Original Article

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

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Summary

Ferulic acid (FA), called also hydroxycinnamic acid, is a polyphenol compound found in many foods such as coffee, apples, rice, oats and wheat. It acts as an antioxidant but its other activities *in vivo* have not been well studied as a whole. The aim of the present study was to investigate the effects of FA on exploratory behavior and locomotor activity in male Wistar rats. FA was administrated orally (20 mg/kg) to different groups of rats for 7, 14, 21 and 30 days. These groups were compared respectively with saline-treated controls. At the end of each experimental period, the changes in exploratory behavior and locomotor activity were recorded in an Opto Varimex apparatus (Columbus Instruments, USA). 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. It was found that FA at all doses for all treatment periods did not significantly affect exploratory behavior and locomotor activity of rats, when compared with the saline-treated controls. At all testing periods, FA did not disturb habituation. As habituation is considered an elementary form of learning, the present study suggested that FA did not disturb the memory and learning processes in rats.
Key words: ferulic acid, exploratory behavior, locomotor activity, rats

Introduction

Ferulic acid (FA) also known as hydroxycinnamic acid is a polyphenol compound (Figure 1). This phenolic acid is widely distributed in nature. The dietary sources are coffee, cereals, especially the wholegrain ones such as wheat and brown rice, some fruits (apples, pineapples, and kiwis) and vegetables (tomatoes, carrots). It is concentrated in the bran of grains, peels of fruits and vegetables, as well as in vegetable roots [1, 2].

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 the treatment for diseases of the central nervous system [3]. The ability of polyphenols and their metabolites to cross the blood brain barrier was demonstrated in 2010 by Janle et al. [4] who found ^{14}C-labeled plant polyphenols in the brain tissue and brain microdialysate. There are findings that polyphenols from berries do accumulate in the brain following long-term consumption [5]. Chang et al. [6] reported that free FA was recovered in the kidneys, lungs, liver, spleen, heart, uterus and brain (~2.6 μg/g) approximately 30 min after oral administration of 521 μ mol/kg FA in rats.

The aim of the present study was to investigate whether FA applied to male Wistar rats for different periods of time has an effect on exploratory behavior and locomotor activity.

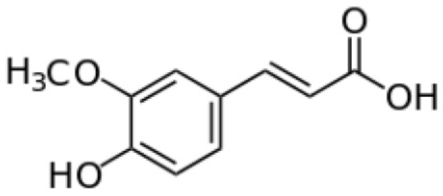

Figure 1. Ferulic (hydroxycinnamic) acid

Materials and Methods

Ferulic acid

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

The experiments were carried out on 80 rats divided into 8 groups of 10 animals each. 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). Rats from FA groups were treated with FA at a dose of 20 mg/kg as a 10 ml/kg solution. The control groups were treated with saline 10 ml/kg. There were two groups of rats for each treatment period: Control and FA.

Testing of exploratory behavior and locomotor activity

Exploratory behavior and locomotor activity of the animals were recorded in an Opto Varimex apparatus (Columbus Instruments, USA). The experimental chamber measured 50 cm×50 cm×25 cm. The apparatus records the number of photobeam interruptions while an 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 FA application. Before each test, the apparatus was wiped clean and dried.

Statistical analysis

Behavioural data were analyzed by analysis of variance (ANOVA). Separate two-way repeated measures ANOVA was used to process the data obtained for horizontal and for vertical movements between subject factors: drug (two levels: saline, FA) and time (five levels: 1st, 2nd, 3rd, 4th and 5th min). Separate one-way repeated measures ANOVA was used to process the data obtained for the total number of horizontal and vertical movements during the whole 5-min and 10-min periods of observation. ANOVA data were further analyzed by post hoc *t*-test. A level of $P<0.05$ was considered significant. GraphPad Prism statistical software was used.

Results

Effect of FA on exploratory behavior

Effect of FA on the horizontal movements during the first 5 min

The horizontal movements of the animals recorded every minute for the first 5-min period are presented on Figures 2A, 3A, 4A and 5A. Separate post-hoc *t*-test comparisons for each minute demonstrated that FA administered at a dose of 20 mg/kg for periods of 7, 14, 21 and 30 days had no significant effect on the number of horizontal movements.

Effect of FA on the vertical movements during the first 5 min

The numbers of vertical movements of the rats recorded every minute for the first 5-min period are presented on Figures 2B, 3B, 4B, and 5B. Separate post-hoc *t*-test comparisons for each minute demonstrated that FA applied at a dose of 20 mg/kg for periods of 7, 14, 21 and 30 days had no significant effect on the number of vertical movements in comparison with the respective saline-treated controls.

Analysis of changes in the number of horizontal and vertical movements per minute during the first 5 min showed that treatment with FA for all the periods (7, 14, 21 and 30 days) did not compromise the habituation of rats to the new environment of the apparatus (Figures 2, 3, 4, 5).

Thus, the results for the horizontal and vertical movements showed that FA applied orally to rats for 7, 14, 21 and 30 days did not significantly affect exploratory behavior and habituation of the animals.

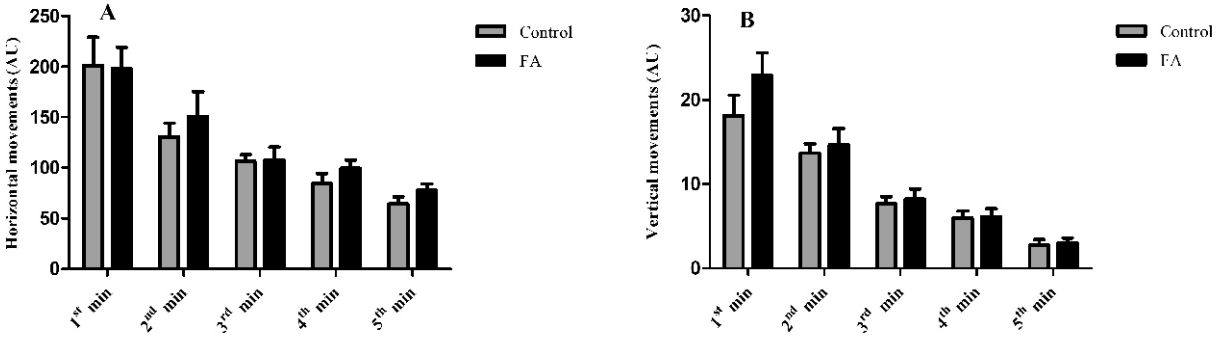

Figure 2. Effect of ferulic acid (FA) at a dose of 20 mg/kg applied orally to rats for 7 days on the number of horizontal (panel A) and vertical (panel B) movements recorded every minute for a 5-min observation period; AU – arbitrary units; n=10

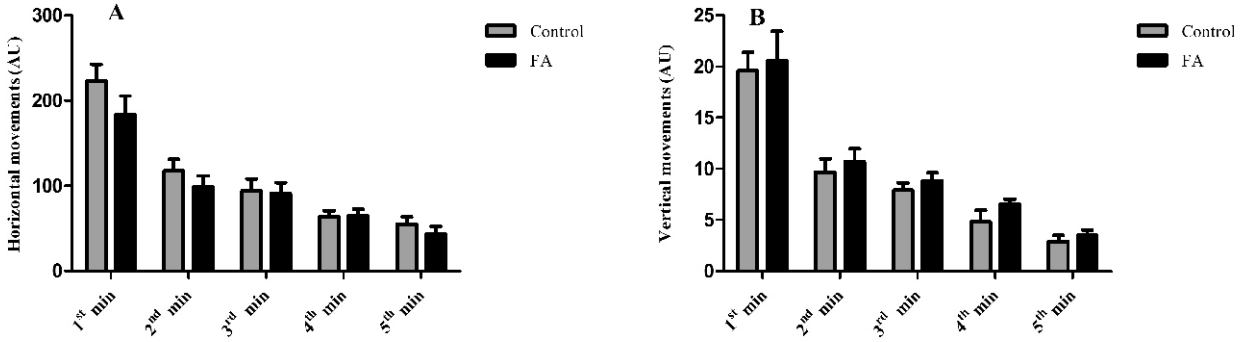

Figure 3. Effect of ferulic acid (FA) at a dose of 20 mg/kg applied orally to rats for 14 days on the number of horizontal (panel A) and vertical (panel B) movements recorded every minute for a 5-min observation period; AU – arbitrary units; n=10

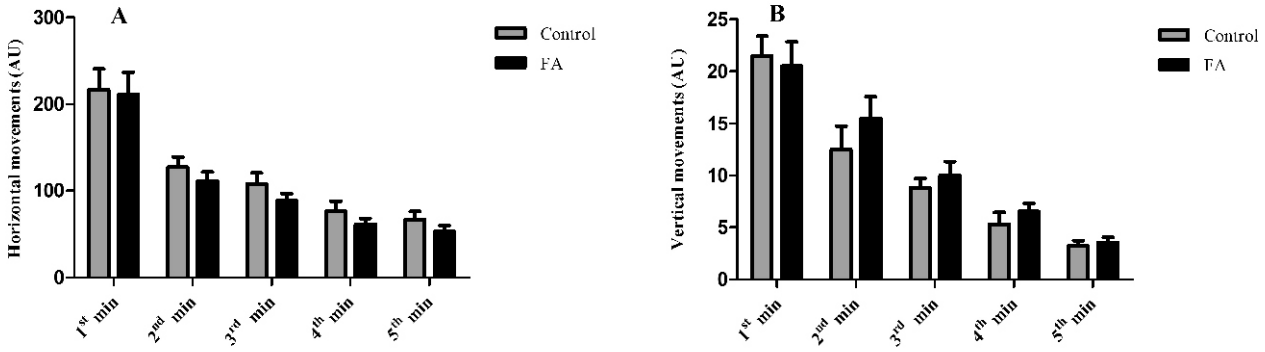

Figure 4. Effect of ferulic acid (FA) at a dose of 20 mg/kg applied orally to rats for 21 days on the number of horizontal (panel A) and vertical (panel B) movements recorded every minute for a 5-min observation period; AU – arbitrary units; n=10

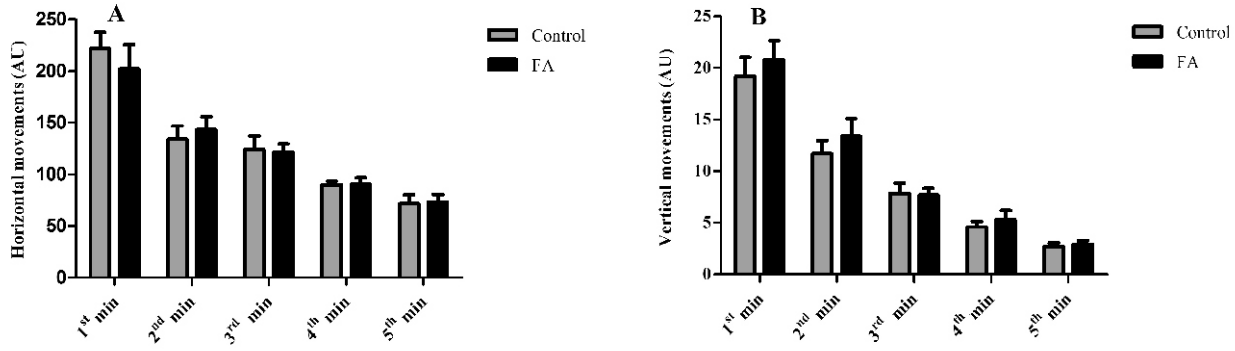

Figure 5. Effect of ferulic acid (FA) at a dose of 20 mg/kg applied orally to rats for 30 days on the number of horizontal (panel A) and vertical (panel B) movements recorded every minute for a 5-min observation period; AU – arbitrary units; n=10

Effect of FA on locomotor activity

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

The total control number of horizontal movements for the first 5 min on the 7th, 14th, 21st and 30th day was respectively 586.4±30.48, 552.8±39.39, 594.8±35.67 and 641.1±32.83. For the FA groups these values were 634±33.09, 482.9±28.44, 525.3±38.34 and 630.1± 0.94, respectively (Figure 6A). The post-hoc *t*-test demonstrated that FA (20 mg/kg) had no significant effect on the total number of horizontal movements for the first 5 min on the 7th day (t= .058, P⩽ 0.304), on the 14th day (t=1.439, P⩽ 0.167), on the 21st day (t=1.327, P⩽0.201) and on the 30th day (t=0.244, P⩽0.81) as compared with the respective saline-treated controls.

After the whole 10-min period of observation, the number of horizontal movements for the control groups was 764.5±25.59, 745.2±66.92, 763.6±45.76 and 812.7±34.23 on the 7th, 14th, 21st and 30th day, respectively. For the FA groups these values were 828.4±41.96, 661.1±27.08, 703.1±48.13 and 801.5±34.85 (Figure 7A). The post-hoc *t*-test revealed that FA administered to rats at a dose of 20 mg/kg for all periods (7, 14, 21 and 30 days) had no significant effect on the horizontal activity recorded during the 10-min observation period.

Effect of FA on the number of vertical movements during the periods of 5 min and 10 min

For the first 5 min, the number of the vertical movements of the control groups was 48.1±2.99, 44.9±2.68, 51.3±3.44 and 46.0±2.69 on the 7th, 14th, 21st and 30th day, respectively. The respective values for the FA groups were 54.8±4.36, 50.1±2.88, 56.0±4.88 and 50.1±4.62. The post-hoc *t*-test comparisons demonstrated

that FA (20 mg/kg) did not significantly affect the total number of vertical movements for the period of 5 min on the 7th day (t=1.268, P⩽ 0.221), 14th day (t=1.322, P⩽ 0.203), 21st day (t=0.787, P⩽ 0.442) and 30th day (t=0.38, P⩽ 0.709) as compared with the respective saline-treated controls (Figure 6B).

After the whole 10-min period, the control number of vertical movements on the 7th, 14th, 21st and 30th day was 64.8±3.84, 61.6±3.68, 67.1±3.79 and 61.1±3.48. For the FA groups these values were 74.0±5.34, 68.9±4.53, 72.7±5.78 and 67.0±5.01 (Figure 7B). Post-hoc *t*-test comparisons demonstrated that FA applied to rats at a dose of 20 mg/kg for all periods (7, 14, 21 and 30 days) did not induce significant changes in the vertical movements for the whole 10-min period of observation.

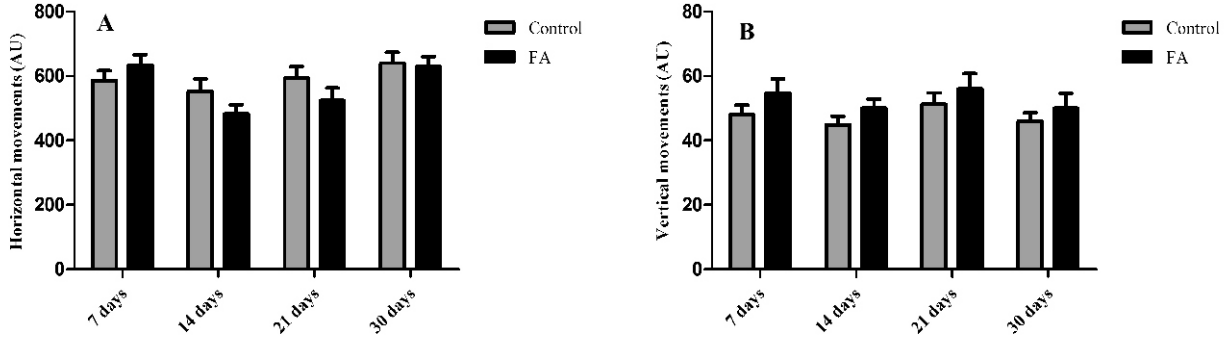

Figure 6. Effect of ferulic acid (FA) at a dose of 20 mg/kg applied orally to rats for 7, 14, 21 and 30 days on the total number of horizontal (panel A) and vertical (panel B) movements recorded for the first 5-min period; AU – arbitrary units; n=10

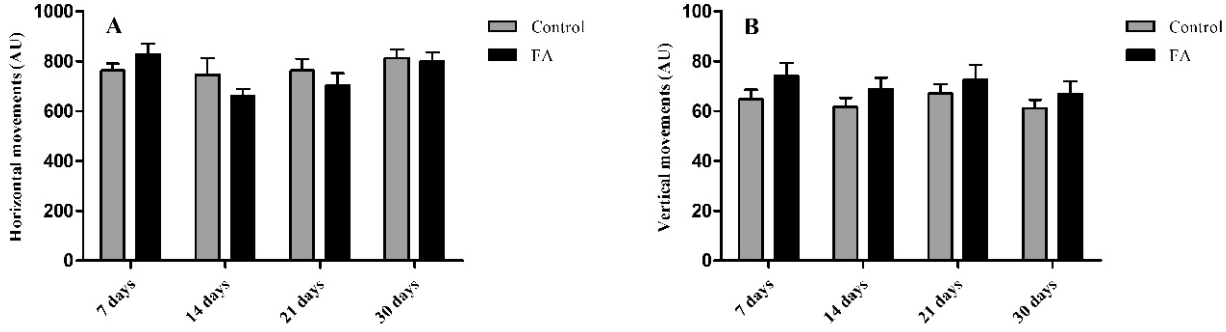

Figure 7. Effect of ferulic acid (FA) at a dose of 20 mg/kg applied orally to rats for 7, 14, 21 and 30 days on the total number of horizontal (panel A) and vertical (panel B) movements recorded for the whole 10-min period. AU – arbitrary units; n=10

Discussion

In the present study, exploratory behavior and locomotor activity of rats were tested when the animals were placed in an unfamiliar environment – the chamber of the Opto Varimex apparatus. This test is a common measure of exploratory behavior and general activity in rodents [7]. The expected pattern of behavior is that animals will tend to highly explore the novel arena initially, and eventually habituate to the environment [8, 9]. Exploration has been defined as active investigation (e.g. locomotion) that might lead to an animal gaining information about its environment [10]. The short length of time emphasizes exploratory behavior [7].

The results from the present study showed that FA at the dose of 20 mg/kg for 7, 14, 21, and for 30 days did not significantly affect the exploratory behavior and locomotor activity of rats. Based on these results we could suggest that the subchronic administration of FA did not cause sedation and motor discoordination, therefore it did not exhibit toxic effects on the central nervous system of rats.

In contradiction with this observation, the study of Tu et al. [11] demonstrated that FA showed dose-dependent sedative effects in normal mice.

The gradual decrease in exploratory behavior with time is the result of the habituation of animals to a novel environment. Habituation is believed to be an elementary form of learning, therefore the decreased exploration is taken as an index of memory [12, 13]. The present study showed that FA treatment for the four different periods did not compromise the habituation of rats to the new environment of the Opto Varimex apparatus. These results suggested that FA did not have any adverse effects on memory. This is in coincidence of our unpublished data that FA at this dose could improve memory. Furthermore, there is much evidence to suggest that polyphenols found in fruits and fruit juices have the capacity to improve memory [14-16]. This beneficial effect on memory could be in relation with the antioxidant activity of FA and its possible neuroprotective effect [17, 18]. There are data that long-term consumption of FA can offer protection from Alzheimer's disease and other cognitive defficits [19, 20].

Conclusions

The results showed that FA administered orally to rats for 7, 14, 21 and 30 days at a dose of 20 mg/kg had no effect on exploratory behavior and locomotor activity. FA at all the doses and testing periods did not disturb habituation. As habituation is considered an elementary form of learning, the present study showed that FA did not disturb the memory and learning processes in rats.

Acknowledgements

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