Explor Anim Med Res,
Vol.5, Issue - 1, 2015, p. 27-32

Research Article

A BEHAVIORAL STUDY ON LEARNING AND MEMORY IN ADULT SPRAGUE DAWLEY RAT IN INDUCED ACETAMIPRID TOXICITY

P. S. Mandal[1*], S. Mondal[1], S.S. Karnam[2], K. Purohit[3]

ABSTRACT: A study was conducted to evaluate the behavioral change related with learning and memory due to the toxicity of Acetamiprid. On 24 healthy male Sprague dawley rats (12 for contextual fear condition and 12 for modified step-down model) the study was performed. Treated animal showed impaired learning and memory in both Contextual fear condition and Modified Step-down model of behavioral assays.

Key word: Learning and memory, Acetamiprid, behavior, Sprague dawley rat.

INTRODUCTION

Recent advancement in agriculture and veterinary sciences expose man and animal to various pesticides undoubtedly. The neonicotinoids, acetamiprid and imidacloprid belong to a new class of insecticide those are used worldwide to protect crops from the pest insects and domestic animal from fleas (Kimura-Kuroda *et al.*, 2012 and Mondal *et al*., 2012). The frequent and continuous use of acetamiprid has resulted in their widespread distribution in environment. Human and animal are exposed indirectly at different level and having long lasting behavioral change. Studies reported memory deficit (Maren *et al.*, 2013) in the exposed animals.

Fear is essential for survival and a way of response to circuitry stimuli. The understanding of brain circuits involved in fear has been achieved by studying the circuits of learning condition for example, an environmental context (a conditioning chamber) may be arranged to deliver a stimuli in form of a footshock, which then leads to conditioned responses to the context, such as freezing behavior in rats (Jiang *et al.*, 2010). The hippocampus, by providing spatial and temporal information, participates in the formation of conditioned fear (Abumaria *et al.*, 2011).

Learning is the acquisition of information, experience and skill related to adjustment to environment or updating to its sustainability.

[1]*Department of Veterinary Pathology, West Bengal University of Animal and Fishery Sciences, 37 KB Sarani, Kolkata-70037, West Bengal, India.*
[2] *Department of Veterinary Pathology, College of Animal Husbandry and Vety Sc. Durg. Madhya Pradesh, India.*
[3]*Assistant Professor, RAJUVAS, Rajasthan, India.*
**Corresponding author. e- mail: parthasarathi.vet@gmail.com*

Retention of this information or pass on new information which is already acquired is called memory (Gupta *et al.,* 2012). Learning and memory has been introduced in neurotoxicological studies on different environmental pollutants (Gilbert *et al.,* 1996) and has proven to be a useful tool for linking behavioral and neurochemical outcomes.

Information is lacking, however, about which of the brain structures could be affected by acetamiprid exposure and thus contribute to these long-lasting behavioral changes. A week long exposure has been made to rat in this experiment with an objective to see the effect of acetamiprid on learning and memory behaviour.

MATERIALS AND METHODS

The present study was conducted on 24 healthy male Sprague dawley rats in two groups where 12 animals were for contextual fear condition and another 12 were for modified step-down method of learning and memory. The rats were procured and housed in cages at department of veterinary pathology, West Bengal University of Animal and Fishery Sciences, Kolkata, India. Three animals per cage were accommodated in polycarbonate cages during the experimental period. Husk was used as bedding material. Husk was strained and sterilized by autoclaving in transparent polythene bag using sterol strip as an indicator of successful sterilization. Changing frequency of bedding material was twice a week. Animals were allowed to acclimatize for a period of 7 days prior to experiment and provided standard feed (Nutri Lab, rodent feed, Vetcare Pvt. Ltd, Bangalore) and allowed water *ad libitum*.

Experimental protocol was approved by institutional animal ethics committee before starting the experiment.

Chemical and formulation

Acetamiprid (CAS No-135410-20-7) was procured from Sigma Aldrich, USA. Acetamiprid was formulated using distilled water as a vehicle. Acetamiprid solution was administered directly in stomach by oral gavages with dose volume of 10 ml/kg (10mg/ml) for 7 days daily. Body weights were recorded before administration of acetamiprid.

A 100mg/kg dose was selected based on some literature precedence (Mondal *et al.*, 2012).

Contextual fear conditioning

This test assesses the fear condition thus providing the indication of strength of learning and memory acquired during training. This paradigm is standard tests of emotional memory. These behaviors rely on the integrity of amygdala, hippocampus, thalamus, and other structures (Packard and Teather, 1997).

Apparatus: CoulBourn habituation instrument, Habitest isolation cubicle, USA, fitted with an electrical grid on the floor of the conditioning chamber consisted of Plexiglas box (25.0X 25.0-cm grid of parallel 0.1-cm caliber stainless steel bars 18 in number spaced 1.5 cm apart). Floor grid was removable. The electrical input can be controlled by the software graphic state. Camera is placed upside to the chamber and animal can be observed from out side in the monitor screen.

Habituation: Animals were habituated or trained for two sessions in each day on day 4 to day 6 to the context of the instrument. Each session period was for 300 second. In the conditioning session (training), rats were placed in the chamber for 5 min for habituation (Middei *et al.,* 2012). During training regimen, after taking out each of animal, an alcohol swabbing

Table 1: Reduced freezing behavior in the acetamiprid treated group (Second).

Vehicle	Acetamiprid
58.67	19.00
45.33	37.33
62.67	23.67
64.67	26.33
54.33	32.67
52.67	17.00
47.00	20.33
51.00	13.00
61.33	18.67
63.00	29.33
54.67	43.33
61.33	31.33

Table 2: Reduced latency time to touch grid floor in the acetamiprid treated group (Second).

Vehicle	Acetamiprid
120	7
120	8
112	12
90	10
116	5
120	8
96	6
120	13
120	12
120	4
118	3
85	9

was done to clean and remove all those dirt and smell of housed animal as so the animal in queue would not be able to assess the cue kept or leave by the previous animal.

Test: On day 6 an electrical shock of 2mA was given for 2 seconds. Animal placing to shock interval was of 180 seconds. Before shock was applied base line freezing time (in seconds) was recorded on day 6. On day 7, total freezing duration in seconds was measured to the specified duration as it was in training session i.e. 300 seconds. Context conditioning was assessed 24 h after the training by placing rat for 5 min in the conditioning chamber (Alvares *et al*., 2010). Rat behavior was videotaped, and fear memory was manually assessed by scoring the total amount of freezing behavior (defined as complete lack of movement, except for respiration) during the 5-min test. Values are reported as percent of time spent freezing. At the end of the procedure, rat returned to their home cage (Walker and Davis, 2000).

Modified Step-down model of learning and memory:

This test assesses the olfactory and visual learning as wel as comfort level analysis in home cage apehention. This paradigm is standard tests of aquiered skill memory.

Apparatus: Apparatus consist of a perplex chamber which was one sided open and 12cmX8cm in size. The chamber was kept at 2 ft distance fom the home cage. At the path in between chamber and home cage a grid floor was fitted to shock devices which deliver scramble foot shock for 5 seconds of 1.5 mA strength.

Habituation: Animal was placed to the chamber and allowed to explore untill it found its home cage in the fist day. Second day training was given for 10 seconds. After taining it was retuned to the home cage.

Test: On third day animal was placed to the

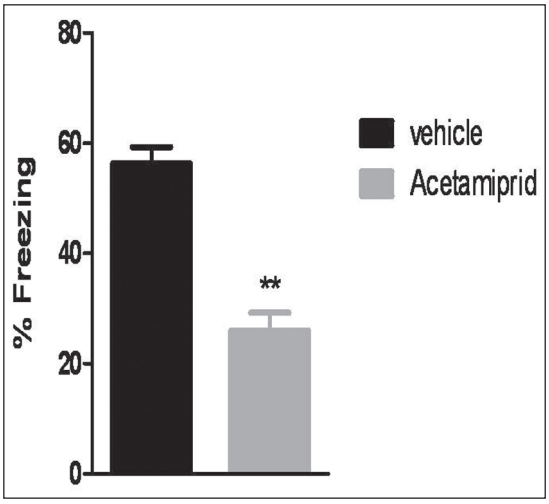

Fig. 1: Reduced freezing behavior in the acetamiprid treated group (p=0.01).

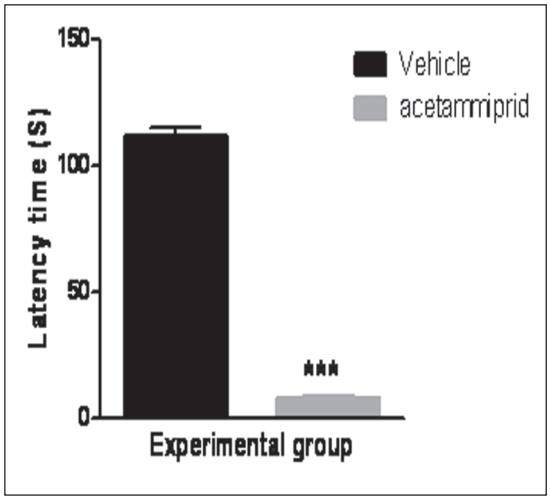

Fig. 2: Reduced latency time to touch grid floor in the acetamiprid treated group (p=0.01).

persplex chamber and allowed to explore. When it was crossing the grid floor, shock was given. Then, it was returned to the home cage manualy. 4th day was the actual test day. The time of latency was measured to touch the grid floor on the way to its home cages (Bermudez-Rattoni *et al.*, 1997).

RESULTS AND DISCUSSIONS

Contextual fear conditioning: Fear to the context i.e. conditional stimuli, was measured as freezing behavior of the individual animal. Data was represented in table 1 and fig. 1. Percent freezing was more in animal of vehicle group than acetamiprid treated animal.

Modified Step-down model of learning and memory:

In this present study the latency time was more in vehicle group (Table: 2 and Fig. 2) than the treated animal which showed that acetamiprid impaired learning and memory in both behavioral model design. Acetamiprid acts agonistically to neural nicotinic acetylcholine receptor (nAChR). Availability of acetamiprid in the hippocampus opens ionotropic receptors and subsequently positive ion flow into the cell causing excitotoxicity. But repetitive opening of nAChR causing desensitization to the receptor and the ion channel and thus not flowing Ca^{2+} across the membrane. Desensitization is a common property of nAChRs (Xiao *et al.*, 2011). Moreover secondary inhibitory neurotransmitters like Y-amino butyric Acid, glycine released at synapse from the synoptosome causing competitive outflow with glutamate. Detrimental effct on GABAergic interneurons leads to learning and memory defiits in mice (Knoferle *et al.*, 2014). NMDA receptor is mostly glutamate mediated, which though opened with high frequency stimulation but can't remain open for long time. From this experiment it was observed that in learning and memory, nicotinic acetylcholine receptors (nAChRs) played a role (Xiao *et al.*, 2011) and nAChR agonist like acetamiprid impaired memory formation. Yang *et al*. (2012)

observed thioredoxin deficit likely plays an important role in the impaired spatial learning and memory in the rats exposed to chronic intermittant hypoxia and may work through the apoptosis of neurons in the hippocampus. Research with rats indicates that spatial memory may be adversely affected by damage to the hippocampus in a way that closely resembles schizophrenia (Lewis and Levitt, 2002).

CONCLUSON

From the present study it was concluded that acetamiprid exposure impaired learning ability of rats as well as hampered working memory.

ACKNOWLEDGEMENTS

Authors are thankful to the Dean, F/O-VAS, WBUAFS and Director, Biology, TCG Lifesciences pvt. Ltd for providing facility and instrument for the experiment.

REFERENCE

Abumaria N, Yin B, Zhang L, Xiang-Yao L, Chen T, Descalzi G, Zhao L, Luo L, Ran C, Zhuo M, Liu G (2011) Effects of elevation of brain magnesium on fear conditioning, fear extinction, and synaptic plasticity in the infralimbic prefrontal cortex and lateral amygdala. J Neurosci 31: 14871–14881.

Alvares LO, Engelke DS, Diehl F, Scheffer-Teixeira R, Haubrich J, Cassini LF, Molina VA, Quillfeldt JA (2010) Stress response recruits the hippocampal endocannabinoid system for the modulation of fear memory. Learn Mem 17: 202-209.

Bermudez-Rattoni F, Introini-Collison I, Coleman-Mesches K, McGaugh JL (1997) Insular cortex and amygdala lesions induced after aversive training impair retention: effects of degree of training. Neurobiol Learn Mem 67: 57-63.

Gilbert ME, Mack CM, Lasley SM (1996) Chronic developmental lead exposure increases the threshold for long-term potentiation in rat dentate gyrus in vivo. Brain Res 736: 118–124.

Gupta A, Hemraj JS, Jindal A, Upmanyu N (2012) Various animal model to check learning and memory- a review. Int J Pharma Sci Res 4: 112-115.

Jiang W, Duysen EG, Hansen H, Shlyakhtenko L, Schopfer LM, Lockridge O (2010) Mice treated with chlorpyrifos or chlorpyrifos oxon have organophosphorylated tubulin in the brain and disrupted microtubule structures, suggesting a role for tubulin in neurotoxicity associated with exposure to organophosphorus agents. Toxicol sci 115: 183–193.

Kimura-Kuroda J, Komuta Y, Kuroda Y, Hayashi M, Kawano H (2012) Nicotine-Like Effects of the Neonicotinoid Insecticides Acetamiprid and Imidacloprid on Cerebellar Neurons from Neonatal Rats. PLoS ONE 7: e32432. doi:10.1371/journal.pone.0032432.

Knoferle J, Yoon SY, Walker D, Leung L, Gillespie AK, Tong LM, Bien-Ly N, Huang Y (2014) Apolipoprotein E4 Produced in GABAergic Interneurons Causes Learning and Memory Deficits in Mice. Neurobiol of Disease 34: 14069-14078.

Lewis DA, Levitt P (2002) Schizophrenia as a disorder of neurodevelopment. Annual Rev Neuroscience 25: 409–432.

Middei S, Spalloni A, Longone P, Pittenger C, O'Mara SM, Marie H, Teule MA (2012) CREB selectively controls learning-induced structural remodeling. Learn. Mem 19: 330-336.

Mondal S, Ghosh RC, Mukhopadhayay SK (2012) Studies on the electrolytes and microelements in Wistar rat following multiple exposures to acetamiprid. Toxicol Ind Health 28: 422-427.

Packard MG, Teather LA (1997) Double dissociation of hippocampal and dorsal-striatal memory systems by posttraining intracerebral injections of 2-amino-5-phosphonopentanoic acid. Behav Neurosci 111: 543-51.

Maren S, Phan KL, Liberzon I (2013) The contextual brain: implications for fear conditioning, extinction and psychopathology. Nature Reviews (Neuroscience) 14: 417-428.

Walker DL, Davis M (2000) Involvement of NMDA receptors within the amygdala in short-versus long-term memory for fear conditioning as assessed with fear-potentiated startle. Behav Neurosci 114: 1019-33.

Xiao C, Rahul S, Ryan MD, Elisha DWM, McIntosh JM, Henry AL (2011) Characterizing functional á6â2 nicotinic acetylcholine receptors in vitro: mutant â2 subunits improve membrane expression, and fluorescent proteins reveal responsive cells. Biochem Pharmacol 82: 852–861. doi:10.1016/j.bcp.2011.05.005.

Yang XH, Liu HG, Liu X, Chen JN (2012) Thioredoxin and impaired spatial learning and memory in the rats exposed to intermittent hypoxia. Chin Med J 125: 3074-80.

***Cite this article as**: Mandal PS, Mondal S, Karnam SS, Purohit K (2015) A behavioral study on learning and memory in adult Sprague Dawley rat in induced Acetamiprid toxicity. Explor Anim Med Res 5(1): 27-32.