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The Behavioural Responses Displayed by Litter Rats After Chronic Administration of Non-Toxic Concentrations of ZnTe to Parent Rats are Mediated Primarily by Te

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Trace elements are an omnipresent group of chemical elements that are found 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, and defensive behaviour. In the second part of this study, Zn and Te were examined to discern which of both elements could be responsible of the behavioural changes observed previously. ZnTe and $ZnCl_2$ were used in chronic administration. All groups of rats were examined under 4 different experimental approaches: (1) general motor activity and motivated exploration; (2) lateralized exploratory activity; (3) defensive behaviour, and (4) social behaviour. Results shown that Te specifically increased motivated behaviour; blocked the spontaneous left-biased exploration, escape and social responses related to territorial challenges. On the other hand, Zn increased the ambulatory activity, rearing and focalized motivated behaviour, and modified differently some behavioural parameters associated to exploration. Present data are in agreement with previous results, and support the concept that trace elements can modulate behaviour by brain mechanisms that appear to be selective.

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

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 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 disease relevance to living systems.[22, 32] For instance, selenium, that initially was considered a toxic element for human health is present in a special type of proteins, the selenoproteins, where it is specifically incorporated into the amino-acid cysteine, forming selenocysteine.[8] It is interesting to note that several selenoproteins are expressed in the brain, suggesting that this type of proteins might be participating in selected brain functions, not specifically elucidated at the present time.[8, 26] Selenium also has been found to be critical to immunological and thyroid functions in vertebrates.[18, 19]

Other examples are illustrated by zinc, which it has been found to be needed for brain development, and its deficiency induces impairment of neuronal precursor cell proliferation in the rat.[10] Zinc, also stabilizes the finger-loop domain in DNA-binding proteins, suggesting a sophisticated molecular control on the genetic code carrier molecule of DNA.[4] Tellurium, another trace element frequently found associated with copper and sulfur-bearing ores,[25] has not been recognized actually as an essential element to living beings in spite that it is present in some plants of the *Alium* family, and specific disorders have

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been associated to ingestion of the metalloid in animals, suggesting a possible role in biological systems.[6, 11, 12, 27] Previous evidence presented by our laboratory has shown that ZnTe in non-toxic concentrations administered in the drinking water to pregnant rats along all gestation, delivery, lactation, weaning and preadolescent periods; exposed litters showed that ZnTe treatment produced two opposing effects.[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 and lateralized behavioural display in a lateralized exploratory labyrinth. In other different test measuring defensive behaviour, natural defensive responses were attenuated in ZnTe treated rats.[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] Such a selective behavioural and molecular change in treated animals poses the question about who of the chemical elements can be the responsible of the observed effects; Zn, Te or both. This is not an apparent trivial question, because what is known about the mechanism of action for both trace elements appears not to be the same.[21, 24, 10, 11, 15, 34, 27] Thus, theoretical projections which could have medical or physiological importance might be misleading if one assumed mechanism for one element is attributed to the other.

The objective of the present work was to discern which of the trace elements in the chronic administration schedule can be responsible of the behavioural effects observed and described previously.[28]

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 an equivalent concentrations of zinc chloride or zinc telluride 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 Zinc chloride ($ZnCl_2$, Dalton Co., Ind. Argentina) were used as trace elements (Diagram 1). As shown in Diagram 1, trace elements treatment applied to experimental groups, Zn chemical element administration was common for the two groups at the same equivalent dose than Te; but only one group received Te.

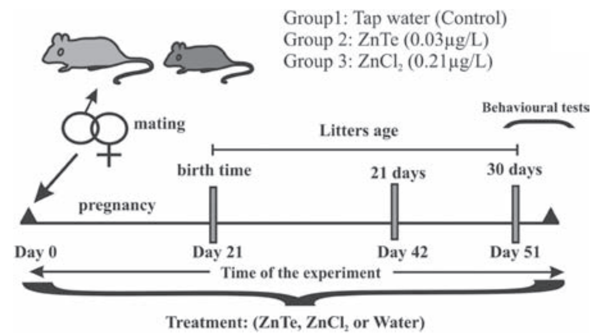

Diagram 1. Simplified scheme showing trace elements administration protocol applied to animals in the different experimental groups. All treatments began after mating the parent rats. Day 0 was considered when sperms were found in vaginal smears. Trace elements were administered in the drinking water. Further details, see Materials and Method Section.

In this way it was possible to compare the relevance of Te compared to Zn.

As shown in Diagram 1, after mating pregnant mothers were exposed to normal tap water (Control, Group 1, $n = 2$); water solutions of 1.55 nmol/L (0.3 μg/L) ZnTe (Group 2, $n = 2$), and 1.55 nmol/L (0.21 μg/L) of $ZnCl_2$ (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, $ZnCl_2$ or water. At birth, pups were standardized to 10 animals per litter trying to maintain whenever when 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 18–20, 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, and a tube rack as novelty object as described in detail previously.[28]

Five variables were selected. Two of them are general estimates of motor and ambulatory activity displaying the

general state of animals, and the other three are indices of motivated exploratory activity. 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. The following variables were measured:
(1) Corridor behavioural activity. All behaviours displayed by rats while they are in the corridor of the labyrinth, such as walking, rearing, head-dipping, and sniffing on the left or right side walls, including non-exploratory behaviours such as grooming and immobilization measured by a digital automatic counter (counting rate 2 counts/sec) monitored by an observer unaware of treatments.
(2) Initial Compartment behavioural activity. It is included in this measure all the behavioural activity displayed by rats while they were in this compartment. This activity was not measured directly and was calculated by subtracting corridor behavioural activity counting from the total counting of the test (3 min = 360 counts).
(3) Lateralized exploration. It is included in this variable all behaviours related to exploration displayed when the animal chooses one side of the corridor during exploration. Behaviours included:
(i) Walking nearby the left or right wall of the corridor, at constant speed, with vibrissae touching the wall.
(ii) Lateral head-dipping.
(iii) Rearing against the left or right walls of the corridor. This score was measured in the same way than Corridor Behavioural Activity.

(4) Non-exploratory activity. It is included in this variable the following behaviours:
(i) Immobilization at any site of the corridor; walking at the center of the corridor not approaching to any side wall.
(ii) Grooming.

Non-exploration activity was calculated by subtracting the lateralized exploratory activity from the corridor behavioral score.

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. Two variables were measured.
(1) Active swimming activity, all the vigorous swimming movements displayed by animals involving all four extremities at approximately constant rate, and motor activity showed during immersion looking for a escape. Activity was measured by digital automatic counting 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

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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. In the two initial min the testing animal (host rat) was put alone in the arena in order to familiarize with the cage. At the beginning of min 3, a different and new rat the same size and sex (intruder rat) was put in one corner of the cage. Behavioural display was recorded until testing period was finished. The following variables were measured:

(1) Latency to interact, time measured by digital counting that the host animal takes to face the intruder. Sniffing, touching, gentle biting, and dragging the intruder were recorded as social behavioural display.

(2) Number of contacts, frequency of contacts displayed by the interacting animals. A contact was defined when the host or the intruder animal displayed any of the behaviours above mentioned during the social interaction.

(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. These include biting, sniffing, touching and dragging. 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.

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

In this experiment the influence of Zn and Te, in single or combined administration on general motor and motivated behaviour induced by novelty was evaluated. The OVM device was used to measure these behavioural activities.

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

In this experiment the influence of Zn and Te, 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 Zn or Te on Defensive Behaviour

In this experiment the influence of Zn and Te, in single or combined administration on defensive behaviour was evaluated. Behavioural activity was measured in the forced swimming test.

2.4.4. Effects of Chronic Administration of Zn or Te on Social Behaviour

In this experiment the influence of Zn and Te, in single or combined administration on social behaviour was evaluated. This behavioural activity was measured by 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. 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 $\pm$ standard error of the mean in the horizontal, ambulatory and non ambulatory activities; the median $\pm$ standard error the median in head dipping, rearing and focalized exploration in Experiment 1. With exception of percentage of animals in Experiment 2 (Fig. 2(C)), in all the other experiments data are expressed as the median $\pm$ 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

As it was previously described, ZnTe and $ZnCl_2$ solutions did not affect daily beverage consumption nor induce any malformations in newborn rats[28] (results not shown).

3.1. Experiment 1

The general motor activity and motivated behaviour of rats chronically exposed to ZnTe, $ZnCl_2$ or water is shown in Figure 1. Horizontal motor activity in those animals treated with ZnTe or $ZnCl_2$ was not modified in the 5 min test in the OVM, since their scores were not statistically different from control (Fig. 1(A)). However, the ambulatory activity of rats receiving ZnTe or $ZnCl_2$ treatment was significantly increased compared to control (Fig. 1(A)). No differences were observed between the three groups in the non ambulatory activity. Regarding the motivated behaviour, results show that head-dipping was significantly increased by the

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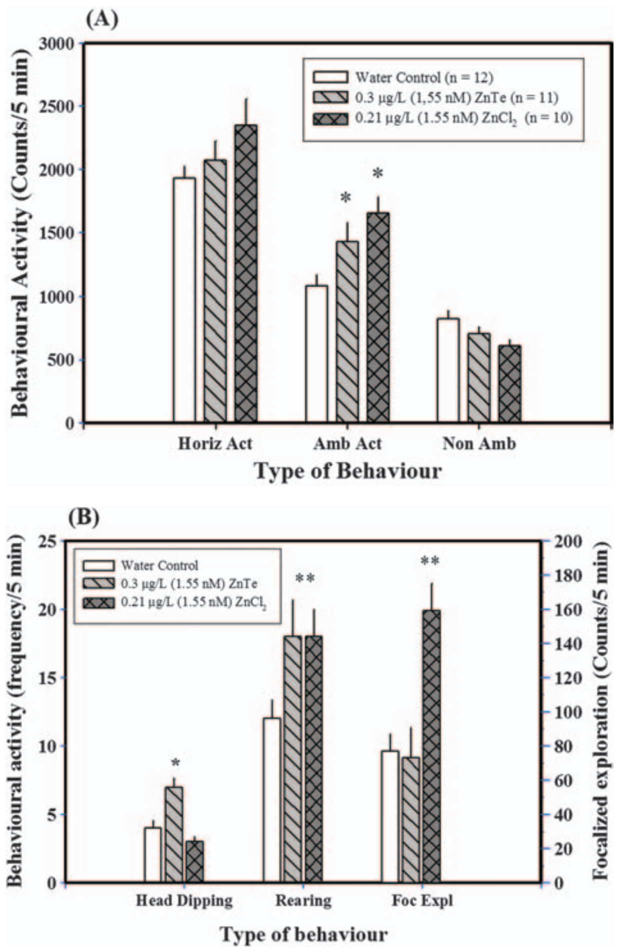

Fig. 1. General motor and motivated spontaneous exploration of rats exposed to ZnTe, $ZnCl_2$ or water treatments measured in the animal automatic detector activity (OVM). *Abbreviations*: (A) Horiz Act = horizontal activity; Amb Act = Ambulatory activity; Non Amb = non ambulatory activity. $^{*}p < 0.05$ versus Control. (B) Foc Expl = focalized exploration. $^{*}p < 0.05$ versus Control. $^{**}p < 0.01$ versus Control. Further details, see Material and Methods section.

ZnTe treatment, but no changes were observed in the score when animals were treated with $ZnCl_2$ (Fig. 1(B)). Rearing and focalized exploration were not affected by the ZnTe treatment. Nevertheless, focalized exploration was significantly increased by the $ZnCl_2$ administration (Fig. 1(B)).

3.2. Experiment 2

The motivated and lateralized behaviour induced by novelty of rats chronically treated with ZnTe, $ZnCl_2$ or water is shown in Figure 2. Behavioural activity displayed by animals in the corridor was significantly decreased by ZnTe or $ZnCl_2$ administration (Fig. 2(A)). At the same time, behavioural activity in the initial compartment was significantly increased by both chemical treatments (Fig. 2(A)). Non exploratory activity compared to control was not affected by ZnTe, but it was significantly decreased by the $ZnCl_2$ treatment (Fig. 2(A)). Lateralized exploratory behaviour showed a statistically bias to

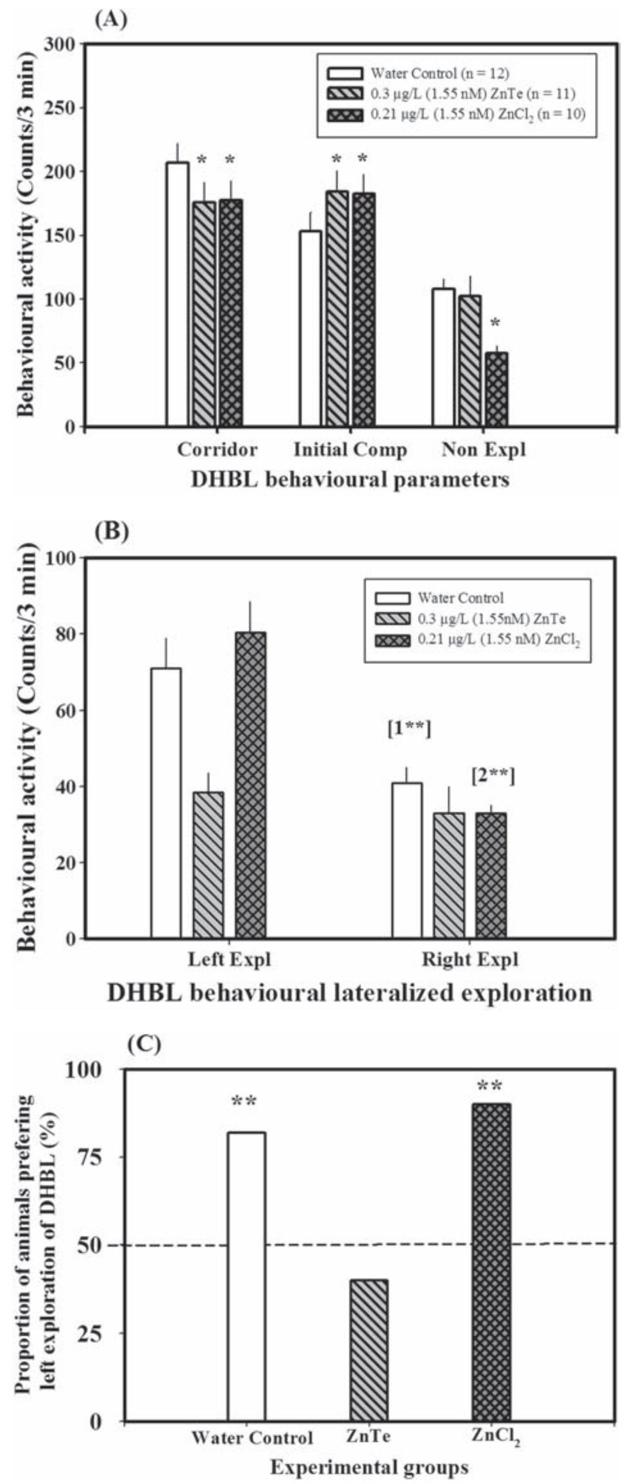

Fig. 2. Motivated and lateralized exploration of rats exposed to ZnTe, $ZnCl_2$ or water treatments measured in the Double Lateral Hole-board Labyrinth (DHBL). *Abbreviations*: (A) Initial Comp = Initial compartment; Non Expl = Non exploratory activity. $^{*}p < 0.05$ versus Control. (B) Left Expl = Exploration of the left side of the labyrinth; Right Expl = Exploration of the right side of the labyrinth. $[1^{**}]$ $p < 0.01$ versus Left exploration of Control group; $[2^{**}]$ $p < 0.01$ versus left exploration of $ZnCl_2$ group. (C) $^{**}p < 0.01$ versus 50% random proportion (horizontal dashed line). Additional details, in the Materials and Methods Section.

the left in control animals (Fig. 2(B)). Treatment with ZnTe abolished completely this left biased exploration (Fig. 2(B)); however, treatment with $ZnCl_2$ did not affect the lateralized exploration. When proportion of animals showing the left lateralized exploration was analyzed in control group, a significant increased proportion of left exploration rats (81.8%) were observed (Fig. 2(C)). Proportion of rats showing these same characteristics in the ZnTe treated animals was not statistically different from 50%, and significantly lower than 81.8% found in control animals (Fig. 2(C)). $ZnCl_2$-treated rats showed the same proportion than control rats, and significantly higher than the percentage found in ZnTe-treated rats (Fig. 2(C)).

3.3. Experiment 3

The defensive behaviour induced by a forced swimming challenge in rats chronically treated with ZnTe, $ZnCl_2$ or water is shown in Figure 3. Control animals showed an active swimming activity about twice the immobilization period. This difference was statistically significant (Fig. 3). ZnTe treatment reduced significantly the active swimming activity, increasing at the same time the immobilization period. Thus, there was no difference between both scores (Fig. 3). However, the $ZnCl_2$ administration did not affect the behavioural defensive display, and the difference between active and immobilization scores in these animals was statistically significant as like the observed in control animals.

3.4. Experiment 4

The social behaviour (intruder-host territorial challenge) in rats chronically treated with ZnTe, $ZnCl_2$ or water is shown in Figure 4. Control animals showed a rather short latency to confront the intruder rat (13 ± 3 Counts/3 min). As shown in the figure, rats treated with ZnTe significantly

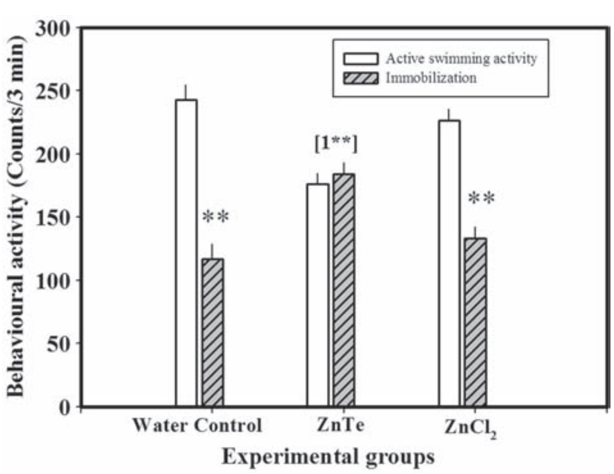

Fig. 3. Defensive behaviour of rats exposed to ZnTe, $ZnCl_2$ or water treatments in the forced swimming test. $^{**}p < 0.01$ versus active swimming activity in the same group; [1**] $p < 0.01$ versus active swimming activity and immobilization of Water Control and $ZnCl_2$ groups respectively. Further details, see Material and Methods Section.

increase this score in the same conditions (Fig. 4(A)). However, those animals treated with $ZnCl_2$, this latency score was similar to that of control rats (Fig. 4(A)). Regarding the number of α contact, control animals showed a median of 8 ± 0.7 in the 3 min period of the test. ZnTe treated rats showed a significant decrease in the score while $ZnCl_2$ treated animals on the contrary displayed a significant increase in the score which was different from control and also from ZnTe group (Fig. 4(B)). As expected, percentage of α contact was around 73% for control group, and about 27% for the ZnTe group. This difference was statistically significant (Fig. 4(C)). $ZnCl_2$ group displayed a similar score than control and statistically different from the ZnTe group (Fig. 4(C)). Finally, duration of α contact in control group was 85 ± 5 Counts/3 min. Animals treated with ZnTe showed a statistical decrease in the score. However, $ZnCl_2$ treated rats displayed a behavioural score similar to the control group and statistical greater than the ZnTe group (Fig. 4(D)).

4. DISCUSSION

Previous results from our laboratory have shown that ZnTe is able to modify the phenotypic behavioural expression when is chronically administered in drinking water to rats.[28] Trace elements affected some general motor behaviour, motivated exploratory display and lateralized exploratory activity.[28] In addition, when the methylation patterns of DNA was examined in the hippocampus of the ZnTe-treated rats; the ratio of non-methylated cytosine to methylated cytosine was significantly increased.[28] This finding is highly suggesting that whatever might be the intrinsic molecular mechanism whereby trace elements are acting, the DNA molecular maintenance and regulation in the cell could be a target of action. Both Zn and Te are trace elements which have biological effects on animals.[21,24,30–32] Thus, the behavioural modifications described in the earlier report might be due to a selected action of any of the trace elements or both. Present results were evaluated considering how the behavioural alterations found with ZnTe are reproduced or not by the Zn treatment alone. It was considered that under this line of reasoning it should be possible to discern the role of Zn and Te.

4.1. Experiment 1

As shown in Figure 1(A), the ambulatory activity, the only behaviour affected by ZnTe administration was also modified by $ZnCl_2$ treatment. This finding suggests that the increase in ambulatory activity observed in rats could be mediated by Zn. If some cooperative action of Te to Zn should exist, it should be expected that the ambulatory activity of animals treated with ZnTe should reach a greater score than that observed with $ZnCl_2$. As shown in Figure 1(A), this difference was not found. Thus, the most prudent conclusion regarding the present result is thinking that the increased ambulation of animals treated with

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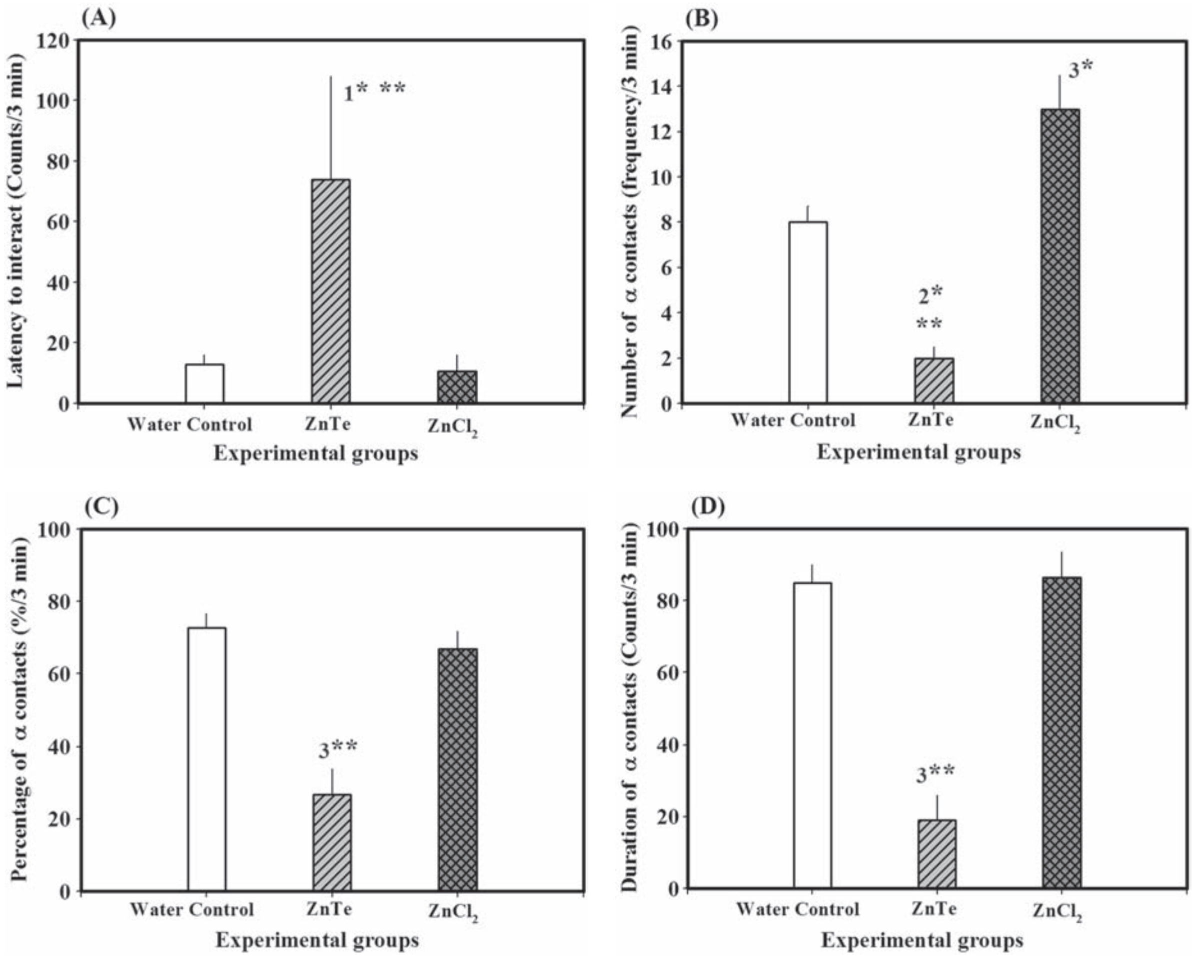

Fig. 4. Social interaction in the intruder-host territorial test of rats exposed to ZnTe, $ZnCl_2$ or water treatments. (A) $1^{*}p < 0.05$ versus Control; $^{**}p < 0.01$ versus $ZnCl_2$ Group. (B) $2^{*}p < 0.05$ versus Control; $^{**}p < 0.01$ versus $ZnCl_2$ Group; $3^{*}p < 0.05$ versus Control. (C) And (D) $3^{**}p < 0.01$ versus Control and $ZnCl_2$.

ZnTe is not due to Te. This conclusion is supported from evidence of other workers which in an experimental setup similar to the presently used, treatment with Te did not affect the locomotor activity measured in an open-field task.[31] On the other hand, regarding the effects of Zn on motor activity, other authors using a different experimental approach related to motor responses in mice with Zn deficiency (tail suspension test) an increase in the immobility time was found.[23] Apparently, the Zn-depending motor activity appears not to be a specific or particular effect of this trace element to mammalian animals. In some species of fish, such as the zebrafish (*Danio rerio*), zinc oxide administered in nanoparticles to hatching specimens induced an increase in locomotor activity of larval animals.[9] Nevertheless, the generality of motor effects of Zn on animal activity should be considered with caution, since recent review about zinc supplementation on motor and higher brain functions in children showed no consistent effect of the trace element on these functional parameters.[14]

It is known that animals exposed to enriched novel environments display various behaviours induced by brain motivation processes.[2,3] As shown in Figure 1(B), only two of these behaviours were affected by ZnTe. The increase in head-dipping activity induced by ZnTe treatment was not reproduced by the $ZnCl_2$ administration in the same conditions (Fig. 1(B)). This result suggests that Te is responsible for influencing this motivated behavioural response. On the contrary, rearing appears to be affected by Zn. Interestingly, focalized exploration was only modified by the $ZnCl_2$, while ZnTe has no effect. This evidence suggests that it is possible that some interaction between Zn and Te exists. It is quite difficult to accept that if Zn has such powerful effect on rearing expression, this influence is not manifested when Zn is administered in combined form with Te. The most reasonable explanation could be that Te might exert some inhibitory or interfering action on Zn chemical action.

4.2. Experiment 2

When animals are exposed to a different novel environment (DHBL), behaviours related to exploration of compartments are modified by the combined treatment of Zn

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and Te (Fig. 2(A)). The decrease in corridor activity and the increase in the initial compartment observed both in the ZnTe and Zn Cl_2 treated animals suggest that it is an effect attributed to Zn (Fig. 2(A)). However, the non exploratory activity displayed by animals when they are in the corridor appears to be modulated by Te, since when this trace element is absent, Zn produced a decrement in the behavioural activity not present when both elements are combined (Fig. 2(A)). Once again, trace elements seem to interact to modulate behaviour. When animals are tested in lateralized exploration activity (Fig. 2(B)), the left biased exploration found in control animals is blocked only in the ZnTe treated rats. Thus, as expected this biological action affected the proportion of rats choosing the left exploration of the labyrinth (Fig. 2(C)). These results suggest that this inhibitory action is specifically due to Te.

4.3. Experiment 3

Animals exposed to stressing situations usually display an escape reaction.[5] 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 several brain structures. As shown in Figure 3, in the forced swimming test control rats displayed an active swimming response about twice the duration of the resting period in the 3 min duration of the test (Fig. 3). The abolishment of the defensive response by ZnTe treatment, and the absence of effect in the $ZnCl_2$ treated animals, suggest that this interference is specifically due to Te (Fig. 3).

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.[33] 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 (α) and confronts the intruder in a very short time after the new animal is put into the cage. As shown in Figure 4(A), control animals showed a median latency of 13 Counts (about 6 seconds) to confront the intruder. This latency was significantly increased in the ZnTe treated rats (Fig. 4(A)), but not affected by the $ZnCl_2$. This result suggests that modification of latency is a specific effect attributed to Te. Not only latency was affected by Te treatment but percentage and duration of α interaction also was modified (Figs. 4(C) and (D)). This evidence gives a stronger support to the idea that these alterations were due to Te. However, decrease in number of α contacts displayed by host rat treated with ZnTe appears to be the consequence of a complex interaction between trace elements (Fig. 4(B)). $ZnCl_2$ administration induced a significant increase in the score while ZnTe produced the opposite effect. As previously found with non-exploratory activity in the DHBL and focalized exploration in the OVM (Figs. 1(B), and 2(A)), once again some type of competing interaction between trace elements appears to be present for selected cognitive behaviours.

5. FINAL REMARKS

Confirming previous results from our laboratory,[28] present data show that alterations in behavioural responses induced by trace elements are diverse but selective. This means that the effects of these chemical elements can not be explained as unspecific actions on brain tissue. On the contrary, it appears that trace elements are able to reach determined neuronal sites in the brain showing some remarking selectiveness. For instance, regarding social behaviour, brain structures such as hippocampus and the amygdala complex have been found to be involved.[13] Such brain structures are well known to have many neurotransmitters and neuromodulators. On this line, vasopressin and ocytocin have been linked to modulate social behaviour in several different species,[17] and abundant evidence has been accumulated showing that the corticotrophin releasing factor system also participate to regulate this behavioural expression.[16] In addition, serotonin and dopamine have been found to be implicated in controlling social interactions in other species rather than mammals.[33] However, in spite of this evidence, it is not known if trace elements, such as Zn or Te, are able to interact with these neurotransmitters systems within the brain structures mentioned before.

Even though some information about the intrinsic, possible target structures and theoretical molecular mechanisms has been described for trace elements,[4,7,10,20,24,27,29,34,35] still its mode of action in the cell is uncertain. Although at first sight, molecular mechanisms attributed to trace elements appear to be diverse, there is some common characteristic linked to the ionic charge that elements have in the cell. For instance, nickel ions increase histone H3 protein methylation, inducing transgene silencing in *in vitro* studies of human lung carcinoma A549 cells.[7] Nickel also can bind to discrete segments of synthetic H4 histone protein tail,[35] suggesting complex interactions between the trace element and DNA. On the other hand, Zn is required to stabilize and induce folding of the DNA-binding domains of some transcription factors present in eucaryotic cells, including facilitation binding of the common transcriptional factor NF-κB to DNA.[20] Regarding Te little is known about its possible role in similar DNA mechanisms. However, molecular evidence in our laboratory suggests that this trace element might be involved in regulatory epigenetic molecular mechanisms of DNA expression.[27,28] The ratio of non-methylated to methylated cytosine in the hippocampal structure of rats treated with

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ZnTe was nearly twice that of control rats.[28] If something is to be said about a theoretical mechanism regarding Te, then its intrinsic actions should be related to some modulation of the methylation mechanism present in the cell.

Finally, one additional interesting feature deserving to mention regarding Zn and Te in the present results, is that found in experiments 1, 2 and 4. Modifications of the behavioural expression in the animal under trace elements treatment appear to be the resultant of some complex unknown interaction between Zn and Te. Although at present, its intrinsic molecular mechanism of action in the cell is not known, it can be deduced that Zn and Te might have a common biochemical step. If some functional protein, transcription factor or intracellular enzyme is involved is a possibility not explored yet. Doubtless, elucidation of this problem will be a point of interest in future research.

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