HHS Public Access

Author manuscript

Neurochem Int. Author manuscript; available in PMC 2023 December 01.

Published in final edited form as:

Neurochem Int. 2022 December ; 161: 105434. doi:10.1016/j.neuint.2022.105434.

SOCIAL ISOLATION INDUCES SUCCINATE DEHYDROGENASE DYSFUNCTION IN ANXIOUS MICE

Saki Watanabe[1], **Alzahra J. Al Omran**[1], **Amy S. Shao**[2], **Zeyu Zhang**[3], **Chen Xue**[1], **Jifeng Zhang**[1], **Junji Watanabe**[3], **Jing Liang**[1]

[1]Titus Family Department of Clinical Pharmacy, University of Southern California, Los Angeles, CA, 90089, USA

[2]Homer Stryker M.D. School of Medicine, Western Michigan University, Kalamazoo, MI, 49007, USA

[3]Translational Research laboratory, School of Pharmacy, University of Southern California, Los Angeles, CA 90033, USA

Abstract

We have previously reported social isolation induces anxiety-like behavior, cognition decline, and reduction in brain ATP levels in mice. These changes were ameliorated by treatment with dihydromyricetin (DHM), a compound that positively modulates γ-aminobutyric A (GABAA) receptor. To gain further insight into the subcellular mechanisms underlying these changes, we utilized a social isolation-induced anxiety mouse model and investigated changes in mitochondrial oxidative capacity via the electron transport chain. We found that 4 weeks of social isolation decreased ATP levels by 43% and succinate dehydrogenase capacity by 52% of the control, while daily DHM (2 mg/kg oral) administration restored succinate dehydrogenase capacity. These results suggest that social isolation decreased mitochondrial capacity to generate ATP. DHM can be developed to be a therapeutic against anxiety and mitochondrial stress.

Keywords

Dihydromyricetin; Succinate Dehydrogenase; Complex II; Social Isolation; Anxiety; Stress

Corresponding author: Jing Liang, MD, PhD, 1985 Zonal Ave, PSC 504, Los Angeles, CA 90089, Tel: 323-442-3118, jliang1@usc.edu.

AUTHOR CONTRIBUTIONS

S.W., A.A.O., and J.L. contributed to the project idea and hypotheses. S.W., A.A.O., A.S.S., C.X., and J.Z. established the mice model. S.W. collected data and generated figures. S.W. and J.L. analyzed data, performed statistical analyses, and revised the manuscript. Z.Z. and J.W. contributed to methods and assisted with majority of lab instruments. A.A.O., Z.Z., C.X., and J.Z. assisted with running experiments throughout. S.W. wrote the original draft. S.W., A.A.O., A.S.S., C.X., J.Z., Z.Z., J.W., and J.L. reviewed and edited the paper. J.L. supervised the project.

COMPETING INTERESTS

The authors declare no financial interests or conflicts of interest.

1. INTRODUCTION

Stress provokes numerous physiological changes and responses as the body attempts to mitigate the stressor and restore homeostasis. Psychological stressors like social isolation induce responses that are associated with psychiatric disorders such as anxiety[1]. Anxiety disorders are one of the leading causes of disability in the United States and its prevalence has been on a significant upsurge since the 2019 pandemic and social isolation mandates[2–5]. Current treatments that aim to alleviate anxious symptoms are ineffective, and this challenge is compounded by the lack of disease mechanism understanding, which hinders effective anxiolytic development[3,6]. Thus, to develop a safe and effective novel anxiolytic, it is critical to uncover the cellular changes associated with anxiety pathogenesis.

Previously, we demonstrated that 4 weeks of social isolation induced significant anxiety-like behaviors and mild cognitive impairment in wild-type C57BL/6 mice[7,8]. In the same mice, we found reduction of γ-aminobutyric acid A receptors ($GABA_AR$) function, gephyrin expression, and ATP levels[9]. Gephyrin is a key ATP-dependent scaffolding protein that assists in proper $GABA_AR$ clustering and functioning[10]. Thus, our findings indicate that social isolation induces anxiety and cognitive impairment via disruption in gephyrin-GABAergic neurotransmission and synapse formation. Dihydromyricetin [(2R,3R)-3,5,7-trihydroxy-2-(3,4,5-trihydroxyphenyl)-2,3-dihydrochromen-4-one (DHM)], a compound that we have identified as a positive allosteric modulator (PAM) of $GABA_ARs$[11,12], was able to improve anxiety-like and cognition-related behaviors, $GABA_AR$ function, ATP levels, and gephyrin protein levels[7–9].

In recent years, the mitochondrion has been gaining research interest as modulators of and therefore a therapeutic target of anxiety[13]. Stemming from our findings that anxiety caused by social isolation leads to changes in ATP levels[9], we questioned the functional involvement of the mitochondria in this mechanism. In the current study, we utilized our social isolation-induced anxiety mouse model to investigate the mechanisms of subcellular (i.e. mitochondrial) responses to DHM and therapeutic effects to restore these changes. ATP is produced via a proton-driven pump, with the proton gradient mainly generated by the electron transport chain (ETC)[14–16]. Thus, we performed an electron flow assay to determine the ETC functionality in the mitochondria.

2. MATERIALS AND METHODS

2.1 Animals

All animal experiments and sacrifice were performed according to the protocols approved by the University of Southern California (USC) Institutional Animal Care and Use Committee (IACUC). All methods were carried out in compliance with relevant guidelines, regulations, and recommendations, including the ARRIVE guidelines. Six-week-old male C57BL/6 mice (Charles River Laboratories, Hollister, CA) were housed in the vivarium under a 12 h light/dark cycle with free access to food and water.

Social isolation-induced anxiety mouse models were established as described earlier[9]. In short, 6- to 8-week-old C57BL/6 mice (Charles River Laboratories, Hollister, CA) were

housed in the vivarium under a 12 h light/dark cycle with free access to food and water. Two to three mice were housed in each cage for group housing. Social isolation mice were housed singly in opaque cages, except for the top and bottom, to minimize interaction with neighboring cages. We minimally handled the mice and provided no environmental stimuli, such as toys, to prevent confounding stress. The groups were randomly divided as follows for a total of four weeks prior to sacrifice.

1. Group-housed mice with sucrose agar treatment (G2+Veh2)
2. Group-housed mice with DHM 2 mg/kg treatment (G2+D2)
3. Isolated mice with sucrose agar treatment (SI2+Veh2)
4. Isolated mice with DHM 2 mg/kg treatment (SI2+D2)

In the first two weeks of isolation or group housing, all groups did not receive any oral treatment, and then added daily oral administration of sucrose agar (vehicle) or DHM 2 mg/kg for two weeks of isolation or group housing. Mice were humanely euthanized via carbon dioxide (CO_2) exposure followed by rapid decapitation.

2.2 Drug preparation and administration

Sucrose agar and 2 mg/kg DHM (HPLC purified ≥98%, Master Herbs Inc., Pomona, CA) preparation as well as their administration were followed as described earlier[7–9]. In short, 3% agar was dissolved in ~90°C water, followed by DHM + 5% sucrose or 5% sucrose only until cooled and solidified. One small cube (0.5 cm^3) per mouse was administered orally in a small weigh boat, once a day for the last two weeks. Treatments were administered during the dark period of the 12-hour light/dark cycle with minimal disturbance to the animal. Complete consumption of the respective treatment was observed each day.

2.3 Behavioral assays

2.3.1 Elevated Plus Maze (EPM)—The EPM apparatus consists of two open arms and two closed arms surrounded by high opaque walls. The arms intersect perpendicularly with an open central area. The maze is elevated around 20–30 cm and illuminated with red light. Testing started by placing the mouse in the center of the maze facing one of the open arms and behavior recorded for 5 mins. Each mouse was scored offline for time spent in the open arm and closed arm.

2.3.2. Open Field (OF) Test—The OF chamber measured 50 cm (length) × 50 cm (width) × 38 cm (height) and was made from white acrylic plastic. The floor was divided (drawn) into 10 × 10 cm squares for a total of 25 squares. Mouse activity was assessed in open field for 10 min. The following parameters were scored offline: pathlength (cm) traveled in the apparatus and freezing time.

All scoring was conducted manually in a double-blind manner, with each recording being observed three times to minimize error.

2.3 ATP bioluminescence assay

Mouse hippocampus was homogenized in ice-cold Tris-acetate EDTA buffer (0.1 M Tris-acetate + 2 mM EDTA, pH 7.8) using Branson Sonifier 150 (Emerson, St. Louis, MO). The homogenate was centrifuged at 10,000 × g in 4°C for 10 minutes. Supernatant was collected, ice-cold Tris-HCl EDTA buffer (pH 7.8) added, and pH readjusted to 7.8. ATP bioluminescence was measured using 100 µl of supernatant, standards, or water with 100 µl of ATP assay mix, following the ATP luciferin bioluminescence assay kit according to the manufacturer's protocol (Thermo). Luciferase light production was measured using the Synergy H1 Hybrid Multi-Mode Reader (BioTek). Relative luminescence of three measurements from each group were normalized, percentage was calculated relative to the control, and mean and SEM were calculated.

2.4 Seahorse assay

Methods were adapted from recently published protocols for measuring mitochondrial respiration from previously frozen tissues[17,18] as well as Seahorse assay guidelines by Agilent.

2.4.1 Preparation of solutions—Mitochondria isolation buffer (MSHE+BSA) was prepared with 210 mM mannitol, 70 mM sucrose, 5 mM HEPES, 1 mM EGTA, and 0.5% (w/v) fatty acid-free BSA and pH was adjusted to 7.4. 1X mitochondria assay solution (MAS) was prepared with 200 mM mannitol, 70 mM sucrose, 10 mM KH2PO4, 5 mM MgCl2, 2 mM HEPES, 1 mM EGTA, and 0.2% w/v fatty acid-free BSA and pH was adjusted to 7.4. The buffer used in the 96-well assay plate was 1X MAS supplemented with 10 µg/ml cytochrome c and 10 µg/ml alamethicin (MASCA).

Stock injection solutions were prepared as follows: 10 mM NADH in 1X MAS, 0.5 M succinate in 1X MAS, 4 mM rotenone in DMSO, 2 mM antimycin A in DMSO, 10 mM ascorbate in 1X MAS, 5 mM TMPD in 10 mM ascorbate, and 0.5 M azide in 1X MAS. All stock injection solutions were stored in −20°C except for NADH, ascorbate, and TMPD, which were prepared fresh on the day of the assay. All chemicals used for the Seahorse assay were purchased from Millipore Sigma.

2.4.2 Seahorse XFe96 assay—Mice hippocampi were collected and immediately homogenized in ice-cold MSHE+BSA. The homogenates were centrifuged 800 × g in 4°C for 15 minutes, and supernatants were collected and centrifuged an additional 10,000 × g in 4°C for 15 minutes to isolate the mitochondria. Isolated mitochondria were resuspended in minimal amounts of MSHE+BSH, and protein concentration was determined by BCA Protein Assay kit (Thermo) according to the manufacturer's protocol. The samples were stored in −80°C until further analysis.

Oxygen consumption rates (OCR) were measured using the Seahorse XFe96 Analyzer (Agilent Technologies, Santa Clara, CA). 10 µg of mitochondria (in 20 µL of MASCA) were seeded per well, except for the blanks in which only MASCA was added, and the microplate was centrifuged at 2,000 × g for 20 minutes. Additional 130 µl of pre-warmed (37°C) MASCA was added to each well. The loaded injection ports were A) 20 µl of 1 mM

NADH for complex I or 5 mM succinate + 2 μM rotenone for complex II; B) 22 μl of 4μM antimycin A + 2 μM rotenone; C) 24 μl of 5 mM TMPD + 10 mM ascorbate; and D) 26 μl of 50 mM azide.

Following calibration and equilibration of the XFe96 instrument, customized mix/measure protocol was followed (Table 1). The protocol was designed on Agilent Wave Software.

Measurements after antimycin A injection represent respiratory activity not produced by the non-mitochondrial respiration of the ETC complexes, while the measurements after azide injection represent any non-mitochondrial respiratory activity. These two measurements serve as correction values for NADH/succinate + rotenone and TMPD + ascorbate measurements, respectively[17].

2.5 Western blot

2.5.1 SDS-PAGE gel—Mice hippocampus was homogenized in pre-cooled lysis buffer (RIPA + 1% protease inhibitor) using Branson Digital Sonifier 150. Homogenized samples were centrifuged, and supernatant was collected and quantified using the BCA Protein Assay kit (Thermo) according to the manufacturer's guidance. 20 μg of hippocampal lysate was loaded into 10% precast polyacrylamide gels (Bio-Rad Laboratories, Hercules, CA) and gel electrophoresis was performed in Mini-PROTEAN (Bio-Rad) under constant voltage.

2.5.2 Transfer and imaging—Proteins were transferred to 0.2 μm PVDF Trans-Blot Turbo membranes (Bio-Rad) using Trans-Blot Turbo (Bio-Rad). Membranes were blocked with 6% (w/v) skim milk (Bio-Rad) in PBST and incubated with primary antibody diluted 1:1000 in PBST + 3% (w/v) BSA overnight at 4°C. Membranes were probed with respective HRP-conjugated secondary antibodies (Bio-Rad 1706515 and 1706516) diluted 1:3000 in PBST + 6% (w/v) skim milk. Blots were detected using Pierce ECL Western Blotting substrate (Thermo) and iBright FL1000 Imager (Thermo). Band density was quantified using FIJI Gel Plugin (NIH). The following primary antibodies were used: rabbit anti-mouse TOM20 mAb (Cell Signaling 42406) and mouse anti-mouse β-actin mAb (Cell Signaling 4970).

2.6 Statistical analysis

The following equations were used to analyze results from the Seahorse assay:

1. Maximal respiratory capacity (MRC) thru complex I = OCR NADH − OCR antimycin A
2. MRC through complex II = OCR succinate/rotenone − OCR antimycin A
3. MRC through complex IV = OCR TMPD/ascorbate − OCR azide

The ratio of MRC through complex I or II to MRC through complex IV was calculated for additional relative measure of respiratory capacity.

Bars in the graphs represent mean ± SEM. Significance for all data obtained was determined by two-way ANOVA with multiple comparisons, Holm-Sidak method. $P \leq 0.05$ was

considered as significant with the asterisks denoting statistical significance. Data were analyzed with Agilent Wave Software and SigmaPlot V14.5.

3. RESULTS

3.1 Social isolation induces anxiety-like behavior

Consistent with our previous findings, the animals in our social isolation anxiety model exhibited anxiety-like behavior, based on elevated plus maze (EPM) and open field (OF) tests (Fig. 1).

3.2 Social isolation reduces ATP levels

Previous work in our lab has shown a reduction in hippocampal ATP levels in socially-isolated mice and recovery with DHM treatment[9]. Mice in this present study also displayed lower ATP levels to 43.431% of control (G2+Veh2 group) (Fig. 2A), confirming the effect of social isolation-induced anxiety on ATP levels. We indirectly measured and compared the number of mitochondria across the groups by probing for translocase of the outer membrane (TOM20). Based on this protein, we found no differences in TOM20 expression and therefore no differences in mitochondrial density (Fig. 2B).

3.3 DHM restores the oxidative capacity of succinate dehydrogenase in socially isolated mice

Next, we utilized an electron flow assay to determine any dysfunctions among the complexes that may be leading to ATP reduction. The electron flow assay protocol was based on findings reported by Acin-Perez, R. (2020) and Osto, C. (2020). Seahorse XF instruments measure oxygen consumption and can therefore indirectly measure ETC activity from complex I (supplied by NADH) or complex II (supplied by succinate/rotenone) through complex IV. By sequentially supplying electrons to each complex of the ETC while inhibiting preceding ones, we can assess their oxidizing ability[18].

Maximal respiratory capacity (MRC) through complex II was decreased in isolated mice (15.189 ± 12.806 pmol/min) compared to control (29.310 ± 9.115 pmol/min), and isolated DHM (30.135 ± 10.531) (Fig. 3A, right). These changes were somewhat reflected in the ratio with complex IV, where capacity was decreased in isolated mice (1.243 ± 1.273) compared to control (4.343 ± 4.027, $P = 0.064$) and isolated DHM (4.613 ± 1.268, $P=0.036$) (Fig. 3B, right). Based on this assay, there were no functional deficits in complex I (Fig. 3A, B, left).

4. DISCUSSION

In this study, even with just four weeks of social isolation, we could detect significant reductions in ATP levels and succinate dehydrogenase capacity. ATP bioluminescence assay confirmed that social isolation-induced anxiety mice exhibited reduced ATP (Fig. 2A). This reduction was not due to reduction in mitochondrial density (Fig. 2B), but likely a reduction in mitochondrial function. Relatedly, our present data demonstrate mitochondrial damage in

socially isolated mice, with a normal oxidizing function in respiratory chain complex I, but an impairment in complex II (succinate dehydrogenase) (Fig. 3B, C).

Succinate dehydrogenase is a unique enzyme that participates in both the ETC and citric acid cycle[16], and therefore plays an important role in ATP production and mitochondrial health[19,20]. This enzyme is the only membrane-bound component of the citric acid cycle, and is the smallest and only complex that is not involved in directly pumping protons[21]. In a similar study, high-anxious Wistar rats displayed lower mitochondrial complex I and II levels and respiratory capacity as well as lower ATP levels compared to low-anxious counterparts[13]. Another study reported the degeneration of succinate dehydrogenase following stress [22]. Thus, reduced capability of succinate dehydrogenase is likely to contribute to the reduction in ATP.

Since ATP is involved in many important functions in cells[23], its reduction can cause damage at all levels, including proper neurotransmission[23]. From our previous studies utilizing the same anxiety mouse model, we found a reduction of GABAergic neurotransmission, gephyrin (a scaffolding protein that programs and supports $GABA_AR$ clustering) protein levels, and cognitive function[7,8]. Combined with the present findings, succinate dehydrogenase dysfunction could explain the ATP reduction, which leads to reduced ATP-dependent gephyrin function and therefore reduced $GABA_AR$ scaffolding and neurotransmission. Reduced GABAergic neurotransmission then leads to mild cognitive impairment as well as anxiety-like behaviors[9].

This study may be subject to some limitations. Future studies will incorporate a larger sample size to increase the confidence of these findings. While DHM recovered ATP levels in our previous study[9], the current findings did not show a significant recovery, but it could be seen that the tendency of recovery is consistent with previously discovery. This discrepancy is likely also related to the small sample size, and future studies will verify the effect of DHM on ATP levels.

Additionally, the findings in this study provide only a small snapshot of mitochondrial dysfunction in anxiety induced by social isolation. While we found complex II dysfunction via Seahorse assay, ongoing studies are incorporating other aspects of mitochondrial function and viability to provide a bigger picture of the role of this organelle in the mechanism of anxiety. However, our findings reinforce the notion that social isolation, even short-term, can induce changes in mitochondrial function and that this organelle is a critical player in proper neurotransmission.

5. CONCLUSION

In this report, we present important findings that social isolation induces substantial cellular changes, that cause damage to complex II of the electron transport chain. Decreased mitochondrial function could lead to the disruption of gephyrin-mediated GABAAR clustering, resulting in loss of neurotransmission and cognition decline. Our findings suggest that mitochondrial functions play a critical role in the development of anxiety.

ACKNOWLEDGEMENTS

This continuing research was supported by the National Institute of Health grants AA017991 (to J.L.), University of Southern California Good-Neighbor Foundation (to J.L.), and Army Health Professions Scholarship Program (HPSP) (to A.S.S.), Saudi Arabian Cultural Mission (SACM) (to A.J.A). We would like to thank Dr. Richard W. Olsen, Distinguished Professor at the David Geffen School of Medicine at UCLA, for his comments. We would like to thank Dr. Ronald Irwin at the Department of Pharmacology and Pharmaceutical Sciences at USC for his advice on isolated mitochondrial work. We would also like to thank Dr. Xuesi (Max) Shao at the Department of Neurobiology at UCLA for his comments.

REFERENCES

1. Mumtaz F, Khan MI, Zubair M, Dehpour AR. Neurobiology and consequences of social isolation stress in animal model—A comprehensive review. Biomedicine & Pharmacotherapy. 2018-09-01 2018;105:1205–1222. doi:10.1016/j.biopha.2018.05.086 [PubMed: 30021357]
2. Kasper S, den Boer J, Ad Sitsen J. Handbook of Depression and Anxiety: A Biological, Approach Handbook of Depression and Anxiety: A Biological, Approach Marcel Dekker Inc., New York 2003.
3. Craske MG, Stein MB, Eley TC, et al. Anxiety disorders. Nature Reviews Disease Primers. 2017-12-21 2017;3(1):17024. doi:10.1038/nrdp.2017.24
4. Hossain MM, Rahman M, Trisha NF, et al. Prevalence of anxiety and depression in South Asia during COVID-19: A systematic review and meta-analysis. Heliyon. 2021-04-01 2021;7(4):e06677. doi:10.1016/j.heliyon.2021.e06677 [PubMed: 33898819]
5. Pappa S, Ntella V, Giannakas T, Giannakoulis VG, Papoutsi E, Katsaounou P. Prevalence of depression, anxiety, and insomnia among healthcare workers during the COVID-19 pandemic: A systematic review and meta-analysis. Brain, Behavior, and Immunity. 2020-08-01 2020;88:901–907. doi:10.1016/j.bbi.2020.05.026 [PubMed: 32437915]
6. NIMH. Anxiety Disorders. NIMH; 2018. https://www.nimh.nih.gov/health/topics/anxiety-disorders/index.shtml
7. Watanabe S, Omran AA, Shao AS, et al. Dihydromyricetin improves social isolation-induced cognitive impairments and astrocytic changes in mice. Scientific Reports. 2022/04/07 2022;12(1):5899. doi:10.1038/s41598-022-09814-5 [PubMed: 35393483]
8. Al Omran AJ, Shao AS, Watanabe S, et al. Social isolation induces neuroinflammation and microglia overactivation, while dihydromyricetin prevents and improves them. Journal of Neuroinflammation. 2022/01/04 2022;19(1):2. doi:10.1186/s12974-021-02368-9 [PubMed: 34983568]
9. Silva J, Shao A, Shen Y, et al. Modulation of Hippocampal GABAergic Neurotransmission and Gephyrin Levels by Dihydromyricetin Improves Anxiety. Front Pharmacol, https://doiorg/103389/fphar202001008. 2020;
10. Choii G, Ko J. Gephyrin: a central GABAergic synapse organizer. Experimental & Molecular Medicine. 2015/04/01 2015;47(4):e158–e158. doi:10.1038/emm.2015.5 [PubMed: 25882190]
11. Shen Y, Lindemeyer AK, Gonzalez C, et al. Dihydromyricetin As a Novel Anti-Alcohol Intoxication Medication. Journal of Neuroscience. 2012-01-04 2012;32(1):390–401. doi:10.1523/jneurosci.4639-11.2012 [PubMed: 22219299]
12. Liang J, Kerstin Lindemeyer A, Shen Y, et al. Dihydromyricetin Ameliorates Behavioral Deficits and Reverses Neuropathology of Transgenic Mouse Models of Alzheimer's Disease. Neurochemical Research. 2014-06-01 2014;39(6):1171–1181. doi:10.1007/s11064-014-1304-4 [PubMed: 24728903]
13. Filiou MD, Sandi C. Anxiety and Brain Mitochondria: A Bidirectional Crosstalk. Trends in Neurosciences. 2019;42(9):573–588. doi:10.1016/j.tins.2019.07.002 [PubMed: 31362874]
14. Spinelli JB, Haigis MC. The multifaceted contributions of mitochondria to cellular metabolism. Nature Cell Biology. 2018-07-01 2018;20(7):745–754. doi:10.1038/s41556-018-0124-1 [PubMed: 29950572]

15. Patergnani S, Suski JM, Agnoletto C, et al. Calcium signaling around Mitochondria Associated Membranes (MAMs). Cell Communication and Signaling. 2011-01-01 2011;9(1):19. doi:10.1186/1478-811x-9-19 [PubMed: 21939514]

16. Rutter J, Winge DR, Schiffman JD. Succinate dehydrogenase - Assembly, regulation and role in human disease. Mitochondrion. 2010;10(4):393–401. doi:10.1016/j.mito.2010.03.001 [PubMed: 20226277]

17. Osto C, Benador IY, Ngo J, et al. Measuring Mitochondrial Respiration in Previously Frozen Biological Samples. Current Protocols in Cell Biology. 2020-12-01 2020;89(1)doi:10.1002/cpcb.116

18. Acin-Perez R, Benador IY, Petcherski A, et al. A novel approach to measure mitochondrial respiration in frozen biological samples. The EMBO Journal. 2020-07-01 2020;39(13)doi:10.15252/embj.2019104073

19. Rustin P, Munnich A, Rötig A. Succinate dehydrogenase and human diseases: new insights into a well-known enzyme. European Journal of Human Genetics. 2002/05/01 2002;10(5):289–291. doi:10.1038/sj.ejhg.5200793 [PubMed: 12082502]

20. Jardim-Messeder D, Caverzan A, Rauber R, de Souza Ferreira E, Margis-Pinheiro M, Galina A. Succinate dehydrogenase (mitochondrial complex II) is a source of reactive oxygen species in plants and regulates development and stress responses. 10.1111/nph.13515. New Phytologist. 2015/11/01 2015;208(3):776–789. doi:10.1111/nph.13515 [PubMed: 26082998]

21. Moosavi B, Berry EA, Zhu X-L, Yang W-C, Yang G-F. The assembly of succinate dehydrogenase: a key enzyme in bioenergetics. Cellular and Molecular Life Sciences. 2019/10/01 2019;76(20):4023–4042. doi:10.1007/s00018-019-03200-7 [PubMed: 31236625]

22. Marchi S, Patergnani S, Missiroli S, et al. Mitochondrial and endoplasmic reticulum calcium homeostasis and cell death. Cell Calcium. 2018-01-01 2018;69:62–72. doi:10.1016/j.ceca.2017.05.003 [PubMed: 28515000]

23. Patel A, Malinovska L, Saha S, et al. ATP as a biological hydrotrope. Science. 2017-05-19 2017;356(6339):753–756. doi:10.1126/science.aaf6846 [PubMed: 28522535]

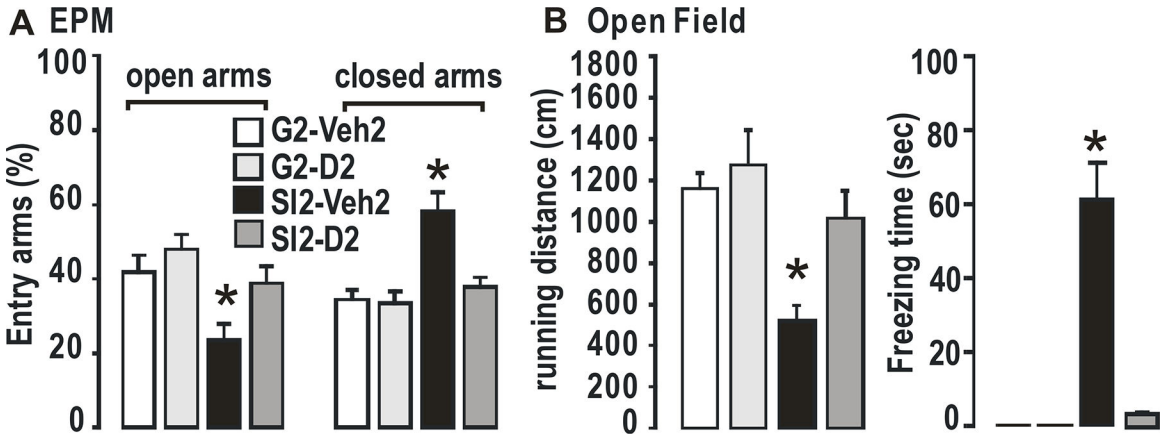

Fig. 1. Anxiety behavioral tests.

A. Elevated plus maze. **B.** Open field. N = 4 per group. Two-way ANOVA with multiple comparisons, Holm Sidak method, *, $p \leq 0.01$ vs G2+Veh2.

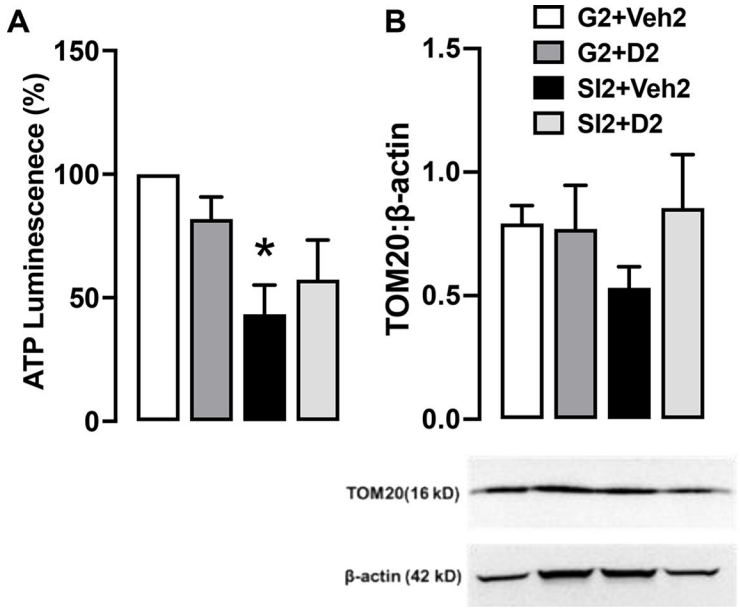

Fig. 2. ATP and TOM20.

A. Normalized hippocampal ATP bioluminescence shown as percentage in comparison to the control. N = 3 per group. **B.** TOM20 blotted on the same gel as β-actin. Blot is shown as different parts of the same gel. Bars show quantification of TOM20 with β-actin as the loading control. N = 4 per group. Two-way ANOVA with multiple comparisons, Holm Sidak method, *, $p \leq 0.01$ vs G2+Veh2.

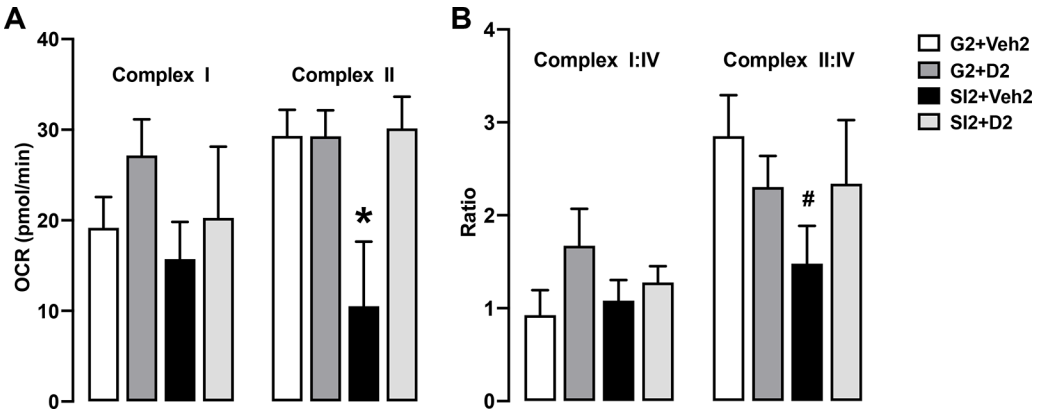

Fig. 3.

A, B. Maximal respiratory capacity (MRC) of isolated hippocampal mitochondria for complex I and complex II. N = 7 per group. **C, D.** MCR ratio to complex IV. N = 7 per group. Two-way ANOVA with multiple comparisons, Holm-Sidak method. *, $p \leq 0.01$ vs. G2+Veh2; #, $p \leq 0.10$ vs G2+Veh2.

Table 1.

Seahorse XFe96 protocol

Action	Time (mins)
Mix	0.5
Measure	4
INJECTION A	
Mix	0.5
Measure	4
Mix	0.5
Measure	4
INJECTION B	
Mix	0.5
Measure	4
INJECTION C	
Mix	0.5
Measure	4
Mix	0.5
Measure	4
INJECTION D	
Mix	0.5
Measure	4
Mix	0.5
Measure	4