/ml), and (c) anti-NMDAR1 (0.4 μg/ml) antibodies (Chemicon). The antibodies were diluted in blocking buffer, and the blots were incubated overnight at 4°C. After washing, the blots were incubated for 1 h at room temperature with affinity-purified HRP-labeled goat anti-rabbit IgG (DAKO, Glostrup, Denmark) and diluted 1:1,000 in blocking buffer. Detection of labeled proteins was performed using the ECL detection reagents according to conditions recommended by the manufacturers (Amersham, Little Chalfont, UK).

**RESULTS**

**Course of the Disease**

Upon examination at 10 days p.i., *T. b. brucei* were found in the blood of all rats in which the parasites had been injected. The rats did not display signs of disease during the first 30 days after infection. After 30–35 days p.i., the rats started to lose weight, and by day 40–45 p.i., the weight loss was marked; otherwise, the animals used in this study showed no overt signs of disease. In other series of experiments, we have observed that most

**Sprague-Dawley rats infected with the presently used stable of the parasites do not show severe signs of disease until they become moribund and die 55–60 days p.i.**

**Electrophysiological Recordings**

In the analysis of spontaneous activity generated by SCN neurons performed with extracellular recordings, the firing rate of neurons from control uninfected animals recorded between CT 5–12 in the first set of experiments showed a peak at CT 7.1 ± 0.6 h (p < 0.05, one-way ANOVA) (Fig. 1A), with a mean frequency at the peak of 6.05 ± 0.31 Hz. No peak of activity between CT 5–12 could be observed in any of the slices sampled from infected animals.

To analyze whether peaks of activity occurred at other time points in the infected SCN, continuous recordings were performed in the second set of experiments between CT 0 and CT 23 in slices from infected animals. Also in this set of experiments, a peak of activity occurred at CT 7.1 ± 0.9 h (p < 0.05, one-way ANOVA) in slices from uninfected rats (Fig. 1B), while occurred at CT 4.3 ±
0.4 h (p < 0.01, one-way ANOVA) in slices from infected animals (Fig. 1C, D), indicating a phase advance of 2.8 h (p < 0.05, Student’s t-test). The data of recordings from single slices indicated that the time peak of spontaneous activity recorded from different slices almost coincided (Fig. 1A, C). No other peaks of activity could be observed in the recordings from infected animals.

The mean firing rate of neurons recorded in slices from infected animals was reduced between CT 4-11 as compared with slices from control animals. This was most evident in the second set of experiments (3.4 Hz ± 0.18 SEM; 165 neurons analyzed, and 5.6 Hz ± 0.21 SEM; 122 neurons analyzed, in slices from infected and control rats, respectively; p < 0.0001, two-way ANOVA analysis). The mean firing rates of the peaks also differed between the 2 groups in this set of experiments (5.33 ± 1.03 Hz at CT 4.3 and 6.88 ± 0.68 Hz at CT 7.1 in slices from infected and control animals, respectively). However, the amplitude and duration of the recorded action potentials did not differ significantly between the infected and control animals, indicating that passive membrane properties of SCN neurons were preserved during the infection. The number of firing cells identified during the sampling period did not decrease in slices from infected animals as compared with control slices.

**Tract Tracing**

After unilateral injections of B-HRP into the eye in vivo, labeled fibers densely filled the optic nerve and chiasm in both the control and infected rats (Fig. 2). A similar degree of labeling, density and distribution of the anterogradely labeled retinal preterminal fibers and terminal-like elements in the SCN, concentrated in the ventrolateral part of the nucleus (Fig. 2), was observed in both groups of animals. Thus, no obvious structural alterations of retino-hypothalamic afferents were evident at the light microscopical level in the SCN at this stage of trypanosome infection.

**Glutamate Receptors**

The immunolabeling with the antibodies in the SCN in normal rats was similar to that described previously (26, 27). The immunoreactivity to GluR1 resulted in diffuse staining of the SCN neuropil, and in labeling of cells in the most dorsal aspect and along the lateral borders of the nucleus. GluR1 immunostaining did not markedly differ between control and infected rats, as confirmed by the densitometric evaluation that did not reveal significant differences between the 2 groups. In control rats, GluR2/3-immunoreactive neurons were concentrated in
the ventral portion of the SCN, in which the neuropil immunostaining was very intense (Fig. 3A). At variance with the pattern of immunoreactivity observed in control rats, in the infected animals Glu2/3 immunopositivity was markedly decreased, and only a limited number of faintly immunoreactive cell bodies were detected in the ventral part of the SCN (Fig. 3B). Accordingly, denisitometric evaluation resulted in significantly lower values in the infected rats (48.6 ± 1.6 % and 61.5 ± 3.2 % relative units in infected vs control animals; p < 0.01; Student’s t-test). As a qualitative observation, GluR2/3 immunoreactivity in other diencephalic structures, such as the thalamic midline nuclei and the supraoptic nucleus, was not lower in the sections from infected rats than in the sections from control subjects.

Immunopositivity to the NMDAR1 receptor subtype was intensely expressed by numerous cells throughout the SCN in control rats (Fig. 3C, E), but a significantly lower number of labeled cell bodies was evident in the infected rats (Figs. 3D, F; 4). The denisitometric evaluation also showed a significant decrease of NMDAR1 immunopositivity in the SCN of infected rats (36.8 ± 2.2 % and 43.6 ± 2.0 % relative units in infected and controls, respectively; p < 0.05). In the same sections, immunopositivity to NMDAR1 was not decreased elsewhere in the diencephalon (e.g. throughout the thalamus, in the supraoptic nucleus, and in the magnocellular portion of the hypothalamic paraventricular nucleus) of infected rats. The quantitative evaluation of NMDAR1-immunostained cells in the supraoptic nucleus did not reveal any significant difference in this structure in the infected vs control animals.

**Immunoblot Analyses**

Immunoblots showed major bands at 108 kDa when probed with GluR1 or GluR2/3 antibodies and a major band at 105 kDa when probed with the anti-NMDAR1 antibodies (Fig. 5). The bands of GluR2/3 and NMDAR1 of SCN sampled from infected rats consistently showed a lower immunointensity as compared with those from uninfected rats, indicating a decrease in the expression of these receptors.

**DISCUSSION**

The present study points out that a *T. b. brucei* infection in rats causes alterations in the circadian timing system, with a decrease of the SCN oscillatory activity and a phase advance of the peak of spontaneous firing frequency of SCN neurons, indicating a dysregulation of their circadian oscillation. In addition, the present investigation provides evidence in vivo of a downregulation of glutamate receptor subunits responsible for clock resetting and, in particular, for photic entrainment.

The observed alterations in the oscillatory activity of the SCN during trypanosome infection may derive from alterations in the presynaptic retinal input to the SCN, in nonphotic or behavioral stimuli, and/or in the SCN neurons. In the eye, trypanosomes are not present in the retina, even at late stages of the infection, but localize to the choroid (28). Although factors released by the parasites in the choroid may have influenced retinal ganglion cells, the present data indicate that during the infection, these cells could take up and transport a tracer normally through their axons, which did not show obvious structural changes at the light microscopical level. Thus, our data do not point to major alterations in the organization of the retina-hypothalamic pathway in the infected animals. Photic cues entraining the SCN are also conveyed along indirect retinal afferents that derive from the intergeniculate leaflet (7). However, the present evidence of a lack of structural diencephalic alterations does not favor the occurrence of lesions in the indirect photic input to the SCN during trypanosome infection. On the other hand, rats at later stages of disease may have a reduced locomotor activity as previously documented (13), and this could as an altered behavioral stimulus influence the rhythm of neurons of the circadian pacemaker, since the level of an animal’s activity can exert a feedback effect on the SCN (29).

Regarding primary alterations in SCN neurons as a cause of the activity changes, their neurophysiological membrane properties appeared to be intact and structural changes at the light microscopic level and cell loss were not detected in the hypothalamus of the infected rats in the present study, consistent with the absence of overt neuronal degeneration in both experimental and human
trypanosomiasis (5, 28). The distribution of the immunostaining to glutamate receptor subunits observed in the SCN was also consistent with that reported in previous studies (30). However, there was a downregulation of the ionotropic glutamate receptor subunits AMPA GluR2/3 and NMDAR1, which suggests that excitatory neurotransmission is severely altered in the SCN during the infection. The immunoreactivity to the glutamate receptor antibodies employed in the present investigation, as well as NMDA receptor binding, are unaffected in the rat SCN by different phases of the circadian cycle (30, 31). It is, therefore, unlikely that the sampling times could have accounted for the changes in the immunopositivity we detected in an advanced stage of experimental trypanosome infection. The role of NMDA receptors in mediating photic influence on the SCN is still under discussion (32). However, several lines of evidence have indicated that a blockade of glutamatergic neurotransmission disrupts the response of SCN neurons to optic nerve stimulation in vitro, and cellular and behavioral responses to
TRYPANOSOMES DYSREGULATE THE CIRCADIAN CLOCK

Fig. 4. Effect of trypanosome infection on the number of NMDAR1-immunopositive neurons in the SCN. Horizontal bars: SD; ** = p < 0.01.

Fig. 5. Immunoblots of SCN whole cell extract probed with anti-GluR1 antibodies (lane 1: control; lane 2: infected), anti-GluR2/3 antibodies (lane 3: control; lane 4: infected) and anti-NMDAR1 antibodies (lane 5: control; lane 6: infected). Note the reduced intensities of the bands from extracts of infected brains labeled by the anti-GluR2/3 (lane 4) and anti-NMDAR1 (lane 6) antibodies as compared with uninfected brains (lanes 3 and 5, respectively).

light in vivo. Of particular interest in the present context is that both glutamate and NMDA can cause light phase shifts in the peak of circadian firing rhythms in slice preparations of the SCN (20–22). Furthermore, NMDA receptor antagonists can block the induction in the SCN of the immediate early gene c-fos in response to a light pulse (33–35). Thus, the present finding of a diminished expression of glutamate receptor subunits could underlie the impairment of light-induced Fos protein expression previously documented in T.b. brucei-infected rats (16).

The factors responsible for the alterations in the expression of glutamate receptor subunits in the SCN remain to be elucidated, and no data are available at present on glutamate release from retinal terminals in the infected animals. The SCN neurons could be affected by the molecules released during the interactions between the trypanosomes and the host. In previous studies we have described that the parasites release a molecule, TLTF (Trypanosomereleased Lymphocyte Triggering Factor), which selectively binds to the CD8 molecule of T-lymphocytes and triggers them to a prompt production of interferon-gamma (IFN-γ) (36). This cytokine is a key immunoregulatory molecule, but also serves as a growth-stimulating factor for trypanosomes (37), establishing a remarkable bidirectional signaling. TLTF may be the first example of a “trypanokine,” i.e. a factor released by the parasite to modulate the cytokine network of the host to the benefit of the parasite (38). The parasites do not penetrate the blood-brain barrier, but trypanosomes, as well as CD8+ T cells, can be detected in areas of the brain outside the barrier, such as the choroid plexus and the circumventricular organs, in the vicinity of the SCN (28, 39). Therefore, SCN neurons could be influenced through diffusible molecules released into the cerebrospinal fluid or from adjacent areas devoid of a blood-brain barrier, such as the organum vasculosum of the lamina terminalis, during the molecular interactions between trypanosomes and T-cells. In support to this pathogenetic mechanism, we have previously described that parasites and lymphocytes in the median eminence exert an effect on the para-ventricular and supraoptic nuclei of the hypothalamus (39) and that intracerebroventricular injections of IFN-γ can functionally activate SCN neurons to express Fos protein (40).

The present finding of astrocytic hypertrophy is consistent with previous data reported in experimental trypanosomiasis (41), but our observations point out that the invasion of macrophages/microglial cells during the infection is more prominent than that of astrocytes in the SCN region. An activation of astrocytes has been previously correlated with production of interleukin-1α and tumor necrosis factor-α in the brains of T.b. brucei-infected mice (41). These cytokines are somnogenic (42), but they have not been reported to cause a fragmentation of the sleep pattern as seen in African sleeping sickness, and their eventual action on the SCN function remains to be verified. Furthermore, prostaglandins may be released during T.b. brucei infections (43), and these products of activated macrophages have been implicated in sleep-wake regulation (44).

The changes in the oscillatory activity of SCN neurons were observed in the present study in relatively late stages of the experimental trypanosome infection, in which we have previously observed a phase advance in the body temperature cycle and a fragmentation of the sleep pattern in rats (13, 14). A disruption of sleep has been observed in humans affected by African trypanosomiasis, and in the most severely ill patients, a circadian rhythm of sleep-wakefulness was no longer discernible (2). Thus, African trypanosomiasis is not a state of hypersonnia (2), but instead reflects a loss of the monophasic character of sleeping and waking, so that patients are somnolent during the day and restless during the night (reviewed in 1). Removal of the SCN in rats can lead to a disruption of the sleep-wakefulness cycle with an equal distribution of sleep during the light and dark hours (45). The presently described alterations in the SCN function may therefore play a role in the dysregulation of endogenous rhythms in sleeping sickness, which also include a
loss of circadian rhythmicity in cortisol and prolactin secretion (1–4, 46). The interest to analyze the potential role of IFN-γ in mediating these effects on SCN function is indicated by the evidence of IFN-γ production in patients infected by T.b. gambiense (47) and by the report that plasma levels of IFN-γ, but not of interleukin-1β or -2 or tumor necrosis factor-α, may correlate with the clinical progression of the disease (46).

In conclusion, the present experiments disclosed a severe disturbance in vitro of the rhythmic neuronal activity of the SCN from T.b. brucei-infected rats. As T.b. brucei are extracellular parasites that do not invade neurons, the release of different signaling molecules by the trypanosomes or from activated lymphocytes of the host may account for the downregulation of glutamate receptor subunits in the SCN and eventually for the interference with the electrophysiological properties of SCN neurons observed in the present study. This is to our knowledge the first report of in vitro experiments showing a dysfunction of the circadian oscillator in a disease. Thus, the present findings provide a model for studies of functional and molecular dysregulation of the biological clock that could reveal neural correlates of sleeping sickness.

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