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Enhanced conditioning of adverse memories in the mouse modified swim test is associated with neuroinflammatory changes – Effects that are susceptible to antidepressants

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ABSTRACT

Deficient learning and memory are well-established pathophysiologic features of depression, however, mechanisms of the enhanced learning of aversive experiences associated with this disorder are poorly understood. Currently, neurobiological mechanisms of enhanced retention of aversive memories during depression, and, in particular, their relation to neuroinflammation are unclear. As the association between major depressive disorder and inflammation has been recognized for some time, we aimed to address whether neuroinflammatory changes are involved in enhanced learning of adversity in a depressive state. To study this question, we used a recently described mouse model of enhanced contextual conditioning of aversive memories, the modified forced swim model (modFST). In this model, the classic two-day forced swim is followed by an additional delayed session on Day 5, where increased floating behaviour and upregulated glycogen synthase kinase-3 (GSK-3) are context-dependent. Here, increased time spent floating on Day 5, a parameter of enhanced learning of the adverse context, was accompanied by hypercorticosteronemia, increased gene expression of GSK-3α, GSK-3β, c-Fos, cyclooxygenase-1 (COX-1) and pro-inflammatory cytokines interleukin-1 beta (IL-1β), tumor necrosis factor (TNF), and elevated concentrations of protein carbonyl, a marker of oxidative stress, in the prefrontal cortex and hippocampus. There were significant correlations between cytokine levels and GSK-3β gene expression. Two-week administration of compounds with antidepressant properties, imipramine (7 mg/kg/day) or thiamine (vitamin B1; 200 mg/kg/day) ameliorated most of the modFST-induced changes. Thus, enhanced learning of adverse memories is associated with pro-inflammatory changes that should be considered for optimizing pharmacotherapy of depression associated with enhanced learning of aversive memories.

Abbreviations: GSK-3, glycogen synthase kinase; TNF, tumor necrosis factor; IL, interleukin; modFST, modified forced swim test; ROS, reactive oxygen species; NF-κB, nuclear factor-κB; COX-1, cyclooxigenase-1; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; PTSD, post-traumatic stress disorder; HPA, hypothalamic-pituitaryadrenal axis; D, Day

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1. Introduction

While compromised brain plasticity is a well-established pathophysiologic feature of depression, little is known about disorder-associated enhanced cognitive processing. Enhanced cognitive processing of adverse events is one of the most critical elements in the pathology of major depression that can contribute to the development of stress-related depressive symptoms (Clark, Chamberlain, & Sahakian, 2009; de Bitencourt, Pamplona, & Takahashi, 2013; Gold & Korol, 2012; Gold, Licinio, & Pavlatou, 2012). However, its neurobiological mechanisms are currently unclear. At the same time, the association between depressive disorders and inflammation has been recognized for some time (Anthony & Pitossi, 2013; Dantzer, 2009; Leonard & Maes, 2012). However, it remains unclear as to what aspects of this highly heterogeneous disorder inflammation may specifically contribute (Harro, 2019; Michopoulos, Powers, Gillespie, Ressler, & Jovanovic, 2017).

Enhanced acquisition and retention of aversive memories is characteristic of melancholic and anxiety-associated depression (Monzon et al., 2010) and depression that is comorbid with post-traumatic stress disorder (PTSD) (Flory & Yehuda, 2015). Positive correlations between blood levels of C-reactive protein, pro-inflammatory cytokines (such as interleukin-1 beta (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor (TNF)), as well as cyclooxygenase-1 (COX-1) and the enhanced learning of aversive memories has been demonstrated in patients with PTSD (Lindqvist et al., 2017; Miller, Lin, Wolf, & Miller, 2018; Toft et al., 2019). This condition is well known to share many symptoms with major depressive disorder and is highly comorbid with this disease, as well as with generalized anxiety disorder and panic disorder (Michopoulos, Diaz, & Wilson, 2016). Central overexpression of pro-inflammatory cytokines functionally interferes with increased activities of glycogen synthase kinase-3 (GSK-3) and is associated with treatment resistance in depressed patients with comorbidity for PTSD (Bailey, Cordell, Sobin, & Neumeister, 2013; Cortés-Vieyra et al., 2012; Costemale-Lacoste, Guilloux, & Gaillard, 2016). Up-regulation of GSK-3 was shown to be implicated in the processing of aversive memories during PTSD (Lopresto, Schipper, & Homberg, 2016), and emotional and cognitive dysregulation during depressive syndrome (Jope & Roh, 2006).

Up to now, very few mechanistic studies have explored the role of neuroinflammation in the enhanced processing of aversive memories. While translational approaches have been extensively used to study depression-related pro-inflammatory changes in models of stress (Couch et al., 2013, 2016; Patel, Kas, Chattarji, & Buwalda, 2019), helplessness (Chover-Gonzalez, Jessop, Tejedor-Real, Gibert-Rahola, & Harbuz, 2000) and other depression paradigms (Dudek et al., 2019; Mesquita et al., 2008), to date, limited literature described neuroinflammatory mechanisms associated with inappropriate learning of aversive memories in animals. Recently, we established a mouse paradigm of enhanced contextual conditioning of aversive memories, in which the classic two-day swimming test is followed by an additional swim test on Day 5 (Markova et al., 2017; Pavlov et al., 2017; Strekalova et al., 2016).

In this modified forced swim model (modFST), increased floating behaviour and GSK-3β expression were exhibited during the delayed swim session and were found to be context-dependent and reversible by a pre-treatment with antidepressant compounds (Markova et al., 2017; Strekalova et al., 2016). In the modFST, the increase of floating behavior during the delayed test on Day 5 correlates with brain over-expression of GSK-3β and was validated as a biomarker of enhanced learning of adverse context. Both changes were associated with the exposure of animals to the context alone and are reversible by pre-treatment with a low dose of the tricyclic antidepressant imipramine (Strekalova et al., 2016). While imipramine can ameliorate behavioural despair in a classic Porsolt test, low doses do not to affect behavioural outcomes in this test, but in the modFST model they were shown to prevent an increase of floating and over-expression of GSK-3β on day 5 (Markova et al., 2017; Strekalova et al., 2016). The context of forced swimming on day 5 alone was also shown to provoke similar molecular changes with experience of swimming itself, which suggests that classical Pavlovian contextual conditioning operates in our model.

We hypothesized that augmented contextual conditioning in the modFST is accompanied by central pro-inflammatory changes. Therefore, we sought to explore whether expression of IL-1β, TNF and COX-1 is altered in the hippocampus and prefrontal cortex of mice exposed to the modFST. As elevated expression of pro-inflammatory cytokines is often accompanied by increased corticosterone secretion via activation of the hypothalamic-pituitaryadrenal axis (HPA) axis (Uchoa et al., 2014) we also studied blood corticosterone concentrations and c-Fos expression, whose over-expression is associated with increased production of GSK3 and cytokines (Kadry, Abdel-Megeed, El-Meliegy, & Abdel-Hamid, 2018). Elevated expression of pro-inflammatory cytokines in the CNS can also lead to increased oxidative stress (Sato, Takahashi, Sumitani, Takatsu, & Urano, 2010), and we evaluated the level of oxidative stress using protein carbonyl content as a marker (Frijhoff et al., 2015; Vignisse et al., 2017). We applied a two-week pre-treatment with a low dose of imipramine or thiamine (vitamin B1), an important metabolism regulator with antioxidant, anti-stress and anti-inflammatory properties (Bettendorff, Lakaye, Kohn, & Wins, 2014; Gorlova et al., 2019; Mkrtchyan et al., 2015; Pan et al., 2010), whose chronic administration to mice has been shown to prevented increased floating behaviour and GSK-3 function in mice subjected to the modFST (Markova et al., 2017; Pavlov et al., 2017). Imipramine was included as a 'positive' control, besides, it has been reported to have anti-inflammatory activities in variety of models, (Kamel, Gad, Mansour, Safar, & Fawzy, 2019; Simões et al., 2019), and particularly, suppress the over-production of inflammatory cytokines in models of depression and stress (Duda et al., 2019; Iwata, Ishida, Kaneko, & Shirayama, 2016; Mudgal et al., 2019).

2. Methods

2.1. Animals

3-month-old male C57BL6/J mice were obtained from Stolbovaja-RAS, Moscow region (http://www.spf-animals.ru/about/providers/animals) and housed individually under standard conditions (light on 9.00–21.00). Experimental protocols conformed to directive 2010/63/EU, and were approved by the local veterinarian committee (MSMU #11–18-2018; *see Supplementary file*).

2.2. Experimental outline

Animals were evaluated for their floating behaviour in 6-min sessions split by three 2-min intervals on Day 1, Day 2 and Day 5 and had tap water (FST group) or were pre-treated with imipramine or thiamine (Imi-FST and Thi-FST groups) for two weeks, via drinking water; dosing of selected drug concentrations was controlled as described in *Supplementary file* (see also Pavlov et al., 2017). We chose a thiamine concentration of 200 mg/kg/day and an imipramine concentration of 7 mg/kg/day. The concentration of drugs in water was calculated in accordance with daily water intake and the body weight of mice, as described elsewhere (Cline et al., 2012, 2015; Pavlov et al., 2017; Strekalova, Gorenkova Schunk Dolgov, Bartsch, 2006; Vignisse et al., 2017). Previous studies employed HPLC analysis of brain levels of thiamine and its metabolites (Vignisse et al., 2017) and have evaluated the level of imipramine in blood (Strekalova et al., *unpublished data*, 2002) and have reported efficacy for the chosen dosing regime. The regulation of the dose of the selected interventions was controlled as described in the *Supplementary file* (see also Pavlov et al., 2017).

Floating behaviour was defined by the absence of any directed movements of mice and was scored using validated method with CleverSys (Clever Sys Inc, Reston, VA, USA; Malatynska et al., 2012).

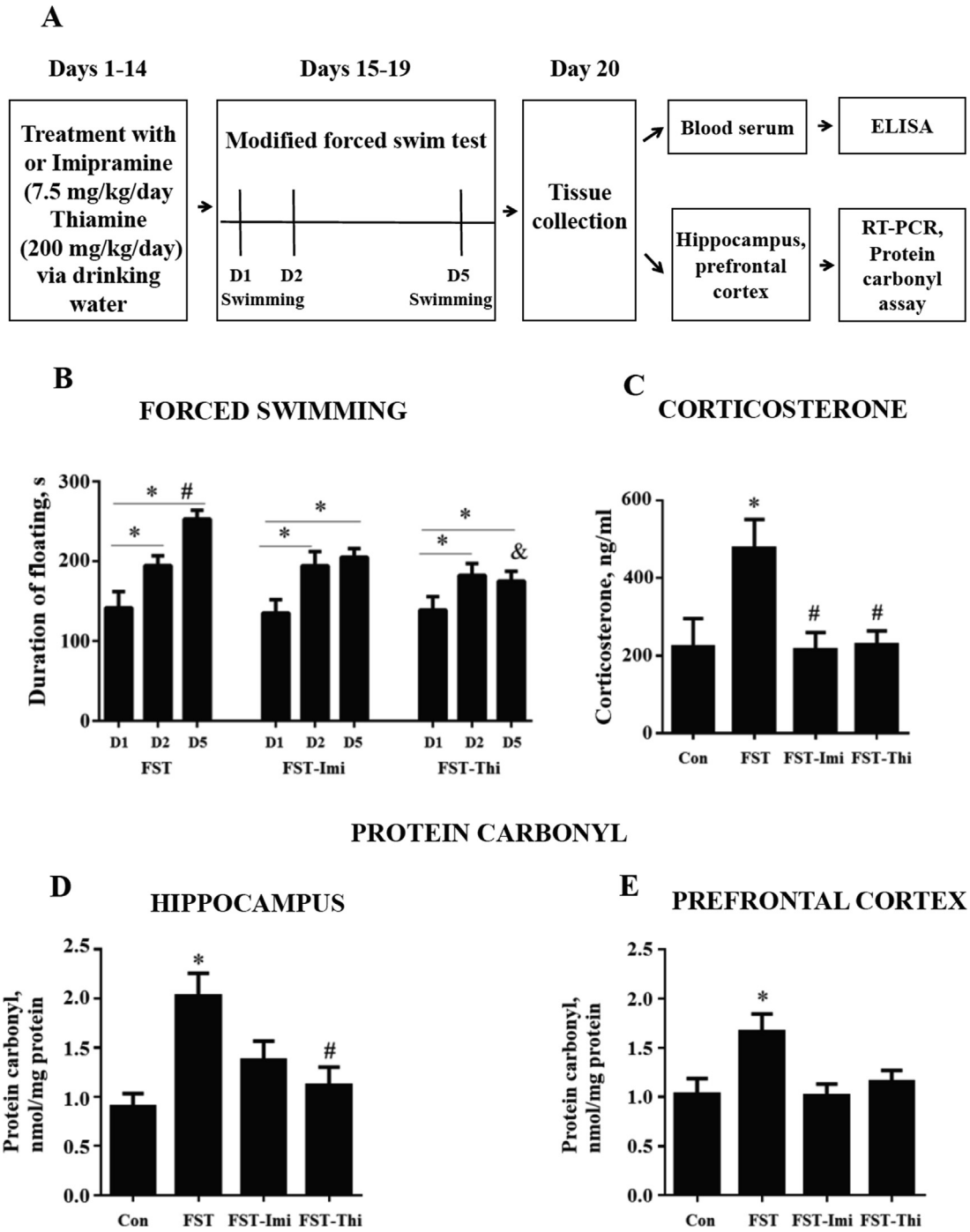

Fig. 1. Experimental outline, potentiated behavioral despair and accompanying brain oxidation and systemic CORT upregulation during delayed swimming session. (A) After pre-treatment with imipramine or thiamine mice were subjected to 5-day modified swim paradigm and killed; their brains were dissected for in vitro assays, including quantitative reverse transcription polymerase chain reaction (qRT-PCR) and measurement of carbonyl protein content; blood was collected for measurement of corticosterone levels. (B) On testing Day 2 and 5, total floating behavior was increased in comparison to the one at Day 1. Total duration of floating on Day 5 was also significantly increased in comparison with Day 2. This parameter was decreased in Thi-FST group in comparison with FST group on Day 5. (C) FST mice on Day 5 displayed increased systemic CORT level in comparison with controls. Thiamine or imipramine counteracted this effect. (D) FST mice on Day 5 displayed increased protein carbonyl content in the hippocampus and (E) in the prefrontal cortex in comparison with controls. Mice treated with thiamine, but not imipramine, did not differ from controls in protein carbonyl content in the hippocampus and displayed significant decrease of this measure in comparison with non-treated group. *$p < 0.05$ vs. testing Day 1, #$p < 0.05$ vs. testing Day 2, &$p < 0.05$ vs. testing Day 5 of FST group, repeated measures and two-way ANOVA, post hoc Tukey's test. D1: Day 1; D2: Day 2; D5: Day 5 of modified swim test paradigm. Each group was comprised of 6–7 mice.

Mice subjected to the modFST were killed 10 min after the swim session on Day 5, which was at the same time as the naïve controls that had not been not subjected to a swim session, but had been handled on each day of the test (as for the modFST mice). Mice were terminally anaesthetized with an intraperitoneal injection of Nembutal (Bayer, Wiesbaden, Germany) and were then transcardially perfused with 10 ml of the ice-cold 0.9% NaCl. Their brains were dissected, and prefrontal cortex and the whole hippocampus were isolated as described elsewhere (Gorlova et al., 2019). Blood serum was collected as described elsewhere (Couch et al., 2016; Pavlov et al., 2019) and stored at −80 °C

for subsequent in vitro assays; each group had 6–7 animals per experimental run (Fig. 1A). The correlation analysis between floating behavior and the other endpoints investigated was carried out; it was only performed on the mice exposed to the modFST. Both vehicle- and drug-treated mice exposed to the modFST were included in the correlation analysis. Data from one run was used for all assays except the correlation analysis between floating behavior and gene expression, for which cDNA from three experimental runs were used, as described elsewhere (Pavlov et al., 2017).

2.3. Biochemical assays

CORT ELISA was performed using ab108821 kit (Abcam, Cambridge, UK) as previously described (Gorlova et al., 2019; Vignisse et al., 2017), for details see *Supplementary file*. For protein carbonyl assay, OxiSelect™ Protein Carbonyl Fluorometric Assay kit (Cell Biolabs, Inc., San Diego, CA, USA) kit was used as described elsewhere (Vignisse et al., 2017; Gorlova et al., 2019, see *Supplementary file*). Extraction of mRNA, cDNA synthesis and quantitative RT-PCR were performed to study brain concentrations of GSK3α/β, IL-1β, TNF, COX-1 and c-Fos mRNA as described elsewhere (Gorlova et al., 2019; Pavlov et al., 2017, 2019). Expression data were normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH), used as housekeeping gene, and calculated as relative-fold changes compared to control mice and in percent from mean control values. Sequences for primers are listed in the *Supplementary Table 1*.

2.4. Statistics

Data were analyzed with GraphPad Prism v.6.0 (San Diego, CA, USA). Repeated measures ANOVA and two-way ANOVA were used to analyze the behavioral and the corticosterone data, one-way ANOVA was applied to analyze the results of the PCR and ELISA assays. Tukey's test was used for post-hoc analysis. Spearman correlation was employed for correlation analysis. The level of confidence was set at 95% ($p < 0.05$), data shown as mean and SEM.

3. Results

3.1. Overproduction of brain protein carbonyl and blood CORT accompany behavioral despair during delayed swim session: effects of imipramine and thiamine

In the modified forced swim test (modFST), a repeated two-way ANOVA with session and treatment as factors revealed significant effects of the sessions on outcome ($F_{2,48} = 14.57$, $p < 0.0001$) and treatment ($F_{2,48} = 4.218$, $p = 0.0114$), on total duration of floating (Fig. 1B). A significant effect of sessions, but not treatment, on duration of floating was found in minutes 1–2 ($F_{2,48} = 10.66$, $p = 0.0001$ and $F_{2,48} = 1.21$, $p = 0.307$, respectively). For minutes 3–4, a significant effect on duration of floating of both sessions ($F_{2,48} = 7.27$, $p = 0.0017$) and treatment ($F_{2,48} = 10.66$, $p = 0.0001$) was revealed. No significant effect of sessions and treatment on duration of floating was revealed in minutes 5–6 ($F_{2,48} = 0.38$, $p = 0.68$ and $F_{2,48} = 2.64$, $p = 0.08$, respectively); there was no significant interaction between these factors ($F_{2,48} = 0.36$, $p = 0.83$). In all groups, duration of floating was significantly elevated during the first two time intervals on Day 2 in comparison to Day 1, that was not shown for the last time interval of the test ($p < 0.05$ and $p > 0.05$, respectively; repeated measures ANOVA and Tukey's test¸ *Supplementary Table 2*). On Day 5, total duration of floating and duration of floating for 1–2 min interval were significantly increased in comparison with values registered on Day 2 in the FST group ($p < 0.05$; repeated measures ANOVA and Tukey's test; Fig. 1B, *Supplementary Table 2*), but not Imi- or Thi-FST groups ($p > 0.05$).

No significant group differences were found in each time interval of testing, except significantly shortened duration of total floating in Thi-FST group in comparison with FST mice (*Supplementary Table 2*). Together, the analysis of the floating behavior in the 2-min intervals indicates that the treatments significantly affected the duration of floating behavior in the first two time intervals of the test, but not during the last 2 min of the swim session. This may be interpreted as possible sign of effects of drugs on cognitive factors in this test, rather than on despair behavior caused by prolonged unescapable swimming. The overall analysis showed significantly elevated total duration of floating during delayed session on Day 5, a measure of enhanced contextual conditioning in this test was observed in pharmacologically naïve mice, but not in groups treated with thiamine or imipramine.

There were significant group differences in CORT levels in blood ($F_{3,38} = 5.48$, $p = 0.003$, one-way ANOVA). Specifically, this measure was increased in FST mice in comparison with controls ($p = 0.04$; Tukey's test; Fig. 1C). In contrast, CORT levels in Thi-FST and Imi-FST mice were not different from control values ($p = 0.56$ and $p = 0.27$, respectively) and were significantly decreased in comparison with FST group ($p = 0.006$ and $p = 0.01$, respectively). We found significant group differences in protein carbonyl contents in the prefrontal cortex ($F_{3,38} = 6.58$, $p = 0.002$, one-way ANOVA; Fig. 1D) and hippocampus ($F_{3,38} = 6.209$, $p = 0.003$, Fig. 1E). There was a significant increase of this measure in the hippocampus and prefrontal cortex of FST group in comparison with controls ($p = 0.025$ and $p = 0.03$, respectively, Tukey's test). No such changes were shown for Imi-FST and Thi-FST groups ($p = 0.44$ and $p = 0.90$, respectively), and the latter but not the former group displayed a significantly lower protein carbonyl concentration in the hippocampus than the FST group ($p = 0.406$ and $p = 0.01$; respectively).

3.2. Brain over-expression of pro-inflammatory factors and c-Fos is associated with increased floating in the modified swim test and precluded by thiamine and imipramine

We found significant group differences in mRNA expression of GSK-3β and GSK-3α in the hippocampus ($F_{3,38} = 4.39$, $p = 0.008$ and $F_{3,38} = 4.13$, $p = 0.01$, respectively) and of GSK-3β, but not GSK-3α, in the prefrontal cortex ($F_{3,38} = 3.92$, $p = 0.03$ and $F_{3,38} = 2.35$, $p = 0.096$, one-way ANOVA). This replicated our previous findings that also described an upregulation of the gene expression of GSK-3 in the brain of mice exposed to the modFST (*see Supplementary Table 3)*. There were significant group differences in IL-1β mRNA expression in the hippocampus ($F_{3,38} = 3.73$, $p = 0.01$), but not in the prefrontal cortex ($F_{3,38} = 0.42$, $p = 0.73$; Fig. 2A and B). We observed a significant increase in the FST group in comparison with control mice in this measure in the hippocampus but not in the prefrontal cortex ($p = 0.04$ and $p = 0.56$, respectively. This effect was counteracted in Imi-FST and Thi-FST groups vs FST group in hippocampus, while no significant differences were displayed in Imi-FST and Thi-FST groups vs FST group in the prefrontal cortex ($p = 0.04$ and $p = 0.02$, respectively, and $p = 0.56$ and $p = 0.72$, respectively; Fig. 2A and B). TNF mRNA expression was significantly different between the groups in the hippocampus ($F_{3,38} = 3.64$, $p = 0.01$) and in the prefrontal cortex ($F_{3,38} = 4.57$, $p = 0.007$). There was a significant increase in the FST group in comparison with control mice in this measure in the hippocampus and in the prefrontal cortex ($p = 0.03$ and $p = 0.01$, respectively) that was not found in Imi-FST and Thi-FST groups ($p = 0.35$ and $p = 0.17$, respectively, and $p = 0.52$ and $p = 0.09$, respectively; Fig. 2C and D).

Significant group differences in mRNA expression of COX-1 was found in the hippocampus ($F_{3,38} = 3.42$, $p = 0.02$), but not in the prefrontal cortex ($F_{3,38} = 0.84$, $p = 0.47$). In the hippocampus, this parameter showed a significant increase in the FST group in comparison with control mice ($p = 0.04$) that was not found in Imi-FST and Thi-FST groups ($p = 0.28$ and $p = 0.14$, respectively; Fig. 2E and F). Finally, significant group differences in mRNA expression of c-Fos were shown

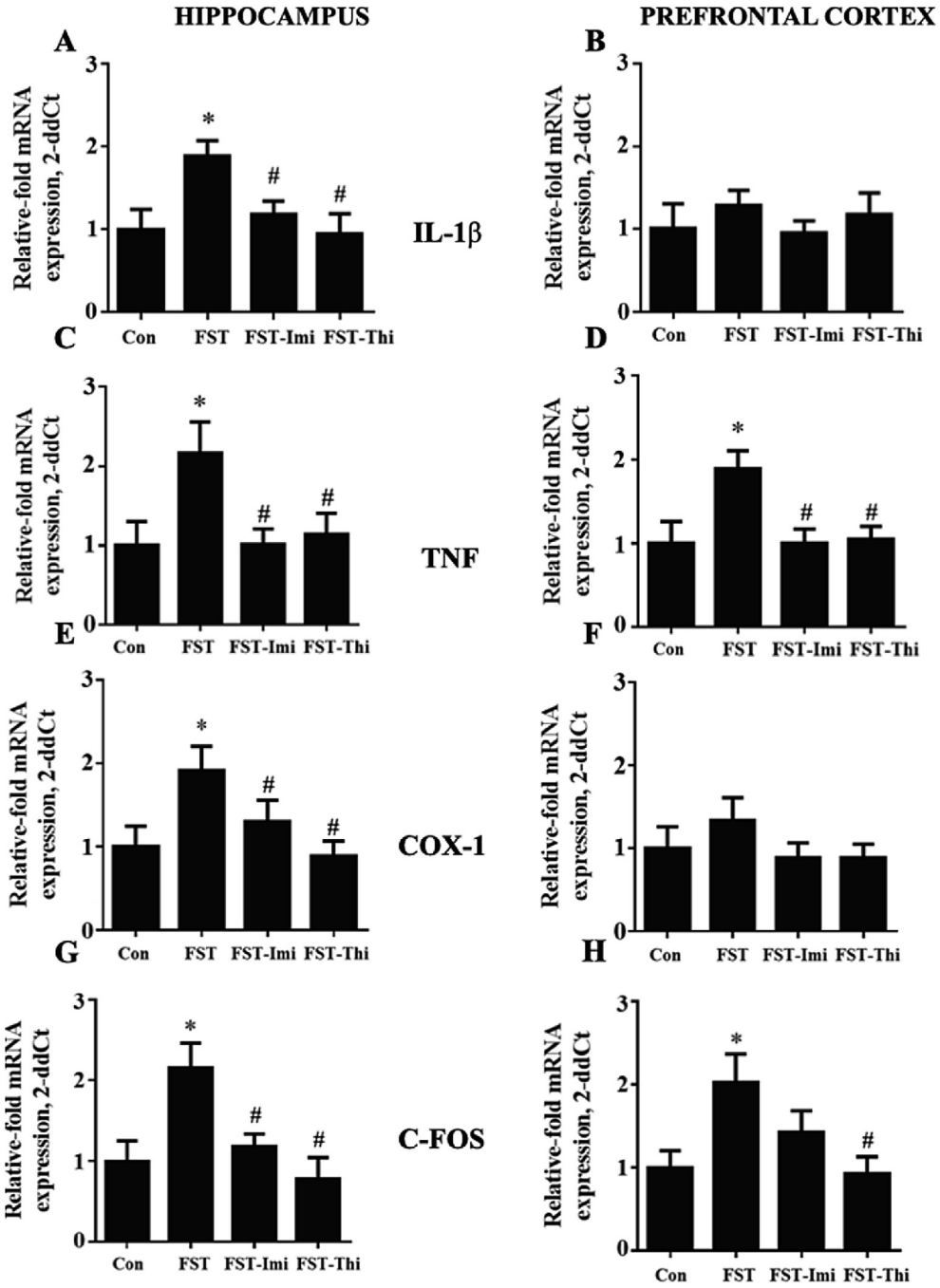

Fig. 2. Pro-inflammatory changes during delayed swimming session. Mice on Day 5 displayed increased (A) IL-1β mRNA expression, (C) TNF expression, (E) COX-1 expression and (G) c-Fos expression in the hippocampus in comparison with controls. Mice treated with imipramine or thiamine did not differ from controls in these parameters and displayed significant decrease of these measures in comparison with FST group. Moreover, on Day 5, these mice displayed increased (D) TNF expression and (H) c-Fos expression in the prefrontal cortex in comparison with controls. Mice treated with imipramine or thiamine did not differ from controls in TNF expression and displayed significant decrease of this measure in comparison with non-treated group. At the same time, only Thi-FST, but not Imi-FST, mice had significantly decreased c-Fos expression in the prefrontal cortex in comparison with FST group. There were no significant differences between groups in (A) IL-1β and (F) COX-1 mRNA levels in the prefrontal cortex. *p < 0.05 vs. controls, #p < 0.05 vs. non-treated mice on Day 5; one-way ANOVA and post hoc Tukey's test. Each group was comprised of 6–7 mice.

both in the hippocampus ($F_{3,38}$ = 5.68, p = 0.002) and prefrontal cortex ($F_{3,38}$ = 3.66, p = 0.01); significant over-expression of this gene was found in FST groups (p = 0.01 and p = 0.03, respectively) but not in Imi- and Thi-FST groups in comparison with controls (p = 0.55 and p = 0.97, respectively and p = 0.63 and p = 0.75, respectively; Fig. 2G and H).

3.3. Correlation analysis of expression of GSK-3 isoforms with behavioral and molecular alterations during delayed swimming session

Significant correlations were found between GSK-3α expression and IL-1β expression in the hippocampus (p = 0.04, r = 0.37, Spearman correlation; Fig. 3A) and TNF expression in the prefrontal cortex (p = 0.01, r = 0.46; Fig. 3G). There were significant correlations between GSK-3β expression and expression of IL-1β in the hippocampus (p = 0.007, r = 0.49; Fig. 3B) and in the prefrontal cortex (p = 0.013, r = 0.22; Fig. 3D), expression of TNF in the hippocampus (p = 0.02, r = 0.43; Fig. 3F) and c-Fos expression in the prefrontal cortex (p = 0.01, r = 0.45; Fig. 3P). No significant correlations were found between GSK-3α expression and expression of IL-1β in the prefrontal cortex (p = 0.26, r = −0.21; Fig. 3C), expression of TNF in the hippocampus (p = 0.48, r = −0.19; Fig. 3E), c-Fos expression in the hippocampus (p = 0.12, r = −0.29; Fig. 3M) and prefrontal cortex (p = 0.99, r = −0.002; Fig. 3K) and expression of COX-1 in the hippocampus (p = 0.079, r = 0.11; Fig. 3I) and prefrontal cortex (p = 0.21, r = 0.24; Fig. 3O). Notably, mRNA expression of IL-1β in the hippocampus positively correlated with total duration of floating (p = 0.03 r = 0.352; data not shown). No other significant correlations were found, including correlations between expression of GSK-3β and other molecules: TNF expression in the prefrontal cortex (p = 0.33,

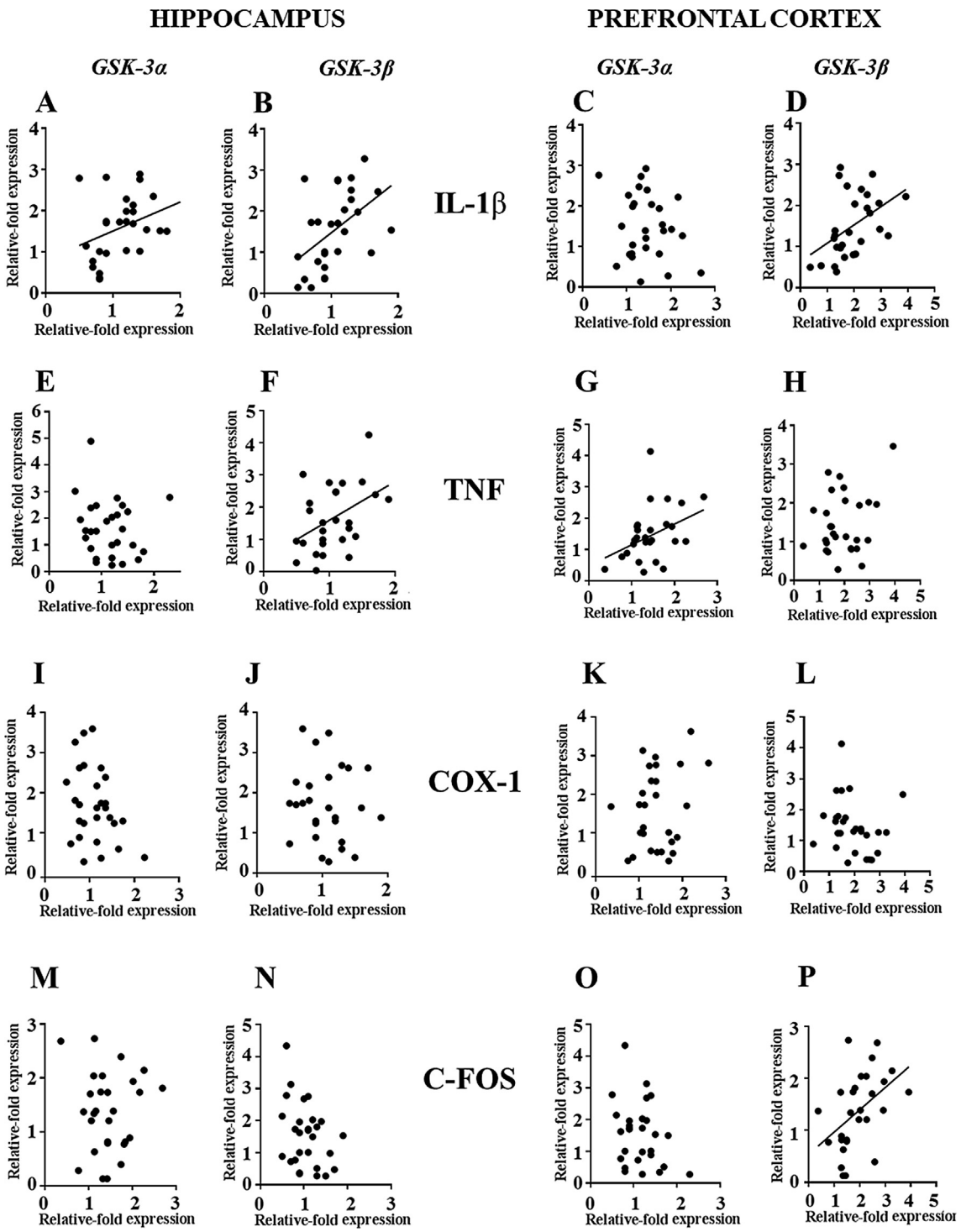

Fig. 3. Correlation analysis between gene expression of GSK-3 isoforms and stress-associated molecules. (A) Significant correlations were found between GSK-3α expression and IL-1β expression in the hippocampus and (G) TNF expression in the prefrontal cortex. (B) Significant correlations were also found between GSK-3β expression and IL-1β expression in the hippocampus and (D) in the prefrontal cortex, (F) TNF expression in the hippocampus and (P) c-Fos expression in the prefrontal cortex. (C) No significant correlations were found between GSK-3α gene expression and IL-1β expression in the prefrontal cortex, (E) TNF expression in the hippocampus, (M) c-Fos expression in the hippocampus and (O) prefrontal cortex, (I) COX-1 expression in the hippocampus and (K) in the prefrontal cortex. (H) There were no significant correlations between GSK-3β expression and TNF expression in the prefrontal cortex, (N) c-Fos expression in the hippocampus, (J) COX-1 expression in the hippocampus and (L) in the prefrontal cortex. $p < 0.05$, trend line indicates significant correlations; Spearman correlation. Each group was comprised of 18–25 mice.

r = 0.19; Fig. 3H), c-Fos expression in the hippocampus (p = 0.06, r = 0.35; Fig. 3N) and expression of COX-1 in the hippocampus (p = 0.68, r = 0.079; Fig. 3J) and prefrontal cortex (p = 0.206, r = 0.13; Fig. 3L).

4. Discussion

To summarize, we found an upregulation of mRNA expression of the pro-inflammatory mediators IL-1β, TNF and COX-1 in the hippocampus

and prefrontal cortex of mice subjected to the modFST, which was prevented by antidepressant treatments with imipramine or thiamine. These findings provide the first evidence for the involvement of pro-inflammatory pathways in an animal model of enhanced learning of aversive memories and the effects of antidepressant treatments on these pathways. Furthermore, we found significant correlations of most of pro-inflammatory changes with key molecular features of the behavioral paradigm that was employed here, particularly the expression of GSK-3α and GSK-3β; the expression of IL-1β also correlated with floating behavior. Pro-inflammatory changes were accompanied by evidence of increased oxidative stress, i.e. increased concentration of protein carbonyl, in the prefrontal cortex and hippocampus. Markers of an increased systemic stress response, the CNS over-expression of c-Fos and hypercorticosteronaemia, were also found in the modFST group. The majority of these neurobiological abnormalities were prevented by chronic pre-treatment with low dose of imipramine and administration of the anti-oxidant and anti-inflammatory thiamine.

We previously reported that in the modFST, increased floating and brain GSK-3β activities during the additional delayed swim session are positively correlated and context-dependent (Pavlov et al., 2017; Strekalova et al., 2016). In accordance with these earlier results, an increased floating duration and brain GSK-3β over-expression were prevented by pre-treatment with imipramine or thiamine (Markova et al., 2017; Pavlov et al., 2017). Detailed behavioural analyses of delayed swimming session revealed that there was a significant increase of floating behavior only during the initial period in comparison with pharmacologically naïve mice. This suggests that exposure to aversive context of swimming, not unescapable swimming itself, was likely to be the cause of this increase. Such increase was not found in pharmacologically-treated groups, which was in keeping with our previous results (Markova et al., 2017; Pavlov et al., 2017).

Given the established links between stress-induced release of glucocorticoids and elevated GSK-3 activities (Beurel, Grieco, & Jope, 2015), both of which were observed in our study, and pro-inflammatory consequences of the latter (Chang et al., 2013; Huang et al., 2009), we suggest that over-expression of IL-6, TNF and COX-1 in the modFST results from these changes. Elevated GSK-3 activities have been previously shown to inhibit the expression of calcium-responsive-element-binding protein (CREB) and the anti-inflammatory cytokine IL-10 (Huang et al., 2009), and promote the expression of proinflammatory cytokines via activation of nuclear factor-κB (NF-κB) (Chang et al., 2013). In contrast, the blockade of Toll-like receptor 4 (TLR4), a major mediator of inflammatory processes (Buchanan, Hutchinson, Watkins, & Yin, 2010) during psychological stress in mice, ameliorated GSK-3 activities and depressive-like symptoms (Cheng et al., 2016).

Over the years, most attention has focused on the role of central IL-1β expression in the generation of sickness behavior, and IL-1β signaling pathways in the brain are accepted as having an important role in the induction of depressive-like changes in behavior (D'Mello & Swain, 2017; Gądek-Michalska, Tadeusz, Rachwalska, & Bugajski, 2013). Over-expression of IL-1β in the brain has been shown to correlate with an individual's predisposition to stress-induced anhedonia in mice (Couch et al., 2013) and accompany the depressive syndrome induced in other models in mice and rats (Cordeiro et al., 2019; Fang et al., 2019), including the ultrasound stress model of "emotional stress" (Gorlova et al., 2019; Morozova et al., 2016; Pavlov et al., 2017). The present study revealed the up-regulation of this cytokine both in the pre-frontal cortex and in the hippocampus, and there was a correlation with floating behavior and overexpression of GSK-3β in the prefrontal cortex. Notably, overproduction of IL-1β has been described in individuals with PTSD (Toft et al., 2019; Wang, Caughron, & Young, 2017) indicating its translational potential.

TNF, another cytokine studied in our work here, was over-expressed in the brain of mice exposed to modFST. The potential for TNF to contribute to mood disorders has gained attention based on the basal 'antidepressant' phenotype of TNF and TNF receptor knockout mice (Simen, Duman, Simen, & Duman, 2006), "pro-hedonic" effects of targeted deletion of TNF receptor 2 in sucrose intake test (Yamada et al., 2000) and there is evidence that TNF blockade can reduce signs of depression (Krügel, Fischer, Radicke, Sack, & Himmerich, 2013). The present study revealed a link between TNF over-expression and augmented learning of adverse memories that is in line with clinical data suggesting altered TNF expression during PTSD and depression (Miller et al., 2018; Passos et al., 2015; Yuan, Chen, Xia, Dai, & Liu, 2019).

Our study also revealed an increase in gene expression of hippocampal COX-1, which previously appeared to accompany the development of stress-induced anhedonia in mice (Couch et al., 2013). In line with these findings, mice lacking COX-1 were reported to be resilient to social stress-induced anhedonia (Tanaka et al., 2012). COX-1 was found to be over-expressed in depressed patients as well as in patients with acute PTSD (Michopoulos et al., 2019; Passos et al., 2015; Powers et al., 2019). The correlation between the molecular markers and floating behavior in the modFST does not imply causation, but suggests that a functional relationship is likely to exist between the molecular changes and regulation of the modFST behavior and acquisition of aversive memories. This was particularly marked for the pro-inflammatory cytokines and both isoforms of GSK3, which replicate our previous findings.

Here, we demonstrated that administration of imipramine and thiamine exerted central anti-inflammatory and anti-oxidative stress effects in the modFST. These treatments were previously shown to normalize depressive- and anxiety-like behaviours, GSK-3 expression, markers of oxidative stress and hippocampal cell proliferation during predation stress, the ultrasound model of emotional stress and the modFST (Cline et al., 2012, 2015; Gorlova et al., 2019; Pavlov et al., 2017; Vignisse et al., 2017). As indicated above, imipramine was included as a 'positive' control and a pharmacological reference for comparison with the thiamine intervention, which has also been reported to have anti-inflammatory activities. SSRIs are also reported to have anti-inflammatory effects, and, if the mice had been treated with an SSRI, they may also have had similar, beneficial effects, but has been reported to evoke weak changes in the forced swimming behavior of naïve laboratory rodents (Strekalova et al., 2006).

As over-production of cytokines, including TNF has been shown to stimulate glutamate release from astrocytes (Bezzi et al., 2001) and microglia (Takeuchi et al., 2006) that might result in excessive intracellular calcium, leading to activation of calcium-dependent lipases and up-regulation of reactive oxygen species (Dargelos et al., 2010), these mechanisms could explain the increases in brain oxidative stress markers that appear during the modFST. Administration of thiamine or imipramine ameliorated protein carbonyl levels in the brain, suggesting an improvement in the balance of direct oxidation processes by reactive oxygen species (ROS) of amino acid residues (Cecarini et al., 2007). These data are in keeping with the literature suggesting compromised markers of oxidative stress during depression and comorbid disorders such as PTSD, anxiety disorders and their normalization by antidepressant treatment (Berk et al., 2013; Miller et al., 2018).

Finally, the above-mentioned effects of treatments were accompanied by lowered c-Fos expression in the hippocampus and prefrontal cortex of experimental animals that suggests activity-dependent normalizing changes in these brain structures and may be due to previously reported functional links to oxidative stress, GSK3, and neuroinflammatory mechanisms (Kadry et al., 2018; Rana & Singh, 2018).

5. Conclusions

Taken together, our study suggests that the development of enhanced contextual conditioning of adverse memories in the modFST mouse paradigm is associated with a proinflammatory profile and signs of oxidative stress. These changes are likely to be due to increases in blood glucocorticoid levels and GSK-3 activities (Thoeringer et al., 2012). Given that patients with symptoms of depression and PTSD

disorders displayed signs of neuroinflammation (Michopoulos et al., 2017), it might be anticipated that the treatments investigated here may have therapeutic potential, not only for prevention of associated symptoms, but also for the treatment of the condition. Our findings are in line with clinical data on the pathophysiology of depression, suggesting that depression of distinct forms leads to elevated levels of inflammation and an oxidant/antioxidant imbalance (Ogłodek & Just, 2018), as well as emphasize a role for neuroinflammatory processes during augmented learning of aversive memories (Miller et al., 2018). They further validate the modFST model as a tool for studying inappropriate learning of aversive memory in the pathophysiology of depressive syndromes and highlight future directions for the development of novel therapeutics targeting oxidative stress and inflammation in patients with related psychopathologies.

CRediT authorship contribution statement

Dmitrii Pavlov: Visualization, Investigation, Supervision. **Anna Gorlova:** Visualization, Investigation, Supervision. **Lucien Bettendorff:** Data curation, Writing - original draft. **Allan A. Kalueff:** Data curation, Writing - original draft. **Aleksei Umriukhin:** Data curation, Writing - original draft. **Andrey Proshin:** Visualization, Investigation, Supervision. **Alexander Lysko:** Visualization, Investigation, Supervision. **Rainer Landgraf:** Conceptualization, Methodology. **Daniel C. Anthony:** Conceptualization, Methodology, Writing - review & editing. **Tatyana Strekalova:** Conceptualization, Methodology, Data curation, Writing - original draft, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.nlm.2020.107227.

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