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Long-term memory in the parasitoid *Trichogramma telengai* Sorokina, 1987 (Hymenoptera: Trichogrammatidae)

Долговременная память паразитоидов *Trichogramma telengai Sorokina*, 1987 (Hymenoptera: Trichogrammatidae)

M.A. Fedorova[1]*, S.E. Farisenkov[1], E.S. Shcherbakova[1], A.A. Polilov[1]
М.А. Федорова[1]*, С.Э. Фарисенков[1], Е.С. Щербакова[1], А.А. Полилов[1]

[1] Department of Entomology, Faculty of Biology, Lomonosov Moscow State University, Moscow, 119234 Russia.
[1] Кафедра энтомологии, Биологический факультет, Московский государственный университет имени М.В. Ломоносова, Москва, 119234 Россия.

* Corresponding author. E-mail: mariafedorova1997@gmail.com

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КЛЮЧЕВЫЕ СЛОВА: ассоциативное обучение, память, Trichogrammatidae, *Trichogramma telengai*, микронасекомые, миниатюризация.

ABSTRACT. The process of miniaturization poses a challenge for insects as they need to maintain essential cognitive functions despite substantial reductions in body size. Despite having fewer and smaller neurons, *Trichogramma telengai* Sorokina, 1987 displayed the capacity for associative learning and retention of different kinds of memory. Earlier experiments have shown the presence of short-term memory in *T. telengai.* In this study, we observe memory retention for up to 24 hours, indicating the presence of long-term memory. In this experiment, unlike most studies on the cognitive abilities of parasitoids, we used aversive training to high temperatures instead of olfactory stimuli. This allowed us to observe the capacity for different memory consolidation pathways and to compare the memory retention abilities between insects of different groups.

РЕЗЮМЕ. Вследствие миниатюризации перед насекомыми встает задача сохранить жизненно важные функции организма при значительном уменьшении размеров тела. Несмотря на уменьшенное количество и размер нейронов, *Trichogramma telengai* Sorokina, 1987 показывает способность к сохранению разных форм памяти. Предыдущие эксперименты показали наличие кратковременной памяти у паразитоидов *T. telengai*. В настоящем исследовании мы наблюдаем запоминание вплоть до 24 часов, что свидетельствует о наличии долговременной памяти. В отличие от большинства исследований когнитивных способностей паразитоидов, в этом эксперименте использовали обучение не на ольфакторные стимулы, а более универсальное аверсивное обучение на высокие температуры. Это позволило пронаблюдать способность к другим способам консолидации памяти, а также сделало возможным сравнение способностей к сохранению памяти у насекомых из разных групп.

Introduction

The central nervous system (CNS) of miniature parasitoids undergoes several changes typical of microinsects, including a reduction in the number and volume of neurons, and an overall compactization of the CNS [Makarova, Polilov, 2013]. Despite miniaturization, the long-term optimization by natural selection through compensatory mutations prevents fatal changes in the functioning of the nervous system, changes that would otherwise lead to a decline in cognitive abilities [Bolstad *et al*., 2015]. The structural plan of the head ganglia in parasitoid wasps of the genus *Trichogramma* Westwood, 1833 conforms to the general pattern observed in insects [Makarova, Polilov, 2013; Makarova *et al*., 2021]. The brain of *Trichogramma telengai* Sorokina, 1987 comprises approximately 17,000 neurons with an average diameter of 2.26 ± 0.08 mm (mean ± SE) [Makarova *et al*., 2021]. This reduction in size is achieved through a decrease in cytoplasmic volume, resulting in the nucleus occupying 50–60% of the cell [Makarova, Polilov, 2013]. On average, the brain volume of *T. evanescens* Westwood, 1833 measures 0.46 nl, occupying 7.3% of the total body volume [Makarova, Polilov, 2013]. The body size reduction does not affect the ability to retain

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memory, as both large and small individuals of *Trichogramma* exhibited similar results in experiments involving olfactory and visual stimuli [Woude *et al.*, 2018; Fedorova *et al.*, 2023].

Studies on associative learning in parasitoids are typically conducted using olfactory stimuli [Huigens *et al.*, 2009; Kruidhof *et al.*, 2012; Farahani *et al.*, 2014]. Based on olfactory learning, *Trichogramma* demonstrates the capacity to form short-term memory [Woude *et al.*, 2018], anesthesia-resistant memory, and long-term memory lasting up to 24 hours [Huigens *et al.*, 2009; Kruidhof *et al.*, 2012]. The persistence of protein synthesis-dependent long-term memory in parasitoids was proved by feeding *Lariophagus distinguendus* Förster, 1841 (Hymenoptera: Pteromalidae) with actinomycin D, a transcription blocker, which erased the learned response 24 h after the training [Collatz *et al.*, 2006]. Olfactory stimuli are effective due to their high relevance to the insect; however, they are not universal, requiring the individual selection of odorants for each species. The thermal arena used in our study is applicable to any miniature arthropod species, allowing for the comparison of their learning rates and the duration of memory retention [Fedorova *et al.*, 2022, 2023].

This study continues a series of research on the topic of associative learning in microinsects [Fedorova *et al.*, 2022, 2023]. Previously, the ability of *T. telengai* for associative learning and memory retention for up to 6 hours after training were demonstrated.

Material and methods

Insects

We studied adults of the wasp *Trichogramma telengai* Sorokina, 1987 (Hymenoptera: Trichogrammatidae), a widely distributed egg parasitoid of moths [Sorokina, 1987]. These insects have a body weight of approximately 6.69 ± 0.84 µg (mean ± SE). *Trichogramma* used in the experiments was reared on the eggs of the Angoumois grain moth, *Sitotroga cerealella* Olivier, 1789 (Lepidoptera: Gelechiidae). The insects were kept at 25 °C and a photoperiod of 12 : 12 h. In the experiments, only individuals no older than 3 days were used. Between memory retention tests, the insects were placed in separate vials with a thread soaked in a sugar solution.

To conduct associative learning and memory tests, a universal thermal arena for microinsects was used. It was extensively described in previous studies dedicated to the cognitive abilities of thrips and parasitoid wasps [Fedorova *et al.*, 2022, 2023]. The tested insect was placed on the arena, which was heated to 37 ± 0.5 °C, a temperature uncomfortably high for it. The insect had to locate a cool spot that had a comfortable temperature of 25 °C. An LED screen surrounded the arena. The pattern displayed on the LED screen consisted of vertical stripes, forming the target pattern, and horizontal stripes providing uniform lighting. In the experiments with the test group, the target pattern coincided with the spot of comfortable temperature, allowing the insect to learn to locate that spot based on the pattern displayed on the screen. In the experiments with the control group, the target pattern and the spot of comfortable temperature switched randomly, so the association between them could not form.

Training procedure

Each insect was placed onto the arena and underwent preliminary testing (T_p) to reveal whether or not it is attracted to any spot or pattern initially. The first training session, comprising 10 cycles, was then conducted. Each cycle included three stages: at first an insect searched a cool spot, when found, it spent 1 minute in this spot storing the memory about the visual stimulus, after that a researcher shifted the position of the cooler spot and a pattern on the screen.

Immediately after the first training session, the first memory test (T_0) was conducted to determine whether learning had occurred. Exactly 1 h after the first training session, the second session, consisting of seven cycles, was conducted. The second training session was aimed at obtaining better memory consolidation. After 24 h, the second memory test (T_{24}) was performed.

The experiment was conducted on a total of 58 specimens (37 in the test group and 21 in the control group), of which 41 specimens participated in the final memory test after 24 hours (22 in the test group and 19 in the control group). Individuals unable to locate the cold spot within 5 minutes or to complete all search cycles were excluded.

Data acquisition and analysis

The movement of the insects was recorded using a Moticam 3 digital camera. Coordinates of movement trajectories were obtained using the Tracker 5.0.5 software (https://physlets.org/tracker). Based on these coordinates, the time spent in each of the four sectors was calculated.

The analysis involved examining the distribution of time spent by the insects in each sector. Two criteria were used for data comparison: the percentage of time spent in the target sector (a quarter of the arena corresponding to the target pattern on the screen) and the learning index (the ratio of the difference between the time spent in the target sector and the time spent in the opposite sector to the total time in these two sectors) in the tests T_p, T_0, and T_{24}. The learning index helps to focus on the data related to the target and opposite sectors while discarding information about time spent in neighboring sectors. Sectors do not have exact boundaries on the arena, and it is difficult for miniature insects with low-resolution vision to navigate with great accuracy. Therefore, contrasting the two preceding sectors allows to emphasize more important values and helps to minimize small errors in orientation. This approach is widely used in similar studies, for example with *Drosophila melanogaster* Meigen, 1830 [Ofstad *et al.*, 2011]. Statistical analysis was performed using STATISTICA 12, including t-tests for data analysis.

Results

In the preliminary test (T_p), no significant differences were observed either in terms of the percentage of time spent in the target sector (t-test, df = 49, F = 1.152, t = −0.380, p = 0.706) (Fig. 1a) or in the learning index (t-test, df = 49, F = 2.014, t = 0.175, p = 0.862) (Fig. 1b).

In T_0, both percentage of time spent in the target sector (t-test, df = 61, F = 1.274, t = 2.613, p = 0.011) (Fig. 1a) and learning index (t-test, df = 61, F = 1.587, t = 2.943, p = 0.005) (Fig. 1b) were greater in the test group.

In T_{24} this tendency persisted. The test and the control groups differed significantly both in the percentage of time spent in the target sector (t-test, df = 39, F = 1.030, t = 2.119, p = 0.040) (Fig. 1a) and in the learning index (t-test, df = 39, F = 1.020, t = 2.910, p = 0.006) (Fig. 1b).

In the test group, the time spent in the target sector (t-test, df = 79, F = 1.416, t = 3.436, p = 0.001) (Fig. 1a)

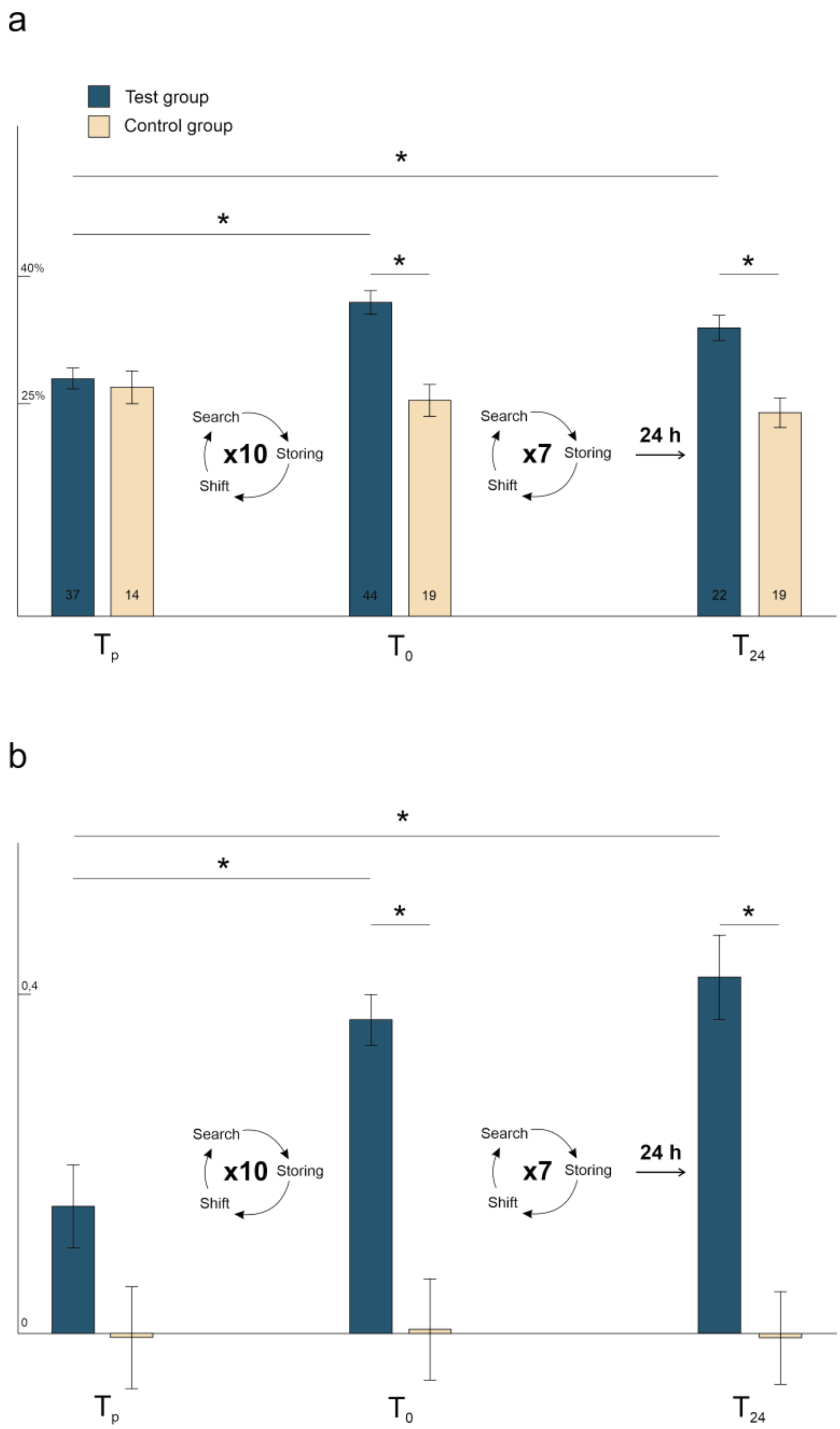

Fig. 1. The performance of *T. telengai* in each of three tests: a — the percentage (%) of time spent by *T. telengai* in the target sector in each of the three tests (M ± SE), *t*-test: $*p < 0.05$. b — the learning index in each of the three tests (M ± SE), *t*-test: $*p < 0.05$. The column headers indicate the sample sizes.

Рис. 1. Показатели перемещения *T. telengai* в каждом из трех тестов: a — процент (%) времени, проведенного *T. telengai* в целевом секторе в каждом из трех тестов (M ± SE), *t*-test: $*p < 0.05$. b — индекс обучения в каждом из трех тестов (M ± SE), *t*-test: $*p < 0.05$. В основании столбцов указаны объемы выборок.

and the learning index (t-test, df = 79, F = 2.364, t = 2.969, $p = 0.004$) (Fig. 1b) were significantly higher in T_0 compared to T_p.

The same results were observed when comparing T_{24} and T_p. The time spent in the target sector (t-test, df = 57, F = 1.177, t = -2.112, $p = 0.039$) (Fig. 1a) and the learning index (t-test, df = 57, F = 1.649, t = -2.613, $p = 0.011$) (Fig. 1b) were significantly higher in T_{24}.

No significant differences were observed comparing T_0 and T_{24} either in the percentage of the time spent in the target sector (t-test, df = 64, F = 1.668, t = 1.023, $p = 0.310$) (Fig. 1a) or in the learning index (t-test, df = 64, F = 1.434, t = -0.563, $p = 0.575$) (Fig. 1b).

Discussion

The success of memorizing a pattern depends on its impact strength on the insect [Menzel, 1968; Menzel, 1979; Hoedjes *et al*., 2012]. It was demonstrated earlier that *Trichogramma* is capable of consolidating long-term memory after olfactory training [Huigens *et al*., 2009; Kruidhof *et al*., 2012]. Olfactory learning involves the octopaminergic pathway of memory consolidation, as observed in *Apis mellifera* Linnaeus, 1758 [Mercer, Menzel, 1982; Hammer, Menzel, 1998; Menzel, 1999; Farooqui *et al*., 2003], *D. melanogaster* [Iliadi *et al*., 2017; Schwaerzel *et al*., 2003], and *Bombus impatiens* Cresson, 1863 [Breslow, 2017]. In this study, we observed the retention of memory during up to 24 hours following spatial aversive training at high temperatures. Memory consolidation under such stimuli occurs via the serotonin pathway, independent of the octopaminergic pathway [Sitaraman *et al*., 2008]. Since memory in this case persists for at least 24 hours, it can be classified as long-term memory, which is supported by other experiments with *T. evanescens*, in which the insects were fed with the translation-inhibitor anisomycin [Huigens *et al*., 2009].

With the help of aversive training to high temperatures we have already discovered the abilities to preserve memory up to 1 h after training in thrips [Fedorova *et al*., 2022] and up to 6 h in *Trichogramma* [Fedorova *et al*., 2023]. The ability of microinsects to handle cognitive tasks regardless of the presented stimuli suggests that miniaturization does not lead to simplification or loss of any mechanisms related to cognitive functions.

Conflict of interest. The authors declare that they have no conflict of interest.

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