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The Altered Behavioural Responses Displayed by Litter Rats After Chronic Administration of Non-Toxic Concentrations of ZnTe to Parent Rats are Reversed by Simultaneous Folic Acid Treatment

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Trace elements are an omnipresent group of chemical elements that are present practically in all types of environments sustaining life. Since its principal characteristic is the very low concentration in ground and water, it was thought that its importance to metabolic processes to the living cell was minimal. However, in the past 15 years knowledge has been accumulated regarding that these chemical elements have important influences on the cell dynamic homeostatic mechanisms. Previous evidence from our laboratory has shown that chronic administration of ZnTe to pregnancy, delivery and subsequent juvenile stages in rats affected several of its behavioural parameters related to motivated, lateralized exploration, social and defensive behaviour. In the third part of this study, the possible effect of folic acid (FolA) on the Te-induced behavioural changes was studied. Three experimental groups were formed, Control, animals treated with tap water; ZnTe, animals treated with the trace elements, and ZnTe + FolA, animals treated with the combination of ZnTe and FolA, in the same way that of the previous experiments. Results show the folic acid treatment did not counteract the increase of motor activity observed in those animals treated with ZnTe. However, in the exploration induced by novelty measured in the Double Lateral Hole-board Labyrinth, the corridor behavioural activity displayed by animals under the combination of FolA and ZnTe was similar to control and significantly different from the ZnTe-treated group. The left exploration bias naturally present in control, and blocked in the ZnTe-treated animals, was restored to control values in the FolA + ZnTe treated animals. A similar observation was found with the percentage of animals with left-bias exploration, where those rats treated with the combination of FolA and ZnTe reached similar values to control and significantly greater than the ZnTe-treated rats which were statistically lower than control. Social behaviour, inhibited by ZnTe was restored to normal in the FolA treated animals. A similar observation was found in the defensive behaviour test. Results are compatible with the idea that FolA, which is known as a methyl donor reagent, can restore the behavioural effects of ZnTe, giving support to previous results suggesting that trace elements could act by molecular mechanisms involving epigenetical modulation of DNA.

Keywords: Defensive Behaviour, Lateralization, Trace Elements, Te, Zn, Folic Acid.

RESEARCH ARTICLE

1. INTRODUCTION

The study of the relationship between the environment and the organisms dates back from many years ago. The importance of animals, water supplies, and vegetation has been extensively investigated. However, the possible influence of very low concentrations of some ubiquitous materials, such as the inorganic chemical elements surrounding the living organisms, has passed practically unnoticed, with the exception of those cases where very high or very low concentrations of elements were found which have toxic or deleterious effects to living systems.[18, 30] Previous evidence from our laboratory has shown that ZnTe, in non-toxic concentrations administered in drinking water to pregnant rats along all gestation, delivery, lactation, weaning and preadolescent periods; exposed offspring showed two opposing main behavioural alterations.[28] On one hand; treated young rats displayed excitatory motor and selective motivated exploration responses in a behavioural automatic activity measuring device, and on the other hand; impairments in motivated lateralized behavioural

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display in a lateralized exploratory labyrinth; impairment of social interactions in a intruder-host territorial test, and altered defensive responses in a swimming test were observed.[28] These behavioural expressions were found to be associated with changes in the methylation patterns of DNA in the hippocampus but not in the prefrontal cortex of ZnTe-treated animals.[28] This evidence suggests that the complex behavioural display observed in the Te-treated animals is the final expression of intrinsic molecular events where DNA methylation and demethylation is some important step. Recent evidence has revealed that these dynamic processes participate in the molecular mechanism whereby transmission of DNA methylation signals after DNA replication is carried on to perpetuate patterns of epigenetic regulation to subsequent cell generations.[9] DNA methylation is a common regulatory process of expression of human DNA, occurring at cytosines within a 5′-CpG-3′ dinucleotide, when a methyl group from S-adenosylmethionine is enzymatically transferred to the 5 position of the pyrimidine molecule, generating 5-methylcytosine.[9,17]

Considerable concern was generated to understand these methylation regulatory molecular events having important effects to the life of organisms, together with increasing interest in those chemical agents that can modulate it. On this line, a surprising finding described for folate, a water soluble vitamin (B9), essential for health through early as advanced age[24] and well known by its vital role for development, regeneration and function of the nervous system was is implication as a methyl donor to methylation regulation of DNA.[8,9,14,16,20,29] Several dysfunctions induced by different stressors such as protein deficiency, drugs, or metabolic states can be reversed with the supplementation of folate.[2,7,33] Taking into consideration that in our studies Te appears to exert its effect by a methylation-demethylation mechanism,[28] and being responsible of the major behavioural alterations independently of Zn,[26] it was considered of interest to evaluate the hypothesis that the altered behavioural expression of the offspring induced by Te might be reversed by folate treatment.

2. MATERIAL AND METHODS

2.1. Animals

Rats of a Holzman-derived colony, weighing 250–300 g, 90 days old and maintained in thermoregulated (22–24 °C) and controlled light conditions (06.00 on–20.00 h off) were used. Standard rat chow and water were available ad libitum for control animals, but trace elements-treated animals received in addition to rat chow ad libitum zinc telluride and Folic acid in drinking water.

2.2. Experimental Design

The experimental protocol used in the present work was described previously.[28] Briefly, chronic exposition to trace elements beginning from birth up to prepuberal maturation stages of litter rats was applied. Zinc telluride (ZnTe, Sigma-Aldrich Co, U.S.A.) and Folic acid (FolA, Parafarm, Buenos Aires, Argentina) were used as chemical agents. As shown in Diagram 1, after mating, pregnant mothers were exposed to normal tap water (Control, Group 1, $n = 2$); water solutions of 0.3 μg/L ZnTe (1.55 nmol/L, Group 2, $n = 2$), and 2.3 mg/L FolA (4 μmol/L, Group 3, $n = 2$). Treatments were applied during all pregnancy, delivery, lactation, weaning and prepuberal periods of maturing rats. Thus, mothers and pups were continuously exposed to ZnTe, combination of ZnTe and FolA or water. At birth, pups were standardized to 10 animals per litter trying to maintain whenever possible the relationship of 1:1 of male to female rats. When maturing rats were 21 day-old (Day 42 of treatment), young rats were weaned and separated from their mothers. At 30 day-old (Day 51 of treatment) young rats of both sexes were subjected to a battery of behavioural tests in order to evaluate general motor activity, motivated exploration, lateralized, social, and defensive behaviour in the same way as previously described.[28] Total number of young rats used in all experiments was 10–15, since some animals were lost for reasons not related to experimental treatments. After ending the behavioural tests, all animals were sacrificed by decapitation.

2.3. Behavioural Tests

The following behavioural tests were used to evaluate exploration of novel environments, lateralization, preferential decisions, and defensive behaviour.

2.3.1. The General Activity and Exploratory Behaviour Detector (OVM)

It consists of rectangular open-field with acrylic walls, equipped with infra-red detectors and digital counting devices for measuring animal activity (Optovarimex instruments, U.S.A). Device was enriched with holes in the floor,

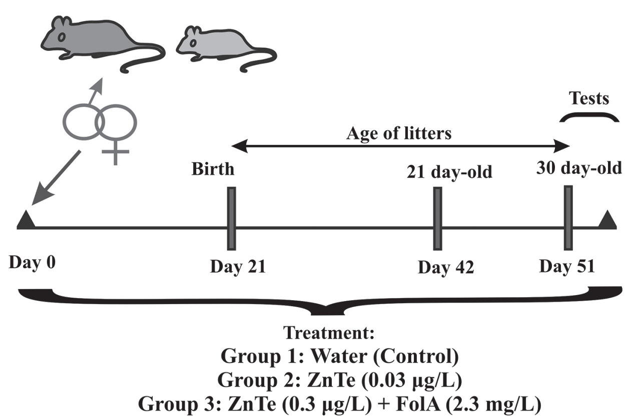

Diagram 1. Experimental setup showing the experimental groups used in the present work. Details, see the text in Section 2.2 (Experimental design).

and a tube rack as novelty object as described in detail previously.[28]

Variables were:

(1) Ambulatory behavioural activity, the motor activity displayed by animals while they move in any direction of the arena during exploration, as measured by automatic digital counting proportional to the time of active movement.

(2) Non ambulatory behavioural activity, all movements that animals display remaining in one position, without displacement as measured by automatic digital counting proportional to the time of behavioural movement. Grooming and sniffing are the main behavioural components of this variable.

(3) Head-dipping, counted as frequency of head dips into any of the four holes of the OVM hole-board when this animal behaviour lasted at least 2 seconds.

(4) Rearing, counted as frequency of animal's rears, standing still on his rear feet and leaned on the walls of the OVM hole-board cage, sniffing to the air for at least 2 seconds.

(5) Focalized exploration, measured by digital counting proportional to time at a rate of 2 Counts/sec when the animal sniffs, touches with its front feet, climbs over the tube rack or explores the holes of the rack.

Variables (3), (4) and (5) were measured by an expert observer unaware of treatments. Test was applied to single animals and had a total duration of 5 min.

2.3.2. The Double Lateral Hole-Board Labyrinth (DHBL)

This labyrinth evaluates motivated exploration that can be expressed in lateralized form, as described previously.[1, 28]

DHBL is made of wood and is composed by a rectangular cage 39 cm wide, 70 cm length and 15 cm height. Inside there are two compartments disposed in 90° each. The first compartment (Initial) has 39 cm length and 15 cm wide with a central entrance to the second compartment (Corridor). Corridor has 55 cm of length, 17 cm wide, and on its side walls there are 4 lateral holes, each 3 cm in diameter. In this test behavioural activity of animals was driven only by exploratory motivation induced by novel environments. Variables measured were:

(1) Corridor behavioural activity. All behaviours displayed by rats while they are in the corridor of the labyrinth measured by a digital automatic counter (counting rate 2 counts/sec) monitored by an observer unaware of treatments as described previously.[28]

(2) Initial Compartment behavioural activity.

(3) Lateralized exploration.

(4) Non-exploratory activity.

In this test, behavioural laterality was considered to be present when the median of lateralized exploration on one side of the walls statistically outnumbers the opposite exploration.

Test was applied to single animals and had a total duration of 3 min.

2.3.3. Forced Swimming Test

This test measures the defensive behavioural response of animals subjected to a stressful situation represented by active swimming in a closed environment having no escape.[28] Device consists of a transparent acrylic tube measuring 50 cm height by 12 cm diameter (internal diameter), filled with water at room temperature up to half of the cylinder height, as described previously.[28] Variables measured were:

(1) Active swimming activity, measured by digital automatic counting device at a rate of 2 Counts/sec monitored by an expert observer unaware of treatments.

(2) Immobilization, the time lapse where animals do not swim, floating without movements or displaying slow motion of its extremities enough to avoid sinking into the water. Since test had a total duration of 3 min (360 Counts), this behavioural activity was obtained by subtracting the active swimming activity from total counting.

2.3.4. The Social Interaction Test

This test (intruder-host territorial test) measures the social display of two interacting rats in a determined territory challenge by an intruder. Test was performed in a rectangular steel cage (26 cm width, 42 cm long and 20 cm height) with wood shavings in the floor. Total duration of testing was 5 min, as described previously.[28] Variables measured were:

(1) Latency to interact, time measured by digital counting that the host animal takes to face the intruder.

(2) Number of contacts, frequency of contacts displayed by the interacting animals

(3) Percentage of "α" contacts, number of "α" episodes displayed by the host to the intruder. An alpha episode is defined as an interaction initiated and addressed to the intruder by the host animal. When the behaviour is reversed, the behavioural display of the host is considered as submissive acceptance and named "β" activity. Percentage was calculated by dividing α activity by total number of contacts $(\alpha + \beta)$. This variable measures the natural capacity of coping and territorial defensive behaviour of animals under testing.

(4) Duration of α contact, time measured by digital counting of the duration of α social interaction displayed by the host animal in the test.

All behavioural tests were filmed by with a digital video camera, and recorded in a DVD player/recorder Phillips, model DVDR3455H.

2.4. Experiments

The following experiments were performed.

RESEARCH ARTICLE

2.4.1. Effects of Chronic Administration of Te or Te + FolA on General Motor and Motivated Behaviour

In this experiment the influence of Zn and FolA, in single or combined administration on general motor and motivated behaviour induced by novelty was evaluated. Measuring of the behavioural activity was performed using the OVM device.

2.4.2. Effects of Chronic Administration of Te or Te + FolA on Lateralized and Motivated Behaviour

In this experiment the influence of Te and Te + FolA, in single or combined administration on lateralized and motivated behaviour induced by novel environment was evaluated. Measuring of the behavioural activity was performed using the LDHB.

2.4.3. Effects of Chronic Administration of Te or Te + FolA on Defensive Behaviour

In this experiment the influence of Te and Te + FolA, in single or combined administration on defensive behaviour was evaluated. Measuring of the behavioural activity was performed in the forced swimming test.

2.4.4. Effects of Chronic Administration of Te or Te + FolA on Social Behaviour

In this experiment the influence of Te and Te + FolA, in single or combined administration on social behaviour was evaluated. This behavioural activity was measured in the intruder-host territorial test.

2.5. Statistical Analysis

Multiple comparisons for behaviours between experimental groups, was made by the Non Parametric Test of Dunn.[6] When comparisons involved paired groups, the Mann-Whitney Test was used. The significance of single percentage differences was analyzed by the Binomial Distribution (The Sign Test). A p value of less than 0.05 was considered as statistical significant. Results are presented as the mean ± standard error of the mean in the horizontal, ambulatory and non ambulatory activities; for head dipping, rearing and focalized exploration in Experiment 1, the median ± standard error the median was used. With exception of percentage of animals in Experiment 2.4.2 (Fig. 2(C)), in all the other experiments data are expressed as the median ± standard error of the median.

2.6. Ethical Care of Animals

The present experimental protocol was revised and approved by the Comité Institucional de Cuidado de Animales de Laboratorio (Institutional Committee of Care and Welfare of Experimental Animals) of the Faculty of Medical Sciences, Universidad Nacional de Cuyo (CICUAL).

3. RESULTS

ZnTe, as previously described and FolA solutions did not affect daily beverage consumption nor induce any malformations in newborn rats.[28]

3.1. Experiment 1

The general motor activity and motivated behaviour of rats chronically exposed to ZnTe, ZnTe + FolA or water in the OVM is shown in Figure 1. Horizontal motor activity was significantly increased in those animals treated with ZnTe compared to control (Fig. 1). Treatment of rats with the combination of FolA and ZnTe did not modify the effect found with ZnTe alone. The ambulatory and the non ambulatory activity in those animals treated with the trace elements or the combination of FolA and ZnTe was also increased compared to control (Fig. 1). The exploratory activity induced by novelty in the three experimental groups in the OVM is shown in Table I. ZnTe induced a significant increase in head-dipping, compared to control ($p < 0.01$), which was not modified by the combination with FolA (Table I). Rearing was also affected by the ZnTe treatment ($p < 0.01$ vs. control) but FolA effectively blocked this effect. Finally, regarding to the focalized exploration, the increase observed in those animals treated with ZnTe was also found in those rats treated with FolA and ZnTe.

3.2. Experiment 2

The motivated and lateralized behaviour induced by novelty of rats chronically treated with ZnTe, (ZnTe + FolA) or water in the DHBL is shown in Figure 2. Behavioural activity displayed by animals in the corridor was significantly decreased by ZnTe; effect that was blocked by

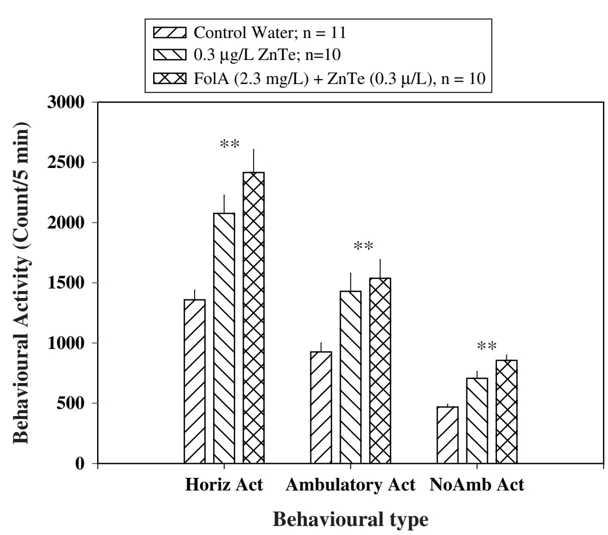

Fig. 1. General motor activity in the OVM of rats subjected to water, ZnTe or (FolA + ZnTe) treatments. Horiz Act = horizontal displacement of rats; Ambulatory Act = ambulation of rats; NoAmb Act = non ambulatory motor display. **Statistically different from control, $p < 0.01$.

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Table I. Motivated exploratory parameters in the OVM in rats subjected to water, ZnTe and FolA + ZnTe treatments.

	Groups		
Behavioural activity	Control ($n = 11$)	ZnTe ($n = 10$)	(FolA + ZnTe) $n = 10$
Head-dipping (frequency/5 min)	4.5 ± 0.6	$7 \pm 0.7^{**}$	7 ± 0.8
Rearing (frequency/5 min)	13 ± 1.4	$18 \pm 2.7^{**}$	9 ± 1.7
Focalized exploration (Counts/5 min)	43 ± 7	$73 \pm 18^{**}$	$113 \pm 19^{**}$

Note: **Significantly different from control, $p < 0.01$.

the simultaneous treatment with FolA (Fig. 2(A)). The non exploratory behaviours displayed by rats in the corridor were not affected by ZnTe or the combination of ZnTe + FolA. Instead, the behavioural activity in the initial compartment was increased by ZnTe or the combination (ZnTe + FolA) compared to control.

In the lateralized exploration, the natural left bias presented in control was blocked by the ZnTe treatment, but it was present in the (ZnTe + FolA) treated animals (Fig. 2(B)). Regarding the proportion of animals showing left-bias exploration in the three experimental groups; control animals showed a percentage significantly above random; ZnTe treated animals showed a percentage not different from 50%, and the (ZnTe + FolA) group showed a percentage significantly higher than 50% (Fig. 2(C)).

3.3. Experiment 3

The defensive behaviour induced by a forced swimming challenge in rats chronically treated with ZnTe, (ZnTe + FolA) or water is shown in Figure 3. Control animals showed an active swimming activity about two times higher than the immobilization activity (Fig. 3). ZnTe treatment inhibited this spontaneous balance, and animals displayed about the same response for both behaviours. (ZnTe + FolA) treatment restored to normal the active swimming response about in the same proportion than in control group (Fig. 3).

3.4. Experiment 4

The social behaviour (intruder-host territorial challenge) in rats chronically treated with ZnTe, (ZnTe + FolA) or water is shown in Figure 4. Control animals showed a rather short latency to confront the intruder (about 10 Counts/3 min) and about 73% of α-behaviour with a total duration of 85 Counts/3 min (Figs. 4(A–C)). The ZnTe group, instead showed an increased latency to confront the intruder; decreased percentage of α-behaviour, and decreased duration in the same activity, significantly different from control. The (ZnTe + FolA) treatment restored to normal all the behavioural parameters in this social interaction test (Figs. 4(A–C)).

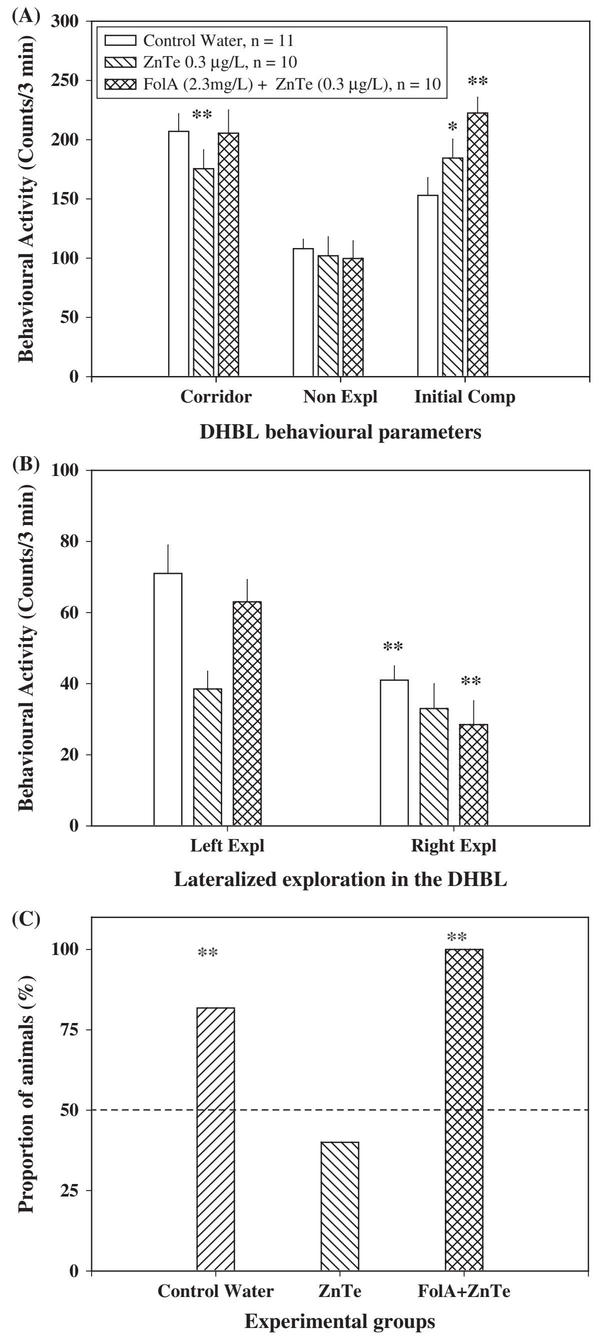

Fig. 2. Behavioural parameters in the DHBL of rats subjected to water, ZnTe or (FolA + ZnTe) treatments. (A) Variables of general exploration; Non Expl = non exploratory activity; Initial Comp = behavioural activity in the initial compartment. See text for details. $^{**}p < 0.01$ versus control; $^{*}p < 0.05$ versus control. (B) Lateralized exploration; Left Expl = exploration of the left side of the labyrinth; Right Expl = exploration of the right side of the labyrinth. $^{**}p < 0.01$ versus left side of the corresponding group. (C) Percentage of animals showing left side exploration preference; $^{**}p < 0.01$ versus 50%, considered random.

4. DISCUSSION

Folic acid (vitamin B9) is a widespread factor in nature composed by three molecular moieties; pterin, *p*-aminobenzoic, and glutamic acid molecules. The pterin

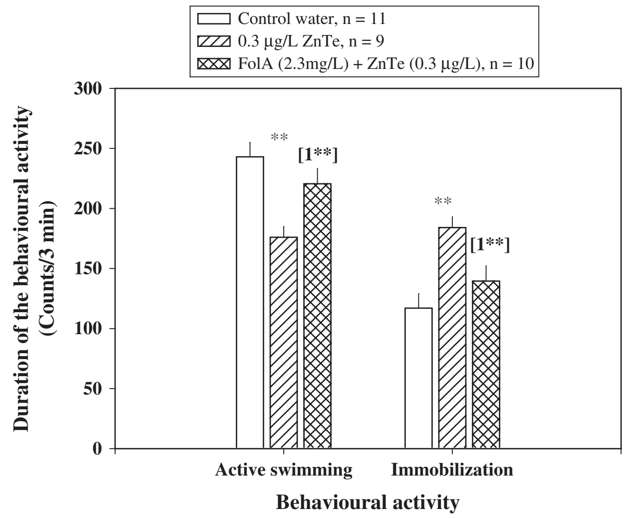

Fig. 3. Defensive behaviour in the forced swimming test of rats subjected to chronic administration of water, ZnTe or (FolA + ZnTe). [1**] $p < 0.01$ versus ZnTe group. ** $p < 0.01$ versus control. Additional details, see Material and Methods section.

moiety is subjected to redox reactions giving several related products which have biological activity and the glutamic acid portion can add several units of the same chemical kind originating polyglutamylated forms which also are bioactive. It has been found that folate metabolic pathway is critical for the development, regeneration cell processes and adequate functioning of the nervous systems.[14] Folate also is considered one of the major intracellular one-carbon carrier, and through several biochemical reactions is the most important molecule maintaining the flux of methyl groups to numerous methylation reactions such as methylation of DNA, RNA, neurotransmitters, phospholipids, and histones.[9, 31] In spite that folic acid is involved in many metabolic reactions, its role in the methylation-demethylation of DNA has called the attention of many workers, since this biochemical process is characteristic of epigenetic mechanisms in the cell.[9, 16, 25] The molecular details of folate mechanisms of action are not well understood, but are in part depending on the molecular activity of the high affinity folate receptor 1, and de novo DNA methyltransferases biosynthesis.[14]

As previously described, the biological effect of non toxic concentrations of Te has in common with the folate actions in the organism, the epigenetic process of methylation of DNA.[28] Thus, under this perspective it is reasonable to interpret the present results in terms of metabolic folate interaction with Te.

4.1. Experiment 1

As shown in Figure 1, all general motor activity parameters were affected by ZnTe treatment, and application of FolA did not modify these behavioural responses. A first impression regarding this finding can be that these behavioural parameters are not depending of Te,[28] or if Te

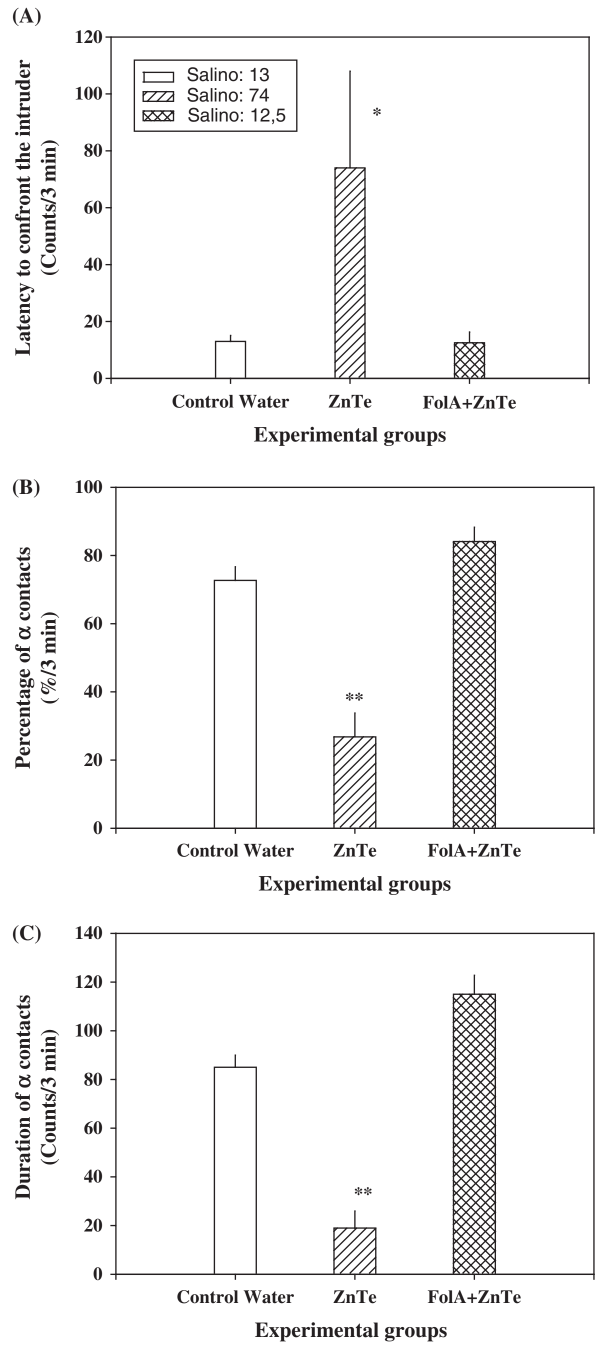

Fig. 4. Social activity of rats subjected to water, ZnTe or (FolA + ZnTe) treatments. (A) * $p < 0.05$ versus control. (B) ** $p < 0.01$ versus control. (C) ** $p < 0.01$ versus control.

participation nevertheless is present it is mediated by some mechanism not depending on demethylation of DNA. This conclusion receives some support from evidence with the same experimental schedule, where the increase in the ambulatory activity observed in the ZnTe treated rats is due to Zn and not to Te,[26] suggesting that possibly the Zn influence on behaviour might have a different mechanism. However, as shown in Table I, head-dipping and rearing increased by ZnTe were blocked by the FolA treatment, suggesting that at least these behaviours might be related to methyl availability processes.

4.2. Experiment 2

As previously described,[28] the behavioural parameters measured in the DHBL globally are evaluating several behaviours related primarily to inspection of novel environments (exploratory motivation). The spontaneous left-biased exploration is the final output of complex mechanisms in the brain related to the differential activation of paired neural structures, processes generally known as functional lateralization.[1,13,28] The existence of this property in animals originate the natural bias for using left or right behavioural alternatives observed in several species.[13] As shown in the present results (Fig. 2), behavioural activity of corridor, initial compartment, left-biased exploration and the proportion of animals showing left-bias exploration were affected by ZnTe in complete agreement with previous results,[28] and restored to normal values in the FolA-treated rats. These data support the idea that the complex mechanisms of Te are depending on methyl-demethylation equilibrium in the cell.

4.3. Experiment 3

Animals exposed to stressing situations usually display an escape reaction.[3] The magnitude or the intensity of this response gives to the animal a higher probability to avoid the noxious stimulus. Escape responses are part of a complex process identified as defensive behaviour, setting in motion many internal mechanisms involving some brain structures such as the amygdala and the hippocampus.[10] It is worthwhile to mention that these behaviours affected by ZnTe (Fig. 3), were restored to control values in the (FolA + ZnTe) group. It is not clear how FolA may affect the defensive response displayed by animals in the forced swimming test. However, it is possible that the increased synaptic transmission of neurons of the brain due to folate administration can change the response level of some behaviours, since it is known that *in vitro* experiments, folate can increase the neuronal excitability.[11] Other way to see these results is to speculate that (FolA + ZnTe) treatment in rats restored the normal epigenetic processes affected by trace elements in the animals.

4.4. Experiment 4

Social behaviour represents one of the most relevant adaptation responses to animals living in communities, generating an optimization of group living.[32] The study of its physiology is quite relevant because its impairment is the main symptom in many psychiatric diseases in humans. In the Intruder-Host Territorial Test, resident rat (the host) displays a "dominant" role, identified as "α" and confronts the intruder in a very short time after the new animal is put into the cage. As shown in Figure 4, latency to confront the intruder; percentage of α contacts and duration of α activity, affected by the ZnTe treatment were restored to normal values by FolA in the present experimental conditions. In spite that these processes have been intensively studied in the past years, still the precise brain mechanisms are not completely understood. Neuromodulators, such as monoamines and neuropeptides can alter the functional properties of some brain circuits changing the firing activity of neurons provoking excitatory or inhibitory states which at last influence the social behaviour.[12,19] It is not know if folate influence on the restoration of social behaviour in the present study might be due to the direct action of folate on neuron excitability in selected brain structures, or to a complex consequence of methylation-demethylation processes. If this later possibility is true, it can be interpreted as restoration of the epigenetic control in the (FolA + ZnTe) group. Additional complementary evidence will be gained with the molecular biology of DNA methylation patterns. Blood and brain samples have been obtained from the three experimental groups and biochemical processing is under way. It is expected that in the near future a more definitive answer can be available.

5. FINAL REMARKS

Although there is agreement that FolA is an essential vitamin for health during most of life period of organisms,[7,14,15,24] there is some evidence that folate administration can induce deleterious effects,[20,21,23] giving rise to a problem of clinical concern regarding if FolA treatment is or is not appropriate. Some studies have found that FolA did not alter the usual physiological parameters in ovine fetuses in uterus, where it is well known that FolA has a vital role.[5,22] It is possible that part of the divergent results might be due to different experimental models, administration route and doses of FolA.

In the present study, animals were exposed to an approximate dose of 46 ± 2 μg of FolA/day/rat (4 μmol/day/rat), as estimated by the amount of water solution ingested daily by the animals. This amount is well in excess compared with the offer of ZnTe, as initially designed in the experiments of competence between ZnTe and FolA. From a theoretically point of view, it was considered that this unbalanced ratio of FolA/ZnTe, even if disparities in biological activity in molar basis might exist, it would be enough to overcome one action over the other. It is difficult to decide if dose of FolA administered in the present experimental design is a "very high toxic dose" for rats. In some nutritional studies, it was considered that 100 μg/100 g body weight is a "physiological" or adequate dose for pregnant rats, which can be supplemented up to 500 μg/100 g body weight.[4] In other studies, where moderate high folic supplementation was sufficient to induce some adverse effects in rats, a dose of 2500 μg/100 g body weight was used.[23] In the present work, rats received an approximate dose of 57 μg/100 g body weight. On this line of reasoning,

RESEARCH ARTICLE

it appears that dose of FolA was not enough in our rats to provoke effects that can be considered toxic.

Thus, the most significant conclusion regarding the experiments performed in this study was the notion that the altered behavioural effects observed in those animals treated with non-toxic concentrations of ZnTe, supposedly mediated by alterations of methylation patterns of DNA[28] have been in great part reversed by the simultaneous administration of folate which has been described to act on the methylation-demethylation equilibrium in the cell. Since the cognitive, social and defensive tests used in the present study are animal models to alike behavioural display in humans as already described in previous studies,[28] the magnitude of the FolA results give an interesting perspective about environment and epigenesis regulation.

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RESEARCH ARTICLE

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