Physiology & Behavior 165 (2016) 28–34

Contents lists available at ScienceDirect

Physiology & Behavior

journal homepage: www.elsevier.com/locate/phb

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

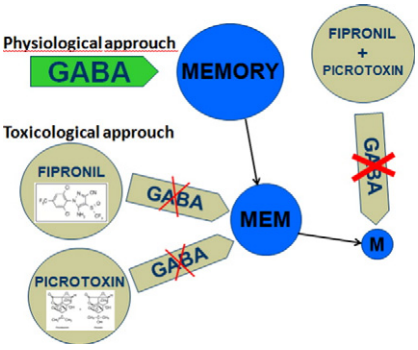
Antonio Francisco Godinho *, Ana Carolina de Oliveira Souza, Caio Cristóvão Carvalho, Daniel França Horta, Daniel De Fraia, Fabio Anselmo, João Leandro Chaguri, Caique Aparecido Faria

Center of Toxicological Assistance (CEATOX), Biosciences Institute, Paulista State University (UNESP), Campus of Botucatu, Rua Prof. Dr. Antonio Celso Wagner Zanin, s/n, CEP 18618-689, Botucatu, SP., Brasil

HIGHLIGTHS

- 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



ARTICLE INFO

Article history:
Received 4 April 2016
Received in revised form 27 June 2016
Accepted 28 June 2016
Available online 30 June 2016

Keywords:
fipronil
picrotoxin
pesticide
memory behavior
GABA

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.

1. Introduction

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

* Corresponding author.
E-mail address: godinho@ibb.unesp.br (A.F. Godinho).

http://dx.doi.org/10.1016/j.physbeh.2016.06.035
0031-9384/

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 LD_{50} 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 (15^{th} 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) (*Vacuntainer* 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

memory task, the animal was placed in the end of a prefixed arm, different from that of training period, and should meet and interact with food. As memory parameters were recorded: a) latency time to find food, b) number of arms visited incorrectly, c) number of arms revisited. 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.

2.2.2. Locomotor activity was assessed utilizing an open field arena according [16]

The open field task was assessed using a wooden box measuring 97x32.5 cm (diameter x height), as described previously [16]. The box was divided into three concentric circles, which were subdivided by painted black lines into 18 similar spaces. Briefly, for locomotor activity observation, each rat was placed in the center of the arena and for the next 3 min was scored ambulation frequency (number of floor units entered with the four paws). 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.

Tasks in all apparatus (ORT, 8-RAM, and OF) were filmed for subsequent quantification of each behavior exhibited by animals.

2.3. Determination of fipronil blood levels

Whole-blood levels of fipronil were determined by liquid-liquid extraction (LLE) and high-performance liquid chromatography with ultraviolet detection (HPLC-UV) system (Prominence Shimadzu®, Kyoto, Japan), according to the method proposed by Xavier et al. [16] and adapted from Chaguri et al. [17]. Briefly, whole blood samples were subjected to extraction by acetonitrile (Merck, Germany) with stirring and filtering. After reaching room temperature, the filtered material was evaporated and re-suspended with acetonitrile and passed with hydrophilic syringe filter with 13mm diameter and pore 0,22μm (PTFE membrane, VWR, Atlanta, GA, USA). A volume of 10 μl was injected into HPLC-UV, using a chromatographic column (C18). The fipronil blood levels were expressed in μg/mL.

2.4. Statistical comparisons

Results obtained were analyzed using GraphPad Instat Software (San Diego, Califórnia, USA). Data were compared by the two-way analysis of variance (ANOVA); a Tukey–Kramer post hoc test was used for comparisons between means when ANOVA was significant at $p<0.05$ level [18].

3. Results

3.1. Pilot experiment

Fig. 1 shows that animals exposed to 30 mg/kg fipronil (F30) but not 10 mg/kg fipronil (F10) exposure have significant ($p<0.05$) decreases in the novel object recognition index in relation to controls in both, short-term (A) and long-term (B) memory assessments.

In the 8-RAM task (Fig. 2), we observed that exposure to fipronil 30, but not fipronil 10, significantly ($p<0.05$) increases the latency to find food in relation to control and F10 treatment (A). The number of arms visited incorrectly (B) and number of arms revisited (C) were increased significantly ($p<0.05$) in F30-treated rats in relation to the control group.

3.2. Picrotoxin experiments

The amounts of food and water ingested by animals in all treatment groups were unchanged ($p>0.05$) during the exposure period (data not shown). Fig. 3 indicates that fipronil exposure significantly decreases weight gain in treated animals in relation to controls, whereas picrotoxin did not significantly alter weight gain ($p>0.05$). Animals receiving fipronil and picrotoxin had a significant ($p<0.05$) decrease in weight gain in relation to controls and picrotoxin–treated animals.

Fig. 4 shows that both fipronil and picrotoxin exposure significantly decrease ($p<0.05$) the novel object recognition index in relation to controls for both short-term (4A) and long-term (4B) memory. When animals receiving fipronil were also exposed to picrotoxin, there was a significant ($p<0.05$) enhancement of the effects of fipronil on the novel object recognition index for short-term memory, but not for long-term memory. Assessment of total object exploration time (4C and 4D) indicates that control animals tend to explore ($p<0.05$) the novel objects significantly more, while animals treated with fipronil or picrotoxin tend to explore the novel objects significantly less ($p<0.05$) in both, short-(4C) and long-term (4D) tests. We also observe that when animals are exposed to fipronil and picrotoxin, there is a significant ($p<0.05$) intensification of their effects on the exploration of novel objects in the short-term test (4C), but not in the long-term test (4D).

In the 8-RAM task (Fig. 5A), we observed that fipronil, but not picrotoxin, significantly increased ($p<0.05$) the latency to find food in relation to control animals. When animals received fipronil and picrotoxin, the latency was significantly higher ($p<0.05$) in relation to controls and fipronil- and picrotoxin-treated mice. Fig. 5B shows that fipronil, but not picrotoxin, significantly increases ($p<0.05$) the number of incorrectly visited arms in relation to controls. When animals

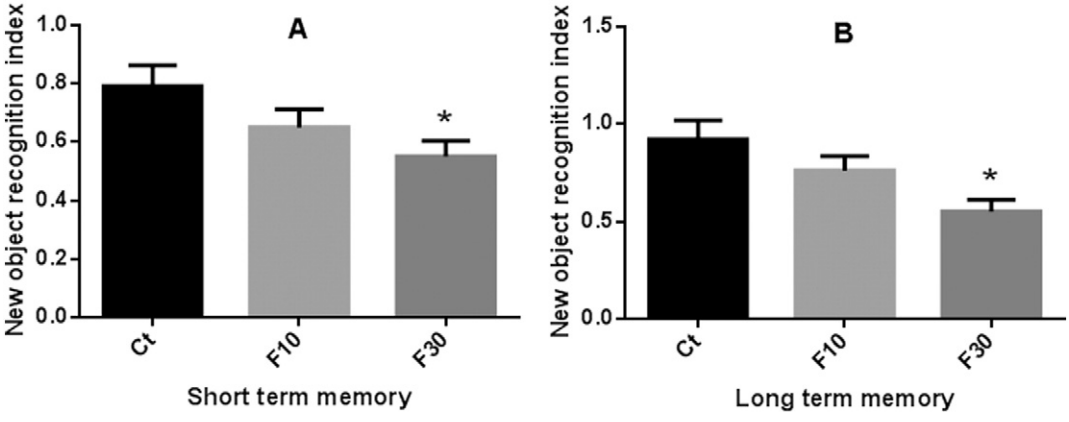


Fig. 1. Novel object recognition index in animals in the different treatment groups for short- (A) and long-term (B) memory. Values represent the mean ± S.E.M. of 10 animals per treatment group. Ct = control, F10 = fipronil 10mg/kg, F30 = fipronil 30 mg/kg. *$p<0.05$ vs. control (ANOVA).

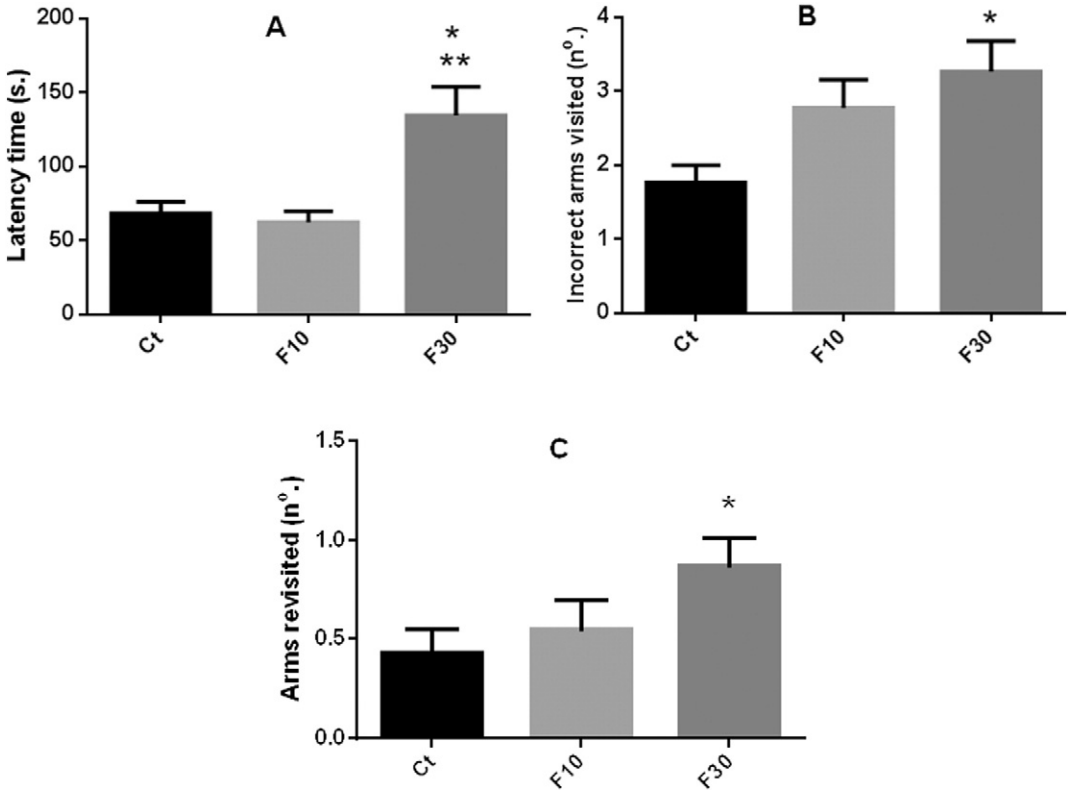


Fig. 2. Results of 8-RAM assessment in animals in the different treatment groups. Values represent the mean ± S.E.M. of 10 animals per treatment group. Ct = control, F10 = fipronil 10mg/kg, F30 = fipronil 30 mg/kg. *p<0.05 vs. control; **p<0.05 vs. F10 (ANOVA).

received fipronil and picrotoxin, the number of arms visited incorrectly increased significantly ($p<0.05$) in relation to controls. Fig. 5C shows that fipronil and picrotoxin significantly increase ($p<0.05$) the number of arms revisited in relation to the control group. When animals received fipronil and picrotoxin, the number of arms revisited significantly increased ($p<0.05$) in relation to controls and also in relation to fipronil- and picrotoxin-treated rats.

Fig. 6 indicates that the level of fipronil in total blood in treated animals is significantly higher ($p<0.05$) in comparison to control animals. This demonstrates that treatment with gavage is a very good tool for the study of fipronil intoxication using the dose chosen based on the pilot experiment.

The results of the open field task indicate that fipronil, picrotoxin, and fipronil and picrotoxin treatment do not modify locomotor activity in relation to controls (data not shown).

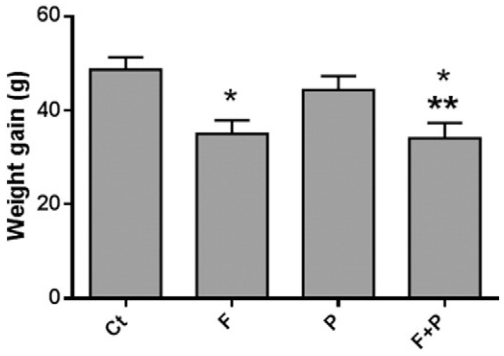


Fig. 3. Weight gain in animals in the different treatment groups. Values represent the mean ± S.E.M. of 15 animals per treatment group. Ct = control, F = fipronil, P = picrotoxin. *p<0.05 vs. control; **p<0.05 vs. picrotoxin (ANOVA).

4. Discussion

Analysis of the animals' weights during the treatment period showed that there was a reduction in weight gain in animals treated with fipronil and in those co-exposed with fipronil and picrotoxin. This result confirms previous reports indicating that chronic fipronil exposure in mice eating food containing fipronil leads to decreased weight gain, decreased food consumption, and decreased food conversion efficiency [19].

GABA can increase or decrease food ingestion depending on its site of action. When microinjected into the medial hypothalamus, GABA leads to an increase in food ingestion. However, when injected into the lateral hypothalamus, GABA leads to a decrease in food ingestion [20]. GABA antagonists, such as picrotoxin and fipronil, can block the appetite stimulation promoted by 2-deoxyglucose [21], suggesting that GABAergic neurons participate in this regulation mechanism.

Picrotoxin is a non-competitive antagonist of GABAergic receptors and acts by blocking and modulating chlorine channels [22]. Administration of picrotoxin did not significantly reduce weight gain in animals throughout the treatment period. However, it led to changes in cognitive and spatial memory. We can thus suggest that the dose of picrotoxin used in our study was not high enough to induce weight loss.

Our results indicate that fipronil exposure leads to changes in memory in animals, in a similar manner as picrotoxin exposure. When animals were co-exposed with fipronil and picrotoxin, changes in behavior were intensified, suggesting synergistic action. Both fipronil and picrotoxin antagonize the $GABA_A$ receptor. Thus, our results strongly suggest that the memory-related changes can be linked to GABA neurotransmission.

The 8-RAM and object recognition test (ORT) are especially suitable for testing the effects of pharmacological interventions and chemical agents on learning and memory. New objects induce excitement and lead to motivational effects, which are used as reinforcements to induce learning [23]. The ORT, which was conducted to test the cognitive

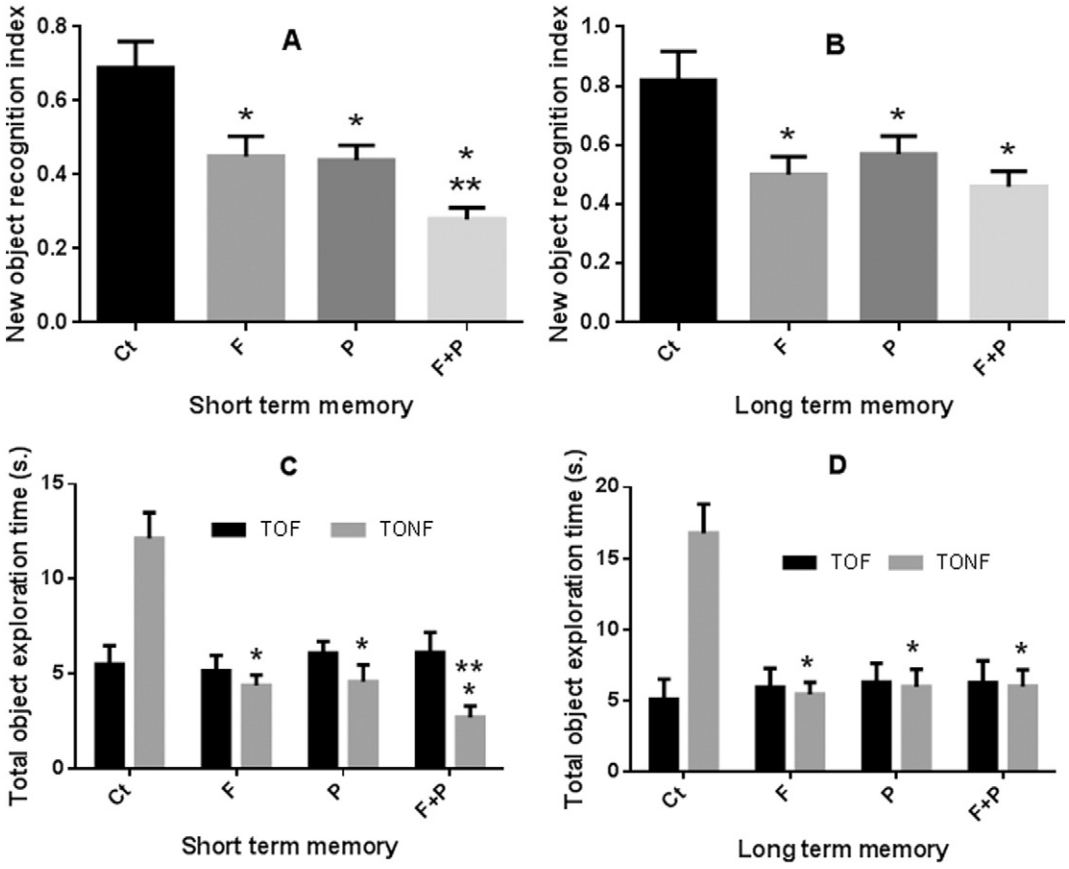


Fig. 4. Novel object recognition index and total object exploration time in animals in the different treatment groups for short- (A) and long-term (B) memory. Values represent the mean ± S.E.M. of 15 animals per treatment group. Ct = control, F = fipronil, P = picrotoxin, TOF = time in familiar object, TONF = time in not familiar object. *p<0.05 vs. control; **p<0.05 vs. fipronil and picrotoxin (ANOVA).

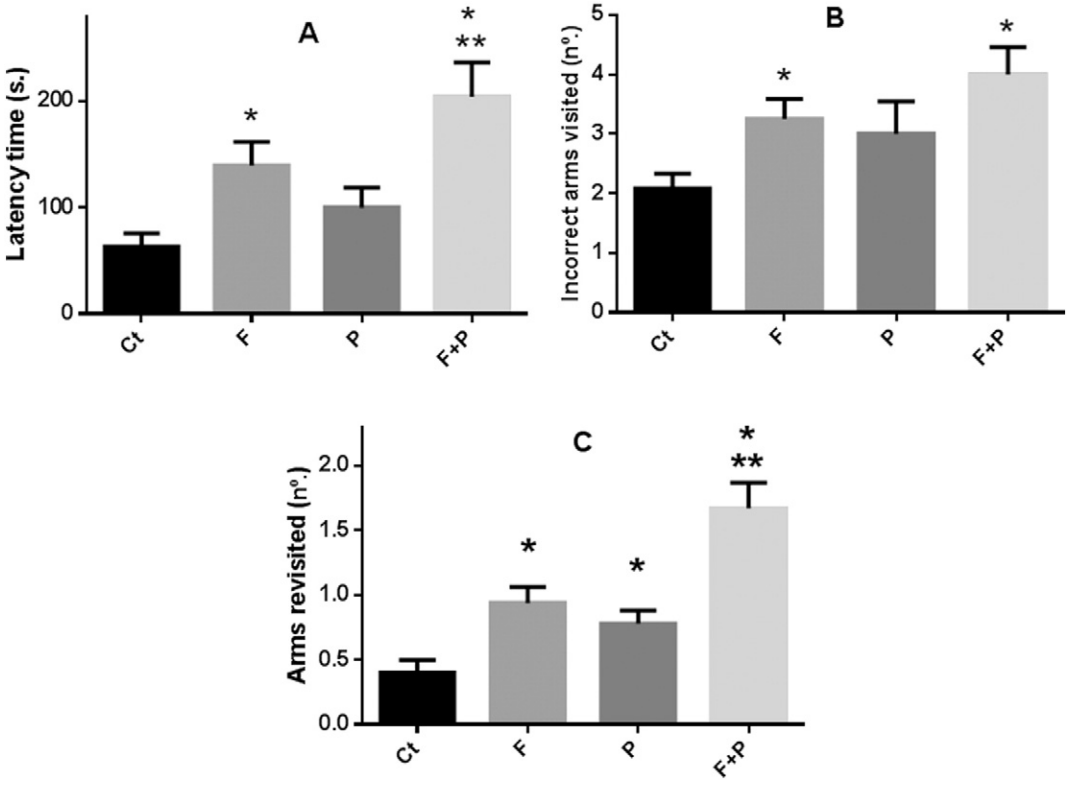


Fig. 5. Results of the ORT in animals in the different treatment groups. Values represent the mean ± S.E.M. of 15 animals per treatment group. Ct = control, F = fipronil, P = picrotoxin. *p<0.05 vs. control; **p<0.05 vs. fipronil and/or picrotoxin (ANOVA).

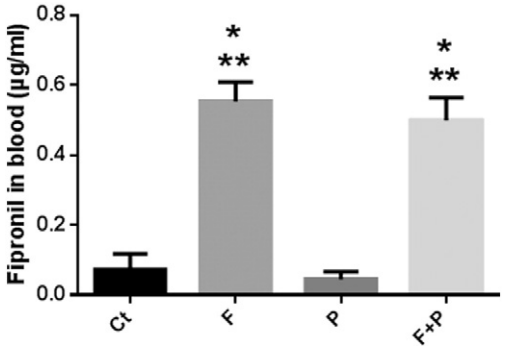


Fig. 6. Fipronil levels in blood of animals in the different treatment groups. Values represent the mean ± S.E.M. of 8 animals per treatment group. Ct = control, F = fipronil, P = picrotoxin. *p<0.05 vs. control; **p<0.05 vs. picrotoxin (ANOVA).

memory of animals exposed to fipronil and picrotoxin, indicates that exposure to the pesticide causes a deficit in memory, both in the short-term and in the long-term.

Tests used to assess memory for recognizing novel objects in rodents are based on the natural tendency of animals to explore new objects [24]. In behavioral testing, it is possible to calculate an index of discrimination based on the proportion of time that the animal spends investigating the novel object compared to the familiar object.

Evidence obtained in studies using lesions in rodents indicates that the perirhinal cortex is the brain region required for the recognition of a novel object based on visual cues [25]. New object detection based on spatial cues requires the use of the hippocampus, the parahippocampal cortex, and the entorhinal cortex [26]. Using this line of reasoning, we can conclude that our results indicate that fipronil and picrotoxin affect the perirhinal cortex, as our test utilized visual cues.

Drugs that activate the GABAergic system lead to deficits in memory formation when administered into the lateral ventricle, the basal forebrain, the hippocampus, or the amygdala [27]. Our results seem to suggest the opposite, as fipronil and picrotoxin are inhibitors of the GABA system. The observed differences may be due to the method of administration, since McEown and Treit [27] directly administered the drug to specific locations in the central nervous system (CNS), while we used gavage to treat the animals with fipronil and intraperitoneal injections to treat the animals with picrotoxin. Thus, our methods of administration led to systemic actions of the agents used.

Results of the 8-RAM indicated increases in the latency to find food, the number of arms visited incorrectly, and the number of arms revisited, clearly demonstrating a loss of memory in animals exposed to fipronil and picrotoxin. There was synergism and an even more exacerbated response in co-treated animals, indicating that co-treatment enhances spatial memory deficits. Memory is defined as the retention of learned information [28] and is a process that requires the integrated activity of different brain regions and neurotransmitter systems [29]. Learning can be defined as the mechanism by which new information or knowledge is acquired. Thus, learning is the acquisition of new information, which is the first stage of memory [28].

Toxic lesions in the CNS affect the acquisition, consolidation, and evocation phases of memory. As memory consolidation occurs primarily in the hippocampus [23], which is a GABAergic system critical for the development and maintenance of memory [30], our results with fipronil and picrotoxin, both of which are GABA receptor antagonists, suggest that the toxic actions of the two substances may be related to the hippocampus.

Interestingly, the ORT results indicate that the synergism between fipronil and picrotoxin is most evident in the evaluation of short-term memory, which may indicate a greater action of these agents on the memory acquisition phase, rather than the consolidation phase.

Changes in the dynamics and the activities of several neurotransmitters within the hippocampal formation indicate that some mediators of learning and memory, including glutamate, acetylcholine, and GABA, play important roles in memory formation in the hippocampus. The roles of these neurotransmitters were discovered when learning or memory were impaired or prevented following the administration of agonists or antagonists of their respective receptors [31].

Data from the literature indicate that the central GABAergic system has a key role in cognition, including roles in the formation and consolidation of memory [32–35]. In addition, a large body of evidence from preclinical literature indicates that changes in memory are mediated by changes in GABAergic areas of the brain [35].

Additionally, data from neurophysiological studies, including injury and electrophysiological evaluations in humans and animals, show that certain areas of the brain rich in GABA receptors (e.g. amygdala, septum, hippocampus, and entorhinal cortex) play important roles in memory [35,36]. Our results corroborate those of previous studies.

The observation that fipronil significantly decreases weight gain in treated animals suggests that this alone may have had a major impact on the changes in cognitive performance observed in the ORT and 8-RAM tasks, especially as a food reward is used to motivate the subject in the 8-RAM task. Since the GABA antagonist picrotoxin failed to alter weight in treated animals, this effect it seems to be specific to fipronil, which is a toxic substance.

An important question is whether these animals experienced generalized toxic effects due to fipronil exposure. To help us address this issue, the locomotor activity of animals in all groups were examined in the open field arena. We observed that none of the treatments, including fipronil exposure, changed this behavior. This indicates that the health/behavior of the animals was not affected by fipronil exposure.

On the other hand, data from short-term and long-term toxicity studies of fipronil in rats, rabbits, mice, and dogs do not suggest the presence of endocrine disruption [37]. However, in long-term studies, fipronil was shown to decrease thyroid hormone levels in rats. Researchers concluded that this effect resulted from increased clearance, rather than a direct effect of fipronil on the thyroid. Fipronil may act as an endocrine disrupter in glands such as the thyroid by altering hormones levels and changing endocrine functions, which could then lead to weight changes in the animals, even in the absence of reduced food intake. These effects should be investigated in the future.

GABA is important to the mechanisms underlying memory, and we observed that fipronil and picrotoxin act as antagonists of the $GABA_A$ receptor. We thus suggest that the $GABA_A$ receptor, which is affected by exposure to environmental agents with the same mechanism of action as fipronil, may underlie changes in or the abnormal modulation of processes involved in learning, and those involved in the formation and consolidation of cognitive and spatial memory.

Our experiments highlight the importance of GABA and the $GABA_A$ receptor to the processes underlying cognitive and spatial memory in experimental animals. This study thus contributes to a better understanding of the suggested link between environmental contamination by agents such as pesticides and increased incidences of neurodegenerative diseases, such as Parkinson's, Alzheimer's, and multiple sclerosis, all of which involve memory deterioration.

5. 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.

Acknowledgment

This study was partially supported by scholarships (2014/18429-6 and 2014/18433-3) from Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP, Brazil).

References

[1] C.C. Tingle, J.A. Rother, C.F. Dewhurst, S. Lauer, W.K. King, Fipronil: environmental fate, ecotoxicology, and human health concerns, Rev. Environ. Contam. Toxicol. 176 (2003) 1–66.

[2] J. Tang, A. Usmani, E. Hodgson, R.L. Rose, In vitro metabolism of fipronil by human and rat cytochrome P450 and its interactions with testosterone and diazepam, Chem. Biol. Interact. 147 (2004) 319–329.

[3] T. Narahashi, X. Zhao, T. Ikeda, K. Nagata, J.Z. Yeh, Differential actions of insecticides on target sites: basis for selective toxicity, Hum. Exp. Toxicol. 4 (2007) 361–366.

[4] T. Narahashi, X. Zhao, T. Ikeda, V.L. Salgado, J.Z. Yeh, Glutamate-activated chloride channels: Unique fipronil targets present in insects but not in mammals, Pestic. Biochem. Physiol. 97 (2) (2010) 149–152.

[5] D. Jackson, C.B. Cornell, B. Luukinen, K. Buhl, D. Stone, Fipronil technical fact sheet, National Pesticide Information Center, Oregon State, 2009 1–3.

[6] X. Zhao, J.Z. Yeh, V.L. Salgado, T. Narahashi, Sulfone metabolite of fipronil blocks gamma-aminobutyric acid- and glutamate-activated chloride channels in mammalian and insect neurons, J. Pharmacol. Exp. Ther. 314 (1) (2005) 363–373.

[7] C.C.D. Tingle, J.A. Rother, C.F. Dewhurst, S. Lauer, W.J. King, Fipronil: Environmental Fate, Ecotoxicology, and Humam Health Concerns, Rev. Environ. Contam. Toxicol. 176 (2003) 1–66.

[8] R.J. Katz, L. Liebler, GABA involvement in memory consolidation: evidence from post trial aminooxyacetic acid, Psychopharmacology 56 (2) (1978) 191–193.

[9] L. Menzies, C. Ooi, S. Kamath, J. Suckling, P. Mckenna, P. Fletcher, E. Bullmore, C. Stephenson, Effects of gamma-aminobutyric acid - modulating drugs on working memory and brain function in patients with schizophrenia, Arch. Gen. Psychiatry 64 (2) (2007) 56–67.

[10] L. Michels, E. Martin, P. Klaver, R. Edden, F. Zelaya, D.J. Lythgoe, R. Luchinger, D. Brandeis, R.L. O'Gorman, Frontal GABA levels change during working memory, PLoS One 7 (2012) e3193.

[11] M.S. Udo, T.M. Sandini, T.M. Reis, M.M. Bernardi, H.S. Spinosa, Prenatal exposure to a low fipronil dose disturbs maternal behavior and reflex development in rats, Neurotoxicol. Teratol. 45 (2014) 27–33.

[12] V.C. Moser, N. Stewart, D.L. Freeborn, J. Crooks, D.K. Macmillan, J.M. Hedge, et al., Assessment of serum biomarkers in rats after exposure to pesticides of different chemical classes, Toxicol. Appl. Pharmacol. 282 (2) (2015) 161–174.

[13] L. Heredia, M. Torrente, M.T. Colomina, J.L. Domingo, Assessing anxiety in C57BL/6J mice: A pharmacological characterization of the open-field and light/dark tests, J. Pharmacol. Toxicol. Methods 69 (2014) 108–114.

[14] S.L. Dix, J.P. Aggleton, Extending the spontaneous preference test of recognition: evidence of object-location and object-context recognition, Behav. Brain Res. 99 (1999) 191–200.

[15] D.S. Olton, The Radial Arm Maze as a tool in the behavioral pharmacology, Physiol. Behav. 40 (1987) 793–797.

[16] P.R. Terçariol, A.F. Godinho, Behavioral effects of acute exposure to the insecticide fipronil, Pestic. Biochem. Physiol. 99 (2011) 221–225.

[17] J.L. Chaguri, A.F. Godinho, D.F. Horta, V.H. Gonçalves-Rizzi, J.S. Possomato-Vieira, R.A. Nascimento, C.A. Dias-Junior, Exposure to fipronil elevates systolic blood pressure and disturbs related biomarkes in plasma of rats, Environ. Pharmacol. Toxicol. 42 (2016) 63–68.

[18] G.W. Snedecor, W.G. Cochran, Statistical Methods, eighth ed. Wiley, Iowa, 1991.

[19] United States Environmental Protection Agency (US.EPA), New Pesticide Fact Sheet - Fipronil; EPA 737-F-96-005, Office of Prevention, Pesticides and Toxic Substances, Office of Pesticide Programs, U.S. Government Printing Office Washington, DC, 1996 1–10 (gov/fedrgstr/EPA-PEST/199ay-12/pr736DIR/Facts/Factsheet.txt.html).

[20] S. Tsujii, G.A. Bray, GABA-related feeding control in genetically obese rats, Brain Res. 540 (1991) 48–54.

[21] J.E. Ahlskog, B.G. Hoebel, Overeating and obesity from damage to a noradrenergic system in the brain, Science 182 (1992) 166–169.

[22] R. Shin, S. Ikemoto, Administration of the GABA receptor antagonist picrotoxin into rat supramammillary nucleus induces c-Fos in reward-related brain structures. Supramammillary picrotoxin and c-Fos expression, BMC Neurosci. 11 (2010) 101–114.

[23] C.K.B. Silveira, C.R.G. Furini, F. Benetti, S.C. Monteiro, I. Izquierdo, The role of histamine receptors in the consolidation of object recognition memory, Neurobiol. Learn. Mem. 103 (2013) 64–71.

[24] A. Ennauceur, One-trial object recognition memory in rats and mice: methodological and theoretical issues, Behav. Brain Res. 215 (2) (2010) 244–254.

[25] M.M. Albasser, R.J. Chapman, E. Amin, M.D. Iordanova, D.S. Vann, J.P. Aggleton, New behavioral protocols to extend our knowledge of rodent object recognition memory, Learn. Mem. 17 (8) (2010) 407–419.

[26] G.R.E. Barker, E.C. Warburton, When is the hippocampus involved in recognition memory? J. Neurosci. 31 (29) (2011) 10721–10731.

[27] K. Mceown, D. Treit, Inactivation of the dorsal or ventral hippocampus with muscimol differentially affects fear and memory, Brain Res. 1353 (2010) 145–151.

[28] M.A. Lynch, Long-term potentiation and memory, Physiol. Rev. 84 (2004) 87–136.

[29] Y. Hu, Z. Xia, Q. Sun, A. Orsi, D.A. Rees, A new approach to the pharmacological regulation of memory: Sarsasapogenin improves memory by elevating the low muscarinic acetylcholine receptor density in brains of memory-deficit rat models, Brain Res. 1060 (2005) 26–39.

[30] E.M. Stanley, M.A. Wilson, J.R. Fadel, Hippocampal neurotransmitter efflux during one-trial novel object recognition in rats, Neurosci. Lett. 511 (2012) 38–42.

[31] E. Dere, J.P. Huston, M.A. De Souza Silva, The pharmacology, neuroanatomy and neurogenetics of one-trial object recognition in rodents, Neurosci. Biobehav. Rev. 31 (5) (2007) 673–704.

[32] J.A. Gray, S. Quintero, J. Mellanby, C. Buckland, M. Fillenz, S.C. Fung, Some biochemical, behavioural and electrophysiological tests of the GABA hypothesis of anti-anxiety drug action, in: N. Bowery (Ed.), Actions and Interactions of GABA and Benzodiazepines, Raven Press, New York 1984, pp. 237–262.

[33] I. Izquierdo, J.H. Medina, $GABA_A$ receptor modulation of memory: The role of endogenous benzodiazepines, Trends Pharmacol. Sci. 121 (1991) 260–265.

[34] I. Izquierdo, J.H. Medina, Correlation between the pharmacology of long-term potentiation and the pharmacology of memory, Neurobiol. Learn. Mem. 63 (1995) 19–32.

[35] M. Davis, The role of the amygdala in emotional learning, Int. Rev. Neurobiol. 36 (1994) 225–266.

[36] Do hippocampal lesions produce amnesia in animals? in: J.N.P. Rawlins, S.M. Stahl, S.D. Iversen, E.C . (Eds.), Goodman, Cognitive Neurochemistry, Oxford University Press, Oxford 1987, pp. 73–89.

[37] D. Jackson, C.B. Cornell, B. Luukinen, K. Buhl, D. Stone, Fipronil Technical Fact Sheet, National Pesticide Information Center Oregon State University Extension Services, 2009 1–11.