



EFFECTS OF GALLIC ACID ON EXPLORATORY BEHAVIOR AND LOCOMOTOR ACTIVITY IN RATS

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ABSTRACT

PURPOSE: The present study investigated the effects of GA on exploratory behavior and locomotor activity in male Wistar rats. **METHODS:** GA (20 mg/kg) or saline were administrated orally to different groups of rats for 7, 14, 21 and 30 days. At the end of each experimental period, the changes in rat behavior were recorded in an Opto Varimex apparatus. The number of horizontal and vertical movements recorded every minute served as a measure of exploratory activity and habituation. The total number of movements was used as a measure of locomotor activity. **RESULTS:** It was found that after 7 days of treatment, GA did not significantly affect exploratory behavior and locomotor activity of rats compared to the saline-treated controls. After the 14 day treatment period, GA did not significantly affect horizontal activity but reduced the number of vertical movements. After 21 and 30 days, GA significantly reduced both horizontal and vertical movements of rats compared to the controls. At all testing periods, GA did not disturb habituation which is considered as an elementary form of learning. **CONCLUSION:** The present study suggested that GA applied subchronically to rats has a sedative effect and does not disturb the memory and learning processes.

Key words: Wistar rats, Opto Varimex apparatus, movements, habituation, sedative effect, learning, memory

INTRODUCTION

Galic acid (GA) also known as hydroxybenzoic acid is a phenolic acid, a polyphenol compound. The dietary sources are olives (1), berries, mango, plums, grapes, nuts, wine (2, 3). Many types of tea (black, green, white) are also an important source of GA (2,3). Polyphenols are represented by four main classes of substances – flavonoids, phenolic acids, stilbens and lignans. Plant polyphenols are able to access the brain via the blood brain barrier and represent novel therapeutic agents in central nervous system diseases (4). The ability of polyphenols and their metabolites to cross the blood brain barrier was demonstrated in 2010 by Janle et al. (5) as they found ¹⁴C-labeled plant

polyphenols in the brain tissue and brain microdialysate. There are findings that grape seed polyphenols, namely GA, catechin and epicatechin, do accumulate in the brain following long-term consumption (6).

The aim of the present study was to investigate the effects of the subchronic administration of GA on exploratory behavior and locomotor activity in male Wistar rats.

MATERIALS AND METHODS

Galic acid

Galic acid from the laboratories of Sigma Aldrich was used in the experiment.

Animals and treatment

Male Wistar rats (180-220 g at the beginning of experiments) were housed in polypropylene boxes with free access to food and drinking water. The experiments were carried out according to the rules of the Ethics Committee of the Institute of Neurobiology, Bulgarian Academy of Sciences, in compliance with the

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national policies and the EEC Directive of 1986 (86/609/EEC).

80 rats divided into 8 groups of 10 animals each were used for the experiments. The rats were treated orally through an orogastric cannula in the course of 7 days (one week), 14 days (2 weeks), 21 days (three weeks) or 30 days (one month). There were two groups of rats for each treatment period: Control and GA. The control groups were treated with saline 10 ml/kg. Rats from GA groups were treated with GA at a dose of 20 mg/kg as a 10 ml/kg solution.

Testing of exploratory behavior and locomotor activity

Exploratory behavior and locomotor activity were recorded in an Opto Varimex apparatus (Columbus Instruments, USA), according to the method of Köhler & Lorens (7). The experimental chamber was 50 cm × 50 cm × 25 cm. This apparatus records the number of photobeam interruptions while the animal moves. It provides selective counting of the number of horizontal movements (ambulation) and vertical movements (rearings) in arbitrary units (AU). The information obtained was automatically recorded every minute for the first 5 min of the test and for the next 5 min thereafter. The number of horizontal and vertical movements recorded every minute for the first 5 min served as a measure of exploratory activity and habituation to the new environment. The total number of movements during the first 5 min and during the whole 10-

min period of observation was used as a measure of locomotor activity. The experiments were carried out at the same time (between 9:00 a.m. and 1:00 p.m.). The rats were placed in the central quadrant of the activity monitor. The different groups were tested on the 7th, 14th, 21st and 30th day 60 min after the last treatment. Before each test, the apparatus was wiped clean and dried.

Statistical analysis

Separate t-tests were used to process the data obtained for horizontal and for vertical movements for the 1st, 2nd, 3rd, 4th and 5th min and for the whole 5-min and 10-min periods of observation. A level of p < 0.05 was considered significant. GraphPad Prism statistical software was used.

RESULTS

Effect of GA on exploratory behavior

Effect of GA on the horizontal movements for every minute during the first 5 min

After 7 and 14 days of treatment GA at a dose of 20 mg/kg had no significant effect on the number of horizontal movements for the 1st, 2nd, 3rd, 4th and 5th min as compared with the respective saline-treated controls. After the 21-days treatment period GA significantly reduced the number of horizontal movements for the 2nd (p < 0.05), 3rd, 4th and 5th min (p < 0.01). After the 30-days treatment period GA significantly reduced the number of horizontal movements for the 1st, 2nd and 3rd min (p < 0.05) (Figure 1).

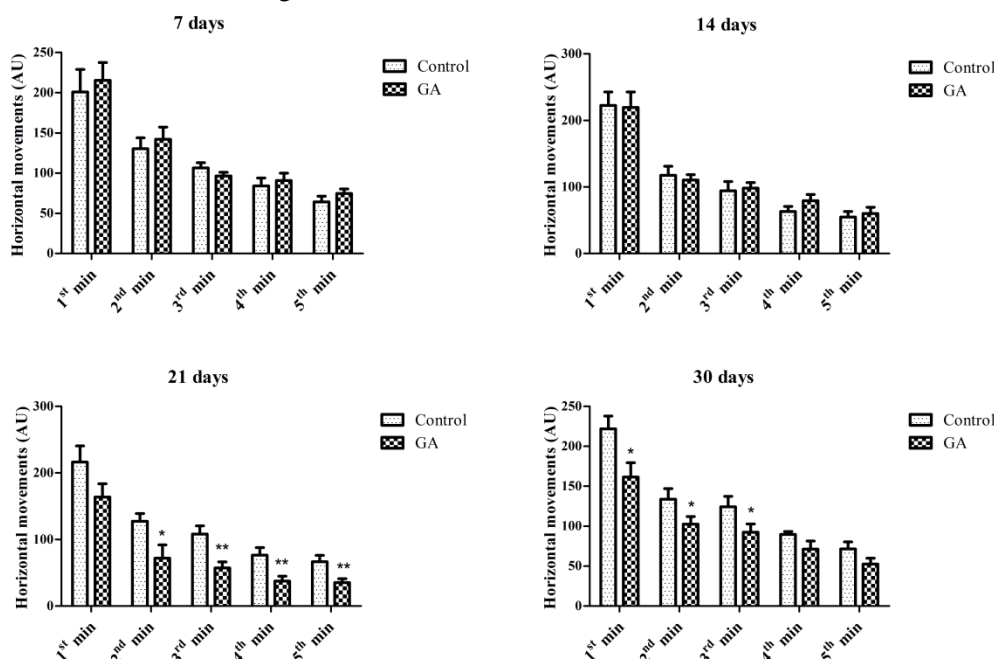


Figure 1. Effect of gallic acid (GA) at a dose of 20 mg/kg applied orally to rats for 7, 14, 21 and 30 days on the number of horizontal movements recorded every minute for a 5-min observation period; AU – arbitrary units; n = 10; *p < 0.05 vs control; **p < 0.01 vs control.

The number of horizontal movements is gradually reduced with time in every group independent of the kind and duration of treatment which shows that the habituation to the new environment is not disturbed (**Figure 1**).

Effect of GA on the vertical movements for every minute during the first 5 min

GA at a dose of 20 mg/kg had no significant effect on the number of vertical movements for the 1st, 2nd, 3rd, 4th and 5th min after 7 days of treatment as compared with the respective saline-treated controls. After the 14-days

treatment period GA significantly reduced the number of vertical movements for the 1st, 3rd and 5th min ($p < 0.05$). After 21 days of treatment GA significantly reduced the number of vertical movements for the 1st, 4th and 5th min ($p < 0.05$). After the 30-days treatment period GA significantly reduced the number of vertical movements for the 1st, 2nd, 3rd, 4th and 5th min ($p < 0.05$) (**Figure 2**).

The number of vertical movements gradually reduced with time for every group and every period of treatment which is a marker for habituation to the new environment (**Figure 2**).

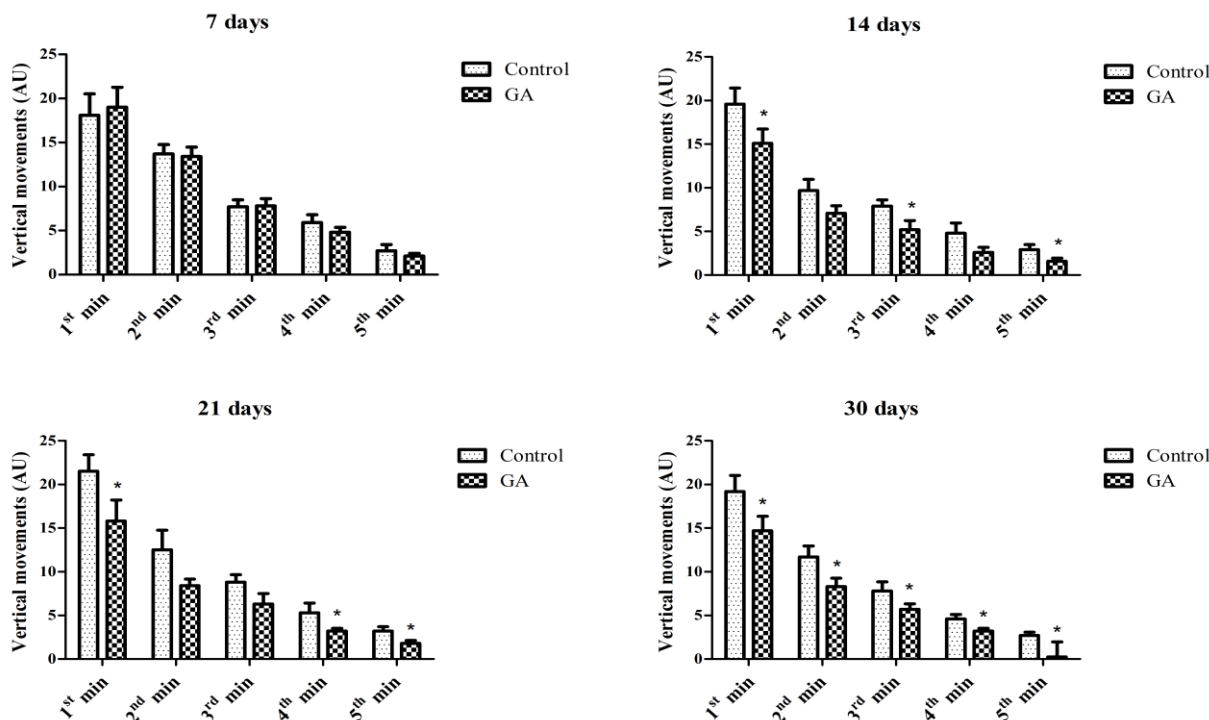


Figure 2. Effect of gallic acid (GA) at a dose of 20 mg/kg applied orally to rats for 7, 14, 21 and 30 days on the number of vertical movements recorded every minute for a 5-min observation period; AU – arbitrary units; n = 10; * $p < 0.05$ vs control.

Effect of GA on locomotor activity

Effect of GA on the number of horizontal movements for the periods of 5 min and 10 min

The post-hoc t-test revealed that GA administered to rats at a dose of 20 mg/kg for 7 and 14 days had no significant effect on the horizontal locomotor activity recorded during the first 5 min and during the whole 10-min observation period. It significantly reduced the number of horizontal movements for the 5-min period ($p < 0.001$) and for the whole 10-min period ($p < 0.01$) after 21-day treatment. After 30 days of treatment GA also significantly reduced the number of horizontal movements for the 5-min period and for the whole 10-min period of observation ($p < 0.01$) (**Figure 3**).

Effect of GA on the number of vertical movements for the periods of 5 min and 10 min

The post-hoc t-test revealed that GA administered to rats for 7 days had no significant effect on the vertical activity recorded during the first 5 min and during the whole 10-min observation period. It significantly reduced the number of vertical movements for the 5-min period and for the whole 10-min period ($p < 0.001$) when applied to rats for 14 days. GA also significantly reduced the number of vertical movements for the 5-min period and for the whole 10-min period ($p < 0.001$) after 21-day treatment period and significantly reduced the number of vertical movements for the 5-min period ($p < 0.01$) and for the whole 10-min period of observation ($p < 0.001$) after 30 days of treatment (**Figure 3**).

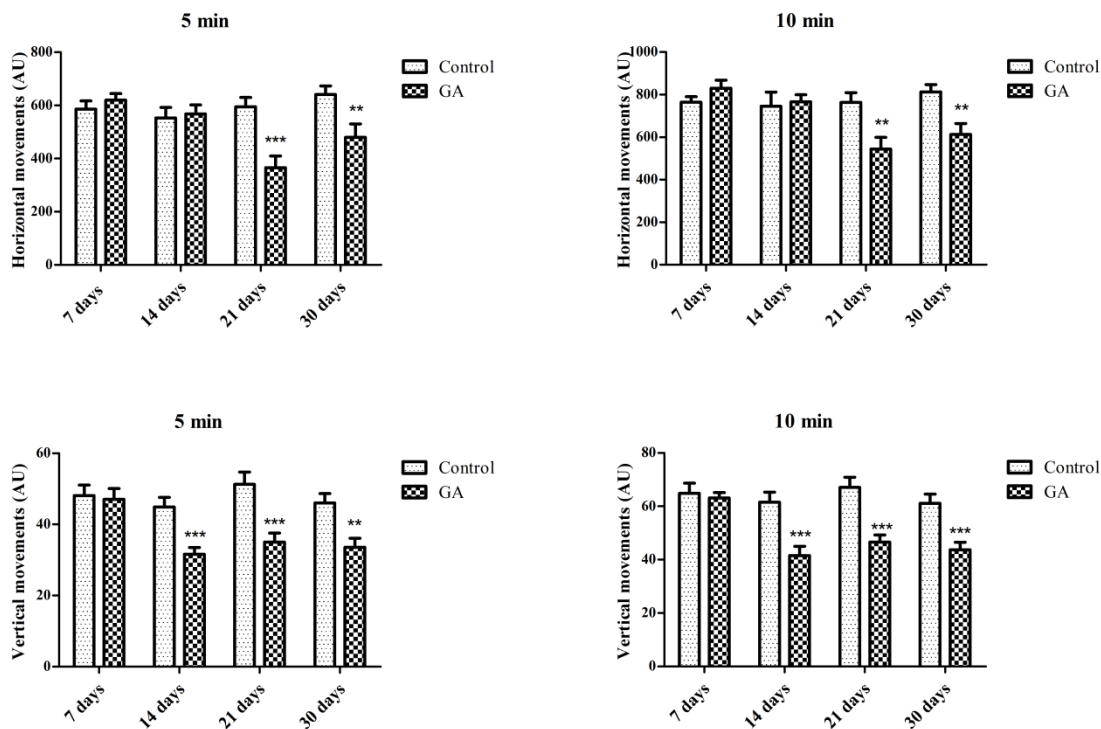


Figure 3. Effect of gallic acid (GA) at a dose of 20 mg/kg applied orally to rats for 7, 14, 21 and 30 days on the total number of horizontal and vertical movements recorded for the first 5-min period and for the whole 10-min period of observation; AU – arbitrary units; n = 10; **p < 0.01 vs control; ***p < 0.001 vs control.

DISCUSSION

In the present study, exploratory behavior and locomotor activity of rats were tested using an Opto Varimex apparatus. This test is a common measure of exploratory behavior and general activity in rodents (8). Its concept lies in the natural pattern of behavior of animals to highly explore an unfamiliar environment (the chamber of the apparatus) initially, and eventually habituate to it (9, 10). Exploration has been defined as active investigation (e.g., locomotion) that might lead to an animal gaining information about its environment (11). The short length of time emphasizes exploratory behavior (8).

The results from the present study showed that GA applied at a dose of 20 mg/kg for 14, 21 and 30 days reduced the locomotor activity of rats compared with the respective saline-treated controls. These changes in locomotor activity may be due to a sedative effect of GA. This is in some contradiction with the results of Mansouri et al. (2014) who found that GA in doses 30-300 mg/kg had an anxiolytic-like but not sedative effect (12). In the same study GA caused sedation in a dose of 500 mg/kg. The anxiolytic-like effect was comparable to that of benzodiazepine (BDZ) drug diazepam

and 5-HT_{1A} agonist buspirone. These findings suggest that the sedative effect of GA might be due to activation of BDZ receptors.

Habituation to a novel environment is considered an elementary form of learning, therefore the decreased exploration is taken as an index of memory (13, 14). The present study showed that GA for the four treatment periods did not compromise the habituation of rats to the new environment of the Opto Varimex apparatus. These results suggested that GA did not disturb memory. This is in coincidence with the data of Korani et al. (2014) who found that GA ameliorated the spatial memory deficit in a model of vascular dementia (15). Mansouri et al. (2013) found that GA prevented the cognitive deficit in a model of Alzheimer's disease (16). This beneficial effect on memory could be related to the antioxidant activity of GA and a possible neuroprotective effect (17, 18).

The accumulation of polyphenolic substances in the brain following long-term consumption could explain the absence of effect in the animals treated for 7 days and its onset after 14-day treatment with GA (19).

CONCLUSION

The results from the present study show that after subchronic administration GA reduced the locomotor activity and exploratory behavior of rats which might be due to a sedative effect. GA did not disturb the habituation of the animals to the new environment and thus did not adversely affect this elementary form of learning in young/healthy rats.

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