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Original article

# Mitochondrial proteomics investigation of frontal cortex in an animal model of depression: Focus on chronic antidepressant drugs treatment

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ABSTRACT

*Background:* Alteration in the brain mitochondrial functions have been suggested to participate, as a relevant factor, in the development of mental disorders. Therefore, the brain mitochondria may be a crucial therapeutic target in the course of depression.
*Methods:* Our goal was to find out the impact of two antidepressant drugs with various mechanisms of action – imipramine and fluoxetine, on the frontal cortex mitochondria-enriched fraction in an animal model of depression based on the prenatal stress procedure.
*Results:* Our results confirmed that the prenatal stress caused depressive-like disturbances in the adult offspring rats, which were normalized by the chronic imipramine and fluoxetine administration. For the first time, using 2D-LC–MS/MS, we demonstrated nine differentially expressed proteins after the imipramine administration. Of these proteins, the up-regulation of the 2′,3′-cyclic-nucleotide 3′-phosphodiesterase enzyme and down-regulation of the Hypoxanthine-guanine phosphoribosyltransferase (HPRT), Ras-related proteins (Rap-1A and Rap-1B) and Transgelin-3 (NP25) were the most striking. In contrast, after the chronic fluoxetine treatment, we observed differential expression in five proteins, including the enhanced expression of component of pyruvate dehydrogenase complex and diminished of Glutathione *S*-transferase P (Gstp-1), as well as Maleylacetoacetate isomerase.
*Conclusions:* These results overcome the interesting data that brain mitochondria in the frontal cortex may constitute the target for pharmacotherapy. The multifaceted profile of both antidepressant drugs action makes difficult to elucidate the exact mechanism of imipramine and fluoxetine action in the brain mitochondria. Further study of mitochondrial dysfunction in psychiatric disorders will be base to know the possible biological consequences of our observations.


## Introduction

Despite many years of studies the biological background of depression is not defined yet. Recently prevails opinion that it has a multifaceted origin that involves molecular, cellular and functional malfunctions in brain areas, mainly frontal cortex and hippocampus [1]. Among others, a lot of attention to the potential influence of mitochondrial dysfunction on the onset of this disease is dedicated. In the brain neuronal cells energy production is basically dependent on mitochondrial oxidative phosphorylation [2] which makes them especially vulnerable to mitochondrial malfunction. Apart from energy production, mitochondria participate in the metabolism of reactive oxygen species (ROS), calcium homeostasis and apoptosis regulation [3]. Importantly, unequal role of brain mitochondria is related to neurotransmitter release and neuroplasticity processes: cell differentiation, neurite outgrowth, dendritic remodeling – necessary for the specific development and functioning of the nervous tissue. Therefore, in line with "the mitochondrial dysfunction hypothesis" it may be postulated that impaired mitochondrial functions may lead to various psychiatric conditions, including depression [4].

In fact, a lot of data showed that depression is related with frontal hypometabolic function together with hypermetabolism in some limbic areas [3]. Electron microscopy study showed

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alteration in the mitochondria distribution and dimension in brain, which may be linked to the energy deficits in patients [5]. Furthermore, a malfunction in mitochondrial morphology, an increase in the levels of mtDNA mutations and mitochondrial DNA (mtDNA) polymorphisms has been found. What is more, the down-regulation of nuclear mRNA molecules and proteins that are involved in mitochondrial respiration, decreased high-energy phosphates as well as a diminished pH has been described in the course of depression [6].

Recent data pointed that mitochondrial disturbances in depression disease may result from early unfavorable life experiences, that lead to the long-lasting metabolic, neuroanatomical and functional changes in the brain. In experimental studies the prenatal stress procedure in rats is a well-documented animal model of depression. A lot of studies verified this model, showing that prenatally stressed rats exhibit long-lasting behavioural changes [7,8]. Moreover, stress during pregnancy in rats leads to prolonged neurobiological deficits as well as in hypothalamo-pituitary-adrenal (HPA) axis activity [8–10], in neurotransmitter network [11], and malfunction in the immune system [12]. Recently, our study found that the prenatally stressed rats exhibit deficits in metabolic activity [13] and disturbances in mitochondrial biogenesis as well as changes in the brain mitoproteome in adulthood [14]. Stress altered the respiratory chain function, decreased ATP production, led to structural changes in mitochondria and apoptosis [15]. The oxidized mtDNA and other harmful factors derived from "stressed mitochondria" potentiate the inflammatory processes [16].

Overall, taking into account mentioned data, mitochondrial dysfunction should be considered as an important target for therapeutic intervention in stress-related pathology [3,17]. In fact the understanding the effects of antidepressant drugs on the brain mitochondrial function may improve the therapeutic strategies [18,19]. Unfortunately, available data concerning the impact of antidepressant drugs on mitochondrial function remain ambiguous and controversial. Some of them pointed that antidepressants have suppressive [20,21], while other stimulatory effects [22] on brain mitochondrial functioning. This conflicting data may result from various types of mitochondria from a single brain area, as well as of metabolic heterogeneity of areas in the brain [23]. Moreover, recent data emphasize that the antidepressants action may depend on the mitochondria metabolic localization in pre- or post-synaptic compartments that have different energetic requirements.

Since a lot of data pointed that proteomics studies may contribute to a better understanding of mitochondrial dysfunction-related brain illnesses, the purpose of this study was, using two-dimensional electrophoresis, coupled with tandem mass spectrometry to determine the impact of chronic treatment of imipramine and fluoxetine on the mitochondria-enriched fraction in the frontal cortex of adult prenatally stressed rat.

Notably, for our study we chose two antidepressant drugs with different mechanism of action. Fluoxetine belongs to the selective serotonin re-uptake inhibitor family (SSRI) and is commonly used in clinic in treatment of depression. Importantly, recent data demonstrated that fluoxetine penetrates cells through the plasma membranes, and 60–70% of 18F-fluoxetine was showed to interact with mitochondrial brain fractions [24,25], therefore the potential therapeutic impact of fluoxetine on the brain mitochondria should be under consideration. Contrary to fluoxetine, imipramine, which is a dibenzazepine-derived tricyclic antidepressant (TCA), acts mainly as a serotonin and norepinephrine reuptake inhibitor in the brain [26]. Although side effects may significantly limit the widespread use of imipramine, it still remains a powerful drug of choice for the treatment of drug-resistant depression, hence we postulate that better understanding of the effect of imipramine on the mitochondria in the brain appears to be important.

## Materials and methods

### *Animals*

Sprague-Dawley rats (200–250 g) were purchased from Charles River Corporation (Germany), and kept in an animal housing facility at a room temperature of 22 ± 1 °C and 12/12 h light/dark cycle with food and water available *ad libitum*. Vaginal smears from females were collected daily in order to determine pro-estrous cycle phase. During proestrus, rats were placed with males for 12 h, and next day morning vaginal smears were tested for the presence of sperm. Next, pregnant females were assigned randomly to: control and stress groups (n = 10 per group). All experiments were approved by the Local Ethics Committee in Kraków, Poland.

### *Stress procedure*

The prenatal stress was conducted as previously described [7,8,27,28]. Briefly, pregnant females were subjected to stress sessions daily beginning on the 14th day of pregnancy until

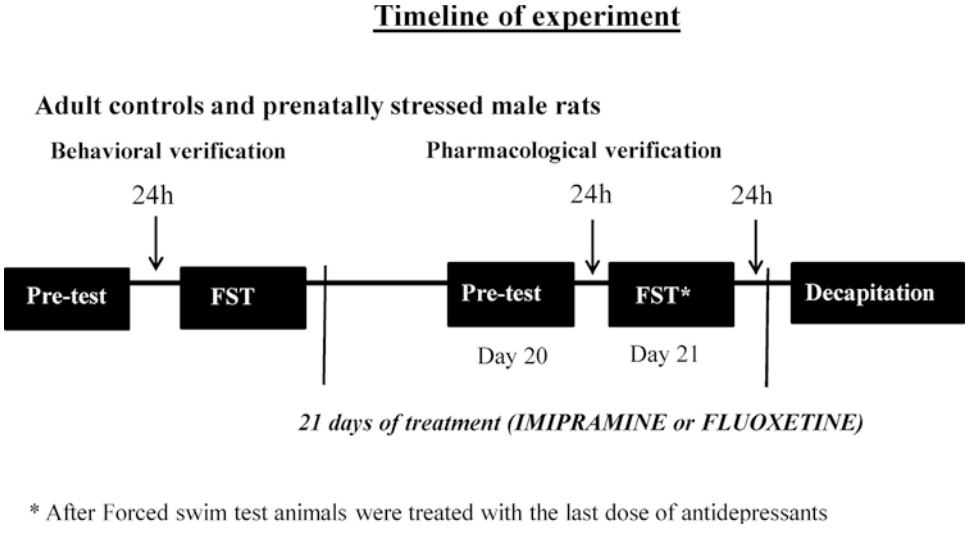


**Fig. 1.** Schematic representation of the timeline of the experiment.

delivery (at 09:00 am 12:00 pm and 5:00 pm). During sessions, animals were placed in plastic cylinders (7/12 cm) and exposed to a bright light for 45 min. Control pregnant females were left undisturbed in their home cages. Next, male offspring were selected from 21-day-old litters, and housed in groups of four animals per cage (1–2 animals from each litter). At 3 months of age, the offspring of the control and stressed mothers underwent the first behavioural verification (forced swim test – see Fig. 1).

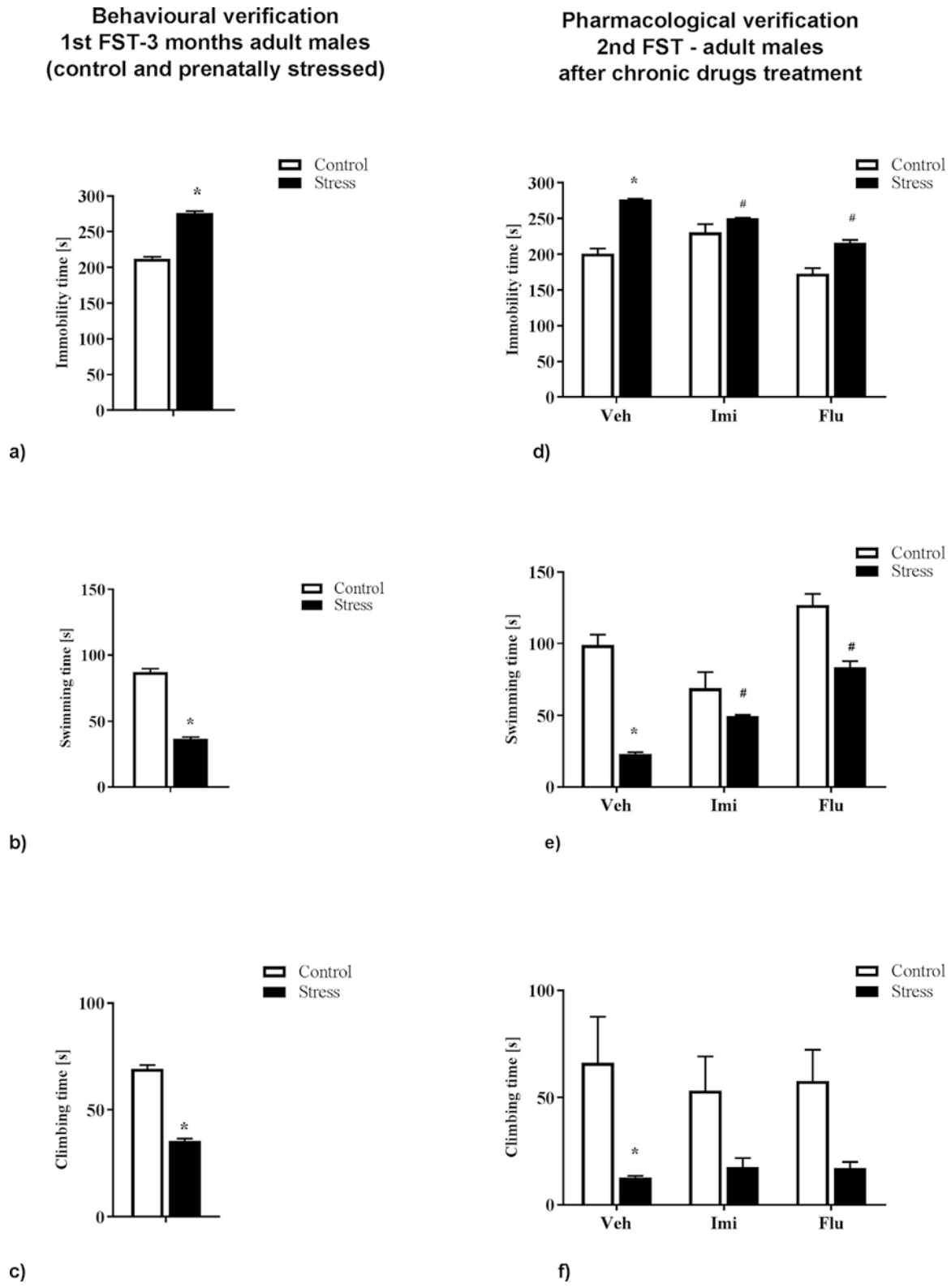


**Fig. 2.** The impact of prenatal stress on the behavioural parameters in the forced swim test (a–c). The effects of prenatal stress and chronic imipramine or fluoxetine administration on the immobility (d), swimming (e) and climbing (f) times (in seconds) in the forced swim test. The data are presented as the means ± SEM with n = 6 in each group; ANOVA (two-way), followed by Duncan's test. $^{*}p \leq 0.05$ compared with the control Veh group; # $p \leq 0.05$ compared with the prenatally stressed Veh group.

*Forced swim test (FST, Porsolt test)*

FST was performed according to the method described previously [12,29]. Rats (n = 18 per group) were individually subjected to two trials in which they were forced to swim in a cylinder (44 cm high, 22.5 cm in diameter) filled with water (23 °C) up to a height of 35 cm. There was a 24-h interval between the first and second trial. The first trial lasted 15 min, and the second 5 min. The total durations of immobility, mobility (swimming) and climbing were measured throughout second trial [29,30].

*Antidepressant drug treatment*

After the first FST test, control and prenatally stressed rats were divided into 6 groups (CONTROL+ VEHICLE, CONTROL+ IMIPRAMINE, CONTROL+ FLUOXETINE, STRESS+ VEHICLE, STRESS+ IMIPRAMINE, and STRESS+ FLUOXETINE; n = 6 animals, in each group). Animals were treated chronically (21 days) with antidepressants or vehicle. Imipramine (Sigma Aldrich, St. Louis, MO, USA), fluoxetine (Eli Lilly, France) or vehicle (0.9% saline, Polpharma, Poland) were injected *ip* once daily. The drugs were injected at a dose of 10 mg/ml/kg. For the pharmacological verification of the animal model of depression, the animals underwent the forced swim procedure again on the last days of the chronic antidepressant drug treatment (see diagram illustrated on Fig. 1). Twenty-four hours after the final injection, the animals were sacrificed by rapid decapitation.

*Preparation of subcellular fractionation*

At 4 °C, from freshly-harvested frontal cortices the mitochondria-enriched fraction was isolated (from the same animals also hippocampi were collected and investigated – [19]). Afterwards the brain structure was homogenized in a buffer of 250 mM sucrose and 1 mM EGTA at a pH of 7.8 and a mixture of 1 mM PMSF and a protease inhibitor (Sigma, USA). The nuclei and unbroken cells were pulled-down by centrifugation at 1,000 *g* for 10 min, while mitochondrial fraction was received by centrifugation of the supernatant at 12,000 *g* for 10 min. The mitochondria-enriched pellet was purified using 3 cycles of resuspension, homogenization and centrifugation (at 12,000 *g* for 15, 20, and 15 min), while cytosolic fraction was obtained by further centrifugation of the supernatant (90 min at 125,000 *g*, 4 °C). Samples were collected and stored at −80 °C until assayed.

*Two-dimensional electrophoresis (2-DE), gel image analysis, LC–MS/MS*

Two-dimensional electrophoresis, gel image analysis and LC–MS/MS of mitochondria enriched pellets from frontal cortex were performed as described previously [19]. All details of these procedures were put in Supplementary data.

*Immunoblotting*

The Western blot analyses of cytochrome c oxidase (COX-IV) and cyclophilin A were conducted to determine the purity of fractions, as we described previously [19]. For more details see Supplementary data.

*Statistical analysis*

The results were analysed using Statistica 10.0 software (StatSoft, Tulsa, USA). The behavioural data were analysed using one-way or two-way analysis of variance (ANOVA) (pharmacological verification; with prenatal stress and treatment as the factors), next the significance of the differences between the means was evaluated by *post hoc* test. The PDQuest™ 8.0.1 software was used for the normalization and comprehensive gel image analysis. The Student's *t-test* embedded in the PDQuest software was used to reveal the statistically significant changes in the expression of the mitochondrial proteins represented on the 2-DE gels as silver-stained spots.

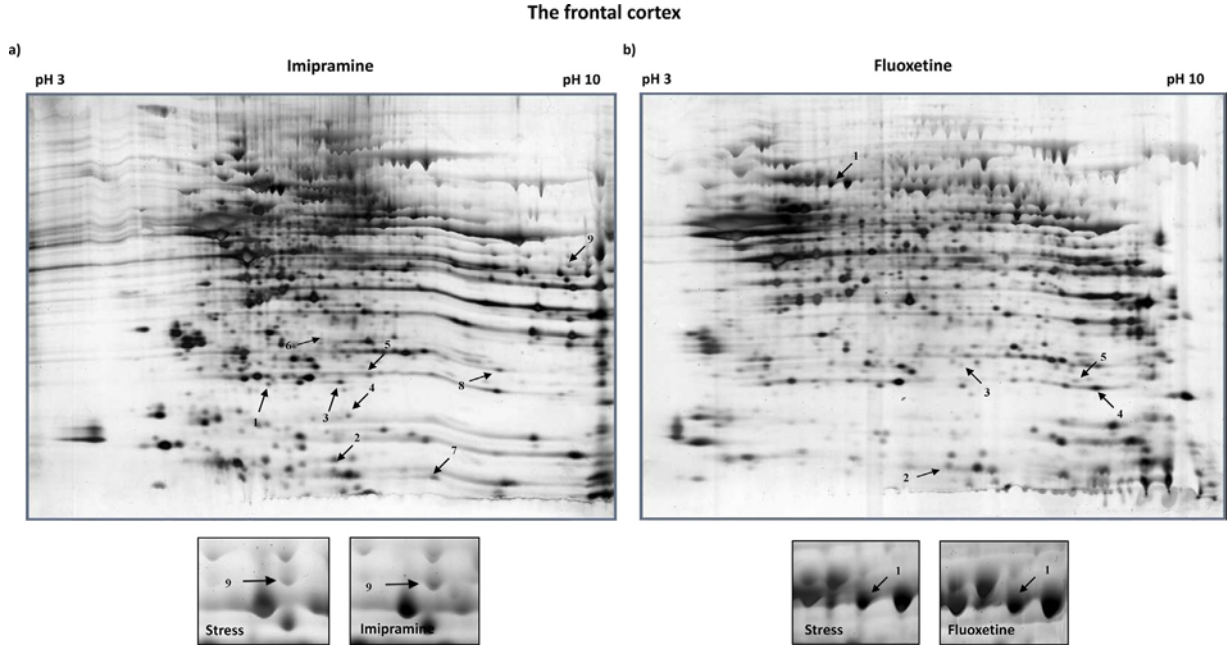


**Fig. 3.** A. Representative 2-D map of the mitochondrial proteins expressed in the frontal cortex of prenatally stressed rats that were treated with imipramine compared with prenatally stressed rats, and the magnifications of the spot pairs correspond to 2′,3′-cyclic-nucleotide 3′-phosphodiesterase. B. Representative 2-D map of the mitochondrial proteins expressed in the frontal cortex of prenatally stressed rats treated with fluoxetine compared with that in prenatally stressed rats, and the magnifications of the spot pairs correspond to the component of the pyruvate dehydrogenase complex.

## Results

### Behavioural study

*Effect of the prenatal stress procedure and chronic imipramine or fluoxetine treatment on the behaviour of adult offspring*

We assessed the impact of prenatal stress on the animals' depression-like behaviours in the Porsolt test. We confirmed that prenatally stressed rats displayed a longer immobility time ($F_{(1,34)} = 338{,}70$; $p < 0.05$; Fig. 2a) but shorter swimming ($F_{(1,34)} = 336{,}37$; $p < 0.05$; Fig. 2b) and climbing ($F_{(1,34)} = 274{,}52$; $p < 0.05$; Fig. 2c) times – given before [19].

In the following part of experiments, we examined the impact of a chronic imipramine and fluoxetine treatment on the behavioural changes evoked by the prenatal stress procedure by re-performing the Porsolt test. As previously, we demonstrated an increase in the immobility time ($F_{(1,29)} = 69{,}84$; $p < 0.05$) and a decrease in the swimming ($F_{(1,29)} = 69{,}84$; $p < 0.05$) and climbing ($F_{(1,29)} = 19{,}57$; $p < 0.05$) times, which confirmed that the changes induced by the prenatal stress procedure are long-term (Fig. 2d–f). Moreover, we found a significant effect of drugs ($F_{(2,29)} = 31{,}11$; $p < 0.05$) and a significant stress x drug interaction ($F_{(2,29)} = 9{,}14$; $p < 0.05$; Fig. 2d) on the immobility time. The Duncan test indicated that imipramine ($p < 0.05$) and fluoxetine ($p < 0.05$) shortened the immobility time in the prenatally stressed offspring (Fig. 2d). We also observed effect of drugs ($F_{(2,29)} = 31{,}11$; $p < 0.05$) and a significant stress x drug interaction on the swimming time ($F_{(2,29)} = 9{,}14$; $p < 0.05$). The *post hoc* test revealed that imipramine ($p < 0.05$) and fluoxetine ($p < 0.05$) extended the swimming time in the offspring of the stressed females (Fig. 2e) – given before [19].

### Biochemical study

*Effect of chronic imipramine treatment on the mitochondria-enriched sub-proteome in the frontal cortex of prenatally stressed rats*

The effect of imipramine (IMI) on the mitochondria protein expression in the frontal cortex was explored using 2-D electrophoresis coupled with tandem MS. Representative 2-D gel images of the mitochondrial proteins from the prenatally stressed offspring injected with IMI and selected pairs of spots showing the changes between the prenatally stressed offspring and prenatally stressed animals treated with the drug are shown in Fig. 3a. The LC MS/MS analysis of the numbered spots as well as the associated differences we compiled in Table 1. The quantification of the significant changes in expression of the mitochondrial proteins is visualized on Fig. 4a.

We found nine diversely expressed proteins in the probes from the prenatally stressed rats that were imipramine injected. As illustrated (Table 1A and Fig. 4a), the levels of four of them were increased (Histidine triad nucleotide-binding protein 1, Macrophage migration inhibitory factor, ES1 protein homologue, and 2′,3′-cyclic-nucleotide 3′-phosphodiesterase). Contrary the levels of five of these proteins were diminished (Ras-related protein Rap-1b, Ras-related protein Rap-1A, ADP-ribosylation factor 3, Transgelin-3 and Hypoxanthine-guanine phosphoribosyltransferase).

*Effect of chronic fluoxetine treatment on the mitochondria-enriched sub-proteome in the frontal cortex of prenatally stressed rats*

On Fig. 3b we demonstrated the representative 2-D gel images of mitochondrial proteins from the prenatally stressed animals that were injected with fluoxetine (FLU) as well as selected pairs of spots indicating the differences between the prenatally stressed and prenatally stressed offspring after chronic FLU administration. The LC MS/MS analysis of the numbered spots and the associated differences are shown in Table 1. The quantification of the statistically significant changes in the expression of the mitochondrial proteins is illustrated in Fig. 4b.

We identified five differentially expressed proteins in the frontal cortex of the prenatally stressed offspring after chronic fluoxetine (FLU) treatment using PDQuest™, and analysed by mass spectrometry. Three proteins were down-regulated (Maleylacetoacetate isomerase, Superoxide dismutase [Mn], and Glutathione S-transferase P), while two proteins were up-regulated (Component of pyruvate dehydrogenase complex and D-dopachrome decarboxylase) (Table 1B, Fig. 4b).

## Discussion

Of the well-accepted animal models of mood disorders, the prenatal stress procedure is often used. The application of this procedure has been confirmed by reports of compliance with the requirements for the construct and face and predictive validity

**Table 1**
Differentially expressed proteins in the frontal cortex of prenatally stressed rats treated with imipramine (a) or fluoxetine (b) compared with the prenatally stressed rats. $p < 0.05$; n = 3 biological replicates per group.

| No. | Protein | UniProtKB accession number | Molecular mass (Da) | pI[a] | Unique peptides | Total peptides | Protein coverage (%) | Fold change |
|---|---|---|---|---|---|---|---|---|
| A) IMPRAMINE | | | | | | | | |
| 1 | Ras-related protein Rap-1b | Q62636 | 20785 | 5.65 | 3 | 10 | 18.50% | −4.56 |
| 2 | Histidine triad nucleotide-binding protein 1 | P62959 | 13768 | 6.36 | 6 | 30 | 29.40% | 1.54 |
| 3 | Ras-related protein Rap-1A | P62836 | 20974 | 6.39 | 2 | 10 | 11.40% | −1.92 |
| 4 | ADP-ribosylation factor 3 | P61206 | 20588 | 6.84 | 12 | 65 | 41.40% | −8.8 |
| 5 | Transgelin-3 | P37805 | 22486 | 6.84 | 13 | 85 | 46.70% | −2.52 |
| 6 | Hypoxanthine-guanine phosphoribosyltransferase | P27605 | 24462 | 6.07 | 5 | 17 | 28.00% | −12.8 |
| 7 | Macrophage migration inhibitory factor | P30904 | 12469 | 6.8 | 5 | 17 | 23.50% | 1.88 |
| 8 | ES1 protein homolog | P56571 | 28155 | 9.11 | 11 | 88 | 30.50% | 1.62 |
| 9 | 2′,3′-cyclic-nucleotide 3′-phosphodiesterase | P13233 | 47239 | 9.03 | 16 | 91 | 30.20% | 2.7 |
| B) FLUOXETINE | | | | | | | | |
| 1 | Component of pyruvate dehydrogenase complex | P08461 | 67123 | 8.76 | 21 | 56 | 37.70% | 2.49 |
| 2 | D-dopachrome decarboxylase | P80254 | 13125 | 6.09 | 7 | 18 | 76.30% | 2.14 |
| 3 | Maleylacetoacetate isomerase | P57113 | 23946 | 7.63 | 10 | 18 | 46.30% | −3.79 |
| 4 | Superoxide dismutase [Mn] | P07895 | 24659 | 8.96 | 9 | 33 | 35.10% | −1.23 |
| 5 | Glutathione S-transferase P | P04906 | 23424 | 6.89 | 10 | 26 | 43.80% | −2.07 |

Differentially expressed proteins in the frontal cortex of prenatally stressed rats treated with imipramine (A) or fluoxetine (B) compared with prenatally stressed rats.

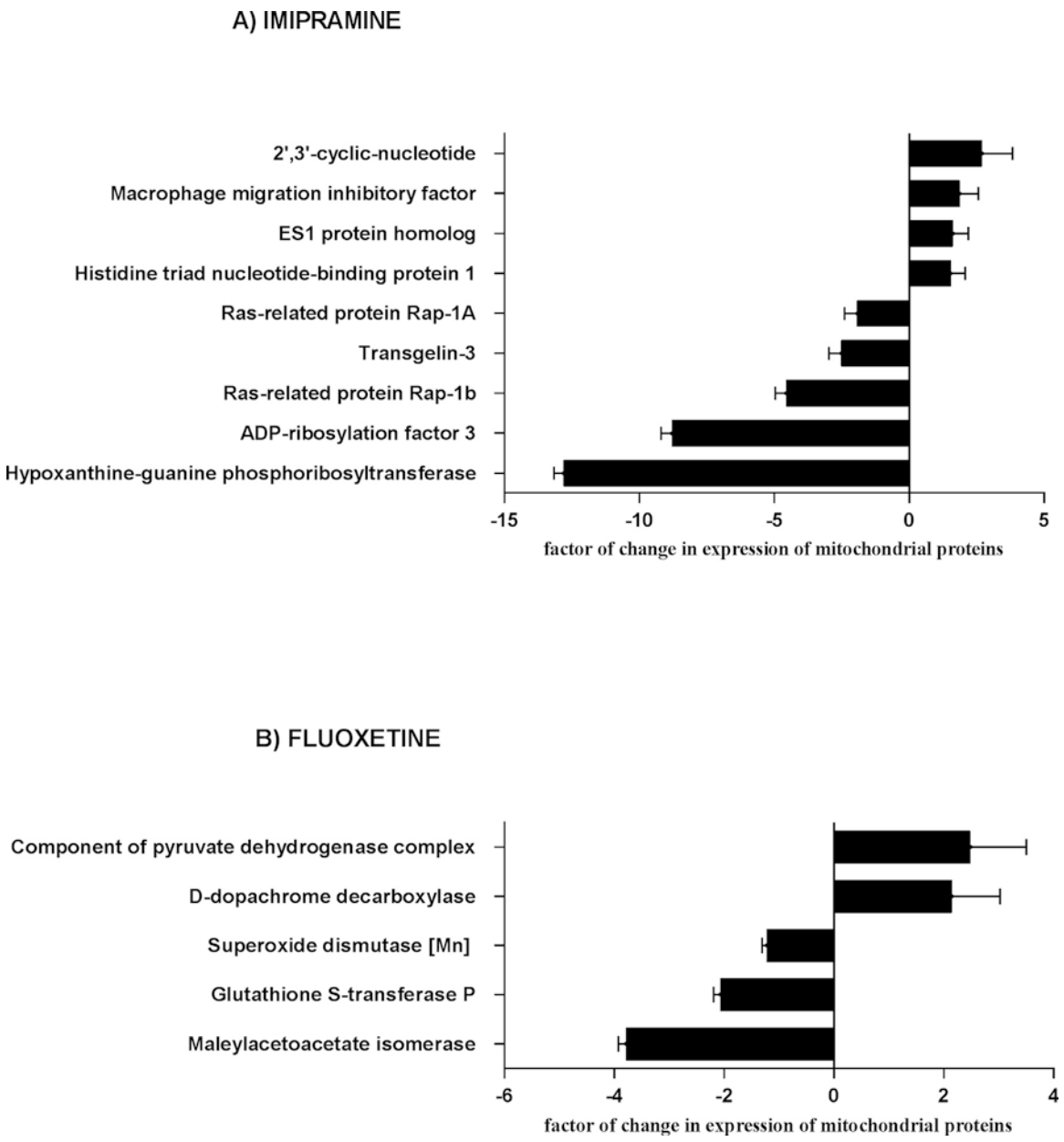


**Fig. 4.** Relative changes in the expression of mitochondrial proteins in the frontal cortex of prenatally stressed rats that were treated with imipramine (a) or fluoxetine (b) compared with the prenatally stressed rats. $p < 0.05$; n = 3 biological replicates per group.

[28]. As expected, this study confirmed that behavioural disturbances are present in the offspring of rat females that were stressed daily during the last week of pregnancy. The adult prenatally stressed offspring exhibit a prolonged immobility time in the Porsolt test and shortened swimming as well as climbing times. Furthermore, our results indicated that the chronic imipramine and fluoxetine treatment shortened the prolonged immobility that was caused by the prenatal stress and extended the swimming times in the Porsolt test, thereby normalizing the behavioural disturbances that were evoked by the prenatal stress [19]. Interestingly, we demonstrated for the first time that in addition to the beneficial impact on the behavioural disturbances, the chronic imipramine and fluoxetine administration discreetly affected the protein expression profile in the cortical mitochondria of adult prenatally stressed offspring.

The first finding is the observation that the chronic injection with the classical drug imipramine changed the frontal cortex mitochondria-enriched sub-proteome in prenatally stressed rats. The analysis revealed 9 differentially expressed proteins after the chronic imipramine treatment. Notably, in our study, we used imipramine as a reference drug, which is commonly used in the clinic to treat drug-resistant depression [31]. Moreover, its direct impact on mitochondria in the frontal cortices is still controversial [32,33]. It is worth emphasizing that our previously published studies in the same experimental paradigm [19] found that chronic imipramine administration affected with various potency also the hippocampal mitoproteome.

Anyway, among the changes evoked by the imipramine administration in frontal cortex, we observed a nearly 3-fold increase in the level of 2′,3′-cyclic-nucleotide 3′-phosphodiesterase (CNPase). This enzyme catalyses the phosphodiester hydrolysis of 2′,3′-cyclic nucleotides to 2′-nucleotides, but a cohesive understanding of its specific physiological functions remains unclear. Previous data have revealed that CNPase is expressed at

extremely high levels in oligodendrocytes, and its deficiency correlates with the increased influx of calcium into these cell [34]. Moreover, the oligodendrocyte CNPase appears to be dependent on the IGF-1/insulin pathway, and the dysfunction in this pathway, that is typically observed in prenatally stressed animals, was normalized by the antidepressant treatment [27]. CNPase knock-out mice have been shown to develop progressive axonal degeneration and motor deficits, leading to death [35]. In addition, other studies have suggested that the down-regulation of CNPase leads to an increase in the mitochondrial membrane permeability and apoptosis [34]. In the context of our study, it is important to note the observation that the down-regulation of CNPase is implicated in the pathogenesis of depression, schizophrenia [36] and Alzheimer disease [37]. This finding may suggest that the favourable imipramine effect is due to its action on the expression of this enzyme in the cortical mitochondria in adult prenatally stressed offspring. Recently, studies have demonstrated that CNPase exhibits neuroprotective properties by down-regulating inflammatory responses, particularly by indirectly acting on iNOS and pro-inflammatory cytokines, including IL-1β and TNF-α [34]. Therefore, it should be considered that imipramine reduced the over-activation of microglia which is present in the adult prenatally stressed rats, by impacting the expression of CNPase; however this hypothesis requires further investigation.

Of the proteins that were down-regulated after the chronic imipramine treatment in present study, the expression of the Ras-related proteins (Rap-1A and Rap-1B) was notable. These are two small proteins that are related to the Rap1 family members, namely, Rap1a and Rap1b, and while these proteins are encoded by separate genes, they share a 95% amino acid identity and may have complementary biological functions [38]. The Ras-related proteins play a key roles in proliferation, differentiation, regulation of actin polymerization and cell spreading [39,40]. Interestingly, in neuronal cells, the Ras-related proteins plays pivotal role in synaptic depression. In fact, Rap1 has been shown to be a key factor in the long-term depression (LTD) of synaptic transmission in hippocampal synapses [41,42], leading to the inhibition of the glutamatergic system [43], whereas Rap2 is required for the modulation of AMPA receptors [44]. In contrast, a deficiency in the Ras-related proteins in the frontal cortex has been shown to result in an impairment in synaptic plasticity and glutamatergic transmission in mice [45]. Since our previous data obtained in hippocampus of prenatally stressed rats demonstrated the down-regulation of 14-3-3 protein, it is worth mentioning that some of the proteins from Ras-related GTP-binding family can be modulate by the 14-3-3 (i.e RGK proteins) [46]. Furthermore, 14-3-3 proteins may play a role in the regulation of GTP-ase activity, which in neurons control axonal and dendritic process shape, extension and navigation [47]. Taking into account the impact of chronic imipramine treatment on the down-regulation of 14-3-3 in the hippocampus and Ras-related proteins in the frontal cortex, the hypothetical target of this drug action may be considered, although it is currently difficult to predict the biological consequences of imipramine impact on the mitoproteome in prenatally stressed offspring.

In the present study we also demonstrated a 2.5-fold decrease in the level of Transgelin-3 after the imipramine administration. Transgelin-3, which is also known as NP25, is widely expressed in the central and peripheral nervous systems in rats [48]. Since transgelin-3 belongs to the calponin family and co-localizes with actin and tubulin, it has been speculated that this protein is important in neuronal plasticity [49]. Thus far, studies exploring the effect of stress and antidepressant drug treatment on the expression of transgelin-3 are scare and divergent. Consistently with our study but using a chronic mild stress model of depression, other authors [50] found that transgelin-3 is down-regulated in the hippocampus of stressed rats after an oleamide treatment (a fatty amide with anti-anxiety effects). However, transgelin-3 was enhanced in the frontal cortex in rats that were chronically treated with fluoxetine [51]. Further studies are required to explore not only the exact impact of antidepressant drugs but also the biological importance of the modulation of NP25.

We observed that the imipramine administration lead to a diminished expression of hypoxanthine- guanine phosphoribosyl-transferase (HPRT) in the mitochondria-enriched frontal cortex of the prenatally stressed rats. HPRT is an enzyme that is needed for the effective recycling of purine nucleotides; therefore, the reduced activity of this enzyme converts purine metabolism and leads to elevated levels of the breakdown product urea. Moreover, the diminished HPRT activity disrupts brain functioning as evidenced in HPRT knock-out animals, which display changes in the brain that are complies with the disruption of purine recycling, nucleotide metabolism and enhanced levels of the metabolite citrate as well as reduced levels of lipids and fatty acids. Interestingly, an HPRT deficit also entails a reduction in dopamine levels [52].

In our comparative research we also evaluated the impact of chronic fluoxetine treatment on the mitoproteome in the frontal cortex of prenatally stressed offspring. Of the changes evoked by the fluoxetine treatment, the increase in the level of the pyruvate dehydrogenase component – E2 subunit is the most striking. The E2 subunit of this complex is responsible for the transfer of an acetyl group from acetyl lipoamide to coenzyme A (CoA) and the formation of acetyl-CoA. Previous studies have shown that the pyruvate dehydrogenase activity is strictly controlled by several mechanisms [53]. Among them, insulin acts by increasing the de-phosphorylation of the pyruvate dehydrogenase complex and accelerating the conversion of pyruvate to acetyl-CoA [54]. Interestingly, our published data demonstrated a tendency to reduction in the activity of the pyruvate dehydrogenase complex in brain homogenates from stressed animals [13], suggesting that the beneficial effect of fluoxetine on the brain changes caused by the prenatal stress procedure are partially related to the stimulatory property of this drug on the expression of this key metabolism regulator.

Regarding the impact of fluoxetine on the frontal cortex mitoproteome profile, we noted an inhibitory influence on the expression of Glutathione *S*-transferase P (Gstp1) and Maleylacetoacetate isomerase (known as Zeta class glutathione transferase). Glutathione is a tripeptide that forms complexes with electrophilic compounds with the participation of glutathione transferases. Many studies noted that the above mentioned enzymes are oxidative stress markers that play important protective roles in neurodegenerative diseases – the reduced Gstp1 activity was observed in the brain and cerebrospinal fluid in Alzheimer's disease [55]. Recently, studies have indicated that tricyclic antidepressants, including imipramine and amitriptyline, diminished the level of Gstp1 [56] by directly impacting drug-enzyme interactions via the non-specific binding of antidepressants to Gstp1. However, Gstp1 in the brain was found to attenuate the therapeutic activity of tricyclic antidepressant drugs [57]. Considering the suppressive impact of the chronic fluoxetine administration in present study on Gstp1 in the cortical mitoproteome in the animal model of depression, it is likely that fluoxetine has a similar mechanism of action. Since it is difficult to interpret our present observation in which fluoxetine diminished the level of *S*-transferase Glutathione P, future studies are needed. It has to be mentioned that our present study as well as results obtained in the hippocampus of prenatally stressed rats, did not show direct correlations, which clearly indicates, that fluoxetine impact on the mitoproteome depends on the particular area of the brain. Therefore further studies are required to determine the exact biological consequences of presented results.

Summing up, we confirm that prenatal stress procedure lead to behavioural depression-like deficits in adult male offspring, while chronic imipramine or fluoxetine administration normalized most of these deficits. Using 2-D electrophoresis coupled with tandem mass spectrometry, we found that the chronic treatment of both antidepressant drugs modulates the mitochondrial protein profile in the frontal cortex of adult prenatally stressed offspring. Although our research shows data pointing that the chronic administration of imipramine and fluoxetine, which have different mechanisms of action, exerts a varied, multifaceted impact on the mitochondria-enriched sub-proteome in the frontal cortex, additional experiments are required to elucidate the possible biological consequences underlying the effects mediated by these antidepressants.

## Conflict of interest

All authors have no financial interests or potential conflicts of interests to declare.

## Contribution statement

A.B.-K. and R.O. were responsible for the conception and design of the study. J.Ś., K.Ch., E.T. and K.K. assisted with the rat behavioural analyses. K.G., A.S. and M.S. were responsible for the analyses of the samples and the interpretation of the data. A.B.-K. and K.G. drafted the manuscript. All authors revised the paper critically for important intellectual content and provide final approval of the version to be published.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.pharep.2017.11.016.

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