Paternal Exposure to Cigarette Smoke alters Behavior and Gene and miRNA Expression in Offspring

PRABAGARAN ESAKKY (prabhuhoxbes2@gmail.com)
Washington University School of Medicine in Saint Louis: Washington University in St Louis School of Medicine https://orcid.org/0000-0003-4688-2427

Andrea M Drury
Washington University In Saint Louis: Washington University in St Louis

Paul Felder
Washington University In St Louis: Washington University in St Louis

Alexandra Medvedeva
Washington University In St Louis: Washington University in St Louis

Kelle H Moley
Washington University In St Louis: Washington University in St Louis

---

Research

Keywords: Cigarette smoke condensate, caudal sperm, miRNA, fetal brain, F1 offspring, q-RTPCR, neurobehavioral changes, prepulse inhibition of startle, neuronal receptors, nicotine, F0, serum, paternal exposure, intergenerational inheritance, and phenotype

Posted Date: April 27th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-423467/v1

Abstract

Cigarette smoking causes neurobehavioral disorders such as anxiety, addiction, and attention-deficit hyperactivity disorder (ADHD). Smokers have higher rate (53%) of major depressive disorder than non-smokers (6%). Smokers absorb nearly 10% of nicotine from each cigarette (~10 –15 mg) and tar or CSC (cigarette smoke condensate; 5.3 mg of nicotine / ml). Exposure to nicotine in utero causes tremors and startle responses in newborns, and ADHD by age 6. However, studies that demonstrate paternal-mediated neurobehavioral changes in offspring are limited. We set out to determine whether paternal smoking alters neurobehavioral outcomes in offspring and underlying molecular mechanisms. Our male mouse model demonstrates that the CSC exposure in adult mice leads to altered emotionality, hyperactive and anxiety-like nature, reduced sensorimotor gating, and increased anxious depression in their F1 adolescents. This was preceded by dysregulation of neuronal receptors at protein and mRNA levels and their targeting miRNAs in fetal (e18.5) brain. These intergenerational molecular changes in F1 offspring seem to be mediated through CSC-altered miRNAs in F0 caudal sperm. In addition, the sub-chronic CSC treatment elevates miRNA levels in sire serum without hindering the postnatal growth of progeny. Thus, the paternal exposure to CSC causes behavioral and molecular changes in offspring possibly by altering the F0 sperm-borne miRNAs.

Full Text

This preprint is available for download as a PDF.

Figures

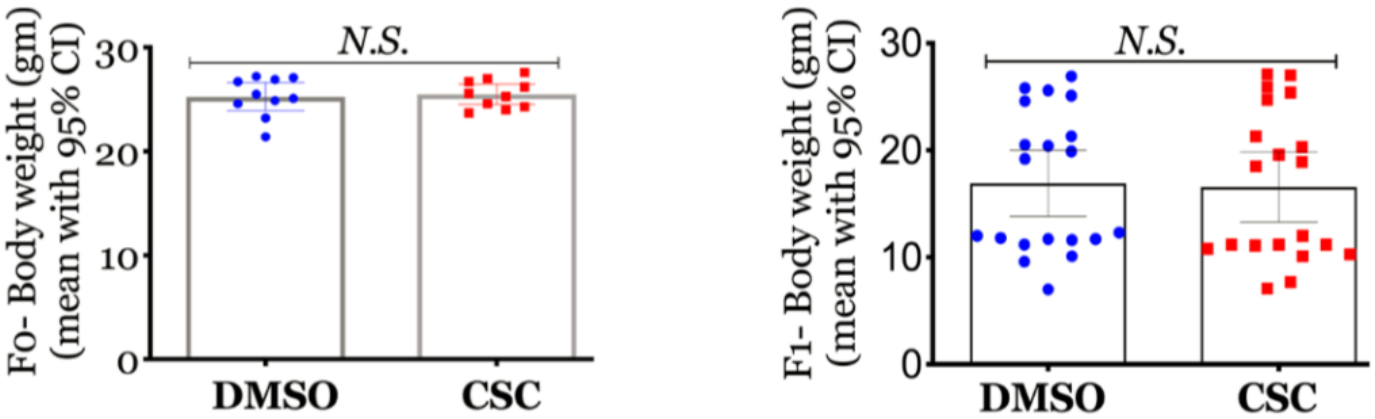

Figure 1

Effect of paternal CSC exposure on offspring growth. Histograms show the body weight of F0 males (left) and its F1 offspring (right) born to males exposed to CSC or DMSO daily for 40 consecutive days.

Approximately 10-20 mice per treatment group from 7-8 litters were used. The data were analyzed by unpaired nonparametric two-tailed Mann-Whitney t-test to represent mean difference of total body weight (gm) between treatment groups with 95% CI, a <0.05 and p values. N.S: Not significant.

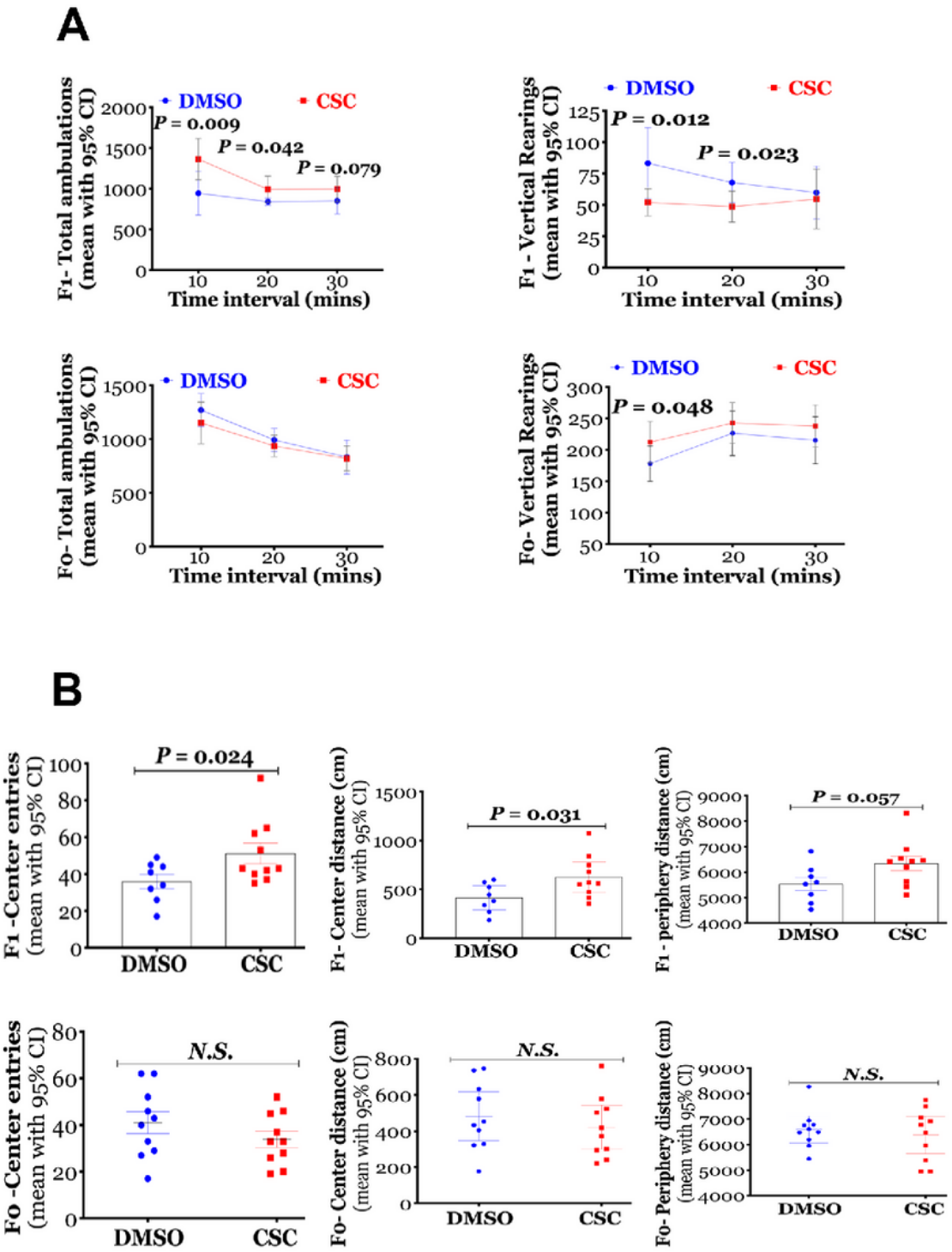

Figure 2

Locomotor activity/open-field behavior test. 2A, Locomotor activity assay: Total ambulations (left panels) and vertical rearings (right panels) of F1 offspring (top), and DMSO-, and CSC-exposed F0 males

(bottom). Approximately 10-20 mice per treatment group from 7-8 litters were used. Mice were subjected to 30-min tests with 10-minute intervals. The data were analyzed by two-way ANOVA and Sidak's multiple comparisons test to represent mean difference between treatment groups with 95% CI and a P <0.05. GraphPad Prism 8.1.1 statistical software was used for data analysis. 2B, Open field behavior test: Number of entries to center, distance travelled from the center (cm), and in the peripheral zone (cm) of the open field by the F1 offspring (top panels) and treated F0 males (bottom panels). Approximately 10-20 mice per treatment group from 7-8 litters were subjected to 10-minute assay. The data were analyzed by unpaired two-tailed t-test to represent mean difference between treatment groups with 95% CI and a P <0.05. GraphPad Prism 8.1.1 statistical software was used for data analysis. N.S: Not significant.

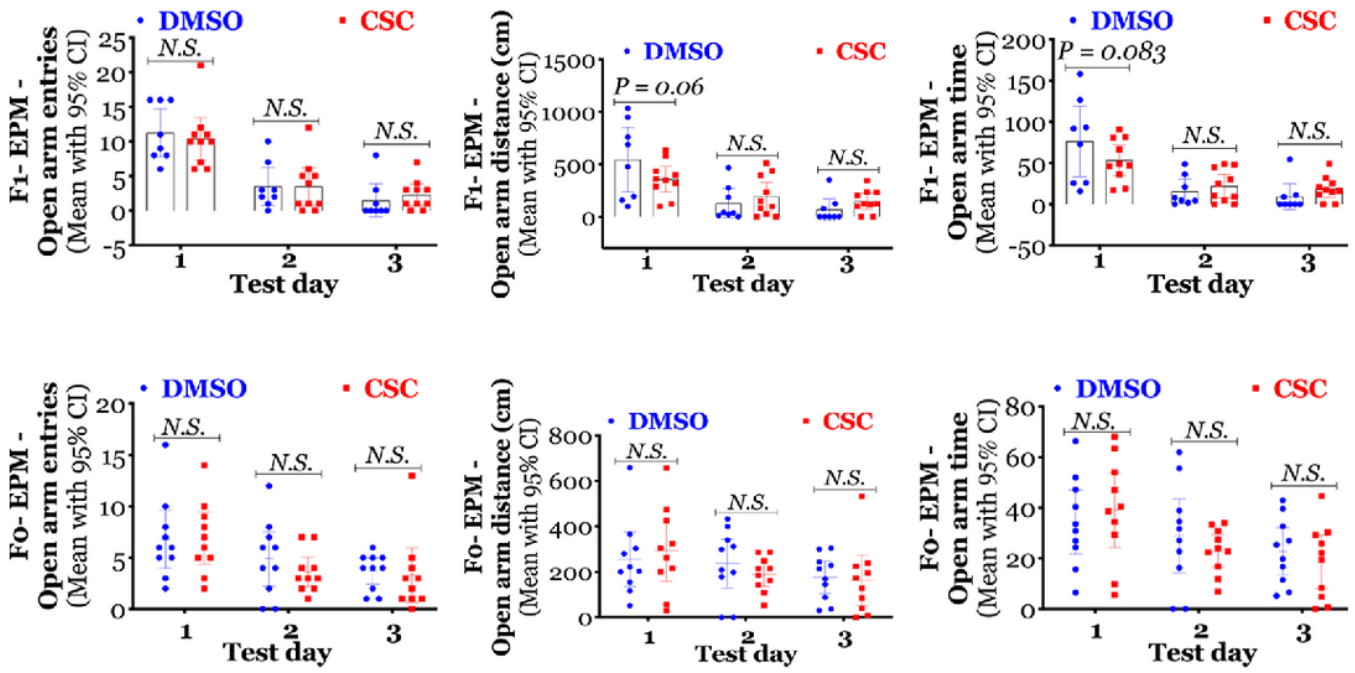

Figure 3

Elevated plus maze (EPM) test. Number of entries made, time spent (sec), and distance traveled (cm) into the open and closed arms and center area of elevated plus maze by F1 offspring (top panels), and exposed F0 sires males (bottom panels) were quantified following a five-minute test session. The conditioned avoidance of the open arms by the mice in a dimly lit room was evaluated over 3 consecutive days. Approximately 10-20 mice per treatment group from 7-8 litters were assessed. The data were analyzed by two-way ANOVA and Sidak's multiple comparisons test to represent mean difference between treatment groups with 95% CI and a P <0.05. GraphPad Prism 8.1.1 statistical software was used for data analysis. N.S: Not significant.

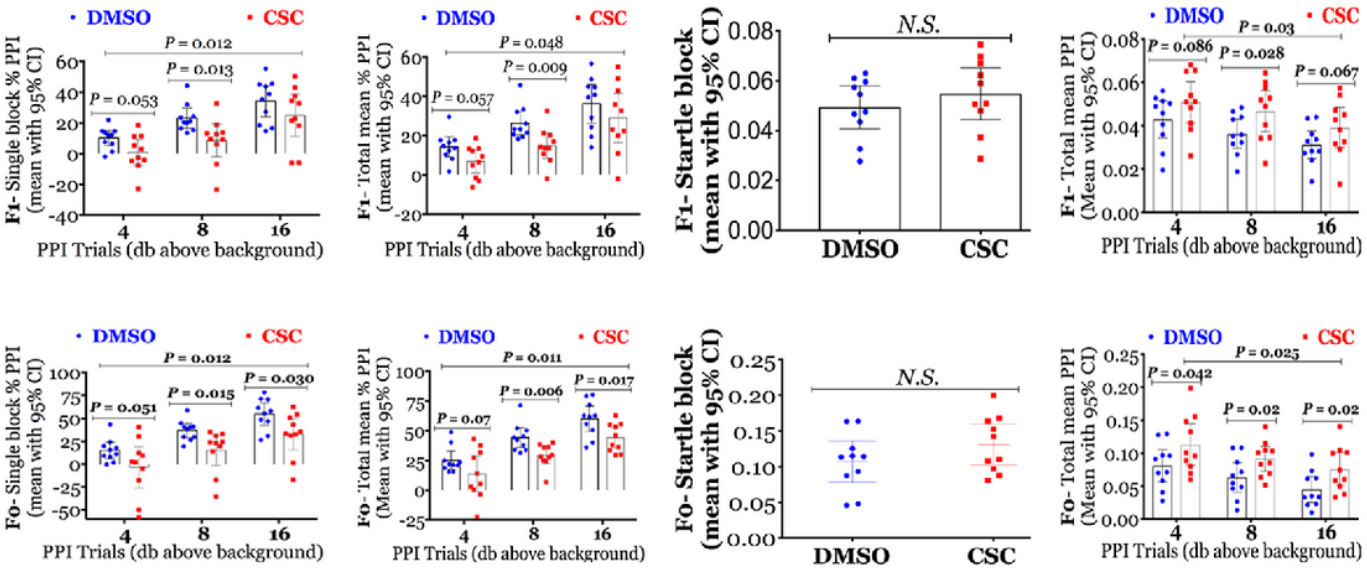

Figure 4

Acoustic startle/prepulse inhibition of startle (PPI) Test. The mean percent of acoustic single block % PPI, total mean % PPI, mean startle block, and total mean PPI of F1 offspring (top panels), and CSC-, and DMSO exposed F0 males (bottom panels) by setting the decibels (db) at 4, 8, and 16 above the background. The amount of PPI is expressed as the percent decrease in the amplitude of the startle reactivity due to presentation of the prepulse (% PPI). Approximately 10-20 mice per treatment group from 7-8 litters were used. The data were analyzed by two-way ANOVA and Sidak's multiple comparison test to represent mean difference between treatment groups with 95% CI and a P <0.05. N.S: Not significant.

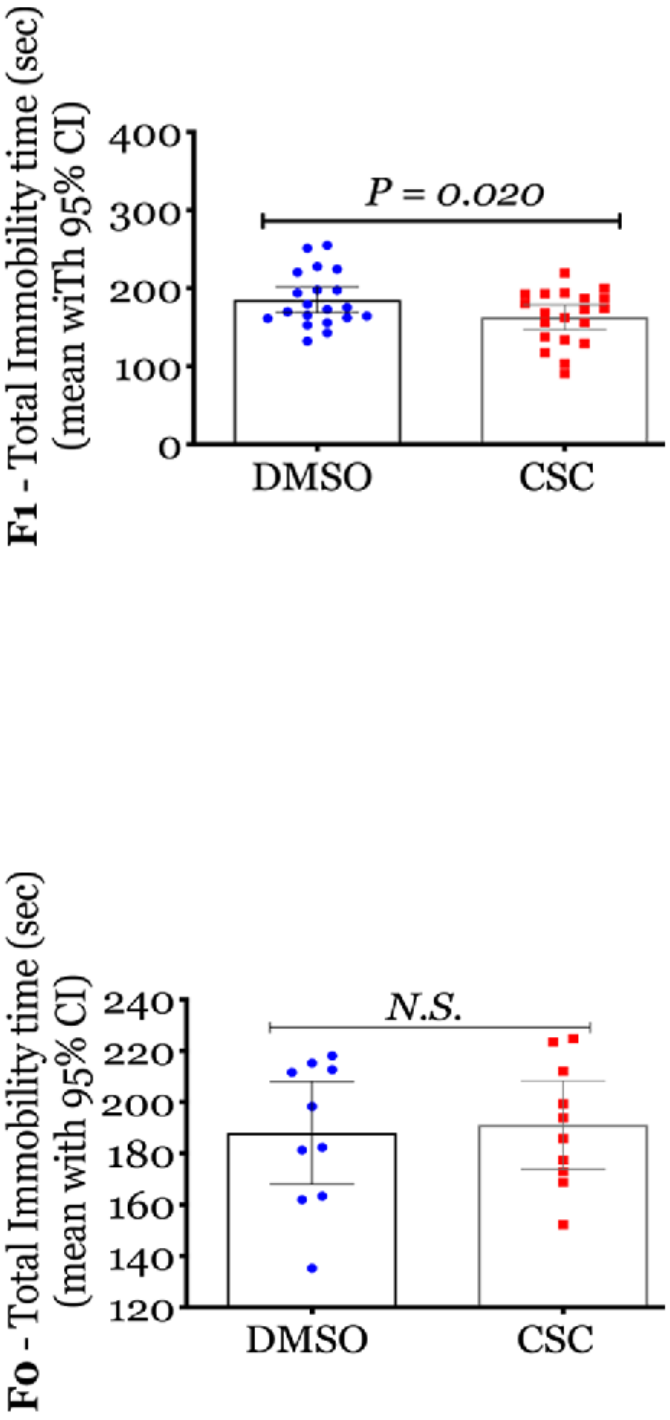

Figure 5

Tail suspension test. F1 offspring (top), and their DMSO-, and CSC- exposed F0 males (bottom). Approximately 10-20 mice per treatment group from 7-8 litters were used. The data were analyzed by Wilcoxon matched-pairs t-test to represent mean difference of total immobility time (sec) between treatment groups with 95% CI, and P <0.05. N.S: Not significant.

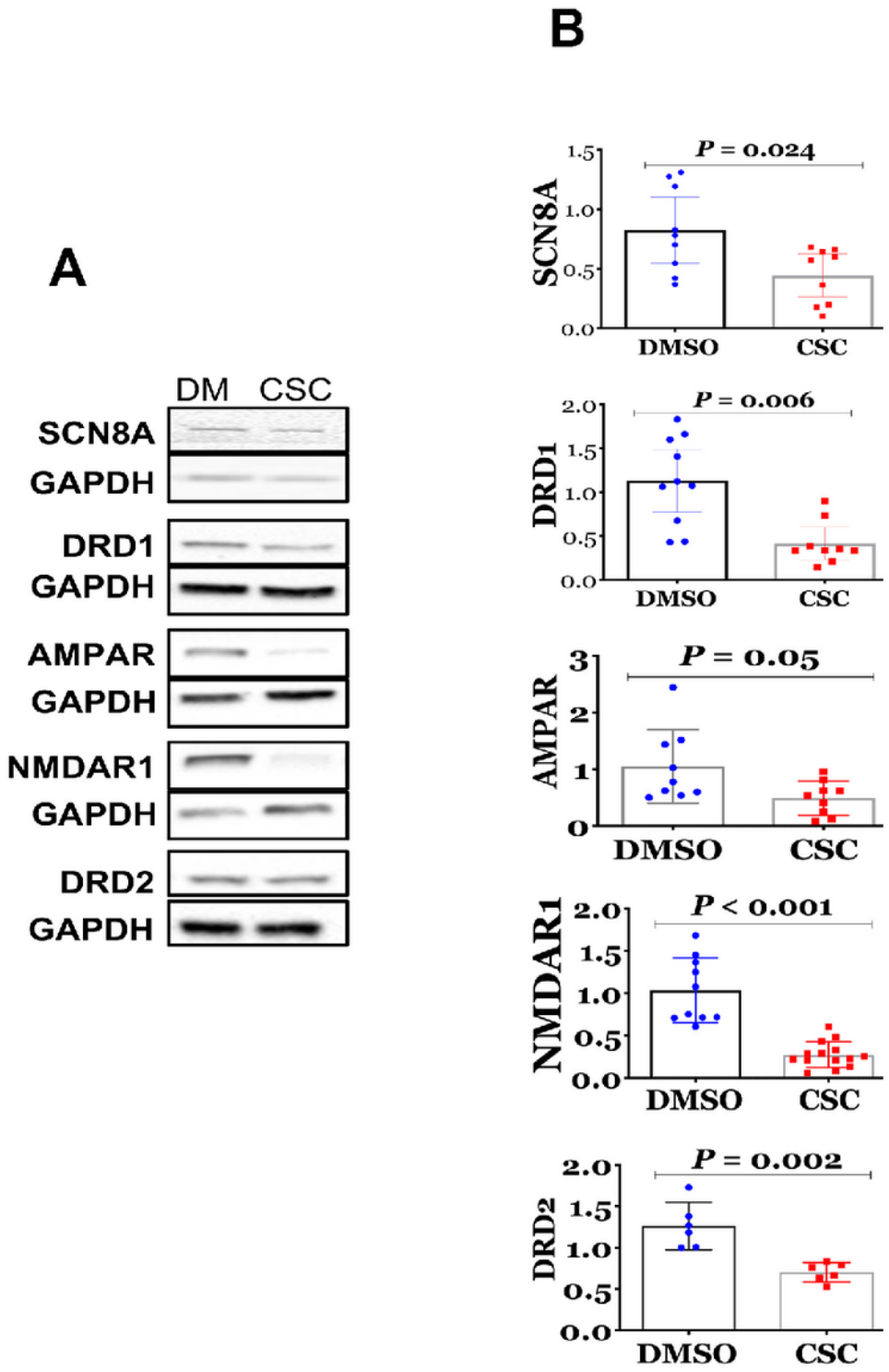

Figure 6

Expression of neuronal receptor proteins in F1 fetal (e18.5) brain. 6A: Western blot analysis of neuronal receptors such as voltage gated sodium channel protein (SCN8A), dopamine receptors (DRD1, DRD2), GRIA2 (glutamate ionotropic receptor AMPA type subunit 2 / AMPAR), and GRIN1 (glutamate ionotropic receptor NMDA type subunit 1 / NMDAR1) in F1 fetal (e18.5) brain. Aliquots of 40 µg of protein from DMSO- (DM), and CSC-treated brain samples separated by SDS-PAGE were probed with primary

antibodies to selected proteins and respective secondary antibodies. SuperSignal West Femto Maximum Sensitivity Chemiluminescence Substrate (Pierce) was used for detection, and the blots were normalized to GAPDH (1:2000, Cell signaling). 6B: Histogram represents the mean fold changes in respective target protein levels between DMSO vs CSCexposed F1 fetal brain samples in densitometric units normalized to GAPDH. Approximately, 5-6 mice / treatment group were used from 7-8 litters. Mann-Whitney unpaired two-tailed t-test used to represent mean with 95% CI, and P <0.05.

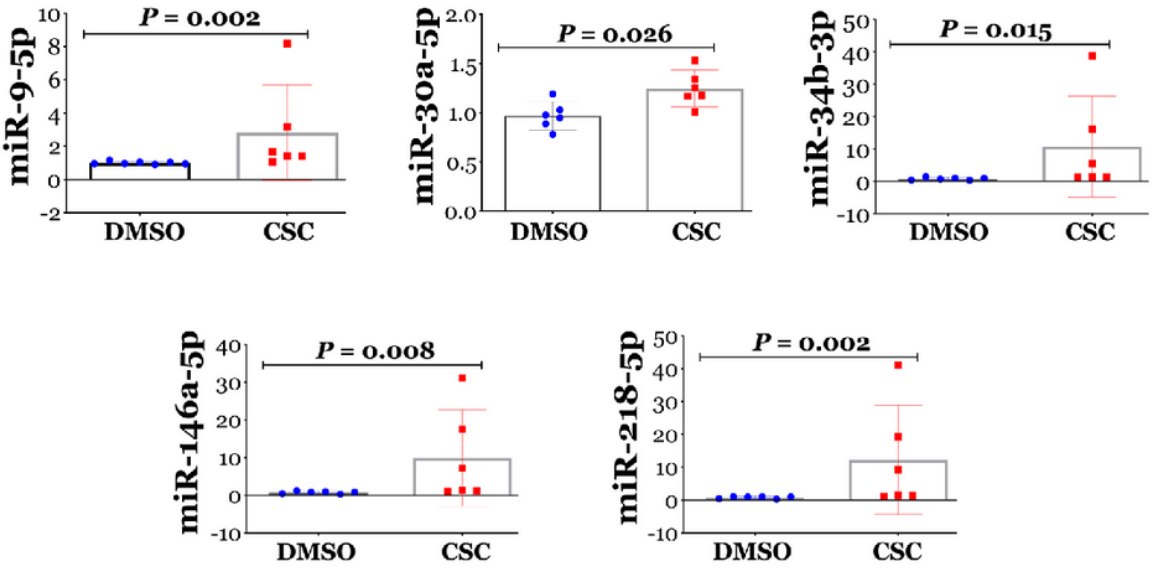

Figure 7

Determination of putative miRNAs expression in F1 fetal (e18.5) brain. Q-RTPCR analysis was performed to assess the expression of miR-9a-5p, miR-30a-5p, miR-34b-3p, miR-146a5p, and 218-5p in F1 fetal (e18.5) brain of DMSO or CSC-exposed sires. The data were normalized and analyzed as described and the histograms reflect the relative fold change in miRNAs between the treatment groups. Approximately, 5-6 mice / treatment group were used from 5-6 litters. Mann-Whitney unpaired two-tailed t-test used to represent mean with 95% CI, and P <0.05.

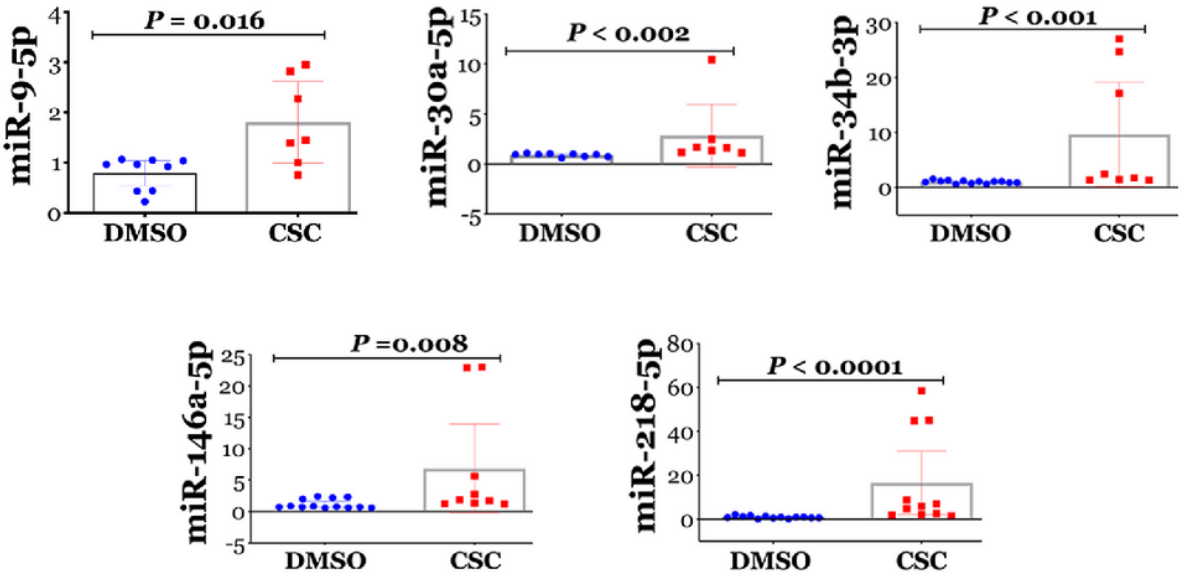

Figure 8

Effect of CSC on candidate miRNAs expression in F0 caudal sperm. Q-RTPCR analysis was performed to determine the expression of miR-9a-5p, miR-30a-5p, miR-34b-3p, miR146a-5p, and 218-5p in Fo caudal sperm of 40-day DMSO or CSC-injected sires. The data were normalized and analyzed as described and the histograms reflect the relative fold change in miRNAs between the treatment groups. Approximately 10-20 mice / treatment group were used. Mann-Whitney unpaired two-tailed t-test used to represent mean with 95% CI, and P <0.05.

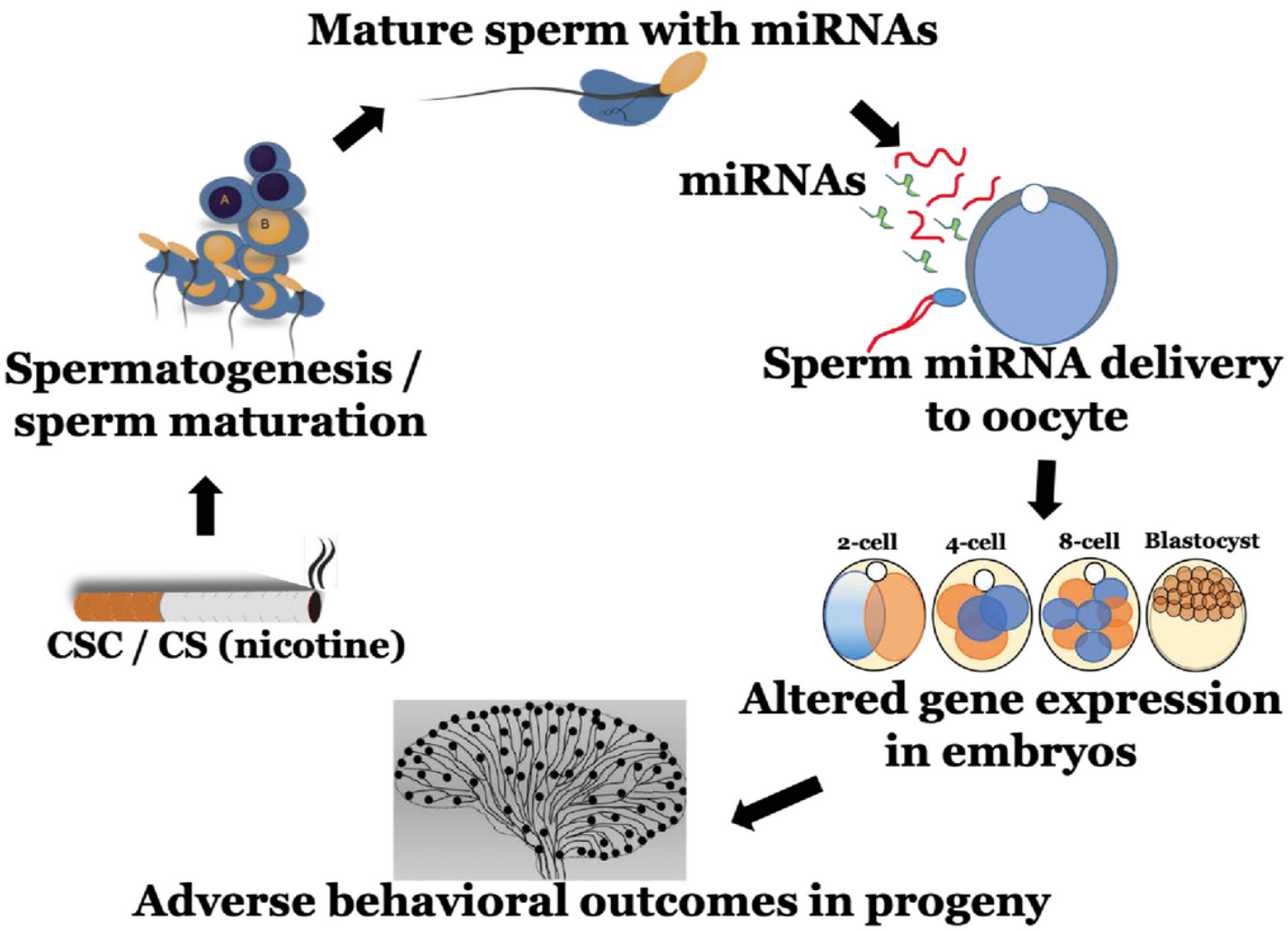

Figure 9

Proposed schematic of paternal cigarette smoke mediated neurobehavioral outcome in offspring. Paternal cigarette smoke (CS) exposure can alter sperm miRNA levels during spermatogenesis or maturing sperm can acquire them during epididymal sojourn. Sperm delivers CSC-altered miRNAs to oocytes during fertilization. Sperm-delivered miRNAs in fertilized oocytes may dysregulate maternal mRNAs mostly at early stages of embryo development. Changes in target mRNAs expression associated with neuronal signaling lead to adverse neurobehavioral and neurobiological outcomes in progeny. CSC / CS: Cigarette smoke condensate / cigarette smoke.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- PrabaEsakkyFigsandTablesMB.pdf