PNAS
www.pnas.org

Supplementary Information for

Serotonin regulates mitochondrial biogenesis and function in rodent cortical neurons via the 5-HT$_{2A}$ receptor and SIRT1-PGC-1α axis

Sashaina E. Fanibunda[1,2], Sukrita Deb[1], Babukrishna Maniyadath[1], Praachi Tiwari[1], Utkarsha Ghai[1], Samir Gupta[1], Dwight Figueiredo[1], Noelia Weisstaub[3], Jay A. Gingrich[4], Ashok D.B. Vaidya[2], Ullas Kolthur-Seetharam[1*], Vidita A. Vaidya[1*]

Corresponding authors-

Ullas Kolthur-Seetharam[1*] Email: ullas@tifr.res.in
Vidita A. Vaidya[1*] Email: vvaidya@tifr.res.in

This PDF file includes:

Supplementary Materials and Methods
Supplementary Figures: Figs. S1 to S5 with legends
Tables S1 to S4
References for SI reference citations
Abbreviations

Supplementary Information

Supplementary Materials and Methods

Mouse Lines

The 5-HT_{2A} receptor knockout mouse line (1) contains a neo-stop cassette between two unidirectional lox P sites, introduced into the 5' untranslated region of the *htr2a* gene, thus providing the possibility of tissue specific restoration of the 5-HT_{2A} receptor using Cre recombinase. The Emx1-IRES-Cre knockin mice (Jax-mice-ID 005628) (2) expresses the Cre recombinase from the endogenous *Emx1* locus. $SirT1^{lox/lox}$ mice were generated by insertion of a flanked loxP neomycin cassette exerted into exon 4 of Sirt1 gene (Jax-mice-ID: 008041), (3). Emx1-Cre;$Sirt1^{-/-}$ (Sirt1cKO) mice and the appropriate control Emx1-Cre;$Sirt1^{+/+}$ (WT) bigenic lines were generated by crossing Emx1-Cre and $SirT1^{lox/lox}$ mice or Emx1-Cre and $Sirt1^{+/+}$ mouse lines respectively. The hM3Dq line (RC::PDq line) expresses the hM3Dq DREADD under the control of a chicken β-actin promoter targeted to the (Gt)ROSA26Sor locus and exhibits Cre-dependent expression (kind gift from Patricia Gaspar) (4). The Pet1-Cre mouse line (generated by the Deneris lab) exhibits Cre mediated activity in a serotonergic-specific manner, with activity restricted to 5-HT neurons (kind gift from Patricia Gaspar) (5). The bigenic hM3Dq/Pet1-Cre line was generated by crossing the hM3Dq (RC::PDq line) mouse line with the Pet1-Cre mouse line. The Ai9 mouse line (Jax-mice-ID: 007909) is a Cre reporter line that expresses tdTomato fluorescence following Cre-mediated recombination and excision of the loxP-flanked STOP cassette. A bigenic reporter mouse line, Pet1-Cre/Ai9, was generated via crossing the Ai9 reporter mouse line with Pet1-Cre mice.

Animals

Timed pregnant Sprague-Dawley dams were bred in the Tata Institute of Fundamental Research (TIFR) animal facility and embryos derived at embryonic day 18.5 (E18.5) were

used for all cortical culture experiments, with the exception of experiments with cortical cultures established from specific mouse mutant lines. Mouse cortical cultures were generated from E18.5 embryos derived from wild-type and serotonin$_{2A}$ receptor knockout (5-HT$_{2A}^{-/-}$) dams, with the possibility of Cre-mediated rescue of 5-HT$_{2A}$ receptor expression (1, 6). Mouse cortical cultures were also generated from embryos derived from bigenic E18.5 Emx1-Cre;Sirt1$^{+/+}$ (wild-type: WT), Emx1-Cre;Sirt1$^{-/-}$ (Sirt1cKO) and SirT1$^{lox/lox}$ dams (Emx1-Cre: Jax-mice-ID 005628) (SirT1$^{lox/lox}$: Jax-mice-ID 008041) (3). For *in vivo* experiments, male Sprague Dawley rats (5-6 months), WT and Sirt1cKO mice (15 months), bigenic hM3Dq/Pet1-Cre mice (15 months) generated using the Pet1-Cre line (5) and the hM3Dq line (RC::PDq line) (4), and C57BL/6NCrl/Cri mice (derived originally from the C57BL/6NCrl strain from Charles River, and maintained at the Cancer Research Institute (CRI) ACTREC, Mumbai from where the line was obtained and bred in the TIFR animal facility) (5-6 months) were used. Intracortical infusion of 5-HT was carried out via delivery through osmotic minipumps (Alzet 2001) implanted surgically in Sprague-Dawley rats. All animals were group housed, maintained on a 12 h light–dark cycle with *ad libitum* access to food and water. Experimental procedures were in accordance with the guidelines of the Committee for Supervision and Care of Experimental Animals (CPCSEA), Government of India, and were approved by the TIFR Institutional Animal Ethics committee (CPCSEA-56/1999).

Primary cortical culture

Dams were euthanized with CO_2 and embryos were collected in ice cold Ca^{2+} and Mg^{2+} free Hanks' balanced salt solution (HBSS) supplemented with 300 mM HEPES. Cortices were dissected in ice cold minimum essential medium, enzymatically dissociated in 0.05% trypsin/EDTA for 10 min, and triturated to obtain a single cell suspension in Neurobasal medium supplemented with 2% B27 and 0.5 mM L-glutamine. Cells were seeded

in plates coated with poly-D-lysine (0.1 mg/ml, Sigma-Aldrich, USA) at a density of 1 million cells per 9.6 cm^2. Neurons were cultured at 37°C with 5% CO_2 and 95% humidity with a half- medium change every 2 days. Cortical neurons were allowed to differentiate for at least seven days *in vitro* (DIV 7) prior to treatment (7, 8). All reagents were purchased from Thermo Fisher Scientific, (USA) unless specified.

For specific sets of experiments cortical cultures generated from 5-$HT_{2A}^{-/-}$ mouse embryos were transduced with rAAV8-CaMKIIα-GFP-Cre (UNC Vector Core facility, NC, USA) on DIV 2 to restore *Htr2a* expression (5-$HT_{2A}^{-/-Res}$). Cortical cultures derived from $SirT1^{lox/lox}$ mouse embryos were transduced *in vitro* with rAAV8-CaMKIIα-GFP or rAAV8-CaMKIIα-GFP-Cre on DIV 2, to yield control cortical neurons ($SirT1^{lox/lox}$) or cortical neurons with loss of function of SIRT1 ($SirT1^{co/co}$) respectively.

Drug treatment paradigms

For dose response studies, rat cortical neurons were treated with 5-HT (10, 50 and 100 µM) for six days. For mitochondrial marker analysis, mitotracker imaging experiments and reactive oxygen species (ROS) measurements, rat cortical cultures were treated with 5-HT (100 µM) for six days. For time-point analysis, cortical neurons were treated with 5-HT (100 µM) for durations of 4 h, 6 h, 8 h, 24 h, 48 h, 72 h, and 6 days. Cortical cultures were also treated with norepinephrine or dopamine (10, 100 µM) for six days to assess influence of other monoamines. Mouse cortical cultures from 5-$HT_{2A}^{-/-}$, 5-$HT_{2A}^{-/-Res}$, SIRT1cKO, $SirT1^{co/co}$ and their respective controls were treated with 5-HT (100 µM) for six days. In experiments with 5-HT receptor antagonists, specific inhibitors of kinase pathways or the SIRT1 inhibitor, rat cortical neurons were treated with 5-HT (100 µM) for 72 h in the presence of the 5-HT_{2A} receptor antagonist, MDL100,907 (10 µM), the 5-HT_{1A} receptor antagonist, WAY100,635 (10 µM), MEK inhibitor U0126 (10 µM), PLC inhibitor U73122 (5 µM), PI3-kinase inhibitor LY294002 (10 µM) or SIRT1 inhibitor EX-527 (10 µM). For

experiments with the 5-HT_{2A} receptor agonist, rat cortical neurons were treated with DOI (5, 10 µM) for six days or Lisuride (10 µM) for 72 h. For mitochondrial respiration analysis, cortical neurons were seeded at an equal density of 10 x 10^4 cells per well of the Seahorse XF24 cell culture microplate (Seahorse Bioscience, Agilent Technologies, CA, USA), and treated with 5-HT (100 µM) or DOI (10 µM) for 72 h. For studies addressing the influence of 5-HT in protection against excitotoxic or oxidative stress, cell viability and cellular reactive oxygen species (ROS) were assessed in cortical neuron cultures pretreated with 5-HT (50, 100 µM, 6 days) followed by challenge with kainate (100, 200 µM; 3.5 h) or H_2O_2 (100, 200 µM; 7 h). The contribution of SIRT1 to the effects of 5-HT on neuronal viability following kainate (10 to 1000 µM) or H_2O_2 (10 to 1000 µM) treatment was determined using (1) the SIRT1 inhibitor EX-527 (10 µM), or (2) cortical neurons derived from Sirt1cKO and WT mice. The influence of DOI (10 µM) in neuroprotection against kainate (100, 200 µM; 3.5 h) or H_2O_2 (100, 200 µM; 7 h) was determined by assessing cell viability and the contribution of SIRT1 in these effects of DOI was determined using EX-527 treatment (10 µM). Controls involved treatment of cultures with vehicle (water), with the exception of U73122, U0126, LY294002, EX-527, Lisuride and DOI where the vehicle was 0.1% DMSO.

For *in vivo* experiments, Sprague-Dawley rats, Sirt1cKO and WT mice received intraperitoneal administration of DOI (2 mg/kg) or vehicle (0.9 % saline) once daily for four days and were sacrificed two hours after the last treatment. Sprague-Dawley rats received intracortical infusion of vehicle (Phosphate buffered saline) or 5-HT (10 µM) via stereotactic delivery through an osmotic minipump (Alzet Model 2001) for 7 days. Bigenic hM3Dq/Pet1-Cre mice were generated from the Pet1-Cre (5) and hM3Dq (RC::PDq line) (4) mouse lines, and received intraperitoneal administration of vehicle or clozapine-N-oxide (CNO, 3 mg/kg), once daily for 10 days prior to sacrifice 2 h post the final CNO injection. All compounds were purchased from Tocris Bioscience (United Kingdom), except 5-HT, DOI,

norepinephrine, WAY100,635, EX-527, H_2O_2 and kainate (Sigma-Aldrich, USA). The concentration of neurotransmitters used was based on prior studies (9–14).

Quantitative real time polymerase chain reaction

RNA was extracted from cortical neurons using the commercially available RNeasy Micro/Mini kit (Qiagen). 50 ng of RNA per sample was reverse transcribed to cDNA using random hexamers and the Superscript IV reverse transcription kit (Invitrogen, USA). Quantitative real time PCR was performed using gene specific primers and cDNA was amplified in a Light Cycler 96 (Roche Applied Science, Switzerland) real time PCR system using KAPA SYBR® FAST Universal 2X qPCR Master Mix (Kapa Biosystems). Gene expression levels were normalized to the endogenous 18S ribosomal RNA per sample, and the relative expression levels between control and treated samples were computed by the ΔΔCt method, as described previously (15). Data are represented as fold change ± SEM as compared to control.

Mitochondrial DNA levels

Mitochondrial DNA (mtDNA) levels were compared in control versus treated cortical neurons by quantitative real time PCR. Total DNA was extracted from cells using the commercially available All Prep DNA/RNA Mini kit (Qiagen, Germany). Levels of cytochrome B - a mitochondrial genome encoded gene were normalized to levels of a nuclear encoded gene cytochrome C. Relative mitochondrial DNA levels between groups were quantified by the ΔΔCt method as described previously (16).

Immunofluorescence

For immunostaining, cortical neurons were fixed in 4% paraformaldehyde, permeabilized with 0.2% Triton X-100, followed by blocking in 10% horse serum. Cells were then incubated with primary antibodies, which included the rabbit anti-VDAC (1:200, Abcam, MA, USA) or goat anti-5-HT_{2A} (1:250, Santacruz Biotechnologies, CA, USA),

incubated along with the pan neuronal marker mouse anti-MAP2 (1:500, Sigma-Aldrich) overnight at 4°C. Cells were washed and incubated with secondary antibodies, Alexa Fluor 488 conjugated anti-rabbit (1:250, Molecular probes) or Alexa Fluor 488 conjugated anti-goat (1:250, Molecular probes, CA, USA) or Alexa Fluor 568 conjugated anti-mouse (1:250, Molecular probes, CA, USA) respectively for 2 hours, followed by washes. The nuclei were counterstained using Hoechst, mounted in Vectashield (Vector laboratories, CA, USA) and images were captured on the Olympus Fluoview 1000 confocal laser scanning microscope. For immunofluorescence, coronal sections (50 μm) through the dorsal and median raphe (DRN, MRN) were incubated with primary antibodies, that included mouse anti-Tph2 (1:1000, Sigma-Aldrich), rabbit anti-cFos (1:500, Cell Signaling Technology, MA, USA) either alone or coincubated with rat anti-5-HT (1:250, Abcam). This was followed by incubation with secondary antibodies Alexa Fluor 488 conjugated anti-mouse (1:500, Molecular probes) or Alexa Fluor 488 conjugated anti-rabbit (1:500, Molecular probes, CA, USA) or Alexa Fluor 568 conjugated anti-rat (1:250, Molecular probes, CA, USA) respectively and images were captured on the Olympus Fluoview 1000 confocal laser scanning microscope.

Mitotracker Staining

Cortical neurons were loaded with mitotracker green (20 nM, Invitrogen) for 45 min, and images were recorded using an Olympus Fluoview 1000 confocal laser scanning microscope. The intensity of staining in neurites was quantitated over a distance of 10 μm commencing from the point proximal to the cell body using ImageJ® software (NIH, USA) in 15-20 neurons per treatment condition per experiment.

Western blot analysis

Control and treated cortical neuron cultures were lysed in ice cold Radioimmunoprecipitation assay (RIPA) buffer (10 mM Tris-Cl (pH 8.0), 1 mM EDTA, 0.5

mM EGTA, 1% NP-40, 0.1% sodium deoxycholate, 0.1% SDS, 140 mM NaCl), supplemented with protease and phosphatase inhibitors (Roche Applied Science). Lysates were centrifuged at 12000 rpm for 30 min and supernatant was collected. Protein estimation was performed using the QuantiPro BCA (Bicinchoninic Acid) assay kit (Sigma-Aldrich) and lysates were resolved by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Proteins were transferred onto polyvinylidene fluoride (PVDF, Merck Millipore, MA, USA) membranes, blocked in 5% milk in Tris Buffered Saline-Tween (0.1%) and probed with primary antibodies in 5% BSA (bovine serum albumin) overnight at 4°C. Primary antibodies included mouse anti-PGC-1α (1:300, Calbiochem), rabbit anti-TFAM (1:1000, Sigma-Aldrich), rabbit anti-VDAC (1:250, Abcam), mouse anti-Cyt C (1:250, Abcam), mouse anti-ATP5A (1:1000, Abcam), mouse anti-SIRT1 (1:250, Sigma-Aldrich), mouse anti-actin (1:4000, Sigma-Aldrich), rabbit anti-βIII-tubulin (1:1000), rabbit anti-phospholipase C (PLC) beta 3 (1:1000), rabbit anti-phospho PLC beta 3 (1:1000), rabbit anti-Akt (1:2000), rabbit anti-phospho Akt (1:1000), rabbit anti-p44/42 MAPK (ERK1/2) (1:1000), and rabbit anti-phospho p44/42 MAPK (ERK1/2) (1:500) (Cell Signaling Technology, MA, USA). Blots were washed and incubated with goat anti-rabbit IgG peroxidase labelled or rabbit anti-mouse IgG peroxidase labelled (1:7000, Sigma-Aldrich) secondary antibodies for 2 hours at room temperature. Signal was detected using a chemiluminescence kit (Thermo Fisher Scientific), and bands were visualized on x-ray films (Fiji films) or the GE Amersham Imager 600. Actin or tubulin were used as loading controls for normalization as indicated, and the relative density of bands was quantitated using ImageJ software (NIH, USA).

Cellular ATP

Cortical neurons were lysed in boiling water and lysate was centrifuged at 12,000 rpm for 20 min at 4°C. The ATP levels in the supernatant were quantified using the ATP

bioluminescent assay kit (Sigma-Aldrich), by mixing the luciferin substrate and luciferase enzyme mix with equal amounts of supernatant in a 96 well plate. The light emitted is proportional to the ATP consumed in the reaction, and was measured using a luminometer (Berthold Technologies, Germany). ATP levels were normalized to protein content of each sample, estimated using a BCA protein assay kit (Sigma-Aldrich) and expressed as fold change of treated over control.

Mitochondrial Respiration Analysis

Cortical neurons were seeded at an equal density of 10 x 10^4 cells per well of the Seahorse XF24 cell culture microplate and differentiated in Neurobasal medium. Cells were treated with 5-HT or DOI for 72 h, with 4-5 replicate wells per group. On the day of the assay, control and treated neurons were washed twice in assay medium, and 500 µL of assay medium pre-warmed to 37°C, was added in each well. Assay medium comprised of Seahorse XF base medium supplemented with 25 mM glucose (Sigma), 1 mM pyruvate (Sigma), 2 mM glutamine (Thermo Fisher Scientific) pH 7.4. 2% B27 (Thermo Fisher Scientific) was added prior to the addition of the medium to the wells. The plate was equilibrated in a non–CO_2 incubator for 45 min at 37°C, and thereafter loaded into the Seahorse XF24 analyzer. Oxygen Consumption rate (OCR) (pmol min^{-1}) was measured baseline in assay medium, and thereafter cells were exposed to sequential injections of oligomycin (1 µM, ATP synthase complex V inhibitor), FCCP (2 µM, collapses the proton gradient, disrupts the mitochondrial membrane potential and uncouples oxygen consumption from ATP production) and rotenone (0.5 µM, complex I inhibitor), with three consecutive OCR measurements taken after each injection. OCR measurements at baseline and following the three serial injections, were used to calculate basal respiration, ATP production, maximal respiration, non-mitochondrial respiration and spare respiratory capacity. OCR measurements were normalized to blank wells and cell count in each well, and expressed as pmol/min/10^4 cells. Experiments were

repeated thrice and values from all the repeats were collated when calculating mitochondrial parameters. A representative trace of OCR per treatment condition is depicted.

Isolation of mitochondria and bioenergetic measurements

Mitochondria were isolated from the prefrontal cortex (PFC) of vehicle (saline) treated and DOI injected Sprague Dawley rats (5 - 6 months), by modifying the differential centrifugation protocol as described in Rogers et al., 2011 (17). In brief, tissue was homogenized in ice-cold mitochondrial isolation buffer (MSHE+BSA, containing 210 mM mannitol, 70 mM sucrose, 5 mM HEPES, 1 mM EGTA, and 0.5% (w/v) fatty acid-free BSA, pH 7.2) using a dounce homogenizer (4 strokes). Following centrifugation at 800 g for 10 minutes (4°C), the supernatant was collected in a separate tube and centrifuged at 8,000 g for 10 minutes (4°C). The pellet was suspended in MSHE+BSA, and the centrifugation procedure was repeated. Finally, the pellet was resuspended in a small volume of ice-cold mitochondrial assay buffer (MAS+BSA, containing 220 mM mannitol, 70 mM sucrose, 10 mM KH_2PO4, 5 mM $MgCl_2$, 2 mM HEPES, 1 mM EGTA and 0.5% (W/V) fatty acid-free BSA, pH 7.2). Protein concentrations were determined using BCA estimations using BSA as standard, and 10 µg of mitochondrial preparations were used for bioenergetic analysis immediately after the isolation. Mitochondria were plated (10 µg/50 µl) in Seahorse XFe24 micro-plates in MAS+BSA buffer supplemented with substrates, pyruvate (10 mM) and malate (2 mM), and centrifuged at 2000 g for 20 min (4ºC). Prior to the assay, 450 µl of substrate containing MAS+BSA was added to each well. Basal State-2 respiration, primarily Complex-I dependent, under limiting endogenous ADP and succinate concentrations, was measured initially. Following this, Complex II dependent state 3 respiration was measured post injection of 2 µM rotenone + 10 mM Succinate + 4 mM ADP. Oligomycin (2µM) induced state 4 respiration was measured next, which was followed by FCCP (8 µM) induced maximal respiration and non-mitochondrial respiration post antimycin A (8 µM) injections.

State-2 (Complex-I/-II dependent) and State-3 (Complex-II dependent) OCR are depicted graphically and Oligomycin sensitive reduction in OCR was computed as the ATP production rate.

Cellular ROS Measurement

To assess levels of cellular reactive oxygen species (ROS), carboxy-H_2DCFDA (25 μM) was added to the medium for 30 min at 37°C. Cells were then washed twice and images were captured using the Olympus Fluoview 1000 confocal laser scanning microscope. Alternatively, to quantitate the fluorescence, cells were lysed in RIPA buffer and fluorescence was read per treatment per well at excitation/emission of 495/529nm, and normalized to protein content per well. Protein was estimated by BCA method (Sigma).

Osmotic Minipump Surgery

Sprague-Dawley rats (males, 5-6 months) were anaesthetized using isoflurane (1%-1.5%) in oxygen. Animals were placed in a stereotactic apparatus (Stoelting Instruments, USA) and cannulae were implanted in the cortex (relative to Bregma: 0.9 mm posterior, 2 mm lateral and 2 mm ventral) and secured using dental cement and anchor screws. Osmotic minipumps (Alzet model 2001; flow rate 1 μl/h, 7 d pump) were primed according to the procedure described by the manufacturer with either Phosphate buffered saline (PBS) or 10 μM 5-HT (in 200 μl PBS). In brief, all pumps were placed in beakers containing sterile PBS and primed overnight at 37ºC in an incubator. Pumps were attached to the cannulae, and inserted in a subcutaneous pocket made at the back of the animal, and animals were closely monitored for post surgical recovery.

Data Availability Statement

Detailed protocol, raw data and materials will be made available upon request. This manuscript does not contain any omics datasets.

Supplementary Figures

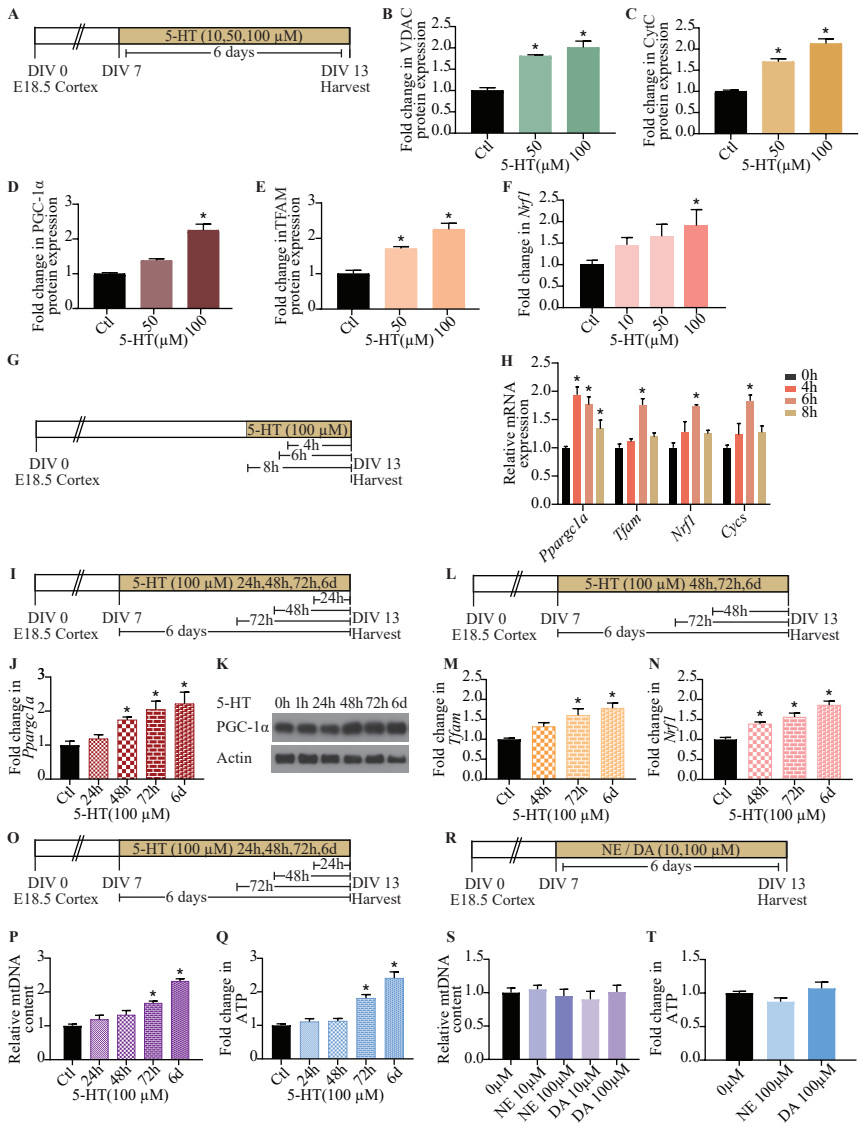
A
5-HT (10,50,100 μM)
6 days
DIV 0
E18.5 Cortex
DIV 7
DIV 13
Harvest
B
Fold change in VDAC protein expression
Ctl
50
100
5-HT(μM)
C
Fold change in CytC protein expression
D
Fold change in PGC-1α protein expression
E
Fold change in TFAM protein expression
F
Fold change in Nrf1
10
G
5-HT (100 μM)
4h
6h
8h
H
Relative mRNA expression
0h
4h
6h
8h
Ppargc1a
Tfam
Nrf1
Cycs
I
5-HT (100 μM) 24h,48h,72h,6d
24h
48h
72h
J
Fold change in Ppargc1a
24h
5-HT(100 μM)
K
5-HT
0h 1h 24h 48h 72h 6d
PGC-1α
Actin
L
5-HT (100 μM) 48h,72h,6d
M
Fold change in Tfam
N
Fold change in Nrf1
O
P
Relative mtDNA content
Q
Fold change in ATP
R
NE / DA (10,100 μM)
S
Relative mtDNA content
0μM
NE 10μM
NE 100μM
DA 10μM
DA 100μM
T
Fold change in ATP

Figure S1-Supplementary data for Figure 1: *Mitochondrial biogenesis and function are regulated by 5-HT*

(A) Shown is a schematic depicting treatment of neurons with increasing doses of 5-HT (10, 50, 100 µM) starting at DIV 7. (B-E) Quantitative densitometric analysis of VDAC (B), Cyt C (C), PGC-1α (D) and TFAM (E) immunoblots from control (Ctl) and 5-HT treated cortical neuron cultures. Results are expressed as fold change of control ± SEM (Compiled results from n = 5-11 per treatment group/N = 2-3, $*P < 0.05$ as compared to control, one-way ANOVA, Tukey's *post-hoc* test). (F) Quantitative qPCR analysis of *Nrf1* expression from control and 5-HT treated cortical neuron cultures, results are represented as fold change of control ± SEM. (Representative results from n = 4 per treatment group/N = 2, $*P < 0.05$ as compared to control, one-way ANOVA, Tukey's *post-hoc* test). (G) Shown is a schematic depicting the treatment paradigm of neurons with 5-HT (100 µM) for 4 h, 6 h and 8 h and lysed synchronously at DIV 13. (H) Quantitative qPCR analysis of *Ppargc1a, Tfam, Nrf1* and *Cycs* expression levels in cortical neurons *in vitro* in the 5-HT time-course experiment at 4 h, 6 h and 8 h are represented as fold change of control ± SEM. (Representative results from n = 4 per treatment group/N = 2, $*P < 0.05$ as compared to control, one-way ANOVA, Tukey's *post-hoc* test). (I) Shown is a schematic depicting 5-HT (100 µM) treatment of cortical neurons for 24 h, 48 h, 72 h or 6 days and lysed synchronously at DIV 13. (J) qPCR analysis of *Ppargc1a* expression levels in the 5-HT time-course experiment, results are represented as fold change of control ± SEM. (Representative results from n = 4 per treatment group/N = 2, $*P < 0.05$ as compared to control, one-way ANOVA, Tukey's *post-hoc* test). (K) Shown is a representative immunoblot for PGC-1α with actin as the loading control in the 5-HT time-course experiment at indicated time-points. (L) Shown is a schematic depicting the treatment paradigm of neurons with 5-HT (100 µM) for 48 h, 72 h or 6 days and lysed synchronously at DIV 13. (M and N) qPCR analysis of *Tfam* (M) and *Nrf1* (N) expression levels in the 5-HT

time-course experiment at indicated time-points, and results are represented as fold change of control ± SEM. (Representative results from n = 4 per treatment group/N = 2, *P < 0.05 as compared to control, one-way ANOVA, Tukey's *post-hoc* test). (O) Shown is a schematic depicting 5-HT (100 µM) treatment of cortical neurons for 24 h, 48 h, 72 h or 6 days and lysed synchronously at DIV 13. (P and Q) Graphs depict quantitation of mtDNA and ATP levels in the 5-HT time-course experiment at indicated time-points, and results are represented as fold change of control ± SEM. (Representative results from n = 4 per treatment group/N = 2, *P < 0.05 as compared to control, one-way ANOVA, Tukey's *post-hoc* test). (R) Schematic depicting treatment of neurons with different doses of norepinephrine (NE: 10, 100 µM) or dopamine (DA: 10, 100 µM) from DIV 7 to DIV 13. (S and T) Graphs depict quantitation of relative mtDNA (S) and ATP (T) levels in neurons treated with NE or DA. Results are expressed as fold change of control ± SEM. (Representative results from n = 4 per treatment group/N = 2, *P < 0.05 as compared to control, one-way ANOVA, Tukey's *post-hoc* test).

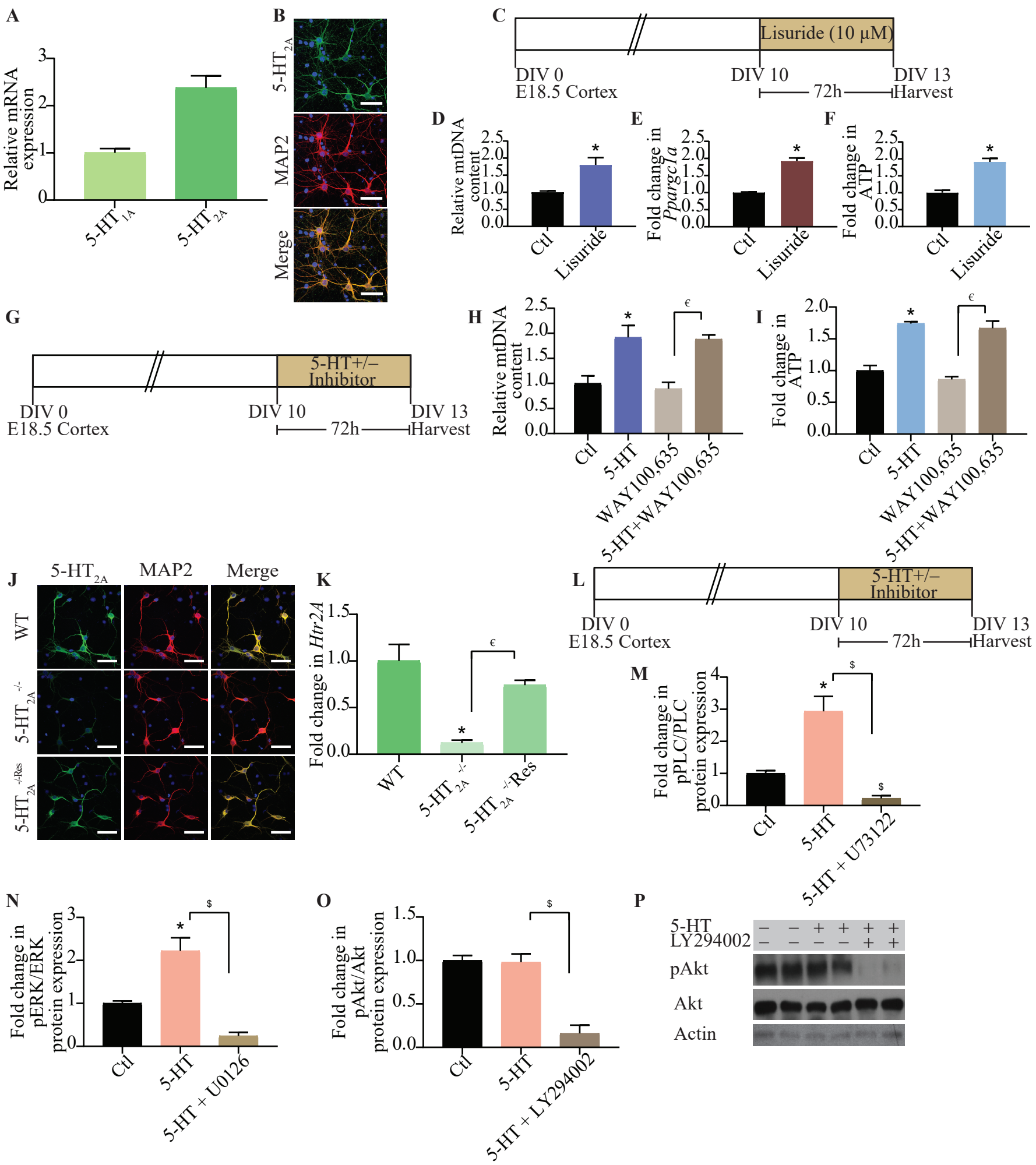
A
Relative mRNA expression
5-HT1A
5-HT2A
B
5-HT2A
MAP2
Merge
C
Lisuride (10 µM)
DIV 0
E18.5 Cortex
DIV 10
72h
DIV 13
Harvest
D
Relative mtDNA content
Ctl
Lisuride
E
Fold change in Ppargc1a
F
Fold change in ATP
G
5-HT+/– Inhibitor
H
5-HT
WAY100,635
5-HT+WAY100,635
I
J
WT
5-HT2A -/-
5-HT2A -/-Res
K
Fold change in Htr2A
L
M
Fold change in pPLC/PLC protein expression
5-HT + U73122
N
Fold change in pERK/ERK protein expression
5-HT + U0126
O
Fold change in pAkt/Akt protein expression
5-HT + LY294002
P
LY294002
pAkt
Akt
Actin

Figure S2-Suplementary data for Figure 2: *Mitochondrial effects of 5-HT are mediated via the 5-HT$_{2A}$ receptor*

(A) Quantitative qPCR analysis of mRNA expression of 5-HT$_{1A}$ and 5-HT$_{2A}$ receptor in cortical neuron cultures. (Representative results from n = 4 per treatment group/N = 2). (B) Shown are representative images for 5-HT$_{2A}$ receptor immunofluorescence (green), neuronal marker MAP2 (red) and merge (yellow) from cortical neuron cultures. Scale bar: 50 µm. Magnification: 60X. (C) Shown is a schematic depicting the treatment paradigm for cortical neuron cultures with Lisuride (10 µM), for 72 h commencing DIV 10. (D-F) Quantitation of mtDNA (D), *Ppargc1a* mRNA (E) and ATP (F) levels in cortical neurons treated with Lisuride are represented as fold change of control (Ctl) ± SEM. (Representative results from n = 4-8 per treatment group/N = 2, **P* < 0.05 as compared to control, unpaired Student's *t*-test). (G) Shown is a schematic depicting the treatment paradigm of cortical neuron cultures with 5-HT in the presence or absence of the selective 5-HT$_{1A}$ receptor antagonist, WAY100,635 commencing DIV 10. (H and I) Graphs depict quantitative analysis for mtDNA (H) and ATP (I) levels with results expressed as fold change of control (Ctl) ± SEM. (Representative results from n = 4 per treatment group/N = 2, **P* < 0.05 as compared to control, $^{\epsilon}$*P* < 0.05 as compared to WAY100,635, two-way ANOVA, Tukey's *post-hoc* test). (J) Shown are representative images for 5-HT$_{2A}$ receptor immunofluorescence (green), MAP2 (red) and merge (yellow) in WT, 5-HT$_{2A}^{-/-}$ and 5-HT$_{2A}^{-/-Res}$ cortical neuron cultures. Scale bar: 50 µm. Magnification: 60X. (K) Quantitative analysis of *Htr2A* mRNA levels in cortical neuron cultures from WT, 5-HT$_{2A}^{-/-}$ and 5-HT$_{2A}^{-/-Res}$ indicate reduced *Htr2A* levels in 5-HT$_{2A}^{-/-}$ cultures, with rescue of expression in the 5-HT$_{2A}^{-/-Res}$ group. Results are expressed as the fold change of WT ± SEM. (Representative results from n = 4 per treatment group/N = 2, **P* < 0.05 as compared to WT, $^{\epsilon}$*P* < 0.05 as compared to 5-HT$_{2A}^{-/-}$, one-way ANOVA, Tukey's *post-hoc* test). (L) Shown is a schematic depicting the treatment paradigm of cortical

neurons with 5-HT (100 µM) in the presence or absence of signaling inhibitors for PLC (U73122, 5 µM), MEK (U0126, 10 µM) and PI3K (LY294002, 10 µM). (M-P) Quantitative densitometric analysis of pPLC/PLC levels (M), pERK/ERK levels (N) and pAkt/Akt (O) levels with ratios normalized to actin or tubulin as loading controls. Results are expressed as fold change of control ± SEM. (Compiled results from n = 4-5 per treatment group/N = 3, $*P < 0.05$ as compared to control, $^{\$}P < 0.05$ as compared to 5-HT treated group, one-way ANOVA, Tukey's *post-hoc* test). (P) Shown is a representative immunoblot of pAkt and Akt in control and 5-HT treated cells in the presence of absence of the PI3K inhibitor, LY294002 which resulted in a robust inhibition of pAkt levels in 5-HT treated cortical neurons.

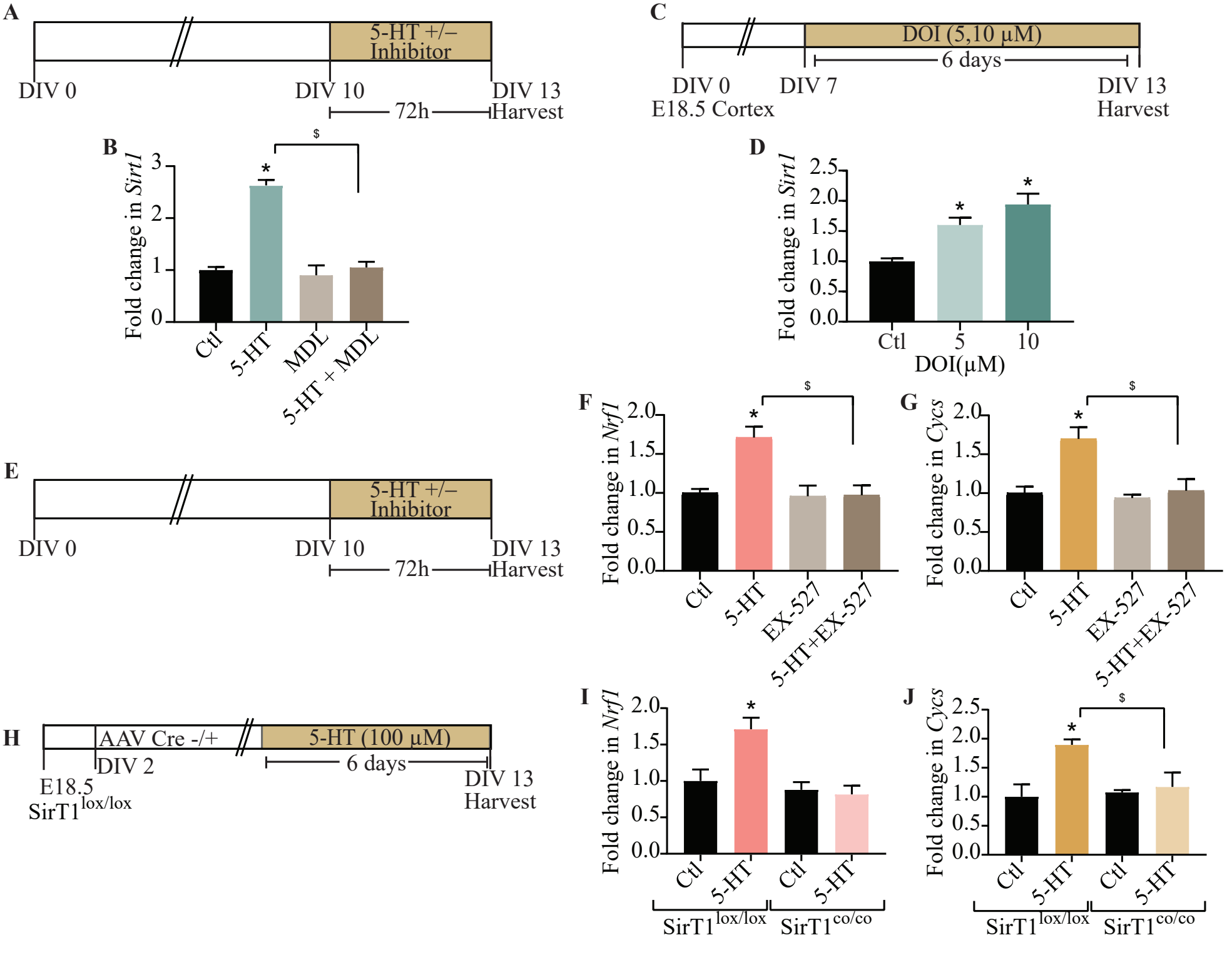
A
5-HT +/–
Inhibitor
DIV 0
DIV 10
DIV 13
Harvest
72h
B
Fold change in Sirt1
Ctl
5-HT
MDL
5-HT + MDL
C
DOI (5,10 µM)
6 days
DIV 0
E18.5 Cortex
DIV 7
DIV 13
Harvest
D
Fold change in Sirt1
Ctl
5
10
DOI(µM)
E
5-HT +/–
Inhibitor
DIV 0
DIV 10
DIV 13
Harvest
72h
F
Fold change in Nrf1
Ctl
5-HT
EX-527
5-HT+EX-527
G
Fold change in Cycs
Ctl
5-HT
EX-527
5-HT+EX-527
H
AAV Cre -/+
5-HT (100 µM)
6 days
DIV 2
E18.5
SirT1lox/lox
DIV 13
Harvest
I
Fold change in Nrf1
Ctl
5-HT
Ctl
5-HT
SirT1lox/lox
SirT1co/co
J
Fold change in Cycs
Ctl
5-HT
Ctl
5-HT
SirT1lox/lox
SirT1co/co

Figure S3-Suplementary data for Figure 3: *SIRT1 is required for the effects of 5-HT on mitochondria*

(A) Shown is a schematic depicting the treatment paradigm of cortical neurons with 5-HT (100 µM) in the presence or absence of the 5-HT_{2A} receptor antagonist MDL100,907 (MDL) (10 µM) commencing DIV 10. (B) Graph depicts quantitation of *Sirt1* mRNA levels in cortical neurons treated with 5-HT in the presence or absence of MDL100,907 (MDL), and expressed as fold change of control ± SEM. (Representative results from n = 4 per treatment group/N = 2, $^{*}P < 0.05$ as compared to control, $^{\$}P < 0.05$ as compared to 5-HT treated group, two-way ANOVA, Tukey's *post-hoc* test). (C) Shown is a schematic depicting the treatment paradigm of neurons with increasing doses of DOI (5, 10 µM) commencing DIV 7. (D) Quantitative qPCR results for *Sirt1* mRNA levels are expressed as fold change of control ± SEM. (Representative results from n = 4 per treatment group/N = 2, $^{*}P < 0.05$ as compared to control, one-way ANOVA, Tukey's *post-hoc* test). (E) Shown is a schematic depicting the treatment paradigm of neurons with 5-HT (100 µM) in the presence or absence of the Sirt1 inhibitor, EX-527 (10 µM). (F and G) Quantitation of mRNA expression of *Nrf1* (F) and *Cycs* (G) in cortical neurons treated with 5-HT in the presence or absence of EX-527 are expressed as fold change of control ± SEM. (Representative results from n = 4 per treatment group/N = 2, $^{*}P < 0.05$ as compared to control, $^{\$}P < 0.05$ as compared to 5-HT treated group, two-way ANOVA, Tukey's *post-hoc* test). (H) Shown is a schematic depicting the treatment paradigm for cortical neuron cultures derived from $SirT1^{lox/lox}$ embryos and then transduced *in vitro* on DIV 2 with rAAV8-CamKIIα-GFP (AAV Cre-) or rAAV8-CamKIIα-GFP-Cre (AAV-Cre+) to yield control cortical neurons ($SirT1^{lox/lox}$) or cortical neurons with loss of function of SIRT1 ($SirT1^{co/co}$). $SirT1^{lox/lox}$ and $SirT1^{co/co}$ cortical neuron cultures were then treated with vehicle or 5-HT (100 µM) commencing DIV 7. (I and J) Graphs depict quantitation of *Nrf1* (I) and *Cycs* (J) mRNA expression in $SirT1^{lox/lox}$ and $SirT1^{co/co}$ cortical

neurons, in the presence or absence of 5-HT and represented as fold change of control ± SEM. (Representative results from n = 6 per treatment group/N = 2, $*P < 0.05$ as compared to Ctl ($SirT1^{lox/lox}$), $^{\$}P < 0.05$ as compared to 5-HT ($SirT1^{lox/lox}$), two-way ANOVA, Tukey's *post-hoc* test).

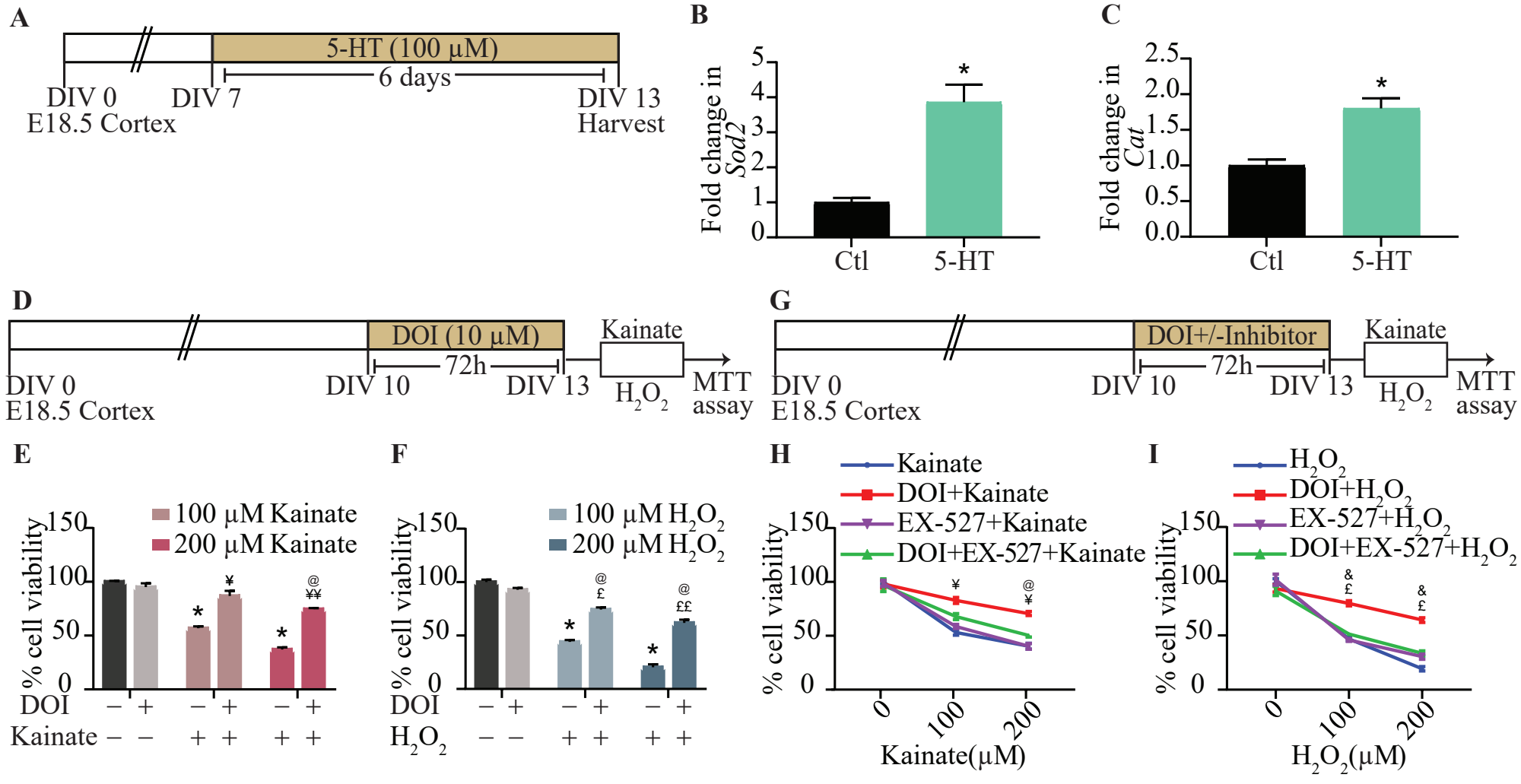
A
5-HT (100 µM)
6 days
DIV 0
E18.5 Cortex
DIV 7
DIV 13
Harvest
B
Fold change in Sod2
Ctl
5-HT
C
Fold change in Cat
Ctl
5-HT
D
DOI (10 µM)
72h
Kainate
DIV 0
E18.5 Cortex
DIV 10
DIV 13
H2O2
MTT assay
G
DOI+/-Inhibitor
72h
Kainate
DIV 0
E18.5 Cortex
DIV 10
DIV 13
H2O2
MTT assay
E
100 µM Kainate
200 µM Kainate
% cell viability
DOI
Kainate
F
100 µM H2O2
200 µM H2O2
% cell viability
DOI
H2O2
H
Kainate
DOI+Kainate
EX-527+Kainate
DOI+EX-527+Kainate
% cell viability
Kainate(µM)
I
H2O2
DOI+H2O2
EX-527+H2O2
DOI+EX-527+H2O2
% cell viability
H2O2(µM)

Figure S4-Supplementary data for Figure 4: *5-HT_{2A} receptor activation exerts neuroprotective effects against excitotoxic and oxidative stress*

(A) Shown is a schematic depicting the treatment paradigm of neurons with 5-HT (100 µM) commencing DIV 7. (B and C) qPCR analysis of *Sod2* and *Cat* mRNA levels in 5-HT treated cortical neurons are expressed as fold change of control (Ctl) ± SEM. (Representative results from n = 4 per treatment group/N = 2, $^{*}P < 0.05$ as compared to control, unpaired Student's *t*-test). (D) Shown is a schematic depicting the treatment paradigm of cortical neurons with the 5-HT_{2A} receptor agonist, DOI (10 µM) commencing DIV 10 followed by a challenge with kainate (100, 200 µM) or H_2O_2 (100, 200 µM) on DIV 13 and analysis of cell viability. (E) Graph depicts cell viability assessed by the MTT assay in cortical neurons challenged with kainate (100, 200 µM) with or without pretreatment with DOI (10 µM). Results are expressed as percent of control cell viability ± SEM. (Representative results from n = 3 per treatment group/N = 2, $^{*}P < 0.05$ as compared to control, $^{¥}P < 0.05$ as compared to 100 µM kainate treated group, $^{¥¥}P < 0.05$ as compared to 200 µM kainate treated group, $^{@}P < 0.05$ as compared to DOI treated group, two-way ANOVA, Tukey's *post-hoc* test). (F) Graph depicts cell viability assessed by the MTT assay in cortical neurons challenged with H_2O_2 (100, 200 µM) in cortical neurons with or without pretreatment with DOI (10 µM). Results are expressed as percent of control cell viability ± SEM. (Representative results from n = 3 per treatment group/N = 2, $^{*}P < 0.05$ as compared to control, $^{£}P < 0.05$ as compared to 100 µM H_2O_2 treated group, $^{££}P < 0.05$ as compared to 200 µM H_2O_2 treated group, $^{@}P < 0.05$ as compared to DOI treated group, two-way ANOVA, Tukey's *post-hoc* test). (G) Shown is a schematic depicting the treatment paradigm of neurons with DOI (10 µM) in the presence or absence of the SIRT1 inhibitor, EX-527 (10 µM), followed by challenge with kainate or H_2O_2 (100, 200 µM) and analysis of cell viability using the MTT assay. (H) Shown is a line graph for cell viability of cortical neurons in response to kainate (100, 200 µM) with

treatment groups of kainate (blue), DOI + kainate (red), EX-527 + kainate (purple), DOI + EX-527 + kainate (green) expressed as per cent of control cell viability ± SEM. (Representative results from n = 3 per treatment group/N = 2, $^{¥}P < 0.05$ as compared to kainate treatment group, $^{@}P < 0.05$ as compared to DOI + EX-527 + kainate treated group, three-way ANOVA, Tukey's *post-hoc* test). (I) Shown is a line graph for cell viability of cortical neurons in response to H_2O_2 (100, 200 µM) with treatment groups of H_2O_2 (blue), DOI + H_2O_2 (red), EX-527 + H_2O_2 (purple), DOI + EX-527 + H_2O_2 (green) expressed as per cent of control cell viability ± SEM. (Representative results from n = 3 per treatment group/N = 2, $^{£}P < 0.05$ as compared to H_2O_2 treated group, $^{\&}P < 0.05$ as compared to DOI + EX-527 + H_2O_2 treated group, three-way ANOVA, Tukey's *post-hoc* test).

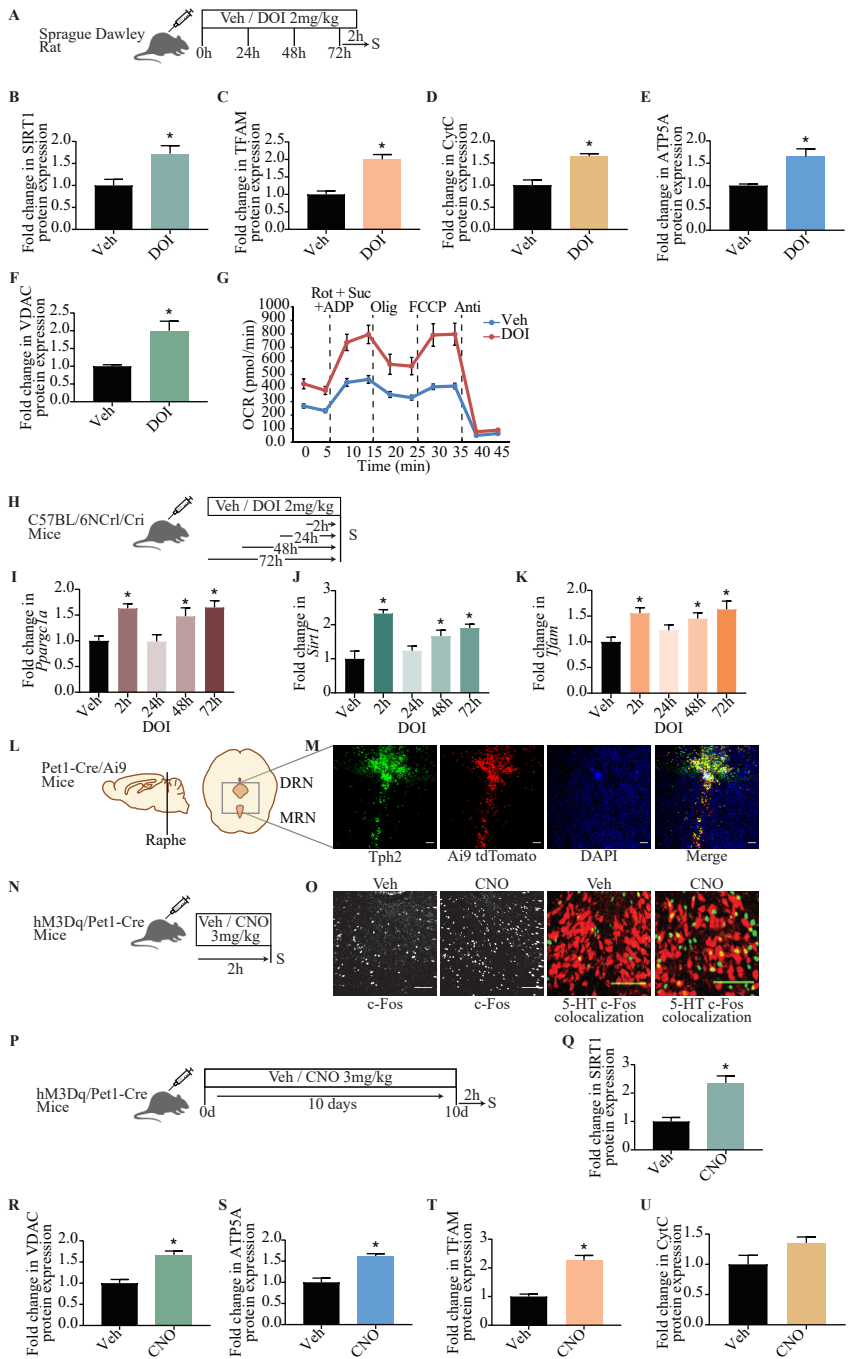
A
Sprague Dawley Rat
Veh / DOI 2mg/kg
0h
24h
48h
72h
2h
S
B
Fold change in SIRT1 protein expression
C
Fold change in TFAM protein expression
D
Fold change in CytC protein expression
E
Fold change in ATP5A protein expression
F
Fold change in VDAC protein expression
Veh
DOI
G
Rot + Suc
+ADP
Olig
FCCP
Anti
Veh
DOI
OCR (pmol/min)
Time (min)
H
C57BL/6NCrl/Cri Mice
Veh / DOI 2mg/kg
2h
24h
48h
72h
S
I
Fold change in Ppargc1a
J
Fold change in Sirt1
K
Fold change in Tfam
DOI
L
Pet1-Cre/Ai9 Mice
Raphe
DRN
MRN
M
Tph2
Ai9 tdTomato
DAPI
Merge
N
hM3Dq/Pet1-Cre Mice
Veh / CNO 3mg/kg
2h
S
O
Veh
CNO
c-Fos
5-HT c-Fos colocalization
P
hM3Dq/Pet1-Cre Mice
Veh / CNO 3mg/kg
0d
10 days
10d
2h
S
Q
Fold change in SIRT1 protein expression
R
Fold change in VDAC protein expression
S
Fold change in ATP5A protein expression
T
Fold change in TFAM protein expression
U
Fold change in CytC protein expression

Figure S5-Supplementary data for Figure 5: *In vivo regulation of cortical mitochondrial DNA Content, gene and protein expression, mitochondrial OCR, and ATP by 5-HT*

(A) Shown is a schematic of the treatment paradigm for Sprague-Dawley rats with the 5-HT_{2A} receptor agonist, DOI (2 mg/kg) or vehicle (Veh; saline). (B – F) Quantitative densitometric analysis of SIRT1 (B), TFAM (C), Cyt C (D), ATP5A (E) and VDAC (F) immunoblots from cortices derived from vehicle (Veh) and DOI treated Sprague Dawley rats. Results are expressed as fold change of vehicle ± SEM (n = 4 per treatment group/N = 1, **P* < 0.05 as compared to vehicle, unpaired Student's *t*-test). (G) Shown is a representative Seahorse plot for isolated mitochondria (10 µg) derived from the PFC of vehicle and DOI-treated Sprague-Dawley rats, with measurements of basal State-2 respiration, primarily Complex-I dependent, under limiting endogenous ADP and succinate concentrations, followed by Complex II dependent state 3 respiration measured post injection of 2 µM rotenone (Rot) + 10 mM Succinate + 4 mM ADP. Further, oligomycin (Olig) (2µM) induced state 4 respiration, FCCP (8 µM) induced maximal respiration and non-mitochondrial respiration post antimycin A (Anti) (8 µM) injections are also shown. (H) Shown is a schematic depicting the treatment paradigm for time course analysis of gene expression in C57BL/6NCrl/Cri mice administered vehicle or DOI (2mg/kg), once daily and sacrificed at 2, 24, 48 and 72 h. (I-K) Quantitative qPCR analysis for *Ppargc1a* (I), *Sirt1* (J) and *Tfam* (K) mRNA expression is expressed as fold change of vehicle ± SEM. (Representative results from n = 9 per treatment group/N = 1, **P*< 0.05 as compared to vehicle, one-way ANOVA, Tukey's *post-hoc* test). (L) Shown is a schematic representing a sagittal section from a Pet1-Cre/Ai9 reporter mouse line, with a raphe coronal section indicating the dorsal raphe nucleus (DRN) and median raphe nucleus (MRN). (M) Shown is the expression of the tdTomato reporter in Tph2 immunopositive serotonergic neurons in representative sections from bigenic Pet1-Cre/Ai9 mice at the level of the DRN. Scale bar: 100 µm. Magnification: 20X.

(N) Shown is a representative paradigm for chemogenetic activation of serotonergic neurons using hM3Dq/Pet1-Cre bigenic mice and systemic administration of the DREADD agonist clozapine-N-oxide (CNO, 3mg/kg) with sacrifice 2 h post CNO administration. (O) Representative sections from vehicle and CNO treated hM3Dq/Pet1-Cre mice indicate the DREADD-mediated activation of raphe neurons as revealed by increased c-Fos, a marker for neuronal activity. c-Fos positive cells also exhibit colocalization with 5-HT immunofluorescence in confocal stack images, demonstrating DREADD-mediated activation of serotonergic neurons in the raphe. Scale bar: 100 μm. Magnification: 20X and digitally zoomed. (P) Shown is a representative paradigm for chemogenetic activation of serotonergic neurons using hM3Dq/Pet1-Cre bigenic mice and systemic administration of the DREADD agonist clozapine-N-oxide (CNO, 3mg/kg, once daily for 10 days) with sacrifice 2 h following the final CNO administration. (Q-U) Quantitative densitometric analysis of SIRT1 (Q), VDAC (R), ATP5A (S), TFAM (T) and Cyt C (U) immunoblots from the PFC of vehicle (Veh) and CNO treated hM3Dq/Pet1-Cre mice. Results are expressed as fold change of vehicle ± SEM (n = 4 per treatment group/N = 1, $*P < 0.05$ as compared to vehicle, unpaired Student's *t*-test).

List of Primers

Table S1: Primer sequences used for quantitative PCR analysis of rat cDNA

Gene name	Description	Primer	Sequence (5'-3')
Ppargc1a	peroxisome proliferator-activated receptor gamma, coactivator 1 alpha	Forward	TGAACTACGGGATGGCAACT
		Reverse	GAAGAGCAAGAAGGCGACAC
Nrf1	nuclear respiratory factor 1	Forward	CACACACAGCATAGCCCATC
		Reverse	TTTGTTCCACCTCTCCATCA
Tfam	transcription factor A, mitochondrial	Forward	GCTAAACACCCAGATGCAAA
		Reverse	GCTTCCTTCTCTAAGCCCATC
Sirt1	sirtuin 1	Forward	AGAACCACCAAAGCGGAAA
		Reverse	ACAGCAAGGCGAGCATAAA
Cycs	cytochrome c, somatic	Forward	ACCAGCCCGGACCGAATTTA
		Reverse	GTGTAAGAGAATCCAGCAGCCT
Cat	catalase	Forward	TGTTGAATGAGGAGGAGAGG
		Reverse	TTCTTAGGCTTCTGGGAGTTG
Sod2	superoxide dismutase 2	Forward	TACAGATTGCCGCCTGCTC
		Reverse	TTCTCCCAGTTGATTACATTCC
Htr1a	5-hydroxytryptamine receptor 1A	Forward	GGACTCCGTGCACTAATGGG
		Reverse	AGAGGAAGGTGCTCTTTGGAGTT
Htr2a	5-hydroxytryptamine receptor 2A	Forward	AGAACCCCATTCACCACAGC
		Reverse	AGGCAGCTCCCCTCCTTAAA
18S rRNA	18S ribosomal RNA	Forward	TTTCGAGGCCCTGTAATTGG
		Reverse	CCCAAGATCCAACTACGAGC

Table S2: Primer sequences used for relative mitochondrial DNA (mtDNA) analysis in rat

Gene name	Description	Primer	Sequence (5'-3')
mt-Cytb	cytochrome b, mitochondrial	Forward	ACGCTCCATTCCCAACAAAC
		Reverse	GTTGGCCTCCGATTCATGTT
Cycs	cytochrome c, somatic	Forward	AGGCTGCTGGATTCTCTTACA
		Reverse	GTCTGCCCTTTCTCCCTTCT

Table S3: Primer sequences used for quantitative PCR analysis of mouse cDNA

Gene name	Description	Primer	Sequence (5'-3')
Ppargc1a	peroxisome proliferator-activated receptor gamma, coactivator 1 alpha	Forward	GTGGATGAAGACGGATTGCC
		Reverse	GCTGAGTGTTGGCTGGTGCC
Tfam	transcription factor A, mitochondrial	Forward	CAAGTCAGCTGATGGGTATGG
		Reverse	TTTCCCTGAGCCGAATCATCC
Sirt1	sirtuin 1	Forward	GTAACCCTGTAAAGCTTTCAG
		Reverse	CAGAAGAGTCTTGTGGTACAG
Cycs	cytochrome c, somatic	Forward	ACACTGTGGAAAAGGGAGGC
		Reverse	GCACTGGTTAACCCAAGCAA
18S rRNA	18S ribosomal RNA	Forward	TTTCGAGGCCCTGTAATTGG
		Reverse	CCCAAGATCCAACTACGAGC
Htr2a	5-hydroxytryptamine receptor 2A	Forward	CGTGTCCATGTTAACCATCC
		Reverse	TCAGGAAGGCTTTGGTTCTG

Table S4: Primer sequences used for relative mitochondrial DNA (mtDNA) analysis in mouse

Gene name	Description	Primer	Sequence (5'-3')
mt-Cytb	cytochrome b, mitochondrial	Forward	TGCATACGCCATTCTACG
		Reverse	ATGGGTGTTCTACTGGTTG
Cycs	cytochrome c, somatic	Forward	CAGTGCAGAATTACCAGGTGTG
		Reverse	GGTCTGCCCTTTCTCCCTTCT

References

1. Weisstaub N V., et al. (2006) Cortical 5-HT2A receptor signaling modulates anxiety-like behaviors in mice. *Science* 313(5786):536–40.
2. Gorski JA, et al. (2002) Cortical excitatory neurons and glia, but not GABAergic neurons, are produced in the Emx1-expressing lineage. *J Neurosci* 22(15):6309–14.
3. Li H, et al. (2007) SirT1 modulates the estrogen-insulin-like growth factor-1 signaling for postnatal development of mammary gland in mice. *Breast Cancer Res* 9(1):1–12.
4. Teissier A, et al. (2015) Activity of Raphé Serotonergic Neurons Controls Emotional Behaviors. *Cell Rep* 13(9):1965–1976.
5. Scott MM, et al. (2005) A genetic approach to access serotonin neurons for in vivo and in vitro studies. *Proc Natl Acad Sci U S A* 102(45):16472–7.
6. González-Maeso J, et al. (2007) Hallucinogens Recruit Specific Cortical 5-HT2AReceptor-Mediated Signaling Pathways to Affect Behavior. *Neuron* 53(3):439–452.
7. Banker G, Goslin K (1998) Culturing nerve cells. *Cellular and Molecular Neuroscience Series*, eds Banker G, Goslin K (MIT University Press,Cambridge, MA), pp 339–370. 2nd Ed.
8. Pacifici M, Peruzzi F (2012) Isolation and culture of rat embryonic neural cells: a quick protocol. *J Vis Exp* (63):e3965.
9. Kondoh M, Shiga T, Okado N (2004) Regulation of dendrite formation of Purkinje cells by serotonin through serotonin1A and serotonin2A receptors in culture. *Neurosci Res* 48(1):101–9.
10. Lavdas AA, Blue ME, Lincoln J, Parnavelas JG (1997) Serotonin promotes the differentiation of glutamate neurons in organotypic slice cultures of the developing cerebral cortex. *J Neurosci* 17(20):7872–80.

11. Riccio O, et al. (2009) Excess of serotonin affects embryonic interneuron migration through activation of the serotonin receptor 6. *Mol Psychiatry* 14(3):280–90.

12. Bunin MA, Wightman RM (1998) Quantitative evaluation of 5-hydroxytryptamine (serotonin) neuronal release and uptake: an investigation of extrasynaptic transmission. *J Neurosci* 18(13):4854–60.

13. Bunin MA, Wightman RM (1999) Paracrine neurotransmission in the CNS: involvement of 5-HT. *Trends Neurosci* 22(9):377–82.

14. Daws LC, Toney GM (2007) *High-Speed Chronoamperometry to Study Kinetics and Mechanisms for Serotonin Clearance In Vivo* eds C.Borland A, M.Michael L (Boca Raton (FL): CRC Press/Taylor & Francis) doi:NBK2570 [bookaccession].

15. Schmittgen TD, Livak KJ (2008) Analyzing real-time PCR data by the comparative CT method. *Nat Protoc* 3(6):1101–1108.

16. Ghosh S, Banerjee KK, Vaidya VA, Kolthur-Seetharam U (2016) Early Stress History Alters Serum Insulin-Like Growth Factor-1 and Impairs Muscle Mitochondrial Function in Adult Male Rats. *J Neuroendocrinol* 28(9). doi:10.1111/jne.12397.

17. Rogers GW, et al. (2011) High Throughput Microplate Respiratory Measurements Using Minimal Quantities Of Isolated Mitochondria. *PLoS One* 6(7):e21746.

List of abbreviations

1. 1,4-Diamino-2,3-dicyano-1,4-bis[2-aminophenylthio]butadiene - U0126
2. 1-[6-[[(17β)-3-Methoxyestra-1,3,5(10)-trien-17-yl]amino]hexyl]-1H-pyrrole-2,5-dione - U73122
3. 2-(4-Morpholinyl)-8-phenyl-4H-1-benzopyran-4-one hydrochloride - LY294002
4. 2,5-dimethoxy-4-iodoamphetamine - DOI
5. 5-(and-6)-carboxy-2',7'-dichlorodihydrofluorescein diacetate - carboxy-H_2DCFDA
6. 6-Chloro-2,3,4,9-tetrahydro-1H-Carbazole-1-carboxamide - EX-527
7. Brain-derived neurotrophic factor - BDNF
8. Carbonylcyanide-p-trifluoromethoxyphenylhydrazone – FCCP
9. Clozapine-N-oxide – CNO
10. Dimethyl sulfoxide – DMSO
11. Designer Receptors Exclusively Activated by Designer Drugs – DREADDs
12. Extracellular-signal-regulated kinase - ERK (also called MAPK)
13. Hydrogen peroxide - H_2O_2
14. Microtubule Associated Protein 2 – MAP2
15. Mitochondrial DNA - mtDNA
16. Mitogen-activated protein kinase - MAPK or MAP kinase (also called ERK)
17. Mitogen-activated protein kinase kinase - MEK
18. N-[2-[4-(2-Methoxyphenyl)-1-piperazinyl]ethyl]-N-2-pyridinylcyclohexanecarboxamide - WAY100,635
19. Oxidative phosphorylation - OXPHOS
20. Phosphatidylinositol 3-kinase - PI3K
21. Phospholipase C - PLC
22. Protein kinase B (PKB) - Akt

23. R-(1)-a-(2,3-dimethoxyphenyl)21-[2-(4-flurophenylethyl)]24-piperidine-methanol - MDL100,907
24. Reactive oxygen species - ROS
25. Serotonin (5-hydroxytryptamine) - 5-HT