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Thiamine deficiency decreases glutamate uptake in the prefrontal cortex and impairs spatial memory performance in a water maze test

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Abstract

Using an animal model of Wernicke–Korsakoff syndrome, in which rats were submitted to a chronic ethanol treatment with or without a thiamine deficiency episode, the glutamate uptake in the prefrontal cortex and spatial memory aspects were studied. It was found that (i) thiamine deficiency, but not chronic ethanol consumption, induced a significant decrease of glutamate uptake; (ii) thiamine-deficient subjects showed an impaired performance in the water maze spatial memory test though these animals were able to learn the task during the acquisition. In spite of the fact that thiamine deficiency affects both glutamate uptake and spatial reference memory, there was no significant correlation between these two data. The present results show that, although prefrontal cortex is considered by some authors a not vulnerable area to lesions caused by thiamine deficiency, this vitamin deficiency does cause a neurochemistry dysfunction in that region.

Keywords: Thiamine deficiency; Chronic ethanol; Glutamate uptake; Prefrontal cortex; Spatial learning; Spatial memory; Rat

1. Introduction

Several lines of evidence indicate that chronic ethanol consumption associated with thiamine deficiency can culminate in the Wernicke–Korsakoff syndrome (WKS), a neurodegenerative disease (Thomson, 2000). In this syndrome, the neurodegenerative alterations that can occur at functional and/or structural levels contribute to learning and memory deficits observed (Halliday et al., 1994). These patients also tend to persevere when faced with shifts in the task demands (Janowsky et al., 1989; Joyce and Robbins, 1991; Brokate et al., 2003; Hildebrandt et al., 2004). It has been recognized for many years that chronic alcoholism is associated with cognitive impairments, but there are still conflicting data about the pathogenesis related to memory deficits caused by chronic ethanol consumption (Lishman, 1990; Homewood et al., 1997). The debate involves the role of nutritional deficiency in inducing the lesions usually observed in alcoholic patients. Chronic alcoholism can result in thiamine deficiency through several mechanisms, including inadequate food intake, reduced absorption, or a decrease in the rate of conversion of this vitamin to its active form, thiamine pyrophosphate (Langlais, 1995). It has been shown that both ethanol consumption and thiamine deficiency per se can cause cerebral lesions (Hakim and Pappius, 1983; Miguel-Hidalgo et al., 2002). Experimental studies using animal models have addressed the investigation of the mechanisms that underlie the deleterious effects of thiamine deficiency and chronic ethanol, and the interaction between them (Palencia et al., 1994; Homewood et al., 1997; Ciccia and Langlais, 2000).

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Certain brain structures show a selective vulnerability to degeneration in WKS, for instance, thalamus and mammillary bodies (Troncoso et al., 1981; Witt, 1985; Mair et al., 1991; Langlais, 1995; Cook et al., 1998). Some authors have not found morphological lesions in pre frontal cortex in patients with WKS diagnosis (Murata et al., 2001), although neuronal cell loss has been documented in the PFC of alcoholics (Reed et al., 2003; Krill et al., 1997). Diencephalon dysfunctions were described in thiamine deficient animals (Langlais et al., 1987; Mair et al., 1988; Langlais and Zhang, 1993; Savage et al., 2003). These neurochemical alterations, along with the white matter loss that occurs in key fiber tracts from the diencephalon after PTD treatment (Langlais and Zhang, 1997) suggest that thiamine deficiency likely causes system-level dysfunction, which probably underlie the deficits in learning and memory documented in the PTD model. Among these systems, the glutamatergic circuit seems to have an important role in cognitive processes (Steckler et al., 2005; Zhao et al., 2005; Antzoulatos and Byrne, 2004); and there are evidences that thiamine deficiency can cause a disruption in glutamatergic system (Todd and Butterworth, 1998, 2001; Savage et al., 1999).

The glutamatergic neurotransmission in the frontal cortex is required to induce both long-term potentiation (LTP) and long-term depression (LTD), which seem to play important roles in cognitive processes (for review, see Linden, 1999). Besides, glutamatergic activation of the prefrontal cortex by a direct input from the hippocampus seems to be essential as part of the mechanisms of spatial memory (Vickery et al., 1997; Lee and Kesner, 2003).

The concentration of glutamate in synaptic cleft is regulated by a fine balance between glutamate release and reuptake (Danbolt, 2001). Glutamate uptake is carried out by transporters located in neural and glial cells (O'Shea, 2002). In WKS this balance is disrupted in some brain areas and glutamate reaches a high extracellular concentration (Butterworth, 1993, 1995). The immediate consequence is an over-stimulation of glutamate receptors, which leads to excitotoxicity and neuronal death (Meldrum and Garthwaite, 1990).

In the present work we aimed, using an experimental animal model of Wernicke Korsakoff Syndrome, to study the effects of chronic ethanol consumption associated or not to thiamine deficiency on (i) glutamate uptake in the prefrontal cortex, (ii) spatial memory and (iii) operant extinction, and also (iv) to assess the correlation between these neurochemical and behavioral data.

2. Material and methods

2.1. Experimental subjects and treatment

Forty-eight male Wistar 3-month-old rats, weighing 200–250 g, were housed individually under a 12-h light–dark cycle with continuous access to commercial chow. The animals were divided into two groups, which were treated during 36 days with water (W, $n=24$) or 20% v/v ethanol solution (E, $n=24$) ad libitum as their only source of drinking fluid. Body weight was recorded every 15 days throughout the treatment. Chow and fluid consumption were recorded weekly and daily, respectively. After 36 days, the groups W and E were redivided into 2 subgroups, resulting in a total of 4 subgroups ($n=12$). The animals from each subgroup were treated as follows: (i)WS: water and standard diet (without thiamine deficiency); (ii) WD: water and thiamine-deficient diet; (iii) ES: ethanol and standard diet; and (iv) ED: ethanol and thiamine-deficient diet. Animals from groups WD and ED, along with thiamine-deficient diet, received daily i.p. injections (0.25 mg/kg) of pyrithiamine (Sigma Chemical Co.) which is an inhibitor of the enzyme that is responsible for the production of the active form of thiamine. The subgroups WS and ES received i.p. saline injections. All intraperitoneal injections were given at the same time each day. During the episode of thiamine deficiency the amount of chow and fluid consumed and the body weight were recorded daily. Approximately 15 days after the beginning of the thiamine deficiency episode, when the last signs (loss of righting reflex and/or seizure) of this vitamin deficiency were observed, the animals received i.p. thiamin hydrochloride (100 mg/kg, i.p.) injections. After a recovery period of four months, in which the rats from ethanol groups continued to receive the ethanol solution, the animals were submitted to the behavioral tests. After that, all animals were killed by decapitation and their brain used for the biochemical studies. The care and use of animals in this study were done according to the National Institute of Health Guide for Care and Use of Laboratory Animals (National Research Council, 1985).

Ethanol withdrawal could result in a withdrawal syndrome that could have both neurochemical and behavioral consequences (Homewood et al., 1997). Therefore, a control experiment was carried out in order to verify whether the observed effects of ethanol were chronic or acute. A group of animals received i.p. injections of saline ($n=4$) or 60% v/v ethanol solution ($n=4$; 1 g/kg) 15 min before being trained in the Water Maze. In the first day after the last session of the behavioral tests, 15 min after the injections the rats were killed for biochemical studies.

2.1.1. Ethanol blood levels

The tail blood samples (40 μl) were collected, approximately 5 min before the animals were killed by decapitation, in GC vials containing 30 μl of the anticoagulant heparin (25 000 UI, Roche). Ethanol blood levels were determined by headspace gas chromatography equipped with flame ionization detector (Varian CP-3380). The GC conditions were: column temperature, 60 °C; injection temperature, 100 °C; detection temperature, 120 °C. After heating the vial at 60 °C for 5 min, a 50 μl volume of the headspace vapor was injected into the CG port. For GC quantification, the peak area of each compound was used and the concentrations were calculated by using an external calibration curve: the linear regression for the curves always yielded $r>0.99$. Both samples and standards were submitted to the same procedures. Blood ethanol levels were expressed as milligrams of ethanol per deciliter of blood.

2.2. Behavioral assessment

2.2.1. Apparatus

The behavioral test was carried out in the Morris Water Maze (MWM) (Morris, 1984). The apparatus consisted of a circular

pool, 180 cm in diameter and 43 cm in height. The pool was filled with tap water, 27 cm deep, maintained at a temperature of 23 ± 1 °C and made opaque by addiction of powdered milk. The pool was divided into four imaginary quadrants (SE, SW, NE and NW). A 12-cm diameter invisible escape platform was placed in the center of SE, the target quadrant, 2 cm below the surface of the water. This pool was placed in a 9-m^2 square room. There was no visible cue within the pool, that the rats could use to locate the hidden platform; thus, they were required to learn its location relative to distal room cues (e.g., the furniture, the video monitor and posters on the wall). Trials were recorded by a video camera placed in the ceiling and connected to a videocassette recorder.

2.2.2. Procedure

Behavioral assessment began 126 days after the end of the episode of thiamine deficiency and 177 days after the beginning of chronic ethanol treatment. It consisted of 5 daily training sessions, four trials a day, in the water maze. In each session, the platform was kept in the center of the target quadrant (SE). On each trial, the rat was put into the pool, facing towards the wall in one of the four quadrants, randomly chosen, and allowed to swim until it found the platform. If the rat did not find the platform within 60 s, it was gently guided to it. In both conditions, the animal was allowed to remain on the platform for 15 s. For each animal, the latency to find the hidden platform was recorded, and the data expressed as the average performance of each group.

In the day following the last training session, a 120-s probe trial was carried out, in which the platform was removed. Afterwards, using the recorded video images, the subject's performance was assessed as the distance between the animal and the place where the platform had been located during the training sessions. For that, the videotape was played and paused at 2-s intervals and the distance was measured in the TV screen. The mean distance for the first (d_1) and the second (d_2) minute was then computed for each rat. The performance in the first minute was used to express the reference memory. Extinction was also measured, and it is defined as a decrease of a behavior when the reinforcement maintaining it is removed. In the water maze test, this process can be observed when an animal, in the absence of the platform, swims away from the target and starts to look for the platform in other places. So, an index of extinction (EI) was worked out by dividing d_2 by d_1.

2.3. Synaptosomal fraction preparation

The experimental subjects were decapitated 1 day after the behavioral test. Synaptosomal fractions were obtained as previously described (Dunkley et al., 1988). The brain was separated from the cranial cavity and the PFC was rapidly dissected and homogenized in ice-cooled solution (0.32 M sucrose, 1 mM EDTA, 0.25 mM dithiotreitol, pH 7.4). Homogenates were submitted to differential centrifugation and the synaptosomal fraction was obtained in a discontinuous isotonic Percoll gradient at 4 °C. The synaptosomal protein concentration was determined as described by Lowry et al. (1951). In order to evaluate the synaptosomes' integrity, lactate dehydrogenase (LDH) activity in the synaptosomal preparation was carried out (Kubowitz and Ott, 1943).

2.4. Glutamate uptake

Glutamate uptake was determined as described by Mafra et al. (2002) with some modifications. The synaptosomes were, initially, incubated in a depolarizating Krebs–Ringer–Hepes (KRH) medium, pH 7.4, in which NaCl was replaced isosmotically by KCl at a final concentration of 20 mM, at 37 °C for 15 min, to allow the release of endogenous glutamate. After, the synaptosomal suspension was centrifuged (10,000×g, 10 min, 4 °C) and the pellet was resuspended in ice-cooled KRH medium, pH 7.4, containing (in mM): 124.0 NaCl, 4.0 KCl, 1.2 $MgSO_4$, 1.0 $CaCl_2$, 10.0 glucose and 25.0 *N*-[2-hydroxyethyl]piperazine-*N*′-[2-ethane-sulfonic acid] (HEPES). This procedure was repeated twice to remove any residual glutamate. The resulting pellet was resuspended in KRH. Samples of 200 μg/ml of this suspension were pre-incubated under shaking for 5 min at 37 °C. Following that, the incubation was started by addition of 0.4 μCi/μl of [^{3}H]-glutamate (New England Nuclear, specific activity 44 Ci/mmol; 9.0 nM final concentration) and maintained at 37 °C for 10 min. After the incubation, the samples were filtrated by a vacuum system through Whatman GF/B filters. Filters were washed three times with 10 ml of KRH medium containing 6 mM of non-labeled glutamate. The filters containing synaptosomes were placed in scintillation vials with Dioxan Scintillation Solution (DSS) and their radioactivity was determined by spectrophotometry (Liquid Scintillation Analyzer – Packard).

2.5. Statistical analysis

The biochemical experiments were performed in duplicates, and the mean was used for statistical analysis comparisons. All values are expressed as mean ± standard error of the mean (S.E.M.). The biochemical parameters and water maze probe trial data were analyzed using a 2 × 2 (chronic ethanol × thiamine deficiency) ANOVA. Body weight, food and fluid intake and Morris Water Maze training data were analyzed using 2 × 2 × r (chronic ethanol × thiamine deficiency × time) ANOVA with repeated measures in the last element (Winer, 1962). The post hoc test used was the Newmann–Keuls paired comparison (Winer, 1962). Linear regression (Winer, 1962) was used in order to determine the degree to which biochemical and behavioral parameters were linearly correlated. The experimenter was not aware about from which animal's group a sample came. The significant level for all analyses was set at $p < .05$.

3. Results

3.1. Treatment data

Fig. 1 shows animals' weight before, during and after thiamine deficiency episode. Before this episode, ANOVA 2 × 2 × r showed significant main effect only for time [$F(1,36) = 25.20$,

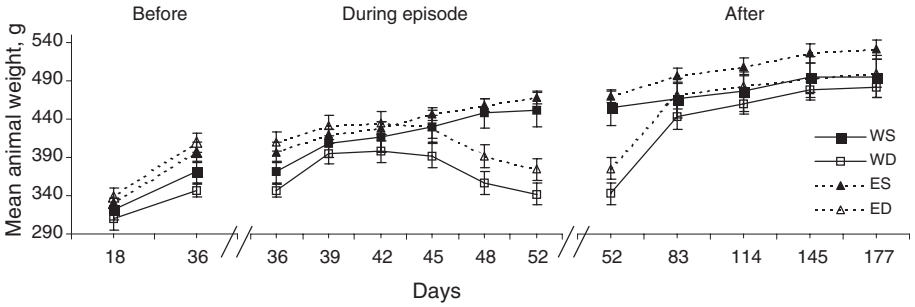

Fig. 1. Weight (mean±S.E.M.) of animals from the four experimental groups, before, during and after thiamine deficiency episode. The groups are denominated by associating characters as follows: W and E mean water and ethanol; S and D mean standard and thiamine-deficient diets, respectively.

$p<0.01$]. During the episode, there were significant main effects of thiamine deficiency [$F(1,36)=7.40$, $p<0.025$], chronic ethanol [$F(1,36)=4.23$, $p<0.05$] and time [$F(5,180)=27.94$, $p<0.01$]. Thiamine deficiency treatments caused significant reductions of body weights. There were also significant interactions between time and thiamine deficiency [$F(5,180)=56.71$, $p<0.01$]. After the thiamine deficiency episode, there were significant main effects of thiamine deficiency [$F(1,36)=5.13$, $p<0.05$] and time [$F(4,144)=282,77$, $p<0.01$]. There was also a significant interaction between time and thiamine deficiency [$F(4,144)=66.79$, $p<0.01$]. These results on weight body are in line with those of chow consumption (data not shown) for which ANOVA also showed significant thiamine deficiency and time main effects and a significant interaction between them.

The animals from both ethanol-treated groups, ES and ED, showed large individual variability in ethanol consumption (data not shown) and this was reflected in their ethanol blood levels, which ranged from 17.0 to 31.2 mg/dl with a mean value of 24.5 mg/dl. There was no significant difference between the averages of blood ethanol level of animals submitted to acute and chronic treatments.

3.2. Acquisition of spatial learning

The performance of the animals from the four groups along all sessions of the spatial task is shown in panel A of Fig. 2. ANOVA ($2\times2\times5$) showed significant main effects for thiamine deficiency [$F(1,36)=11.64$, $p<0.01$] and time [$F(4,144)=52.79$, $p<0.01$). ANOVA (2×2) for each session shows significant main effect for thiamine deficiency in the second [$F(1,42)=16.5$, $p<0.01$] and forth sessions [$F(1,42)=4.87$, $p<0.05$]. In these sessions, animals from groups WD and ED showed a worse performance compared to those animals from WS and ES groups. There was no significant interaction between the treatments.

3.3. Spatial memory and extinction

Probe trial results are shown in Fig. 2. Two-way ANOVA indicated a significant main thiamine deficiency effect [$F(1,42)=4.99$, $p<0.05$] on the mean distance kept from the target in the first minute of probe trial reference memory (panel B). Animals from groups WS and ES showed better performances compared to those animals submitted to thiamine deficiency (WD and ED groups). Two-way ANOVA also indicated significant main effect of thiamine deficiency [$F(1,41)=5.11$, $p<0.05$] on extinction index (panel C). Animals from groups WS and ES showed a higher extinction index compared to those animals submitted to thiamine deficiency (WD and ED groups).

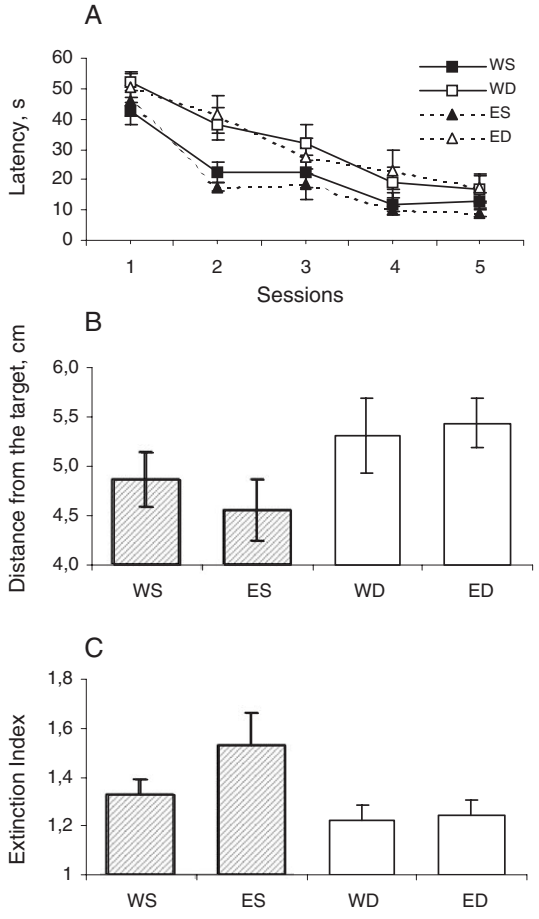

Fig. 2. Behavior of animals in spatial water maze (mean±S.E.M.). (A) Animal performance during training, expressed as escape latencies to find the hidden platform. (B and C) Animal performance during the first minute of probe trial: (B) reference memory – distance from the target; (C) extinction index. The groups are denominated by associating characters as follows: W and E mean water and ethanol; S and D mean standard and thiamine-deficient diets, respectively.

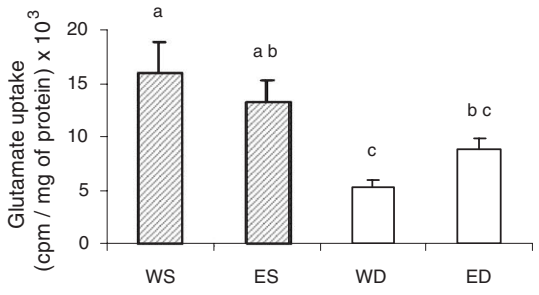

Fig. 3. Glutamate uptake in pre frontal cortex synaptosomes (mean ± S.E.M.) for the four groups. The groups are denominated by associating characters as follows: W and E mean water and ethanol; S and D mean standard and thiamine-deficient diets, respectively. Different characters above bars mean significant differences.

3.4. Glutamate uptake

The effects of thiamine deficiency and/or chronic ethanol consumption on glutamate uptake in prefrontal cortex synaptosomes are shown in Fig. 3. ANOVA 2×2 showed significant thiamine deficiency main effect [$F(1,41)=13.79$, $p<0.01$]. Post hoc Newman–Keuls analysis showed that glutamate uptake by synaptosomes obtained from prefrontal cortex of animals from group WS was significantly bigger compared to that of animals from groups WD ($p<0.01$), and ED ($p<0.05$) but was not significantly different from that of animals from group ES. On the other hand, the average glutamate uptake for the animals from groups ES and ED did not significantly differ.

The statistical analysis of data obtained from the control experiments, in which the animals were treated with acute ethanol, showed that this treatment had no effect on the biochemical and behavioral parameters assessed (data not shown).

No significant correlation was found between biochemical and behavioral parameters (data not shown). The correlation indexes for extinction and memory performance, both versus glutamate uptake were −0.074 ($p>0.05$) and −0.057 ($p>0.05$), respectively.

4. Discussion

The thiamine-deficient animals used in the present study showed the same signs of the pyrithiamine-induced thiamine deficiency (PTD) described in the literature, which include a progressive weight loss and a series of neurological signs that culminate in the loss of the righting reflex and/or tonic seizures (Langlais et al., 1987, 1996). They also showed those neurochemical dysfunctions and lesions that persist after the interruption of PTD treatment. The significant weight loss during the episode of thiamine deficiency was due to a significant decrease in food ingestion (data not shown), as expected, since one of the first clinical signs is anorexia (Plaitakis et al., 1982). These animals recovered the body weight after the reversion of the deficiency.

The chronic ethanol treatment used in the present work resulted in an ethanol blood level similar to that found by other authors who used a similar animal model (Pereira et al., 1998; Chakkalakal et al., 2002; Pronko et al., 2002). We have failed to detect any significant effect related to chronic ethanol consumption on both behavioral assessment and glutamate uptake in PFC. It was maybe due a large variability in ethanol consumption and the relatively low ethanol blood levels when compared to others studies which describes spatial memory impairments associated to chronic ethanol consumption (Santín et al., 2000).

As there were no differences among the four groups in the fifth session of water maze training, one could say that both thiamine-deficient and non-deficient animals were able to learn the task. It should be pointed out that, in the second and forth sessions, animals from the thiamine-deficient groups showed an impaired performance, indicating a delay in learning. This result is consistent with previous findings obtained by our group (Pires et al., 2005) and other authors (Mumby et al., 1999; Langlais and Savage, 1995) in which thiamine-deficient rats showed spatial leaning deficits.

In probe trial, the thiamine-deficient animals (from groups WD and ED) showed performance impairment during the first minute of the test. As stated, there was no significant difference between performances of thiamine-deficient and non-deficient animals at the end of training, but a difference appeared in probe trial, when there was no platform in the pool. Considering that these animals were able to learn the task, a question could be raised: could it be that thiamine-deficient rats used an alternative learning strategy? According to Brandeis et al. (1989), three different strategies can be used to reach the escape platform. By using a learned sequence of body movements (praxis strategy); by using proximal cues, like odor, for example (taxis strategy); or by using distal cues to form a spatial configuration (mapping or spatial strategy). An impaired performance on probe trial could thus indicate a deficit in employing the mapping strategy. For example, during the training sessions, the thiamine-deficient subjects could have merely learned the distance between the platform and the pool wall and swam at that distance from the border, in clock- or counter-clock-wise direction. Or, perhaps, they could have detected some proximal cue related to the platform (for example, a shadow or an odor). Maybe the last hypothesis is less probable as the water was made turbid and was whirled after each trial. In any case, neither of these strategies would work during a probe trial. The difference between the animal performance in the fifth training session and the probe trial suggests that the animals from the thiamine-deficient groups used a non-spatial strategy to locate the hidden platform during learning.

The extinction indexes of thiamine-deficient animals were smaller when compared to those of control animals. This means that during the second minute, the animals from the standard diet groups swam away from the target and started to look for the platform in other places, while thiamine-deficient animals kept searching for the platform in the same place. This behavior could be explained by the fact that these animals were already far away from the target in the first minute of the trial; hence, there were no means to get farther away. But in a previous study (Pires et al., 2005) it was found that even thiamine-deficient animals, that showed a good performance in the first minute, persisted in searching for the platform where it was formerly placed. This perseveration probably indicates a lack of behavioral flexibility, which is a phenomenon also found in Wernicke–Korsakoff syndrome patients. These individuals tend to persevere when faced

with shifts in the task demands (Janowsky et al., 1989; Joyce and Robbins, 1991; Brokate et al., 2003). If extinction is considered not a destruction of previous learning, but a new learning superimposed on an old one (Bouton, 2002), perhaps the failure of thiamine-deficient rats to extinguish is related to their difficulty in learning the spatial task.

It is worthwhile to mention that, in the present study, the decrease in glutamate uptake in the PFC observed in thiamine-deficient animals was greater for animals from WD group. This decrease could be due to a dysfunction or down-regulation of the neuronal glutamate transporters. Some studies have reported that thiamine deficiency induces down-regulation of the astrocyte glutamate transporters (Hazell et al., 2001, 2003).

Another point to be considered is that the observed dysfunction in glutamate uptake in PFC could result in an increase of cortical extra-cellular glutamate concentration that, in turn, could lead to cell death. In fact, Langlais and Savage (1995) demonstrated that thiamine deficiency can cause cortical tissue loss in PFC. On the other hand, other authors have not observed changes in the frontal parietal cortex of PTD subjects, but found an increase of extracellular glutamate in some areas that are affected by thiamine deficiency, such as thalamic nuclei and hippocampus (Langlais and Zhang, 1993; Todd and Butterworth, 1998, 2001; Kruse et al., 2004). In accordance with these studies, Harper and Butterworth (1997), based in neuropathological evaluation, described extensive neuronal cell loss and panecrosis in the thalamic nuclei, mammillary bodies and cerebellum, with relative sparing of cerebral cortical structures. So, from these results, these authors have considered the frontal cortex a non-vulnerable region. In a previous morphological study, using the same WKS animal model, our group observed lesions in thalamic nuclei, but not in the cerebral cortex (data not published). In WKS patients, lesions are often documented in diencephalic region (Kopelman, 1995; Visser et al., 1999; Caulo et al., 2005), albeit some authors have found tissue loss in the cerebral cortex (Kril et al., 1997; Reed et al., 2003). Considering the present results, it was supposed that the reason for this non-vulnerability could be that: (i) the decrease in the glutamate uptake is not severe enough to engender neuronal death; or (ii) there is a specific protective mechanism in the cerebral cortex to prevent glutamate excitotoxicity, which could be absent in vulnerable areas. Some authors have observed fewer NMDA-displaceable binding sites in cerebral cortex of PTD rats and they have proposed that this selective loss of NMDA binding sites is a possible explanation for the relative non-vulnerability of cerebral cortex (Peterson et al., 1995), since NMDA receptor actually plays a role in the pathogenesis of neuronal loss.

We also found that the ethanol treatment, when not associated to thiamine deficiency, did not affect the glutamate uptake in PFC synaptosomes. This result is in accordance with data obtained by other authors, who showed that chronic ethanol treatment had effects neither on cortical synaptosome glutamate uptake nor on levels of glutamate transporters in cerebral cortex (Keller et al., 1983; Devaud, 2001). However, some authors have demonstrated that acute and chronic ethanol exposure in vitro alters, in a concentration-dependent manner, both glutamate uptake in rat astrocytes and the excitatory amino acid transporter (EAAT3) expression in *Xenopus oocytes*. These effects are reversed by withdrawal of ethanol from the incubation medium, by protein kinase C inhibitors and is not associated with an increase in the expression of glutamate transporters (Smith, 1997; Othman et al., 2002; Kim et al., 2003).

Some studies have reported that neuromorphological alterations caused by prolonged ethanol intake or by exposure to ethanol during early phases of development were prevented by a high dose of thiamine (Wenisch et al., 1996; Ba et al., 1996). In the present study, we observed an opposite effect; that is, ethanol seems to protect against thiamine deficiency effect. Compared to the significant reduction in the WD group, the observation that the average glutamate uptake from groups ES and ED did not differ significantly suggests that ethanol may have provided some protective effect. Conversely, other authors also showed results that indicate a potential protective effect of ethanol consumption during episodes of thiamine deficiency (Ciccia and Langlais, 2000). A similar effect on a cholinergic parameter was observed in a study recently carried out by our group, using a mild thiamine deficiency associated to chronic ethanol treatment in rats, in which the thiamine deficiency treatment per se decreased the acetylcholinesterase (AChE) activity in hippocampus, and this effect was reverted when thiamine deficiency was associated to chronic ethanol treatment (data not published). Since there is evidence that cortical glutamatergic system is modulated by the cholinergic system (Aoki and Kabak, 1992; Sher et al., 2004), a hypothesis could be raised that these effects of thiamine deficiency associated to ethanol, on those systems, are related. A change in the ACh synthesis could indirectly affect the glutamatergic system, since it is well known that the ACh release in the PFC and subsequent activation of nicotinic ACh receptors (nAChRs) can interfere with a number of neurotransmitters release, including glutamate release (Vidal, 1994; Lambe et al., 2003; Rao et al., 2003). So, one possibility is that a decrease in cholinergic activity could lead to a decrease of glutamate release in PFC, and this could result in a down-regulation of glutamate uptake, as observed in the present study.

Recent lesion studies with rodents have suggested that the PFC contributes to the temporal ordering of spatial and non-spatial events, to the level of attentional selection as well as to the organization and planning of responses (Ragozzino et al., 1999; Delatour and Gisquet-Verrier, 2000; Dias and Aggleton, 2000). Besides, cholinergic cortical projections are thought to play a fundamental role in cortical processing and to affect attentional and memory processes, including extinction (Mason, 1983; Robbins, 1997). Prefrontal cortex lesioned rats were impaired in switching from using a spatial to a non-spatial strategy (Granon et al., 1996) and also showed inefficient navigational strategies in the spatial water maze tasks (Kolb et al., 1983; Granon and Poucet, 1995). In a recent experiment, it was shown that mice with ectopias (misplaced clusters of neurons), specifically located in the PFC, showed difficulties to switch from using extra maze cues to intra maze cues and vice versa in the MWM (Hyde et al., 2002). The hippocampus is crucial for spatial memory (Squire, 1993; Astur et al., 2002). The prelimbic area in the PFC receives a direct glutamatergic input from the hippocampal CA1/subiculum region (Férino et al., 1987; Takita et al., 1999). The hippocampus–PFC pathway likely provides an essential circuit involved in spatial

information transport, which can be integrated into cognitive and motor-planning processes mediated by the PFC (Doyère et al., 1993; Degenetais et al., 2003). Furthermore, the finding that prefrontal cortex lesions alter the spatial responsivity of hippocampal place cells indicates that this area normally modulates spatial responses in the hippocampus (Kyd and Bilkey, 2003). However, in the present study, no correlation was observed between the decrease in glutamate uptake in the PFC and memory deficit and extinction. So, the decrease in glutamate uptake was not responsible for these animal performance deficits. Possibly, other neuroanatomical structures and/or neurophysiological processes may be involved. Besides, in future studies, a correlation between glutamate uptake in other brain areas and specific cognitive deficits should be addressed.

A point to be emphasized is that, although some authors have found that PFC is not vulnerable to lesions caused by thiamine deficiency, the present study shows evidence that this vitamin deficiency does cause a neurochemistry dysfunction in that region. This dysfunction could be correlated to behavioral deficits in other cognitive aspects, such as working spatial memory and attention processes. So, future studies should address these issues.

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