

Influence of environmental enrichment on the behavior and physiology of mice infected by *Trypanosoma cruzi*

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Abstract

Introduction: Enriched environments normally increase behavioral repertoires and diminish the expression of abnormal behaviors and stress-related physiological problems in animals. Although it has been shown that experimental animals infected with microorganisms can modify their behaviors and physiology, few studies have evaluated how environmental enrichment affects these parameters. This study aimed to evaluate the effects of environmental enrichment on the behavior and physiology of confined mice infected with *Trypanosoma cruzi*. **Methods:** The behaviors of 20 *T. cruzi*-infected mice and 20 non-infected mice were recorded during three treatments: baseline, enrichment, and post-enrichment. Behavioral data were collected using scan sampling with instantaneous recording of behavior every 30s, totaling 360h. Plasma TNF, CCL2, and IL-10 levels and parasitemia were also evaluated in infected enriched/non-enriched mice. Behavioral data were evaluated by Friedman's test and physiological data by one-way ANOVA and area under the curve (AUC) analysis. **Results:** Results showed that environmental enrichment significantly increased exploratory behaviors and diminished inactivity. The use of environmental enrichment did not diminish circulating levels of TNF and IL-10 but diminished circulating levels of CCL2 and parasitemia. **Conclusions:** Positive behavioral and physiological effects of environmental enrichment were observed in mice living in enriched cages. Thus, environmental enrichment improved the welfare of these animals.

Keywords: Chagas disease. Inflammation. Laboratory animals. Welfare.

INTRODUCTION

Stress can be defined as the biological response elicited when an individual perceives a threat to its homeostasis¹. Chronic stress provoked by poor captive environments should be avoided, as it can change animal behavior. Specifically, animals may express more stereotypical abnormal behaviors (e.g. repetitive behaviors that have no apparent function)². Moreover, chronic stress may diminish animals' immune responses, which is associated with the development of pathologies that affect not only physical³ but also emotional status (e.g., increased anxiety and fear)⁴. Since stress can influence the immune system, as well as the physiology and behavior of animals, results originating from studies with animals housed under such conditions can be biased and conclusions wrongly interpreted⁵⁻⁷.

One approach for avoiding, decreasing, or eliminating stress-related problems when researching captive animals is environmental enrichment⁸. Environmental enrichment is a method that involves the introduction of different items into enclosures to stimulate physical and psychological activities that improve animal welfare⁹. In other words, enrichment transforms a monotonous environment into a dynamic, complex, and unpredictable one, resulting in improvements in animal health¹⁰.

Experimental animals used in studies involving infection with pathogenic organisms can exhibit modified behavior in the presence of these infective agents¹¹. If these animals are captive, they can also suffer from a lack of stimuli and the high predictability of their environment^{12,13}. The synergistic effects of parasitism and living in a non-enriched captive environment may rapidly diminish the welfare of the animals¹⁴, increasing the morbidity and mortality rates of the experimentally infected animals¹⁵.

Trypanosoma cruzi is a flagellated protozoan and the causative agent of Chagas disease in humans. It is largely used in experimental infections to study the inflammatory response and chemotherapy. After invasion of mammalian

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hosts, this parasite disseminates through the blood system, promoting systemic and local activation of the immune response and provoking pathologies mainly in the heart and central nervous system¹⁶⁻¹⁹. Experimental mice infected with *T. cruzi* normally exhibit diminished locomotive-explorative behaviors, showing a tendency to run in a circular pattern and displaying a humpback walk¹¹. In this initial phase of infection, the antigenic molecule of *T. cruzi* promotes the activation and release of inflammatory mediators and oxygen and/or nitrogen intermediary radicals^{20,21}. In particular, IFN- γ (gamma interferon) released by T lymphocyte and NK (natural killer) cells is the main cytokine responsible for controlling parasitism during the acute phase and is pivotal in intensifying the activation of phagocyte cells and other immune cells. Meanwhile, interleukin-10 (IL-10) regulates the host immune response^{22,23}. Of similar importance, chemokines are known for their capacity to induce leukocyte migration and activation of immune system cells, driving these cells to parasite infection sites²⁴. The chemokines CCL2 [chemokine (C-C motif) ligand 2] (MCP-1 – monocyte chemoattractant protein-1) and CCL5 [chemokine (C-C motif) ligand 5] (RANTES – Regulated on Activation Normal T cell Expressed and Secreted) are directly linked to lymphocyte migration and control of parasite replication, respectively²⁵. Similarly, the lack of CCR5 (C-C chemokine receptor type 5) eliminates the ability of lymphocytes to migrate to the infection site and control parasite replication²⁶. Thus, the balance between cytokine and chemokine production during this initial phase might determinate the intensity and severity of pathogenesis^{27,28}, affecting the behavior and survival of these animals.

Since environmental enrichment enhances animal welfare, we hypothesize that enriched environment-reared animals will display more locomotive-explorative behaviors and fewer abnormal behaviors compared to animals reared in conventional cages. We also speculate that modulation of the immune response through reductions in TNF (Tumor Necrosis Factor), CCL2, and IL-10 will allow enriched environment-reared animals to better manage *T. cruzi* infection²⁹. Thus, the aim of this study was to evaluate whether different physical environmental enrichment items affected the behavioral and immune responses of mice infected with *T. cruzi*.

METHODS

Animals and maintenance

Forty healthy male C57BL/6 mice aged 9-10 weeks and weighing 16-18g from the Center of Animal Science of the Federal University of Ouro Preto were used in this study.

Ethical considerations

The study was approved by the Animal Ethics Committee of the Federal University of Ouro Preto under license number 2012-60.

Mice were kept in standard autoclavable polypropylene boxes (30.3 × 19.3 × 12.6cm; Beira-Mar®) with a stainless steel lid, arranged on conventional shelves, placed in a room with a

controlled temperature (22°C), and submitted to a 12 light/12 dark cycle (white light on from 07:00 to 19:00; acclimation period: 4 weeks). Food pellets (Nuvilab CR1®) and a bottle of filtered water were provided *ad libitum*. Autoclaved sawdust (Oeste®) was used as bedding and changed twice a week. Five mice were housed together in each box. All boxes were autoclaved before use.

Mice were infected with 5,000 blood trypomastigotes of the VL-10 (Virgem da Lapa) *T. cruzi* strain, which belongs to the TcII discrete typing unit (DTU)³⁰ and presents cardiac tropism and slight fibrosing inflammation in C57BL/6 mice³¹. Blood was diluted in 0.9% saline solution to adjust the concentration, and it was injected into mice intraperitoneally. Infected and non-infected animals were exposed to either standard or enriched housing environment (n = 10 animals in each group). After experiments, mice were euthanized in a CO₂ chamber (10L/min of CO₂)³², and hearts were collected and weighed.

Experimental protocol

Three structural enrichment items were used in this study: PVC (polyvinyl chloride) pipes, egg boxes, and straw nests. The PVC pipe was cleaned every week with 70% alcohol and reused whenever needed. Straw nests and egg boxes were autoclaved and exchanged twice a week. The order of items for testing was defined following the Latin square design, and all items were provided separately.

The study was divided into three treatments: 1) baseline, in which data were collected before the introduction of the environmental enrichment items; 2) enrichment, in which data were collected when the enrichment items were offered; 3) post-enrichment, in which data were collected after the removal of the enrichment items when the conditions returned to those of baseline treatment. Thirty hours of behavioral data distributed over six weeks were collected for each treatment (10h for each enrichment item during enrichment treatment). Data were collected from Monday to Friday, with 1-week intervals between treatments.

Behavioral data were collected using scan sampling with instantaneous recording of behaviors every 30s³³. Each behavioral sampling lasted 30 min, totaling 360h of behavioral data; samplings occurred from 12:00 to 12:30 and from 16:00 to 16:30, since mice were most active during these hours based on 20h of preliminary observations (**Figure 1A**). All data were collected with the researcher 1m from the boxes to avoid researcher interference in mouse behavior.

Ethogram

An ethogram for mice was built based on 20h of preliminary observation using an *ad libitum* recording method³³ and consultation with the scientific literature³⁴ (**Table 1**).

Parasitemia

Comparisons between the number of circulating parasites in blood samples of enriched and non-enriched mice were performed. For this, blood was collected from another 20 male C57BL/6 mice aged 9-10 weeks and weighing 16-18g from the Center of Animal Science of the Federal University of

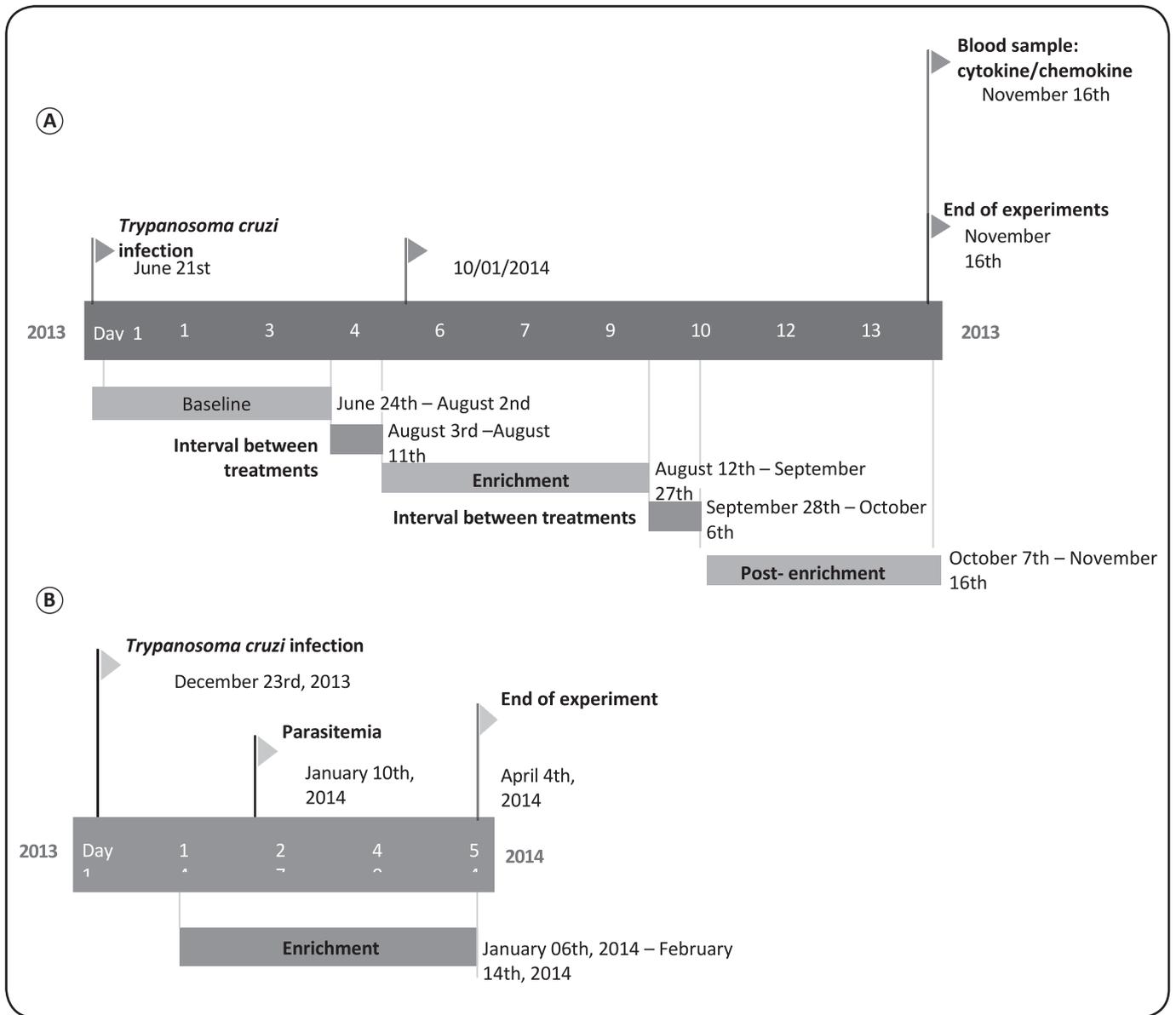


FIGURE 1 - Graphic timeline for the recording of behavioral and physiological data of C57BL/6 mice. A. Timeline for behavioral and physiological data recording. B. Timeline for parasitemia data recording.

TABLE 1
Ethogram for the behaviors recorded for C57BL/6 mice.

Behavior	Acronym	Description
Allogrooming	AG	Mouse cleans the fur of another mouse.
Grooming	GRO	Mouse cleans its own fur.
Exploration	EXP	Mouse explores the box, sniffing and walking, without exploring the roof of the box.
Roof exploration	EXR	Mouse explores the roof of the box, sniffing and hanging on lid.
Inactivity	IN	Mouse stands still (inactive) or sleeps.
Negative social interaction	IS-	Mouse intimidates, attacks, or bites another mouse, mutilating it.
Abnormal behaviors	ABN	Mouse bites the lid or jumps in a repetitive or stereotypical way or exhibits negative allogrooming of another mouse (when the grooming is so intense and repetitive that the fur of the groomed mouse is plucked).
Not visible	NV	Mouse not visible.

AG: allogrooming, **GRO:** grooming, **EXP:** exploration; **EXR:** roof exploration; **IN:** inactivity; **IS-:** negative social interaction; **ABN:** abnormal behaviors; **NV:** not visible.

Ouro Preto. These mice were maintained under the same conditions as the other animals (acclimation period: 4 weeks). New animals were required since the peak of parasitemia occurred 27 days after infection; in the original group, this peak occurred before the enrichment treatment, preventing assessment of parasitemia³⁵. Parasitemia analysis was initiated 19 days after infection of mice with *T. cruzi*, according to Brener's technique³⁶; this was 4 days after the implementation of environmental enrichment. Parasitemia was evaluated during enrichment treatment only, and the behaviors displayed by mice were grouped for analyses (**Figure 1B**). Blood (5 μ L) extracted from each mouse's caudal vein was placed on a microscope slide with a 22 \times 22mm glass slide coverslip. Microscope slides were analyzed under a 4,000-factor microscope at 40 \times magnification. The slides were divided into 50 random fields for parasite counts. After counting, the number of parasites was multiplied by the microscope's factor to achieve the true parasitemia value. Parasitemia curves for enriched and non-enriched mice were compared.

Inflammatory mediators

Plasma samples from mice collected from the orbital venous sinus were also used to assess circulating levels of monocyte chemoattractant protein (CCL2) (Mouse CCL2/JE/MCP-1 DuoSet Elisa Kit, R&D Systems, MN, USA), IL-10 (Mouse interleukin 10, IL-10 Elisa kit, BioSource, CA, USA), and TNF (Mouse TNF-alpha quantitative Elisa kit, R&D Systems, MN, USA). All procedures followed manufacturer protocols. Blood samples for cytokine/chemokine analysis were collected from subject mice at 14:00 on the last day of the post-enrichment treatment as a terminal procedure, 148 days after infection by *T. cruzi*. All samples were measured simultaneously in duplicate.

Statistical analysis

Behavioral data did not follow a normal distribution according to the Anderson-Darling normality test; thus, non-parametric statistics were used throughout. The Friedman test with Dunn's post-hoc analysis was used to evaluate behavioral differences among the three treatments and the three environmental enrichment items; Bonferroni correction was applied, and thus significant values were achieved at $\alpha = 0.02$. A Mann-Whitney test was used to evaluate behavioral differences between infected and non-infected mice and between enriched and non-enriched mice (for enriched mice, behavioral data collected for all three environmental enrichment items were pooled and analyzed together). These analyses were run with a confidence level of 95% ($\alpha = 0.05$) using Minitab 16 and Prisma 5.0³⁷.

Physiological data were normally distributed, according to the Anderson-Darling normality test; thus, parametric tests were used throughout. One-way ANOVA was used to evaluate differences in inflammatory mediators and heart weight among the experimental groups. An independent sample *t*-test was used to evaluate differences in parasitemia in the experimental groups. Areas under the curve (AUCs) were calculated to determine if numbers of blood parasites differed between enriched and non-enriched mice. The AUC was derived from parasite concentration and time, measuring how long parasites

remain in mouse blood. All tests were run with a confidence level of 95% ($\alpha = 0.05$) using Minitab 16 and Prisma 5.0³⁷.

RESULTS

Behavioral data

Mouse behavior was significantly affected by enrichment treatment. Inactivity was significantly more common in the non-enriched group than in the enriched group, both for infected and non-infected mice (**Figure 2 A, B**). In addition, both infected and non-infected mice showed an increase in cage exploration during enrichment treatment, but this increase was only significant for the non-infected group (**Table 2**). Invisibility increased during enrichment treatment in both groups, while roof exploration decreased during enrichment treatment only in the infected group (**Table 2**). Grooming was less frequent in both the non-infected and infected groups during enrichment treatment (**Table 2**).

Allogrooming occurred at different rates in the enriched and non-enriched groups. Among enriched mice, it was more commonly performed in the infected group than in the non-infected group, while among non-enriched mice, allogrooming was more common among non-infected than infected mice (**Figure 2 C, D**). Allogrooming was significantly more common in the non-enriched group, both among infected and non-infected mice (**Figure 2 C, D**). Negative social interactions were also exhibited significantly more frequently by the non-enriched group, both among infected and non-infected mice (**Figure 2 E**).

Abnormal behaviors in infected mice did not significantly differ between the enriched and non-enriched groups, although they were recorded more often in the non-enriched group (**Figure 2 F**). Among non-infected mice, the expression of abnormal behaviors was significantly lower in the enriched group than in the non-enriched group (**Figure 2 F**). Abnormal behaviors diminished post-enrichment only in the infected group (**Table 2**).

In comparing the three enrichment treatments, grooming occurred more frequently when the PVC pipe was used for enrichment and less frequently when the egg box was used in both infected and non-infected groups [infected: $F = 20.68$, $p < 0.01$; non-infected: $F = 18.00$, $p < 0.01$; $n = 20$, degrees of freedom (DF) = 2 for both tests]. Infected mice became more inactive when the straw nest was offered, and non-infected mice became more inactive when the PVC pipe was offered (infected: $F = 19.30$, $p < 0.01$; non-infected: $F = 21.19$, $p < 0.01$; $n = 20$, DF = 2 for both tests). The category *not visible* was recorded more frequently when the egg box was offered in both groups (infected: $F = 30.10$, $p < 0.01$; non-infected: $F = 30.10$, $p < 0.01$; $n = 20$, DF = 2 for both tests).

Physiological data

For most days, the parasitemia curves of enriched mice were lower than those of non-enriched mice, but this was only significant on day 33 (*t*-test = 2.1, $p = 0.04$, DF = 18). The numbers of parasites in the enriched mice were significantly lower than those of the non-enriched mice (AUC enriched: 2,910; AUC non-enriched: 3,089).

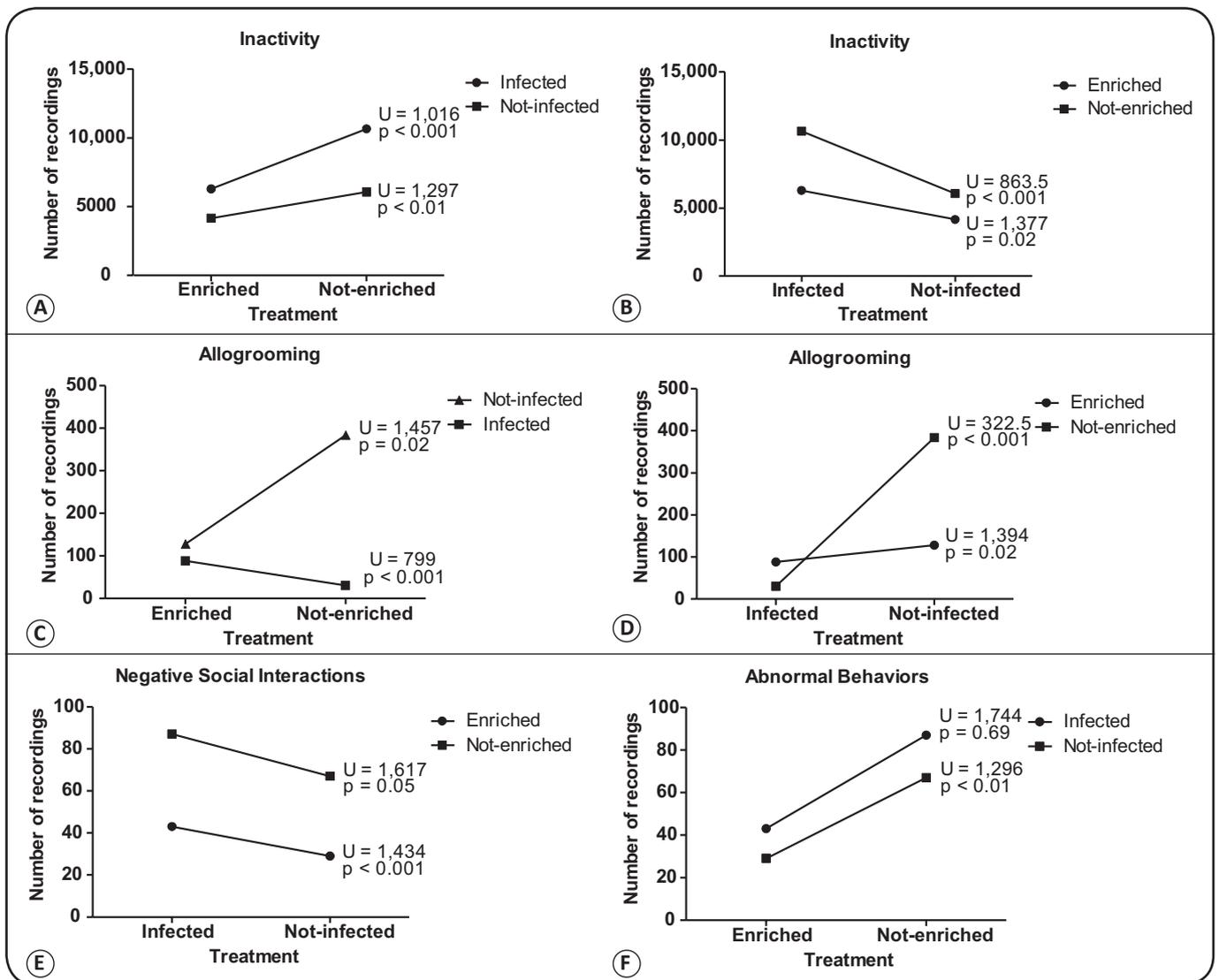


FIGURE 2 - Behavioral comparisons of the effects of *Trypanosoma cruzi* infection on the enriched and non-enriched groups and between the effects of enrichment on the infected and non-infected groups. U: Mann-Whitney test, n = 40, DF = 2.

Infected mice produced more TNF than non-infected mice, especially under non-enrichment conditions (Figure 3A). Generally, enriched mice produced less TNF than non-enriched mice in both infected and non-infected groups; however, these differences were not statistically significant (Figure 3A). Environmental enrichment decreased the production of CCL2 both in infected and non-infected mice (Figure 3B). While no significant differences were found among groups in terms of IL-10 production, there was slightly reduced production of this cytokine in the enriched groups (Figure 3C).

Finally, no differences were found in heart weight among the groups ($F = 1.39$, $p = 0.26$, $n = 10$, $DF = 3$), with an average weight (\pm SD) of $5.2 \times 10^{-3} \pm 2.3 \times 10^{-4}$ in infected and enriched mice, $5.4 \times 10^{-3} \pm 5.4 \times 10^{-4}$ in infected and non-enriched mice, $4.0 \times 10^{-3} \pm 6.2 \times 10^{-4}$ in non-infected and enriched mice, and $4.0 \times 10^{-3} \pm 6.2 \times 10^{-4}$ in non-infected and non-enriched mice.

DISCUSSION

In this study, we found that environmental enrichment decreased inactivity in both *T. cruzi*-infected and non-infected mice and decreased roof exploration in the infected group. The non-infected group showed an increase in cage exploration and a reduction in grooming. Abnormal behaviors decreased only for the infected group and only in the post-enrichment period. Similar increases in activity and decreases in inactivity have been observed in many studies of environmental enrichment^{8,38-40}. With an increase in stimulation inside the cage, mice have more opportunities to explore items, resulting in more activity and less inactivity. Increased activity levels benefit captive animals by helping them avoid boredom, a symptom normally seen in non-enriched environments⁸. However, enriched mice can spend more time interacting with the enrichment items than moving through the cage⁴¹. Notably, one study showed that mice kept

TABLE 2

Comparisons between the behaviors expressed by the infected and non-infected C57BL/6 mice during the baseline, enrichment, and post-enrichment treatments (mean ± standard error).

Behaviors	Treatments			Friedman statistic	p-value
	baseline	enrichment	post-enrichment		
GRO EI	31.08 ± 1.67	25.13 ± 2.47	44.13 ± 3.12	5.70	0.06
GRO ENI	36.10 ± 2.69 ^a	19.52 ± 2.05 ^b	37.12 ± 2.61 ^{a,c}	28.46	< 0.0001*
EXP EI	24.77 ± 2.99	29.50 ± 4.49	20.78 ± 3.31	1.32	0.52
EXP ENI	25.53 ± 2.15 ^a	53.28 ± 5.55 ^b	54.00 ± 6.34 ^{b,c}	27.58	< 0.0001*
EXR EI	14.00 ± 2.35 ^a	6.58 ± 0.51 ^b	3.93 ± 0.96 ^{b,c}	22.34	< 0.0001*
EXR ENI	10.47 ± 1.18	11.57 ± 1.66	8.13 ± 1.38	4.10	0.13
IS- EI	0.08 ± 0.05	0.13 ± 0.09	0.00 ± 0.00	2.00	0.37
IS- ENI	0.02 ± 0.02	0.75 ± 0.28	0.07 ± 0.05	7.82	0.02*
IN EI	169.70 ± 8.21 ^a	104.70 ± 12.85 ^b	201.80 ± 8.80 ^{a,c}	26.53	< 0.0001*
IN ENI	175.30 ± 7.32 ^a	69.18 ± 10.06 ^b	141.50 ± 9.28 ^c	46.02	< 0.0001*
ABN EI	0.57 ± 0.18 ^a	0.73 ± 0.33 ^a	0.03 ± 0.03 ^b	8.000	0.02*
ABN ENI	0.52 ± 0.24	0.43 ± 0.16	0.40 ± 0.16	0.1600	0.92
NV EI	8.53 ± 1.04 ^a	88.28 ± 14.30 ^{a,b}	1.97 ± 0.51 ^c	59.23	< 0.0001*
NV ENI	9.10 ± 1.30 ^a	79.57 ± 11.91 ^b	1.15 ± 0.49 ^c	63.17	< 0.0001*

GRO: grooming; **EXP:** exploration; **EXR:** roof exploration; **IS-:** negative social interaction; **IN:** inactive; **ABN:** abnormal behaviors; **NV:** not visible; **EI:** enriched and infected; **ENI:** enriched and non-infected. ^{a,b,c} Different superscript letters indicate statistical differences. *Significant values after Bonferroni correction.

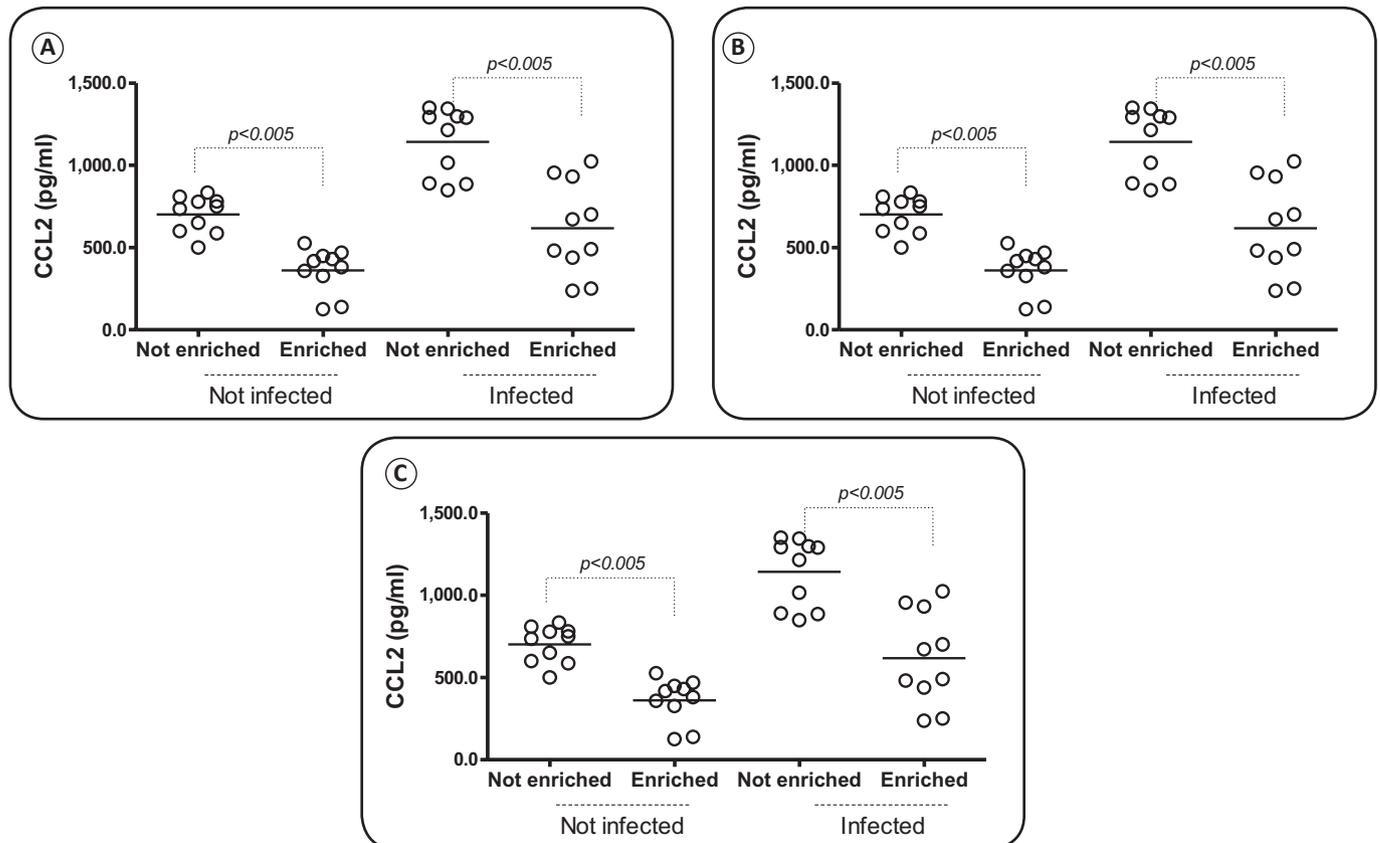


FIGURE 3 - Comparisons of the production of inflammatory mediators in infected and non-infected and enriched and non-enriched groups. (A): TNF (Tumor Necrosis Factor). (B): CCL2 [chemokine (C-C motif) ligand 2]. (C): IL-10 (interleukin-10).

in non-enriched cages presented higher activity levels than mice kept in enriched cages⁴². The authors argued that mice explore cages more when no novelty is provided or maintain higher activity levels due to the impossibility of building nests or sleeping or due to increased attempts to escape from the cage. In the present study, when an item was available, mice explored the item more than an empty cage. However, infected mice showed only a slight increase in activity, and this could be a direct reflection of infection with *T. cruzi*, as this parasite increases lethargy in animals⁴³.

Environmental enrichment techniques are known to diminish the expression of abnormal behaviors in many animal species^{8,38,44-47}. In the present study, abnormal behaviors did not decrease significantly during enrichment treatment in infected groups, showing that the items used may not be adequate for this. A significant reduction in the expression of abnormal behaviors during the post-enrichment period in the infected group, however, showed that the benefits of environmental enrichment can be enduring. For the non-infected groups, environmental enrichment decreased the expression of abnormal behaviors.

Egg boxes elicited more activity from mice than the other enrichment items and could also be used as hiding places. According to the Royal Society for the Prevention of Cruelty to Animals⁴⁸, hiding places are crucial for the maintenance of rodents in laboratories and captivity, increasing their welfare by making them feel safe. The mice in the present study used the egg boxes to hide, reducing the visibility of mice more than the other enrichment items. PVC pipes are cold, poorly ventilated items that are difficult to manipulate, and these characteristics may have induced the mice to avoid them³⁸. Straw nests and egg boxes, in contrast, are well-ventilated and more easily manipulated items, permitting all individuals to use them concomitantly, and these characteristics may have been more attractive to animals.

The increase in social interactions in the infected and enriched group suggests a positive influence of environmental enrichment in decreasing the depressive profile generated by the parasite *T. cruzi*^{11,49}. The non-enriched non-infected group exhibited higher levels of allogrooming, corroborating the results of a study⁴² in which mice kept in conventional environments expressed more allogrooming behaviors than mice kept in enriched environments. Similarly, we found that overall, infected mice exhibited less allogrooming than non-infected mice. A further reduction in allogrooming in infected and enriched mice was due to the use of the enrichment items by the animals.

Negative social interactions were recorded significantly less frequently in infected mice than in non-infected mice. Infected animals were less aggressive to other individuals, and this was probably due to their depressive profiles¹¹. These animals initiated negative social interactions but soon gave up the confrontations, remaining immobile and inactive, which was also previously reported⁴³. Some studies showed that enriched C57BL/6 mice exhibited more aggressive behaviors than non-enriched mice and concluded that environmental enrichment

may exacerbate welfare problems^{50,51,52}. However, since the expression of negative social interactions did not increase during enrichment treatment in our study, we conclude that the use of enriched environments does not increase stress or aggression⁴⁹.

Trypanosoma cruzi infection alters the immune system of the host, increasing the concentrations of the cytokine TNF and chemokine CCL2^{53,54}. While environmental enrichment significantly decreased the production of CCL2, TNF concentrations were not significantly altered, although they did decrease in the enriched groups; both are inflammatory mediators that recruit and activate cells to the inflammatory site²⁰. IL-10 did not decrease with enrichment, which was expected since expression of this cytokine normally changes only when it is necessary to regulate the inflammatory response²¹. The regulatory cytokine IL-10 acts mainly to control the parasite in the acute phase of infection, inhibiting the action of TNF, which intensifies the inflammatory process⁵⁵. Thus, it is assumed that the presence of TNF at the beginning stage of infection is essential for controlling parasite infection, but its intensification and persistence during longer periods of infection would prejudice the host due to the intensification of the tecidual inflammatory response and consequent functional organ sequel⁵⁶. By decreasing the TNF concentration, environmental enrichment enhanced the immune responses of the mice⁵⁶.

The chemokine CCL2 promotes the attraction of inflammatory cells to inflammatory sites, and together with TNF, it is responsible for the myocarditis induced by parasitism¹⁷. However, in this study, environmental enrichment did not alter TNF production in infected animals. In contrast, via an unknown pathway, the chemokine CCL2 was reduced in infected mice under environmental enrichment. This pattern is, at least, a precondition for reducing the inflammation in tissues.

The parasitic loads of the enriched mice were also lower than those in the non-enriched mice, showing that environmental enrichment allowed animals to better cope with the parasites by increasing their welfare. Mice infected with *Babesia microti* and exposed to environmental enrichment took longer to reach a peak of parasitemia, probably due to changes in behavioral, hormonal, and immunocompetence parameters⁴⁹. Environmental enrichment, in this case, promoted more resistance to the parasite. Similar results were found for turkeys (*Meleagris gallopavo*)⁵⁸. The authors found a detrimental effect of environmental enrichment offered during the first two weeks of life in turkeys, showing that they become more susceptible to bacterial infections later in life. In contrast, two studies found beneficial effects of environmental enrichment on mice infected with encephalitis and influenza virus^{57,59}. Finally, environmental enrichment does not compromise the immune response in mice chronically infected with *Mycobacterium avium*⁶⁰. These studies highlight the importance of evaluating different physiological and behavioral parameters to better understand the effects of environmental enrichment on animals infected with parasites.

In conclusion, environmental enrichment improved the behavioral profile of *T. cruzi*-infected mice, reducing inactivity and increasing cage exploration. Although the expression

of abnormal behaviors decreased in the infected group during enrichment treatment, the items used were unable to stimulate mice to eliminate or significantly reduce these behaviors. Environmental enrichment also improved the physiological profile of *T. cruzi*-infected mice, reducing the production of some inflammatory mediators and parasitemia. Thus, enriched mice should be used preferentially in parasitological studies, as it enhances the quality of the results. This should facilitate the development of better treatments for diseases, consequently benefitting public health.

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Conflict of interest

The authors declare that have no conflicts of interest.

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