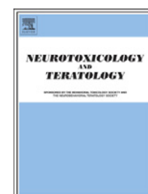
Neurotoxicology and Teratology xxx (2011) xxx–xxx

Contents lists available at ScienceDirect

Neurotoxicology and Teratology

journal homepage: www.elsevier.com/locate/neutera



The effect of thiamin tetrahydrofurfuryl disulfide on behavior of juvenile DBA/2J mice

Judith I. Hills [a,1], Mari S. Golub [b], Lucien Bettendorff [c], Carl L. Keen [a,*]

[a] Department of Nutrition, One Shields Ave., University of CA, Davis, CA 95616, USA
[b] BMB/CNPRC, University of CA, Davis, CA 95616, USA
[c] GIGA-Neurosciences, University of Liège, B-4000 Liège Belgium

ARTICLE INFO

Article history:
Received 11 February 2010
Received in revised form 26 May 2011
Accepted 19 July 2011
Available online xxxx

Keywords:
Thiamin
Behavior
Learning
Social
Activity
Acoustic startle prepulse inhibition

ABSTRACT

Due to genetic defects or illness some individuals require higher amounts of thiamin than are typically provided by the diet. Lipid-soluble thiamin precursors can achieve high blood levels of thiamin and result in increased concentrations in the central nervous system. High intakes of thiamin have been reported as beneficial in children with autism and attention deficit/hyperactivity disorder. The current study examined the effect of thiamin tetrahydrofurfuryl disulfide (TTFD), a lipophilic precursor, on behavior in the juvenile male DBA/2J mouse. Mice given by oral gavage deionized water or deionized water providing 100 mg or 340 mg TTFD/kg body weight daily for 17 days, starting at postnatal day 18, were tested for effects on operant learning, social interaction, general activity level, and prepulse inhibition of acoustic startle, as well as effects on growth and select organ weights. Results indicate lower activity and altered social interaction at both treatment levels and decreased acoustic startle at the 100 mg/kg level. Compared to controls, percent weight gain was lower in the TTFD-treatment groups, but percent body length increase was not affected by TTFD treatment. TTFD treatment did not influence percent organ weights as percentage of body weights. TTFD treatment resulted in increased whole brain thiamin concentrations. These results support the concept that lipophilic thiamin precursors provided during early development can affect a number of behavioral parameters. In clinical trials with children with behavior disorders, attention should be given to preventing possible adverse gastrointestinal irritant effects associated with TTFD therapy.

© 2011 Published by Elsevier Inc.

1. Introduction

Thiamin, vitamin B-1, has several known functions in the body that have the potential to affect brain activity and behavior. As thiamin diphosphate (ThDP) it serves as a cofactor for enzymes involved in energy metabolism and formation of essential body constituents (McCormick, 2000) as well as the degradation of 3-methyl branched chain fatty acids and 2-hydroxy straight chain fatty acids (Casteels et al., 2007). Thiamin triphosphate (ThTP) activates Cl^- uptake through maxi chloride channels in excised patches of neuroblastoma cells, is involved in nicotinic receptor clustering at the neuromuscular junction, and has been hypothesized to play a role in brain cell signaling and protection against mitochondrial oxidative stress (reviewed by Bettendorff and Wins, 2009). Cell signaling functions have also been proposed for adenosine thiamin triphosphate (AThTP) and adenosine thiamin diphosphate (AThDP) (Frédérich et al., 2009). Other reported or hypothesized functions include regulation of enzyme expression (e.g., (Pekovich et al., 1998a)); alteration of neuronal membrane ion channels that result in prolonged depolarization responses (Houzen and Kanno, 1998; Tallaksen and Tauboll, 2000); maintenance of nerve membrane potentials (Itokawa, 1996); alteration of neurotransmitter release (Yamashita et al., 1993) or uptake (Thomson and Marshall, 2006); and antioxidant activity of unphosphorylated thiamin (reviewed by (Gibson and Blass, 2007)).

The signs of thiamin deficiency are protean and manifest differently depending on an individual's age, dietary deficiencies and relative amounts of dietary carbohydrate, disease status, and genetic makeup (Inouye and Katsura, 1965). Cells differ in their ability to uptake thiamin, the amounts that are needed, and regulation of the different forms of thiamin and their compartmentalization (Bettendorff, 1995; Pekovich et al., 1998b). Specialized transporters limit the rate of thiamin uptake

Abbreviations: ASPPI, acoustic startle prepulse inhibition; AThTP, adenosine thiamin triphosphate; AThDP, adenosine thiamin diphosphate; b.i.d., twice daily; BxD, recombinant cross of C57Bl/6 with DBA/2 mice; HACTV, horizontal locomotor activity beam breaks; dB, decibels; HFHL, high frequency hearing loss; ln, natural log; LnSt, starting body length at postnatal day 18; LRM, localized, non-ambulatory, repetitive movement beam breaks; LSMeans, least squares means; mAChR, muscarinic acetylcholine receptor; MBR, mean baseline response; MSR, mean startle response; nAChR, nicotinic acetylcholine receptor; PctCtr, percent of time in arena center; PctLnChg, percent length change since postnatal day 18; PctWtChg, percent weight change since postnatal day 18; PND, postnatal day; q.i.d., 4 times daily; q.i.d., four times daily; RT, resting time; sqrt, square root; T0, 0 mg thiamin tetrahydrofurfuryl disulfide/kg body wt; T100, 100 mg thiamin tetrahydrofurfuryl disulfide/kg body wt; T340, 340 mg thiamin tetrahydrofurfuryl disulfide/kg body wt; ThDP, thiamin diphosphate; ThMP, thiamin monophosphate; ThTP, thiamin triphosphate; TTFD, thiamin tetrahydrofurfuryl disulfide; Tx, TTFD treatment; VACTV, vertical (rearing) activity beam breaks; WtCur, current body weight; WtSt, starting weight at postnatal day 18.

* Corresponding author. Tel.: +1 530 752 6331; fax: +1 530 752 8966.
E-mail addresses: judyhills@gmail.com (J.I. Hills), msgolub@ucdavis.edu (M.S. Golub), l.bettendorff@ulg.ac.be (L. Bettendorff), clkeen@ucdavis.edu (C.L. Keen).
[1] Permanent address: 43677 Montgomery Ave., Davis, CA 95618, USA.

0892-0362/$ – see front matter © 2011 Published by Elsevier Inc.
doi:10.1016/j.ntt.2011.07.006

ARTICLE IN PRESS

(reviewed by (Bettendorff and Wins, 2009)); therefore, conditions affecting these transporters can influence thiamin requirements. Thus, in disease states that result in defects of upstream factors, such as enzymes or other proteins that interact with thiamin, a means of bypassing thiamin transport can be of value. Thiamin tetrahydrofurfuryl disulfide (TTFD) can be taken orally and absorbed without need for passage through thiamin transporters (Mitoma, 1973; Suzuoki et al., 1968). TTFD has been used clinically in Japan and the U.S. (Lonsdale, 2006), and is generally considered safe (Baker and Frank, 1976; Lonsdale, 1987a; Mizutani et al., 1971). The oral LD50 in mice is 2200 mg/kg (Anon, 1982).

Thiamin or TTFD has shown promise in the treatment of two neurological disorders in children. A pilot human study (Lonsdale et al., 2002) investigating treatment of young autistic children with 50 mg b.i.d. by rectal suppository suggested positive results with respect to improvements in behavior, speech, and sleep. A beneficial effect of high-dose thiamin was also reported in children with hyperkinesis (Brenner, 1982) where 8 of 100 children responded favorably to 100 mg q.i.d.; 4 of the children required supplementation long term, a finding that suggested a genetic basis to their high thiamin requirement. Researchers (Lonsdale, 1987a, 1982b, 1990, 2006) have reported other multifaceted behavioral and somatic disorders in children that have responded to thiamin or lipophilic thiamin precursor administration. In adults, lipophilic forms of thiamin have been used to treat psychobehavioral inhibition and asthenia, enhance memory in elderly patients, and improve cognitive function and reduce anxiety in university students with severe psychosomatic fatigue (reviewed by (Van Reeth, 1999)), as well as a number of other disorders which will be reviewed below in Section 4.4.

This present study was undertaken to focus specifically on behavioral effects of pharmacologic doses of thiamin provided via oral TTFD. The test animal was the juvenile male DBA/2J mouse, an inbred strain that has been widely studied and characterized. The possibility that this mouse may have a defect in thiamin utilization has been advanced, though not substantiated (Eudy et al., 2000; Lonsdale, 1982a). This mouse experiences rapid age-related hearing loss (Johnson et al., 2008). TTFD treatment reportedly extends juvenile DBA/2J susceptibility to audiogenic seizures (Lonsdale, 1982a), a finding that could indicate a change in the advance of their hearing loss. The present study used juvenile mice in order to simulate effects of supplementation in young children with behavioral disorders.

To assess behavioral effects of TTFD, we developed a rapid, sequential test battery including operant learning, social dyadic interaction, monitoring of activity levels over a 24-h period, and prepulse inhibition of acoustic startle. Low response rates in the juvenile mice during the evaluation of operant learning and technical difficulties with the apparatus minimized the ability to draw conclusions from this assessment, thus these data are not presented.

2. Methods and materials

2.1. Animals and animal care

The animal protocol was approved by the UC Davis Animal Care and Use Administrative Advisory Committee. Eighteen-day-old male DBA/2J mice were purchased from Jackson Laboratory West (Sacramento, CA vivarium) in thirteen cohorts that each included 6 to 8 mice randomly distributed among treatment groups (control and TTFD) plus an equal number used as stimulus mice for the social dyadic interaction test. Assignment to treatment group was done upon arrival, using one mouse per litter per treatment group. Treatment groups in each cohort were subdivided into 2 'squads' because of limitations in testing equipment. Treatment groups and squads were balanced for body weight of the mice.

All experimental and stimulus mice were caged with littermates until postnatal day (PND) 21, at which time experimental mice were individually caged, whereas stimulus mice were then paired with a non-littermate, with change to a different non-littermate each day until social dyadic testing was completed. This re-pairing of stimulus mice prevented frequent rearing and jumping (escape) behavior seen in preliminary studies when stimulus mice were continuously caged with littermates.

Mice were housed under temperature (20–22 °C) and light-controlled (reverse phase, lights on 21:15–09:15) conditions and fed a complete, purified egg white protein based diet (Dyets modified AIN-93G) and deionized water ad lib throughout the study period, except as follows: for experimental mice, food was restricted 4-h prior to the 2-h training session for operant learning and the 2-h operant learning test itself. As is common in nutritional studies, treatments for experimental mice were initiated upon receipt of the mice.

From PND 18 to PND 34 experimental mice were given daily oral gavage (at 09:00 for squad 1, at 11:30 for squad 2) with 5 μl fluid/g body weight. Gavage treatments were deionized water (control, T0, n = 24), 100 mg TTFD/kg body weight in deionized water (T100, n = 23), or 340 mg TTFD/kg body weight in deionized water (T340, n = 24). These dosages correspond on a thiamin molar basis to lipophilic forms of thiamin used in previous studies with mice (Lonsdale, 1982a; Micheau et al., 1985).

With the exception of the 24-h activity test, the tests were conducted approximately 3-h after gavage, during the first half of the dark cycle, a time mice are naturally active. Mice were transported to and from test locations in a dark, insulated container.

Mice used for tissue analysis were divided into the same three treatment groups (n = 5–6/group), reared under similar conditions as the mice used for the behavioral work (without behavior testing), provided deionized water and a similar diet (Kwik-Uribe et al., 2000) supplemented with additional thiamin to bring the thiamin content to the same level (5 mg/kg diet) as provided to the experimental mice and as meets the recommended intake level for mice (N.R.C.U.S.S.o.L.A, 1995). After 12 days of gavage treatment, the mice were euthanized by CO_2 inhalation and whole brain was removed for thiamin analysis.

2.2. Study design

The timeline for the behavioral study is given in Table 1.

2.3. Growth and organ weights

Experimental mice were weighed daily before gavage, and body length (nose to rump) was determined at the start of the study and before necropsy. Mice were observed at both the start and end of the study to detect any changes in general activity, ambulation, posture,

Table 1
Timeline for study[a].

Postnatal day	Animal care and testing
18	Receive mice. Weigh, measure length, observe, assign to squad and treatment
21	Individually cage experimental mice. Pair cage stimulus mice with non-littermate.
22–28	Re-pair stimulus mice
25	Dipper training for operant test
26	Operant test
27	Social dyadic interaction, session 1
28	Social dyadic interaction, session 2
29	Test of 24-h activity, squad 1
30	Test of 24-h activity, squad 2
32	Prepulse inhibition of acoustic startle test
34	Weigh, measure length, observe, necropsy for organ weights

[a] n = 24 T0 (control), 23 T100 (100 mg TTFD/kg body weight), 24 T340 (340 mg TTFD/kg body weight)

Please cite this article as: Hills JI, et al, The effect of thiamin tetrahydrofurfuryl disulfide on behavior of juvenile DBA/2J mice, Neurotoxicol Teratol (2011), doi:10.1016/j.ntt.2011.07.006

ARTICLE IN PRESS

appearance, or behavior. On PND 34, 3 h after gavage, mice were euthanized by CO_2 inhalation, and the brain, testes, liver, spleen, kidneys, and heart were rapidly removed and weighed.

2.4. Behavior tests

2.4.1. Social dyadic interaction test

Mice proceeded to this test after completing the operant behavior test, which is not discussed due to procedural difficulties with that test (unpublished data). Social behaviors were studied by pairing each experimental mouse with a DBA/2J stimulus mouse (a mouse of the same age and sex that did not receive gavage treatment with TTFD or water) on two consecutive days. Experimental and stimulus mice were ranked and paired according to weight. Stimulus mice were used once on each of the consecutive test days and were paired with different experimental mice on the two days. Prior to starting the test, the stimulus mouse was marked with a black marker for identification then both mice were placed in separate Plexiglas holding chambers (3.1 cm^2) identical to the test chamber and allowed to acclimate for 5 min. Following acclimation, both mice were placed in the test chamber at the same time, and videotaped for 10 min under low illumination. The chambers were cleaned before testing each pair of mice.

An experienced observer, blinded to the experimental treatment, scored the number and duration of focal (experimental) mouse behaviors using Noldus Observer 5.0 software (Wageningen, the Netherlands) according to categories adapted from Terranova and Laviola (Terranova and Laviola, 2001). Behaviors were grouped into categories that reflected activity level and orientation of activity (toward the stimulus mouse vs. the environment) (Table 2).

2.4.2. 24-h activity monitoring

Activity monitoring was conducted in an enclosed, automated 'open field' (Integra, Accuscan, Columbus, OH) as previously described (Golub et al., 2004), over a 24 h period, with data collected in 3-min time bins. Each mouse was placed in the apparatus chamber (36 cm^2, Plexiglass box) containing access to food and water approximately 1¾ h prior to the end of the light cycle (which was uniformly set for the same time for each cohort), after being weighed and receiving its gavage treatment. The first 30 min of activity in the arena (data collected and analyzed in ten 3-min time bins) was used to determine adaptation to a novel environment and assess emotionality. For the remainder of the 24-h period, 3-min time bins were synchronized with respect to day/night cycle by using the time stamp on each 3-min time bin. Four hundred fifty one synchronized 3-min time bins (1353 min total) exclusive of the adaptation period were obtained for each mouse and divided into 23 time bins, the first consisting of 33 minutes, and the remaining time bins consisting of 1 h each. Means of activities for each time bin were obtained for each mouse. Activity rhythms were also summarized for 75 min (25 3-min time bins) following the beginning of the dark cycle, the time of peak activity.

Table 2
Social dyadic interaction: behavior groups used for ANCOVA.

Behavioral group	Component behaviors
Social passive: includes mild-mannered association with the stimulus mouse	Social inactive, push past, cuddle, social receptive, turn away
Social active: includes vigorous interaction with the stimulus mouse	Groom partner, push under, crawl over/under, follow
Total active: includes both social active behavior and other vigorous activity directed toward the environment	Groom partner, push under, crawl over/under, follow, explore, jump
Other: includes behaviors that were less active or of uncertain intent regarding the stimulus mouse	Approach, social sniff, groom self, rear

Component behaviors were grouped into larger behavioral groups (i.e., social passive, social active, total active, other) that reflected activity level and orientation of activity (toward the stimulus mouse vs. the environment). Analyses were conduced on these behavior groups.

2.4.3. Acoustic startle/prepulse inhibition (ASPPI)

This procedure tests the degree to which presentation of a brief low intensity sound (the prepulse) provided 30–500 ms prior to a sudden intense startle-producing sound (the pulse) inhibits the resulting startle reflex. The prepulse normally reduces the startle response and is an operational measure of sensorimotor gating, a process by which an animal filters out extraneous information and protects against sensory overload for (review see (Swerdlow et al., 2008)). Deficits in sensory prepulse inhibition (PPI) are studied with reference to several disorders, including schizophrenia, panic disorder, bipolar disorder, obsessive compulsive disorder, comorbid Tourette syndrome/attention deficit hyperactivity disorder, and Huntington's disease (for review see Swerdlow et al., 2008). In rodents the startle response itself is commonly used to assess emotional reactivity and the effects of anti-anxiety drugs (Bourin et al., 2007; Grillon, 2008; McCaughran et al., 2000). Species and strains within species differ in their regulation of startle and PPI (Swerdlow et al., 2008).

A commercial startle reflex system (SR-LAB, San Diego Instruments, San Diego, CA), previously described (Berman et al., 2008), was used. The mouse was allowed to acclimate in the dark chamber for 5 min before testing commenced. The 10-min test session consisted of 50 stimulus trials presented in a pseudo random manner, separated by inter-trial intervals of 5- to 20-s (5 s steps). Testing was divided into 10 blocks, each consisting of five trial combinations: (i) 120-dB, 40 ms startle alone, (ii) 120-dB, 40 ms startle preceded by 74-dB prepulse, (iii) 120-dB, 40 ms startle preceded by 82-dB prepulse, (iv) 120-dB, 40 ms startle preceded 90-dB prepulse, and (v) no stimulus (background white noise only), as previously described (Berman et al., 2008).

2.5. Necropsy and tissue analysis for thiamin and thiamin phosphates

Following euthanasia by CO_2 inhalation, the whole brain (including olfactory bulb and brainstem) was rapidly excised, immediately frozen in liquid nitrogen, and stored at minus 80 °C until extracted and analyzed by HPLC according to published methods (Bettendorff et al., 1991). The remaining pellet was dissolved in 2 ml 1 N NaOH in a warm water bath, then analyzed for protein content by the Bradford method (Bradford, 1976) using fatty acid-free bovine serum albumin as the protein standard.

2.6. Chemicals

Chemical sources were as follows: TTFD from Cardiovascular Research, Ltd (Concord, CA, USA); thiamin, ThMP, ThDP, tricholoroacetic acid (99+%, ACS), and bovine serum albumin from Sigma Aldrich (St. Louis, MO, USA). Diethyl ether and stabilizer-free tetrahydrofuran were from Biosolve (Valkenswaard, The Netherlands). ThTP and AThTP were prepared as previously described (Bettendorff et al., 2003 and Frédérich et al., 2009, respectively). Purified water was obtained using a Barnstead NANO-pure system (Van Nuys, CA).

2.7. Statistical analysis

Analysis of variance (ANOVA) or covariance (ANCOVA) was conducted with SAS 9.2 for Windows (SAS Institute, Inc., Cary, NC) using the Mixed Procedure with Tukey–Kramer post hoc comparisons. Cohort was used as the random effect. For repeated measures over time an auto regressive structure [AR(1)] was used. The group option was included where appropriate to optimize model fit. Differences in all analyses were considered significant at $P<0.05$. P values have been rounded to 0.05, 0.01, 0.005, 0.001, 0.0005, or 0.0001, as appropriate. The results of analyses showing significance of TTFD effects are

Please cite this article as: Hills JI, et al, The effect of thiamin tetrahydrofurfuryl disulfide on behavior of juvenile DBA/2J mice, Neurotoxicol Teratol (2011), doi:10.1016/j.ntt.2011.07.006

ARTICLE IN PRESS

presented in tables, along with details of the analyses. Where interactions of treatment with covariates occurred, between-group significance of treatment was examined at the 25th, 50th, and 75th percentiles of the covariate.

Because mice grow rapidly during the juvenile period, weight was measured at several time points during treatment, and the gain in weight from the pretreatment baseline to necropsy (percent weight change) and growth in length from baseline to necropsy were used as growth endpoints. Organs as percent of body weight were determined for each mouse at necropsy.

Lower weight gain (represented as percent weight change in analyses) may be an indicator of generally delayed development that could be reflected in behavior. Because weight gain was found to differ between TTFD treatment groups at early stages of behavioral testing, further analyses were conducted for those behavioral endpoints which showed direct effects of both TTFD and weight gain. These analyses produced a measure of total effect of treatment, derived from path analysis, which takes into account how treatment directly affects behavior as well as how it indirectly affects behavior through its effect on weight gain. Comparison of direct and total effects (not shown) indicated that although some behavioral effects of TTFD occurred partially through effects on weight or weight gain, that component was much smaller than the direct effect.

For the social dyadic interaction test, the duration and number of episodes for each behavior group for the two sessions were analyzed by repeated measures ANCOVA, and ANCOVA was also performed on the mean of the two sessions for each behavior. Analysis of episode and duration data yielded similar results; only the duration data group comparisons are presented.

For the activity test adaptation period, data were analyzed by repeated measures ANCOVA across ten 3-min time samples. Repeated measures ANCOVA of variables over the 24-h period (time bins synchronized for the light/dark cycle, excluding the adaptation period) was conducted using each subject's means for 23 time bins described in Section 2.4.2. Spline graphs were used to examine the rhythm of several activity measurements during the light→dark transition period. Mean values of these variables for each of the twenty-five 3-min time samples following onset of the dark cycle were plotted using sm50 interpolation and analyzed with polynomial mixed models. When interactions of treatment × sample were significant and the model with those interactions showed better fit than the model without the interactions, treatment was considered to significantly affect the activity pattern.

For the acoustic startle prepulse inhibition test, means for baseline (i.e., no stimulus) response, startle response, and startle response following each prepulse level were obtained for each mouse. Acoustic startle prepulse inhibition (ASPPI) was calculated as ((1 − (startle-following-prepulse / startle-without-prepulse)) * 100). ANCOVA was performed on mean baseline response, mean startle response, and mean acoustic startle prepulse inhibition using both concurrent weight (a mechanical effect) and percent weight change (a developmental effect) as covariates. Since preliminary analyses indicated the mechanical effect of current weight showed greater effects than the developmental effect of percent weight change, only the results with the former covariate are presented. Due to a significant effect of treatment on current weight, path analyses were performed, and the total effects of treatment are presented in the table and figures for this test.

3. Results

3.1. Growth and organ weights (Table 3)

A between-group difference in percent weight change was significant by PND 26, the time of the operant test ($F_{2,10.4} = 4.04$, $P = 0.0503$, T340 < T0, $P < 0.05$, a 28% decrease). At study end (PND 34), compared to T0 percent weight change of both T100 and T340 mice was lower (Fig. 1A), but growth in length did not differ between treatment groups (Fig. 1B). Greater starting weight (at PND 18) and greater starting length were associated with lower weight gain and lower length gain, respectively. At the time of the acoustic startle prepulse inhibition (ASPPI) test, compared to T0 current weight was less in T100 ($P < 0.01$) and T340 ($P < 0.05$) mice. Current weight was also significantly positively associated with starting weight. No significant between-group differences were found in organ weights (brain, liver, spleen, heart, kidneys, testes) when expressed as percent of body weight (analysis data not shown) (Table 3).

3.2. Behavior tests

3.2.1. Social dyadic interaction test

Severe aggressive behavior occurred with two dyads (aggression by a control mouse in one instance and by a T340 mouse in the other instance), preventing observation of other normal behaviors. These two dyads were removed from the analysis (Fig. 2). Q1

Repeated measures ANCOVA of data from the two observation sessions (Table 4) showed treatment effects for duration of behavior in the categories Social Passive (T0 less than T340, $P = 0.01$), Social Active (T0 greater than T100 and T340, $P < 0.01$ each), and Total Active (T0 greater than T100 and T340, $P < 0.0005$ and $= 0.0001$, respectively) but not the Other behavior category. ANCOVA of the mean activities from the two sessions also showed significant treatment effects, and the direction of between-group differences was similar (Table 4, Fig. 4). Mean episodes of social passive behavior were significantly lower (13%) in the second test session, indicating adaptation to the test for that behavioral category (data not shown).

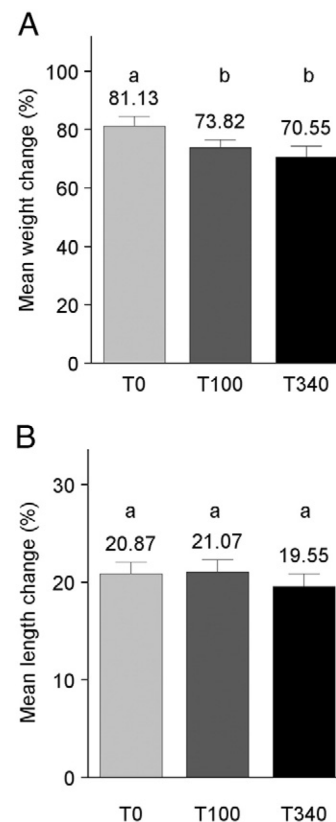


Fig. 1. Percent weight change gain (PctWtChg) and percent length change (PctLnChg) (Table 3). Changes in weight and length (nose to rump) between study start and study finish were computed for each mouse. (A) ANCOVA for PctWtChg showed T0 > T100 and T340, $P < 0.05$ each. (B) ANCOVA for PctLnChg showed no between-group differences. Between-group differences are indicated by a vs. b notation. Error bars represent S.E.M. T0 = control (n = 19), T100 = 100 mg TTFD/kg body weight (n = 20), T340 = 340 mg TTFD/kg body weight (n = 23).

UNCORRECTED PROOF

Please cite this article as: Hills JI, et al, The effect of thiamin tetrahydrofurfuryl disulfide on behavior of juvenile DBA/2J mice, Neurotoxicol Teratol (2011), doi:10.1016/j.ntt.2011.07.006

Table 3
Statistical analysis results for growth[a].

Transformed variable	ANCOVA[b] fixed effects	F test, Pr>F for treatment and significant covariates	
Ranked WtCur	Tx|WtSt	Tx	$F_{2,66}=5.82$, $P=0.0047$
(at ASPPI test)		WtSt	$F_{1,66}=84.78$, $P<0.0001$
Squared PctWtChg	Tx|WtSt	Tx	$F_{2,67}=4.23$, $P=0.0186$
(at necropsy)		WtSt	$F_{1,69}=14.67$, $P=0.0003$
Ranked PctLnChg	Tx|LnSt|PctWtChg	Tx	$F_{2,60.9}=0.30$, $P=0.7443$
(at necropsy)		LnSt	$F_{1,60.1}=30.49$, $P<0.0001$

WtCur = current weight, ASPPI test = test for prepulse inhibition of acoustic startle, PctWtChg = percent change in weight from study start to necropsy, PctLnChg = percent change in body length from study start to necropsy, Tx = treatment, WtSt = body weight at study start (PND 18), LnSt = body length (nose to rump) at study start (PND 18), rPctWtChg = residual from regression of percent weight change on treatment.

[a] n = 19 T0, 20 T100, 23 T340

[b] Vertical bars (|) indicate that significance of all indicated effects and their interactions was tested; however, as noted, the F test and significance levels are only listed for treatment (whether or not it reached significance) and other effects and interactions that reached significance.

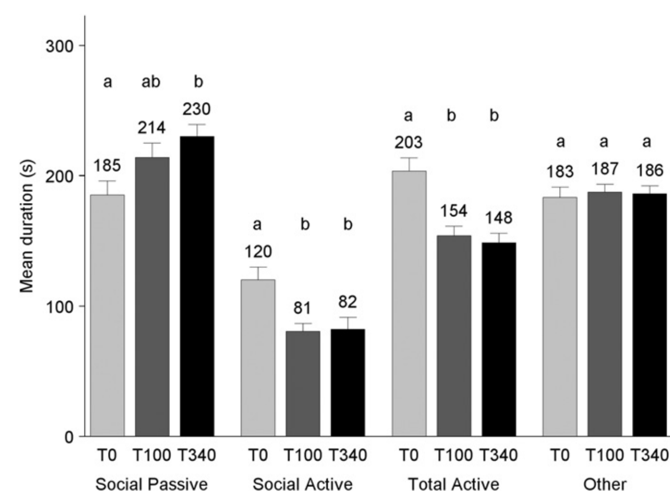


Fig. 2. Duration of activity in four behavior categories during social dyadic interaction (Table 4). Behaviors were quantified in two 10-min sessions for each mouse, and means of the two sessions are shown in the figure. ANCOVA of session means showed TTFD-treated groups differed from controls in three behavior categories: Social Passive, T340>T0 ($P=0.01$); Social Active, T0>T100 and T340 ($P<0.005$ and 0.01, respectively); and Total Active T0>T100 and T340 ($P<0.0005$ each). Between-group differences are indicated by a vs. b notation. Error bars represent S.E.M. T0 = control (n = 19), T100 = 100 mg TTFD/kg body weight (n = 20), T340 = 340 mg TTFD/kg body weight (n = 23).

Table 4
Statistical analysis results for duration of social dyadic behaviors[a].

Transformed variable	ANCOVA[b] fixed effects	F test, Pr>F for treatment and significant covariates	
Repeated measures			
Social passive behavior	Tx|Session	Tx	$F_{2,55.4}=4.37$ $P=0.0173$
sqrt Social active behavior	Tx|Session	Tx	$F_{2,57.3}=7.00$, $P=0.0019$
sqrt Total active behavior	Tx|Session	Tx	$F_{2,59}=12.32$, $P<0.0001$
Other behavior	Tx|Session	Tx	$F_{2,59}=0.10$, $P=0.9065$
Session means			
Social passive behavior	Tx	Tx	$F_{2,59}=4.50$, $P=0.0150$
sqrt Social active behavior	Tx	Tx	$F_{2,59}=7.20$, $P=0.0016$
sqrt Total active behavior	Tx	Tx	$F_{2,59}=12.40$, $P<0.0001$
cubed other behavior	Tx	Tx	$F_{2,59}=0.03$, $P=0.9752$

Analyses were conducted on behavior categories using (1) repeated measures on 2 test sessions and (2) the means of the 2 sessions.

Tx = treatment.

[a] n = 19 T0, 20 T100, 23 T340

[b] Vertical bars (|) indicate that significance of all indicated effects and their interactions was tested; however, as noted, the F test and significance levels are only listed for treatment (whether or not it reached significance). Other effects and interactions did not reach significance.

3.2.2. Activity monitoring

During the first 30 min (adaptation) of the monitoring period, TTFD-treated mice differed from control on three activity variables (Table 5, Fig. 3). Compared to T0, percent time in the arena center was reduced in both the T100 and T340 mice (Fig. 3B); localized repetitive movement was significantly reduced for T340 mice (Fig. 3C); and resting time was increased for both T100 and T340 mice (Fig. 3D). Treatment did not significantly affect horizontal locomotor activity (Fig. 3A). Habituation to the testing environment is indicated by significant time (3-min sample) effects for each behavior. Greater percent weight change (PctWtChg) was overall associated with greater horizontal locomotor activity.

For the remainder of the 24-h period that was synchronized for the light/dark cycle (Table 5, Fig. 4), significant treatment effects occurred

Table 5
Statistical analysis results for activity monitoring (adaptation and 24-h light cycle synchronized) [a].

Transformed variable	ANCOVA[b] fixed effects	F test, Pr>F for treatment and significant covariates	
Adaptation activity[c]			
sqrt HACTV	Tx|3-min sample|rPctWtChg	Tx	$F_{2,53.9}=1.54$, $P=0.2228$
		3-min sample	$F_{9,318}=63.13$, $P<0.0001$
		rPctWtChg	$F_{1,73.8}=7.44$, $P=0.0080$
ln PctCtr	Tx|3-min sample|rPctWtChg	Tx	$F_{2,52.6}=7.07$, $P=0.0019$
		3-min sample	$F_{9,105}=2.46$, $P=0.013$
sqrt LRM	Tx|3-min sample|rPctWtChg	Tx	$F_{2,97.3}=3.44$, $P<0.0361$
		3-min sample	$F_{9,462}=12.68$, $P<0.0001$
Cubed RT	Tx|3-min sample|rPctWtChg	Tx	$F_{2,242}=146.1$, $P<0.0001$
		3-min sample	$F_{2,541}=12.08$, $P<0.0001$
24-h activity[c]			
sqrt HACTV	Tx|Time bin|rPctWtChg	Tx	$F_{2,337}=3.35$, $P=0.0363$
		Time bin	$F_{22,1212}=85.09$, $P<0.0001$
ranked PctCtr	Tx|Time bin|rPctWtChg	Tx	$F_{2,101}=3.52$, $P=0.0332$
		Time bin	$F_{22,1183}=35.42$, $P<0.0001$
		Tx*rPctWtChg	$F_{2,101}=3.11$, $P=0.0491$
sqrt LRM	Tx|Time bin|rPctWtChg	Tx	$F_{2,179}=5.90$, $P=0.0033$
		Time bin	$F_{22,1125}=68.83$, $P=0.0001$
		Tx*Time bin*rPctWtChg	$F_{44,1072}=1.45$, $P=0.0301$
ranked RT	Tx|Time bin|rPctWtChg	Tx	$F_{2,868}=4.80$, $P=0.0084$
		Time bin	$F_{22,479}=231.78$, $P<0.0001$

HACTV = horizontal locomotor activity beam breaks, PctCtr = percent of time in the arena center, LRM = localized repetitive movement, RT = resting time, Tx = treatment, 3-min sample = time bins in which data were collected for analysis during adaptation, rPctWtChg = residual from regression of percent weight change on treatment, Time bin = composite time samples used for light cycle-synchronized 24-h analysis (see Section 2.4.2), * = interaction between effects.

[a] n = 20 T0, 21 T100, 22 T340

[b] Vertical bars (|) indicate that significance of all indicated effects and their interactions was tested; however, as noted, the F test and significance levels are only listed for treatment (whether or not it reached significance) and other effects and interactions that reached significance.

[c] Repeated measures analyses were conducted on behaviors during (1) the 30-min adaptation period and (2) the remainder of the 24-h period that was synchronized for onset of the dark cycle.

ARTICLE IN PRESS

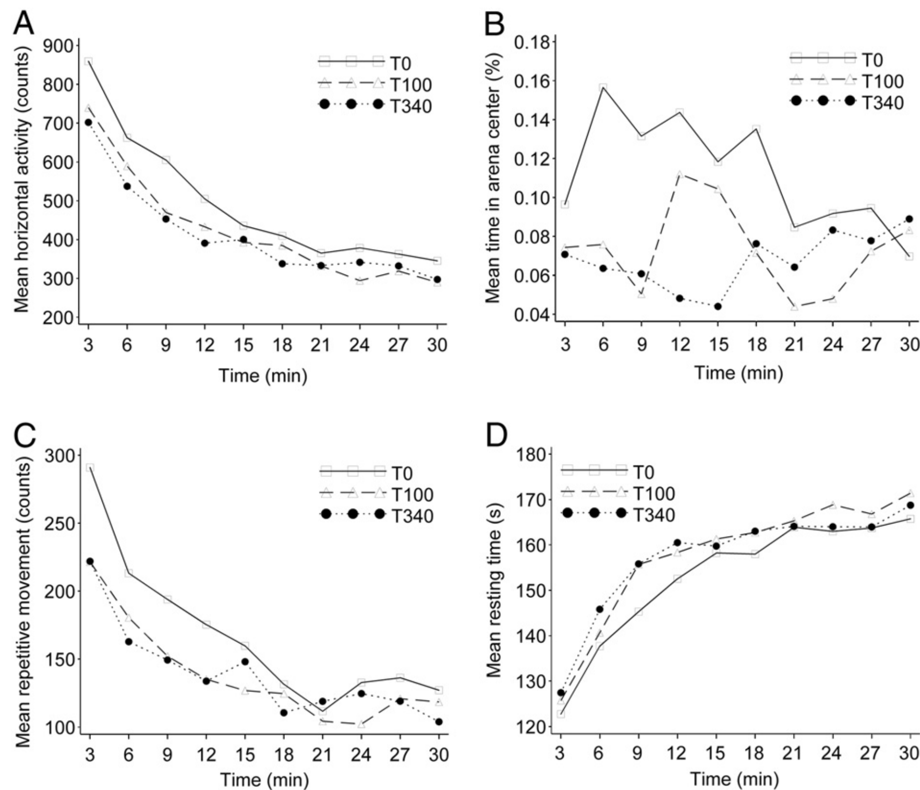


Fig. 3. Activity during open field adaptation (Table 5). (A–E) Plots depict mean levels of activities for each 3-min time segment during the first 30-min in the open field chamber. (A) Treatment did not significantly affect horizontal activity horizontal activity (HACTV). Significant treatment effects occurred for (B) percent time in arena center (PctCtr), with T0>T100 and T340 (P<0.05 and <0.01, respectively); (C) localized repetitive movements (LRM), with T340<T0 (P<0.05); and (D) resting time (RT), with T0<T100 and T340 (P<0.0005 and P<0.0001, respectively). Significant time effects occurred for ABCDE, indicating habituation. T0 = control (n = 20), T100 = 100 mg TTFD/kg body weight (n = 21), T340 = 340 mg TTFD/kg body weight (n = 22).

for mean horizontal locomotor activity (Fig. 4A), with T0 greater than T100; for percent time in the arena center (Fig. 4B), with T100 greater than T0 at covariate means; for mean localized repetitive movement (Fig. 4C), with T0 greater than T100; and resting time (Fig. 4D), with T100 greater than T0. In the analysis of percent time in the arena center, the interaction of treatment with rPctWtChg was evidenced by significant between-group effects (T100 greater than T0) at the 25th (P = 0.001) and 50th (P<0.05), but not the 75th , percentiles of PctWtChg. Close examination of subset analyses and figures of localized repetitive movement data did not clarify the nature of the 3-way interaction (rPctWtChg*Treatment*Time bin*). Significant time bin effects occurred for all activity measurements (Table 6).

During the day→night transition period (Fig. 5A–D, analysis data not shown), significant treatment effects occurred for horizontal locomotor activity, localized repetitive movement, percent time in center, and resting time. The spline plot for resting time shows a more marked decrease and slower rebound for controls compared to TTFD groups, and plots for the other measurements show decreased response amplitude for both TTFD treatment groups and delayed peaks for T100 mice relative to control.

3.2.3. Acoustic startle/prepulse inhibition (ASPPI) (Table 7)

Compared to control (T0), mean baseline (no stimulus) response (MBR) was greater for T100 and T340 (Fig. 6A), and greater current body weight (WtCur) was significantly positively associated with MBR. The mean startle response of T100 was lower than that of T0. For the 82-dB prepulse, percent startle inhibition of T100 was lower than that of T0 and T340. The analysis was repeated using mice matched for magnitude of startle response to pulse alone (n = 10 per treatment group). These analyses showed no between-group differences in startle inhibition; the lower startle inhibition by the T100 group in the larger data set was due to their lower startle response. Treatment did not affect startle inhibition by the 74-dB or 90 dB prepulses. Although a 3-way interaction (Treatment*rMBR*rWtCur) occurred in the 74-dB analysis, no between-group differences were found at the 25th, 50th, or75th percentile combinations of the covariates.

3.3. Brain thiamin and thiamin phosphates

TTFD treatment affected whole brain thiamin concentrations (Table 8, Fig. 7). Significantly higher concentrations of thiamin occurred in the T100 and T340 treatment groups compared to the T0 group. No significant differences in brain tissue levels of the phosphorylated thiamin derivatives ThDP or ThMP were observed, and levels of ThTP and AThTP were too low for accurate quantification.

4. Discussion

4.1. Growth and organ weights

TTFD treatment resulted in a reduction in percent body weight gain in both the T100 and T340 groups but there was no change in percent body length gain. The effect on percent weight gain was evident in the T340 group by the time of the first behavioral test. The lower percent weight gain of TTFD-treated mice was not anticipated. A previous study in which 14–16 week-old BALB/c mice were administered 300 mg of the lipophilic thiamin sulbutiamine daily by oral intragastric intubation for 10 d did not report relative changes in body weight (Micheau et al., 1985). Rodents given food supplemented with another lipophilic thiamin precursor, thiamin propyl disulfide, were reported to increase in body weight faster than those receiving water soluble thiamin salts or no thiamin supplement (Shimazono and Katsura, 1965).

Please cite this article as: Hills JI, et al, The effect of thiamin tetrahydrofurfuryl disulfide on behavior of juvenile DBA/2J mice, Neurotoxicol Teratol (2011), doi:10.1016/j.ntt.2011.07.006

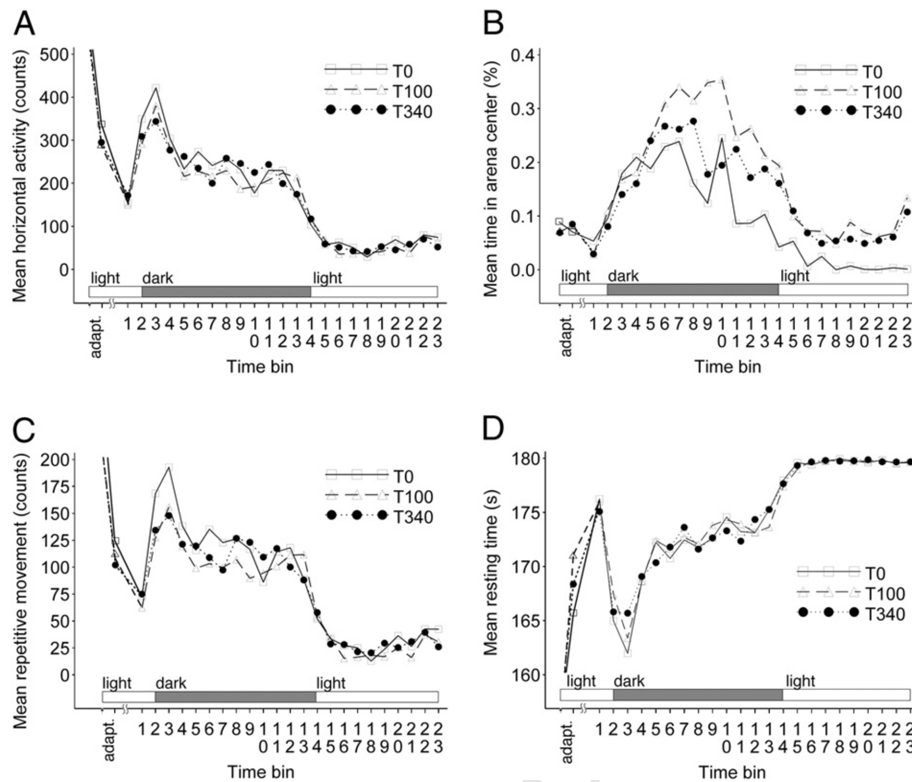


Fig. 4. 24-h open field activity, with time bins synchronized for the light/dark cycle (Table 6). The arrow indicates placement of mice into the chambers, which was immediately followed by the 30-min adaptation period presented in Fig. 3. The last adaptation measurement mean is indicated; the first adaptation measurement mean is indicated when the Y axis for the remaining light synchronized time period permitted. The discontinuity on the X axis represents the variable time elapsed to permit synchronization of the time bins following the adaptation period. The first time bin was 33 min long; the remaining time bins were 1 h long. Behavior means for each mouse were computed for each time bin and used for repeated measures ANCOVA. The figures represent treatment group means derived from individual means. Significant treatment effects occurred for (A) horizontal activity (HACTV) (T0>T100, P<0.05), (B) percent time in arena center (PctCtr) (T0<T100, P<0.05 at covariate means), (C) localized repetitive movement (LRM) (T0>T100, P<0.005 at covariate means), and (D) resting time (RT) (T0<T100, P<0.01). A significant time bin effect occurred for each measurement (P<0.0001 each). Interactions of covariates occurred for (B) and (C), as discussed in Section 3.2.2. T0 = control (n = 20), T100 = 100 mg TTFD/kg body weight (n = 21), T340 = 340 mg TTFD/kg body weight (n = 22).

Two possible explanations for the lower body weight gain in the TTFD groups are (1) an irritant effect of the treatment on the GI tract, leading to reduced food intake (Lonsdale et al., 2002; Mizutani et al., 1972) or (2) a metabolic stimulant effect of TTFD via enhanced noradrenaline secretion and thermogenesis (Oi et al., 1999). TTFD-treated mice used for tissue analysis were observed to eat less, as indicated by the frequency with which their food cups required filling, which suggests that decreased food intake, possibly due to an irritant effect of TTFD gavage or decreased appetite, contributed to the lower weight gain observed in these animals.

The dosages of TTFD used in this study were selected based on previously published studies with lipophilic thiamin derivatives in mice. However, one of those studies (Micheau et al., 1985) used older mice, whose GI tracts may have been more robust, and a different lipophilic thiamin (sulbutiamine) was used. In the second (Lonsdale et al., 2002), TTFD was administered intraperitoneally. Should gavage delivery of TTFD be causing irritation of the gastrointestinal tract, an alternative method of delivery would need to be considered in future studies. Lower dosages of TTFD could also be considered. TTFD therapy in children (Lonsdale, 1987a, 2006, 2001, 2004; Lonsdale et al., 1982, 2002) used doses lower than those used in the present study. Attention to the route of administration or to buffering agents may be needed in human studies.

Treatment resulted in a lower current body weight in both T100 and T340 mice at the time of the acoustic startle prepulse inhibition (ASPPI) test. Because current weight can affect the mechanism of startle detection, current weight was used as a covariate in the path analysis model for components of that test.

Table 6
Statistical analysis results for prepulse inhibition of acoustic startle[a].

Transformed variable	ANCOVA[b] fixed effects	F test, Pr>F for treatment and significant covariates	
sqrt MBR	Tx|rWtCur	Tx	$F_{2,66}=8.62$, P = 0.0005
		rWtCur	$F_{1,66}=10.27$, P = 0.0021
ln MSR	Tx|rWtCur|rMBR	Tx	$F_{2,67}=3.23$, P = 0.0457
74-dB ASPPI	Tx|rWtCur|rMBR	Tx	$F_{2,58}=0.34$, P = 0.7166
		rMBR*Tx	$F_{2,58}=3.74$, P = 0.0298
		rWtCur*rMBR*Tx	$F_{2,58}=4.87$, P = 0.0111
82-dB ASPPI	Tx|rWtCur|rMBR	Tx	$F_{2,67}=7.23$, P = 0.0014
cubed 90-dB ASPPI	Tx|rWtCur|rMBR	Tx	$F_{2,52.2}=1.41$, P = 0.2530

MBR = mean baseline (no stimulus) response, MSR = mean startle response to pulse alone, dB = decibels of sound, ASPPI = acoustic startle prepulse inhibition, Tx = treatment, rWtCur = residual from regression of current weight on treatment, rMBR = residual from regression of mean baseline response on treatment | rWtCur, * = interaction between effects.

[a] n = 23 T0, 24 T100, 23 T340

[b] Vertical bars (|) indicate that significance of all indicated effects and their interactions was tested (however, as noted, the F test and significance levels are only listed for treatment (whether or not it reached significance) and other effects and interactions that reached significance.

4.2. Behavior tests

4.2.1. Social dyadic interaction

Control and TTFD-treated mice spent similar amounts of time with the stimulus mouse, but the nature of their social interaction differed. Compared to control, TTFD-treated mice showed more passive (cuddling-type) interaction and less boisterous interaction with the

ARTICLE IN PRESS

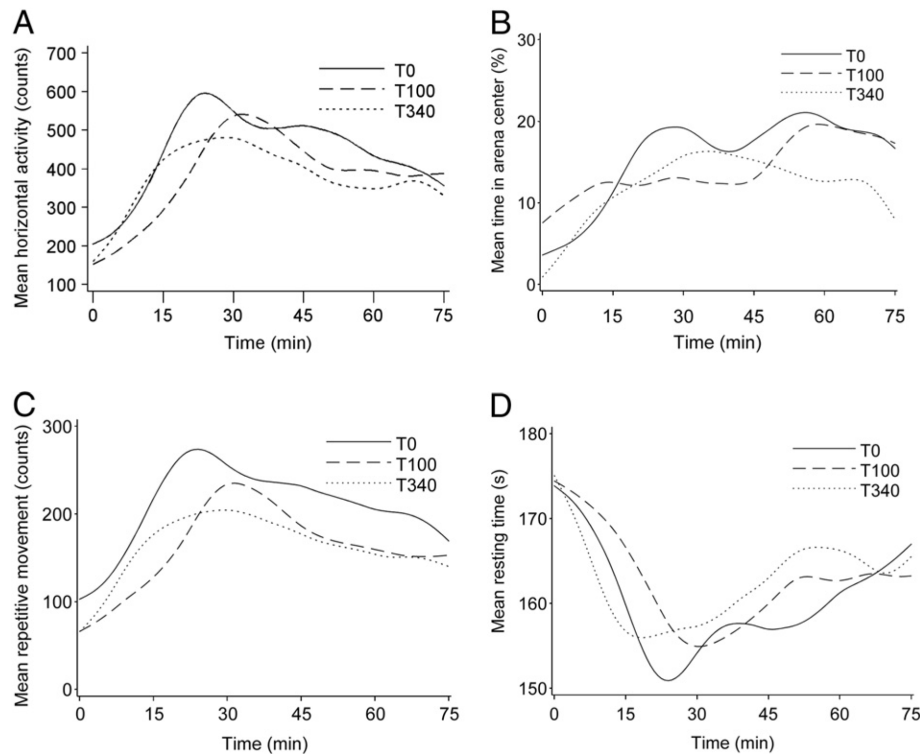


Fig. 5. Open field activity following onset of dark cycle. (A, B, C, D, E) Mean levels of activities were computed for each 3-min interval for 75 min following onset of the dark cycle and are plotted with sm50 interpolation. Polynomial mixed model analysis (explained in Section 2.7) indicated a significant treatment effect for each activity shown, with treatment × 3-min sample significant ($P \leq 0.05$) at one or more levels of interaction in each case (data not shown). Active behaviors were decreased in amplitude for both T100 and T340, and were delayed in the T100 group. T0 = control (n = 20), T100 = 100 mg TTFD/kg body weight (n = 21), T340 = 340 mg TTFD/kg body weight (n = 22).

stimulus mouse. Results of the test suggest a dose-related lower total activity level in TTFD-treated mice.

Social proximity has previously been observed to be rewarding for the DBA/2J mouse (Moy et al., 2007; Panksepp and Lahvis, 2007). Further study is needed to determine whether the altered social activity observed in the TTFD-treated mice extends to animal models of childhood behavior disorders that are characterized by hyperactivity and disruptive interactions with peers. It has been suggested that nicotinic acetylcholine receptor (nAChR) function is involved in regulation of social behavior (Granon et al., 2003), and a cholinergic mechanism underlying thiamin effects has been proposed (see Section 4.2.2). Some childhood cases of hyperactivity have responded to high-dose thiamin (Brenner, 1982). Improved behavior has also been reported in autistic children treated with TTFD, but the nature of the improvements was not described (Lonsdale et al., 2002).

4.2.2. 24-h activity monitoring

Open field testing yielded 3 main findings: (1) activity levels were generally lower in TTFD-treated mice than in controls, (2) different activities were altered in the adaptation period vs. the 24-h period, and (3) during the light-dark transition period TTFD-treated groups showed a dose-related decrease in peak amplitudes of active behaviors and the T100 group showed a delay in active behaviors relative to controls.

During the adaptation period, for all treatment groups active behaviors generally decreased over time while resting time increased. Compared to control, the overall higher resting time for both TTFD groups, as well as lower localized repetitive movement for the T340 group, suggests decreased activity with TTFD treatment. Locomotor difficulties were not observed in TTFD-treated mice in the social dyadic test, suggesting innate motor deficits probably did not underlie decreased activity. The decrease in percent of time in the arena center for TTFD groups could signify increased anxiety or decreased risk taking, or it may have been a result of overall lower activity. The latter explanation may apply since center time was higher than T0 in the T100 group during the 24-h period. Also, decreased acoustic startle response in T100 compared to T0 mice in the ASPPI test may possibly indicate decreased (rather than increased) anxiety (discussed below). Further behavioral experiments could clarify whether thigmotaxis (reduced center time in the open field) signified increased anxiety vs. decreased risk taking during the adaptation period and whether there were coordination problems that may not have been detected in the current testing regimen (Curzon et al., 2009).

The 24-h data again indicate overall lower activity in TTFD-treated mice compared to control, but in different components. Here control mice showed greater horizontal activity than T100 mice (vs. no between-group differences during adaptation); control mice showed greater localized repetitive movement than only T100 (vs. T0 greater than T340 during adaptation); and the average resting time for controls was less than that of the T100 group (vs. T0 less than both T100 and T340 during adaptation). Percent time in the arena center was increased for T100 mice compared to controls (indicating adaptation to that area with longer exposure), a result contrasting to that found in the adaptation period where time in center was greater for controls than for the T100 and T340 groups. Thus, the dosage of TTFD resulted in differing effects on activity during each activity period (adaptation and 24-h), and effects were not always dose related.

The mechanism(s) underlying TTFD's effects on activity are unknown, but several lines of evidence suggest that altered cholinergic function could play a role. Previous experimental animal and human studies have proposed that stimulation of cholinergic function by TTFD could underlie its effect on brain function (Lonsdale, 1987a, 1987b, 1982a; Micheau et al., 1985; Mimori et al., 1996). In normal human volunteers high-dose thiamin has been reported to counteract hippocampal behavioral deficits induced by the non-selective mAChR antagonist scopolamine (Meador et al., 1993). A number of behavioral deficits seen in thiamin-deficient rodents are

ARTICLE IN PRESS

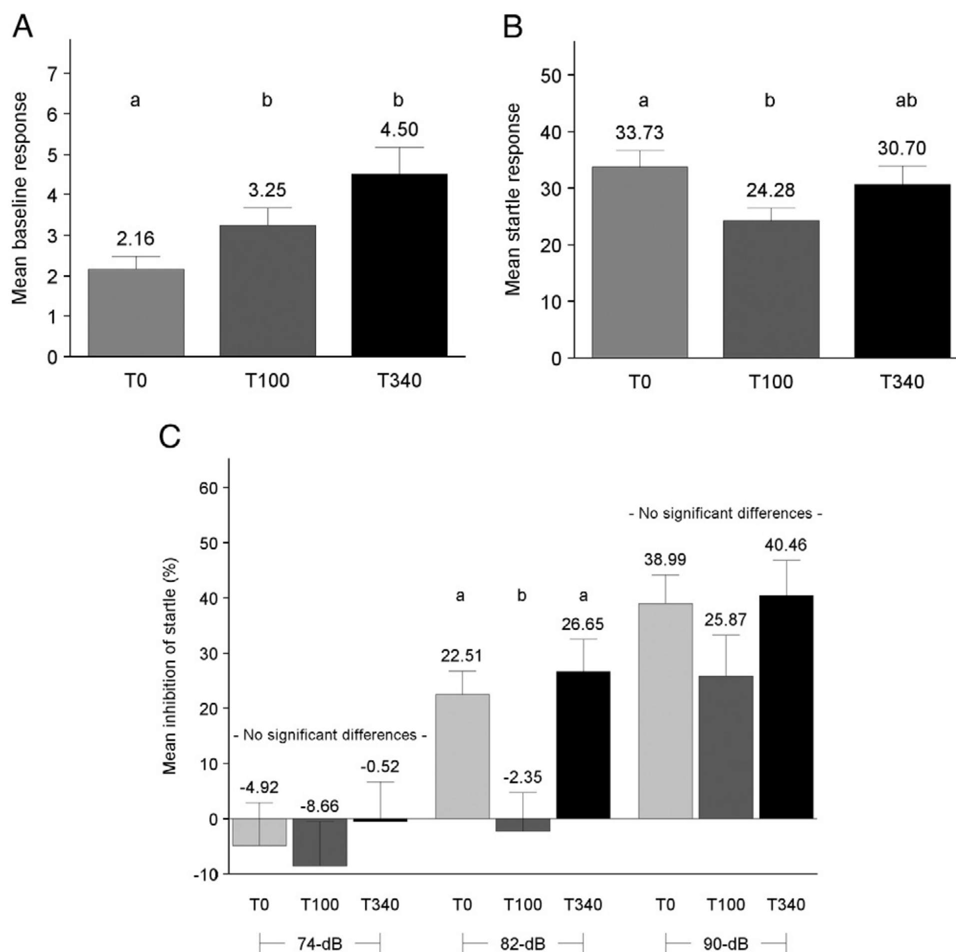


Fig. 6. Acoustic startle/prepulse inhibition (Table 7). Behavior means for each mouse were computed for each measurement and used for ANCOVA. The figures represent treatment group means derived from individual means. Significant treatment effects occurred for (A) mean baseline (no stimulus) response (MBR) with only background noise in the acoustic startle apparatus (T0<T100 and T340, P<0.05 and P<0.0005, respectively); (B) mean startle response to pulse alone (MSR) (T100<T0, P<0.05); and (C) startle inhibition by the 82-dB prepulse (ASPPI) (T100<T0 and T340, P<0.01 each). For the 74-dB and 90-dB prepulses, no significant treatment effect occurred. Between-group differences are indicated by a vs. b notation. Error bars represent S.E.M. T0 = control (n = 23), T100 = 100 mg TTFD/kg body weight (n = 23), T340 = 340 mg TTFD/kg body weight (n = 23).

remediated by pro-cholinergic agents (Nakagawasai et al., 2001, 2000, 2007, 2004). Thiamin can affect acetylcholine levels by (1) increasing levels of acetylcholine precursors via its cofactor roles in the pyruvate dehydrogenase complex (acetyl Co-A production) and transketolase (NADPH/antioxidant protective effect) (Gibson and Blass, 2007; Gloire et al., 2006; Jones, 2000; McGrane, 2000; Salminen and Kaarniranta, 2010; Sheline and Wei, 2006) and increasing the rate of neuronal high affinity uptake of choline (Micheau et al., 1985), and (2) preventing (via antioxidant protective effects) reduction of nerve growth factor induced transcription of choline acetyltransferase, the enzyme responsible for synthesis of acetylcholine (Toliver-Kinsky et al., 2000). Thiamin may differentially affect acetylcholine receptors; for example, thiochrome, an oxidation product and metabolite of thiamin, enhances the binding and actions of acetylcholine at muscarinic M4 relative to other muscarinic receptors (Lazareno et al., 2004).

Table 7
Statistical analysis results for HPLC analysis of whole brain content of thiamin and thiamin phosphate (per mg protein)[a].

Transformed variable	ANOVA fixed effect	F test, Pr>F for treatment
1/Thiamin	Tx	$F_{2,5.62}=27.05$, P = 0.0013
ranked ThMP	Tx	$F_{2,13}=0.48$, P = 0.6317
1/cubed ThDP	Tx	$F_{2,10}=0.85$, P = 0.1383

ThMP = thiamin monophosphate, ThDP = thiamin diphosphate
[a] n = 5 T0, 5 T100, 6 T340

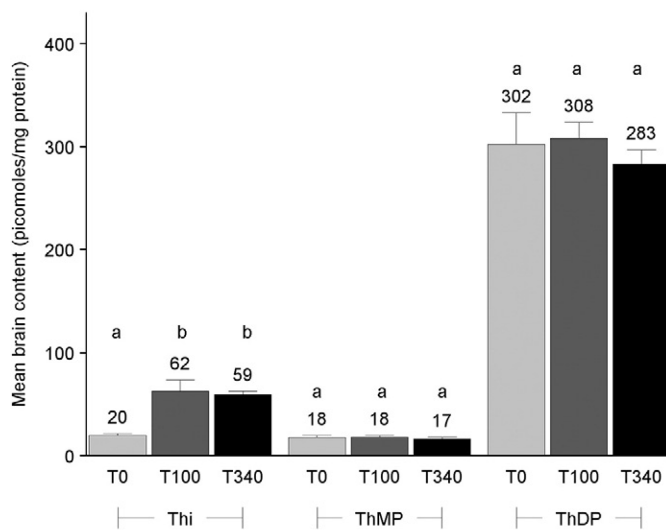


Fig. 7. Effect of treatment on the thiamin and thiamin phosphate content of whole mouse brain (Table 8). Tissue analysis showed between-group differences in the level of thiamin (Thi) (T0<T100, P<0.01; T0<T340, P<0.001), but no between-group differences in levels of thiamin monophosphate (ThMP) or thiamin diphosphate (ThDP). Levels of thiamin triphosphate (ThTP) and adenosine thiamin triphosphate (AThTP) were too low to quantify accurately. Between-group differences are indicated by a vs. b notation. Error bars represent S.E.M. T0 = control (n = 5), T100 = 100 mg TTFD/kg body weight (n = 23), T340 = 340 mg TTFD/kg body weight (n = 6).

Please cite this article as: Hills JI, et al, The effect of thiamin tetrahydrofurfuryl disulfide on behavior of juvenile DBA/2J mice, Neurotoxicol Teratol (2011), doi:10.1016/j.ntt.2011.07.006

ARTICLE IN PRESS

Thiamin-related cholinergic enhancement may also be involved in regulation of circadian rhythm for reviews see (Datta, 2010; Rosenwasser, 2009; Turner et al., 2010), which could explain differences observed here during the light/dark transition. Subclinical dietary thiamin deficiency altered circadian rhythm in 6 week-old C57BL/6J mice (Bennett and Schwartz, 1999). Studies using other species have shown circadian activity effects of the lipophilic thiamin precursor sulbutiamine (Van Reeth, 1999). Limited human reports suggest effects of thiamin deficiency (Wilkinson et al., 1997) and thiamin augmentation via TTFD (Lonsdale et al., 2002) on sleep.

Further study is needed to elucidate the mechanism of the effect of TTFD on activity, sleep, and body rhythms and to determine if lipophilic thiamin precursors might benefit disorders of these functions in humans.

4.2.3. Acoustic startle/prepulse inhibition (ASPPI)

Although the DBA/2J mouse suffers juvenile-onset high frequency hearing loss (HFHL), previous tests demonstrated that the acoustic startle response is independent of HFHL in juvenile mice when the prepulse is broad-band white noise rather than pure tones (McCaughran et al., 1999). Our test protocol used broad-band noise in 32-d old mice.

Our ASPPI study yielded 4 main findings regarding TTFD effects: (1) TTFD produced a dose-related increase in mean baseline response (the no stimulus response during only broad-band background noise); (2) the response to the startle pulse alone was lower for T100 compared to T0 mice; (3) prepulse inhibition with the 82 dB prepulse was reduced for T100 compared to both T0 and T340 mice; and (4) when mice were matched for startle response, no change in 82-dB prepulse inhibition was observed.

Mean baseline (no stimulus) response increased with increasing current weight over the entire group of mice and also within each treatment group. Yet, despite their lower mean body weights, the T100 and T340 groups showed higher mean baseline response than controls, findings that suggest the increase in mean baseline response was not due primarily to the TTFD effect on weight. A rising baseline response in adult DBA/2 mice has been reported in response to high doses of stimulants (Flood et al., 2010) which was attributed to hyperactivity, such as increased turning behavior in the test cylinders, or finer stereotypic movements. An increased general activity in the startle chamber has also been noted in nicotine withdrawn DBA/2 mice (Semenova et al., 2003) which was suggested to reflect increased body tremor or agitation. The accentuated response in the confined environment by TTFD-treated mice contrasted with their decreased activity in the open field test and the social dyadic interaction test. A confined, isolated environment, such as the restraint cylinder used for acoustic startle testing, may solicit unique behaviors. Observation of control and TTFD-treated mice in tightly restrained containers would shed light on what behaviors are involved and whether T100 and T340 mice demonstrate less anxiety-induced freezing behavior.

T100, but not T340, mice showed decreased startle response compared to T0 mice, a finding that suggests activation of different neurotransmitter pathways depending on dosage. Pre-clinical thiamin deficiency in rodents has been shown to increase the startle response to electric shock and was correlated with reduced activity of erythrocyte transketolase, an enzyme for which thiamin diphosphate is a cofactor (Peskin et al., 1967). Increased startle response was attributed to neurological hyperexcitability and was thought to correlate with reported observations of increased spontaneous activity in preclinically thiamin-deficient rats. We know of no previous reports of supranormal thiamin intake decreasing auditory startle or startle due to other sensory input, however. In rodents the startle response is commonly used to assess emotional reactivity and the effects of anti-anxiety drugs (Bourin et al., 2007; Grillon, 2008; McCaughran et al., 2000). How TTFD affects various neurotransmitter systems impinging on startle and whether decreased startle in T100 mice indicates an anxiolytic effect at that dosage merits further study.

Compared to several other mouse strains, the DBA/2 strain has shown spontaneously low auditory PPI (McCaughran et al., 1997; Paylor and Crawley, 1997) and has been proposed as a model for testing drugs intended for psychiatric conditions that demonstrate PPI deficits (Olivier et al., 2001). Our study showed no improvement in PPI with TTFD treatment. TTFD doesn't appear to offer potential for treatment of disorders with disrupted sensory gating if PPI facilitation is used as the criterion.

4.3. Whole brain analysis for thiamin and thiamin phosphates

TTFD treatment markedly increased the level of thiamin in whole brain, but had no significant effect on concentrations of ThMP or ThDP. Levels of ThTP and AThTP are extremely low in mice compared to rats (Frédérich et al., 2009), and improved methods of detection are needed.

Two recent studies, one in rats (Nozaki et al., 2009) and the other in mice (Pan et al., 2010), also showed elevated levels of thiamin, but not ThMP or ThDP with TTFD treatment. Results (unpublished) in our laboratory suggest that ThDP levels in brainstem (medulla, pons, inferior colliculi) of DBA/2J mice may be marginally increased by TTFD administered via drinking water. Necropsy of a larger number of mice is needed to obtain pooled samples of various brain regions for analysis. Turnover of coenzyme-bound ThDP is slow (Bettendorff et al., 1994), but it is possible that higher intracellular thiamin could increase flux through the rapid turnover pools of ThDP and ThTP without increasing the ThDP level.

4.4. Other considerations

Through studies in humans, animals, and cell cultures, highly absorbable thiamin precursors have been shown to have beneficial effects via a variety of mechanisms: e.g., on complications of diabetes mellitus (e.g., (Du et al., 2010; Hammes et al., 2003; Karachalias et al., 2010)), vascular endothelial dysfunction (Verma et al., 2010), cognitive function (Bizot et al., 2005; Micheau et al., 1985; Mimori et al., 1996; Pan et al., 2010), endotoxin induced uveitis and lipopolysaccharide-induced cytotoxic effect (e.g., (Yadav et al., 2010)), other inflammatory conditions (e.g., (Matsui et al., 1985)), toxicity due to heavy metals and various chemicals (Fujiwara, 1965; Lonsdale et al., 2002; Reddy et al., 2010), alcoholic and nutritional polyneuropathies and myopathies (Djoenaidi et al., 1992; Woelk et al., 1998), dysautonomic symptoms (Lonsdale, 2009), infant brainstem dysfunction and apnea (Lonsdale, 2001), postinfectious asthenia (Shah, 2003), psychobehavioral inhibition occurring during major depression (Loo et al., 2000), and various disorders possibly associated with thiamin dependency that are expressed particularly under conditions of physical or emotional stress (Lonsdale, 1987a, 2006).

Thiamin requirements are not only influenced by various disease conditions, as mentioned above, but by individual differences in thiamin utilization. While a few notable examples of genetic disorders influencing thiamin requirements have been well-studied [e.g. Leigh disease and West syndrome, thiamin responsive megaloblastic anemia with diabetes and deafness, and neuropathy and bilateral striatal necrosis with exacerbation during febrile illnesses (Ames et al., 2002; Spiegel et al., 2009), others that produce more subtle behavioral changes or susceptibility to disease may well be awaiting discovery and may underlie case reports of beneficial effects of pharmacologic use of thiamin or its lipophilic derivatives (Lonsdale, 2006). Low frequency missense alleles of many different enzymes that result in impaired function are hypothesized to be common and may be nutrient sensitive (Marini et al., 2008). Combinations of nutrients may be required in cases where vitamin function is compromised (Ames et al., 2002). Also, when a pharmacologic dose of a nutrient is used, downstream shifts in metabolic pathways may require adjustment in the dietary intake of other nutrients (Lonsdale, 1987a, 1990).

Please cite this article as: Hills JI, et al, The effect of thiamin tetrahydrofurfuryl disulfide on behavior of juvenile DBA/2J mice, Neurotoxicol Teratol (2011), doi:10.1016/j.ntt.2011.07.006

ARTICLE IN PRESS

Even without underlying disease conditions or metabolic abnormalities that may increase thiamin requirement, children in Western nations may be at risk of inadequate thiamin nutriture. Because of its role in oxidative metabolism, the requirement for thiamin is increased with higher carbohydrate intake. Whereas the normal rodent diet contains high levels of thiamin relative to rodent requirements, the typical human diet does not (Fleming et al., 2003). Body stores of thiamin are limited, and the requirement for thiamin in infancy and childhood is relatively high (I.o.M. (U.S.), 2002). Concern has been expressed that in Western cultures relative thiamin deficiency may occur due to diets high in calories from refined carbohydrates, and that treatment of resulting functional disorders with physiological doses of thiamin provided in multivitamin preparations may not be sufficient to address defective enzyme/cofactor bonding that results from prolonged poor dietary habits (Lonsdale, 2006).

Apart from human case studies, long-term effects of TTFD on a range of behaviors have not been systematically studied to our knowledge. Alterations in morphology and neurotransmission during development can have long-term behavioral effects, even when the initiating nutrient or pharmaceutical is discontinued (e.g., Stevens et al., 2008). Study of behavioral effects of TTFD at different life stages with follow-up to assess residual effects on behavior is needed.

5. Conclusion

Behavioral and growth effects of diet supplementation with a lipophilic thiamin precursor, TTFD, were studied in the juvenile male DBA/2J mouse. TTFD was administered by gavage (100 mg/kg and 340 mg/kg body weight). Compared to control, dose-related reduction in weight gain occurred. Treatment did not affect gain in body length or organ weights as percent of body weight. A sequential battery of behavioral tests was conducted, and data were analyzed taking into account treatment effects on weight gain. TTFD-treated mice showed decreased locomotor activity in solitary open field testing and also when interacting with a conspecific. During social interaction TTFD-treated mice engaged in more passive (cuddling-type) as opposed to vigorous play-type behavior. Mice treated with the lower dosage of TTFD showed decreased startle response to loud noise. Both treatment groups showed a significant increase in whole brain levels of thiamin, but no change in levels of the phosphorylated derivatives ThMP and ThDP. Further work is needed to ascertain the mechanisms underlying behavioral effects and to determine the potential for beneficial effects in treating children with behavioral disorders.

Conflict of interest statement

The authors certify that there is no financial conflict of interest between any of the authors and any company or product that is part of this research.

Acknowledgments

This research was supported by a University of CA, Davis, M.I.N.D. Institute Pre-Doctoral Scholar Fellowship to Judith I. Hills. Lucien Bettendorff is Research Director at the F.R.S.-FNRS, Belgium. Behavior testing was conducted at the University of CA, Davis, Murine Behavior Assessment Laboratory under direction of M.S. Golub.

References

Drugs in Japan 6 (1982) 99.
I.o.M. (U.S.). P.o. Micronutrients., Dietary reference intakes for thiamin, rboflavin, niacin, vitamin B6, folate, vitamin B12, pantothenic acid, biotin, and choline. A report of the Standing Committee on the Scientific Evaluation of Dietary Reference Intakes and its Panel on Folate, Other B Vitamins, and Choline and Subcommittee on Upper Reference Levels of Nutrients. Food and Nutrition Board, Institute of Medicine, Editoin Edition. Washington, D.C: National Academy Press; 2002.
Ames BN, Elson-Schwab I, Silver EA. High-dose vitamin therapy stimulates variant enzymes with decreased coenzyme binding affinity (increased K(m)): relevance to genetic disease and polymorphisms. Am J Clin Nutr 2002;75:616–58.
Baker H, Frank O. Absorption, utilization and clinical effectiveness of allithiamines compared to water-soluble thiamines. J Nutr Sci Vitaminol (Tokyo) 1976;22(Suppl):63–8.
Bennett MR, Schwartz WJ. Altered circadian rhythmicity is an early sign of murine dietary thiamine deficiency. J Neurol Sci 1999;163:6–10.
Berman RF, Pessah IN, Mouton PR, Mav D, Harry J. Low-level neonatal thimerosal exposure: further evaluation of altered neurotoxic potential in SJL mice. Toxicol Sci 2008;101:294–309.
Bettendorff L. Thiamine homeostasis in neuroblastoma cells. Neurochem Int 1995;26: 295–302.
Bettendorff L, Nghiem HO, Wins P, Lakaye B. A general method for the chemical synthesis of gamma-P-32-labeled or unlabeled nucleoside 5′-triphosphates and thiamine triphosphate. Anal Biochem 2003;322:190–7.
Bettendorff L, Peeters M, Jouan C, Wins P, Schoffeniels E. Determination of thiamin and its phosphate esters in cultured neurons and astrocytes using an ion-pair reversed-phase high-performance liquid chromatographic method. Anal Biochem 1991;198:52–9.
Bettendorff L, Wins P. Thiamin diphosphate in biological chemistry: new aspects of thiamin metabolism, especially triphosphate derivatives acting other than as cofactors. FEBS J 2009;276:2917–25.
Bettendorff L, Wins P, Lesourd M. Subcellular localization and compartmentation of thiamine derivatives in rat brain. Biochim Biophys Acta 1994;1222:1–6.
Bizot J-C, Herpin A, Pothion S, Pirot S, Trovero F, Ollat H. Chronic treatment with sulbutiamine improves memory in an object recognition task and reduces some amnesic effects of dizocilpine in a spatial delayed-non-match-to-sample task. Prog Neuropsychopharmacol Biol Psychiatry 2005;29:928–35.
Bourin M, Petit-Demouliere B, Dhonnchadha BN, Hascoet M. Animal models of anxiety in mice. Fundam Clin Pharmacol 2007;21:567–74.
Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1976;72:248–54.
Brenner A. The effects of megadoses of selected B complex vitamins on children with hyperkinesis: controlled studies with long-term follow-up. J Learn Disabil 1982;15: 258–64.
Casteels M, Sniekers M, Fraccascia P, Mannaerts GP, Van Veldhoven PP. The role of 2-hydroxyacyl-CoA lyase, a thiamin pyrophosphate-dependent enzyme, in the peroxisomal metabolism of 3-methyl-branched fatty acids and 2-hydroxy straight-chain fatty acids. Biochem Soc Trans 2007;35:876–80.
Curzon P, Zhang M, Radek RJ, Fox GB. The behavioral assessment of sensorimotor processes in the mouse. Acoustic startle, sensory gating, locomotor activity, rotarod, beam walking; 2009.
Datta S. Cellular and chemical neuroscience of mammalian sleep. Sleep Med 2010;11:431–40.
Djoenaidi W, Notermans SL, Dunda G. Beriberi cardiomyopathy. Eur J Clin Nutr 1992;46:227–34.
Du Y, Kowluru A, Kern TS. PP2A contributes to endothelial death in high glucose: inhibition by benfotiamine. Am J Physiol Regul Integr Comp Physiol 2010.
Eudy JD, Spiegelstein O, Barber RC, Wlodarczyk BJ, Talbot J, Finnell RH. Identification and characterization of the human and mouse SLC19A3 gene: a novel member of the reduced folate family of micronutrient transporter genes. Mol Genet Metab 2000;71:581–90.
Fleming JC, Tartaglini E, Kawatsuji R, Yao D, Fujiwara Y, Bednarski JJ, et al. Male infertility and thiamine-dependent erythroid hypoplasia in mice lacking thiamine transporter Slc19a2. Mol Genet Metab 2003;80:234–41.
Flood DG, Zuvich E, Marino MJ, Gasior M. The effects of d-amphetamine, methylphenidate, sydnocarb, and caffeine on prepulse inhibition of the startle reflex in DBA/2 mice. Psychopharmacology (Berl) 2010;211:325–36.
Frédérich M, Delvaux D, Gigliobianco T, Gangolf M, Dive G, Mazzucchelli G, et al. Thiaminylated adenine nucleotides. FEBS J 2009;276:3256–68.
Fujiwara M. Absorption, excretion and fate of thiamine and its derivatives in human body. In: Shimazono N, Katsura E, editors. Review of Japanese literature on beriberi and thiamine. Kyoto: Vitamin B Research Committee of Japan; 1965. p. 179–213.
Gibson GE, Blass JP. Thiamine-dependent processes and treatment strategies in neurodegeneration. Antioxid Redox Signal 2007;9:1605–19.
Gloire G, Legrand-Poels S, Piette J. NF-kappaB activation by reactive oxygen species: fifteen years later. Biochem Pharmacol 2006;72:1493–505.
Golub MS, Germann SL, Lloyd KC. Behavioral characteristics of a nervous system-specific erbB4 knock-out mouse. Behav Brain Res 2004;153:159–70.
Granon S, Faure P, Changeux JP. Executive and social behaviors under nicotinic receptor regulation. Proc Natl Acad Sci USA 2003;100:9596–601.
Grillon C. Models and mechanisms of anxiety: evidence from startle studies. Psychopharmacology (Berl) 2008;199:421–37.
Hammes HP, Du X, Edelstein D, Taguchi T, Matsumura T, Ju Q, et al. Benfotiamine blocks three major pathways of hyperglycemic damage and prevents experimental diabetic retinopathy. Nat Med 2003;9:294–9.
Houzen H, Kanno M. Thiamine and its derivatives inhibit delayed rectifier potassium channels of rat cultured cortical neurons. Neuropharmacology 1998;37:313–22.
Inouye K, Katsura E. Clinical signs and metabolism of beriberi patients. In: Shimazono N, Katsura E, editors. Review of Japanese literature on beriberi and thiamine. Kyoto: Vitamin B Research Committee of Japan; 1965. p. 29–63.
Itokawa Y. Thiamine and nervous system function: an historical sketch. Metab Brain Dis 1996;11:1–7.
Johnson KR, Longo-Guess C, Gagnon LH, Yu H, Zheng QY. A locus on distal chromosome 11 (ahl8) and its interaction with Cdh23 ahl underlie the early onset, age-related hearing loss of DBA/2J mice. Genomics 2008;92:219–25.

UNCORRECTED PROOF

Please cite this article as: Hills JI, et al, The effect of thiamin tetrahydrofurfuryl disulfide on behavior of juvenile DBA/2J mice, Neurotoxicol Teratol (2011), doi:10.1016/j.ntt.2011.07.006

ARTICLE IN PRESS

Jones DN. Detoxification and protective functions of nutrients. In: Stipanuk MH, editor. Biochemical and physiologica aspects of human nutrtiion. Philadelphia: W.B Saunders Company; 2000. p. 901–16.
Karachalias N, Babaei-Jadidi R, Rabbani N, Thornalley PJ. Increased protein damage in renal glomeruli, retina, nerve, plasma and urine and its prevention by thiamine and benfotiamine therapy in a rat model of diabetes. Diabetologia 2010;53:1506–16.
Kwik-Uribe CL, Golub MS, Keen CL. Chronic marginal iron intakes during early development in mice alter brain iron concentrations and behavior despite postnatal iron supplementation. J Nutr 2000;130:2040–8.
Lazareno S, Dolezal V, Popham A, Birdsall NJ. Thiochrome enhances acetylcholine affinity at muscarinic M4 receptors: receptor subtype selectivity via cooperativity rather than affinity. Mol Pharmacol 2004;65:257–66.
Lonsdale D. A nutritionist's guide to the clinical use of vitamin B-1. Editoin Edition. Tacoma: Life Sciences Press; 1987a.
Lonsdale D. Dysautonomia, a heuristic approach to a revised model for etiology of disease. Evid Based Complement Altern Med 2009;6:3–10.
Lonsdale D. Effect of thiamine tetrahydrofurfuryl disulfide on audiogenic seizures in DBA/2J mice. Dev Pharmacol Ther 1982a;4:28–36.
Lonsdale D. Functional dysautonomia and nutrition. J Am Coll Nutr 1982b;1. 125–125.
Lonsdale D. Hypothesis and case reports: possible thiamin deficiency. J Am Coll Nutr 1990;9:13–7.
Lonsdale D. Review of the biochemistry, metabolism and clinical benefits of thiamin(e) and its derivatives. Evid Based Complement Altern Med 2006;3:49–59.
Lonsdale D. Sudden infant death syndrome requires genetic predisposition, some form of stress and marginal malnutrition. Med Hypotheses 2001;57:382–6.
Lonsdale D. The effect of thiamine tetrahydrofurfuryl disulfide (TTFD) on blood-pressure in spontaneously hypertensive rats (SHR). J Am Coll Nutr 1987b;6. 79–79.
Lonsdale D. Thiamine tetrahydrofurfuryl disulfide: a little known therapeutic agent. Med Sci Monit 2004;10:RA199–203.
Lonsdale D, Nodar RH, Orlowski JP. Brainstem dysfunction in infants responsive to thiamine disulfide: preliminary studies in four patients. Clin Electroencephalogr 1982;13:82–8.
Lonsdale D, Shamberger RJ, Audhya T. Treatment of autism spectrum children with thiamine tetrahydrofurfuryl disulfide: a pilot study. Neuro Endocrinol Lett 2002;23:303–8.
Loo H, Poirier MF, Ollat H, Elatki S. Effects of sulbutiamine (Arcalion 200) on psycho-behavioral inhibition in major depressive episodes. Encéphale 2000;26:70–5.
Marini NJ, Gin J, Ziegle J, Keho KH, Ginzinger D, Gilbert DA, et al. The prevalence of folate-remedial MTHFR enzyme variants in humans. Proc Natl Acad Sci USA 2008;105:8055–60.
Matsui K, Nakahara H, Watanabe H, Tamatsu H, Nakazawa M, Nakagawa Y, et al. Inhibition by thiamine tetrahydrofurfuryl disulfide (TTFD) of the arachidonic acid cascade-line activation as evidenced in the heart-lung preparation of the dog. Jpn J Pharmacol 1985;39:375–9.
McCaughran Jr J, Bell J, Hitzemann R. On the relationships of high-frequency hearing loss and cochlear pathology to the acoustic startle response (ASR) and prepulse inhibition of the ASR in the BXD recombinant inbred series. Behav Genet 1999;29:21–30.
McCaughran Jr J, Mahjubi E, Decena E, Hitzemann R. Genetics, haloperidol-induced catalepsy and haloperidol-induced changes in acoustic startle and prepulse inhibition. Psychopharmacology (Berl) 1997;134:131–9.
McCaughran Jr JA, Bell 111 J, Hitzemann RJ. Fear-potentiated startle response in mice: genetic analysis of the C57BL/6J and DBA/2J intercross. Pharmacol Biochem Behav 2000;65:301–12.
McCormick DB. Niacin, riboflavin, and thiamin. In: Stipanuk, editor. Philadelphia: W.B. Saunders Company; 2000. p. 458–82.
McGrane MM. Carbohydrate metabolism—synthesis and oxidation. In: Stipanuk MH, editor. Biochemical and physiological aspects of human nutrition. Phladelphia: W.B Saunders Company; 2000. p. 158–210.
Meador KJ, Nichols ME, Franke P, Durkin MW, Oberzan RL, Moore EE, et al, Loring DW. Evidence for a central cholinergic effect of high-dose thiamine. Ann Neurol 1993;34:724–6.
Micheau J, Durkin TP, Destrade C, Rolland Y, Jaffard R. Chronic administration of sulbutiamine improves long term memory formation in mice: possible cholinergic mediation. Pharmacol Biochem Behav 1985;23:195–8.
Mimori Y, Katsuoka H, Nakamura S. Thiamine therapy in Alzheimer's disease. Metab Brain Dis 1996;11:89–94.
Mitoma C. Metabolic disposition of thiamine tetrahydrofurfuryl disulfide in dog and man. Drug Metab Dispos: Biol Fate Chem 1973;1:698–703.
Mizutani M, Ihara T, Kanamori H, Takatani O, Kajiwara K. Effect of thiamine tetrahydrofurfuryl disulfide upon developing fetuses of mice and rats. J Takeda Res Lab 1971;30:131–7.
Mizutani M, Ihara T, Kaziwara K. Effects of orally administered thiamine tetrahydrofurfuryl disulfide on foetal development of rabbits and monkeys. Jpn J Pharmacol 1972;22:115–24.
Moy SS, Nadler JJ, Young NB, Perez A, Holloway LP, Barbaro RP, et al. Mouse behavioral tasks relevant to autism: phenotypes of 10 inbred strains. Behav Brain Res 2007;176:4–20.
Nakagawasai O, Tadano T, Hozumi S, Taniguchi R, Yamadera F, Tan-No K, et al. Involvement of muscarinic receptor on the impairment of avoidance learning in mice fed a thiamine-deficient diet. Biog Amines 2001;16:199–210.
Nakagawasai O, Tadano T, Niijima F, Tan-No K, Kisara K. Immunohistochemical estimation of rat brain somatostatin on avoidance learning impairment induced by thiamine deficiency. Brain Res Bull 2000;51:47–55.
Nakagawasai O, Yamadera F, Iwasaki K, Asao T, Tan-No K, Niijima F, et al. Preventive effect of kami-untan-to on performance in the forced swimming test in thiamine-deficient mice: relationship to functions of catecholaminergic neurons. Behav Brain Res 2007;177:315–21.
Nakagawasai O, Yamaderea F, Iwasaki K, Arai H, Taniguchi R, Tan-no K, et al. Effect of Kami-Untan-To on the impairment of learning and memory induced by thiamine-deficient feeding in mice. Neuroscience 2004;125:233–41.
Nozaki S, Mizuma H, Tanaka M, Jin G, Tahara T, Mizuno K, et al. Thiamine tetrahydrofurfuryl disulfide improves energy metabolism and physical performance during physical-fatigue loading in rats. Nutr Res 2009;29:867–72.
N.R.C.U.S.S.o.L.A. Nutrition, Nutrient requirements of laboratory animals, Editoin Edition. Washington, D.C: National Academy of Sciences; 1995.
Oi Y, Shishido C, Wada K, Odaka H, Ikeda H, Iwai K. Allylthiamindisulfide and related compounds enhance thermogenesis with increasing noradrenaline and adrenaline secretion in rats. J Nutr Sci Vitaminol (Tokyo) 1999;45:643–53.
Olivier B, Leahy C, Mullen T, Paylor R, Groppi VE, Sarnyai Z, et al. The DBA/2J strain and prepulse inhibition of startle: a model system to test antipsychotics? Psychopharmacology (Berl) 2001;156:284–90.
Pan X, Gong N, Zhao J, Yu Z, Gu F, Chen J, et al. Powerful beneficial effects of benfotiamine on cognitive impairment and beta-amyloid deposition in amyloid precursor protein/presenilin-1 transgenic mice. Brain 2010;133:1342–51.
Panksepp JB, Lahvis GP. Social reward among juvenile mice. Genes Brain Behav 2007;6:661–71.
Paylor R, Crawley JN. Inbred strain differences in prepulse inhibition of the mouse startle response. Psychopharmacology (Berl) 1997;132:169–80.
Pekovich SR, Martin PR, Singleton CK. Thiamine deficiency decreases steady-state transketolase and pyruvate dehydrogenase but not alpha-ketoglutarate dehydrogenase mRNA levels in three human cell types. J Nutr 1998a;128:683–7.
Pekovich SR, Poggi V, Martin PR, Singleton CK. Sensitivity to thiamine deficiency in cultured human cells is dependent on cell type and is enhanced in cells from thiamine-responsive megaloblastic anemia patients. J Nutr Biochem 1998b;9:215–22.
Peskin MR, Newton G, Brin M. Thiamine deficiency, infantile manipulation and startle response in rats. J Nutr 1967;91:20–4.
Reddy SY, Pullakhandam R, Dinesh Kumar B. Thiamine reduces tissue lead levels in rats: mechanism of interaction. Biometals 2010;23:247–53.
Rosenwasser AM. Functional neuroanatomy of sleep and circadian rhythms. Brain Res Rev 2009;61:281–306.
Salminen A, Kaarniranta K. Glycolysis links p53 function with NF-kappaB signaling: impact on cancer and aging process. J Cell Physiol 2010;224:1–6.
Semenova S, Bespalov A, Markou A. Decreased prepulse inhibition during nicotine withdrawal in DBA/2J mice is reversed by nicotine self-administration. Eur J Pharmacol 2003;472:99–110.
Shah SN. Adjuvant role of vitamin B analogue (sulbutiamine) with anti-infective treatment in infection associated asthenia. J Assoc Physicians India 2003;51:891–5.
Sheline CT, Wei L. Free radical-mediated neurotoxicity may be caused by inhibition of mitochondrial dehydrogenases in vitro and in vivo. Neuroscience 2006;140:235–46.
Review of Japanese literature on beriberi and thiamine, Editoin Edition. In: Shimazono N, Katsura E, editors. Kyoto: Vitamin B Research Committee of Japan; 1965.
Spiegel R, Shaag A, Edvardson S, Mandel H, Stepensky P, Shalev SA, Horovitz Y, et al. SLC25A19 mutation as a cause of neuropathy and bilateral striatal necrosis. Ann Neurol 2009;66:419–24.
Stevens KE, Adams CE, Yonchek J, Hickel C, Danielson J, Kisley MA. Permanent improvement in deficient sensory inhibition in DBA/2 mice with increased perinatal choline. Psychopharmacology (Berl) 2008;198:413–20.
Suzuoki Z, Tominaga F, Matsuo T, Sumi Y, Miyakawa M. Metabolism of thiamine and thiamine tetrahydro-furfuryl disulfide to 4-methylthiazole-5-acetic acid in conventional and germfree rats under various dosing conditions. J Nutr 1968;96:433–44.
Swerdlow NR, Weber M, Qu Y, Light GA, Braff DL. Realistic expectations of prepulse inhibition in translational models for schizophrenia research. Psychopharmacology 2008;199:331–88.
Tallaksen CME, Tauboll E. Excitatory effect of thiamin on CA1 pyramidal neurones in rat hippocampal slices in vitro. Eur J Neurol 2000;7:693–8.
Terranova ML, Laviola G. delta-Opioid modulation of social interactions in juvenile mice weaned at different ages. Physiol Behav 2001;73:393–400.
Thomson AD, Marshall EJ. The natural history and pathophysiology of Wernicke's encephalopathy and Korsakoff's psychosis. Alcohol Alcohol 2006;41:151–8.
Toliver-Kinsky T, Wood T, Perez-Polo JR. Nuclear factor kappaB/p49 is a negative regulatory factor in nerve growth factor-induced choline acetyltransferase promoter activity in PC12 cells. J Neurochem 2000;75:2241–51.
Turner J, Hughes LF, Toth LA. Sleep, activity, temperature and arousal responses of mice deficient for muscarinic receptor M2 or M4. Life Sci 2010;86:158–69.
Van Reeth O. Pharmacologic and therapeutic features of sulbutiamine. Drugs Today (Barc) 1999;35:187–92.
Verma S, Reddy K, Balakumar P. The defensive effect of benfotiamine in sodium arsenite-induced experimental vascular endothelial dysfunction. Biol Trace Elem Res 2010;137:96–109.
Wilkinson TJ, Hanger HC, Elmslie J, George PM, Sainsbury R. The response to treatment of subclinical thiamine deficiency in the elderly. Am J Clin Nutr 1997;66:925–8.
Woelk H, Lehrl S, Bitsch R, Kopcke W. Benfotiamine in treatment of alcoholic polyneuropathy: an 8-week randomized controlled study (BAP I Study). Alcohol Alcohol 1998;33:631–8.
Yadav UC, Kalariya NM, Srivastava SK, Ramana KV. Protective role of benfotiamine, a fat-soluble vitamin B1 analogue, in lipopolysaccharide-induced cytotoxic signals in murine macrophages. Free Radic Biol Med 2010;48:1423–34.
Yamashita H, Zhang YX, Nakamura S. The effects of thiamin and its phosphate esters on dopamine release in the rat striatum. Neurosci Lett 1993;158:229–31.

Please cite this article as: Hills JI, et al, The effect of thiamin tetrahydrofurfuryl disulfide on behavior of juvenile DBA/2J mice, Neurotoxicol Teratol (2011), doi:10.1016/j.ntt.2011.07.006