LATERAL GENICULATE NUCLEUS HISTOPATHOLOGY IN THE RAT EXPERIMENTAL MODEL OF AFRICAN TRYPANOSOMOSIS

Maina1*, C. I. and Ng’wena2, G. M.

1. Egerton University, Department of Biological Sciences, P.O. Box 536, 20115-Egerton, Kenya.
2. Maseno University, Department of Medical Physiology, Private Bag, Maseno, Kenya

* Correspondence: Charles Irungu Maina, Egerton University, Department of Biological Sciences, P.O. Box 536, 20115-Egerton, Kenya. Tel. +254728425209. Email; cimainah@gmail.com.

ABSTRACT

Trypanosomosis is an infectious disease of humans and animals characterized by sleep/wake disturbances and disruptions in other circadian rhythm activities. The disease is caused by protozoan parasites of the genus Trypanosoma and transmitted by the bite of infected tsetse flies of the Glossina species. Although trypanosomosis has a well known etiology, histopathological studies on brain regions involved in the control of circadian rhythms are scanty. Lateral geniculate nucleus works in conjunction with the suprachiasmatic nucleus, the master circadian rhythm pacemaker, in regulating circadian rhythms. The purpose of this study was to investigate the effect of T. b. brucei infection on the histology of the lateral geniculate nucleus, a brain region that can serve as an alternative secondary circadian rhythm pacemaker when the master pacemaker fails. Twelve control and twelve experimental male albino rats were used in this study. The experimental rats were inoculated intraperitoneally with 0.2ml of infected blood containing 1 x 10^4 T. b. brucei parasites. The infected animals were allowed to go through the full course of infection and sacrificed when they were in extremis. Each rat was decapitated and the brain immediately extracted from the skull. The brain was fixed in 10% buffered neutral formalin for at least 48 hours. The brain was later removed from the formalin solution and a coronal section made. The coronal section was processed histologically and stained using the haematoxylin and eosin method. The stained slides were observed under a microscope and photomicrographs taken. Histological alterations, including tissue degeneration, infiltration and proliferation of cells, and perivascular cuffing were observed in the lateral geniculate nucleus of infected rats. Lateral geniculate nucleus cannot, therefore, serve as an alternative secondary circadian rhythm pacemaker during trypanosome infection.

Keywords; Trypanosomosis, Lateral geniculate nucleus, Histopathology, Circadian rhythm

HISTOPATHOLOGIE DU NOYAU GENICULAIRE LATERAL DANS LE MODELE EXPERIMENTAL DE RAT DE LA TRYPANOSOMOSE AFRICAINE

Maina1*, C. I. and Ng’wena2, G. M.

1. EgertonUniversity, Département des sciences biologiques, P.O. Encadré 536, 20115-Egerton, Kenya.
2. Université Maseno, Département de physiologie médicale, Private Bag, Maseno, Kenya

* Correspondence: CharlesIrungu Maina, Egerton University, Département des sciences biologiques, P.O. Encadré 536, 20115-Egerton, Kenya. Tél. +254728425209. Email; cimainah@gmail.com.

ABSTRAIT

La trypanosomose est une maladie infectieuse des humains et des animaux caractérisée par des troubles du sommeil / sillage et des perturbations dans d’autres activités de rythme circadien. La maladie est causée par des parasites protozoaires du genre Trypanosoma et transmis par la morsure des mouches tsé-tsé infectées de l’espèce Glossina. Bien que la trypanosomose ait une étiologie bien connue, les études histopathologiques sur les régions cérébrales impliquées dans le contrôle des rythmes circadiens sont rares. Le noyau géniculé latéral travaille en conjonction avec le noyau suprachiasmatisque, le stimulateur cardiaque du rythme circadien, dans la régulation des rythmes circadiens. Le but de cette étude était d’étudier l’effet de l’infection par T. b. brucei sur l’histologie du noyau géniculaire latéral, une région du cerveau qui peut servir de stimulateur cardiaque secondaire circadien secondaire lorsque le stimulateur maître échoue. Dans cette étude, on a utilisé douze rats albinos mâles expérimentaux et douze témoins. Les rats expérimentaux ont été inoculés par voie intrapéritonéale avec 0.2 ml de sang.
INTRODUCTION

Trypanosomosis, also known as sleeping sickness in humans and Nagana in cattle, occurs only in 36 sub-Saharan Africa countries where there are tsetse flies that can transmit the disease. The disease continues to be a major health problem in sub-Saharan Africa where it threatens the health and productivity of humans and livestock, causes massive economic losses and severely constraints the continent’s socio-economic development. The disease is caused by protozoan parasites of the genus Trypanosoma and is transmitted by the bite of an infected tsetse fly of the genus Glossina(1).

The disease progresses in two distinct stages. The first or early stage of the disease, also known as the haemolymphatic phase, is defined by the restriction of the trypanosomes to the blood and lymph system. The second or late stage of the disease, also known as the neurological phase, is characterized by the presence of the parasites in the cerebrospinal fluid(2). In the absence of treatment, trypanosomosis is invariably fatal to both humans and livestock.

The causative agents of trypanosomosis, the Trypanosoma parasites, show early invasion in brain areas that lack a blood-brain barrier, such as the pineal gland and median eminence(3). From here, the trypanosomes invade other brain regions including the thalamus and hypothalamic where they cause inflammatory responses that may lead to disruptions in endogenous circadian rhythms like the sleep/wake cycle.

The lateral geniculate nucleus, located in the thalamus, is the primary relay centre for visual information received from the retina of the eye. It receives input directly from the retina via the retinohypothalamic tract (4). Besides being a major visual processing centre, the lateral geniculate nucleus also participates in the regulation of circadian rhythms through its projections, via the geniculo-hypothalamic tract, to the supra-chiasmatic nucleus, the master circadian rhythm pacemaker in the hypothalamus (5).

Thus, the lateral geniculate nucleus, through the geniculo-hypothalamic tract, provides a secondary, indirect photic input to the suprachiasmatic nucleus as well as an alternate input which has an important role in entrainment of circadian rhythms (6). This is further supported by other studies (7-11) that have reported that the lateral geniculate nucleus is an important component of the circadian timing system and is responsible for the integration of photic and non-photonic information to modify suprachiasmatic nucleus activity. Since the histology and functioning of the suprachiasmatic nucleus is altered during trypanosomosis (12), this study investigated the effect of T.b. brucei on the histology of lateral geniculate nucleus in an attempt to find out if this brain centre can serve as an alternative circadian rhythm pacemaker during trypanosome infection.

MATERIALS AND METHODS

Experimental Setup

Twenty four male albino rats, aged 3-3½ months and weighing 200-220g, were used in this study. The rats were randomly divided into two groups, control and experimental, of twelve rats each. The rats were housed at room temperature in the mini-laboratory animal house in the Department of Biological Sciences, University of Eldoret, Kenya, where the study was carried out. They were housed three per cage and were exposed to 12/12 hours of light/dark cycle throughout the study period. The rats had access to food (mice pencil, Unifeed Millers Ltd, Kisumu, Kenya) and clean water ad libitum.

Two weeks prior to data collection, the rats were observed and accustomed to routine handling. They were also screened for ectoparasites and each rat was injected subcutaneously with 0.01ml of Ivermectin (Ivermin®, Sinochem Ningbo Ltd., China), a broad spectrum parasiticide that effectively controls both ectoparasites and endoparasites(13). The experimental protocol got the approval of the Department of Biological Sciences ethics committee on care and use of animals for research purposes.
Infection of Experimental Rats

An isolate of the parasite *T. b. brucei* (ILTat1.4) was obtained from the International Livestock Research Institute (ILRI), Nairobi, Kenya. The parasite was originally obtained from the blood of a naturally infected cow in Uhombo, Kenya. The isolate was injected intraperitoneally into a donor rat for the purpose of expanding the stabilitate for subsequent inoculation into the experimental group rats. The donor rat was put in a cage and transported to the animal house at the University of Eldoret.

The donor rat was monitored for the presence of parasites daily by direct microscope observation of trypanosomes in wet smears of blood samples obtained from tail bleeds. When parasitaemia was established five days post-infection, the donor rat was anaesthetized with ether and 2ml of blood obtained from it through cardiac puncture. One millilitre (1ml) of this blood was diluted with 2ml of phosphate buffered saline solution (pH 7.4). Then, 0.2ml of this blood, containing about 1.0 x 10^4 live *T. b. brucei* parasites was injected intra-peritoneally to each of the twelve rats in the experimental group. The number of parasites was determined using the Neubauer haemocytometer method(14). Rats in the control group were, concurrently, injected intra-peritoneally with 0.2ml normal saline.

Organ Harvesting and Histological Studies

All the twelve infected experimental rats were allowed to go through the full course of infection and sacrificed when they were in extremis. For every experimental rat sacrificed, a control rat was sacrificed too. Each rat was anaesthetized with ether and then decapitated. A firm cut along the midline of the skull (through both parietal and frontal bones) was made using a sharp knife. Both parietal and frontal bones were tilted thus exposing the brain. The brain was then gently lifted out of the skull and immediately put in 10% buffered neutral formalin where it was fixed for at least 48 hours.

One week later, three brains were randomly selected from each group for further processing. Each of the selected brains was removed from the formalin solution and a coronal section of the brain, across the thalamus, was made. The coronal section was processed histologically using an automated tissue processor(Global Medical Instrumentation Inc., USA). Paraffin blocks were sectioned with a manual rotary microtome (Leica Biosystems, Germany) at 5µm thickness. The thin sections of the coronal section were mounted on glass slides and stained using the standard staining technique of haematoxylin and eosin (15). The stained slides were observed under a light microscope (Euromex, Holland) and photomicrographs taken using a camera (Canon EOS, Canon Inc., Japan) attached to the microscope.

RESULTS

Parasite Detection and Physical Observation of the Rats

Parasites were detected in the tail blood of experimental rats five to eight days post-infection. The experimental rats showed no signs of disease for the first fifteen days post-infection. Thereafter, they showed apparent fatigue, decreased activity, lack of appetite, discharge from the eyes and nose, and paralysis of limbs and tail. On the other hand, the control rats showed no signs of infection throughout the study period. They were also of normal behaviour, appetite, and general activity.

The lateral geniculate nucleus of control rats showed normal architecture composed of normal neurons and glial cells. The nuclei of the neurons were round and quite distinct. On the other hand, the lateral geniculate nucleus of experimental rats showed neurons with smaller and shrunken nuclei, and inflamed and enlarged blood vessels (Figure 1).
DISCUSSION
Although the lateral geniculate nucleus is one of the main components of the visual pathway and an important component of the circadian timing system, it seems to be one to which least attention is paid in histopathological studies. In the present study, the neuronal nuclei in the LGN of experimental rats were smaller and shrunken (pyknotic) compared to those of their matched controls. In addition, the blood vessels became inflamed and enlarged (perivascular cuffing) and there was marked infiltration and proliferation of cells notably glial cells, lymphocytes, plasma cells and macrophages. Such histological alterations could have affected the neuronal network between the lateral geniculate nucleus and the supra-chiasmatic nucleus. The integration role of the lateral geniculate nucleus in modifying supra-chiasmatic nucleus activity was, consequently, affected. The entrainment to a 12/12h light/dark cycle was disrupted in the experimental rats and this could explain the disruption of the sleep/wake cycle observed in these animals.

A study by Watts et al. (16) reported that lesions in the lateral geniculate nucleus result in destruction of not only the geniculo-hypothalamic tract, but also the hypothalamo-geniculate projection originating in the supra-chiasmatic nucleus and terminating in the lateral geniculate nucleus. Similarly, damage to the lateral geniculate nucleus has been reported to impair performance on brightness discrimination tasks (7). These findings support the view that the integrity of the lateral geniculate nucleus is necessary for entrainment of circadian rhythms (8).

The findings of the present study indicate that trypanosomosis causes histological changes in the lateral geniculate nucleus of infected rats. The integration role of the lateral geniculate nucleus in modifying the activity of the supra-chiasmatic nucleus, and in synchronization of circadian rhythms, is hence affected. Lateral geniculate nucleus cannot, therefore, act as an alternative secondary circadian rhythm pacemaker during trypanosomosis.

Acknowledgements: The authors are very grateful to ILRI, Nairobi, Kenya for generously donating the trypanosome isolate and to the Department of Biological Sciences, University of Eldoret, for allowing us to use the department’s facilities.

Conflict of Interest: The authors declare that there are no competing interests.
REFERENCES

1. WHO. Trypanosomiasis, human African (sleeping sickness). 2016. www.who.int/mediacentre/factsheets/fs259/en/. Accessed 10 June 2016

2. Kennedy, F.G. Clinical features, diagnosis, and treatment of human African trypanosomiasis (sleeping sickness. Lancet Neurol2013;12(2):186-194

3. Schultzberg, M., Ambatsis M., SamuelssonE.B.,Krist ensson, K., VanMeirvenne N. Spread of Trypanosomabrucei to the nervous system: early attack on the circumventricular organs and sensory ganglia. J Neurosci Res1988;21:51-61

4. Francl, J.M., Kaur, G., Glass, J.D. Roles of light and serotonin in the regulation of gastrin-releasing peptide and arginine vasopressin output in the SCN circadian clock. Eur J Neurosci2010;32(7):1170-1179

5. Moore, R.Y., Card,J.P. Intergeniculate leaflet; an anatomically and functionally distinct subdivision of the lateral geniculate complex. J Comp Neurol1994;344:403-430

6. Reghunandanan, V., Reghunandanan, R. Neurotransmitters of the suprachiasmatic nuclei. J Circadian Rhythms 2006;4:2-12

7. Harrington, M.E. The ventral lateral geniculate nucleus and the intergeniculate leaflet: interrelated structures in the visual and circadian systems. NeurosciBiobehav Rev 1997;21:705–727

8. Edelstein, K., Amir,S. The Role of the Intergeniculate Leaflet in Entrainment of Circadian Rhythms to a Skeleton Photoperiod. JNeurosci1999;19(1):372-380

9. Menet, J., Vuillez, P., Jacob, N., Pevet,P. Intergeniculate leaflets lesion delays but does not prevent the integration of photoperiodic change by the suprachiasmatic nuclei. Brain Res 2001;6(1-2):176-179

10. Morin, P., Allen,C.N. The circadian visual system. Brain Res Rev 2006;51(1):1-60

11. Gillette, M.U., Abbott,S.M. (2009) Biological timekeeping. Sleep Med Clin2009;4(2):99-110

12. Maina C.I., Ng’wena, G.M., Mwaniki, D.M., Oicho, A.O. Histopathological effects of Trypanosomabrucei infection on the master circadian rhythm pacemaker in rats. Egerton J Sci & Technol 2013;13: 95-114

13. Sutherland, I.H. Veterinary use of ivermectin. Acta Leiden1990;59(1-2): 211-2160

14. Herbert,W.J., Lumsden,W.H.R. Trypanosomabrucei: A rapid “matching” method for estimating the host’s parasitaemia. Exp Parasitol1976;40:427-431

15. Ellis, R. Hematoxylin and Eosin (H&E) Staining Protocol. 2011 https://www.ihcworld.com/_protocols/specialstains/h&e_ellis.htm. Accessed 10 February 2015

16. Watts, A.G., Swanson, L.W., Sanchez-watts,G. Efferent projections of the suprachiasmatic nucleus. I. Studies using anterograde transport of Phaseolus vulgaris-leucoagglutinin in the rat. J Comp Neurol1987;258:204-229