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Supplemental Material

Neuroinflammatory and Behavioral Outcomes Measured in Adult Offspring of Mice Exposed Prenatally to E-Cigarette Aerosols

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Table S1. Individual litter data.

Treatment	LitterID	# Pups	Male	Female	Fraction male pups
FA	P01	10	4	6	0.40
	P02	10	4	6	0.40
	P05	10	6	4	0.60
	P08	10	7	3	0.70
	P09	8	6	2	0.75
	P12	10	8	2	0.80
	P16	10	4	6	0.40
	P19	9	7	2	0.78
	P22	10	7	3	0.70
	P24	10	3	7	0.30
PG/VG	P04	10	6	4	0.60
	P07	10	4	6	0.40
	P11	8	6	2	0.75
	P13	10	6	4	0.60
	P15	10	4	6	0.40
	P18	10	5	5	0.50
	P23	10	3	7	0.30
	P25	10	4	6	0.40
	P33	9	2	7	0.22
+ Nic	P03	10	2	8	0.20
	P06	9	6	3	0.67
	P10	10	3	7	0.30
	P14	9	7	2	0.78
	P21	10	5	5	0.50
	P28	10	4	6	0.40
	P30	10	3	7	0.30
	P36	10	3	7	0.30

FA: Filtered Air; PG/VG: Propylene Glycol/Vegetable Glycerin; +Nic: Propylene Glycol/Vegetable Glycerin plus Nicotine

Table S2. Maternal Weight

Variance (Random Effects) σ^2	Model 1	Model 2	Model 3	Model 4
Residual r_{ij}	23.53	5.97	5.73	5.50
Litter (Dam) μ_{0j}	<0.001	<0.001	<0.001	<0.001
Estimate of Model Fit	Model 1	Model 2	Model 3	Model 3
AIC	1937.30	1247.07	1249.49	1252
χ^2		692.23**	1.58 ^{n.s.}	6.66 ^{n.s.}

n.s. Not Significant ** $p < 0.01$ from Likelihood Ratio Test

Model 1: Random effects only

Model 2: Random effects polynomial model for gestational day

Model 3: Addition of fixed main effect of treatment

Model 4: Addition of interactions for treatment effect

Summary of polynomial model (Model 2)

Fixed Effects	Estimate	Std. Error	95% CI	t-value	p-value
Intercept	28.39**	0.48	(27.45, 29.34)	58.66	<0.001
Day	-0.65**	0.07	(-0.79, -0.52)	-9.48	<0.001
Day ²	0.13**	0.003	(0.12, 0.13)	38.16	<0.001

** $p < 0.01$ from t-test of beta coefficients

Table S3. Offspring Weight (Postnatal Day 21)

Variance (Random Effects) σ^2	Model 1	Model 2	Model 3
Residual r_{ij}	3.66	3.68	3.63
Litter μ_{0j}	8.00	7.34	7.42
Estimate of Model Fit	Model 1	Model 2	Model 3
AIC	1073.29	1057.87	1060.53
χ^2		21.42 ^{**}	1.35 ^{n.s.}

n.s. Not Significant from Likelihood Ratio Test

Model 1: Random effects Only

Model 2: Addition of fixed main effects

Model 3: Addition of interactions for fixed effects

Summary of Fixed Effects (Model 2)

Fixed Effects	Estimate	Std. Error	95% CI	t-value	p-value
Intercept	13.81 ^{**}	0.89	(11.29, 14.78)	15.54	<0.0001
PG/VG	0.28	1.29	(-2.35, 2.92)	0.22	0.83
+Nic	-1.77	1.31	(-4.46, 0.92)	-1.35	0.19
Sex	-0.78 ^{**}	0.17	(-1.12, -0.44)	-4.52	<0.0001

^{**} $p < 0.01$ from t-test of beta coefficient

PG/VG: Propylene Glycol/Vegetable Glycerin; +Nic: Propylene Glycol/Vegetable Glycerin plus Nicotine

Table S4: Offspring Weight (12 weeks)

Variance (Random Effects) σ^2	Model 1	Model 2	Model 3
Residual r_{ij}	83.06	38.57	36.18
Litter μ_{0j}	<0.001	7.09	7.48
Estimate of Model Fit	Model 1	Model 2	Model 3
AIC	913.18	839.68	837.15
χ^2		79.50 **	6.53*

* $p < 0.05$, ** $p < 0.01$ from Likelihood Ratio Test

Model 1: Random effects only

Model 2: Addition of fixed main effects

Model 3: Addition of interactions for fixed effects

Summary of Fixed Effects (Model 3)

Fixed Effects	Estimate	Std. Error	95% CI	t-value	p-value
Intercept	52.17**	1.61	(49.06, 55.28)	32.46	<0.0001
PG/VG	1.91	2.36	(-2.84, 6.67)	0.81	0.43
+Nic	-1.36	2.36	(-6.12, 3.40)	-0.57	0.57
Sex	-14.33**	1.85	(-17.91, -10.74)	-7.74	<0.0001
PG/VG:Female	0.64	2.68	(-4.54, 5.82)	0.24	0.81
+ Nic:Female	6.51*	2.77	(1.14, 11.88)	2.35	0.021

Post Hoc Comparisons	Difference	t-value	p-value
Sex:Treatment			
Male			
Filtered Air vs PG/VG	-1.55	-0.58	0.83
Filtered Air vs +Nic	1.23	0.46	0.89
PG/VG vs +Nic	2.78	1.01	0.58
Female			
Filtered Air vs PG/VG	-2.38	-1.21	0.46
Filtered Air vs +Nic	-5.30*	-2.52	0.049
PG/VG vs +Nic	-2.92	-1.38	0.37

* $p < 0.05$, ** $p < 0.01$ from t-test of beta coefficients

PG/VG: Propylene Glycol/Vegetable Glycerin; +Nic: Propylene Glycol/Vegetable Glycerin plus Nicotine

Table S5. Elevated Plus Maze: Percent Open Arm Exploration

Variance (Random Effects) σ^2	Model 1	Model 2	Model 3
Residual r_{ij}	226.92	226.28	223.94
Litter μ_{0j}	90.67	86.76	87.76
Estimate of Model Fit	Model 1	Model 2	Model 3
AIC	1129.91	1135.10	1136.17
χ^2		0.81 ^{n.s.}	2.93 ^{n.s.}

n.s. Not Significant from Likelihood Ratio Test

Model 1: Random effects Only

Model 2: Addition of fixed main effects

Model 3: Addition of interactions for fixed effects

Table S6. Elevated Plus Maze: Time in Open Arm

Variance (Random Effects) σ^2	Model 1	Model 2	Model 3
Residual r_{ij}	670.28	634.56	621.14
Litter μ_{0j}	374.16	353.94	354.17
Estimate of Model Fit	Model 1	Model 2	Model 3
AIC	1302.62	1305.99	1306.56
χ^2		2.63 ^{n.s.}	3.43 ^{n.s.}

n.s. Not Significant from Likelihood Ratio Test

Model 1: Random effects Only

Model 2: Addition of fixed main effects

Model 3: Addition of interactions for fixed effects

Table S7. Elevated Plus Maze: Open Arm Entries

Variance (Random Effects) σ^2	Model 1	Model 2	Model 3
Residual r_{ij}	19.72	19.46	19.33
Litter μ_{0j}	3.93	3.47	3.44
Estimate of Model Fit	Model 1	Model 2	Model 3
AIC	792.36	795.22	798.31
χ^2		3.14 ^{n.s.}	0.90 ^{n.s.}

n.s. Not Significant from Likelihood Ratio Test

Model 1: Random effects Only

Model 2: Addition of fixed main effects

Model 3: Addition of interactions for fixed effects

Table S8. Elevated Plus Maze: Distance Traveled

Variance (Random Effects) σ^2	Model 1	Model 2	Model 3
Residual r_{ij}	36361.87	35348.13	35344.67
Litter μ_{0j}	9144.42	5112.24	5109.46
Estimate of Model Fit	Model 1	Model 2	Model 3
AIC	1788.23	1783.37	1787.35
χ^2		10.86*	0.02 ^{n.s.}

$p < 0.05$, ** $p < 0.01$ from Likelihood Ratio Test

Model 1: Random effects Only

Model 2: Addition of fixed main effects

Model 3: Addition of interactions for fixed effects

Summary of Fixed Effects (Model 2)

Distance Traveled					
Fixed Effects	Estimate	Std. Error	CI	t-value	p-value
Intercept	730.79**	38.70	(655.21, 806.37)	18.88	<0.0001
PG/VG	64.60	51.87	(-40.83, 170.02)	1.25	0.23
+Nic	164.26**	53.97	(54.57, 273.96)	3.04	0.006
Sex	-54.03	33.48	(-119.41, 11.35)	-1.61	0.11

** $p < 0.01$ from t-test of beta coefficient

PG/VG: Propylene Glycol/Vegetable Glycerin; +Nic: Propylene Glycol/Vegetable Glycerin plus Nicotine

Table S9. Open Field: Time in Center

Variance (Random Effects) σ^2	Model 1	Model 2	Model 3
Residual r_{ij}	812.29	753.76	753.70
Litter μ_{0j}	20.40	12.18	11.87
Estimate of Model Fit	Model 1	Model 2	Model 3
AIC	1239.42	1234.73	1238.67
χ^2		10.69**	0.06 ^{n.s.}

n.s. Not Significant, ** $p < 0.05$ from Likelihood Ratio Test

Model 1: Random effects Only

Model 2: Addition of fixed main effects

Model 3: Addition of interactions for fixed effects

Summary of Fixed Effects (Model 2)

Fixed Effects	Distance Traveled				
	Estimate	Std. Error	CI	t-value	p-value
Intercept	52.91**	4.69	(43.76, 62.07)	11.28	<0.0001
PG/VG	-3.88	6.08	(-16.24, 8.48)	-0.64	0.53
+Nic	-10.95	6.29	(-23.73, 1.82)	-1.74	0.09
Sex	13.85**	4.93	(4.23, 23.47)	2.81	0.006

** $p < 0.01$ from t-test of beta coefficient

PG/VG: Propylene Glycol/Vegetable Glycerin; +Nic: Propylene Glycol/Vegetable Glycerin plus Nicotine

Table S10. Open Field: Distance Traveled

Variance (Random Effects) σ^2	Model 1	Model 2	Model 3
Residual r_{ij}	2462307.1	2317704.0	2268428.9
Litter μ_{0j}	407194.9	308157.9	320803.2
Estimate of Model Fit	Model 1	Model 2	Model 3
AIC	2283.59	2282.84	2285.64
χ^2		6.75 ^{n.s.}	1.20 ^{n.s.}

n.s. Not Significant from Likelihood Ratio Test

Model 1: Random effects Only

Model 2: Addition of fixed main effects

Model 3: Addition of interactions for fixed effects

Table S11. Forced Swim Test: Swimming

Variance (Random Effects) σ^2	Model 1	Model 2	Model 3
Residual r_{ij}	812.74	796.23	795.77
Litter μ_{0j}	286.25	114.61	113.23
Estimate of Model Fit	Model 1	Model 2	Model 3
AIC	1233.59	1224.92	1228.73
χ^2		14.67**	0.195 ^{n.s.}

n.s. not significant, ** $p < 0.01$ from Likelihood Ratio Test

Model 1: Random effects only

Model 2: Addition of fixed main effects

Model 3: Addition of interactions for fixed effects

Summary of Fixed Effects (Model 2)

Swimming					
Fixed Effects	Estimate	Std. Error	CI	t-value	p-value
Intercept	61.34**	6.13	(49.37, 73.32)	10.00	<0.0001
PG/VG	0.28	7.99	(-15.94, 16.49)	0.03	0.97
+Nic	29.71**	8.23	(13.00, 46.42)	3.61	0.001
Sex	-5.86	5.19	(-15.99, 4.27)	-1.13	0.26

** $p < 0.01$ from t-test of beta coefficient

PG/VG: Propylene Glycol/Vegetable Glycerin; +Nic: Propylene Glycol/Vegetable Glycerin plus Nicotine

Table S12. Forced Swim Test: Immobility

Variance (Random Effects) σ^2	Model 1	Model 2	Model 3
Residual r_{ij}	2384.38	2362.02	2257.94
Litter μ_{0j}	673.87	349.68	365.15
Estimate of Model Fit	Model 1	Model 2	Model 3
AIC	1365.75	1362.23	1361.53
χ^2		9.52*	14.22*

* $p < 0.01$ from Likelihood Ratio Test

Model 1: Random effects only

Model 2: Addition of fixed main effects

Model 3: Addition of interactions for fixed effects

Summary of Fixed Effects (Model 3)

Fixed Effects	Estimate	Std. Error	CI	t-value	p-value
Intercept	118.04**	12.20	(94.40, 141.68)	9.67	<0.0001
PG/VG	16.36	18.00	(-19.90, 52.62)	0.91	0.37
+Nic	-50.47**	17.97	(-86.66, -14.29)	-2.81	0.009
Sex	-4.54	14.41	(-32.45, 23.37)	-0.31	0.75
PG/VG:Female	-1.57	21.01	(-42.27, 39.12)	-0.07	0.94
+Nic:Female	40.65	21.74	(-1.45, 82.76)	1.87	0.064

Post Hoc Comparisons	Difference	t-value	p-value
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Sex:Treatment

Male

Filtered Air vs PG/VG	-13.1	-0.77	0.73
Filtered Air vs +Nic	52.2*	3.07	0.016
PG/VG vs +Nic	65.3*	3.70	0.004

Female

Filtered Air vs PG/VG	-16.5	-0.91	0.64
Filtered Air vs +Nic	10.2	0.52	0.86
PG/VG vs +Nic	26.7	1.35	0.38

* $p < 0.05$, ** $p < 0.05$ from t-test of beta coefficients

PG/VG: Propylene Glycol/Vegetable Glycerin; +Nic: Propylene Glycol/Vegetable Glycerin plus Nicotine

Table S13. Familiarization Phase: Total Object Sniff Time

Variance (Random Effects) σ^2	Model 1	Model 2	Model 3
Residual r_{ij}	279.44	276.85	271.49
Litter μ_{0j}	72.92	74.25	74.92
Estimate of Model Fit	Model 1	Model 2	Model 3
AIC	1120.27	1125.47	1127.38
χ^2		0.80 ^{n.s.}	2.09 ^{n.s.}

^{n.s.} Not Significant from Likelihood Ratio Test

Model 1: Random effects only

Model 2: Addition of fixed main effects

Model 3: Addition of interactions for fixed effects

Table S14. Novel Object Recognition: Novel Object Score

Variance (Random Effects) σ^2	Model 1	Model 2	Model 3
Residual r_{ij}	77.87	75.82	75.70
Litter μ_{0j}	11.87	3.05	3.10
Estimate of Model Fit	Model 1	Model 2	Model 3
AIC	948.41	941.17	945.04
χ^2		13.23**	0.13

** $p < 0.01$ from Likelihood Ratio Test

Model 1: Random effects only

Model 2: Addition of fixed main effects

Model 3: Addition of interactions for fixed effects

Summary of Fixed Effects (Model 2)

Fixed Effects	Estimate	Std. Error	CI	t-value	p-value
Intercept	60.71**	1.59	(57.61, 63.81)	38.23	<0.0001
PG/VG	-5.19*	2.03	(-9.33, -1.07)	-2.56	0.017
+Nic	-6.97**	2.11	(-11.25, -2.68)	-3.30	0.003
Sex	-2.67	1.56	(-5.72, 2.70)	-1.71	0.091

* $p < 0.05$, ** $p < 0.01$ from t-test of beta coefficient

PG/VG: Propylene Glycol/Vegetable Glycerin; +Nic: Propylene Glycol/Vegetable Glycerin plus Nicotine

Table S15. Novel Object Recognition: Object Sniff Time

Variance (Random Effects) σ^2	Model 1	Model 2	Model 3	Model 4
Residual r_{ij}	120.13	68.32	56.07	52.78
Litter μ_{0j}	12.81	25.93	31.13	32.33
Estimate of Model Fit	8.58	6.76	6.03	5.65
AIC	Model 1	Model 2	Model 3	Model 4
χ^2	1984.20	1952.60	1943.12	1950.19
Residual r_{ij}		33.59**	17.48**	4.93 ^{n.s.}

** $p < 0.01$ from Likelihood Ratio Test

Model 1: Random effects only

Model 2: Addition of object

Model 3: Addition of treatment and interaction

Model 4: Addition of sex main effect and interaction

Summary of Fixed Effects at Each Treatment Level (Model 3)

Treatment	Fixed Effects	Estimate	Std. Error	CI	t-value	p-value
FA	Intercept	26.47**	1.40	(23.68, 29.26)	18.87	<0.0001
	Novel Object	12.45**	1.50	(9.46, 15.43)	8.29	<0.0001
PG/VG	Intercept	29.80**	1.84	(26.13, 33.47)	16.20	<0.0001
	Novel Object	3.91*	1.89	(0.14, 7.68)	2.07	0.045
+Nic	Intercept	32.04**	2.48	(27.02, 37.06)	12.94	<0.0001
	Novel Object	3.36	2.25	(-1.21, 7.93)	1.49	0.14

* $p < 0.05$, ** $p < 0.01$ from t-test of beta coefficient

PG/VG: Propylene Glycol/Vegetable Glycerin; +Nic: Propylene Glycol/Vegetable Glycerin plus Nicotine

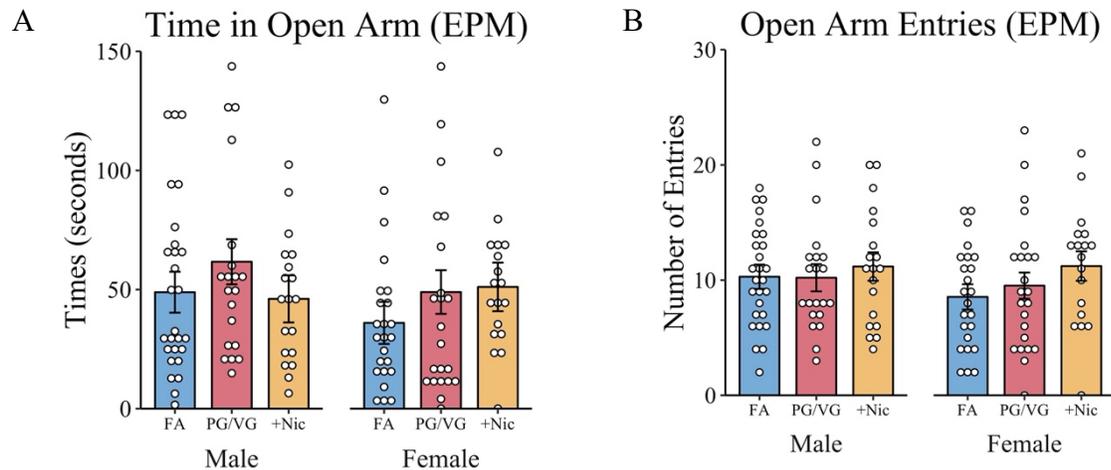


Figure S1. Open arm exploration in the Elevated Plus Maze in male and female adult offspring of mice treated with filtered air (FA), E-cigarette aerosol (PG/VG), or E-cigarette aerosol with nicotine (+nic). Pregnant female mice were exposed to either filtered air or e-cigarette aerosol (PG/VG) with or without nicotine, and offspring were tested using the elevated plus maze (A, B) Reported is (A) the total time offspring spent in the open arm of the elevated plus maze; (B) The number of entries made into the open arm of the maze during the the 5-minute elevated plus maze task; Linear mixed effects modeling with treatment and sex as fixed effects and litters as random effects. Plots represent individual mice; bars represent marginal means \pm SE. FA (10 litters) males $n = 27$, females $n = 24$; PG/VG (9 litters) males $n = 21$, females $n = 23$; + Nic (8 litters) males $n = 19$, females $n = 18$. FA: filtered air, PG/VG: propylene glycol/vegetable glycerin, +Nic: propylene glycol/vegetable glycerin plus nicotine, EPM: elevated plus maze