

Memory impairment due to fipronil pesticide exposure occurs at the GABA_A receptor level, in rats



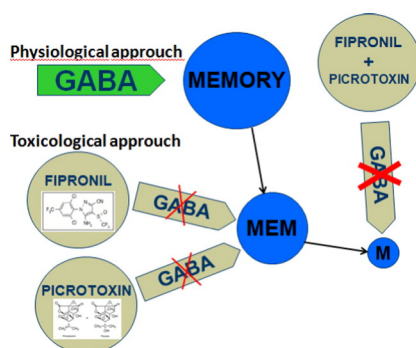
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HIGHLIGHTS

- Fipronil pesticide exposure decreased memory behavior.
- Picrotoxin exposure decreased memory behavior.
- Fipronil + Picrotoxin co-exposure enhances damage on memory behavior.
- Fipronil + Picrotoxin effects occurs with interplay of GABA_A receptors.

GRAPHICAL ABSTRACT



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ABSTRACT

Fipronil (F) a pesticide considered of second generation cause various toxic effects in target and non-target organisms including humans in which provoke neurotoxicity, having the antagonism of gamma-amino butyric acid (GABA) as their main mechanism for toxic action. GABAergic system has been involved in processes related to the memory formation and consolidation. The present work studied the importance of GABA to the mechanisms involved in the very early development of fipronil-induced memory impairment in rats. Memory behavior was assessed using new object recognition task (ORT) and eight radial arm maze task (8-RAM) to study effects on cognitive and spatial memory. Locomotor behavior was assessed using open field task (OF). The dose of fipronil utilized was studied through a pilot experiment. The GABA antagonist picrotoxin (P) was used to enhance fipronil effects on GABAergic system. Fipronil or picrotoxin decrease memory studied in ORT and 8-RAM tasks. Additionally, F and P co-exposure enhanced effects on memory compared to controls, F, and P, suggesting strongly a GABAergic effect. Weight gain modulation and fipronil in blood were utilized as animal's intoxication indicators. In conclusion, here we report that second-generation pesticides, such as fipronil, can have toxic interactions with the CNS of mammals and lead to memory impairment by modulating the GABAergic system.

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1. Introduction

Fipronil [(±)-5-amino-1-(2,6-dichloro- α , α , α -trifluoro-p-tolyl) - 4 trifluoromethylsulfinylpyrazole-3-carbonitrile] is the first member of

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the phenylpyrazole insecticide class, which has a broad spectrum of activity against insects [1]. Fipronil is considered a second-generation insecticide [2] and was initially developed to replace organophosphates pesticides due to its effectiveness against resistant pest strains [3]. It was thus, rapidly adopted as an insecticide used in agriculture.

The low LD₅₀ value of fipronil in houseflies (0.13 mg/kg) [4] and a suggested no observed adverse effect level (NOAEL) for acute oral dosing in rats of 2.5 mg/kg [5] suggest that fipronil is highly specific for the target species. These results, were confirmed by Zhao et al. [6] who showed that glutamate-activated chloride channels are unique fipronil targets and are present in insects, but not in mammals.

However, fipronil's toxic effects are not restricted to those mediated by glutamate-activated chloride channels, as it also targets gamma-aminobutyric acid GABAergic receptors [3,6]. In this sense, the mechanism of action of fipronil is similar to those of groups of insecticides, such as type II pyrethroids and organochlorinated cyclodienes (aldrin, endrin, and dieldrin). These chemicals compounds also affect GABA neurotransmission, although there are differences in the binding sites of the different insecticide classes [7].

Interestingly, in our center for the assistance and control of intoxication, we have received patients subjected to occupational intoxication by fipronil. These patients presented with symptoms typically associated with the blockade of GABAergic receptor function (nausea, headache, and seizures). Surprisingly, they also presented with some memory deficits.

It has been suggested that GABA may be related to processes of memory formation [8] and there are also some studies regarding the relevance of GABA to the processes of learning and memory [9]. Recently, it was demonstrated that the reduction of GABA in the prefrontal cortex causes a delay in cognitive tasks in monkeys [10]. Together, these findings point to the importance of further exploring the mechanisms responsible for fipronil-induced intoxication. No previous study had examined the effects of short-term exposure to low-concentrations of fipronil on memory, and to our knowledge, this is the first study to elucidate the mechanisms involved in the very early development of fipronil-induced memory impairment in rats.

Therefore, in the present work, we expanded on previous reports regarding fipronil neurotoxicity and hypothesized that short-term fipronil exposure (15 days) interferes with GABA neurotransmitter function and is associated with significant changes in memory. The GABA antagonist picrotoxin was used in our experiments to enhance fipronil's effects on memory.

2. Material and methods

2.1. Animals and experimental design

All procedures for animal experimentation were approved by the Ethics Committee, Biosciences Institute of Botucatu, Paulista State University, which is complied with international guidelines of the European Community for the use of experimental animals. Ninety male Wistar rats (250 ± 20 g) were used in this study. The animals were obtained from the colony housed at the Paulista State University and kept in standard rat cages (maximum of four animals per cage) and maintained at 21 ± 2°C, on a 12-hr light/dark cycle, and were given free access to water and rat chow.

The fipronil insecticide utilized in the experiments was the Regent®800WG (BASF- Agro Brazil, Sao Paulo, Brazil, 80% purity). The protocol of fipronil exposure in this study utilizing the via oral was chosen with basis in previous studies, which evaluated the dose range to fipronil [11,12].

The experiments were divided in two parts: first part was designed as a pilot experiment with the objective of to test the effect of two different fipronil doses on memory behavior; second part have as objective to test the GABA antagonist picrotoxin on fipronil effects. The duration for fipronil exposure period in both experimental parts was 15 days. For the

pilot experiment animals were randomly distributed into three groups (N = 10), respectively control (saline solution, gavage), fipronil-exposed group F10 (10mg/kg, daily, gavage), and fipronil-exposed group F30 (30 mg/kg, daily, gavage).

In accord with the results obtained in the pilot experiment, in the second experimental part (picrotoxin experiments), animals were randomly distributed into four groups (N = 15), respectively control (saline solution, gavage), fipronil (30mg/kg, gavage); picrotoxin (Sigma-Aldrich Brazil, 1 mg/kg, i.p.), and fipronil + picrotoxin (co-exposure). The dose of picrotoxin used was chosen based in the experiments of Heredia et al. [13]. During this experimental period were monitored the consumption of food and water, and weight in animals of all treatment groups. At the end of the second experimental protocol (15th day) and 24 hours later, animals were utilized for behavioral test. After behavioral tests rats were anaesthetized with xylazine/ketamine solution (i.p.), having confirmed immobility and loss of righting reflex, rats were killed by exsanguination. The whole-blood samples were collected in lyophilised ethylenediaminetetraacetic acid (EDTA) (Vacutainer Becton-Dickinson, BD, Oxford, UK) and used to fipronil dosage.

2.2. Behavioral tests

2.2.1. For evaluate memory behavior was used the new object recognition task according [14] and the eight radial arm maze task according [15]

The new object recognition task assessment used an open field arena built in white timber, waterproof, measuring 40x25x15cm for young and 58x43x39cm for adults. For tests rats were subjected to a habituation session on the arena for 5 minutes. The following day rats returned to the arena for a new training session for 5 minutes being presented now to a two identical objects of wood (A1 and A2), similar in size, color and texture, and having equal shapes. The objects were positioned in two adjacent corners of the box and at 9cm of the walls. To assess short-term memory retention task, 1.5 hours after the training session, rats were placed to explore the arena for 5 minutes in the presence of two objects: the familiar object A and a novel object B, placed in the same locations as in training period. To assess long-term memory retention task, 24 hours after training session, rats were placed to explore the arena for 5 minutes in the presence of the familiar object A and now a third different object C. Exploration was defined as the time spent in sniffing or touching the object with the nose, and sit on the object was not considered exploration. The same animals were used for assessment of short- and long-term memory. Using the data obtained about the exploration of three distinct objects, a new object recognition index (NORI) for each animal was calculated as the rate TN/TN + TF (TF = time spent exploring the familiar object A, and TN = time spent exploring the novel object B or C) [14]. At the end of each session with a animal apparatus was cleaned with cotton soaked in ethyl alcohol (5%,v/v) to eliminate traces of the animal predecessor.

The eight radial arm maze task assessment used an octagonal radial maze built in white timber, waterproof, and consisted of a central circular platform (20cm high x 47cm diameter) coupled to eight identical arms of the same size (47x11x18cm), symmetrically distributed around it, all covered with transparent acrylic. In the first day animals were placed directly in the central platform of apparatus for five minutes to recognize it. On the second day of training the animals became for 15 minutes in the apparatus independently of the number of visited arms, to recognize it. From the subsequent four days, each animal made training sessions for free arms recognition. The animals were withdrawn from the labyrinth until complete one visit in each arm or have completed 15 minutes in the apparatus. Finally, rats previously placed fasting were trained to find a solid food portion placed at the end of one of the arms (always the same). In the room for experimentation, in around of the 8-RAM apparatus, runways were kept in each wall, which served as the animals spatial orientation for preferential entry into any of the arms. Entry in an arm was considered as walk from the central circular platform until the end of the arm extension. For the

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