Supplementary Online Material

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"Clock Gene Expression during Chronic Inflammation Induced by Infection with *Trypanosoma brucei brucei* in Rats"

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Figure S1

May Grünwald-Giemsa staining of the extracellular parasite *Trypanosoma brucei brucei* in a blood smear sampled from an infected rat. The different forms of the parasite (blue) are shown: Ls; long slender, ss; short stumpy, im; intermediate. Only the Ls forms are dividing.

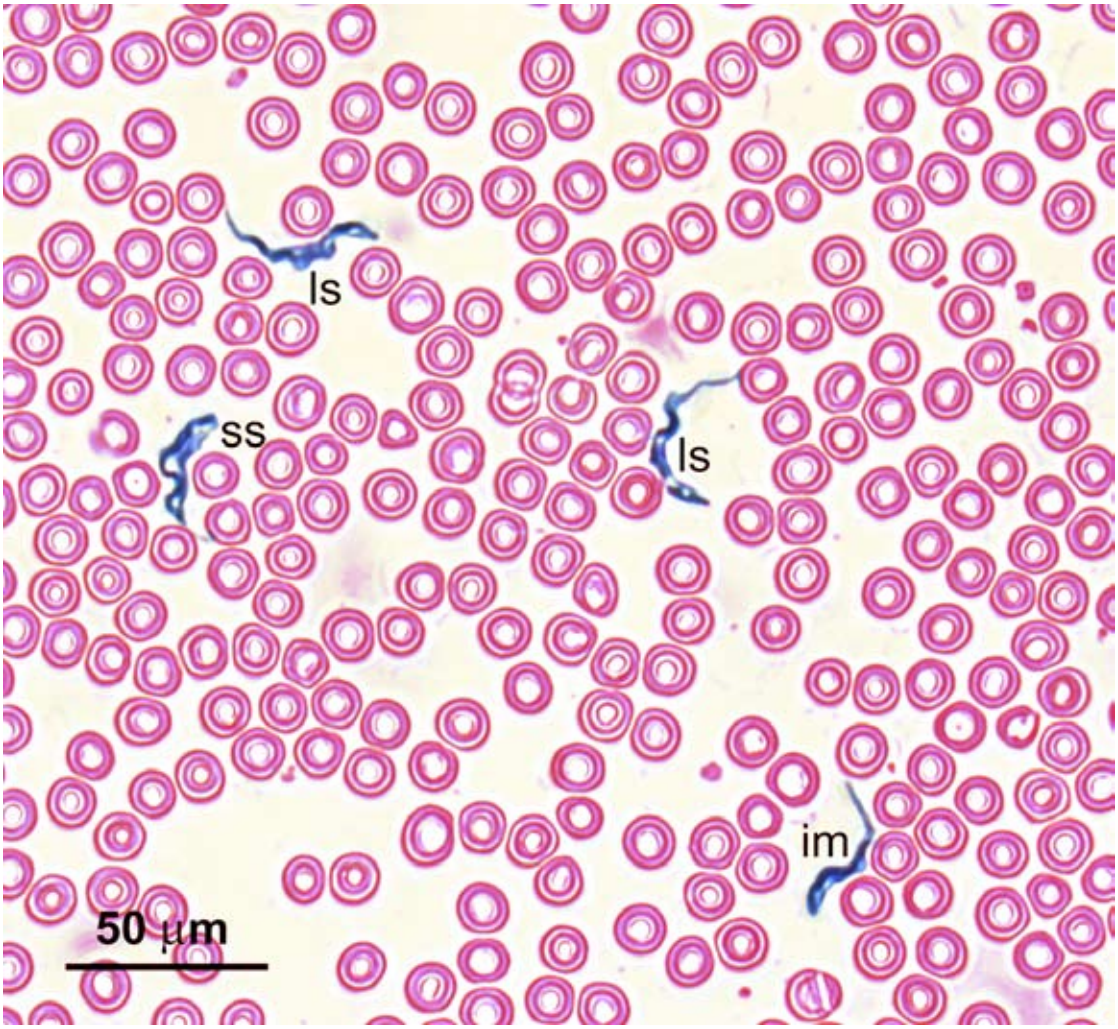
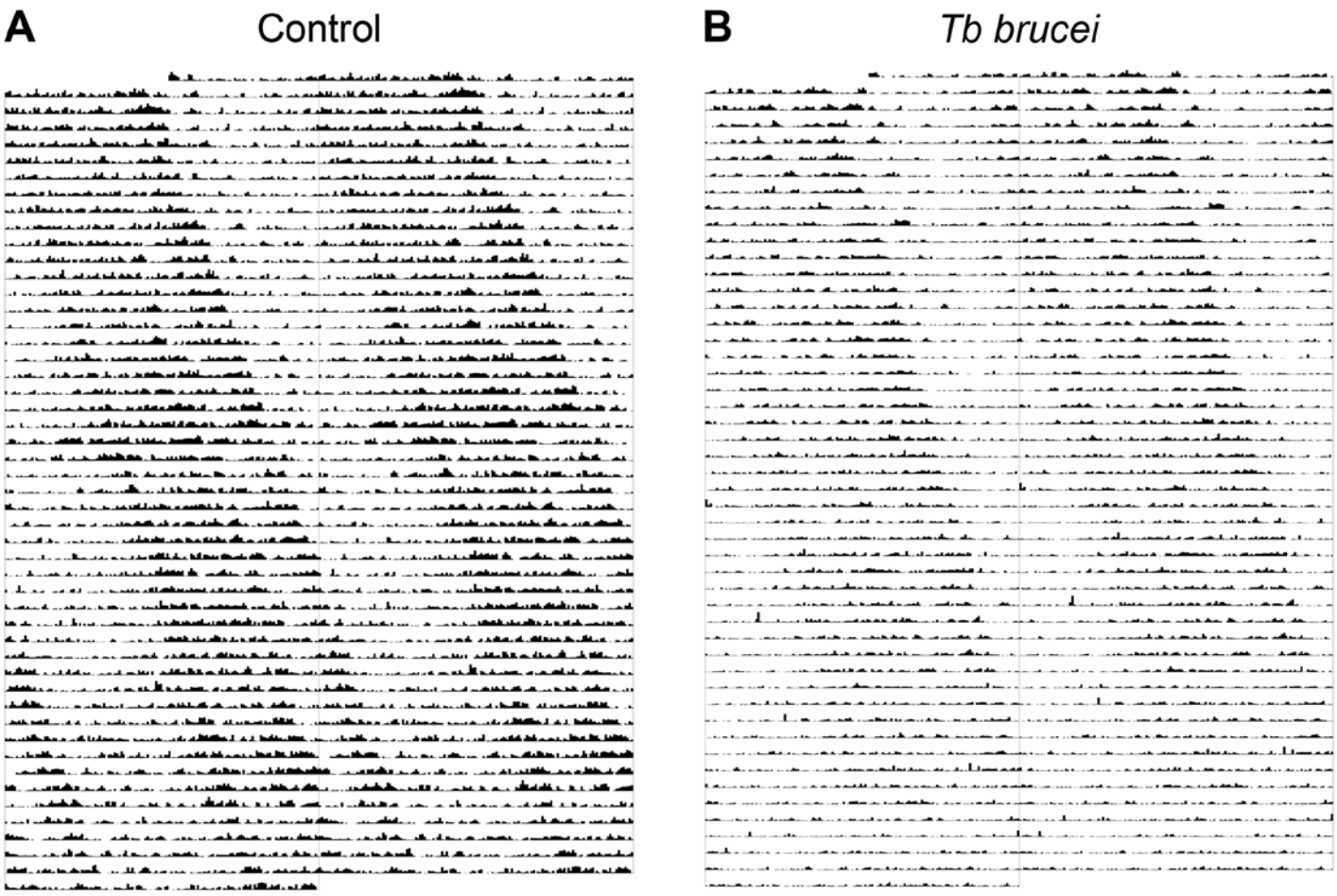


Figure S2

Representative double-plotted actograms of spontaneous locomotor cage activity in constant darkness in a control (A) and trypanosome-infected (B) rat, respectively. The activity rhythm amplitudes from infected rats' activities were significantly lower than controls.



Methods

Culture procedure

Brain tissue was sectioned in the coronal plane at a thickness of 300 μm on a vibrating microtome (NVSL manual advance vibroslicer, WPI Inc., Sarasota, FL). Brain slices were then transferred to chilled HEPES buffered sterile saline (HBSS) and fine scalpels were used to isolate the paired SCN. Pituitary glands were placed in sterile HBSS and the anterior lobe was separated from the neurointermediate lobe. A thin 5 mm^3 fragment of the anterior lobe was then recovered with the aid of a dissecting microscope.). The tissues were positioned on culture membranes (Millicell-CM, PICMORG50, Millipore) that were placed in 35 mm petri dishes containing 1.2 ml culture medium (Dulbecco's modified Eagle's medium, D2902 (Sigma-Aldrich), supplemented with 1X B27 (Invitrogen), 10 mM HEPES, 0.1 mM luciferin (Promega), Pen/Strep 25000U/ml (Invitrogen), D-glucose 0.35% and $NaHCO_3$ 350 mg/L. The culture dishes were sealed with cover glass secured with vacuum grease.

Amplitude calculations of *Per1-luc* rhythms

The amplitude of three consecutive days, beginning with the second day in culture, was divided into two _ cycles; a rise-to-peak amplitude and return-to-trough amplitude. Each of these was referred to as a "cycle" and indicated the decline in amplitude as a function of time in culture, also referred to as the "damping" rate. Damping rate was analyzed with two-factor ANOVA as a function of treatment and "cycle" number followed by pair wise comparisons with Bonferroni's post-hoc test. All comparisons were considered significant at $p < 0.05$.

Primers for real-time PCR

Primers used for the determinations of mRNA were the following: *Per1* forward: TGA GGA GCC AGA GAG GAA AGA GTC, *Per1* reverse: CAA ATA CCT GAG GAT GCT GTC CAG, Clock forward: TTG CTC CAT GGG AAT CCT T, *Clock* reverse: GGA GGG AAA GTG CTC TGT TGT AG, *Bmal1* forward: TGG AGG GAC TCC AGA CAT TC, *Bmal1* reverse: GCT TGA TCC TTG GTC GTT GT, *Cyclophilin* forward: GCT TTT CGC CGC TTG CT, *Cyclophilin* reverse: CTC GTC ATC GGC CGT GAT.