

BEHAVIORAL AND MEMORY CHANGES IN *Mus musculus* COINFECTED BY *Toxocara canis* AND *Toxoplasma gondii*

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SUMMARY

Several researchers have stated that parasites can alter the behavior of their hosts, in order to increase the transmission rate, principally when prey-predator relationships are a reliable way of infection transmission. The aim of this study was to verify the occurrence of changes in anxiety and short-term memory patterns in experimentally infected *Mus musculus* by *Toxocara canis* and/or *Toxoplasma gondii*. Forty male *Mus musculus* (Balb/c) eight-week-old were divided into four groups of 10 mice each. One group was infected with 300 eggs of *Toxocara canis*; a second group was submitted to infection with 10 cysts of *Toxoplasma gondii*; a third group was concomitantly infected with both parasites with the same inoculums and the last group was maintained without infection. The anxiety levels were evaluated using an elevated plus maze and an actometer; the short-term memory was determined by a two-way active avoidance equipment. The determination of anxiety levels were conducted 40 and 70 days after infection and the short-term memory was evaluated 140 days after infection. Mice chronically infected by *Toxoplasma gondii* showed impaired learning and short-term memory, but no significant differences were found in mice infected by *Toxocara canis* or concomitantly infected by *Toxocara canis* and *Toxoplasma gondii* when compared to non infected mice.

KEYWORDS: *Mus musculus*; *Toxocara canis*; *Toxoplasma gondii*; Concomitant infections; Behavior alterations.

INTRODUCTION

According to the manipulation hypothesis, a parasite can alter the behavior of their hosts specifically to increase the transmission. This hypothesis requires that the change of behavior is a sophisticated product resulting in the parasite manipulation of the host, rather than a by-product of other physiological activities of the parasite^{3,32}.

There are many examples in the literature of behavioral changes in insects, crustaceans, and fish acting as intermediate hosts in the life cycle of several species of parasites; however, little is known about behavioral changes in mammals^{1-3,5,21,36}.

Some studies show evidence of behavioral changes in rodents in experimental protocols with single infections; however, in natural conditions, the occurrence of co-infection or multiple parasite infections of the same host should be common^{4,8,26,33,35,40,42}.

Toxoplasma gondii is a protozoan parasite, whose definitive hosts are felines, but other warm-blooded vertebrates may act as intermediate hosts. *Toxocara canis* is a nematode parasite of dogs that eventually infects small mammals, which do not reach maturity. These mammals act as paratenic hosts, because they maintain the larvae in their organs

for a long time and have an important role in the transmission of the parasite through the predator-prey relationship. Both parasites can cross the blood-brain barrier, settling in areas of the central nervous system of rodents related to the control of anxiety and locomotion^{9,16,22}.

QUEIROZ *et al.*³³ investigated the behavior of *Rattus norvegicus* infected by *Toxocara canis* and/or *Toxoplasma gondii*, and concluded that both parasites influenced rodent behavior. However, when the rats were concomitantly infected, a behavior modulation was observed, resulting in slight absence of behavioral alterations.

Due to the high frequency of *Mus musculus* infection by both parasites in natural conditions and the importance of these rodents as paratenic hosts, the aim of the present study was to verify the occurrence of changes in anxiety and short-term memory patterns in experimentally infected *Mus musculus* by *Toxocara canis* and/or *Toxoplasma gondii*.

MATERIALS AND METHODS

Forty male *Mus musculus* (Balb/c) eight-week-old were obtained from the Central Animal Laboratory of the Faculty of Medicine, University of São Paulo. The eggs of *T. canis* were obtained by dissecting female worms, recovered from naturally infected dogs captured by the Center for Zoonosis

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Control of Guarulhos (CCZ/GRU). The cysts of *T. gondii* (ME49 strain cystogenic referring to genotype II) were provided by the Laboratory of Protozoology of the Institute of Tropical Medicine of São Paulo.

The mice were divided into four groups, namely, *Toxocara*: 10 mice infected with 300 eggs of *Toxocara canis*, *Toxoplasma*: 10 mice infected with 10 cysts of *Toxoplasma gondii*; Concomitant infection: 10 mice infected with 300 eggs of *T. canis* and 10 cysts of *T. gondii*, and Control: 10 mice without infection.

The behavior of the mice was assessed by testing in the elevated plus maze, to determine the levels of anxiety, using the technique described by PELLOW & FILE³⁰. The motor activity, another behavioral parameter, was measured using the technique of determination of motor activity in the open field, using an Actometer, as described by SILVA *et al.*³⁴, NASELLO *et al.*²⁹ and GUARALDO *et al.*¹⁸.

The performance evaluation was conducted on two occasions: 40 and 70 days after infection.

To assess learning and memory consolidation, a two-way active avoidance equipment (Ugo Basile, Comerio, Italy) was used on two occasions, as described by KORTE & DE BOER²⁸, 140 days after infection.

At the end of the experiment, all the rats were euthanized and the carcasses were submitted to digestion with HCl 0.5% (XI & JIN, 1998)⁴⁴ and the central nervous system was macerated in saline solution 0.9% for the recovery of *Toxocara canis* larvae and *Toxoplasma gondii* cysts, respectively.

The data were expressed as the mean \pm standard deviation. Statistical comparisons were performed using the Two-way ANOVA for the behavioral variables and the t-Student test for the evaluation of short-term memory. Only probability values which were (*p*) smaller than 0.05 were considered as statistically significant.

All care procedures were performed strictly according to the guidelines for animal experimentation, as stipulated in the Guide for the Care and Use of Laboratory Animals (National Institute of Health Publication Number 86-23, Bethesda, MD). The experimental protocol was approved by the Research Ethics Committee on Animal Experiments of the São Paulo Institute of Tropical Medicine (process no. 2011/098).

RESULTS

The analysis of the variables in the elevated plus maze showed a significant difference ($p < 0.05$) between the group infected by *Toxoplasma gondii* and the control group at 40 dpi; on the other hand, the group with concomitant infections did not show any significant difference in comparison to the non-infected control group (Fig. 1).

On evaluation of the frequency of entries into the closed arms of the elevated plus maze, there was a significant difference ($p < 0.05$) between the groups infected with *T. canis* and *T. gondii*, but not with the group with concomitant infections, in comparison to the control group (Fig. 2).

The same variables observed in an Elevated Plus Maze at 70 days

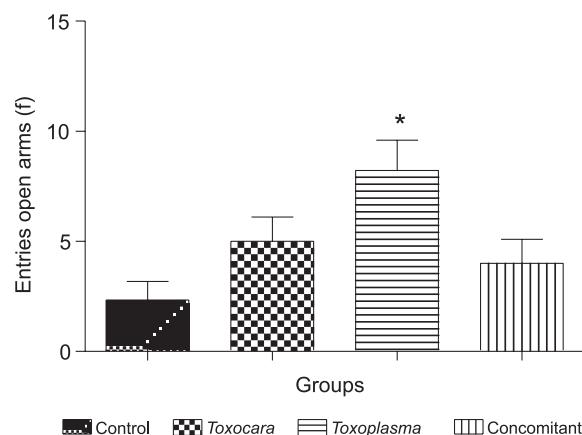


Fig. 1 - Frequency of entries into the open arms of the Elevated Plus Maze *Mus musculus* infected with *Toxocara canis* and/or *Toxoplasma gondii* at 40 dpi. * $p < 0.05$ related to the control.

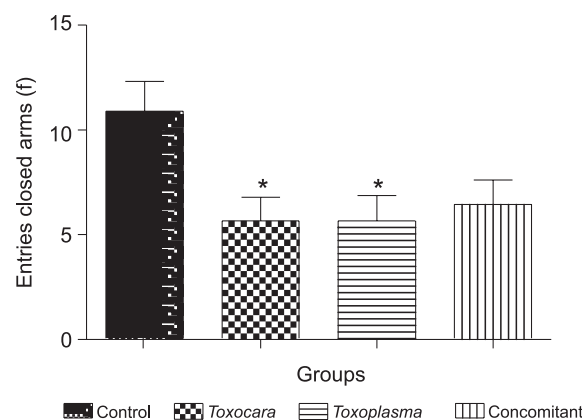


Fig. 2 - Frequency of entries into the closed arms of the Elevated Plus Maze *Mus musculus* infected with *Toxocara canis* and/or *Toxoplasma gondii* at 40 dpi. * $p < 0.05$ related to the control.

post-infection showed no significant difference among all the groups.

In aversive avoidance, there was no significant difference between the groups, however, only the group infected with *T. gondii* showed a difference between the first and second test (Fig. 3).

All mice of the infected groups showed, at least, a *Toxocara canis* larvae and/or *Toxoplasma gondii* cyst into the brain.

DISCUSSION

Since the 70's, interest from researchers has been increasing on the behavioral changes shown by infected rodents^{4,8,15,19,25,27}.

Several studies show changes in the behavior of rodents infected with *Toxocara canis* and *Toxoplasma gondii*. These changes can probably be considered as a means of facilitating the transmission of both parasites to their paratenic hosts by behavioral manipulation^{4,7,10,12-14,20,39}.

Mus musculus plays an important role in the life cycle of *Toxoplasma gondii* and *Toxocara canis*, because it can harbor cysts and larvae,

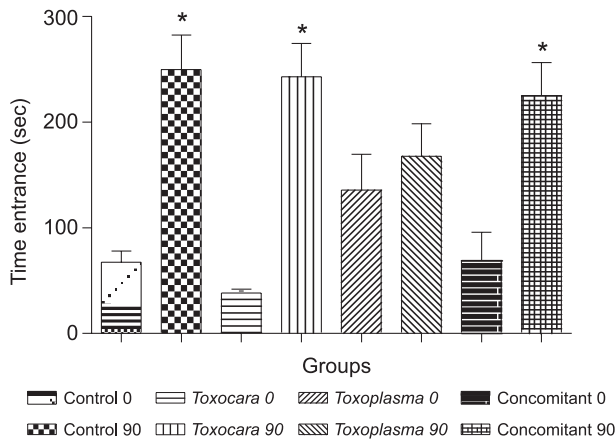


Fig. 3 - Time difference between training and test *Mus musculus* infected with *Toxocara canis* and/or *Toxoplasma gondii* in aversive avoidance test. * = difference between training and control test, *T. canis*, *T. gondii* and concomitant groups. $p < 0.05$.

respectively, in the muscles and other organs, like the central nervous system, for a long time. Moreover, these rodents are part of the food chain of definitive hosts of both parasites^{23,41} and can be transmitted to their definitive host through prey-predator relationships.

COX & HOLLAND¹¹ suggest that the decreased levels of aggression linked to decreased levels of anxiety and lack of inhibition to open environments increases the risk of predation of these rodents and, consequently, could facilitate the transmission of parasites through a prey-predator relationship, a possible way of *Toxocara canis* and *Toxoplasma gondii* transmission, respectively, to dogs and cats.

In the present study, mice infected with *T. gondii* evaluated in the elevated plus maze were less anxious due to higher input frequency in the open arms. However, the same was neither observed in the group infected with *T. canis*, nor in the group concomitantly infected with both parasites. One hypothesis regarding the lack of significance in the behavioral data of the group infected with *T. canis* is the quantity of eggs used for the mice infection. However, QUEIROZ *et al.*³³ had already found similar results in *Rattus norvegicus* concomitantly infected by *Toxocara canis* and *Toxoplasma gondii*, suggesting occurrence of a modulation in behavioral changes when rats were concomitantly infected. On the other hand, COX & HOLLAND^{12,13} found that mice with large amount of *T. canis* larvae in the brain (inoculum of 3,000, embryonated) present increased behavioral alterations than mice infected by smaller quantities of larvae.

Infection with *T. canis* and *T. gondii* can also influence the memory in rodents²⁴. Several surveys conducted on rodents report the presence of *T. canis* larvae in the telencephalon and cerebellum, and cysts of *T. gondii* distributed in various brain regions, but with a higher incidence in the region of the amygdale, areas related to learning, memory, coordination and control of voluntary movements^{17,35,38}.

In this study, no significant differences were observed in the groups infected with *T. canis* and with concomitant infection when the short-term memory was evaluated by aversive avoidance. However, the animals infected with *T. gondii* showed a significant difference in this test. These results support the hypothesis that animals chronically infected with

T. gondii have impaired learning and memory^{31,43} and reinforces the hypothesis of QUEIROZ *et al.*³³ concerning the occurrence of modulation in the behavioral response when rodents were co-infected by both parasites.

RESUMO

Alterações comportamentais e na memória de *Mus musculus* coinfectado por *Toxocara canis* e *Toxoplasma gondii*

Pesquisadores afirmam que parasitos podem alterar o comportamento de seus hospedeiros a fim de aumentar a sua taxa de transmissão. O objetivo deste estudo foi verificar a ocorrência de alterações na ansiedade e padrões de memória de curta duração em *Mus musculus* experimentalmente infectados por *Toxocara canis* e/ou *Toxoplasma gondii*. Utilizaram-se 40 camundongos da espécie *Mus musculus* machos (Balb/c) com oito semanas de idade, divididos em quatro grupos de 10 ratos cada. Um grupo foi infectado com 300 ovos de *Toxocara canis*, um segundo grupo foi submetido à infecção com 10 cistos de *T. gondii*, um terceiro grupo foi infectado concomitantemente com ambos os parasitas e o último grupo foi mantido sem infecção. Os níveis de ansiedade foram avaliados por meio de labirinto em cruz elevado e actômetro, a memória de curta duração foi determinada por esquiva aversiva. A determinação dos níveis de ansiedade foi realizada 40 e 70 dias após infecção e a memória de curto prazo foi avaliada 140 dias após a infecção. Camundongos cronicamente infectados por *Toxoplasma gondii* mostraram deficiência de aprendizagem e memória de curto prazo, mas não foram encontradas diferenças significantes em camundongos infectados por *Toxocara canis* ou concomitantemente infectados por *Toxocara canis* e *Toxoplasma gondii* quando comparados com camundongos não infectados.

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CONFLICT OF INTEREST STATEMENT

The authors declare that there are no conflicts of interest.

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