Neurochemistry International 126 (2019) 210–217

Contents lists available at ScienceDirect

Neurochemistry International

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

Highly aggressive behavior induced by social stress is associated to reduced cytochrome *c* oxidase activity in mice brain cortex

Ana Paula Miranda Mendonça[a,1], Luanda Yanaan Hoppe[b,1], Alessandro Gaviraghi[a], Tânia Cremonini de Araújo-Jorge[b], Gabriel Melo de Oliveira[c], Renata Machado Felippe[b], Marcus F. Oliveira[a,*], Viviane Muniz da Silva Fragoso[b,**]

[a] Institute of Medical Biochemistry Leopoldo de Meis, Federal University of Rio de Janeiro, Av. Bauhínia 400, 21941-590, Ilha do Fundão, Cidade Universitária, Rio de Janeiro, Brazil
[b] Laboratory of Innovations in Therapies, Education and Bioproducts, Instituto Oswaldo Cruz/FIOCRUZ, Av. Brasil 4365, 21045-900, Manguinhos, Rio de Janeiro, Brazil
[c] Laboratory of Cell Biology, Instituto Oswaldo Cruz/FIOCRUZ, Av. Brasil 4365, 21045-900, Manguinhos, Rio de Janeiro, Brazil

ARTICLE INFO

Keywords:
Stress
Energy
Cognition
Mitochondria
Behavior
Metabolism

ABSTRACT

Violence and aggression represent severe social problems, with profound impacts on public health. Despite the development of experimental models to study aggressive behavior is highly appreciated, the underlying mechanisms remain poorly understood. Given the key contribution of mitochondria to central nervous system bioenergetics, we hypothesized that mitochondrial function in brain would be altered by social stress. Using a model of spontaneous aggression, we investigated here the effects of social stress on brain mitochondrial function in prefrontal cortex of Swiss mice. Animals were categorized as highly aggressive, subordinate and non-aggressive (harmonic) after stress induced by regrouping and compared them with non-regrouped animals. Despite social stress did not affect brain cortex oxygen consumption rates and NADH:cytochrome *c* oxidoreductase activity, cytochrome *c* oxidase expression and activity were significantly lower in highly aggressive animals compared to non-regrouped ones. These changes were not observed in ATP synthase and adenine nucleotide translocator content suggesting a selective effect of social stress on cytochrome *c* oxidase. Therefore, aggressive behavior generated upon social stress associates to selective reduction in cytochrome *c* oxidase activity, with potential detrimental effects on brain bioenergetics and function.

1. Introduction

Aggression is defined as the act(s) in which an individual intentionally injures or harms others of their own species (Nelson and Trainor, 2007). In a global scale, death resulted from violence accounts for approximately 1.6 million cases/year, representing an important public health issue (Miczek et al., 2002; World Health Organization, 2002). The incidence of aggression in humans and in other animal species is higher among young males (Blanchard et al., 2013; Nadanovsky et al., 2009). Research on aggression in laboratory animals is implicitly driven by an assumption that results of such studies may be applicable to human behavior as well (Blanchard and Blanchard, 2003).

Abbreviations: AA, Antimycin A; ATP, Adenosine triphosphate; AD, Alzheimer's disease; ADP, Adenosine diphosphate; Agg, Highly aggressive mice; ANT, Adenine nucleotide translocator; ATP5A, ATP synthase subunit 5A; CNS, Central nervous system; COX, Cytochrome *c* oxidase; DTT, Dithiothreitol; EDTA, Ethylenediaminetetraacetic acid; EGTA, Ethyleneglycol-bis(β-aminoethylether)-N,N,N′,N'-: tetraaceticacid; ETS, Electron transport system; FCCP, Carbonyl cyanide p-(trifluoromethoxy) phenylhydrazone; Har, Harmonic mice; High, TST hypermobility; HRR, High resolution respirometry; HRP, Horse radish peroxidase; HPA, Hypothalamic-pituitary-adrenal; KCN, Potassium cyanide; Low, TST low mobility; Med, TST medium mobility; MSA, Model of spontaneous aggression; NR, Non-regrouped mice; O_2, Molecular oxygen; OXPHOS, Oxidative phosphorylation; pmf, Proton motive force; PVDF, Polyvinylidene difluoride; ROS, Reactive oxygen species; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; Sub, Subordinate mice: PBS: Phosphate buffer saline; TMPD, N,N,N′,N′-tetramethyl-p-phenylenediamine; TST, Tail suspension test; wko, Weeks old

* Corresponding authors. Stress Response Biochemistry Laboratory, Institute of Medical Biochemistry Leopoldo de Meis, Health Sciences Center, Federal University of Rio de Janeiro, Av. Bauhinia 400, 21941-590, Ilha do Fundão, Rio de Janeiro, Brazil.
** Corresponding authors. Laboratório de Inovações em Terapias, Ensino e Bioprodutos, IOC, Fiocruz. Pavilhão Cardoso Fontes, 2° andar, sala 64,Av. 4365, 21045-900, Manguinhos, RJ, Rio de Janeiro, Brazil.
E-mail addresses: maroli@bioqmed.ufrj.br (M.F. Oliveira), viviane.fragoso@ioc.fiocruz.br (V.M. da Silva Fragoso).
[1] These authors equally contributed to this work.

https://doi.org/10.1016/j.neuint.2019.03.017
Received 3 December 2018; Received in revised form 28 February 2019; Accepted 21 March 2019
Available online 26 March 2019
0197-0186/

Stress activates the brain, changing its structures and functions, with a metabolic cost that is primarily met by means of oxidative phosphorylation (OXPHOS) in the mitochondria (Morava and Kozicz, 2013). Changes in mitochondrial function are increasingly recognized as key components in altered neuroendocrine stress responses in different species (Adzic et al., 2009; Hollis et al., 2015; Picard et al., 2018; Picard and McEwen, 2018). In rodent models of acute and chronic stresses, altered mitochondrial function, including inhibition of electron transport system (ETS), was associated to redox imbalance and apoptosis (Aboul-Fotouh, 2013; Filipović et al., 2011; Garabadu et al., 2015; Hollis et al., 2015; Picard et al., 2018; Rezin et al., 2008; van der Kooij et al., 2018; Xing et al., 2013). For example, in well-organized societies, aggressive behavior positively correlates with reduced activity of ETS components, parallel to increased glycolysis in the brain in honey bees (Alaux et al., 2009; Chandrasekaran et al., 2015; Li-Byarlay et al., 2014). Structural changes in brain mitochondria, including swelling and decrease of cristae number, were already reported in animal models of stress (Gong et al., 2011; Lobanov et al., 2007; Rosety-Rodriguez et al., 2006). In addition, changes in several neuroendocrine processes are dependent on the integrity of mitochondrial DNA in response to stress (Picard et al., 2015).

Cytochrome *c* oxidase (COX) is a mitochondrial protein complex composed by 13 subunits in vertebrates, that locates at the inner mitochondrial membrane and catalyzes the transfer of four electrons from cytochrome *c* to molecular oxygen (O_2), producing two molecules of water (Capaldi, 1990). During this reaction, part of the energy released from electrons is transduced to generate the proton motive force (*pmf*) across the inner mitochondrial membrane, an essential process to allow ATP synthesis by OXPHOS. COX expression and activity is regulated by several factors including the energy demand (Arnold and Kadenbach, 1999), post-translational modifications (Helling et al., 2012), nitric oxide and carbon monoxide (Brown and Cooper, 1994; Zuckerbraun et al., 2007) and others. Interestingly, dysregulation of COX activity is a hallmark of many neurological conditions, as seen in drug abuse and schizophrenia (Vélez-Hernández et al., 2014; Cavelier et al., 1995).

In an effort to better understand the mechanisms that mediate aggressive behavior triggered by social stress, here we conducted a functional assessment of mitochondria in adult male Swiss Webster mice brain frontal cortex. By using a model of spontaneous aggression (MSA) established by our group (Batista et al., 2012; Fragoso et al., 2016; Oliveira et al., 2014), we observed that aggressive behavior resulted from social stress selectively reduces COX expression and activity in brain frontal cortex. However, no apparent effects on mitochondrial O_2 consumption and NADH:cytochrome *c* oxidoreductase activity were observed in permeabilized slices of brain frontal cortex. These results indicate that selective modulation of COX activity might compromise brain energy demand under social stress conditions, with potential consequences to cognition and behavior.

2. Material and methods

2.1. Animals

Male Swiss Webster mice (3 weeks old, wko) were obtained from Institute of Science and Technology in Biomodels (ICTB/Fiocruz) and maintained at Fundação Oswaldo Cruz animal facility (LBC-LITEB, IOC, Rio de Janeiro). Animals were adapted to the environment for one week in ventilated racks, with temperature (20–22 °C), humidity (40–50%) and photoperiod controlled according to the standard environmental regulations. The animals were maintained under stable conditions of temperature and light, with a 12-h light/dark cycle, and both food and water were available *ad libitum*.

2.2. Model of spontaneous aggression (MSA)

Fig. 1 shows the experimental design of our model. The animals at 3 weeks old (wko) were individually identified and divided into 5 groups of 6 animals each, named clusters (A1 to A5). At 4, 6 and 8 wko, behavioral testing was performed by Tail Suspension Test (TST) (Steru et al., 1985) and aggressive ethogram (number of attacks and lesion extension) (Fragoso et al., 2016; Oliveira et al., 2013). Briefly, in the TST the animals were suspended by the final third of tail at the top of the apparatus and their periods of agitation and immobility were registered during 5 min (Steru et al., 1985). The animals were submitted to ethogram analysis and showed no aggressive attitudes. At 10 wko, the animals were divided into three groups based on the activity pattern determined by TST: *i)* High - hyperactive, ≤ 50 s of immobility, *ii)* Med - medium activity, 51–110 s of immobility, and *iii)* Low - hypoactive, ≥ 110 s of immobility. Then, part of the animals was kept in the same cage (n = 4) and named non-regrouped (NR) (control), and the others were re-grouped (RE: cages R1-R5, 4 animals/cage) based on the activity pattern, containing one animal “High”, one, “Low” and three animals “Med”. At 16 wko, aggressive ethogram analysis was performed, and each individual was classified as harmonic (Har), animals with reduced aggression and discrete or absence of bites/lesions in the body, subordinate (Sub), animals suffering aggression with moderate wounds and lesions on the tail, back and genital region, and aggressive (Agg), highly aggressive mice with higher frequency of intense attacks between individuals. After the behavioral assay, the groups NR, Har, Sub and Agg were euthanized with cervical dislocation, and the cerebral frontal cortex fragment of the right hemisphere was removed for further analyses. This experimental protocol was performed three times, approved by the Ethics Committee for the Use of Animals/IOC under the license 005/2015 in accordance with the guidelines of the National Commission for Research Ethics (CONCEA) and the EU Directive 2010/63/EU for animal scientific use.

2.3. Sample preparation

Frontal cerebral cortex samples were prepared in different media as described elsewhere (Benani et al., 2009). After euthanasia and dissection, the brain was transferred to 2 mL of ice-cold solution A (250 mM sucrose, 1 g/L BSA, 0.5 mM Na_2-EDTA and 10 mM Tris-HCl, pH 7.4) in Petri dishes. The brain was dissected, and the right hemisphere was transferred to another Petri dish containing 1 mL ice cold solution B (BIOPS medium: 20 mM taurine, 15 mM phosphocreatine, 20 mM imidazole, 0.5 mM DTT, 10 mM Ca-EGTA, 5.77 mM ATP, 6.56 mM $MgCl_2$, 50 mM K-MES, pH 7.1). The cerebral cortex tissue of the right hemisphere was dissected into small biopsies of 1 mm × 1 mm x 2 mm size. Solution B was replaced for 2 mL solution B supplemented with 20 µL saponin (5 mg/mL stock solution). The sample remained for 30 min under gentle shaking and was then placed in a Petri dish containing 2 mL of solution C (MiR05 medium: 0.5 mM EGTA, 50 mM K-MES, 3 mM $MgCl_2$, 20 mM taurine, 10 mM KH_2PO_4, 110 mM sucrose, 1 g/L fatty acid free BSA, 20 mM Hepes, pH 7.1). The tissue was kept under gentle agitation for 2 min. The procedure with solution C was repeated 3 times. At the end of the preparation protocol, the samples were incubated in solution C for 2 h at 4 °C under gentle shaking and then the respirometry analysis was performed.

2.4. Respirometry analyses

In order to assess the stimulated respiratory rates of the frontal cerebral cortex tissue of NR, Har, Sub and Agg mice, samples were weighted (≤ 5 mg), and placed into a chamber of a high resolution O2k respirometer (Oroboros Instruments, Innsbruck, Austria) filled with 2 mL of MiR05 medium. Stimulated O_2 consumption rates were determined at 30 °C under constant stirring at 750 rpm. Data were analyzed using DatLab 6.0 software and the O_2 consumption rates were normalized according to tissue mass. After stabilization of O_2 consumption rates under basal conditions, the routine of electron transport system (ETS) activities in permeabilized frontal cerebral cortex was

carried out by following the high resolution respirometry (HRR) analyses coupled to substrate-uncoupler-inhibitor titration (SUIT) protocols established in the literature (Pesta and Gnaiger, 2012). When permeabilized brain cortex samples were incubated only with substrates (glutamate + malate), respiratory rates were < 20 pmol/s/mg. Under this metabolic condition, non-phosphorylating respiratory rates are essentially limited by the magnitude of the *pmf*, and the respiratory rates are compensated by the "proton leak", relieving the inhibitory effect of high *pmf* on the O_2 flux. Increase in the energy demand, experimentally provided by adding 1 mM ADP to respirometer chamber, significantly boosted respiratory rates, as a compensatory mechanism for the reduction of *pmf* consumed by ATP synthase. However, maximal respiratory rates were induced only when *pmf* was completely collapsed by stepwise titration of carbonyl cyanide p-(trifluoromethoxy) phenylhydrazone (FCCP) from 0.1 to 1 μM, which promote the maximal compensatory increase in electron flow at inner mitochondrial membrane. To determine the mitochondrial utilization of O_2 in permeabilized tissue, specific inhibition of complex I and complex III of the ETS by using 0.1 μM rotenone and 1 μg/mL antimycin A (AA), respectively. Upon addition of rotenone and AA, stimulated O_2 consumption rates were drastically reduced, indicating that mitochondria contribute to at least 90% of the O_2utilized by brain frontal cortex in our conditions. Mitochondrial metabolic states were calculated as following: ATP-linked respiration: respiratory rates provided after ADP - (glutamate + malate); Proton leak respiration: respiratory rates provided after (glutamate + malate) - antimycin; Maximal (or uncoupled) respiration: respiratory rates provided after FCCP - antimycin; Residual O_2 consumption (ROX): respiratory rates provided after AA.

2.5. Enzyme activities

Samples of brain frontal cerebral cortex (1 mm × 1 mm x 2 mm) from NR, Har, Sub and Agg mice were collected and homogenized in 200 μL RIPA buffer (25 mM Tris-HCL pH 7.6, 150 mM NaCl, 5 mM EDTA, 1% Triton x-100, 1% deoxycholate sodium, 0.1% SDS) with protease inhibitor (1:100) by using a glass/teflon homogenizer at room temperature. COX activity was assessed in permeabilized brain tissue by HRR through sequential additions of 2 mM ascorbate and 0.5 mM N,N,N′,N′-tetramethyl-p-phenylenediamine (TMPD), upon AA inhibition in experiments described in item 2.4 above. TMPD promoted a huge increase in respiratory rates and specificity of COX activity was determined by inhibiting the enzyme complex with 5 mM potassium cyanide (KCN). Alternatively, brain homogenate samples were freeze-thawed three times and mitochondrial enzyme activities were determined spectrophotometrically using a Shimadzu spectrophotometer model visible 2450 (Shimadzu Scientific Instruments, Tokyo, Japan) following methods described in the literature with slight modifications (Kirby et al., 2007). COX and NADH:cytochrome *c* oxidoreductase activities were determined at room temperature, in 1 mL of hypotonic buffer (25 mM potassium phosphate and 5 mM $MgCl_2$, pH 7.2). COX activity was assessed by following the decrease in absorbance due to the oxidation of ferrocytochrome *c* ($\varepsilon = 18.7\ mM^{-1}\ cm^{-1}$). A volume corresponding to 50 μM reduced cytochrome *c* was added to 1 mL of hypotonic buffer in a spectrophotometer cuvette. Then, a sample corresponding to 50 μg of brain homogenate protein was added, mixed and the absorbance at 550 nm was recorded for about 5 min. Afterwards, a volume corresponding to 1 mM KCN final concentration was added to cuvettes to inhibit COX activity, which was considered as the cyanide-sensitive rate of cytochrome *c* oxidation. NADH:cytochrome *c* oxidoreductase activity was measured as the rotenone-sensitive increase in absorbance at 550 nm due to reduction of ferricytochrome *c*. The reaction was initiated by the addition of 50 μM ferricytochrome *c*, 1 mM KCN, 100 μg of brain homogenate protein and 200 μM NADH. The absorbance was monitored at 550 nm for about 5 min. A sister cuvette was prepared in hypotonic buffer containing 100 μg of brain homogenate protein, 5 μM rotenone, 1 mM KCN and incubated for 10 min at room temperature. Subsequently, 50 μM ferricytochrome *c* and 200 μM NADH were added to the cuvette, mixed and absorbance was monitored at 550 nm for about 5 min. The rates of ferricytochrome *c* reduction in the presence of rotenone was then subtracted from those samples without rotenone and the NADH:cytochrome *c* oxidoreductase activity was considered as the rotenone-sensitive rate of cytochrome *c* reduction. COX and NADH:cytochrome *c* oxidoreductase activities were calculated using the first-order rate constant and expressed as nmols of oxidized/reduced cytochrome *c*/min/mg of total homogenate protein, respectively.

2.6. Western blot

Samples of brain frontal cerebral cortex (1 mm × 1 mm x 2 mm) from NR, Har, Sub and Agg mice were collected and homogenized in 200 μL RIPA buffer (25 mM Tris-HCL pH 7.6, 150 mM NaCl, 5 mM EDTA, 1% Triton x-100, 1% deoxycholate sodium, 0.1% SDS) with protease inhibitor (1:100) for protein extraction. Protein levels from all extracts were determined by the Bradford method and the samples were stored at −80 °C (Bradford, 1976). Protein samples were thawed and separated by denaturing gel electrophoresis. For COXVIIa Western blots, protein samples corresponding to 25 μg were subjected to Tricine sodium dodecyl sulfate-polyacrylamide gel electrophoresis (Tricine-SDS-PAGE) with 16% polyacrylamide gels, and subsequently transferred to polyvinylidene difluoride (PVDF) membranes (Amersham Hybond P 0.2, GE® Healthcare, MA, USA) using a Trans-Blot SD Semi-Dry transfer cell (Biorad, CA, USA). For ATP5A, ANT1 and VDAC2 detection, protein samples corresponding to 25 μg were subjected to glycine sodium dodecyl sulfate-polyacrylamide gel electrophoresis (Glycine-SDS-PAGE) with 10% polyacrylamide gels, and subsequently transferred to nitrocellulose membranes (Biorad, CA, USA) using a Trans-Blot Turbo System (Biorad, CA, USA). After transfer, membranes were stained with 0.2% (w/v) Ponceau S to assess protein loading. Membranes were washed with phosphate buffer saline (PBS) solution, blocked during 2 h with nonfat dry milk (10%), and then incubated for 24 h with anti-COXVIIa (1:8000) (ab110268, Abcam, MA, USA), anti-ATP 5A (1:1000) (ab14748, Abcam, MA, USA), anti-ANT1 (1:1000) (ab110322, Abcam, MA, USA) mouse monoclonal antibodies and anti-VDAC2 (1:5000) (ab47104, Abcam, MA, USA) rabbit polyclonal antibody at 4 °C. After washing with PBS-0.1% Tween 20, membranes were incubated for 1 h at room temperature with goat anti-rabbit IgG H&L horse radish peroxidase (HRP) secondary antibody (1:10,000) (ab97051, Abcam, MA, USA) for anti - VDAC2 antibody, and goat anti-mouse IgG2a heavy chain (HRP) secondary antibody (1:10,000) (ab97245, Abcam, MA, USA) for all other antibodies. The membranes were washed in PBS, and membranes were developed by Imagequant LAS 4000 system (GE® Healthcare, MA, USA). The analysis of the protein content results was normalized by ImageJ and normalized by mitochondrial mass control, VDAC2. The graphs are the result of the analysis of three different gels.

2.7. Statistical analyses

Data were presented as mean ± SD or SE values for each condition. D'Agostino and Pearson normality tests were done for all values to assess their Gaussian distribution. Comparisons between groups were done by non-parametric Mann-Whitney or Kruskal-Wallis tests using the software SPSS version 16,0 (IBM, CA, USA)for data that did not follow normal distribution. Alternatively, one-way ANOVA and *a posteriori* Dunn's tests were employed for data that followed normal distribution by using the GraphPad Prism software version 5.00 for Windows (GraphPad Software, USA). Differences of $p < 0.05$ were considered to be significant.

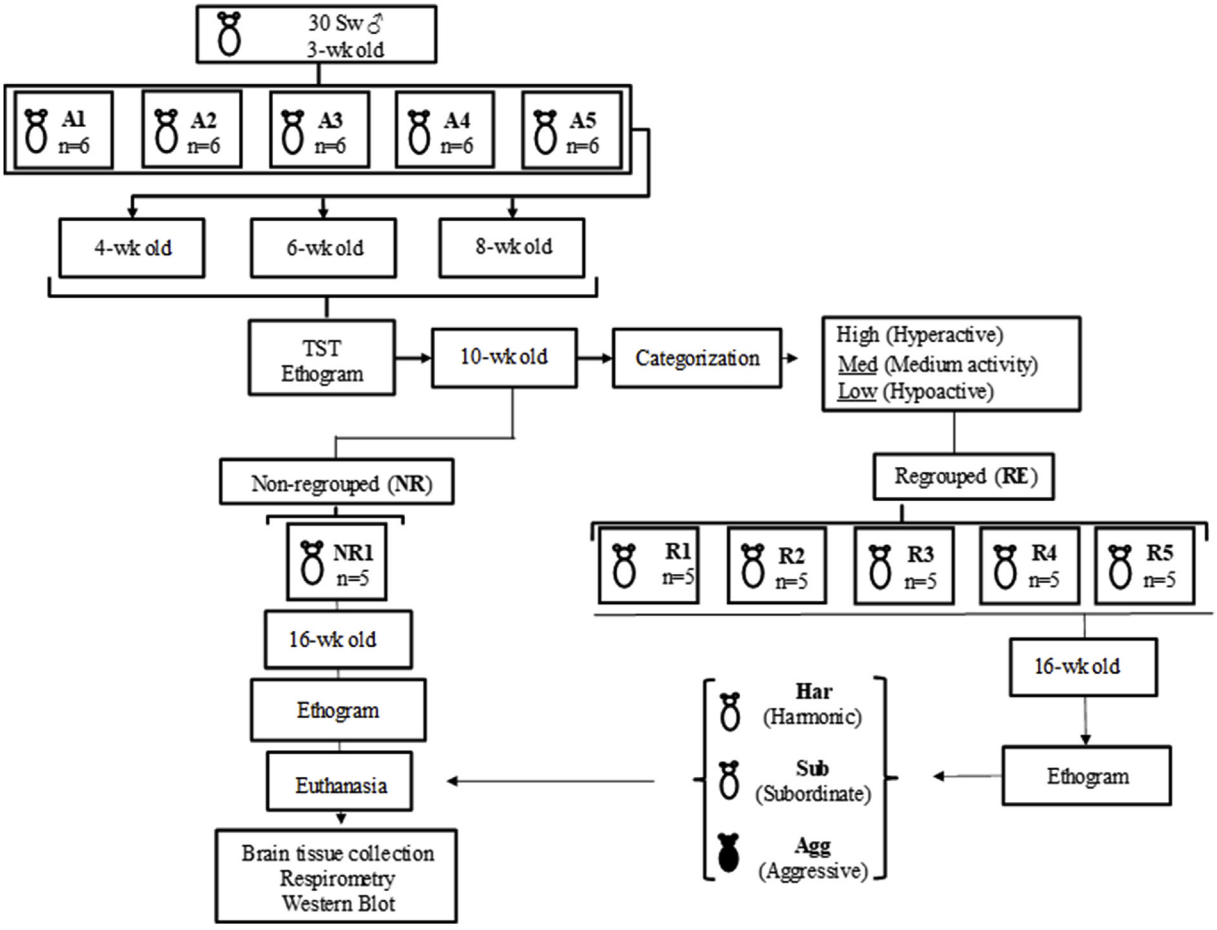

Fig. 1. Experimental design of the model of spontaneous aggression (MSA). The animal grouping (A1-A5) was performed at 3 wko and the behavioral tests at the 4, 6 and 8 wko. At 10 wko, they were classified by TST as High (hyperactive), Med (medium activity), and Low (hypoactive). Then, part of the animals was regrouped (R1-R5) in the proportion of one animal High, one animal Low and three animals Med. Social stress negative control were non-regrouped (NR). At the 16 wko, the aggressive ethogram was repeated and the RE animals were categorized as harmonic (Har, reduced aggression), subordinate (Sub, suffered aggression), and aggressive (Agg, higher frequency of intense attacks). Animals were subsequently euthanized and cerebral frontal cortices from the right hemisphere were removed for HRR, enzyme activities and western blot analyses.

3. Results

3.1. Categorization of animal aggressiveness

The model of spontaneous aggression (MSA) allows the evaluation of the emergence of aggression in male mice during social stress condition promoted by re-grouping (Batista et al., 2012). Through this method, we previously observed an increase in dopamine release in brain prefrontal cortex of highly aggressive animals compared to non-aggressive ones (Oliveira et al., 2014). Also, administration of antipsychotics significantly decreased violent attitudes in aggressive animals (Fragoso et al., 2016). The animals investigated in the present work followed the MSA flowchart, as depicted in Fig. 1. Based on the TST test, 20% of the animals showed High mobility (4 wko: 14.2 ± 11.1; 6 wko: 18.0 ± 14.9 and 8 wko: 12.8 ± 10.2 s); 60%, Med activity (4 wko: 62.3 ± 9.1, 6 wko: 84.5 ± 23.9 and 69.8 ± 11.6 s) and 20% Low activity (4 wko: 125.8 ± 16.8, 6 wko: 124.5 ± 8.3 and 8 wko: 147.0 ± 21.0 s) (Fig. 2A). The aggressive ethogram conducted at 10 wko, confirm that before the regrouping we observed low aggression, independent of the TST activity (4 wko - High: 3.0 ± 2.5; Med: 2.0 ± 2.0 and Low: 2.0 ± 2.0 number attacks/30 min; 6 wko - High: 3.5 ± 1.5; Med: 3.0 ± 2.0 and Low: 2.0 ± 2.0 number attacks/30 min and 8 wko - High: 3.0 ± 2.0; Med: 3.0 ± 2.0 and Low: 4.0 ± 2.5 number attacks/30 min) in all individuals and the absence of lesion due to fights or attacks. At 16 wko, the ethogram analysis determined the aggressiveness level of each animal, and then they were distributed in three categories according to their social behavior: harmonic (Har), subordinate (Sub), and highly aggressive (Agg). Under social stress we observed a significant increase in the number of attacks/30 min in Agg (22.0 ± 1.9) compared to all other groups (NR: 1.5 ± 1.0, Har: 2.0 ± 1.0 and Sub: 0.8 ± 0.3) (Fig. 2B). Lesion extension, a direct consequence of aggressive attacks, revealed that only subordinate mice (Sub) presented extensive lesions (3.0 ± 0.3 cm^2) relative to other groups (Fig. 2C).

3.2. Stimulated respiratory rates were not affected in brain cortex upon social stress

In order to determine whether brain energy metabolism would be affected by social stress, stimulated O_2 consumption rates were assessed in chemically permeabilized frontal cortex slices of male Swiss Webster mice by high resolution respirometry (HRR). A representative stimulated O_2 flux trace during a typical HRR experiment is depicted in Fig. 3A. By using HRR, combined with the complex I-linked substrates glutamate and malate, we determined the stimulated O_2 consumption rates per mass of brain cortex in all four groups (Fig. 3B–E). We observed that social stress did not cause any significant effect on stimulated respiratory rates linked to ATP synthesis (Fig. 3B), proton leak (Fig. 3C), maximal (Fig. 3D), or on residual O_2 consumption (Fig. 3E).

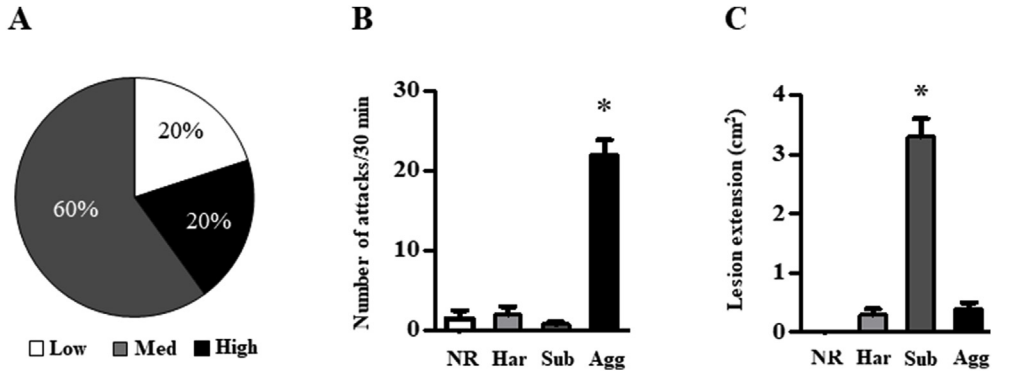

Fig. 2. Assessment of behavioral parameters in social stress. (A) Animals stratification according to TST mobility at 4, 6 and 8 wko in High (hyperactive), Med (medium), Low (hypoactive) and their relative distribution based on the TST test. (B) At 10 wko, ethogram showed low levels of aggressive behavior before regrouping in the all groups, independent of the TST activity. (C) At 16 wko, regrouping led to the categorization into NR (non-regrouped), Har (harmonic), Sub (subordinate) and Agg (aggressive) based on the number of attacks/30 min and (D) Lesion extension (cm^2). Asterisk indicate statistical difference ($p < 0.05$), using the non-parametric Mann-Whitney test, between Agg and the other categories (C) and between Sub and the other categories (D). Data are expressed as mean ± SD of three different experiments with four to six mice/group.

3.3. Cytochrome c oxidase content and activity were specifically decreased in the brain of aggressive animals

We assessed the activity of cytochrome *c* oxidase (COX) in all experimental groups by using two distinct methodologies (Fig. 4). One by HRR assessing the cyanide-sensitive O_2 consumption provided by TMPD + Ascorbate (Fig. 4A), and by spectrophotometric quantification of cyanide-sensitive reduced cytochrome *c* oxidation (Fig. 4B). We observed by HRR a clear and significant reduction close to 43% on COX activity in aggressive animals (73,4 ± 7,5 pmol O_2/s/mg) relative to NR animals (131.4 ± 18.3 pmol O_2/s/mg). Curiously, re-grouping stress differently altered COX activity among groups, as just a slight reduction in Har, but not on Sub animals, were observed in this parameter. These data were strengthened by our observation that COX activity assessed by an independent method, confirmed the significant reduction in enzyme activity (~70% reduction) in aggressive animals relative to NR group (Agg = 46,5 ± 9,8 *vs.* NR = 157,6 ± 41,1 nmol/min/mg) (Fig. 4B). In order to ensure the selectivity by which re-grouping stress affects COX, we then determined NADH:cytochrome *c* oxidoreductase activity in brain homogenates in our experimental setting. We observed that NADH:cytochrome *c* oxidoreductase activity was quite similar in all animal groups, strengthening that COX activity is selectively inhibited by re-grouping stress (Fig. 4C).

Assessment of mitochondrial protein levels by western blot revealed

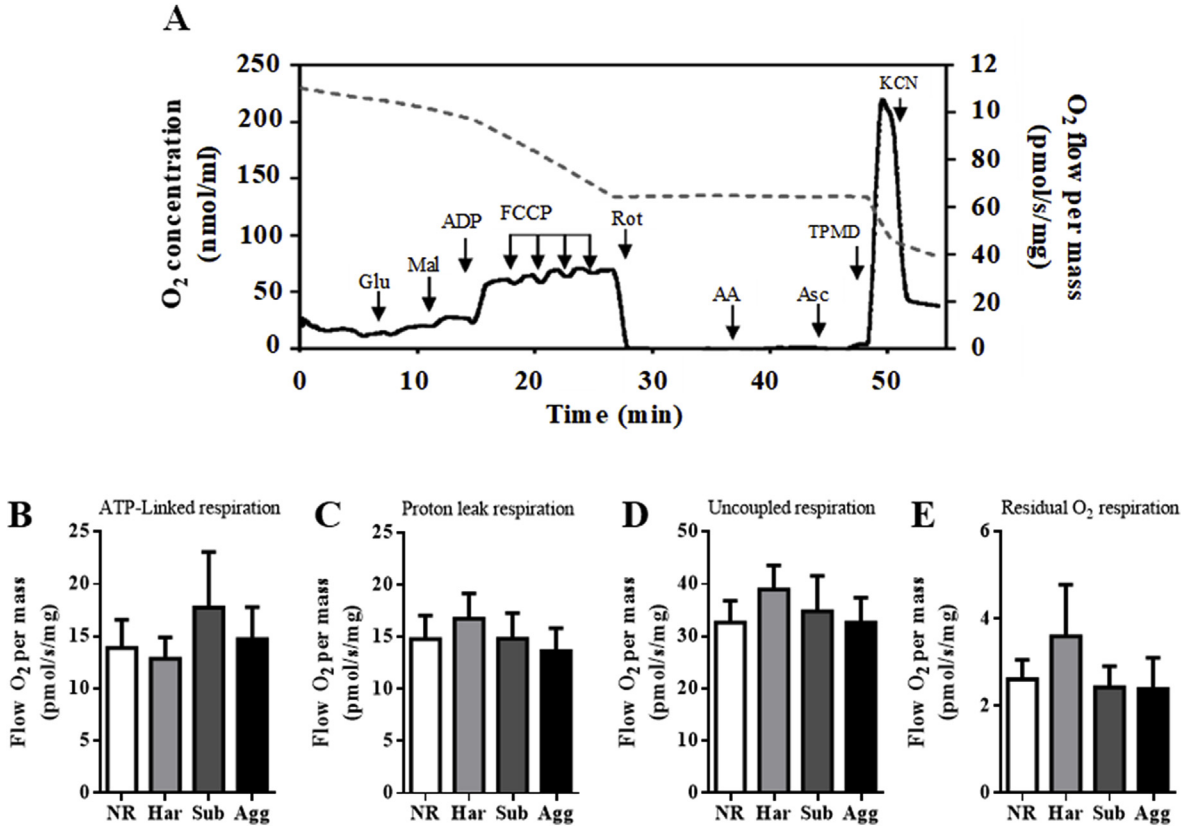

Fig. 3. Social stress does not affect stimulated O_2 consumption in permeabilized brain frontal cortex. (A) Representative traces of stimulated O_2 consumption rates (solid line expressed as pmol/s/mg) and O_2 tension (dashed line expressed as nmol/mL) of permeabilized brain frontal cortex categorized by the MSA. The arrows above trace represents the additions of oxidative phosphorylation modulators as following: 10 mM glutamate (Glu); 2 mM malate (Mal); 1 mM ADP (ADP); 0.1, 0.3, 0.5 and 1 μM FCCP (FCCP); 0.1 μM rotenone (Rot); 1 μg/mL antimycin a (AA); 2 mM ascorbate (Asc); 0.5 mM TMPD (TMPD) and 5 mM KCN (KCN). (B) Proton-leak respiration, (C) ATP-linked respiration, (D) Uncoupled or maximal respiration, (E) Residual O_2 consumption. Data are expressed as mean ± SE of at least five different experiments.

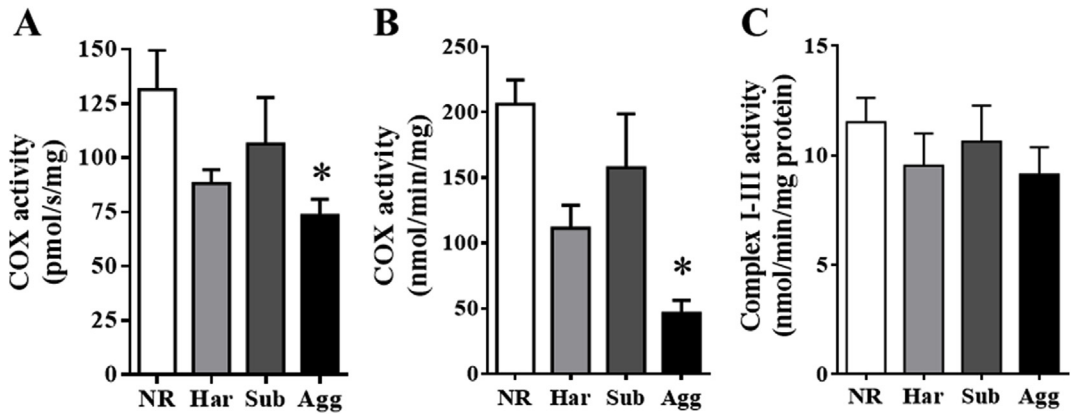

Fig. 4. Cytochrome *c* oxidase activity was selectively and significantly reduced in brain frontal cortex of aggressive mice. COX activity was assessed by high resolution respirometry subtracting the O_2 consumption rates induced by TMPD + ascorbate to that obtained after KCN addition (A) or spectrophotometrically by assessing KCN-sensitive oxidation of reduced cytochrome *c* at 550 nm (B). NADH:cytochrome *c* oxidoreductase activity was determined spectrophotometrically by assessing rotenone-sensitive cytochrome *c* reduction at 550 nm (C). Categories: NR (non-regrouped), Har (harmonic), Sub (subordinate) and Agg (aggressive). Asterisk indicate statistical difference ($p < 0.05$), using the non-parametric Mann-Whitney test, between Agg mice and NR group (A, B). Data are expressed as mean ± SE of at least three different experiments.

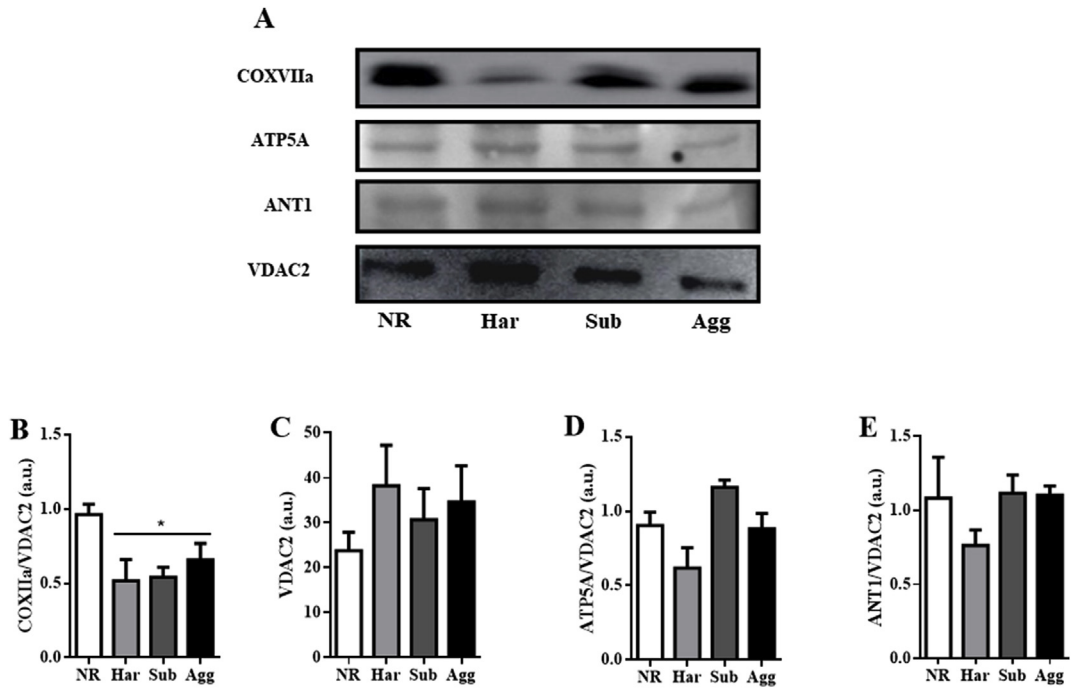

Fig. 5. Cytochrome *c* oxidase content was significantly reduced in brain frontal cortex of aggressive mice. Mitochondrial protein content was determined in cerebral cortex. (A) Western blot analyses of cerebral cortical tissue for COXVIIIa, ATP5A, ANT1 and VDAC2. Constitutive expression of the proteins (B) VDAC2, (C) COXVIIa, (D) ATP5A and (E) ANT1was obtained by the ratio of protein/VDAC2 (mitochondrial mass marker). Categories: NR (non-regrouped), Har (harmonic), Sub (subordinate) and Agg (aggressive). Data are expressed as mean ± SE of at least three different experiments. Asterisk indicates significant differences ($p < 0.05$) of Har, Sub and Agg categories relative to NR group (Kruskal-Wallis test).

that re-grouping stress, by itself, reduced the expression of COX subunit VIIa (Har: 0.52 ± 0.25a.u., Sub: 0.54 ± 0.12 a. u., Agg: 0.66 ± 0.19 a. u. *vs.* NR: 0.96 ± 0.12 a. u.) (Fig. 5A and C). This effect was independent of altered mitochondrial mass since the levels of mitochondrial outer membrane marker VDAC2 was not affected by social stress in brain cortex (Fig. 5A and C). We also quantified ATP synthase subunit 5A and the adenine nucleotide translocator (ANT) levels, which are markers of mitochondrial inner membrane. Fig. 5D and E shows that both ATP synthase and ANT content were not significantly affected by re-grouping stress.

4. Discussion

In the present work, we demonstrate that cytochrome *c* oxidase (COX) activity was selectively and significantly reduced in brain frontal cortex of aggressive animals upon social stress. Although social stress caused no apparent changes in respiratory rates and NADH:cytochrome *c* oxidoreductase activity of brain cortex in all groups tested, we observed significant reductions in COX content just by re-grouping. To our knowledge, this is the first evidence of functional changes in brain mitochondria associated to aggressive behavior in a mammalian model of social stress.

Previous evidence demonstrated a causal link between inhibition of specific brain OXPHOS components with aggressive behavior across

different species (Alaux et al., 2009; Li-Byarlay et al., 2014; Sakata et al., 2005). The abundance and activity of COX follows the brain energy demand, and alterations on COX functionality have direct consequences to CNS energy metabolism. For example, patients with schizophrenia exhibit low metabolic rates and COX activity in different brain regions (Maurer et al., 2001; Wiesel, 1992). In addition, selective reductions in COX activity were reported in brains of AD patients, underscoring that alterations in behavior and cognition are (at least) associated with COX function (Maurer et al., 2000).

In previous studies using the MSA model, we observed a significant increase in corticosterone levels in the Agg group as compared to Sub, suggesting that re-grouping is a stressor factor (Oliveira et al., 2013). Aggressive animals also displayed a significant increase in dopamine production in all brain regions, particularly in the pre-frontal cortex (Oliveira et al., 2014). It is known that corticosterone modulates the CNS, establishing a relationship between the HPA axis and dopamine-mediated behavior responsiveness (Seo et al., 2008; Barzman et al., 2010). Also, the central dopaminergic system exerts positive effects on the HPA axis while glucocorticoids and catecholamines mediated stress-induced alterations (Cabib and Puglisi-Allegra, 1996; Steketee and Kalivas, 2011). Indeed, dopamine modulates electron transport system (ETS) function, inhibiting complex I activity (Przedborski et al., 1993), with direct impacts on ATP-linked respiration, and on COX activity (Czerniczyniec et al., 2007; Glinka and Youdim, 1995; Khan et al., 2005). Despite this, we do not think that increased dopamine levels would *per se* explain the results observed in the present work, as both ATP linked respiration (Fig. 3B) and the NADH:cytochrome *c* oxidoreductase activity (Fig. 4C) were hardly affected by social stress. Therefore, future research is necessary to determine the exact mechanism by which brain COX activity is selectively inhibited by social stress.

In the present work, we observed that COXVIIa protein levels were reduced in all re-grouped animals (Fig. 5B), indicating that a factor released during re-grouping itself caused the specific reduction in this COX subunit. However, significant reductions in COX activity were only observed in aggressive animals (Fig. 4A and B), suggesting that other factors might be involved to further reduce this ETS complex activity. Despite that, ATP-linked and uncoupled respiratory rates in frontal cortex were not affected in aggressive animals (Fig. 3B and D), indicating that the capacity to synthesize ATP was not compromised when mitochondria oxidize glutamate and malate as substrates. Conceivably, reduced COX activity in aggressive animals might accumulate upstream metabolites of TCA cycle and other metabolic pathways, with potential consequences to energy and redox homeostasis in the CNS. In line with this assumption, one might consider that, in normal conditions, respiratory rates *in vivo* are not as high as those measured *ex vivo* in our experimental conditions, which might underscore potentially unassigned roles that limited COX activity might play in brain tissue during social stress. Finally, we do not think that changes in mitochondrial content among groups would explain the differences observed in COX content and activity as social stress caused no significant changes on *i)* respiratory rates provided by glutamate and malate (Fig. 3), *ii)* the levels of voltage dependent anion channel 2 (VDAC 2), as well as ATP synthase and ANT, markers of mitochondrial outer and inner membranes content respectively (Fig. 5) and *iii)* NADH:cytochrome *c* oxidoreductase activity (Fig. 4C). We thus concluded that the effects observed in the present work are not a general consequence of reduced mitochondrial content, by increased mitophagy or disrupted biogenesis, but rather to regulatory changes promoted by social stress.

The structure and function of COX are affected in a wide variety of diseases, and enzyme dysfunction was associated with increased mitochondrial ROS production and cellular toxicity (Srinivasan and Avadhani, 2012; Villani et al., 1998). Conceivably, reduced COX activity in aggressive animals might be a consequence of different factors including: *i)* mitochondrial nitric oxide-dependent inhibition of COX by high dopamine levels (Czerniczyniec et al., 2007); *ii)* protein kinase A-mediated phosphorylation of COX that potentiate the inhibitory effects of ATP (Bender and Kadenbach, 2000; Srinivasan et al., 2013); *iii)* the energy demand, as high ATP/ADP ratio allosterically inhibits COX activity (Arnold and Kadenbach, 1997); *iv)* the presence of carbon monoxide, resulted from heme oxygenase activity (Taskiran et al., 2007). Despite the exact mechanism by which COX activity is downregulated by social stress was not determined in the present work, it is possible that aggressive animals exhibit changes in redox and energy metabolism as a consequence of impaired COX function (Srinivasan and Avadhani, 2012).

5. Conclusions

The results described here indicate that social stress promoted by re-grouping strongly and selectively reduced COX activity in brain cortex in highly aggressive mice. In addition, we observed that re-grouping by itself reduced specifically COX expression in brain cortex of all groups tested. Although mitochondrial stimulated O_2 consumption rates and NADH:cytochrome *c* oxidoreductase activity were not significantly affected, the observations reported here, and the supportive literature evidence (Alaux et al., 2009; Li-Byarlay et al., 2014) raise the possibility that reduced COX activity in brain cortex might induce a shift in energy metabolism towards glycolysis in aggressive animals to maintain brain energy demand. We think that a better understanding of the molecular events that mediate specific reductions in COX activity upon social stress, and the consequences to CNS energy and redox metabolism in highly aggressive animals, might help the development of novel intervention and management strategies to avoid and control the behavioral consequences of social stress. Further research will be necessary to shed a light on these metabolic aspects in the setting of social stress.

Acknowledgements

We would like to thank Drs. Maria de Nazaré Corrêa Soeiro, head of the Laboratório de Biologia Celular (IOC/FIOCRUZ) and Solange Lisboa de Castro for helpful discussions and the logistic support to this research. This work was supported by grants from Conselho Nacional de Desenvolvimento Cientifico e Tecnológico (CNPq), to MFO (#307636/2012-7, 402409/2012-4, 404153/2016-0, 303044/2017-9, INCT_EM, 16/2014), Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ) (#E26/102.333/2013, E-26/203.043/2016), and FAPERJ to GMO (#E26/211.392/2015) and Fundação Oswaldo Cruz (through institutional funding) to VMSF. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.neuint.2019.03.017.

References

Aboul-Fotouh, S., 2013. Chronic treatment with coenzyme Q10 reverses restraint stress-induced anhedonia and enhances brain mitochondrial respiratory chain and creatine kinase activities in rats. Behav. Pharmacol. 24, 552–560.

Adzic, M., Djordjevic, A., Demonacos, C., Krstic-Demonacos, M., Radojcic, M.B., 2009. The role of phosphorylated glucocorticoid receptor in mitochondrial functions and apoptotic signalling in brain tissue of stressed Wistar rats. Int. J. Biochem. Cell Biol. 41, 2181–2188.

Alaux, C., Sinha, S., Hasadsri, L., Hunt, G.J., Guzmán-Novoa, E., DeGrandi-Hoffman, G., Uribe-Rubio, J.L., Southey, B.R., Rodriguez-Zas, S., Robinson, G.E., 2009. Honey bee aggression supports a link between gene regulation and behavioral evolution. Proc. Natl. Acad. Sci. Unit. States Am. 106, 15400–15405.

Arnold, S., Kadenbach, B., 1997. Cell respiration is controlled by ATP, an allosteric inhibitor of cytochrome-c oxidase. Eur. J. Biochem. 249, 350–354.

Arnold, S., Kadenbach, B., 1999. The intramitochondrial ATP/ADP-ratio controls cytochrome c oxidase activity allosterically. FEBS Lett. 443, 105–108.

Barzman, D.H., Patel, A., Sonnier, L., Strawn, J.R., 2010. Neuroendocrine aspects of pediatric aggression: can hormone measures be clinically useful? Neuropsychiatric Dis. Treat. 6, 691–697.
Batista, W.S., Silva, L.C.C.P. da, Demarque, K.C., Oliveira, F.S. de, Acquarone, M., Bôas, F.V., Oliveira, G.M. de, 2012. Aggressive behavior of mice in a vivarium: application of the spontaneous aggressiveness model (MEA). Rev. Soc. Bras. Ciênc. Em Animais Laboratório 1, 322–335.
Benani, A., Barquissau, V., Carneiro, L., Salin, B., Colombani, A.-L., Leloup, C., Casteilla, L., Rigoulet, M., Pénicaud, L., 2009. Method for functional study of mitochondria in rat hypothalamus. J. Neurosci. Methods 178, 301–307.
Bender, E., Kadenbach, B., 2000. The allosteric ATP-inhibition of cytochrome c oxidase activity is reversibly switched on by cAMP-dependent phosphorylation. FEBS Lett. 466, 130–134.
Blanchard, D.C., Blanchard, R.J., 2003. What can animal aggression research tell us about human aggression? Horm. Behav., Special Issue on Aggressive and Violent Behavior 44, 171–177.
Blanchard, D.C., Summers, C.H., Blanchard, R.J., 2013. The role of behavior in translational models for psychopathology: functionality and dysfunctional behaviors. Neurosci. Biobehav. Rev. 37, 1567–1577.
Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254.
Brown, G.C., Cooper, C.E., 1994. Nanomolar concentrations of nitric oxide reversibly inhibitsynaptosomal respiration by competing with oxygen at cytochrome oxidase. FEBS Lett. 356, 295–298.
Cabib, S., Puglisi-Allegra, S., 1996. Stress, depression and the mesolimbic dopamine system. Psychopharmacology (Berl.) 128, 331–342.
Capaldi, R.A., 1990. Structure and function of cytochrome c oxidase. Annu. Rev. Biochem. 59, 569–596.
Cavelier, L., Jazin, E.E., Eriksson, I., Prince, J., Båve, U., Oreland, L., Gyllensten, U., 1995. Decreased cytochrome-c oxidase activity and lack of age-related accumulation of mitochondrial DNA deletions in the brains of schizophrenics. Genomics 29, 217–224.
Chandrasekaran, S., Rittschof, C.C., Djukovic, D., Gu, H., Raftery, D., Price, N.D., Robinson, G.E., 2015. Aggression is associated with aerobic glycolysis in the honey bee brain 1. Genes Brain Behav. 14, 158–166.
Czerniczyniec, A., Bustamante, J., Lores-Arnaiz, S., 2007. Dopamine enhances mtNOS activity: implications in mitochondrial function. Biochim. Biophys. Acta 1767, 1118–1125.
Filipović, D., Zlatković, J., Inta, D., Bjelobaba, I., Stojiljkovic, M., Gass, P., 2011. Chronic isolation stress predisposes the frontal cortex but not the hippocampus to the potentially detrimental release of cytochrome c from mitochondria and the activation of caspase-3. J. Neurosci. Res. 89, 1461–1470.
Fragoso, V.M. da S., Hoppe, L.Y., de Araújo-Jorge, T.C., de Azevedo, M.J., Campos, J.D. de S., Cortez, C.M., de Oliveira, G.M., 2016. Use of haloperidol and risperidone in highly aggressive Swiss Webster mice by applying the model of spontaneous aggression (MSA). Behav. Brain Res. 301, 110–118.
Garabadu, D., Ahmad, A., Krishnamurthy, S., 2015. Risperidone attenuates modified stress-Re-stress paradigm-induced mitochondrial dysfunction and apoptosis in rats exhibiting post-traumatic stress disorder-like symptoms. J. Mol. Neurosci. MN 56, 299–312.
Glinka, Y.Y., Youdim, M.B.H., 1995. Inhibition of mitochondrial complexes I and IV by 6-hydroxydopamine. Eur. J. Pharmacol. Environ. Toxicol. Pharmacol. 292, 329–332.
Gong, Y., Chai, Y., Ding, J.-H., Sun, X.-L., Hu, G., 2011. Chronic mild stress damages mitochondrial ultrastructure and function in mouse brain. Neurosci. Lett. 488, 76–80.
Helling, S., Hüttemann, M., Ramzan, R., Kim, S.H., Lee, I., Müller, T., Langenfeld, E., Meyer, H.E., Kadenbach, B., Vogt, S., Marcus, K., 2012. Multiple phosphorylations of cytochrome c oxidase and their functions. Proteomics 12, 950–959.
Hollis, F., van der Kooij, M.A., Zanoletti, O., Lozano, L., Cantó, C., Sandi, C., 2015. Mitochondrial function in the brain links anxiety with social subordination. Proc. Natl. Acad. Sci. U. S. A 112, 15486–15491.
Khan, F.H., Sen, T., Maiti, A.K., Jana, S., Chatterjee, U., Chakrabarti, S., 2005. Inhibition of rat brain mitochondrial electron transport chain activity by dopamine oxidation products during extended in vitro incubation: implications for Parkinson's disease. Biochim. Biophys. Acta 1741, 65–74.
Kirby, D.M., Thorburn, D.R., Turnbull, D.M., Taylor, R.W., 2007. Biochemical assays of respiratory chain complex activity. Methods Cell Biol. 80, 93–119.
Li-Byarlay, H., Rittschof, C.C., Massey, J.H., Pittendrigh, B.R., Robinson, G.E., 2014. Socially responsive effects of brain oxidative metabolism on aggression. Proc. Natl. Acad. Sci. Unit. States Am. 111, 12533–12537.
Lobanov, S.A., Danilov, A.V., Danilov, E.V., Asaeva, S.K., Arslanova, G.F., 2007. Morphofunctional changes in mitochondria during stress. Bull. Exp. Biol. Med. 144, 849–852.
Maurer, I., Zierz, S., Möller, H.J., 2000. A selective defect of cytochrome c oxidase is present in brain of Alzheimer disease patients. Neurobiol. Aging 21, 455–462.
Maurer, I., Zierz, S., Möller, H., 2001. Evidence for a mitochondrial oxidative phosphorylation defect in brains from patients with schizophrenia. Schizophr. Res. 48, 125–136.
Miczek, K.A., Fish, E.W., de Bold, J.F., de Almeida, R.M., 2002. Social and neural determinants of aggressive behavior: pharmacotherapeutic targets at serotonin, dopamine and γ-aminobutyric acid systems. Psychopharmacology (Berl.) 163, 434–458.
Morava, E., Kozicz, T., 2013. Mitochondria and the economy of stress (mal)adaptation. Neurosci. Biobehav. Rev. 37, 668–680.
Nadanovsky, P., Celeste, R.K., Wilson, M., Daly, M., 2009. Homicide and impunity: an ecological analysis at state level in Brazil. J. Public Health 43, 733–742.
Nelson, R.J., Trainor, B.C., 2007. Neural mechanisms of aggression. Nat. Rev. Neurosci. 8, 536–546.
Oliveira, F. da S., Rodrigues, F.V.B., Demarque, K.C., Rangel, J.A., Alvarenga, T.A., Batista, W.S., Gameiro, L. dos S., Oliveira, G. de, 2013. Characterization of dominant and subordinate social status and the structure of the social hierarchy in swiss webster mice. J. Braz. Soc. Sci. Lab. Anim. 2, 136–146.
Oliveira, F. da S., Demarque, K.C., Rangel, J.A., Batista, W.S., Gameiro, L. dos S., Oliveira, G. de, 2014. Description by HPLC of the concentration of norepinephrine, dopamine and serotonin in different brain regions of highly aggressive Swiss Webster mice. J. Braz. Soc. Sci. Lab. Anim. 2, 254–263.
Pesta, D., Gnaiger, E., 2012. High-resolution respirometry: OXPHOS protocols for human cells and permeabilized fibers from small biopsies of human muscle. Methods Mol Biol. 810, 25–258.
Picard, M., McEwen, B.S., 2018. Psychological stress and mitochondria: a conceptual framework. Psychosom. Med. 80, 126–140.
Picard, M., McManus, M.J., Gray, J.D., Nasca, C., Moffat, C., Kopinski, P.K., Seifert, E.L., McEwen, B.S., Wallace, D.C., 2015. Mitochondrial functions modulate neuroendocrine, metabolic, inflammatory, and transcriptional responses to acute psychological stress. Proc. Natl. Acad. Sci. U. S. A 112, E6614–E6623.
Picard, M., McEwen, B.S., Epel, E.S., Sandi, C., 2018. An energetic view of stress: focus on mitochondria. Front. Neuroendocrinol. 49, 72–85.
Przedborski, S., Jackson-Lewis, V., Muthane, U., Jiang, H., Ferreira, M., Naini, A.B., Fahn, S., 1993. Chronic levodopa administration alters cerebral mitochondrial respiratory chain activity. Ann. Neurol. 34, 715–723.
Rezin, G.T., Cardoso, M.R., Gonçalves, C.L., Scaini, G., Fraga, D.B., Riegel, R.E., Comim, C.M., Quevedo, J., Streck, E.L., 2008. Inhibition of mitochondrial respiratory chain in brain of rats subjected to an experimental model of depression. Neurochem. Int. 53, 395–400.
Rosety-Rodriguez, M., Ordonez, F.J., Rosety, I., Frias, L., Rosety, M.A., Rosety, J.M., Rosety, M., 2006. 8-weeks training program attenuates mitochondrial oxidative stress in the liver of emotionally stressed rats. Histol. Histopathol. 21, 1167–1170.
Sakata, J.T., Crews, D., Gonzalez-Lima, F., 2005. Behavioral correlates of differences in neural metabolic capacity. Brain Res. Brain Res. Rev. 48, 1–15.
Seo, D., Patrick, C.J., Kennealy, P.J., 2008. Role of serotonin and dopamine system interactions in the neurobiology of impulsive aggression and its comorbidity with other clinical disorders. Aggress. Violent Behav. 13, 383–395.
Srinivasan, S., Avadhani, N.G., 2012. Cytochrome c oxidase dysfunction in oxidative stress. Free Radic. Biol. Med. 53, 1252–1263.
Srinivasan, S., Spear, J., Chandran, K., Joseph, J., Kalyanaraman, B., Avadhani, N.G., 2013. Oxidative stress induced mitochondrial protein kinase a mediates cytochrome C oxidase dysfunction. PLoS One 8, e77129.
Steketee, J.D., Kalivas, P.W., 2011. Drug wanting: behavioral sensitization and relapse to drug-seeking behavior. Pharmacol. Rev. 63, 348–365.
Steru, L., Chermat, R., Thierry, B., Simon, P., 1985. The tail suspension test: a new method for screening antidepressants in mice. Psychopharmacology (Berl.) 85, 367–370.
Taskiran, D., Nesil, T., Alkan, K., 2007. Mitochondrial oxidative stress in female and male rat brain after ex vivo carbon monoxide treatment. Hum. Exp. Toxicol. 26, 645–651.
van der Kooij, M.A., Hollis, F., Lozano, L., Zalachoras, I., Abad, S., Zanoletti, O., Grosse, J., Guillot de Suduiraut, I., Canto, C., Sandi, C., 2018. Diazepam actions in the VTA enhance social dominance and mitochondrial function in the nucleus accumbens by activation of dopamine D1 receptors. Mol. Psychiatr. 23, 569–578.
Vélez-Hernández, M.E., Padilla, E., Gonzalez-Lima, F., Jiménez-Rivera, C.A., 2014. Cocaine reduces cytochrome oxidase activity in the prefrontal cortex and modifies its functional connectivity with brainstem nuclei. Brain Res. 1542, 56–69.
Villani, G., Greco, M., Papa, S., Attardi, G., 1998. Low reserve of cytochrome c oxidase capacity in vivo in the respiratory chain of a variety of human cell types. J. Biol. Chem. 273, 31829–31836.
Wiesel, F.A., 1992. Glucose metabolism in psychiatric disorders: how can we facilitate comparisons among studies? J. Neural Transm. Suppl. 37, 1–18.
World Health Organization, 2002. World Report on Violence and Health: Summary. Geneva. . https://www.who.int/violence_injury_prevention/violence/world_report/en/.
Xing, G., Barry, E.S., Benford, B., Grunberg, N.E., Li, H., Watson, W.D., Sharma, P., 2013. Impact of repeated stress on traumatic brain injury-induced mitochondrial electron transport chain expression and behavioral responses in rats. Front. Neurol. 4, 196.
Zuckerbraun, B.S., Chin, B.Y., Bilban, M., d'Avila, J. de C., Rao, J., Billiar, T.R., Otterbein, L.E., 2007. Carbon monoxide signals via inhibition of cytochrome c oxidase and generation of mitochondrial reactive oxygen species. FASEB J. 21, 1099–1106.