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Research article

# Neurocognitive domains and neuropathological changes in experimental infection with *Trypanosoma brucei brucei* in Wistar rats

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ABSTRACT

*Trypanosoma brucei brucei* causes animal trypanosomiasis in several vertebrates and human African trypanosomiasis. Previous studies have only explored the incidence, clinical symptoms, haematology and biochemical changes associated with the disease. The behavioral manipulation hypothesis posits that parasites alter the behavior of host to increase the reproductive abilities of such parasites. Hence, the present study was carried out to investigate changes in behavior and cognition following experimental infection of *T. brucei brucei* in rat model.

This study involved two groups of animals (uninfected control and *T. brucei* infected) with 8 rats per group. After confirmation of parasitaemia in the infected rats both groups were assessed to investigate if infection led to behavioral alterations and neuropathological changes using the open field, social interaction and forelimb suspension tests. Immunohistochemistry was performed on brain tissues using glial fibrillary acidic protein and anticalbindin-D28k, antibodies.

We demonstrated that *T. brucei* infection triggered a significant decrease in exploratory activity, anxiety-like behavior, altered recognition of social novelty and reduced hanging latency in the hanging wire test. Immunohistochemistry revealed significant astrocytosis, loss of dendritic spines and reduction of Purkinje cell layer of the cerebellum.

These results demonstrate that *T. brucei* infection induce signs of anxiety, impaired motor co-ordination with degeneration and loss of Purkinje cells.

## 1. Introduction

*Trypanosoma brucei* is one of the protozoan parasites that cause animal trypanosomiasis in several vertebrates and human African trypanosomiasis (Dorn et al., 2011; WHO, 2017). They are transmitted by bites from tsetse fly (*Glossina* genus) which have acquired infection from animals or humans harbouring the pathogenic parasites. The disease has resulted in three major epidemics in the 20th century. In cattle, the disease is called *Nagana* (Connor, 1994). Infection with *Trypanosoma brucei* causes considerable economic loss and illness in animals and humans globally (Murray et al., 2012; Andrews et al., 2014; Kassebaum et al., 2016).

In humans during the first stage of infection, the trypanosomes proliferate in subcutaneous tissues, lymph and blood. This is also called the haemo-lymphatic stage, which entails bouts of fever, headaches, enlarged lymph nodes, joint pains and itching. In the second stage, the parasites cross the blood-brain barrier to infect the central nervous system. This is known as the neurological or meningo-encephalic stage. The ''behavioral manipulation'' hypothesis affirms that a parasite can change host behavior specifically to increase its own transmission and reproductive efficiency (Laperchia et al., 2016; Masocha et al., 2007; Thomas et al., 2005). The persistence of the parasite *T.* brucei migration to the brain has been associated with changes in behavior leading to confusion, sensory disturbances and poor coordination in an infected host. The sleep cycle is also disrupted by the parasite hence the disease is also known as sleeping sickness (WHO, 2017). Without treatment, sleeping sickness is considered fatal although cases of healthy carriers have been reported (WHO, 2017).

It has been documented that trypanosomes cross the blood-brain barrier (BBB) and attack the central nervous system (CNS) invariably leading to coma and death (Maclean et al., 2013), however, there is a dearth of information on altered behavior following such invasion. Up till

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now, most experimental studies on African trypanosomiasis have only focused on clinical symptoms, hematology and biochemical changes. For instance, Amrouni et al. (2011) had documented changes in dimethylarginine dimethylaminohydrolase activity in rat infected with *T. brucei.* These biochemical changes have been linked to neuro-inflammatory response leading to variation in expression of inducible nitric oxide synthase (iNOS) by neurons and neuroglia (Cespuglio et al., 2019) causing a disruption in cellular elements involved in brain inflammatory processes. During neuroinflammation, glia cells such as astrocytes are exposed simultaneously to a plethora of stimuli leading to a complex network of intracellular events. Astrocytes are critical regulators of immune responses in the injured central nervous system (CNS). During an insult to the CNS, astrocyte activity may exacerbate inflammatory reactions and tissue damage, or promote immunosuppression and tissue repair. In the cerebellum, astrocytes are generated through a tightly regulated process and can be identified based on their morphology and specific layer in the cerebellum (Cerrato et al., 2018, 2019). They are important for cerebellar development and functioning. In the cortical layers, different astrocyte types interact with distinct neuronal subsets and, therefore, possibly develop neuron-specific functional properties. In the Purkinje cell layer, astrocytes are tightly connected with the entire dendritic tree and soma, thus they function to support cerebellar development and Purkinje cell synaptogenesis (Buffo and Rossi, 2013). Insults to the CNS are characterized by activation of astrocytes, which is usually described as up-regulation of glial fibrillary acidic protein (GFAP). Astrocytes can also drive the induction and the progression of the inflammatory state due to their Ca2+ signals and that it is strongly related to the disease severity/state. Nevertheless, previous studies did not examine behavioral alterations and astrocytic changes associated with documented neuro-inflammatory responses associated with *T brucei brucei* infection.

Therefore there is a need to investigate changes in behavior, cognition and neuropathology following experimental infection of *T. brucei* in rat model.

## 2. Materials and methods

The experimental procedures were performed as approved by the Institutional Animal Care and Use Ethics Committee (UI-ACUREC/18/0026) adhering to National Institute of Health guidelines for the care and use of Laboratory animals. Efforts were made to minimize the number of animals used and suffering.

### 2.1. Experimental rats

Male and female Wistar rats aged 12 weeks weighing between 200 and 300 g were purchased from the Experimental Animal Unit, Faculty of Veterinary Medicine, University of Ibadan, Nigeria. They were acclimatised and kept in a pathogen-free environment for 2 weeks prior to the commencement of study.

The animals had free access to food and water and were maintained under a 12 h: 12 h light/dark cycle. The experiment involved two groups of animals (uninfected control and *T. brucei* infected) with 8 rats per group in an acute study.

#### 2.1.1. Trypanosoma brucei strain

*T. brucei brucei* (Bassa/Quanpan strain (MBOS/NG/94/NITOR) isolate used in the study was obtained from Nigerian Institute for Trypanosomiasis and Onchocerciasis Research (NITOR), Vom and maintained in the Department of Veterinary Physiology and Biochemistry by serial passage in rats.

### 2.2. Inoculation of parasites

The tail of a *T. brucei brucei* infected rat was scrubbed with damp cotton wool and the tip was cut with a sterile pair of scissors. Thereafter, whole blood was collected into 0.5 ml normal saline solution and thoroughly mixed before drawing into a 1 ml syringe. A drop of this solution was subsequently placed on a microscope slide and observed under a light microscope to ascertain the presence of the parasite in the solution. Experimental infection was done by inoculating rats intraperitoneally when the parasite suspension contained 105–110 trypanosomes/ml at x100 magnification in the donor rats. The experimental rats were thereafter inoculated intraperitoneally with 0.2 ml of this suspension as described by Adeyemi et al. (2012).

#### 2.2.1. Parasitaemia (parasite count)

Parasitaemia was verified daily in experimentally infected rat by counting the number of trypanosomes per view under light microscope at x100 magnification from a thin blood smear obtained from the tip of the tail of an infected rat. Behavioral tests were performed at 6 and 7-day post infection in the animals. The parasitaemia was recorded at 12 h interval until when the parasiteamia level was above 110 trypanosme/ml post infection. (This was therefore selected as end-point of the study as we observed from previous monitoring that several of the rats were found dead once the parasiteamia was above 110 trypanosme/ml post infection). The rats were anesthetized with ketamine/xylazine injection and thereafter transcardially perfused with ice-cold 0.9% saline followed by formaldehyde solution (obtained dissolving paraformaldehyde in 0.1 M phosphate buffer, pH 7.2, at a 4% concentration). Brains were removed and stored in 10% neutral buffered formalin for paraffin embedding.

### 2.3. Open field test

Exploration and mobility in the experimental animals were measured in an open field at day 6 post *T. brucei* infection. At the commencement of the trial, each rat was placed at the center of an open field maze (72 × 72 × 36 cm) whose floor was divided into 16 equal squares by black lines. The movement of the rat was recorded by a USB webcam (Logitech HD-1820p) during the 5-minutes test. The recorded video file was further analyzed to determine some variables of motor activity such as the number of line crossings, rearing, freezing, grooming and stretch attend posture. The open field was then cleaned with 70 % ethyl alcohol and allowed to dry between trials. The behavioral scoring as previously described by Brown et al. (1999) includes;

1. Line Crossing: frequency with which the rats crossed one of the grid lines with all its four limbs
2. Rearing: frequency with which the rat stood on its hind limbs in the maze without the aid of a wall.
3. Stretch Attend Posture: Frequency with which the rat demonstrated forward elongation of the head and shoulders followed by retraction to its original position.
4. Grooming: frequency in which the rat spent licking or scratching itself while immobile.
5. Freezing: duration in which the mouse was totally motionless.

The test and the analyses were performed by an observer blinded to the treatment conditions.

### 2.4. Social interaction test

Social interaction in control and *T. brucei*-infected rats was measured once during the animal's light cycle at day 7 post infection. We used a modified version of the Crawley's sociability and preference for social novelty test that has been previously described (Kaidanovich-Beilin et al., 2011; Torres et al., 2018). The same box used for the open field test was used to assess social behavior in the experimental rats by placing two similar cups in opposite sides of the box. The test was divided into two sessions (each session lasted for ten minutes). In the first session, a rat (stranger rat 1) was placed under one of the wired cups, while the second cup located in the opposite side was left empty. The duration of the active

contacts between the test rat and both the empty cup and the cup containing stranger rat 1 was recorded. Each time the rat touched the cup with its snout or paws was recorded and noted as the active contact. In the second session, a second (new) rat was placed under the cup that was initially left empty during the first session. The duration of active contacts between the test rat and both the familiar and new rat was recorded. The test rat and stranger rats were of identical strain, sex, weight, age but different litters. Both sessions were recorded and manually analyzed by an observer blinded to the treatment conditions.

### 2.5. Forelimb suspension test

This was carried out using a metallic wire (2 mm (diameter) x 80 cm (length)) suspended at a height of 50 cm over a cushion support. The rats were placed on the wire with their forelimbs only and the duration in which the rat held the wire was recorded. Each rat was tested twice with at least 30 min inter-trial interval according to description by Shabani et al. (2012). The time in which the animal held the wire without falling is regarded as the hanging latency, a maximum of 2 min was allowed for this test.

### 2.6. Immunohistochemistry

Immunohistochemistry for anti-GFAP and anti-calbindin was performed on paraffin sections (4 μm in thickness). Each section was dewaxed, rehydrated and rinsed in distilled water. Antigen retrieval was done in 10 mM citrate buffer (pH = 6.0) for 25 min at a temperature range of 98–100 °C, with subsequent peroxidase quenching in 3% H2O2/methanol.

The sections were then pre-incubated in a blocking buffer solution consisting of 2% casein, for 1hr under gentle shaking at room temperature. Following preincubation, the sections were later probed with rabbit anti-Glial Fibrillary Acidic Protein antibody (z0334, Agilent Technologies® USA, dilution of 1:1000) and anticalbindin rabbit monoclonal antibody (CB-38, Swant® Switzerland, dilution of 1: 12000) for 18 h at 4 °C. The sections were thereafter rinsed thrice (5 min/rinse) in PBS before incubation with the secondary antibody for 2 h at room temperature. The sections were then reacted in avidin biotin-peroxidase solution (ABC kit, Vectastain, Vector Labs, USA) using 3, 3-diaminobenzidine as chromogen, according to manufacturer's protocol. Images were acquired using a light microscope (Leica LAS-EZ® with Leica software application suite version 3.4) equipped with a digital camera.

### 2.7. Quantification of astrocytosis and Purkinje cells in control and T. brucei-infected

Manual counts of astrocytes were performed and expressed as the percentage of the total number of GFAP positive cells counted in different regions of the brain. Additionally, the entire number of Purkinje cells was also counted in control and *T. brucei*-infected sections, scanning the entire length of the Purkinje cell layer. Purkinje cells were identified based on calbindin positivity and their specific location. The length of the Purkinje cell layer was traced and measured in each section to account for the difference in section sizes and plane of cutting. This was used to obtain the percentage of Purkinje cells per square millimeter. Additionally, the Purkinje cells counted were subdivided into intact and pyknotic cells depending on the condensation of chromatin in the nucleus. Counts were performed using the ImageJ "analyze particles" function and expressed as cells per square millimeter.

Based on morphometric principles already described in literature (Bahaei et al., 2018) we analysed GFAP-positive cells by measuring their surface area and process length using ImageJ as earlier described (Rasband, 2011).

### 2.8. Statistical analysis

Data were expressed as mean ± standard error of mean (SEM), comparison was performed by the student's t-test using Graphpad Prism version 5.00 for Windows software. Data from the social interaction test was analyzed by two-way ANOVA followed by Bonferronis post hoc test to correct for multiple comparisons. A probability value of $p \leq 0.05$ was considered statistically significant.

## 3. Results

The results appeared similar between the sexes as we did not observe any statistical difference when results from infected male and female were compared together. Consequently the results are presented as control versus infected.

### 3.1. Parasitaemia

*Trypanosoma brucei* was detected in peripheral blood of all infected rats between 24 and 48 h after inoculation. Parasitaemia increased progressively in all the animals, at 6 day post infection we observed mean of 75 ± 10 trypanosomes/field and by 7 dpi the *T brucei* rats began to die thus we had to terminate the experiment. The control animals remained clinically healthy throughout the experimental period.

### 3.2. T. brucei-infected rats demonstrated reduced exploratory activities with signs of anxiety

*T. brucei* infection in experimental rats led to marked decrease in locomotor activity as seen in the significant ($p = 0.0073$) reduction in the number of lines crossed in the open field test when compared with control animals (66% decreases in the number of lines crossed) as presented in Figure 1A. In contrast to the control group, the frequency of rearing was also significantly decreased ($p = 0.0340$) in the *T. brucei* infected group (Figure 1B). On the other hand, the *T. brucei* group had significantly increased freezing time and frequency of stretch attend posture when compared to the control animals as depicted in Figure 1C and E. The frequency of grooming was however similar ($p = 0.0934$) in both control and *T. brucei*-infected groups (Figure 1D).

### 3.3. T. brucei infection diminished social behavior (interaction) in experimental rats

We set out to investigate if *T. brucei* infection extended to alter social behavior using a modified form of the Crawley's sociability and preference test for social novelty as previously described (Kaidanovich-Beilin et al., 2011).

In the first session of the test, both the control and *T. brucei*-infected groups displayed a preference for the cup with the stranger rat 1 over the empty cup, which is indicative of normal social behavior (Figure 2A). Two-way ANOVA revealed that *T. brucei*-infected rat spent significantly more time with the cup containing stranger mouse 1 over the empty container, also an indication of normal behavior ($F(1,16) = 0.0600$, $p = 0.8154$, Figure 2A). Furthermore, specific two-group comparisons showed that control rat preferred the container with the new rat (stranger rat 2) over the familiar mouse (stranger mouse 1) in the second session of the experiment, and this difference was significant ($p = 0.0229$, two-tailed Student's t test). Two-way ANOVA also revealed that *T. brucei* infection accounts for 52.47% of the total variance ($F(1, 16) = 30.72$, $p < 0.0001$) and this is considered extremely significant. However, *T. brucei*-infected rats did not display a preference for either container, demonstrating an alteration in recognition of social novelty ($p = 0.0636$, two-tailed Student's t test) as shown in Figure 2B.

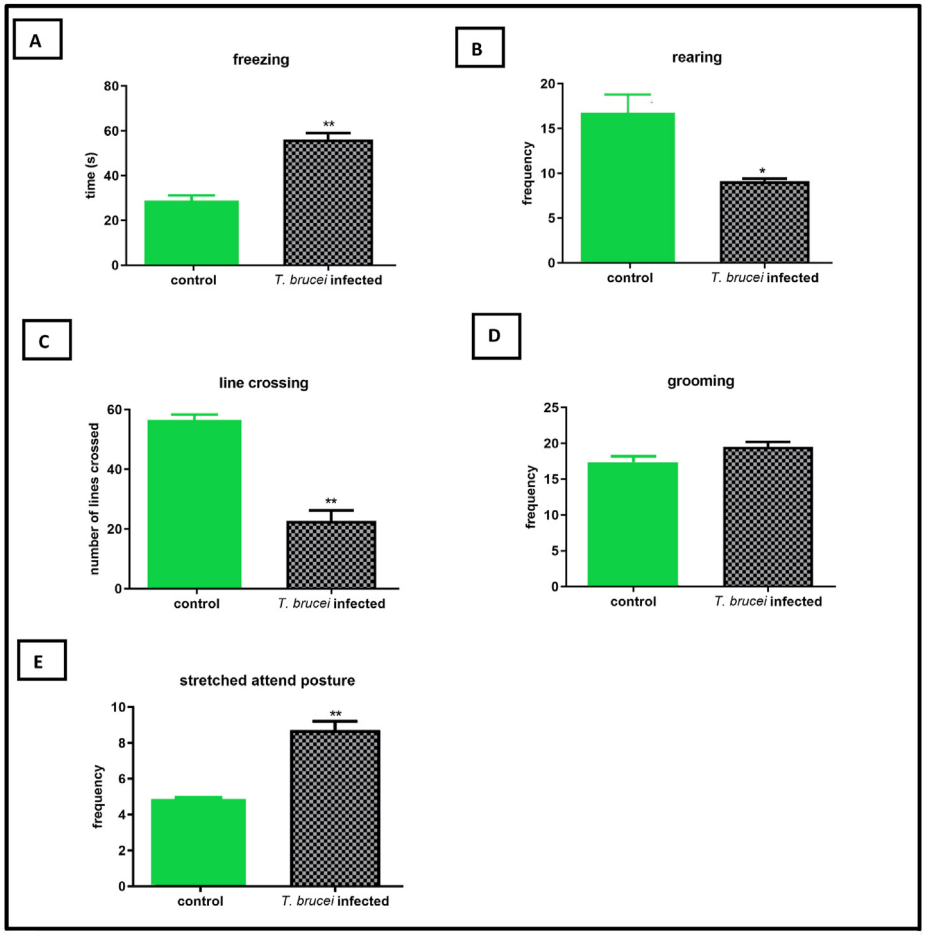


**Figure 1.** The Open field test showing (A) number of line crossings (B) number of rearing (C) duration of freezing (D) grooming (E) Stretch attend posture during the 5-min observation in control and at 6-day post *T. brucei* infection in experimental rats (n = 8 per group), Data are shown as mean ± SEM. * significantly different from the control at $p \leq 0.05$, ** significantly different from the control at $p < 0.01$.

### 3.4. T. brucei decreased latency on the hanging wire

*T. brucei* infected rats had significantly ($p = 0.0102$) reduced hanging latency on the forelimb suspension test at 6-day post infection (Figure 3A). In addition, more than half of the *T. brucei* infected rats had fallen from the suspended wire mid-way into the test (Figure 3B).

### 3.5. T. brucei infected rats had increased astrocytosis with disruption of the Purkinje cell layer of the cerebellum

In the present study we quantified the expression of glial fibrillary acidic protein (GFAP) to measure astrocytic expression as seen in Figure 4. We did not observe any significant difference in astrocytic expression and morphology in the olfactory bulb, cortex and hippocampus. However in the cerebellum, we noticed the astrocytes in the *T. brucei* infected group appear to be enlarged with prominent GFAP staining. Quantification of this GFAP expression revealed a significant increase (p = 0.0017, more than 4-fold) in GFAP immunoreactivity in the *T. brucei*-infected group relative to control (Figure 4E).

There was significantly decreased number of intact but increased pyknotic Purkinje cells nuclei in the *T. brucei* infected group when compared with control animals. This loss of Purkinje cell is easy to appreciate in some cerebellar region (Figure 5). On the contrary no loss of Purkinje cell was observed in the control. Some of the Purkinje cell in the *T. brucei* infected also had a less flask-like shape. The mean surface area (671.30 ± 26.30 $\mu m^2$) of GFAP-positive astrocytes in *T. brucei* infected group was significantly higher that than of the control (316.30 ± 17.70 $\mu m^2$). Similarly the length of the astrocytic process was significantly longer in the infected group (18.20 ± 0.60 μm) compared to the control (8.80 ± 1.04 μm) (Figure 4F and G).

## 4. Discussion

Cognitive impairments in *T. brucei* infection have received less attention than clinical signs manifestations despite the former often have a considerable impact on the progression and outcome of the infection. In the present study, we used arrays of behavioral testing and showed changes in cognitive behavior following experimental infection with *T. brucei* in rat model. In the late-stage of trypanosomes infection, it has been documented that the protozoa cross the blood–brain barrier (BBB), invade the central nervous system (CNS) invariably leading to coma and death if untreated (Maclean et al., 2013). Recent findings have also linked behavioral alterations to parasitic diseases, particularly those with neurological tropism such as toxoplasmosis (Nourollahpour et al., 2016; Torres et al., 2018), human toxocariasis (Alvarado-Esquivel, 2013; Cong et al., 2014) and cysticercosis (Abdoli et al., 2014; Wiwanitkit, 2014). However, to date, no findings have published the association of behavioral alterations and *Trypansoma* despite several reports on its neurotrophism; hence the reason for this study.

The reduced line crossings in the *T. brucei*-infected rats may be related to motor impairments in the infected animals. The decreased line crossing in the infected group was also accompanied by a similar reduction in rearing. Rearing is one of the numerous components of rodent behavior used to measure the general activity (Walsh and Cummins,

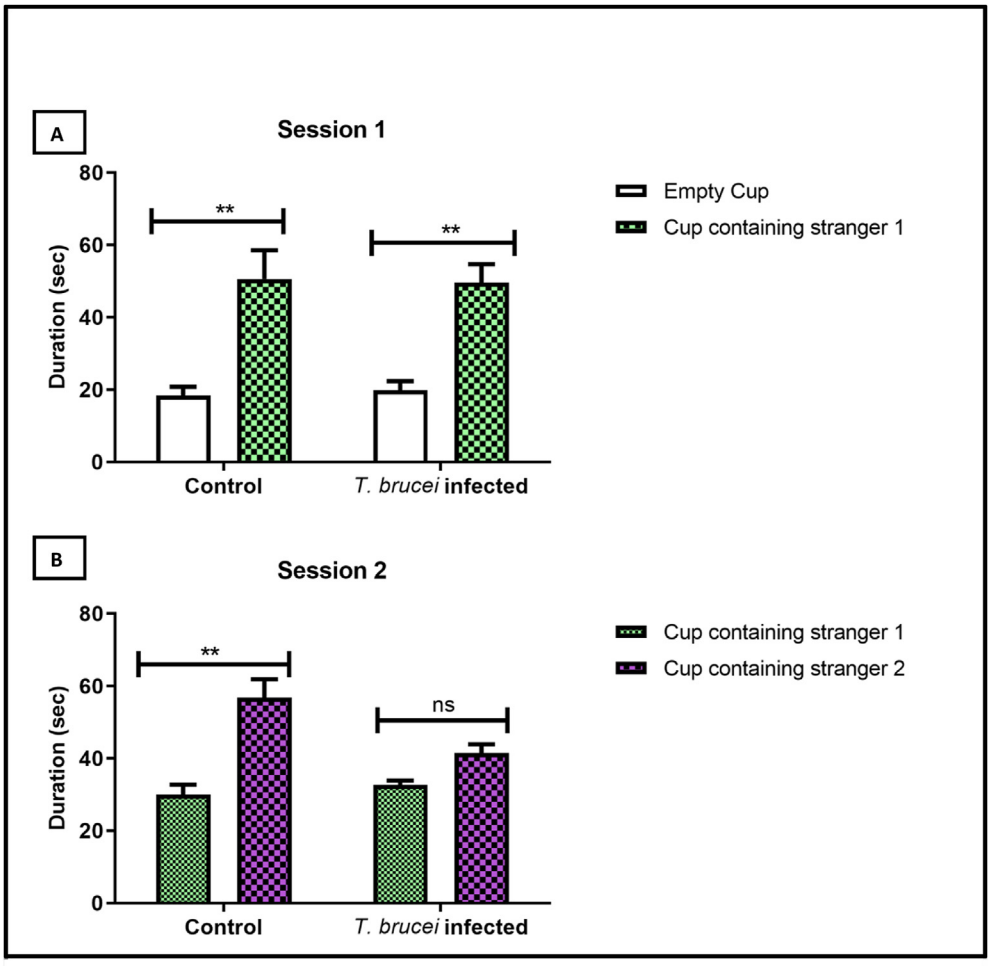


**Figure 2.** *T. brucei* infection altered social novelty recognition in experimental rats. Rats were tested once on day 7 after infection with *T. brucei* (A) The duration of active contacts between the test rat and the empty cup or the cup containing stranger rat 1 is shown (n = 8) (B) Duration of active contacts between the test rat and stranger rat 1 (familiar rat) and stranger rat 2 (new rat). Data are shown as mean ± SEM. * significantly different from the control at $p \leq 0.05$, ** significantly different from the control at $p < 0.01$, ns not significant.

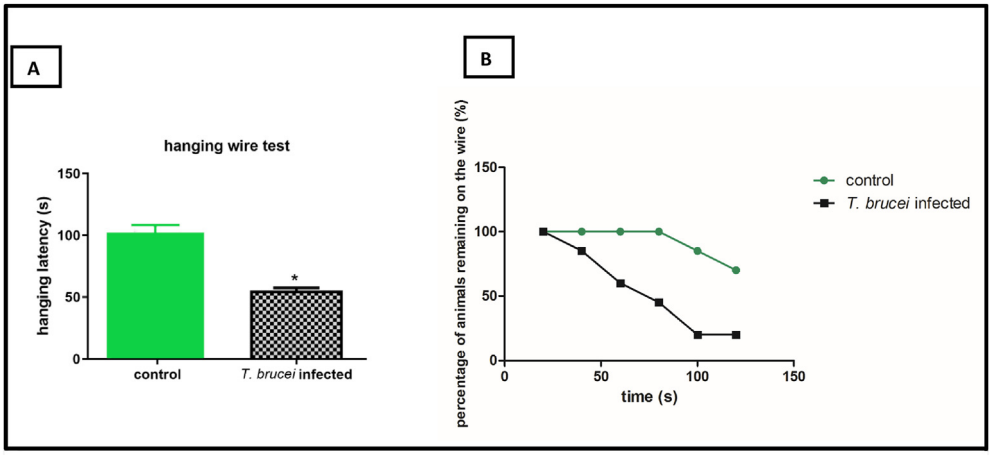


**Figure 3.** Latency of fall (B) percentage of animal remaining on the on the hanging wire testday 6post infection with *T. brucei*. n = 8; Mean ± SEM*significantly different from the control group at $p \leq 0.05$.

1976) of the animals; it is also a part of stereotyped behavior induced in rats by activation of postsynaptic dopaminergic receptors (Randrup and Munkvad, 1974). A decrease in these parameters indicates low exploration and increased anxiety in the infected animals compared to control. Similarly, the increased freezing duration demonstrated as immobility in the *T.brucei*-infected group has also been associated with increased anxiety (Seibenhener and Wooten, 2015) and this has been documented in several neurological disorders (Kar, 2009; Akcali et al., 2010). The stretch attend posture is a behavior used to assess risk in rodent in which an animal is hesitant to move from its present position to a new one (Blanchard et al., 2001) and thus a high frequency of this behavior as seen in the *T. brucei*-infected rats indicates a higher level of anxiety. This anxiogenic effect of *T. brucei* infection correlates positively with the decreased exploratory activities in the infected group. In summary, these results indicate that *T. brucei*-infected rats had impaired motor activity and showed anxiety-like behavior. Wolkmer et al. (2013) has reported no

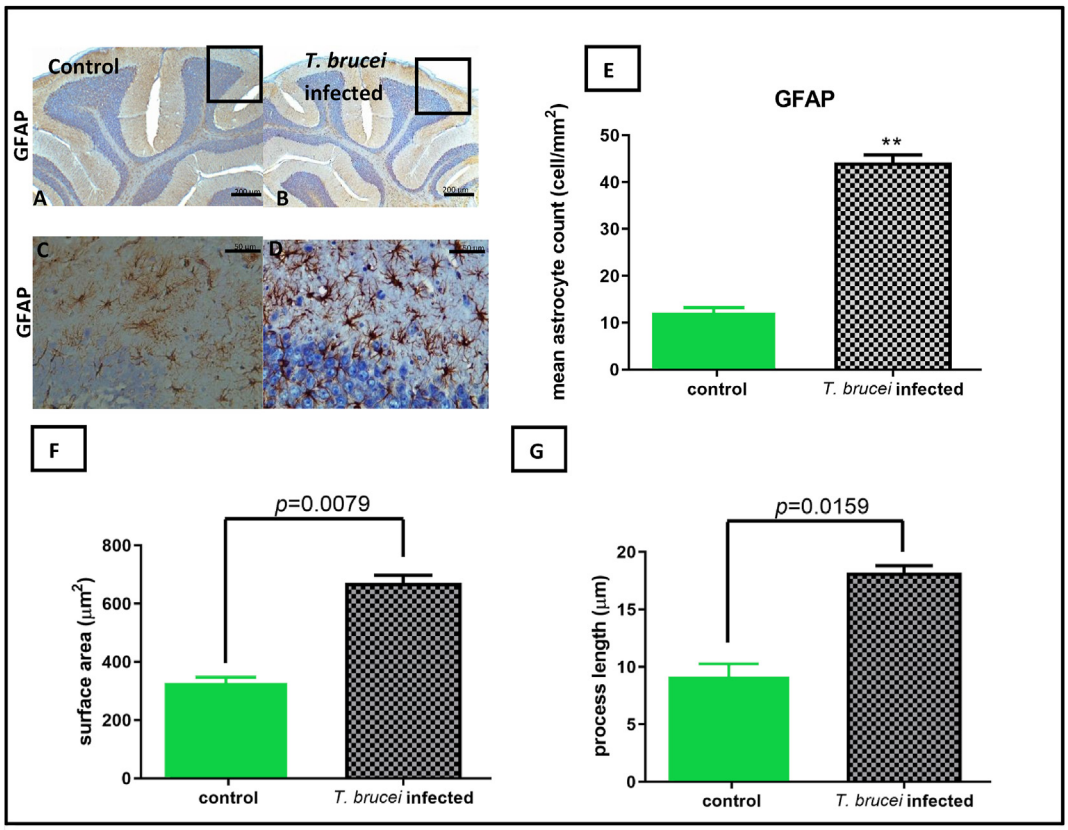


**Figure 4.** *T. brucei* infection increased GFAP expression in the cerebellum of rats. C and D are higher magnification of boxed area in A and B. (A) Brains from control rat and (B) rat intraperitonelly infected with *T. brucei* at 7 days. Brains were collected after intra cardiac perfusion with ice-cold 10% NBF. Brain sections were fixed and stained with anti-GFAP. Whole brain images were scanned using Leica Biosystems. Representative images were taken from the cerebellum × 40 magnification. n = 8 rats per group, Scale bar = 50 µm. At least five different fields of the cerebellum were randomly selected from 2 to 4 rats per group. Representative images of the cerebellum of control and *T. brucei*-infected rats stained with anti- GFAP.(E)The mean intensity of GFAP positive cells at 7 days post infection was quantified using ImageJ software from five different fields (n = 8/group). Morphometric features of GFAP- positive astrocytes showing representative (F) surface area (G) length of astrocytic process. Data are shown as mean ± SEM. *p < 0.05, **p < 0.01 (n = 5/group).

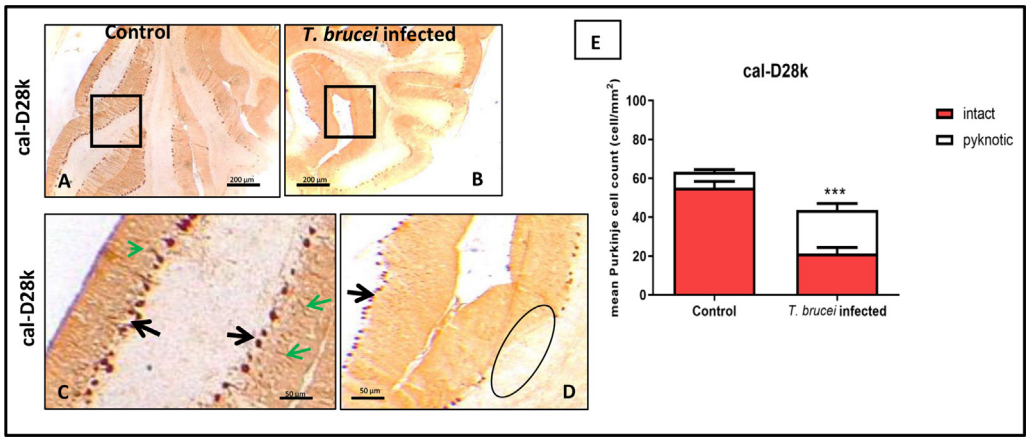


**Figure 5.** Immunoreactivity of calbindin-D28k in Purkinje cells in the cerebellum A. control rat. B. *T. brucei* infected rat. Brains were collected after intra cardiac perfusion with ice-cold 10% NBF (Scale bar 200 µm).C and D are higher magnification (scale Bar 50 µm) of boxed area in A and B. Immune-positive linearly arranged Purkinje neurons (black arrow) and dendritic processes (green arrows) and their terminal arborizations in the molecular layer in the control rats (A and C). (D). A higher magnification of B showing decrease immunoreactivity accompanied by loss of Purkinje neurons and dendrites (oval shape) in the Purkinje cell and molecular layers respectively. (E). Quantitative neuronal cell count in the cerebellum revealed a decrease in Purkinje neurons following infection with *T.brucei* 7 day-post infection, mean pyknotic neurons in the infected rats significantly (***P‹0.001) increased when compared with the control (n = 5/group).

change in locomotor activity in rats infected with *T. evansi,* however, the findings of this study indicate that *T. brucei*-infected rats had reduced locomotion, impaired motor activity and showed anxiety-like behavior. Also, Vilar-Pereira et al. (2015) demonstrated depression, anxiety and loss of motor coordination following *T. cruzi* infection. These differences in observed locomotor activity may be due to the different strains of

*Trypanosoma* and different host species used. It has been documented that the *T. brucei* is more CNS-invasive compared to the *T. evansi* and the involvement of the CNS in *T. brucei* infection is widely reported by the appearance of lesions in the CNS and presence of the parasite in brain tissues. However, our results are in consonance with those of Da Silva et al. (2012) that *T. brucei* entry into the brain during infections leads to lesions in the muscular and peripheral nerve systems reducing locomotion that may lead to paralysis.

We also examined how *T. brucei* infection affects social cognition by measuring social recognition in control and infected rats. Social recognition denotes the ability to differentiate potential social and mating partners, familiar and unfamiliar subjects, and subjects presenting either social rewards or posing threats (Kavaliers and Choleris, 2018). Rodents are extremely social animals and when positioned in an area in which territory has not been established, they socially interact with one another displaying a number of behavioral acts (Sams-Dodd, 1995). The significant changes in social contact by *T. brucei* infected rats in the second session of the social interaction test demonstrate social withdrawal as earlier documented by Sams-Dodd (1996). The failure of the infected rats to recognize social novelty has been linked to increased anxiety (Yizhar, 2012) in rodents. This correlates with the anxiogenic behavior demonstrated in the open field test.

The forelimb suspension is a recognized measure of muscular strength and has been used to screen for neurobehavioral toxicity. Furthermore, changes in grip strength have been interpreted as evidence of motor neurotoxicity (Maurissen et al., 2003). The observed reduction in hanging latency of *T. brucei*-infected rat in the forelimb suspension test confirms muscular weakness following infection in experimental rats leading to altered forelimb grip performance.

The behavioral alterations following *T. brucei* infection in rodent models are rare in literature and thus made comparison with other reports difficult. Thus, our findings provide baseline information on behavioral alterations following *T. brucei* infection in rat model.

The cerebellum is one of the brain structures implicated in playing a role in social interaction (Yizhar et al., 2011), thus we examined structural alteration in this region following acute *T. brucei* infection. Glial fibrillary acidic protein (GFAP) is the major intermediate filament in mature astrocytes. GFAP is a member of the cytoskeletal protein family thought to be important in modulating astrocyte motility and shape by providing structural stability to extensions of astrocytic processes. The overexpression of GFAP as presently seen in the *T. brucei*-infected rat is an indicator of reactive astrocytosis. Though astrocytes are more resistant to various CNS insults than oligodendrocytes and neurons they are nonetheless vulnerable to many harmful processes and sometimes undergo necrosis (Largo et al., 1996). The astrocytic swelling in the *T. brucei*-infected group signifies one of the earliest pathological features associated with CNS injuries. The present study has demonstrated that *T. brucei* elicit astrocytic gliosis as a prominent neuropathologic change leading to astrocytic hypertrophy. Consequently this swelling may impair astrocyte integrity and function. Ultimately, when swelling is severe, the cell membrane may rupture, resulting in cell death and impairs astrocytic functions. Purkinje cells are large, GABAergic neurons that serve as the single output of the cerebellar cortex. The Purkinje cells are characteristically located in a single row between the granular and molecular layers. Their myelinated axons terminate on neurons of the cerebellar nuclei trees, are flattened and oriented perpendicular to the parallel fiber. Cerebellar Purkinje cells (PCs) play a crucial role in motor functions and their progressive degeneration as typified by increased pyknotic nuclei in infected rat is closely associated with apoptosis (Atiba et al., 2021) and motor impairments (Kim et al., 2009). The degeneration of Purkinje cells within the cerebellar cortex may contribute to the loss of motor co-ordination of infected animals in the behavioral tests.

In relation to other neurons, the maintenance of $Ca^{2+}$ homeostasis in Purkinje neurons is critically imperative to their function and survival (Crepel and Jaillard, 1991). Purkinje cells contain large quantities of smooth endoplasmic reticulum (SER) and comparatively high concentrations of cytoplasmic $Ca^{2+}$binding proteins such as calbindin (Llano et al., 1994).

Calbindin is a calcium binding protein highly expressed in Purkinje cells and a marker of Purkinje cells in normal and degenerative cerebellar tissue (Adebiyi et al., 2020). Calbindin expression in Purkinje cells is neuroprotective against degeneration in various acute and chronic disorders. All normal Purkinje cells are calbindin immunopositive (Lal, 1999) but in the present study immunohistochemistry confirms the loss of Purkinje cells in *T. brucei* experimentally infected rats leading to neurodegeneration. Furthermore, motor coordination deficits in infected animals may be explained by changes in Purkinje cell morphology. These Purkinje cell bodies positive for cal-D28k displayed an abnormal morphology, with a less flask-like shape in the infected rats. Loss of Purkinje cells of the cerebellar cortex in *T. brucei* infection may therefore represent an important neurodegenerative mechanism.

## 5. Conclusion

This study indicates that *T. brucei* altered behavior in experimental infection using rat model. It induces signs of anxiety, impaired motor coordination with degeneration and loss of Purkinje cells. This understanding of the behavioral alterations following *T. brucei* infection may provide a foundation to design broad spectrum and novel therapeutic interventions for accurate treatment of this parasitic infection. The loss of Purkinje neurons is prominent and often irreversible. Thus, designing therapies to protect Purkinje cells in *T. brucei* infection and related conditions is vital to preserve cerebellar function.

## Declarations

### *Author contribution statement*

Olamide Elizabeth Adebiyi: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Temidayo Olutayo Omobowale, Mathew Oluwole Abatan: Conceived and designed the experiments.

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### *Data availability statement*

Data included in article/supplementary material/referenced in article.

### *Declaration of interests statement*

The authors declare no conflict of interest.

### *Additional information*

No additional information is available for this paper.

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