Neurochemical mechanisms underlying acute and chronic ethanol-mediated responses in zebrafish: the role of mitochondrial bioenergetics

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Abstract

Ethanol (EtOH) is a socially-accepted drug, whose consumption is a risk factor for non-intentional injuries, development of pathologies, and addiction. In the brain, EtOH affects redox signaling and increases reactive oxygen species (ROS) production after acute and chronic exposures. Here, using a high-resolution respirometry assay, we investigated whether changes in mitochondrial bioenergetics play a role in both acute and chronic EtOH-mediated neurochemical responses in zebrafish. For the first time, we showed that acute and chronic EtOH exposures differently affect brain mitochondrial function. Acutely, EtOH stimulated mitochondrial respiration through increased baseline state, CI-mediated OXPHOS, OXPHOS capacity, OXPHOS coupling efficiency, bioenergetic efficiency, and ROX/ETS ratio. Conversely, EtOH chronically decreased baseline respiration, complex I- and II-mediated ETS, as well as increased ROX state and ROX/ETS ratio, which are associated with ROS formation. Overall, we observed that changes in mitochondrial bioenergetics play a role, at least partially, in both acute and chronic effects of EtOH in the zebrafish brain. Moreover, our findings reinforce the face, predictive, and construct validities of zebrafish models to explore the neurochemical bases involved in alcohol abuse and alcoholism.

Keywords: alcohol-mediated responses; high-resolution respirometry assay; mitochondrial bioenergetics; neuroscience; zebrafish.

1. Introduction

Ethanol (EtOH) is one of the most socially-accepted addictive drug worldwide (Gneiting and Schmitz, 2016). Alcohol consumption is a risk factor for accidents, development of pathologies, as well as addiction and alcoholism (Rehm, 2011). Alcohol-related chronic disorders constitute a substantial health and economic burden due to the occurrence of different types of diseases, including neuropsychiatric conditions (Ridley et al., 2013). These disorders contribute to the alcoholism-related high morbidity and mortality (Shield et al., 2013).

Evidence shows that acute and chronic ethanol exposures affect redox signaling and increase free radicals production in the central nervous system (CNS), which impairs proteins, carbohydrate, and fatty acid metabolism (Manzo-Avalos and Saavedra-Molina, 2010). Mitochondria play a key role in energy production via aerobic metabolism, and mitochondrial electron transport chain has been widely recognized as an endogenous source of reactive oxygen species (ROS) (Bolisetty and Jaimes, 2013). EtOH oxidation can affect mitochondria physiology, which culminates in the overproduction of ROS (Almansa et al., 2009). EtOH also impairs the membrane potential, decreases Ca^{2+} intracellular levels (Goodlett and Horn, 2001), and affects the mitochondrial electron transport system, thereby reducing ATP production and triggering neuronal death (Bailey et al., 1999; Cunningham and Van Horn, 2003; Guo et al., 2013).

In translational neuroscience research, the zebrafish (*Danio rerio*) is an emergent vertebrate tool for modeling human-related disorders (Fontana et al., 2018; Stewart et al., 2015). This species shares a high genomic conservation when compared to humans (Howe et al., 2013), and presents an evolutionarily conserved physiology (Holzschuh et al., 2001; Horzmann and Freeman, 2016; MacRae and Peterson, 2015). In zebrafish,

EtOH-mediated effects on behavior are concentration- and time-dependent, and redox imbalances occur following acute and chronic exposures (Muller et al., 2017; Rosemberg et al., 2012). Although EtOH modulates redox signaling and induces oxidative stress in this species, there are no data regarding whether such responses are due to changes in mitochondrial bioenergetics. Thus, the goal of this study was to verify whether changes in mitochondrial bioenergetics play a role in EtOH-mediated effects on the CNS of zebrafish using a high-resolution respirometry assay.

2. Materials and methods

2.1. Animals

Subjects were adult (4–6 months-old) short fin wild-type zebrafish (*Danio rerio*) of mixed genders (50:50 male:female ratio). Fish were obtained from a local supplier (Hobby Aquários, RS, Brazil) and acclimated in 40-L tanks for two weeks in a maximum density of four fish per liter. Tanks were filled with non-chlorinated water under constant mechanical, biological, and chemical filtration. Water temperature, pH, and conductivity were set at 28 ± 1 °C, 7.2 ± 0.5, and 400 ± 50 μS, respectively. Ammonia, nitrite, and nitrate values were kept lower than 0.2 ppm, 0.05 ppm, and 0.05 ppm, respectively. Animals were kept on a 14/10 light/dark photoperiod cycle (lights on at 7:00 am), water dissolved oxygen equal or above 95% saturation and fed with a commercial flake fish food (Alcon BASIC®, Alcon, Brazil) twice daily. All protocols were approved by the Ethics Commission on Animal Use of the Federal University of Santa Maria (process number 026/2014).

2.2. Alcohol exposure protocols

104 We used two protocols to investigate whether EtOH modulates mitochondrial
105 bioenergetics. To evaluate the acute effects of EtOH, fish were individually exposed to
106 1.0 % (v/v) EtOH (Merck, Darmstadt, Germany) for 1 hour (*n* = 5–7 animals per
107 group), a concentration that induces sedative/depressant-like behavior and impairs
108 oxidant processes in the zebrafish brain (Chatterjee and Gerlai, 2009; Rosemberg et al.,
109 2012). Chronically, EtOH was administered as described previously, using the
110 intermittent exposure protocol (Mathur and Guo, 2011; Muller et al., 2017). Briefly,
111 zebrafish were kept in housing tanks and exposed to 1.0 % (v/v) EtOH for 8 consecutive
112 days (20 min per day) and euthanized at 9th day. For both protocols, control fish were
113 handled in a similar manner, except that no EtOH was added. Importantly, no physical
114 abnormalities were observed during the exposure period. After euthanasia, the brains
115 were dissected and samples were prepared to further biochemical analyses.
116
117 *2.3. Mitochondrial respiration assays*
118 Mitochondrial activity was measured by high-resolution respirometry using an
119 Oxygraph-2k (O2k, Oroboros Instruments, Innsbruck, Austria). Briefly, 2–3 brains (~12
120 mg of tissue) were pooled per sample and homogenized in 120 µL of medium
121 containing 5 mM Tris-HCl (pH 7.4), 250 mM sucrose, and 2 mM EGTA. Samples were
122 homogenized gently with a pestle and 100 µL of homogenate was further transferred to
123 2 mL respiration buffer (115 mM KCl, 10 mM KH_2PO_4, 2 mM $MgCl_2$), 3 mM HEPES,
124 1 mM EGTA, essentially fatty acid-free BSA (0.2%, pH 7.2). The experiments were
125 performed at 28 °C using DatLab 4.0 software (Oroboros Inc., Austria), with continuous
126 stirring at 750 rpm (de Carvalho et al., 2017).
127 Using titration protocols (Aw, 2016; Carvalho et al., 2013), we assessed the
128 influence of various substrates and inhibitors in mitochondrial function as reflected in

129 different respiration states. Glutamate + pyruvate + malate and succinate were used as
130 oxidizable substrates. We determined the changes in mitochondrial respiratory chain
131 complexes, respiratory rates, and the production of oxidative oxygen species.

132 After signal stabilization, the baseline respiration supported by endogenous
133 substrates was measured. The complex I (CI)-mediated leak (LEAK*;* L(n)) respiration
134 was determined using 5 mM pyruvate, 5 mM glutamate and 1 mM malate. CI-mediated
135 oxidative phosphorylation (OXPHOS) was tested using 2.5 mM ADP. The convergent
136 electron flow during the maximal OXPHOS respiration (CI + CII_{OXPHOS}) was
137 determined with substrates of CI and CII (10 mM succinate). To induce LEAK state, we
138 added 2 μg/mL oligomycin, an inhibitor of ATP synthase by blocking its proton
139 channel. The electron transport system (ETS) respiration represents the uncoupled
140 respiration, which was measured using carbonyl cyanide 4-(trifluoromethoxy)
141 phenylhydrazone (FCCP) as uncoupler (optimum concentration reached between 0.5
142 and 1.5 μM); CI + CII-mediated ETS respiration (CI + CII_{ETS}) was determined in the
143 presence of FCCP, while CII-mediated ETS respiration (CII_{ETS}) was measured in the
144 presence of 0.5 μM rotenone. The addition of 2.5 μM antimycin A was performed to
145 inhibit complex III activity, which abolished mitochondrial respiration. Then, the
146 residual oxygen consumption (ROX) with small contributions from electron leak in the
147 uncoupled state was measured. We also determined the magnitude of residual oxygen
148 consumption relative to the maximum oxygen consumption (expressed as fold change
149 of ROX/ETS ratio), ETS/OXPHOS ratio, OXPHOS capacity, and OXPHOS coupling
150 efficiency, which is based on the ratio of free to total OXPHOS capacity (1-L/P).
151 Mitochondrial bioenergetics capacity was quantified by subtracting the ADP-induced
152 CI_{OXPHOS} values from the CI_{LEAK}. Moreover, the respiratory control rates (RCR) were
153 measured as indicators of the mitochondrial coupling state (RCR = CI_{OXPHOS}/CI_{LEAK}

ratio), as well as the succinate control factor ($CI_P/CI+CII_O$, fold change). Substrate control ratio (SCR) (CI_{OXPHOS}/CII_{ETS} ratio) was quantified to evaluate the effects of EtOH on mitochondrial respiratory control.

2.4. Statistics

Normality of data and homogeneity of variances were analyzed by Kolmogorov-Smirnov and Bartlett's tests, respectively. Because results were normally distributed and homoscedastic, data were expressed as means ± standard error of the mean (S.E.M.) and the effects on mitochondrial activity were analyzed by unpaired Student's *t* test, considering $p \leq 0.05$ as significant.

3. Results

3.1. EtOH acutely stimulates mitochondrial O_2 consumption

Fig. 1. depicts the acute effects of 1.0% EtOH exposure on the mitochondrial bioenergetics. EtOH-exposed group showed higher baseline respiration ($t_{(0.05;\ 8)} = 3.991$, $p = 0.004$) and complex I- induced oxidative phosphorylation (CI_{OPHOX}) ($t_{(0.05;\ 8)} = 3.265$, $p = 0.0114$) than control. However, CI_{LEAK} respiration, and complex I- and II-induced oxidative phosphorylation ($CI + CII_{OPHOX}$) did not change between groups. When the respiration was uncoupled by FCCP ($CI + CII_{ETS}$) no differences were observed. Moreover, the C_{IIETS} respiration and ROX values did not show significant differences between groups. EtOH exposure also increased ROX/ETS ratio ($t_{(0.05;\ 8)} = 3.639$, $p = 0.0066$) **(Fig. 2A)** and decreased ETS/OXPHOS ($t_{(0.05;\ 8)} = 6.088$, $p = 0.0006$) **(Fig. 2B)**. The OXPHOS capacity ($t_{(0.05;\ 8)} = 2.391$, $p = 0.0438$) **(Fig. 2C)**, OXPHOS coupling efficiency ($t_{(0.05;\ 8)} = 3.017$, $p = 0.0116$) **(Fig. 2D)**, bioenergetic efficiency ($t_{(0.05;\ 8)} = 2.695$, $p = 0.0273$) **(Fig. 2E)**, RCR ($t_{(0.05;\ 8)} = 2.791$, $p = 0.0235$) **(Fig. 2F)** increased after

EtOH exposure. Succinate control factor was lower ($t_{(0.05;\ 8)}$ = 2.528, p = 0.0353) **(Fig. 2G)** in EtOH group, while SCR increased ($t_{(0.05;\ 8)}$ = 3.037, p = 0.0161) following acute EtOH regimen **(Fig. 2H)**.

3.2. EtOH chronically impairs mitochondrial respiration

Fig. 3 shows the effects of 1.0% chronic EtOH exposure on mitochondrial respiration. EtOH decreased the baseline state ($t_{(0.05;\ 10)}$ = 2.783, p = 0.0193), while no changes in CI_{LEAK} respiration, as well as in complex I- and II-induced oxidative phosphorylation ($CI_{OPHOX,}$ CI + CII_{OPHOX}) were verified. EtOH-exposed fish showed decreased CI + CII_{ETS} ($t_{(0.05;\ 10)}$ = 2.817, p = 0.0183) and C_{IIETS} ($t_{(0.05;\ 10)}$ = 4.048, p = 0.0023) and higher ROX values ($t_{(0.05;\ 10)}$ = 3.84, p = 0.0033) and ROX/ETS ratio ($t_{(0.05;\ 10)}$ = 3.696, p = 0.0031) than control **(Fig. 4A).** Although the ETS/OXPHOS **(Fig. 4B),** OXPHOS capacity **(Fig. 4C),** OXPHOS coupling efficiency **(Fig. 4D),** bioenergetic efficiency **(Fig. 4E)**, RCR **(Fig. 4F)**, and succinate control factor **(Fig. 4G)** did not change, SCR was increased ($t_{(0.05;\ 8)}$ = 2.593, p = 0.0268) after EtOH exposure **(Fig. 4H)**. **Fig. 5** shows a schematic representation of the energy metabolism of acetate from EtOH catabolism **(Fig. 5A)** and the main effects of acute and chronic EtOH exposures on mitochondrial bioenergetics described **(Fig. 5B)**.

4. Discussion

Evidence has shown that EtOH can modulate redox signaling and induce oxidative stress in the zebrafish brain (Muller et al., 2017; Rosemberg et al., 2012). Oxidative stress is one of the main mechanisms associated with the harmful effects of EtOH in the brain (Augustyniak et al., 2005; Pereira et al., 2015; Sun et al., 2001; Sun and Sun, 2001), and mounting data support a crucial role of mitochondrial dysfunction

in alcohol-related neurotoxicity in various animal models (Pereira et al., 2015; Wu and Cederbaum, 2003; Yang and Luo, 2015; Zimatkin et al., 2006). However, there are no data reporting whether redox alterations in the CNS occur due to changes in mitochondrial respiration or via direct action of EtOH and its toxic metabolite acetaldehyde in zebrafish. While the acute EtOH exposure overstimulated mitochondrial O_2 consumption, EtOH chronically decreased mitochondrial respiration by negatively modulating the ETS activity. Therefore, our novel findings demonstrate that both acute and chronic EtOH exposures affect mitochondrial function by different mechanisms depending on the administration protocol.

EtOH acutely stimulated mitochondrial respiration through increased in baseline respiration and CI_{OXPHOS}. OXPHOS capacity (directly related to CI electron flux), coupling efficiency, and bioenergetics efficiency also increased after acute EtOH exposure, reinforcing the EtOH stimulatory effect on mitochondrial O_2 consumption. The enhanced baseline respiration and CI_{OXPHOS} may be related with EtOH metabolism pathway in the brain, which increases NADH levels during the oxidation process (Deitrich et al., 2006; Hipolito et al., 2007). The acetate from EtOH metabolism can be incorporated into acetyl-coenzyme A (acetyl-CoA), a substrate of the Krebs cycle, which increases the formation of reducing equivalents (Deng and Deitrich, 2008; Lieber, 2005). NADH plays a role in ATP generation during the OXPHOS, facilitating ATP production. However, excessive NADH formation may overstimulate CI complex, thereby generating the leak of electrons (Vinogradov and Grivennikova, 2016). This phenomenon may reflect higher mitochondrial O_2 consumption, which facilitates ROS formation (*e.g.*, $O_{2^{\cdot}}$, H_2O_2) (Bailey and Cunningham, 2002; Bailey et al., 1999; Hoek et al., 2002). Importantly, the reduction of NAD^+/NADH ratio as a consequence of EtOH metabolism can disrupt fatty acid oxidation and induce ketogenesis, lactic acidosis, and

hypoglycemia (Cunningham and Bailey, 2001; Haorah et al., 2013; Lieber, 2005; McGuire et al., 2006). Based on our findings, the increased OXPHOS following EtOH exposure does not result from changes in complex II activity. Although EtOH can acutely decrease ATP production (Budd and Nicholls, 1996; Liu et al., 2014), the mitochondrial overstimulation could facilitate ROS formation in the CNS (Hoek et al., 2002), corroborating the higher ROX/ETS ratio observed here. These results support a role of mitochondria in mediating oxidative stress in zebrafish, which showed impaired brain antioxidant enzyme activities and increased lipid peroxidation in our previous report (Rosemberg et al., 2010).

In addition to the acute exposure protocol, we explored the chronic effects of EtOH in zebrafish. Chronically, EtOH-exposed group showed a reduced baseline respiration as well as an impaired ETS, reflected by the lower CI + CII- and CII-mediated ETS. A dysfunction of CII-mediated respiration may overload other mitochondrial complexes, thereby affecting ETS and accentuating endogenous ROS formation. Importantly, the increased SCR suggest a main involvement of complex I in ETS. Moreover, the higher ROX state and ROX/ETS ratio corroborate with the increased ROS levels and pro-oxidant effects in the zebrafish brain described elsewhere (Muller et al., 2017). Thus, we suggest that part of the O_2 is not being consumed by mitochondria, but rather by other EtOH detoxification pathways (*e.g.*, catalase and CYP450 enzymes), which are directly involved in EtOH metabolism (Moghe et al., 2011). Oxidative damage after chronic EtOH exposure can alter the fluidity of the mitochondrial membrane (Kowaltowski et al., 2009; Tapia-Rojas, 2018), disrupt the mitochondrial membrane potential (Karadayian et al., 2015), and reduce the mitochondrial complexes I, III, and IV activities, which are necessary for ATP formation (Bustamante et al., 2012; Karadayian et al., 2015).

In conclusion, our novel findings show that EtOH affects the mitochondrial respiration in the zebrafish brain. Bioenergetic alterations in the CNS could be related to multifactorial mechanisms (*e.g.*, pro-oxidant properties of EtOH, ROS generation, and deregulation of OXPHOS), playing a central role in EtOH-mediated neurotoxicity. Due to the similarity of zebrafish CNS physiology with those of rodents and humans, this species can provide robust and translational data regarding the neurobiological bases of alcohol abuse and addiction, contributing to unravel novel therapeutic strategies. Moreover, this study also demonstrates the feasibility of the high-resolution respirometry assay as a promising strategy to assess mitochondrial bioenergetics in zebrafish models with high predictive, face, and construct validities.

Conflict of interest

The authors declare no conflict of interest.

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422

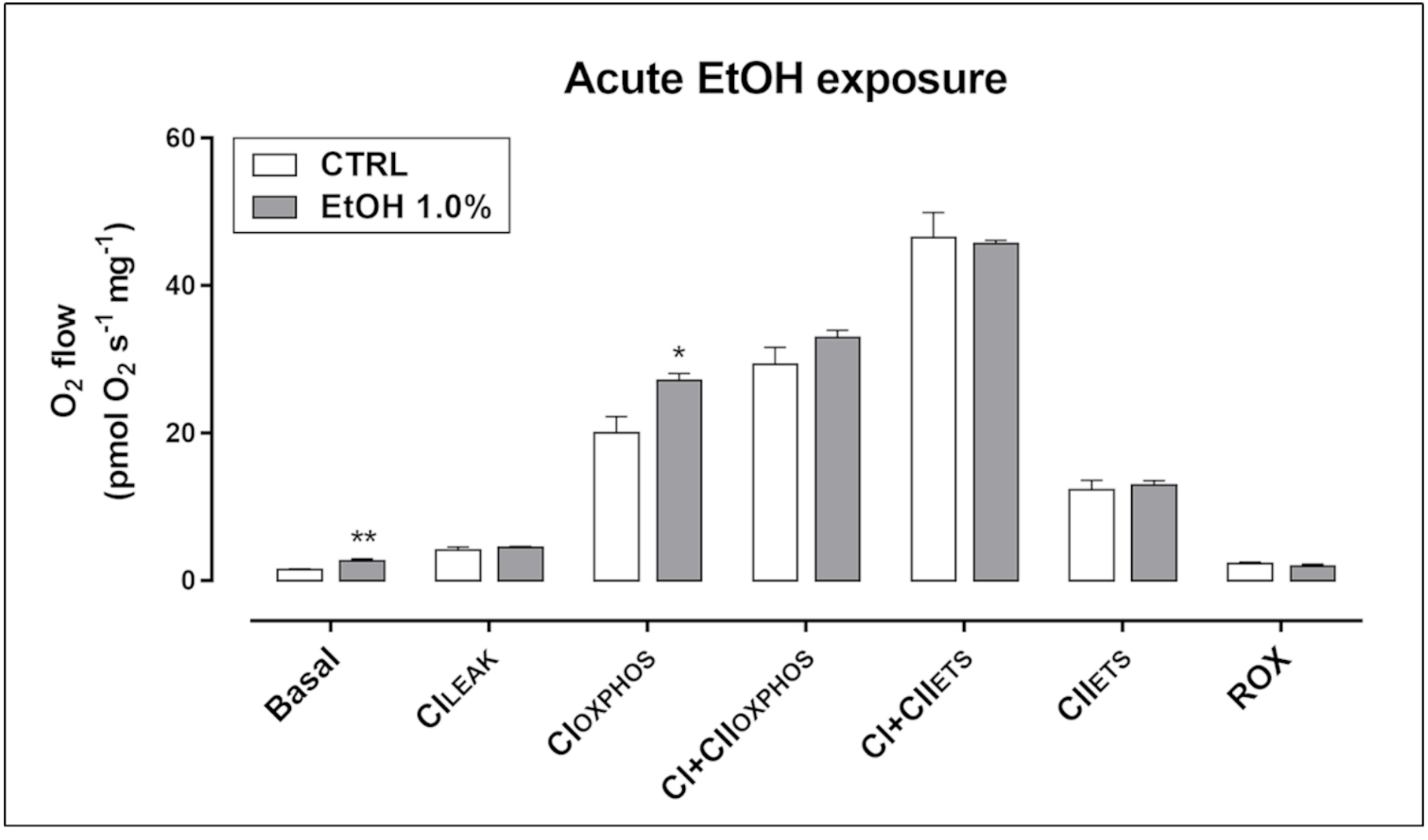
Figure Captions

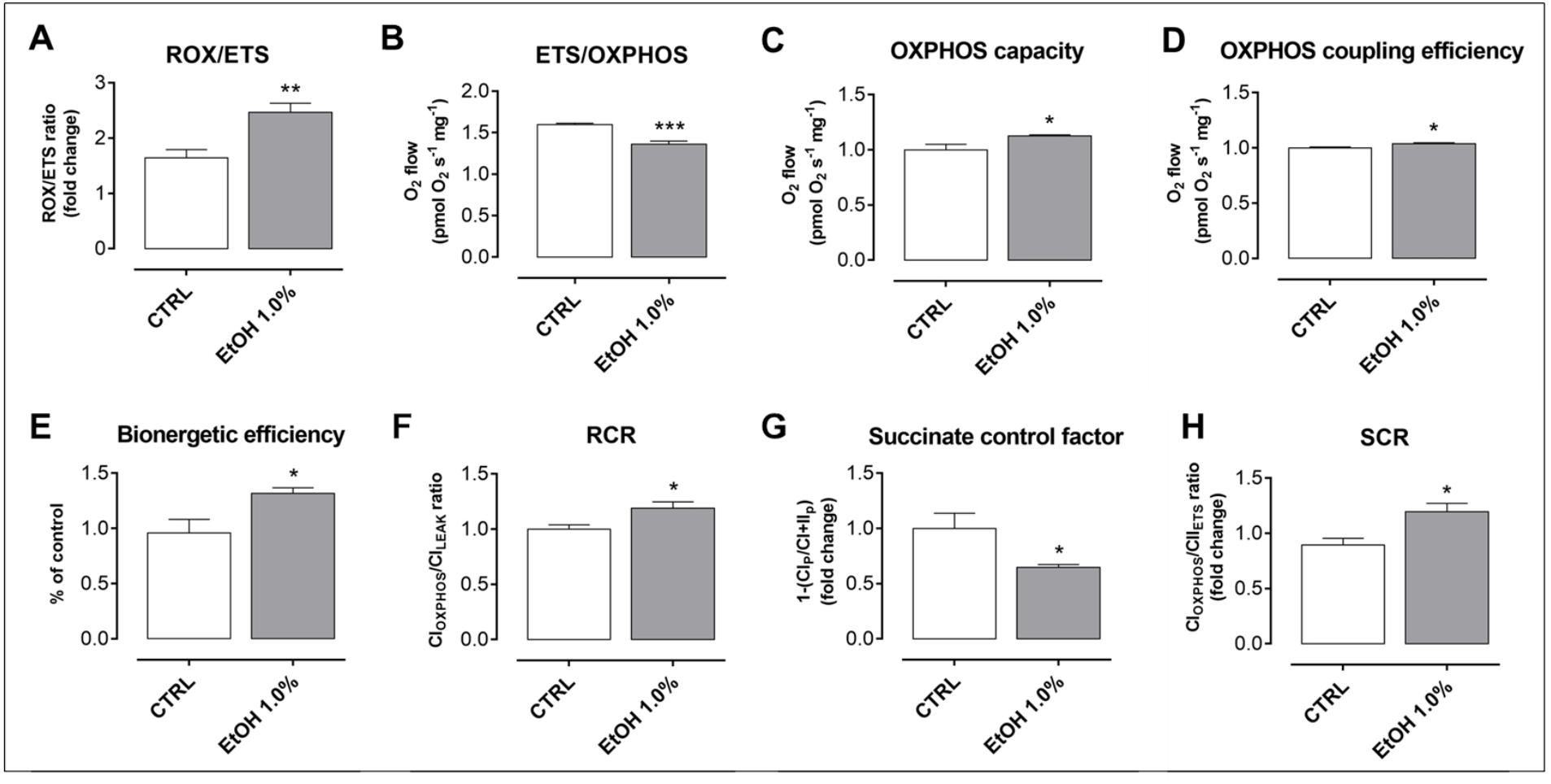
Fig. 1. Mitochondrial O_2 consumption in the zebrafish brain following acute 1.0% (v/v) EtOH exposure. Mitochondrial functions are presented with the abbreviation(s) of the complex(es) involved followed by the state of respiration measured in the presence of endogenous substrates (baseline), pyruvate + malate + glutamate (CI_{LEAK}), + ADP (CI_{OXPHOS}), + succinate (CI+ CII_{OXPHOS}), + FCCP (CI + CII_{ETS}), + rotenone (CII_{ETS}), + antimycin A (Ama) used to correct for residual O_2 consumption (ROX). Data were expressed as mean ± SEM and analyzed by unpaired Student's *t*-test (* $p < 0.05$, $n = 5$ per group).

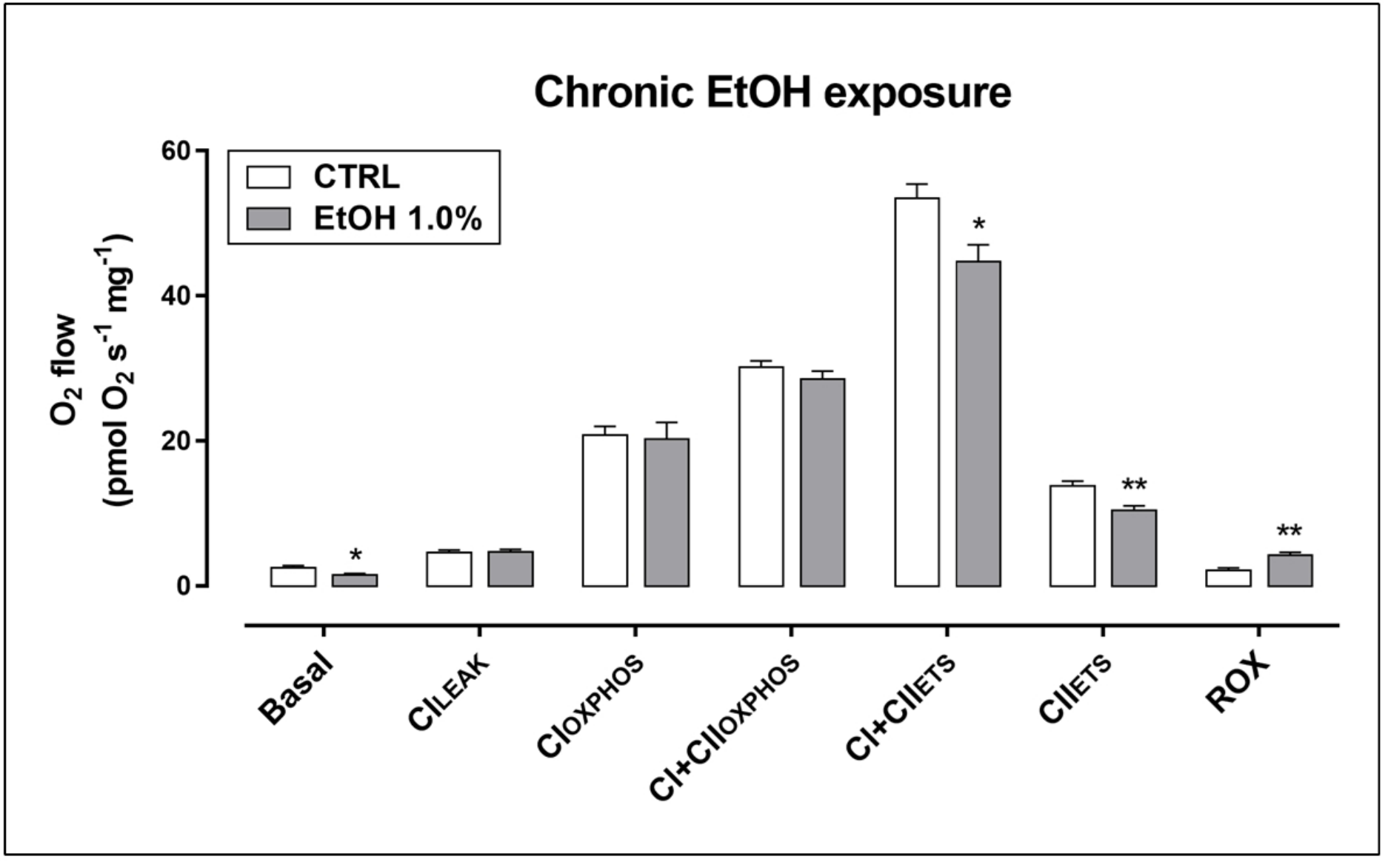
Fig. 2. Acute effects of 1.0% (v/v) EtOH on residual oxygen consumption (ROX/ETS ratio, fold change) **(A)**, ETS/OXPHOS **(B)**, OXPHOX capacity **(C)**, OXPHOS coupling efficiency **(D)**, bioenergetic efficiency (by subtracting the ADP-induced CI_{OXPHOS} values from the CI_{LEAK}) **(E)**, respiratory control rate (RCR = CI_{OXPHOS}/CI_{LEAK} ratio) **(F)**, succinate control ratio (CI_{P}/CI+CII_{O}, fold change) **(G)**, and substrate control ratio (SCR) (CI_{OXPHOS}/CII_{ETS} ratio, fold change) **(H)**. Data were expressed as mean ± SEM and analyzed by unpaired Student's *t*-test (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, $n = 5$ per group).

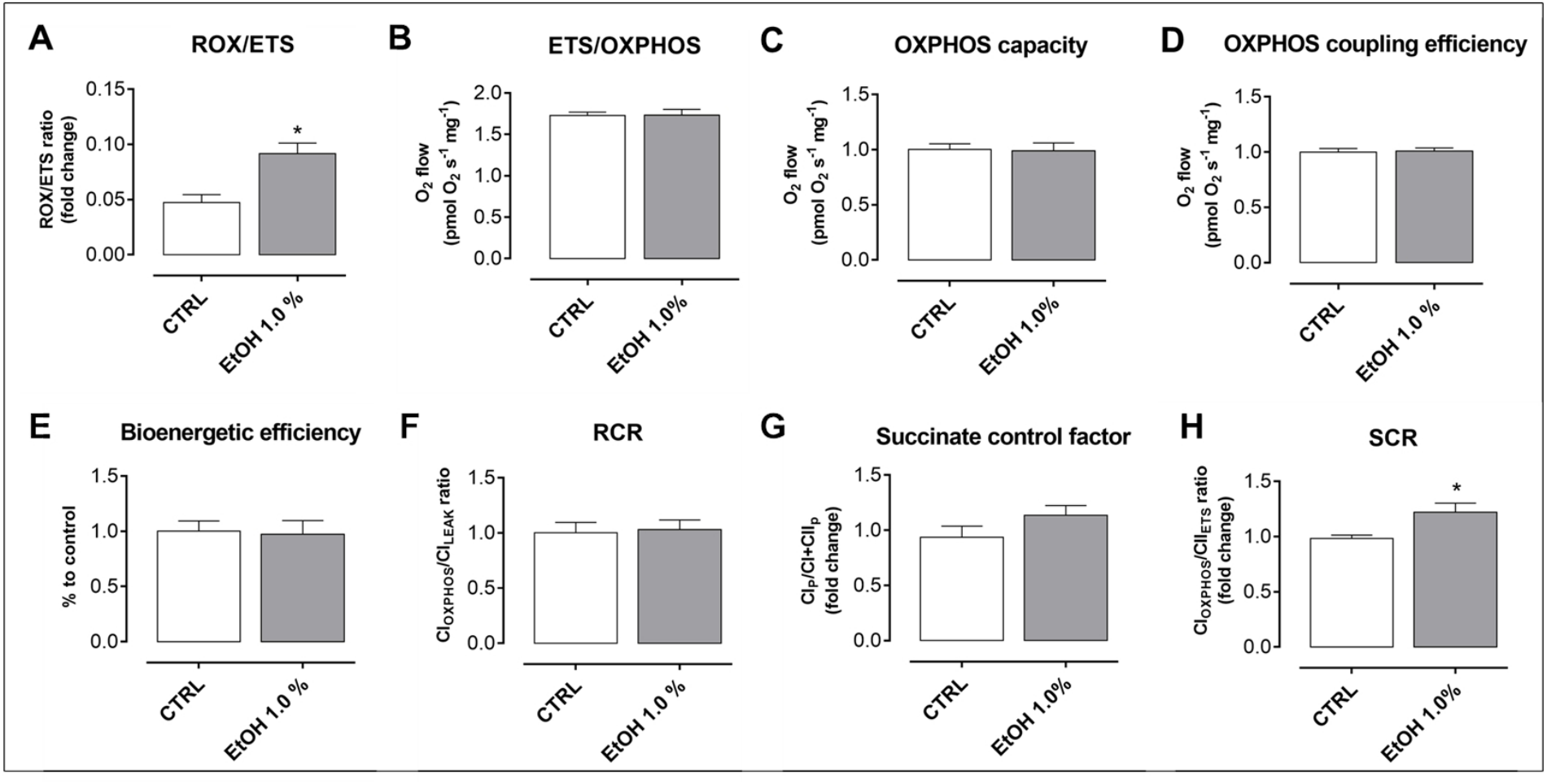
Fig. 3. EtOH (1.0%, v/v) chronically alters mitochondrial O_2 flow in the zebrafish brain. Mitochondrial functions are presented with the abbreviation(s) of the complex(es) involved followed by the state of respiration measured in the presence of endogenous substrates (baseline), pyruvate + malate + glutamate (CI_{LEAK}), + ADP (CI_{OXPHOS}), + succinate (CI+ CII_{OXPHOS}), + FCCP (CI + CII_{ETS}), + rotenone (CII_{ETS}), + antimycin A

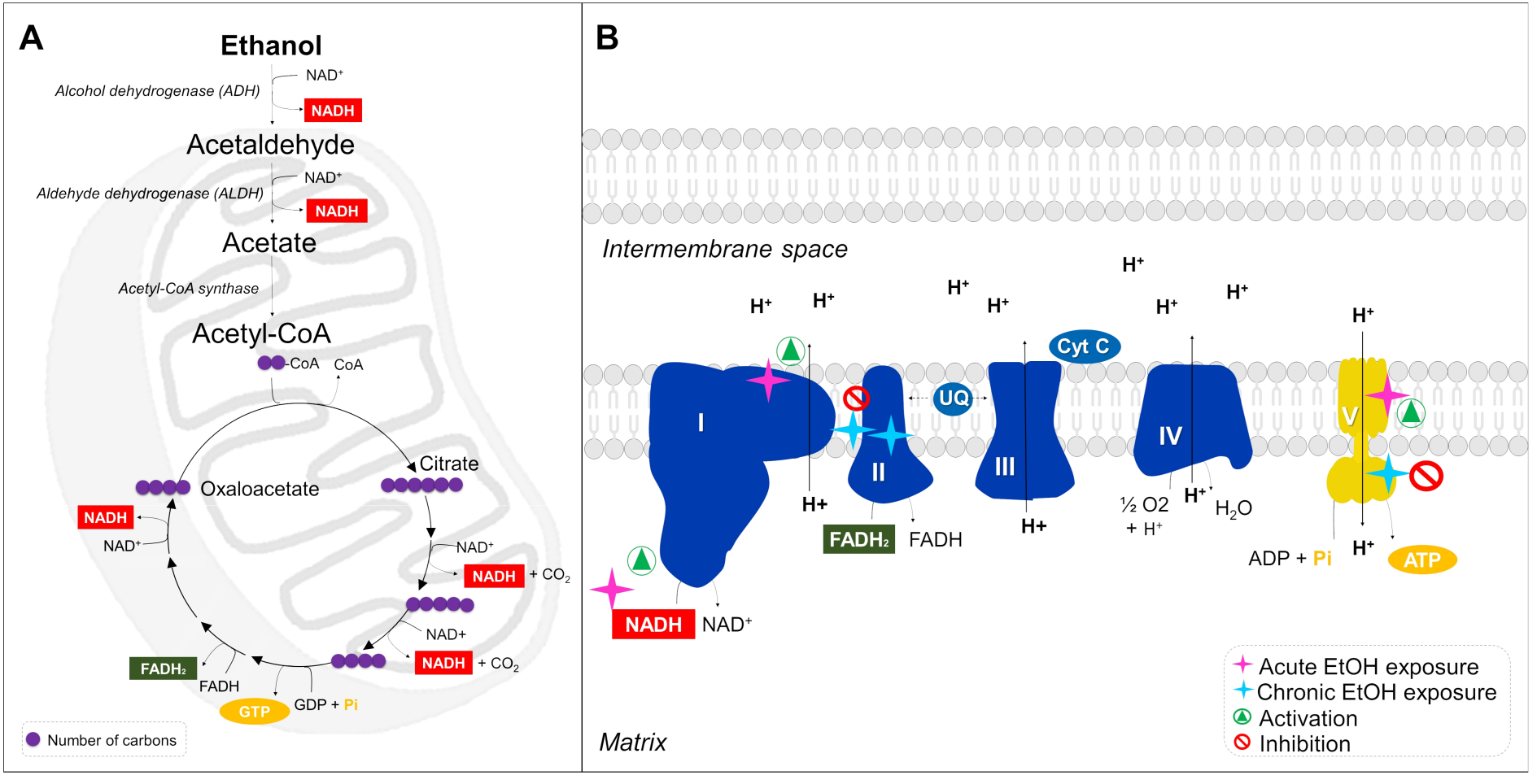
448 (Ama) used to correct for residual O_2 consumption (ROX). Data were expressed as
449 mean ± SEM and analyzed by unpaired Student's *t*-test. Data were expressed as mean ±
450 SEM and analyzed by unpaired Student's *t*-test (* $p < 0.05$, ** $p < 0.01$, $n = 6$ per
451 group).
452
453 **Fig. 4.** Effects of 1.0% (v/v) chronic EtOH exposure on residual oxygen consumption
454 (ROX/ETS ratio, fold change) **(A)**, ETS/OXPHOS **(B)**, OXPHOX capacity **(C)**,
455 OXPHOS coupling efficiency **(D)**, bioenergetic efficiency (by subtracting the ADP-
456 induced CI_{OXPHOS} values from the CI_{LEAK}) **(E)**, respiratory control rate (RCR =
457 CI_{OXPHOS}/CI_{LEAK} ratio) **(F)**, succinate control ratio ($CI_P/CI+CII_O$, fold change) **(G)**, and
458 substrate control ratio (SCR) (CI_{OXPHOS}/CII_{ETS} ratio, fold change) **(H)**. Data were
459 expressed as mean ± SEM and analyzed by unpaired Student's *t*-test (* $p < 0.05$, $n = 6$
460 per group).
461
462 **Fig. 5.** Schematic representation of the production of reduced equivalents via EtOH
463 catabolism **(A)** and mechanisms underlying the effects of acute and chronic EtOH
464 exposure in the zebrafish brain mitochondria **(B)**. EtOH acutely stimulates CI-mediated
465 OXPHOS, while the chronic exposure decreases complex I- and II-mediated ETS.


Acute EtOH exposure
CTRL
EtOH 1.0%
O2 flow
(pmol O2 s-1 mg-1)
60
40
20
0
**
*
Basal
CILEAK
CIOXPHOS
CI+CIIOXPHOS
CI+CIIETS
CIIETS
ROX


A
ROX/ETS
ROX/ETS ratio (fold change)
3
2
1
0
**
CTRL
EtOH 1.0%
B
ETS/OXPHOS
O2 flow (pmol O2 s-1 mg-1)
2.0
1.5
1.0
0.5
0.0
***
CTRL
EtOH 1.0%
C
OXPHOS capacity
O2 flow (pmol O2 s-1 mg-1)
1.5
1.0
0.5
0.0
*
CTRL
EtOH 1.0%
D
OXPHOS coupling efficiency
O2 flow (pmol O2 s-1 mg-1)
1.5
1.0
0.5
0.0
*
CTRL
EtOH 1.0%
E
Bionergetic efficiency
% of control
1.5
1.0
0.5
0.0
*
CTRL
EtOH 1.0%
F
RCR
CIOXPHOS/CILEAK ratio
1.5
1.0
0.5
0.0
*
CTRL
EtOH 1.0%
G
Succinate control factor
1-(CIP/CI+IIP) (fold change)
1.5
1.0
0.5
0.0
*
*
CTRL
EtOH 1.0%
H
SCR
CIOXPHOS/CIIETS ratio (fold change)
1.5
1.0
0.5
0.0
*
CTRL
EtOH 1.0%


Chronic EtOH exposure
CTRL
EtOH 1.0%
O2 flow
(pmol O2 s-1 mg-1)
60
40
20
0
*
*
**
**
Basal
CILEAK
CIOXPHOS
CI+CIIOXPHOS
CI+CIIETS
CIIETS
ROX


A
ROX/ETS
ROX/ETS ratio (fold change)
0.15
0.10
0.05
0.00
*
CTRL
EtOH 1.0 %
B
ETS/OXPHOS
O2 flow (pmol O2 s-1 mg-1)
2.0
1.5
1.0
0.5
0.0
CTRL
EtOH 1.0%
C
OXPHOS capacity
O2 flow (pmol O2 s-1 mg-1)
1.5
1.0
0.5
0.0
CTRL
EtOH 1.0%
D
OXPHOS coupling efficiency
O2 flow (pmol O2 s-1 mg-1)
1.5
1.0
0.5
0.0
CTRL
EtOH 1.0 %
E
Bioenergetic efficiency
% to control
1.5
1.0
0.5
0.0
CTRL
EtOH 1.0 %
F
RCR
CIOXPHOS/CILEAK ratio
1.5
1.0
0.5
0.0
CTRL
EtOH 1.0 %
G
Succinate control factor
CIP/CI+CIIP (fold change)
1.5
1.0
0.5
0.0
CTRL
EtOH 1.0%
H
SCR
CIOXPHOS/CIIETS ratio (fold change)
1.5
1.0
0.5
0.0
*
CTRL
EtOH 1.0%


A
Ethanol
Alcohol dehydrogenase (ADH)
NAD+
NADH
Acetaldehyde
Aldehyde dehydrogenase (ALDH)
NAD+
NADH
Acetate
Acetyl-CoA synthase
Acetyl-CoA
CoA
CoA
Citrate
Oxaloacetate
NADH
NAD+
NAD+
NADH + CO2
NAD+
NADH + CO2
FADH2
FADH
GTP
GDP + Pi
Number of carbons
B
Intermembrane space
H+
I
II
III
IV
V
UQ
Cyt C
FADH2
FADH
½ O2
+ H+
H2O
ADP + Pi
ATP
NADH
NAD+
Matrix
Acute EtOH exposure
Chronic EtOH exposure
Activation
Inhibition