Published in final edited form as:

Behav Brain Res. 2012 July 1; 232(2): 421–425. doi:10.1016/j.bbr.2012.04.004.

Thiamine deficiency degrades the link between spatial behavior and hippocampal synapsin I and phosphorylated synapsin I protein levels

Leticia S. Resende^a, Angela M. Ribeiro^{a,*}, David Werner^b, Joseph M. Hall^b, and Lisa M. Savage^b

^aPrograma de Pós-graduação em Neurociências, Laboratório de Neurociência Comportamental e Molecular, LaNeC, Universidade Federal de Minas Gerais, Belo Horizonte 31270-010, Brazil

^bBehavioral Neuroscience Program, Department of Psychology, State University of New York at Binghamton, Binghamton, NY 13902, USA

Abstract

The links between spatial behavior and hippocampal levels of synapsin I and phosphosynapsin I were assessed in normal rats and in the pyridoxamine-induced thiamine deficiency (PTD) rat model of Wernicke–Korsakoff’s syndrome. Synapsin I tethers small synaptic vesicles to the actin cytoskeleton in a phosphorylation-dependent manner, is involved in neurotransmitter release and has been implicated in hippocampal-dependent learning. Positive correlations between spontaneous alternation behavior and hippocampal levels of both synapsin I and phosphorylated synapsin I were found in control rats. However, spontaneous alternation performance was impaired in PTD rats and was accompanied by a significant reduction (30%) in phosphorylated synapsin I. Furthermore, no correlations were observed between either form of synapsin I and behavior in PTD rats. These data suggest that successful spontaneous alternation performance is related to high levels of hippocampal synapsin I and phosphorylated synapsin I. These results not only support the previous findings that implicate impaired hippocampal neurotransmission in the spatial learning and memory deficits associated with thiamine deficiency, but also suggest a presynaptic mechanism.

Keywords

Pyridoxamine; Thiamine deficiency; Spontaneous alternation performance; Synapsin I and rodent

Thiamine (vitamin B1) is essential for the maintenance of normal cellular function. Thiamine deficiency may lead to the development of Wernicke’s encephalopathy (WE), which is characterized by ataxia (loss of coordinated muscle movement), nystagmus (involuntary eye movement) or ophthalmoplegia (eye movement paralysis) and “confusion” or change in consciousness/mental status [1]. Without restoration of thiamine levels during

*Corresponding author at: Laboratório de Neurociências Comportamental e Molecular - LaNeC, Av. Antônio Carlos, 6627, (FaFiCH) sala F1002, Campus Pampulha, Belo Horizonte, MG, CEP 31270-901, Brazil. Tel.: +55 31 3409 2642; fax: +55 31 3409 2642. angelamr@icb.ufmg.br (A.M. Ribeiro).

WE, the chronic condition of Wernicke–Korsakoff syndrome (WKS) can emerge [2]. Focal brain lesions similar to those that occur in patients with WE/WKS can be experimentally induced in rats using pyridoxamine induced thiamine deficiency (PTD), combined with a diet deficient of thiamine [3].

Studies have shown that PTD rats have deficits in spatial learning and memory [4–7]. These cognitive dysfunctions are mainly attributed to damage to diencephalic structures, primarily thalamus and mammillary bodies [8]. Although little evidence implicates hippocampal overt lesion or cell loss in PTD rats [8], studies suggest this region is functionally impaired and likely contributes to the amnesic state seen in PTD animals. Previously, we have shown reduced hippocampal ACh efflux in the hippocampus of PTD rats is correlated with impaired behavioral performance on a spontaneous alternation task [9]. In addition, neurogenesis was decreased in the hippocampus after PTD treatment [10].

Brain-derived neurotrophic factor (BDNF) is a modulator of synaptic plasticity involved in learning and memory events. In addition, it is known that PTD treatment reduces hippocampal BDNF expression [11]. The ability of BDNF to influence neural function may be intrinsic to its ability to modulate synaptic transmission by regulating synapsin I *via* tyrosine kinase B (TrkB) receptor [12]. Synapsin I tethers small synaptic vesicles to the actin cytoskeleton in a phosphorylation-dependent manner thereby regulating vesicular availability in the nerve terminals and subsequent neurotransmitter release [13]. Pires et al. [14] found alterations in a number of hippocampal phosphorylated proteins in rats following chronic ethanol exposure and thiamine deficiency. Importantly, these studies found changes in a hippocampal 86 kDa protein and was suggested to be synapsin I. Given this implication, the purpose of the present study was to evaluate the effects of thiamine deficiency on hippocampal synapsin I and phosphorylated synapsin I levels and to determine whether these proteins are linked to the learning and memory deficits in the PTD rodent model.

Seventeen Sprague-Dawley male rats (Harlan Co., Indianapolis, IN, USA), approximately 2 months old, were initially housed two per cage during treatment with unlimited access to water and rodent chow in a colony room with a 12-h/12-h light–dark cycle. Rats were randomly assigned to one of the following treatment groups: (1) pair-fed control (PF, $n = 8$), or (2) pyridoxamine-induced thiamine deficiency (PTD, $n = 9$). Subjects in the PTD group were given thiamine-deficient chow *ad libitum* with daily injections (0.25 mg/kg, i.p.) of pyridoxamine hydrobromide (Sigma–Aldrich, St. Louis, MI, USA). On days 14–16 of treatment, the animals displayed loss of righting reflexes and eventual generalized convulsions (seizures). Within 4.25 h after the onset of seizure, PTD treated animals were given an injection of thiamine (100 mg/kg, i.p.) every 8 h until the animals regained their upright posture. PF animals were fed an amount of thiamine-deficient chow equivalent to the average amount consumed by the PTD groups and were given daily injections of thiamine (0.4 mg/kg i.p.). After treatment, all subjects were placed on regular chow and allowed to regain the weight lost during treatment.

Following three weeks of recovery, PTD or PF treatments the animals were tested on a spontaneous alternation task using a 4-arm plus maze. The spontaneous alternation task was chosen because it provides a measure of spatial working memory and does not require any

training or reinforcement [15–17] and PTD-treated rats have consistently demonstrated impairment on this task [9,18], which is dependent on the dentate gyrus [19]. On the day of behavioral testing, the subject was transported to a cue-rich testing room in a covered opaque home cage to minimize exposure to the testing environment. Following a 20 min acclimation period in a covered habituation box (41 cm × 30 cm × 35 cm) located in the testing room, the animal was placed on the center of the 4-arm maze and allowed to explore freely for 18 min. The 4-arm plus-maze was constructed of wood with clear Plexiglas sidewalls (12 cm high) and a floor painted black. All four arms were of equal distance (55 cm) and the apparatus was elevated 80 cm above the floor. The number and sequence of arms entered were recorded to determine alternation scores. A successful alternation was defined as the subject entering each of the arms during a successive 4-arm choice sequence. The percent alteration score is equal to the ratio of: (actual alternations/possible alteration) × 100 [15].

Immediately following the behavioral tests the animals were decapitated and the hippocampus from both hemispheres were rapidly dissected out, frozen on dry ice, and stored at –80 °C until further use. For analysis of synapsin I and phosphorylated synapsin I level in the hippocampus, Western blot analysis was performed as described previously with modifications [20]. In brief, the hippocampal tissue was homogenized in lysis buffer (1% SDS, 1 mM EDTA, 10 mM Tris) containing protease and phosphatase inhibitor cocktails. Protein concentrations were determined using a bicinchoninic acid method (Pierce, Rockford, IL, USA) and compared to bovine serum albumin standards. Hippocampal samples were denatured and separated by electrophoresis on 8–16% sodium dodecyl sulfate polyacrylamide gels (Life Technologies, Grand Island, NY), transferred to a polyvinylidene difluoride membranes (Life Technologies), and blocked overnight in 1% BSA, 0.01% Tween-20 in PBS, followed by incubation overnight with an affinity purified goat polyclonal antibody against the carboxyl terminal of phosphosynapsin I (1:250; Santa Cruz Biotechnology, Santa Cruz, CA). Membranes were then exposed to a peroxidase-conjugated secondary antibody (1:15,000 dilution, anti-goat HRP, Santa Cruz) for 1 h and protein levels were detected with enhanced chemiluminescence (GE Healthcare, Amersham, UK). Membranes were exposed to film under non-saturating conditions. Membranes were subsequently exposed to synapsin I (1:250 Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA) and β -actin (1:500; Millipore, Billerica, MA) for normalization. Importantly, the phosphorylated synapsin I and synapsin I antibodies do not bind to the same motif on the Synapsin I protein.

The mean percent alternation and total number of arm entries during spontaneous alternation were analyzed using Student's *t*-test. Synapsin I and phosphorylated synapsin I protein levels were expressed as a ratio of β -actin and converted to percent of control group and analyzed by Student's *t*-test. Additionally, ratios of total synapsin I to phosphorylated synapsin I were obtained to further assess phosphorylation rate of synapsin I in response to PTD treatment. Additionally, linear regression analysis was performed on the individual samples to evaluate the relation between the levels of synapsin I or phosphorylated synapsin I and alternation behavior data. Statistical differences were considered significant at the level of $p < 0.05$.

PTD-treated rats had lower levels of alternation behavior compared to PF rats ($t(15) = 2.71$; $p = 0.01$, Fig. 1). However, PTD and PF rats did not differ in activity as the number of arms entered during the testing session was not different as a function of treatment ($t(15) = 1.49$; $p = 0.15$).

For phosphorylated synapsin I, a significant decrease in PTD animals was observed compared to PF animals ($t(15) = 2.20$; $p < 0.02$), as well as a trend towards decreased total levels of synapsin I ($t(15) = 1.57$; $p = 0.06$ (Fig. 2A and B). However, PTD and PF animals did not differ in the ratio of synapsin I per phosphorylated synapsin I ($t(15) = 0.46$; $p = 0.32$) (Fig. 2C).

Regression analysis between spontaneous alternation scores and hippocampal synapsin I or phosphorylated synapsin I protein levels were assessed separately for PF and PTD groups (Fig. 3A and B, respectively). Positive correlations between percent alternation and the mean of synapsin I ($r = +0.77$, $p = 0.02$) and phosphorylated synapsin I ($r = +0.88$, $p = 0.007$) were observed in the PF group. However, thiamine deficiency disrupted the correlation between alternation behavioral and synapsin I ($r = -0.42$, $p = 0.25$) and phosphorylated synapsin I ($r = -0.31$, $p = 0.41$) in the PTD group. Regression analysis, after pooling data from the two groups together, also showed no significant correlation for either synapsin I ($r = +0.44$, $p = 0.07$) or phosphorylated synapsin I ($r = +0.38$, $p = 0.13$), likely due to the bidirectional associations for the PF and PTD groups.

Rats recovered from PTD treatment are impaired on several tests of spatial learning/memory and hippocampal function, including delayed alternation [21], spatial matching-and non-matching-to-sample [22], acquisition of a water maze task [6], and both the two arm T-maze [4] and four arm plus-maze [9] versions of spontaneous alternation. Beyond supporting previous studies demonstrating that PTD rats have impaired spontaneous alternation performance, the present study is the first to suggest that thiamine deficiency alters the regulation of a molecule involved with presynaptic vesicular release within the hippocampus. Furthermore, in normal rats there is a significant link between hippocampal synapsin I (non-phosphorylated and phosphorylated) levels and spatial learning that is disrupted by thiamine deficiency.

These data lend further support to previous studies suggesting presynaptic plasticity that evokes the synapsin I cascade is critical for spatial learning. For instance, studies have shown that hippocampal dependent learning stimulated the ERK dependent phosphorylation of synapsin I [23,24] and that deficits in spatial learning could, in part, be attributed to depressed levels of synapsin I [25]. These results are in agreement with our previous study indicating changes in the phosphorylation levels of some proteins in the hippocampus of rats after the PTD treatment, particularly the p86 protein [14]. Moreover, performance on the water maze correlated with the amount of phosphorylation of the p86 protein, of which synapsin I is a possible candidate.

Decreases in both synapsin I and phosphorylated synapsin I in PTD-treated rats are likely related to the fact that PTD treatment chronically reduces BDNF protein expression within the hippocampus [11]. Therefore, it is likely that BDNF impacts the synthesis and

phosphorylation of synapsin I [12]. According to Jovanovic et al. [12] BDNF stimulation of nerve-terminal TrkB causes the downstream activation of MAP kinase, leading to an enhancement of synapsin I phosphorylation at MAP kinase-dependent phosphorylation sites and a concomitant potentiation of neurotransmitter release. Our findings that thiamine deficiency induced an alteration in this pathway may give some insight about the molecular mechanisms underlying the neurochemical dysfunctions in the PTD model.

Decreases in synapsin and phosphosynapsin I observed here may be related to reduce neurotransmitter release in the PTD model. The levels or release of several neurotransmitters are disrupted by thiamine deficiency. For instance, behaviorally-stimulated *in vivo* acetylcholine (ACh) release in the hippocampus is blunted in PTD rats [9] and *in vitro* hippocampal acetyl-cholinesterase activity and ACh release is decreased in a milder form of thiamine deficiency [26]. The glutamatergic system in the hippocampus is also affected. Lê et al. [27] showed that electrically stimulated, Ca^{2+} -dependent release of glutamate from hippocampal slices obtained from symptomatic PTD rats is decreased compared to PF controls. Taken together, neurotransmitter release dysfunction following thiamine deficiency may be related to a more general, rather than specific mechanism. The signaling pathways modulating synapsin I-mediated trafficking of synaptic vesicles between the reserve and readily releasable pools in the presynaptic terminal likely affects multiple neurotransmitter systems. Our data also reveal no differences in synapsin I phosphorylation rate between PTD treated and controls PF rats. Therefore, it is more likely that synapsin I transcriptional/translational processes are decreased following thiamine deficiency.

Although the present results and other studies suggest that synapsin I may contribute to thiamine deficiency, we cannot exclude that other vesicular-associated proteins may also be affected. For instance, it is possible that synapsin II and synapsin III which also anchor vesicles to cytoskeletal elements could also be affected [reviewed in: 13]. Other vesicular-associated proteins such as synaptophysin, synaptotagmin, and synaptobrevin could also be potentially affected thiamine deficiency [13,28]. However, further studies are needed to gain a better understanding of thiamine deficient effects on vesicular release. Nonetheless, the present data, in combination with previous studies, hint at nutritional effects on presynaptic plasticity. For example, a two month diet of saturated fat and refined sugar was sufficient to reduce expression of synapsin I compared to healthy controls [29], displayed spatial learning impairments and decreased hippocampal levels of BDNF. In addition, altered levels of hippocampal synapsin I have been observed in an animal model of traumatic brain injury [30] and in patients with schizophrenia or bipolar disorder [31], further suggestion a role in the modulation of cognitive processes.

Overall, our findings indicate that thiamine deficiency disrupts the normal associations between hippocampal synapsin I and phosphorylated synapsin I levels and hippocampal-dependent behavior. These data extend previous findings implicating the involvement of hippocampal neurotransmission dysfunction in the spatial learning and memory deficits in PTD rats and also provides some insight about the mechanisms underlying altered neurochemical release reported in PTD.

Acknowledgments

Supported by CAPES Foundation, Ministry of Education of Brazil.

A portion of this research was supported by grant NINDS 054272 (LMS). Leticia Resende received scholarship from the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

References

1. Thomson AD, Cook CCH, Guerrini I, Sheedy D, Harper C, Marshall EJ. Wernicke's encephalopathy revisited: translation of the case history section of the original manuscript by Carl Wernicke 'Lehrbuch der Gehirnkrankheiten für Aerzte und Studierende' (1881) with a commentary. *Alcohol and Alcoholism*. 2008; 43:174–179. [PubMed: 18056751]
2. Hazzell AS, Butterworth RF. Update of cell mechanisms in thiamine deficiency: focus on oxidative stress, excitotoxicity and inflammation. *Alcohol & Alcoholism*. 2009; 44:141–147. [PubMed: 19151161]
3. Watanabe I. Pyridoxamine-induced acute thiamine-deficient encephalopathy in the mouse. *Experimental and Molecular Pathology*. 1978; 28:381–394. [PubMed: 648625]
4. Langlais PJ, Savage LM. Thiamine deficiency in rats produces cognitive and memory deficits on spatial tasks that correlate with tissue loss in diencephalon, cortex and white matter. *Behavioural Brain Research*. 1995; 68:75–89. [PubMed: 7619308]
5. Mumby DG, Cameli L, Glenn MJ. Impaired allocentric spatial working memory and intact retrograde memory after thalamic damage caused by thiamine deficiency in rats. *Behavioral Neuroscience*. 1999; 113:42–50. [PubMed: 10197905]
6. Pires RGW, Pereira SRC, Oliveira-Silva I, Franco GC, Ribeiro AM. Cholinergic parameters and the retrieval of learned and re-learned spatial information: a study using a model of Wernicke–Korsakoff Syndrome. *Behavioural Brain Research*. 2005; 162:11–21. [PubMed: 15922063]
7. Carvalho FM, Pereira SRC, Pires RGW, Ferraz VP, Romano-Silva MA, Oliveira-Silva IF, et al. Thiamine deficiency decreases glutamate uptake in the prefrontal cortex and impairs spatial memory performance in a water maze test. *Pharmacology Biochemistry and Behavior*. 2006; 83:481–489.
8. Langlais PJ, Mandel RJ, Mair RG. Diencephalic lesions, learning impairments, and intact retrograde memory following acute thiamine deficiency in the rat. *Behavioural Brain Research*. 1992; 48:177–185. [PubMed: 1616608]
9. Savage LM, Chang Q, Gold PE. Diencephalic damage decreases hippocampal acetylcholine release during spontaneous alternation testing. *Learning & Memory*. 2003; 10:242–246. [PubMed: 12888541]
10. Vetreno RP, Klintsova A, Savage LM. Stage-dependent alterations of progenitor cell proliferation and neurogenesis in an animal model of Wernicke–Korsakoff syndrome. *Brain Research*. 2011; 1391:132–146. [PubMed: 21440532]
11. Vetreno RP, Ramos RL, Anzalone S, Savage LM. Brain and behavioral pathology in an animal model of Wernicke's encephalopathy and Wernicke–Korsakoff Syndrome. *Brain Research*. 2012; 1436:178–192. [PubMed: 22192411]
12. Jovanovic JN, Czernik AJ, Fienberg AA, Greengard P, Sihra TS. Synapsins as mediators of BDNF-enhanced neurotransmitter release. *Nature Neuroscience*. 2000; 3:323–329. [PubMed: 10725920]
13. Greengard P, Valtorta F, Czernik AJ, Benfenati F. Synaptic vesicle phosphoproteins and regulation of synaptic function. *Science*. 1993; 259:780–785. [PubMed: 8430330]
14. Pires RGW, Pereira SRC, Carvalho FM, Oliveira-Silva IF, Ferraz VP, Ribeiro AM. Correlation between phosphorylation level of a hippocampal 86 kDa protein and extinction of a behavior in a model of Wernicke–Korsakoff syndrome. *Behavioural Brain Research*. 2007; 180:102–106. [PubMed: 17395279]
15. McIntyre CK, Pal SN, Marriott LK, Gold PE. Competition between memory systems: acetylcholine release in the hippocampus correlates negatively with good performance on an amygdala-dependent task. *Journal of Neuroscience*. 2002; 22:1171–1176. [PubMed: 11826146]

16. Hughes RN. The value of spontaneous alternation behavior (SAB) as a test of retention in pharmacological investigations of memory. *Neuroscience and Biobehavioral Reviews*. 2004; 28:497–505. [PubMed: 15465137]
17. Lalonde R. The neurobiological basis of spontaneous alternation. *Neuroscience and Biobehavioral Reviews*. 2002; 26:91–104. [PubMed: 11835987]
18. Roland JJ, Mark K, Vetreno RP, Savage LM. Increasing hippocampal acetylcholine levels enhance behavioral performance in an animal model of diencephalic amnesia. *Brain Research*. 2008; 1234:116–127. [PubMed: 18706897]
19. Gobeske KT, Das S, Bonaguidi MA, Weiss C, Radulovic J, Disterhoft JF, et al. BMP signaling mediates effects of exercise on hippocampal neurogenesis and cognition in mice. *PLoS ONE*. 2009; 20:e7506.
20. Werner DF, Kumar S, Criswell HE, Suryanarayanan A, Fetzer JA, Comerford CE, et al. PKC γ is required for ethanol-induced increases in GABAA receptor $\alpha 4$ subunit expression in cultured cerebral cortical neurons. *Journal of Neurochemistry*. 2011; 116:554–563. [PubMed: 21155805]
21. Mair RG, Anderson CD, Langlais PJ, McEntee WJ. Thiamine deficiency depletes cortical norepinephrine and impairs learning processes in the rat. *Brain Research*. 1985; 360:273–284. [PubMed: 4075172]
22. Mair RG, Knoth RL, Rabchenuk SA, Langlais PJ. Impairment of olfactory, auditory, and spatial serial reversal learning in rats recovered from pyriethamine-induced thiamine deficiency. *Behavioral Neuroscience*. 1991; 105:360–374. [PubMed: 1907457]
23. Gómez-Pinilla F, So V, Kesslak JP. Spatial learning induces neurotrophin receptor and synapsin I in the hippocampus. *Brain Research*. 2001; 904:13–19. [PubMed: 11516407]
24. Kushner SA, Elgersma Y, Murphy GG, Jaarsma D, Van Woerden GM, Hojjati MR, et al. Modulation of presynaptic plasticity and learning by the H-ras/extracellular signal-regulated kinase/synapsin I signaling pathway. *Journal of Neuroscience*. 2005; 25:9721–9734. [PubMed: 16237176]
25. Corradi A, Zanardi A, Giacomini C, Onofri F, Valtorta F, Zoli M, et al. Synapsin-I- and synapsin-II-null mice display an increased age-dependent cognitive impairment. *Journal of Cell Science*. 2008; 121:3042–3045. [PubMed: 18713831]
26. Pires R, Pereira S, Pittella J, Franco GC, Ferreiro C, Fernandes PA, et al. The contribution of mild thiamine deficiency and ethanol consumption to central cholinergic parameter dysfunction and rats' open-field performance impairment. *Pharmacology Biochemistry and Behavior*. 2001; 70:227–235.
27. Lê O, Hérox M, Butterworth RF. Pyriethamine-induced thiamine deficiency results in decreased Ca²⁺-dependent release of glutamate from rat hippocampal slices. *Metabolic Brain Disease*. 1991; 6:125–132. [PubMed: 1685763]
28. Janz R, Sudhof TC, Hammer RE, Unni V, Siegelbaum SA, Bolshakov VY. Essential roles in synaptic plasticity for synaptogyrin I and synaptophysin I. *Neuron*. 1999; 24:687–700. [PubMed: 10595519]
29. Molteni R, Barnard RJ, Ying Z, Roberts CK, Gómez-Pinilla F. A high-fat, refined sugar diet reduces hippocampal brain-derived neurotrophic factor, neuronal plasticity, and learning. *Neuroscience*. 2002; 112:803–814. [PubMed: 12088740]
30. Griesbach GS, Hovda DA, Molteni R, Gomez-Pinilla F. Alterations in BDNF and synapsin I within the occipital cortex and hippocampus after mild traumatic brain injury in the developing rat: reflections of injury-induced neuroplasticity. *Journal of Neurotrauma*. 2002; 19:803–814. [PubMed: 12184851]
31. Vawter MP, Thatcher L, Usen N, Hyde TM, Kleinman JE, Freed WJ. Reduction of synapsin in the hippocampus of patients with bipolar disorder and schizophrenia. *Molecular Psychiatry*. 2002; 7:571–578. [PubMed: 12140780]

Fig. 1. Behavioral data (mean $\pm$ SD) from a single session of spontaneous alternation testing for control (PF) and pyridoxine induced thiamine deficiency (PTD) rats. (A) Activity level following PTD or PF treatment. (B) Spontaneous alternation behaviors. * $p < 0.05$ compared to PF controls.

Fig. 2.

Relative levels of phosphorylated (phospho)-synapsin I and synapsin I in the hippocampus of control (PF) rats and pyriithiamine induced thiamine deficiency (PTD) rats. Values were expressed as a ratio of synapsin I or phospho-synapsin I to β -actin (internal control) and then converted to percent of control group as presented in the bar figures. (A) Phospho-synapsin I in PF and PTD-treated animals. (B) Synapsin I in PF and PTD-treated animals. (C) Ratio of synapsin I to phospho-synapsin I. Representative images of synapsin I, phospho-synapsin I and β -actin are shown. Data are presented as mean $\pm$ SEM. The p values for the comparisons between PF and PTD groups for phospho-synapsin I and synapsin I are $p = 0.02$ and $p = 0.06$, respectively. Panel C shows the rate of synapsin I to total phosphorylated synapsin I for each group, $p = 0.32$.

Fig. 3. Scatterplot of behavioral data and the levels of and synapsin I (A) and phospho-synapsin I (B). (●) Control (PF) and (◆) pyridoxamine induced thiamine deficiency (PTD).