Thiamine and benfotamine counteract ultrasound-induced aggression, normalize AMPA receptor expression and plasticity markers, and reduce oxidative stress in mice

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HIGHLIGHTS

● Ultrasound stress elevates aggression, oxidation markers, and suppresses plasticity.
● Over-expression of GluA2 subunit of AMPA receptor accompanies ultrasound-induced aggression.
● Expression of 5-HT6 receptor, GluA1 and GluA3 subunits is lowered during ultrasound.
● Thiamine or benfotamine ameliorates the majority of the ultrasound-induced changes.
● Acute ultrasound exposure had no effect on behavior or oxidative/plasticity markers.

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ABSTRACT

The negative societal impacts associated with the increasing prevalence of violence and aggression is increasing, and, with this rise, is the need to understand the molecular and cellular changes that underpin ultrasound-induced aggressive behavior. In mice, stress-induced aggression is known to alter AMPA receptor subunit expression, plasticity markers, and oxidative stress within the brain. Here, we induced aggression in BALB/c mice using chronic ultrasound exposure and examined the impact of the psychoactive anti-oxidant compounds thiamine (vitamin B1), and its derivative benfotiamine, on AMPA receptor subunit expression, established plasticity markers, and oxidative stress. The administration of thiamine or benfotamine (200 mg/kg/day) in drinking water decreased aggressive behavior following 3-weeks of ultrasound exposure and benfotiamine, reduced floating behavior in the swim test. The vehicle-treated ultrasound-exposed mice exhibited increases in protein carbonyl and total glutathione, altered AMPA receptor subunits expression, and decreased expression of plasticity markers. These ultrasound-induced effects were ameliorated by thiamine and benfotamine treatment; in particular both antioxidants were able to reverse ultrasound-induced changes in GluA1 and GluA2 subunit expression, and, within the prefrontal cortex, significantly reversed the changes in protein carbonyl and polysialylated form of neural cell adhesion molecule (PSA-NCAM) expression levels. Benfotiamine was usually more efficacious than thiamine. Thus, the thiamine compounds were able to counteract ultrasound-induced aggression, which was accompanied by the normalization of markers that have been showed to be associated with

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1. Introduction

Both genetic and environmental factors have been shown to regulate aggressive traits via complex codependent mechanisms (Caspi et al., 2002; Lesch et al., 2012), but a causal role for environmental stress is well documented (Angkaw et al., 2013; Fanning et al., 2015, 2017) and is associated with overt aggression in humans (Kim and Haller, 2007; Kessler et al., 2015). Experimental models have also provided support for this relationship (Veenema and Neumann, 2007; Rammal et al., 2010; Costa-Nunes et al., 2014; Malki et al., 2016). In particular, emotional stress, which is referred to as state that is primarily triggered by the perception and cognitive evaluation of adverse events rather than a disturbance of physical nature (Chrousos and Gold, 1992; Fontes et al., 2014), appears to be the type of stress that most frequently results in overt aggressiveness (Angkaw et al., 2013; Fanning et al., 2015, 2017). We have shown that we can induce behavioural changes that are consistent with emotional stress, including aggression and, at a molecular level, oxidative stress within the hippocampus, using chronic ultrasound exposure (Pavlov et al., 2019). However, hitherto our investigation of emotional-stress-induce aggression has been limited to a small number of outcome measures and we have not sought to target the stress-induced molecular changes such as the use of therapy that reduces oxidative stress. Under stress paradigms have also been shown impact on plasticity markers, but, prior to this study, it was unclear how ultrasound exposure would affect plasticity markers and whether changes would be amenable to therapy. Plasticity markers that have been linked to the regulation of social behavior and emotionality include postsynaptic density protein 95 (PSD95) (Been et al., 2016), polysialylated form of neural cell adhesion molecule (PSA-NCAM) (Angata et al., 2004; Winkler et al., 2017), and β-catenin (Maguschak and Ressler, 2012).

Hans Selye defined stress as “the non-specific response of the body to any demand for change” (Selye, 1974), which, in case of emotional stress, is accompanied by physiological responses to any stimulus of a potentially threatening nature (Simonov, 1997; Clark et al., 2009). The use of the term “stress” extends to other fields of research and now includes stress at a cellular level. For instance, excessive formation of free radicals in mitochondria is described as a form of cellular stress known as “oxidative stress”. Increased oxidative stress within the brain can play a key role in the pathophysiology of emotional stress and in the accompanying behavioural abnormalities, including excessive aggression (Boufleur et al., 2013; Okazawa et al., 2014; Schiavone et al., 2015; Clíne et al., 2015a; Patki et al., 2013, 2014; Fanning et al., 2015). Mitochondrial dysfunction and microglia activation are associated with oxidative stress, and can lead to neuronal dysfunction, compromised brain plasticity, and abnormal emotionality (Block and Hong, 2007; Roy et al., 2008; Marais et al., 2009; Diehl et al., 2012; Boufleur et al., 2013). Oxidative stress markers have been found to be elevated in neuropsychiatric conditions, which are often accompanied by inappropriate aggression, such as autism or attention deficit/hyperactivity disorder (Ng et al., 2008; Gvozdjaková et al., 2014), panic disorder (Küloglu et al., 2002), aging-related neurodegeneration (Okazawa et al., 2014; Salminen and Paul, 2014), and certain forms of affective abnormalities and personality disorders (Fanning et al., 2015; Coccaro et al., 2013, 2016; Schiavone et al., 2015). For example, elevated plasma levels of 8-hydroxy-2′-deoxyguanosine was found in patients with intermittent explosive disorder and correlated with incidence of aggressive episodes both in healthy and affected individuals (Coccaro et al., 2016).

A wide range of therapies that ameliorate oxidative stress have been tested for their potential to improve signs of excessive aggression; for instance, in aggressive young adults (Hamazaki et al., 1996), in patients with autism (Gvozdjaková et al., 2014; Stutzmans and Dopheide, 2015), in mice subjected to stress of group housing (Strekalova et al., 2018), and in rats exposed to the stress of sleep deprivation (Solanki et al., 2016). Heavy metal exposure is also known to induce aggression and amenable to therapy after exposure to toxic concentrations of lead (Ebuehi and Ayinde, 2012), and cadmium (El-Tarras et al., 2016). Thiamine (vitamin B1) and its derivatives, are regulators of mitochondrial function and metabolism (Bettendorff et al., 2014) and are known to exert anti-oxidative and anti-stress effects in in vitro models (Schmid et al., 2008; Bozic et al., 2015; Raj et al., 2018) and in experimental models of stress (Markova et al., 2017; Vignisse et al., 2017; Pavlov et al., 2017), traumatic brain injury (Mkrtychyan et al., 2018) and neurodegeneration (Pan et al., 2010, 2016; Tapias et al., 2018). We have recently shown that a two-week pre-treatment regime in mice with thiamine, or its more bioavailable analogue benfotiamine can prevent increases in the GSK3-beta activity and measures of depressive-like and anxiety-like behavior following both predator stress and modified swim test paradigms (Markova et al., 2017; Vignisse et al., 2017; Pavlov et al., 2017). The compounds also rescued hippocampal neurogenesis and protein carbonyl levels after predation stress (Vignisse et al., 2017). Other work has revealed the beneficial effects of benfotiamine on amyloid pathology (Pan et al., 2010, 2016) and tau pathology mediated via Nrf/2 antioxidant response element (ARE)-dependent gene expression (Tapias et al., 2018). Hitherto, it was unclear how thiamine and benfotiamine might affect aggression in animal models.

A number of neurotransmitter systems and hormones have been linked to aggression including glutamate. Increased glutamatergic tone leads to augmentation of aggressiveness in many species (Bullock and Rogers, 1986; Muñoz-Blanco et al., 1986). In particular, dysregulation of AMPA receptors is likely to play an important role in stress-induced aggression (Arsten, 2009; Coccaro et al., 2013; Strekalova et al., 2018; Khlighatyan et al., 2018) as γ-carboline compounds, designed to target AMPA receptors and 5-HT6 receptors, have been found to have anti-aggressive effects in social stress paradigms in mice (Strekalova et al., 2018). Furthermore, altered gene expression of AMPA receptor subunit GluA2 and 5-HT6 receptors in the brain was found in stressed mice in a heterozygous tryptophan hydroxylase 2 (TPH2) knockout (Bazhenova et al., 2017). Gene association studies have also suggested a link between the 5-HTR6 gene functions and aggression during depression (Azenha et al., 2009), schizophrenia (Tsai et al., 1999) and neurodegeneration (Marcos et al., 2008). AMPA-type glutamate receptors are tetratomers composed of subunits GluA1-4 and they mediate a much of the fast synaptic neurotransmission that is implicated in neuroplastic processes. GluA1, GluA2, GluA3, and GluA4 can be assembled in either homomers or heteromers (Hollmann et al., 1989; Keinänen et al., 1990) and they are widely expressed throughout the brain on neurons and glia with the exception of GluA4 that is expressed at a very low level outside the cerebellum. The subcellular localization of AMPA receptors indicates that 60–70% of the receptors are at intracellular sites, thus forming a reserve pool of receptors unless they possess an unknown duty distinct from the silent synapse theory (Lee et al., 2001). Aggressive behavior has been explored in the AMPA subunit mutants; mice lacking GluA1 exhibit reduced anxiety and male aggression (Adamczyk et al., 2012), GluA2 knockout animals are less aggressive than wild-type controls (Shimshek et al., 2006), and the GluA3 deficient
mouse was found to exhibit increased aggressive behavior (Adamczyk et al., 2012). Furthermore, the administration of AMPA receptor antagonists to Turku Aggressive mice has been shown to decrease the biting component of their aggressive behavior. However, it is clear that aggressive traits are associated with regional variations in AMPA receptor expression. In the amygdala, four weeks of social isolation increased AMPA receptor subunits GluA1 and GluA2, but isolation rearing increases the expression of AMPA receptor subunits (GluA1, GluA2 and GluA3) in the prefrontal cortex, but not in the hippocampus or nucleus accumbens (Araki et al., 2014). The prefrontal cortex is known to regulate social behavior and aggressive responses (Siever, 2008; Machado and Bachevalier, 2006), there is less understanding of the molecular changes that underpin decision to engage in aggression. Reactive aggression, in particular, is associated with altered prefrontal activity where AMPA receptor expression is high, but, hitherto, it was unclear how aggression would affect AMPA subunit expression in the prefrontal cortex in a model of emotional stress. The expression of the AMPA receptors in rodents is also high in the hippocampal subregions CA1, CA3 and dentate gyrus, which is also know to play an important role in deciding whether to attack (Leroy et al., 2018) based on evaluation of memories, including memories of past social interactions. Given these observations, we were interested to discover whether aggression induced in a model of emotional stress would affect the expression of AMPA subunits and 5HT-6 receptor and what would be the effect of the psychoactive antioxidants.

Mice that are subject to recently established model of emotional stress, in which “emotionally negative” and “neutral” randomly alternating frequencies of ultrasound with the range 20–45 kHz (Constantini and D’Amato, 2006) for three weeks display increased aggressive and depressive-like behaviors, decreased hippocampal neurogenesis (Morozova et al., 2016; Pavlov et al., 2019). Here, we sought to investigate what impact chronic ultrasound exposure would have on the expression brain oxidative stress markers, plasticity markers, and AMPA subunit receptor expression. Furthermore, we sought to discover whether the administration of thiamine or its derivative, benfotiamine would normalize ultrasound-induced aggression and other emotional behaviors and whether their administration would ameliorate the molecular changes. Increases in aggression in the mice were accompanied, at a molecular level, by profound changes in markers of oxidative stress, AMPA subunit expression, and in key pathways that regulate emotionality and neural plasticity, including postsynaptic density protein 95 (PSD95), polysialylated form of neural cell adhesion molecule (PSA-NCAM) and β-catenin. These changes were largely reversed by the administration of thiamine or benfotiamine.

2. Materials and methods

2.1. Animals and housing conditions

The study was performed using BALB/c 10-12-week-old male mice that were obtained from certified Charles River provider, Stolbovaja, RAS, Moscow region (http://www.spf-animals.ru/about/providers/animals). Mice were housed individually in the standard cages (27x22x15), under controlled laboratory conditions (22 ± 1°C, 55% humidity) and maintained on a reversed 12-h light/dark cycle (lights on at 19:00), with food and water provided ad libitum. All mice have been tested during the dark phase of light/dark cycle. Laboratory housing conditions and experimental procedures were set up and maintained in accordance with Directive 2010/63/EU of 22 September 2010 and carried out under the approval of the local veterinarian committee (10-02-15N72). All efforts were undertaken to minimize the potential discomfort of animals during the study.

Fig. 1. Experiment design. After (A) 21-day- (chronic) or (B) 24h- (acute) exposure of ultrasound exposure and concomitant dosing with thiamine or benfotiamine, both control and stress ultrasound-exposed groups were tested in a battery of behavioural tests, brains were dissected for a subsequent oxidative stress assay, qRT-PCR and Western blot study. qRT-PCR - quantitative reverse transcription polymerase chain reaction assay.
2.2. Study design

In both chronic and acute ultrasound exposure experiments, mice were weighed and assigned to control and ultrasound-exposed groups (data not shown); ultrasound exposure was applied as described elsewhere (Morozova et al., 2016; Strekalova et al., 2018; Pavlov et al., 2019). Mice were subjected to a 3-week exposure of ultrasound of unpredictably alternating frequencies (see below), and after behavioural testing, were additionally investigated for brain levels of oxidative stress markers, carbonyl protein and total glutathione, gene and protein expression of AMPA receptor subunits GluA1-4, 5-HT6 receptor, and protein expression of PSD95, PSA-NCAM and β-catenin using RT-PCR and Western blot (see below; Fig. 1A). Subgroups of mice from the ultrasound-exposed group were treated with thiamine or benfotiamine at the dose of 200 mg/kg/day via drinking water, as described elsewhere (Markova et al., 2017; Vignisse et al., 2017; Pavlov et al., 2017). Mouse behaviour was investigated twenty-four hours after the termination of ultrasound exposure in a novel cage, a social interaction test and in the forced swim test; they were killed sixteen hours after the behavioural testing, their brains were dissected (see below; Fig. 1A).

To control for possible acute effects of ultrasound exposure on behavior, brain oxidative markers and molecular changes, and to rule out the possibility of acute effects of thiamine and benfotiamine, we performed a study with an acute 24 h ultrasound exposure experiment and administration of thiamine compounds. Mice were treated with either compound used at the dose of 200 mg/kg/day via drinking water starting at the onset of ultrasound exposure; 24 h-fluid intake was measured to control drug intake with drinking liquid. After acute 24 h exposure to the ultrasound exposure, animals were behaviourally studied in the novel cage, a social interaction test and a forced swim test, and they were culled 16 h afterwards and their brains were dissected (see below; Fig. 1B).

Behavioural testing was performed as described elsewhere (Vignisse et al., 2017; Strekalova et al., 2018; Pavlov et al., 2019). Control mice were housed under similar conditions, but not exposed to the ultrasound radiation and studied simultaneously with ultrasound-exposed animals. Animals from the control group were littermates of mice from experimental groups, they were housed in an adjacent (separate) room, in order to eliminate the potential effects of stray ultrasound radiation. All manipulations on control and experimental animals were performed simultaneously to rule out possible confounding effects of seasonal inter-batch and other differences. On average, 7–8 animals per group were used; group sizes are indicated in figure legends.

2.3. Ultrasound radiation

Ultrasound exposure was performed as described elsewhere (Morozova et al., 2016; Strekalova et al., 2018; Pavlov et al., 2019). For a 3-week or a 24 h period, ultrasound radiation of average intensity of 50 ± 5 dB and variable frequencies in a 20–45 Hz range was constantly delivered within a laboratory environment to experimental groups of mice using a random schedule of alternating frequencies via a commercially available device (Weitech, Wavre, Belgium). The range of ultrasound stimulation frequency was alternated every 10 min between frequencies 20–25 kHz, 25–40 kHz and 40–45 kHz; the distribution of the ultrasound radiation was controlled by the ultrasound detector (Discovery Channel, Rochester, NY, USA). The shape of the ultrasound signal was fluctuating, thus, mimicking natural ultrasonic vocalizations of mice (Constantini and D’Amato, 2006). Further details on the ultrasound radiation can be found in the Supplementary material (Supplementary material, Pavlov et al., 2019). The selectivity of the adverse effects of low-frequency ultrasound during the radiation period versus the potential general negative effects of a constant noise accompanying the procedure described here was demonstrated previously (Morozova et al., 2016). As a part of these experiments, when mixed frequencies (“white noise”) at the range of 16–20 kHz were employed, no changes in social and depressive-like behaviours were observed. In addition, it was demonstrated that a shortened period of ultrasound exposure of 1–2-weeks duration were ineffective in producing behavioural changes and increase of serum corticosterone level, as compared with a 3-weeks exposure (Morozova et al., 2016; Pavlov et al., 2019).

Additional validation studies were performed in BALB/c mice that were exposed to different ranges of ultrasound frequency to understand whether the low frequency radiation is responsible for the behavioural changes (Supplemental Fig. 1). BALB/c mice were also exposed for 1, 2 or 3-weeks ultrasound treatment and the levels of corticosterone and changes in depressive-like behaviours were assessed to determine the optimum exposure time required to induce a depression-like phenotype (Supplemental Fig. 2). Further validation studies showed that applied here 3-week ultrasound exposure has evoked anxiety-like behavior in the elevated maze in BALB/c mice (Supplemental Fig. 3). Anxiogenic-like changes in the elevated plus maze, open field, depressive-like behaviours in the tail suspension test and increases in the measures of aggressive behavior were precluded by anxiolytic and antidepressant treatment with buspirone (Supplemental Fig. 3). Finally, CBA male mice exposed to the 3-week ultrasound procedure displayed elevated aggressive behavior in the social interaction and social exploration tests (Supplemental Fig. 4). These changes were accompanied by increased blood corticosterone levels that were not found after 1- or 2-week of ultrasound exposure (Supplemental Fig. 4). Thus, additional validation studies of the 3-week ultrasound exposure evidence its profound negative effects on the emotional behaviors and blood corticosterone levels of mice.

2.4. Behavioural tests

2.4.1. Social interaction test

A social interaction test was employed here and was an adaptation of a previously described methodology (Costa-Nunes et al., 2014; Couch et al., 2016; Strekalova et al., 2018). Mice from the experimental group were placed individually in an observation cage (30x60x30 cm) for 30 min to acclimatize. Thereafter, a previously group-housed naïve male mouse of the same strain and a similar weight and age, was introduced and left with the experimental mouse for 8 min. During this period, mice were scored for the latency, total duration and number of episodes of following measures of aggressive and dominant-like behaviours: attacks, crawl-over behavior, following behavior, and the incidence of tail rattling behavior was also scored. Additionally, these three parameters were measured for two forms of neutral social exploration: nose-nose and nose-anus contacts.

2.4.2. Forced swim test

This test was carried out as described previously (Malatynska et al., 2012; Strekalova et al., 2015; Pavlov et al., 2017). Mice were placed into a plastic transparent pool (diameter of 15 cm and a height of 25 cm) filled with water to the depth for 17 cm; water temperature was +23 °C, light intensity was 15 Lux. Total duration of floating behavior, defined by the absence of any directed movements of animals’ head and body, was scored offline during the 6 min-period at three 2-min intervals using Any-maze software (Stoelting Co, Wood Dale, IL, USA). Latency to float was evaluated as well.

2.4.3. Locomotor behavior

Vertical exploratory activity of mice was studied in the novel cage test under red light as described elsewhere (Strekalova and Steinbusch, 2010; Couch et al., 2013). Mice were placed into a plastic cage, their number of exploratory rears was counted for a 5-min under red light. Additionally, total distance travelled was scored to study their horizontal activity over this time period.
3-WEEK ULTRASOUND EXPOSURE

SOCIAL INTERACTION

A

B

C

D

FORCED SWIM

VERTICAL ACTIVITY

HORIZONTAL ACTIVITY

ACUTE ULTRASOUND EXPOSURE

SOCIAL INTERACTION

E

F

G

H

FORCED SWIM

VERTICAL ACTIVITY

HORIZONTAL ACTIVITY

(caption on next page)
2.5. Brain collection

Mice were terminally anaesthetized with an intraperitoneal injection of Nembutal (Bayer, Wiesbaden, Germany) and then were transcardially perfused with 10 ml of the ice-cold 0.9% NaCl. Their brains were dissected, and prefrontal cortex, hippocampus, amygdala, dorsal raphe and striatum were isolated for measurement of oxidative stress markers: two former structures were used for gene and protein expression studies (Fig. 1A). All samples were immediately frozen at −80 °C on a dry ice.

2.6. Protein carbonyl assay

Determination of protein carbonyls using the OxiSelect™ Protein Carbonyl Fluorometric Assay kit (Cell Biolabs, Inc., San Diego, USA). After dissection, brain tissue was stored at −80 °C until use. Glass-glass homogenization followed by a sonication was performed on ice in 1 ml of 1 × Sample Diluent from the OxiSelect™ kit, centrifuged at 10000 g for 5 min at 4 °C, and the supernatant was removed. The total protein concentration was adjusted to 1–10 mg/ml with 1 × Sample Diluent and protein carbonyls were determined according to the guidelines of the manufacturer using the GloMax Multi Detection System (Promega, Madison, WI, USA) equipped with a fluorescence module (485/540 nm filter set). Results were normalized to protein concentration as described previously (Vignisse et al., 2017; Pavlov et al., 2019).

2.7. Total glutathione assay

Oxidative stress was also estimated by the determination of total glutathione contents using the BioVision™ Glutathione Fluorometric Assay Kit (BioVision, Inc., San Francisco, USA) as described elsewhere (Pavlov et al., 2019). In total, 40 mg of each sample was homogenized by glass-glass homogenization followed by a sonication on ice with 100 μl of ice cold Glutathione Assay Buffer and total glutathione contents were determined according to the guidelines of the manufacturer using the GloMax Multi Detection System (Promega, Madison, WI, USA) equipped with a fluorescence module (340/420 nm filter set).

2.8. Quantitative real-time PCR

Quantitative real-time PCR was carried out as described elsewhere (Couch et al., 2013, 2016; Pavlov et al., 2017). Briefly, 1 μg total RNA was converted into cDNA using random primers and SuperScript III transcriptase (Invitrogen, Carlsbad, CA, USA). qRT-PCR was performed using the SYBR Green master mix (Bio-Rad Laboratories, Philadelphia, PA, USA) and the CFX96 Deep Well Real-Time PCR Detection System (Bio-Rad, Hercules, CA, USA). Further details in the protocol used and sequences of primers can be found in Supplementary material and Supplementary Table 1. The housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was selected as the reference gene since previous experiments demonstrated relatively low variability in GAPDH expression in the limbic structures of the rodents exposed to the ultrasound exposure (Morozova et al., 2016; Pavlov et al., 2019). Data were normalized to GAPDH mRNA expression and calculated as relative-fold changes compared to control mice as described elsewhere (Morozova et al., 2016; Couch et al., 2016; Pavlov et al., 2017, 2019).

2.9. Protein isolation and western blot analysis

Tissue samples were prepared using glass-lass homogenization followed by a sonication in ice-cold buffer (Roche Diagnostics, Indianapolis, IN, USA). Details of the Western blot analysis can be found in the Supplementary material and Supplementary Table 2. Briefly, protein concentration was quantified using the BCA protein assay kit (Pierce, Rockford, IL, USA, see Supplementary material). 25 μg of proteins were treated with SDS-PAGE and electroblotted on the PVDF membrane (EMD Millipore, Billerica, MA, USA). These samples were incubated with primary antibodies overnight at 4 °C (for information on antibodies used, see Supplementary material) that was followed by the incubation with respective secondary antibodies (Sigma-Aldrich, St. Louis, MO, USA) for 2 h at room temperature. Immunoreactive bands were detected using Bio-Rad Molecular Imager Software (BioRad Laboratories, Richmond, CA, USA). β-tubulin was used as a reference protein as low fluctuation of its expression was found previously in similar experiments (Morozova et al., 2016; Pavlov et al., unpublished data); expression of proteins of interest were calculated in fold changes from levels of β-tubulin, as described elsewhere (Morozova et al., 2016; Pavlov et al., 2019).

2.10. Drug administration

Experimental solutions replaced normal drinking water. Thiamine or benfotiamine (Sigma-Aldrich, St. Louis, MO, USA) were dissolved in tap water and were changed every 4–5 days as described elsewhere (Markova et al., 2017; Vignisse et al., 2017). The solutions of benfotiamine and thiamine were adjusted to pH 7.0, and drinking behavior of mice was monitored by evaluating the 24-h liquid intake during the first three days of dosing during chronic experiment and at the end of a 24-h period with acute ultrasound exposure study. We found no group differences in fluid intake suggesting normal drinking behavior in ultrasound-exposed and dosed mice (see Supplemental material, Supplemental Fig. 5). The route and choice of dose was based on previous studies showing that no significant changes in weekly measured total liquid intake is observed during the ultrasound exposure (see Supplemental material, Supplemental Fig. 5) (Markova et al., 2017, Vignisse et al., Pavlov et al., 2017, 2019).

2.11. Statistical analysis

Data analysis was performed using GraphPad Prism software.
version 5.03 for Windows (San Diego CA, USA). Data were treated by a two-way ANOVA test; Tukey's test was used for following post-hoc analysis. Statistical significance was set at $p < 0.05$. Data are shown as mean ± SEM.

3. Results

3.1. Ultrasound exposure induces aggressive and depressive-like behavioural changes in mice

In the social interaction test, a two-way ANOVA revealed a
significant ultrasound × treatment interaction in latency to attack (F2,37 = 3.258, p = 0.0498), total number of attacks (F2,37 = 8.912, p = 0.0007), total duration of attacks (F2,37 = 11.12, p = 0.0002, two-way ANOVA) and total duration of crawl-over behavior (F2,37 = 6.359, p = 0.0042 two-way ANOVA). More specifically, ANOVA revealed significant ultrasound effect for total number of attacks (F1,37 = 6.087, p = 0.0185), total duration of attacks (F1,37 = 4.808, p = 0.0347, two-way ANOVA) and total duration of crawl-over behavior (F1,37 = 4.306, p = 0.045 two-way ANOVA; Fig. 2A), indicating increased ultrasound-induced aggressiveness in the treated mice. Furthermore, ANOVA revealed significant treatment effect for latency to attack (F2,37 = 8.248, p = 0.0011), total number of attacks (F1,37 = 17.25, p < 0.0001), total duration of attacks (F2,37 = 18.57, p < 0.0001, two-way ANOVA) and total duration of crawl-over behavior (F2,37 = 7.992, p = 0.0013 two-way ANOVA; Fig. 2A), which was supportive of an anti-aggressive effect of the psychoactive antioxidant drugs thiamine and benfotiamine.

Post-hoc analysis revealed significant increase of latency to attack in ultrasound-exposed group treated with thiamine (p = 0.0413, Tukey’s test) or benfotiamine (p = 0.0022, Tukey’s test; Fig. 2A) in comparison with ultrasound-exposed non-treated group. It revealed significant increase of number of attacks in ultrasound-exposed non-treated group in comparison with naive mice (p = 0.0002, Tukey’s test) and a significant decrease of this parameter in ultrasound-exposed group treated with thiamine (p < 0.0001, Tukey’s test) or benfotiamine (p < 0.0001, Tukey’s test; Fig. 2A) in comparison with ultrasound-exposed non-treated group. Post-hoc analysis also revealed significant increase of total duration of attacks in ultrasound-exposed non-treated group in comparison with naive mice (p < 0.0001, Tukey’s test) and a significant decrease of this parameter in ultrasound-exposed group treated with thiamine (p < 0.0001, Tukey’s test) or benfotiamine (p < 0.0001, Tukey’s test; Fig. 2A) in comparison with ultrasound-exposed non-treated group. Finally, it revealed significant increase of total duration of crawl-over behavior in ultrasound-exposed non-treated group in comparison with naive mice (p = 0.0020, Tukey’s test) and a significant decrease of this parameter in ultrasound-exposed group treated with thiamine (p = 0.0020, Tukey’s test) or benfotiamine (p = 0.0013, Tukey’s test; Fig. 2A).

Additional analysis of aggressive-like behavior in the social interaction test revealed a significant decrease in latency in crawl-over, total number and total duration of crawl-over behavior and a significant increase in number of followings in vehicle-treated mice (p < 0.05 vs. control, two-way ANOVA and post hoc Tukey’s test, see Supplementary material; Supplemental Figs. 6A and B). Both ultrasound-exposed thiamine-treated and benfotiamine-treated animals exhibited no change from control mice in these parameters and displayed significant differences from the ultrasound-exposed vehicle-treated group (#p < 0.05 vs. ultrasound-exposed group, two-way ANOVA and post hoc Tukey’s test). There were no significant changes between groups in latency to interact and total duration of followings as well as in latency to tail rattling, number of tail rattling and total duration of tail rattling (p > 0.05 vs. ultrasound-exposed group, two-way ANOVA and post hoc Tukey’s test, see Supplementary material; Supplemental Fig. 6C).

In the social interaction test, ultrasound-exposed vehicle-treated mice showed a significant decrease in the number and total duration of social contacts in comparison to controls (*p < 0.05 vs. control, two-way ANOVA and post hoc Tukey’s test; see Supplementary material; Supplemental Fig. 7). Ultrasound-exposed benfotiamine-treated, but not thiamine-treated animals exhibited no change from control mice in these parameters and displayed significant differences from the ultrasound-exposed vehicle-treated group (#p < 0.05 vs. ultrasound-exposed group, two-way ANOVA and post hoc Tukey’s test). There were no significant changes between groups in latency to interact (p > 0.05 vs. ultrasound-exposed group, two-way ANOVA and post hoc Tukey’s test).

In the forced swim test, a two-way ANOVA revealed a significant interaction between ultrasound and treatment, in the latency to float (F2,37 = 3.427, p = 0.0428) and duration of floating behavior (F2,37 = 3.695, p = 0.0342, two-way ANOVA). ANOVA revealed a significant ultrasound effect on the latency to float (F1,37 = 36.03, p < 0.0001) and the duration of floating (F1,37 = 4.405, p = 0.0425, two-way ANOVA; Fig. 2B). Chronic ultrasound exposure resulted in significant prolongation of the duration of floating and shortened latency to float, indicating increased helplessness in the ultrasound-exposed group. ANOVA revealed significant treatment effect for latency to float (F2,37 = 17.7, p < 0.0001) and total duration of floating (F2,37 = 16.9, p < 0.0001, two-way ANOVA; Fig. 2B). When the ultrasound-exposed groups were treated with thiamine or benfotiamine, the exhibited decreased duration of floating and an increased latency to float, which is suggestive of an anti-depressant-like effect on the ultrasound-induced behavioural changes.

Fig. 3. Increased brain oxidative stress markers in mice subjected to the ultrasound exposure and effects of thiamine compounds. (A) Ultrasound-exposed vehicle-treated mice demonstrated increased protein carbonyl contents in the prefrontal cortex in comparison with controls (*p < 0.05 vs. control, two-way ANOVA and post hoc Tukey’s test). Ultrasound-exposed thiamine- and benfotiamine-treated mice displayed no changes from control mice in protein carbonyl contents in prefrontal cortex and displayed significantly lower values for this parameter as compared with the ultrasound-exposed vehicle-treated group (#p < 0.05 vs. ultrasound-exposed group, two-way ANOVA and post hoc Tukey’s test). (B) There were no significant changes between groups in protein carbonyl contents in hippocampus (p > 0.05 vs. ultrasound-exposed group, two-way ANOVA and post hoc Tukey’s test). (C) Benfotiamine-treated, but not thiamine-treated, ultrasound-exposed mice displayed significant differences from ultrasound-exposed vehicle-treated group in protein carbonyl contents in amygdala (*p < 0.05 vs. ultrasound-exposed group, two-way ANOVA and post hoc Tukey’s test). (D) Ultrasound-exposed vehicle-treated mice demonstrated increased protein carbonyl contents in the dorsal raphe in comparison with controls (*p < 0.05 vs. control, two-way ANOVA and post hoc Tukey’s test). Benfotiamine-treated, but not thiamine-treated, ultrasound-exposed mice displayed significant differences from the ultrasound-exposed vehicle-treated group in protein carbonyl contents in dorsal raphe (#p < 0.05 vs. ultrasound-exposed group, two-way ANOVA and post hoc Tukey’s test). (E) Ultrasound-exposed vehicle-treated mice demonstrated increased protein carbonyl contents in the striatum in comparison with controls (*p < 0.05 vs. control, two-way ANOVA and post hoc Tukey’s test). Ultrasound-exposed thiamine- and benfotiamine-treated mice exhibited no changes from control mice in protein carbonyl contents in striatum and displayed significantly lower values of this parameter as compared with ultrasound-exposed vehicle-treated group (#p < 0.05 vs. ultrasound-exposed group, two-way ANOVA and post hoc Tukey’s test; 6–7 animals per group were used). (F) Ultrasound-exposed vehicle-treated mice exhibited significantly increased total glutathione contents in prefrontal cortex in comparison to the controls (*p < 0.05 vs. ultrasound-exposed group, two-way ANOVA and post hoc Tukey’s test). Benfotiamine-treated ultrasound-exposed group exhibited no difference in total glutathione of the prefrontal cortex and showed significant decrease in values as compared with the ultrasound-exposed vehicle-treated group (#p < 0.05 vs. ultrasound-exposed group, two-way ANOVA and post hoc Tukey’s test). (G) Ultrasound-exposed vehicle-treated mice exhibited significantly increased total glutathione contents in hippocampus in comparison to the controls (*p < 0.05 vs. control, two-way ANOVA and post hoc Tukey’s test). (H) Ultrasound-exposed, vehicle-treated mice exhibited significantly increased total glutathione contents in amygdala in comparison to the controls (*p < 0.05 vs. control, two-way ANOVA and post hoc Tukey’s test; 6–7 animals per group were used). Bars are Mean ± SEM.
Post-hoc analysis revealed significant decrease of latency to float in ultrasound-exposed non-treated group in comparison with naïve mice (p = 0.241, Tukey’s test) and a significant increase of this parameter in ultrasound-exposed group treated with benfotiamine (p = 0.0489, Tukey’s test; Fig. 2B), but not thiamine (p = 0.3019, Tukey’s test) in comparison with ultrasound-exposed non-treated group. Post hoc analysis also revealed significant increase of total duration of floating in ultrasound-exposed non-treated group in comparison with naïve mice (p = 0.444, Tukey’s test) and a significant decrease of this parameter in ultrasound-exposed group treated with benfotiamine (p = 0.0019, Tukey’s test; Fig. 2B), but not thiamine (p = 0.0513, Tukey’s test) in comparison with ultrasound-exposed non-treated group.

A comparison of exploratory rearing activity by ANOVA revealed no interaction between ultrasound and treatment (F2,37 = 1.116, p = 0.3383), no significant ultrasound effect (F1,37 = 1.309, p = 0.2599) and no significant treatment effect (F2,37 = 0.8919, p = 0.4185, two-way ANOVA; Fig. 2C), indicating a lack of non-specific changes in vertical locomotor activity. For horizontal activity, there also was no interaction between ultrasound and treatment (F2,37 = 0.6669, p = 0.5193), as well as no significant ultrasound effect (F1,37 = 1.612, p = 0.2122) and no significant treatment effect (F2,37 = 0.4536, p = 0.6388, two-way ANOVA; Fig. 2D). Furthermore, mice exposed to the ultrasound for just 24 h did not show any behavioural changes in any of the parameters measured, regardless of treatment (Fig. 2E–G; see Supplementary material, Supplementary Table 3).

3.2. Increased brain oxidative stress markers in mice with ultrasound-induced aggression and effects of thiamine compounds

Following the behavioural tests, the brains of these mice were studied for protein carbonyl and total glutathione content, and markers of oxidative stress in five brain structures. There was a significant interaction between ultrasound and treatment in protein carbonyl content in prefrontal cortex (F2,31 = 5.154, p = 0.0117), dorsal raphe (F2,31 = 8.279, p < 0.0013) and striatum (F2,31 = 4.824, p < 0.0193), but no significant interaction between ultrasound and treatment in the hippocampus (F2,31 = 1.129, p = 0.3362) and amygdala (F2,31 = 1.254, p = 0.2993, two-way ANOVA). There was a significant ultrasound effect in prefrontal cortex (F2,33 = 4.162, p = 0.049; Fig. 3A), dorsal raphe (F2,31 = 43.05, p < 0.0001; Fig. 3D) and striatum (F2,31 = 24.32, p < 0.00; Fig. 3E), where protein carbonyl content was significantly elevated. Furthermore, ANOVA revealed significant treatment effect in prefrontal cortex (F2,31 = 3.356, p = 0.0479; Fig. 3A), amygdala (F2,31 = 12.16, p = 0.0001; Fig. 3C), dorsal raphe (F2,31 = 13.58, p < 0.0001; Fig. 3D) and striatum (F2,31 = 12.61, p < 0.0001; Fig. 3E). These data show that ultrasound exposure increases oxidative stress in limbic brain structures, which could be ameliorated by treatment with thiamine or benfotiamine. Notably, only ultrasound-exposed mice treated with benfotiamine displayed a significant decrease of protein carbonyl content in amygdala (p = 0.038, post hoc Tukey’s test) and dorsal raphe (p < 0.0001, post hoc Tukey’s test), in comparison with the vehicle-treated control group, suggesting superior effects of benfotiamine over that of thiamine.

Post-hoc analysis revealed significant increase of protein carbonyl content in prefrontal cortex in ultrasound-exposed non-treated group in comparison with naïve mice (p = 0.0413, Tukey’s test) and a significant decrease of this parameter in the ultrasound-exposed group treated with thiamine (p = 0.0161, Tukey’s test) or benfotiamine (p = 0.0138, Tukey’s test; Fig. 3A) in comparison with the ultrasound-exposed non-treated group. Notably, only ultrasound-exposed mice treated with benfotiamine displayed a significant decrease of protein carbonyl content in amygdala (p = 0.038, post hoc Tukey’s test) and dorsal raphe (p < 0.0001, post hoc Tukey’s test), in comparison with the ultrasound-exposed non-treated group, but not in the thiamine-treated ultrasound-exposed mice (p = 0.9543, post hoc Tukey’s test; Fig. 3C).

Post-hoc analysis also revealed significant increase of protein carbonyl content in the dorsal raphe in the ultrasound-exposed non-treated group in comparison with naïve mice (p < 0.0001, Tukey’s test). Again, only ultrasound-exposed mice treated with benfotiamine (p < 0.0001, post hoc Tukey’s test), but not thiamine (p = 0.6789, post hoc Tukey’s test; Fig. 3D) displayed a significant decrease of protein carbonyl content in dorsal raphe, in comparison with the ultrasound-exposed non-treated group, suggesting superior effects of benfotiamine over that of thiamine. There also was a significant increase of protein carbonyl content in prefrontal cortex in ultrasound-exposed non-treated group in comparison with naïve mice (p = 0.0002, Tukey’s test) and a significant decrease of this parameter in ultrasound-exposed group treated with thiamine (p = 0.0002, Tukey’s test) or benfotiamine (p < 0.0001, Tukey’s test; Fig. 3E) in comparison with ultrasound-exposed non-treated group.

There was a significant interaction between stress and treatment in total glutathione content in prefrontal cortex (F2,33 = 3.834, p = 0.0834), and striatum (F2,33 = 4.824, p = 0.0145), and no significant interaction between ultrasound and treatment in hippocampus (F2,33 = 2.661, p < 0.0848) and in amygdala (F2,33 = 4.012, p = 0.06728) and dorsal raphe (F2,33 = 0.1137, p = 0.8929, two-way ANOVA). ANOVA revealed a significant ultrasound effect within the prefrontal cortex (F1,33 = 53.22, p < 0.0001; Fig. 3F), hippocampus (F1,33 = 69.65, p < 0.0001; Fig. 3G), amygdala (F1,33 = 31.51, p < 0.0001; Fig. 3H) and striatum (F1,33 = 24.32, p < 0.0001; Fig. 3J), where protein carbonyl content was significantly elevated in ultrasound-exposed group. Furthermore, ANOVA revealed significant treatment effect in prefrontal cortex (F2,33 = 16.26, p < 0.0001; Fig. 3F) and in striatum (F2,33 = 12.61, p < 0.0001; Fig. 3J). At the same time, ANOVA revealed that there was no significant treatment effect in amygdala (F2,33 = 1.114, p = 0.3402; two-way ANOVA; Fig. 3H), and, though close, hippocampus (F2,33 = 2.878, p = 0.0704; Fig. 3G) and dorsal raphe (F2,33 = 2.783, p = 0.0764; Fig. 3J). Post hoc analysis revealed significant increase of total glutathione content in prefrontal cortex in the ultrasound-exposed non-treated group in comparison with naïve mice (p < 0.0001, Tukey’s test) and a significant decrease of this parameter in the ultrasound-exposed group treated with benfotiamine (p = 0.0001, Tukey’s test), but not thiamine (p = 0.0527, Tukey’s test; Fig. 3F) in comparison with the ultrasound-exposed non-treated group. It also revealed significant increase of total glutathione content in hippocampus (p < 0.0001, Tukey’s test; Fig. 3G) and amygdala (p = 0.0066, Tukey’s test; Fig. 3H) in ultrasound-exposed non-treated group in comparison with naïve mice. There also was a significant increase of total glutathione content in striatum in the ultrasound-exposed non-treated group in comparison with naïve mice (p < 0.0001, Tukey’s test) and a significant decrease of this parameter in the ultrasound-exposed group treated with thiamine (p = 0.0109, Tukey’s test) or benfotiamine (p = 0.0004, Tukey’s test; Fig. 3J) in comparison with ultrasound-exposed non-treated group.

Thus, in a similar manner to the results obtained in the protein carbonyl assays, these data showed an increase of oxidative stress measures after 3-weeks of ultrasound exposure, which could be prevented by treatment with thiamine or benfotiamine. Again, only benfotiamine-treated ultrasound-exposed mice displayed a significant decrease of total glutathione content in prefrontal cortex in comparison with the vehicle-treated control group. These data suggest that benfotiamine seems to have greater efficacy in comparison to thiamine. Mice exposed to the acute 24-h ultrasound exposure and/or administration of thiamine compounds did not show any changes in the parameters measured (Supplemental Fig. 8, Supplementary Table 4).

3.3. Altered brain expression of AMPA receptor subunits and 5-HT6 receptor during ultrasound-induced aggression and effects of thiamine compounds

ANOVA revealed significant interaction between ultrasound and...
treatment for the mRNA levels of GluA1 subunit in the prefrontal cortex ($F_{2,33} = 4.96$, $p < 0.0131$) and hippocampus ($F_{2,33} = 5.337$, $p < 0.0098$, two-way ANOVA). More specifically, the mRNA levels of GluA1 subunit in both prefrontal cortex ($F_{1,33} = 5.081$, $p = 0.0310$; Fig. 4A) and hippocampus ($F_{1,33} = 4.662$, $p = 0.382$, two-way ANOVA; Fig. 4B) was decreased after ultrasound exposure. ANOVA also revealed significant effect of treatment on the mRNA levels of GluA1 subunit in the prefrontal cortex ($F_{2,33} = 7.699$, $p = 0.0018$; Fig. 4A) and hippocampus ($F_{2,33} = 5.785$, $p = 0.007$, two-way ANOVA; Fig. 4B).

ANOVA revealed significant interaction between ultrasound and treatment in the mRNA levels of GluA2 subunit in the prefrontal cortex ($F_{2,33} = 4.816$, $p = 0.0146$) and hippocampus ($F_{2,33} = 4.402$, $p = 0.0202$, two-way ANOVA). Ultrasound exposure increased the mRNA levels of GluA2 subunit in both prefrontal cortex ($F_{1,33} = 15.17$, $p = 0.0005$; Fig. 4C) and hippocampus ($F_{1,33} = 42.86$, $p < 0.0001$, two-way ANOVA; Fig. 4D). Again, there was a significant treatment effect in the mRNA levels of GluA2 subunit in the prefrontal cortex ($F_{2,33} = 4.796$, $p = 0.0148$; Fig. 4C) and hippocampus ($F_{2,33} = 4.438$,
Ultrasound-exposed, vehicle-treated mice displayed a significant decrease in GluA1 mRNA concentrations in the prefrontal cortex (*p < 0.05 vs. control, two-way ANOVA and post hoc Tukey's test). In ultrasound-exposed benfotiamine-treated mice, GluA1 mRNA concentrations in the prefrontal cortex were unchanged as compared with the control and displayed significant differences from the ultrasound-exposed vehicle-treated group (*p < 0.05 vs. ultrasound-exposed group, two-way ANOVA and post hoc Tukey's test). (B) Ultrasound-exposed, vehicle-treated mice displayed a significant decrease in GluA1 mRNA concentrations in the hippocampus (*p < 0.05 vs. control, two-way ANOVA and post hoc Tukey's test). In ultrasound-exposed thiamine- and benfotiamine-treated mice, GluA1 mRNA concentrations in the prefrontal cortex were unchanged as compared with the control and displayed significant differences from the ultrasound-exposed vehicle-treated group (*p < 0.05 vs. ultrasound-exposed group, two-way ANOVA and post hoc Tukey's test). Ultrasound-exposed thiamine- and benfotiamine-treated mice levels of expression were unchanged compared to control mice in GluA2 mRNA concentrations in the prefrontal cortex and displayed significant differences from the ultrasound-exposed, vehicle-treated group (*p < 0.05 vs. ultrasound-exposed group, two-way ANOVA and post hoc Tukey’s test). (D) Ultrasound-exposed, vehicle-treated mice demonstrated a significant increase of GluA2 mRNA concentrations in the hippocampus (*p < 0.05 vs. control, two-way ANOVA and post hoc Tukey’s test). Ultrasound-exposed benfotiamine-treated mice showed significant differences from ultrasound-exposed vehicle-treated group in GluA2 mRNA concentrations in hippocampus (*p < 0.05 vs. ultrasound-exposed group, two-way ANOVA and post hoc Tukey’s test). (E) Ultrasound-exposed vehicle-treated mice demonstrated a significant decrease of GluA3 mRNA concentrations in the prefrontal cortex (*p < 0.05 vs. control, two-way ANOVA and post hoc Tukey’s test). (F) Ultrasound-exposed vehicle-treated mice demonstrated a significant decrease of GluA3 mRNA concentrations in the hippocampus (*p < 0.05 vs. control, two-way ANOVA and post hoc Tukey’s test). (G) There were no significant changes between groups in GluA4 mRNA concentrations in the prefrontal cortex (p > 0.05 vs. ultrasound-exposed group, two-way ANOVA and post hoc Tukey’s test). (H) There were no significant changes between groups in GluA4 receptor mRNA concentrations in hippocampus (p > 0.05 vs. ultrasound-exposed group, two-way ANOVA and post hoc Tukey’s test; 6–7 animals per group were used). Bars are Mean ± SEM.

There was no significant interaction between ultrasound and treatment in the mRNA levels of GluA3 subunit in the prefrontal cortex (F(2,33) = 2.274, p = 0.1118) and hippocampus (F(2,33) = 0.6422, p = 0.5326, two-way ANOVA). Although, ANOVA revealed a significant ultrasound effect on the mRNA levels of GluA3 subunit in prefrontal cortex (F(1,33) = 8.58, p = 0.0061; Fig. 4E) and also hippocampus (F(33) = 19.86, p < 0.0001, two-way ANOVA; Fig. 4F), where GluA3 expression was decreased after ultrasound exposure. At the same time there was no significant treatment effect in the mRNA levels of GluA3 subunit in the prefrontal cortex (F(33) = 1.778, p = 0.1848; Fig. 4E).

ANOVA revealed no significant interaction between ultrasound and treatment in the mRNA levels of GluA4 subunit in the prefrontal cortex (F(2,33) = 0.7632, p = 0.4742) and hippocampus (F(2,33) = 0.6422, p = 0.5326, two-way ANOVA) where expression is known to be very low. Furthermore, ANOVA revealed no significant ultrasound effect on the mRNA levels of GluA4 subunit in both prefrontal cortex (F(1,33) = 0.2391, p = 0.6281; Fig. 4G) and hippocampus (F(33) = 0.09418, p = 0.7609, two-way ANOVA; Fig. 4H). There was no significant treatment effect in the mRNA levels of GluA4 subunit in the prefrontal cortex (F(2,33) = 0.8071, p = 0.4548; Fig. 4G) and hippocampus (F(33) = 0.4546, p = 0.865, two-way ANOVA; Fig. 4H).

Post hoc analysis revealed significant decrease of GluA1 expression level in prefrontal cortex in ultrasound-exposed non-treated group in comparison with naïve mice (p = 0.0077, Tukey’s test) and a significant increase of this parameter in the ultrasound-exposed group treated with thiamine (p = 0.0269, Tukey’s test) or benfotiamine (p = 0.0069, Tukey’s test; Fig. 4A) in comparison with ultrasound-exposed non-treated group. There also was a significant decrease of GluA1 expression level in hippocampus in ultrasound-exposed non-treated group in comparison with naïve mice (p = 0.0187, Tukey’s test) and a significant increase of this parameter in ultrasound-exposed group treated with benfotiamine (p = 0.0224, Tukey’s test), but not thiamine (p = 0.1301, Tukey’s test; Fig. 4B) in comparison with ultrasound-exposed non-treated group. There also was a significant increase of GluA2 expression level in hippocampus in ultrasound-exposed non-treated group in comparison with naïve mice (p = 0.0025, Tukey’s test) and a significant decrease of this parameter in ultrasound-exposed group treated with thiamine (p = 0.0446, Tukey’s test) or benfotiamine (p = 0.0110, Tukey’s test; Fig. 4C) in comparison with ultrasound-exposed non-treated group. Post-hoc analysis revealed a significant increase of GluA2 expression level in hippocampus in ultrasound-exposed non-treated group in comparison with naïve mice (p = 0.0019, Tukey’s test) and a significant decrease of this parameter in ultrasound-exposed group treated with benfotiamine (p = 0.0165, Tukey’s test), but not thiamine (p = 0.0999, Tukey’s test; Fig. 4D) in comparison with ultrasound-exposed non-treated group. Finally, post-hoc analysis revealed a significant decrease of GluA3 expression level in prefrontal cortex (p = 0.0155, Tukey’s test; Fig. 4E) and hippocampus (p = 0.0131, Tukey’s test; Fig. 4F) in ultrasound-exposed non-treated group in comparison with naïve mice.

While there was an interaction between ultrasound and treatment at the mRNA level, this was not true for GluA2 protein expression in the prefrontal cortex (F(2,33) = 2.394, p = 0.1128) or hippocampus (F(2,33) = 0.1222, p = 0.8855, two-way ANOVA). However, ANOVA did reveal a significant main effect of ultrasound effect in the GluA2 protein expression in the prefrontal cortex (F(1,33) = 8.929, p = 0.0064), but not in the hippocampus (F(1,33) = 0.1504, p = 0.7016, two-way ANOVA). There was no significant treatment effect in the GluA2 protein expression in the prefrontal cortex (F(2,33) = 1.57, p = 0.2286) and hippocampus (F(2,33) = 1.814, p = 0.1846, two-way ANOVA; Supplemental Fig. 9A). Post-hoc analysis revealed a significant increase of GluA2 protein expression in prefrontal cortex in ultrasound non-treated group in comparison with naïve mice (p = 0.004, Tukey’s test). However, immunohistochemical analysis of the distribution of the subunits that exhibited expression changes after ultrasound exposure (GluA1, GluA2, and GluA3) revealed that the subunits were internalized in in ultrasound-exposed animals compared the controls for GluA1 and GluA2, but not for GluA3. ultrasound-exposed, vehicle-treated mice also exhibited a significant decrease of GluA2 and GluA3, but not GluA1, on the cell surface, in CA1 zone of the hippocampus (Supplemental Figs. 12 and 13). Thus the ultrasound had an impact on the distribution of all of the subunits except GluA4.

There was no significant interaction between ultrasound and treatment for 5-HT6R mRNA levels in the prefrontal cortex (F(2,33) = 0.05771, p = 0.0944) and hippocampus (F(2,33) = 1.292, p = 0.2883). There was also no significant interaction between ultrasound and treatment in 5-HT6 receptor relative fold protein concentrations in the prefrontal cortex (F(2,30) = 1.756, p = 0.19). Furthermore, ANOVA revealed no significant ultrasound effect in 5-HT6R mRNA concentrations in the prefrontal cortex (F(1,33) = 0.1272, p = 0.7236) and hippocampus (F(1,33) = 0.4828, p = 0.4920; Supplemental Fig. 10A), but, at the same time, ANOVA did reveal that there was a significant ultrasound effect in 5-HT6 receptor relative fold protein concentrations in the prefrontal cortex (F(1,30) = 7.351, p = 0.011) and hippocampus (F(1,30) = 6.281, p = 0.0179, two-way ANOVA; Supplemental Fig. 10B). Finally, ANOVA revealed no significant treatment effect in 5-HT6R mRNA concentrations in the prefrontal cortex (F(2,33) = 0.2007, p = 0.8191) and hippocampus.
Fig. 5. Suppressed expression of plasticity factors resulting from the ultrasound and effects of thiamine drugs. (A) Ultrasound-exposed vehicle-treated mice displayed a significant decrease of PSD95 relative fold protein levels in the prefrontal cortex (*p < 0.05 vs. control, two-way ANOVA and post hoc Tukey’s test). (B) Ultrasound-exposed vehicle-treated mice exhibited a significant decrease of PSD95 relative fold protein levels in the hippocampus (*p < 0.05 vs. control, two-way ANOVA and post hoc Tukey’s test). Benfotiamine-treated mice displayed no changes from control mice in PSD95 relative fold protein levels in the hippocampus and displayed significant differences from ultrasound-exposed vehicle-treated group (#p < 0.05 vs. ultrasound-exposed group, two-way ANOVA and post hoc Tukey’s test). (C) Ultrasound-exposed vehicle-treated mice displayed a significant decrease of PSA-NCAM relative fold protein levels in the prefrontal cortex (*p < 0.05 vs. control, two-way ANOVA post hoc and Tukey’s test). Thiamine- and benfotiamine-treated mice exhibited no change from control mice in PSA-NCAM relative fold protein levels in the prefrontal cortex and displayed significant differences from ultrasound-exposed vehicle-treated group (#p < 0.05 vs. ultrasound-exposed group, two-way ANOVA and post hoc Tukey’s test). (D) Ultrasound-exposed vehicle-treated mice displayed a significant decrease of PSA-NCAM relative fold protein levels in the hippocampus (*p < 0.05 vs. control, two-way ANOVA and post hoc Tukey’s test). Benfotiamine-treated mice exhibited significant differences in PSA-NCAM relative fold protein levels in hippocampus from ultrasound-exposed vehicle-treated group (#p < 0.05 vs. ultrasound-exposed group, two-way ANOVA and post hoc Tukey’s test). (E) Ultrasound-exposed vehicle-treated mice had a significant decrease of the β-catenin relative fold protein levels in prefrontal cortex (*p < 0.05 vs. control, two-way ANOVA and post hoc Tukey’s test). Benfotiamine-treated mice had no changes from control mice in the β-catenin relative fold protein levels in the prefrontal cortex and displayed significant differences from the ultrasound-exposed vehicle-treated group (#p < 0.05 vs. ultrasound-exposed group, two-way ANOVA and post hoc Tukey’s test). (F) There were no significant changes between groups in the β-catenin relative fold protein levels in the hippocampus (p > 0.05 vs. ultrasound-exposed group, two-way ANOVA and post hoc Tukey’s test; 6 animals per group were used). Bars are Mean ± SEM.
(F2,33 = 0.6105, p = 0.5491; Fig.10B of Supplementary file), as well as in 5-HT6 receptor relative fold protein concentrations in the prefrontal cortex (F2,33 = 1.247, p = 0.302) and hippocampus (F2,33 = 0.9648, p = 0.3926, two-way ANOVA; Supplementary Fig. 10B). Post-hoc analysis revealed significant decrease of 5-HT6 protein expression in prefrontal cortex (p = 0.0124, Tukey’s test) and hippocampus (p = 0.0427, Tukey’s test) in ultrasound-exposed non-treated group in comparison with naïve mice. Thus, a 3-week long ultrasound exposure resulted in altered expression of the different subunits of the AMPA receptor and some changes in the expression of 5HT-6 receptor in the limbic structures of the brain. Treatment with thiamine or benfotiamine prevented some of these ultrasound-induced changes, but the lack of effect of thiamine or benfotiamine on 5HT-6 receptor expression suggests that it is not contributing to the aggressive behavior in these animals. Mice exposed to the acute 24 h ultrasound exposure and/or administration of thiamine compounds did not show any changes in any of the parameters measured (see Supplementary material, Supplementary Table 5).

3.4. Suppressed expression of plasticity factors resulting from the ultrasound exposure and effects of thiamine drugs

ANOVA revealed a significant interaction between ultrasound and treatment in the protein concentration of PSD95 in hippocampus (F2,30 = 5.024, p = 0.0131), but not in the prefrontal cortex (F2,30 = 1.816, p = 0.1802, two-way ANOVA). Furthermore, ANOVA revealed a significant ultrasound effect in both prefrontal cortex (F1,30 = 9.017, p = 0.0054; Fig. 5A) and hippocampus (F1,30 = 5.252, p = 0.0291, two-way ANOVA; Fig. 5B) as ultrasound-exposed mice displayed a significantly decreased protein concentration of PSD95. There also was a significant treatment effect in the hippocampus (F2,30 = 3.326, p = 0.0496, Fig. 5B) but, though close, not in the prefrontal cortex (F2,30 = 3.177, p = 0.0561, Fig. 5A), indicating the efficacy of treatment with thiamine and benfotiamine against ultrasound-induced changes.

ANOVA revealed a significant interaction between ultrasound and treatment in protein concentration of PSA-NCAM in hippocampus (F2,30 = 4.335, p = 0.0222). Furthermore, ANOVA revealed a significant ultrasound effect in both prefrontal cortex (F1,30 = 4.49, p = 0.0425; Fig. 5C) and hippocampus (F1,30 = 7.071, p = 0.0124, two-way ANOVA; Fig. 5D) where ultrasound-exposed mice exhibited significantly decreased protein concentration of PSA-NCAM. There also was a significant treatment effect in both prefrontal cortex (F2,30 = 5.324, p = 0.0105, Fig. 5C) and hippocampus (F2,30 = 3.633, p = 0.0386, Fig. 5D), once again indicating the effectiveness of treatment with thiamine and benfotiamine against ultrasound-induced changes. Remarkably, only benfotiamine-treated ultrasound-exposed mice demonstrated a significant increase of hippocampal concentrations of PSA-NCAM in comparison with the ultrasound-exposed vehicle-treated group in hippocampus (p = 0.0166, post hoc Tukey’s test).

ANOVA revealed a significant interaction between ultrasound and treatment in protein concentration of β-catenin (F2,30 = 5.297, p = 0.0107), a significant ultrasound effect (F2,30 = 4.424, p = 0.0439) and also a significant treatment effect (F2,30 = 3.753, p = 0.0351, two-way ANOVA, Fig. 5E) in prefrontal cortex. At the same time there was no interaction between ultrasound and treatment in protein concentration of β-catenin (F2,30 = 0.7935, p = 0.4615) or, though close, an ultrasound effect (F2,30 = 3.542, p = 0.0696) or treatment effect (F2,30 = 2.577, p = 0.0928; two-way ANOVA; Fig. 5F) in the hippocampus. Post-hoc analysis revealed significant decrease of PSD95 protein concentration in prefrontal cortex in ultrasound-exposed non-treated group in comparison with naïve mice (p = 0.0471, Tukey’s test; Fig. 5A). There was a significant decrease of PSD95 protein concentration in hippocampus in ultrasound-exposed non-treated group in comparison with naïve mice (p = 0.0121, Tukey’s test) and a significant increase of this parameter in ultrasound-exposed group treated with benfotiamine (p = 0.0041, Tukey’s test), but not thiamine (p = 0.2333, Tukey’s test; Fig. 5B) in comparison with ultrasound-exposed non-treated group.

There was a significant decrease of PSA-NCAM protein concentration in prefrontal cortex in ultrasound-exposed non-treated group in comparison with naïve mice (p = 0.0363, Tukey’s test) and a significant increase of this parameter in ultrasound-exposed group treated with thiamine (p = 0.0438, Tukey’s test) or benfotiamine (p = 0.0111, Tukey’s test; Fig. 5C) in comparison with ultrasound-exposed non-treated group. There also was a significant decrease of PSA-NCAM protein concentration in hippocampus in ultrasound-exposed non-treated group in comparison with naïve mice (p = 0.0060, Tukey’s test) and a significant increase of this parameter in ultrasound-exposed group treated with thiamine (p = 0.0464, Tukey’s test) or benfotiamine (p = 0.0166, Tukey’s test; Fig. 5D) in comparison with ultrasound-exposed non-treated group. Finally, there was a significant decrease of β-catenin protein concentration in hippocampus in ultrasound-exposed non-treated group in comparison with naïve mice (p = 0.0149, Tukey’s test) and a significant increase of this parameter in ultrasound-exposed group treated with benfotiamine (p = 0.0026, Tukey’s test), but not thiamine (p = 0.0512, Tukey’s test; Fig. 5E) in comparison with ultrasound-exposed non-treated group.

Mice exposed to the acute 24 h ultrasound exposure and/or the administration of thiamine compounds did not show any changes in any of the parameters measured (see Supplementary material, Supplementary Table 6).

4. Discussion

We found that mice subjected to the chronic ultrasound exposure exhibit aggressive behavior and, at a molecular level, increased expression in the brain of the oxidative stress markers protein carbonyl and total glutathione. These effects were found in vehicle-treated ultrasound-exposed mice, but not in the thiamine-treated and benfotiamine-treated ultrasound-exposed groups. Ultrasound-exposed vehicle-treated mice displayed elevated gene expression of the GluA2 subunit, although cell surface protein expression is reduced, and decreased protein expression of 5-HT6 receptor and the plasticity factors PSD95, PSA-NCAM and β-catenin, in the prefrontal cortex and the hippocampus. Again, these ultrasound effects were not observed in the thiamine-treated and benfotiamine-treated animals. Control experiments showed that a 24-h acute ultrasound exposure and/or administration of either compound did not affect any of the behavioural and molecular parameters measured in the mice.

Thus, the present study has revealed generalized increases of oxidative stress markers in the brain to parallel elevated scores of aggressive behavior in mouse model emotional stress and these changes were reversed by the administration of thiamine and benfotiamine. Treatment with benfotiamine had a greater effect on reversing brain oxidative stress than thiamine, supporting previous observations that there may be increased bioavailability for benfotiamine in comparison to thiamine (Pan et al., 2010; Vignisse et al., 2017). The presence of increased brain oxidation stress markers with aggression in mice is in keeping with previous findings obtained in rodent models of naturalistic adversity (Diehl et al., 2012; Patki et al., 2013, 2014). For instance, Sprague Dawley rats subjected to social defeat stress, exhibited elevated concentrations of oxidative stress markers that correlated with their scores of aggressive behavior (Patki et al., 2014). Wistar rats subjected to maternal separation stress displayed elevated antioxidant enzymes activities in the hippocampus and exhibited impaired hippocampus-dependent behaviours (Diehl et al., 2012). Physical exercise was shown to counteract stress-induced effects in a rat model of maternal separation (Daniels et al., 2012). Other types of stress, that potentiate aggressiveness in small rodents, including chronic mild stress, were shown to increase oxidative stress measures in the brain in the periphery (Rammal et al., 2010; Costa-Nunes et al., 2014; Cline et al., 2013).
2015a; Malki et al., 2016).

Chronin administration of thiamine and benfotiamine prevented pro-oxidative effects of the ultrasound exposure. Previous studies that employed the same dosing regime, and were of similar duration, reported an absence of a change in the brain content of thiamine mono-phosphate and thiamine di-phosphate, where the latter is an essential cofactor of brain metabolism (Volfvet al., 2008; Pan et al., 2010; Vignisse et al., 2017). Surprisingly, both treatments resulted in similar changes in brain thiamine concentrations, while bioavailability of benfotiamine is known to significantly exceed that of thiamine (Vignisse et al., 2017). From this outcome, it can be assumed that the present ameliorative effects of these compounds are not primarily reliant of the regulation of brain energy metabolism, to and more likely to be dependent on their non-co-enzyme extracellular effects. Indeed, studies of recent years suggest that there are wide spectrum of functions for thiamine in the CNS that are dependent on non-enzymatic mechanisms (Bunik, 2013; Bettendorff et al., 2014; Mkrtchyan et al., 2012; Diehl et al., 2012; Patki et al., 2013, 2016; Clive et al., 2015a,b). These effects are likely to have mediated the anti-oxidative and neuroprotective action of thiamine and benfotiamine reported here.

Thiamine is essential for normal brain function and its deficiency causes metabolic impairment, oxidative damage, and reduced adult hippocampal neurogenesis. While we suspect that the reversal of the AMPA subunit expression profile by thiamine and benfotiamine is likely to be a downstream consequence of the treatment, the Ca²⁺ permeability of AMPA receptors is determined by the presence of the GluA2 subunit (Hume et al., 1991). GluA2 is a critical subunit in determining many of the biophysical properties of AMPARs, including Ca²⁺ permeability, and AMPARs lacking GluA2 are more permeable to Ca²⁺ and Zn²⁺. Thiamine deficiency leads to increased Ca²⁺ entry via GluA2, which is thought to be a consequence of reduced thiamine-dependent pre-mRNA editing that silences GluA2 (ADAR2-dependent RNA editing of GluA2 is involved in thiamine deficiency-induced alteration of calcium dynamics). Thus the ultrasound exposure-induced increase in GluA2 mRNA is likely to result in increased AMPA signaling and treatment with thiamine and benfotiamine may increase pre-mRNA editing to suppress GluA2 mediated calcium currents.

While GluA2 mRNA was increased, GluA1 and GluA3 the levels of the transcript and cell surface protein expression were reduced in ultrasound exposed mice. Surprisingly, the cell surface expression of GluA2 protein was also decreased. This seeming disparity probably reflects increased turnover of GluA2 protein in neuronal populations, which may have resulted in an increased demand for transcript. However, the discrepancy may also reflect increased expression in GluA2 mRNA expression in non-neuronal cell populations. Homomeric GluA2 subunits are responsible for the low conductance of AMPAR in activated microglia. GluA2 levels of expression are increased in microglia by the injections of LPS into mice and we know that stress activates microglia. The GluA2 subunits of the AMPARs, also expressed in astrocytes, are crucial determinants in controlling the calcium permeability and function (Fan et al., 1999). However, while GluA3 is increased in reactive astrocytes GluA2, where they have been measured, is unchanged (Newcombe et al., 2008). Concerning the differences that we observed between the regulation of subunit expression reported here in our study, several studies have suggested that these receptor subunits may play distinct roles in the regulation of AMPA receptor trafficking and synaptic plasticity. Thus, the ability for their expression to be differentially regulated, depending on demand and environmental conditions, is unsurprising. The GluA1 subunit is required for NMDA receptor-dependent synaptic delivery of AMPA receptors. In contrast, GluA2/GluA3 receptors seem to play a complementary role in the constitutive delivery pathway. Furthermore, in studies using GluA1 or GluA2 KO mice, it is clear that GluA1 and GluA2 act differentially in LTP formation (Toyoda et al., 2009). GluA2 incorporation into AMPA receptors seems to be particularly important in synaptic scaling, and, thus, changes in the ratios of GluA1 and GluA2 suggest that synaptic plasticity is likely to be altered following ultrasound exposure.

Whatever the significance of the changes in levels of expression, what is very clear from our study is that treatment normalized receptor subunit expression.

An important non-enzymatic mechanism that may be important here is the previously described action of thiamine and benfotiamine on GSK-3β activities (Pan et al., 2010; Pavlov et al., 2017; Markova et al., 2017). This kinase phosphorylates Nrf2, a regulator of antioxidant response, that suppresses expression of Nrf2-dependent genes, and, consequently, the protection of neurons from oxidative stress (Rada et al., 2012; Beurel et al., 2015). A decrease of GSK-3β activity may then results in a reduction of oxidative stress, which may underpin the effects of the thiamine compounds on protein carbonyl and total glutathione concentrations in brains of the ultrasound-exposed mice.

Pro-oxidative changes in rodent stress models were previously reported to result in impaired social behaviours, elevated aggressiveness, depressive-like changes and suppression of brain plasticity (Daniels et al., 2012; Diehl et al., 2012; Patki et al., 2013, 2016; Clive et al., 2015a,b). In line with these data, we found decreased expression of plasticity factors in the hippocampus and in the prefrontal cortex. A reduction of morphological and molecular markers of brain plasticity was previously shown to accompany excessive oxidative stress and aggression measures in clinical and pre-clinical studies (Marais et al., 2009; Boufler et al., 2013; Okazawa et al., 2014; Salminen and Paul, 2014). Here, we found suppressed gene and protein expression in plasticity markers whose function is also associated with brain oxidative stress and aggressive behavior.

For instance, ultrasound-exposed vehicle-treated mice had lowered expression of β-catenin, a plasticity factor that triggers oxidative stress cascades via forkhead box O (FOXO), an element of GSK-3β pathway, which known as pivotal modulator of neuroinflammation and apoptosis (Arnold et al., 2001; Lento et al., 2014; Beurel et al., 2015). Ultrasound-exposed vehicle-treated mice exhibited also decreased brain expression of PSD95, a scaffolding protein regulating synaptic development and remodeling, whose expression was recently demonstrated to be altered in rodent models of aggression (Been et al., 2016). Diminished expression of PSD95, as well as another important plasticity factor, NCAM and its functionally important polysialylated form PSA-NCAM, have been found to be associated with higher levels of aggression and territoriality in mice (Angata et al., 2004; Calandrau et al., 2010; Winkler et al., 2017), which is in keeping with the results reported here. These data support recently published findings showing that the chronic exposure of BALB/c mice to the ultrasound of alternating frequencies resulted in decreased densities of Kif67- and DCX-positive cells in the hippocampus, altered expression of BDNF together with its receptor TrkB, increased density of Iba-1, a marker of microglial activation, elevated levels of IL-1β and IL-6 in the hippocampus and serum, elevated activities of GSK-3β/Akt/FOXO3a cascade as well as other changes (Pavlov et al., 2019).

Compromised plasticity mechanisms, resulting from stress experiences are suggested to hamper cognitive flexibility and ability to control behavior, thereby leading to excessive aggression (Fanning et al., 2017). In addition to other actions, thiamine and benfotiamine have also been reported to improve cognition and memory. Pro-cognitive drugs developed for Alzheimer disease, such as dimebon and DF302, have also been shown to reduce aggression and this would be in keeping with the actions of thiamine and benfotiamine observed here (Vignisse et al., 2011; Markova et al., 2017; Strezkalova et al., 2018). The results with the pro-cognitive interventions resulted in modulation of AMPA- and 5-HT6 receptor-mediated neurotransmission, which was also observed after treatment with the thiamine compounds.

The role of AMPA- and 5-HT6 receptor-mediated neurotransmission in excessive aggression has been highlighted in previous rodent studies with corticosterone administration (Sun et al., 2018), naturalistic stress paradigms (Partyka et al., 2016; Shimizu et al., 2016), genetic manipulations (Vekovischeva et al., 2004, 2007; Adamczyk et al., 2012), chronic stress (Costa-Nunes et al., 2014; Zhang et al., 2015), pharmacological experiments (Upton et al., 2008; Yun and Rhim, 2011) and in
clinical post-mortem and association studies (Tsai et al., 1999; Garcia-Alloza et al., 2004; Marcos et al., 2008; Azenha et al., 2009; Ramirez, 2013; Zhang et al., 2018). For instance, treatment with antagonists of AMPA/kainate receptor and manipulations of the 5-HT6 receptors were found to attenuate the aggressive behavior of mice subjected to the isolation stress (Adamczyk et al., 2012; Araki et al., 2014; Partyka et al., 2016; Shimizu et al., 2016) and social stress (Vekovischeva et al., 2007). Of note, regulatory effects of GSK-3β on AMPA receptor have also been reported recently (Xhlghatyan et al., 2018) that leads us to speculate that this pathway, in particular, may be responsible for the normalizing action of thiamine and benfotiamine on gene and protein expression of brain AMPA receptor observed in the present study.

The AMPA receptor GluA2, as well as 5-HT6 receptor, are regarded as having a central role in brain plasticity (Song and Huganir, 2002; Garcia-Alloza et al., 2004; Henley and Wilkinson, 2013; Zhang et al., 2015, 2018; Pereira et al., 2015; Meneses, 2017). Thus it seems probable that the anti-aggressive effects of the thiamine compounds are mediated by ameliorating the ultrasound-induced inhibition of plasticity mechanisms in the brain. This view is supported by the positive effects that have been observed for thiamine and benfotiamine on hippocampal plasticity in a rat stress model (Markova et al., 2017), as well as on the induction of neuroprotective mechanisms after CNS trauma (Boyko et al., 2018; Mkrichyan et al., 2018), neurotoxicity (Benton and Donohoe, 1999), insulin resistance (Abdou and Hazell, 2015) and neurodegeneration (Pan et al., 2010; 2016; Tapias et al., 2018).

Finally, it is of note that benfotiamine exerted more pronounced normalizing changes on protein carbonyl, total glutathione and expression of 5-HT6 receptor in the brain of ultrasound-exposed mice than treatment with the same concentration of thiamine. These data are in line with the previously reported superior effects of benfotiamine on hippocampal neurogenesis during predation stress (Vignisse et al., 2017) and in a model of tau-pathology (Tapias et al., 2018) compared to thiamine. These differences may be due to the increased bioavailability of benfotiamine, but similar brain levels of thiamine and its metabolites have reported after treatment with benfotiamine (Vignisse et al., 2017). Thus the differences may reflect altered activity of the non-enzymatic effects of benfotiamine, the analogue-specific mechanisms underlying these differences remain to be explored.

5. Conclusions

Together, current work suggests the potential for therapeutic application of thiamine and its precursors, in particular, benfotiamine, in prevention and treatment of neuropsychiatric conditions associated with increased brain oxidative stress, including symptoms of excessive aggression. Apart from the glutamatergic, serotonergic and brain plasticity mechanisms reported here, other mechanisms could also be responsible for the effects reported here, such as anti-inflammatory activities and the biosynthesis of monoamines that were previously described for thiamine and benfotiamine (Sanchez-Ramirez et al., 2006; Shoeb and Ramana, 2012; Abdou and Hazell, 2015). Given the accumulated clinical evidence of therapeutic efficacy and tolerability of thiamine and benfotiamine (Bunik, 2013; Ghaleiha et al., 2016; Pan et al., 2016), their usefulness in the prevention and therapy for overt aggression and other associated symptoms would come with very little associated risk and full translation, if successful, might be expected to have significant benefit at low cost.

Declaration of interests

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.neuropharm.2019.02.025.

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