The Role of the Nuclear Factor-κB Transcriptional Complex in Cortical Immune Activation in Schizophrenia

Supplemental Information

Supplemental Methods

Quantitative PCR

Frozen tissue blocks containing the middle portion of the right superior frontal sulcus were confirmed to contain PFC area 9 using Nissl-stained, cryostat tissue sections for each subject (1, 2). The gray-white matter boundary of PFC area 9 in a tissue block from each subject was carefully scored with a scalpel blade in locations where the gray matter had uniform thickness and the gray-white matter boundary was easily discerned. The scored gray matter region of the tissue block was then digitally photographed, and the number of tissue sections (40 μm) required to collect ~30 mm^3 of gray matter was determined for each subject. The calculated number of required tissue sections for each subject was then cut by cryostat, and gray matter was separately collected into a tube containing TRIzol reagent in a manner that ensured minimal white matter contamination and excellent RNA preservation (3). Standardized dilutions of total RNA for each subject were used to synthesize cDNA. All primer pairs (Supplemental Table S2) demonstrated high amplification efficiency (>90%) across a wide range of cDNA dilutions and specific single products in dissociation curve analysis. Control studies in which the cDNA template was not included in the quantitative PCR reaction resulted in the absence of amplification.

Poly(I:C)-exposed mice

As previously described (4), timed pregnant C57BL/6J mice (n=12/condition; The Jackson Laboratory, Bar Harbor, Maine) received intraperitoneal injections of polyriboinosinicpolyribocytidilic acid potassium salt [poly(I:C); 20 mg/kg pure form; Sigma, St. Louis, Missouri], a synthetic analogue of double stranded RNA that induces an immune response (5-7), or an equivalent volume of normal saline daily for three days at mid- (E11-13) or late- (E15-17) gestation (Supplemental Table S3). Non-pregnant adult female mice also received similar injections of poly(I:C) 20 mg/kg (n=8) or normal saline (n=8) daily for three days in parallel with the timed pregnant mice. The non-pregnant mice were euthanized 3 hours after the last injection (random estrous cycle) and trunk blood was collected after decapitation. A second set of non- pregnant adult female mice also received injections of poly(I:C) (n=4) or normal saline (n=4) daily for three days at a separate time and underwent transcardial perfusion with normal saline 3 hours after the last injection. Serum IL-6 levels were confirmed to be massively elevated in poly(I:C)- exposed mice (725.1 ± 276.6 pg/ml; t_{14}=7.3, p<.0001) relative to normal saline-exposed mice (8.6 ± 14.3 pg/ml) using the Mouse IL-6 Quantikine ELISA Immunoassay (R&D Systems, Minneapolis, Minnesota) as previously reported (4).

Fresh, frozen brains from one 8-week-old male and/or female offspring per poly(I:C)-injected mother (n=7-8/sex/condition; Supplemental Table S3) and from the non-pregnant adult female mice that received daily poly(I:C) or normal saline injections for three days were included in the study. RNA was isolated from homogenates of frontal cortex tissue sections (12 µm) collected consecutively from Bregma +2.8 to 2.1 mm (excluding sub-rhinal fissure olfactory tissue; (8)) into TRIzol. Quantitative PCR assessment of NF-κB-related markers (Supplemental Table S2) was performed as described for the human studies with the following exception: primer efficiency testing found that transcript levels for TLR4, TNFR2, CD40, and IκBϵ in mouse frontal cortex were insufficient for analysis by quantitative PCR.

All animal studies followed the NIH Guide for the Care and Use of Laboratory Animals and were approved by the University of Pittsburgh's Institutional Animal Care and Use Committee.

Supplemental References

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4. Volk DW, Chitrapu A, Edelson JR, Roman KM, Moroco AE, Lewis DA (2015): Molecular mechanisms and timing of cortical immune activation in schizophrenia. *Am J Psychiatry*. 172:1112-1121.

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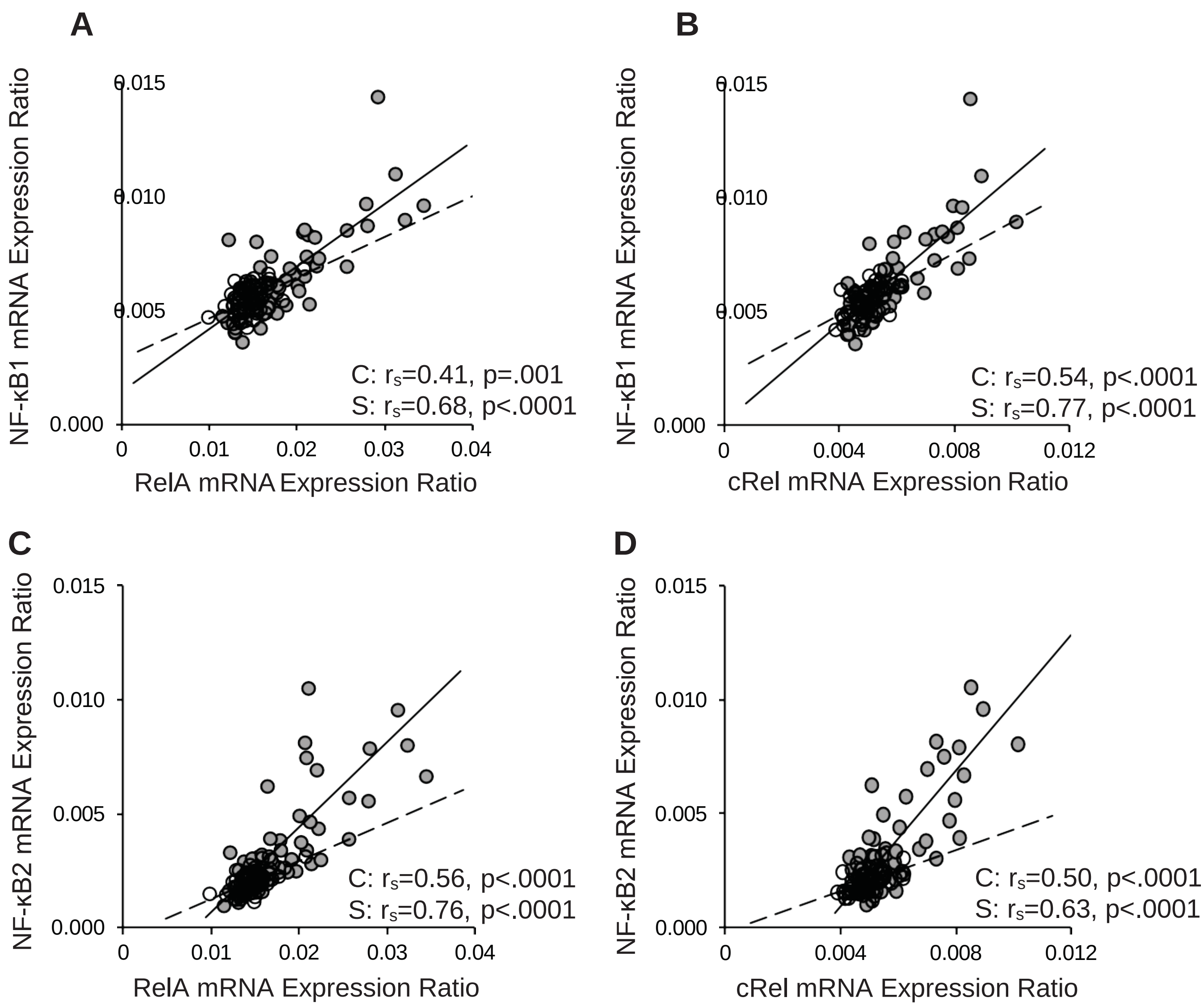
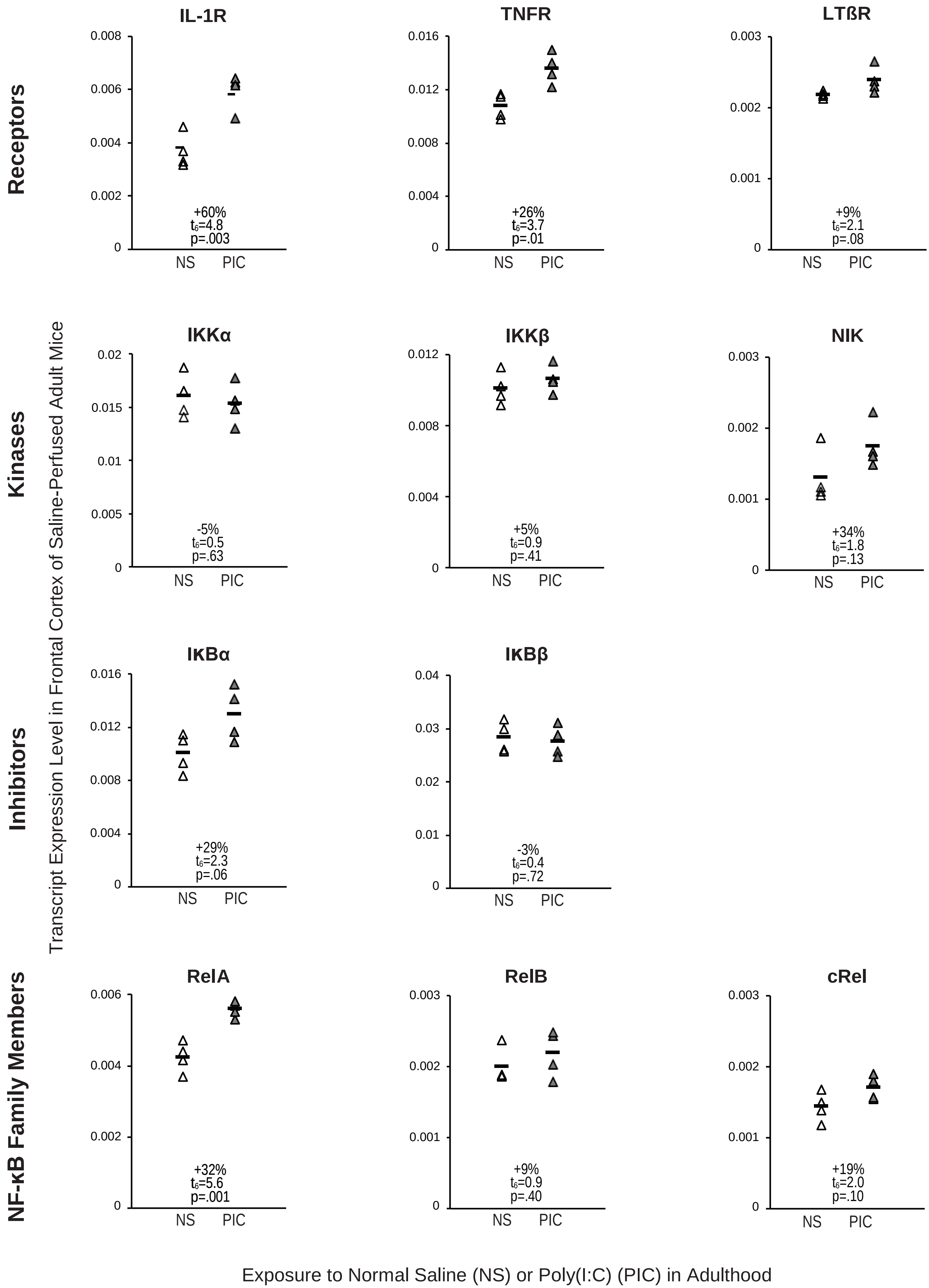


Supplemental Figure S1. Correlations between transcript levels for NF-κB family members in the prefrontal cortex of unaffected comparison subjects and schizophrenia subjects. NF- κB1 and NF-κB2 mRNA levels were positively correlated with mRNA levels for RelA (**A, C,** respectively) and cRel (**B, D,** respectively) in both schizophrenia subjects (S; grey circles; solid line) and unaffected comparison subjects (C; open circles; dashed line).

Figure S2. NF-κB-Related mRNAs in Saline-Perfused Mice with Subacute Exposure to Poly(I:C) in Adulthood



Supplemental Figure S2. Transcript levels for NF-κB-related mRNAs in the frontal cortex of adult mice exposed to poly(I:C), then saline-perfused. To examine whether residual blood cells in brain parenchyma may have contributed to the effects of subacute poly(I:C) exposure on NF-κB-related mRNAs in the frontal cortex, an additional group of adult non-pregnant female mice was exposed to poly(I:C) (n=4; gray triangles) or normal saline (n=4; open triangles) daily for three days then underwent transcardial perfusion with normal saline. Transcript levels for NF-κB family members, activation receptors, kinases, and inhibitors were then quantified in the frontal cortex. Black bars indicate mean mRNA levels for each condition. The pattern of altered NF-kB-related mRNA levels seen in the saline-perfused mice with daily poly(I:C) exposure was highly similar to that seen in mice that were not saline-perfused (Figure 4). For example, transcript levels were also significantly higher for IL-1R, TNFR, and RelA in the frontal cortex of poly(I:C)-exposed mice that underwent transcardial perfusion relative to normal saline-exposed mice that also underwent transcardial perfusion. IκBα mRNA levels were also higher in the poly(I:C)-exposed mice; however, this difference did not quite achieve statistical significance (p=.06). Transcript levels for LTβR, IKKα, IKKβ, NIK, IκBβ, and cRel were similarly unchanged in poly(I:C)-exposed mice relative to normal saline-exposed mice. These findings suggest that any residual blood cells in brain parenchyma likely do not explain the effects of subacute poly(I:C) exposure on NF-κB-related mRNAs in the frontal cortex.

Table S1. Demographic, postmortem, and clinical characteristics of human subjects used in this study.

Unaffected Comparison Subjects									**Subjects with Schizophrenia**														
Pair	**Case**	**Sex/ Race**	**Age**	**PMI[a]**	**Storage Time[b]**	**RIN**	**pH**	**Cause of Death**	**Case**	**DSM IV diagnosis**	**Sex/ Race**	**Age**	**PMI[a]**	**Storage Time[b]**	**RIN**	**pH**	**Cause of Death**	**NSAID ATOD**	**Tobacco ATOD**	**Anti-psychotic ATOD**	**Anti-depressant ATOD**	**Benzodiazepine/ Anticonvulsant ATOD**	**Living Independently ATOD**
1	592	M/B	41	22.1	225	9.0	6.7	ASCVD	533	Chronic undifferentiated schizophrenia	M/W	40	29.1	235	8.4	6.8	Accidental Asphyxiation	No	Unknown	Typical	No	No	No
2	567	F/W	46	15.0	229	8.9	6.7	Mitral valve prolapse	537	Schizoaffective disorder	F/W	37	14.5	234	8.6	6.7	Suicide by hanging	No	Unknown	None	No	No	Yes
3	516	M/B	20	14.0	237	8.4	6.9	Homicide by gun shot	547	Schizoaffective disorder	M/B	27	16.5	233	7.4	7.0	Heat Stroke	No	Unknown	Typical	Yes	Yes	No
4	630	M/W	65	21.2	219	9.0	7.0	ASCVD	566	Chronic undifferentiated schizophrenia; AAR	M/W	63	18.3	230	8.0	6.8	ASCVD	No	Yes	Atypical	Yes	Yes	No
5	604	M/W	39	19.3	223	8.6	7.1	Hypoplastic coronary artery	581	Chronic paranoid schizophrenia; ADC; OAC	M/W	46	28.1	227	7.9	7.2	Accidental combined drug overdose	No	Unknown	Typical	No	Yes	Yes
6	546	F/W	37	23.5	233	8.6	6.7	ASCVD	587	Chronic undifferentiated schizophrenia; AAR	F/B	38	17.8	226	9.0	7.0	Myocardial hypertrophy	No	Yes	Atypical	No	Yes	No
7	551	M/W	61	16.4	232	8.3	6.6	Cardiac tamponade	625	Chronic disorganized schizophrenia; AAC	M/B	49	23.5	220	7.6	7.3	ASCVD	No	Yes	Typical	Yes	No	No
8	685	M/W	56	14.5	212	8.1	6.6	Hypoplastic coronary artery	622	Chronic undifferentiated schizophrenia	M/W	58	18.9	220	7.4	6.8	Right MCA infarction	No	Unknown	None	No	No	No
9	681	M/W	51	11.6	213	8.9	7.2	Hypertrophic cardio-myopathy	640	Chronic paranoid schizophrenia	M/W	49	5.2	218	8.4	6.9	Pulmonary embolism	No	Unknown	Atypical	Yes	No	No
10	806	M/W	57	24.0	191	7.8	6.9	Pulmonary embolism	665	Chronic paranoid schizophrenia; ADC	M/B	59	28.1	216	9.2	6.9	Intestinal hemorrhage	No	Yes	Typical	Yes	No	Yes
11	822	M/B	28	25.3	189	8.5	7.0	ASCVD	787	Schizoaffective disorder; ODC	M/B	27	19.2	195	8.4	6.7	Suicide by gun shot	No	No	Typical	No	No	No
12	727	M/B	19	7.0	206	9.2	7.2	Trauma	829	Schizoaffective disorder; ADC; OAR	M/W	25	5.0	187	9.3	6.8	Suicide by salicylate overdose	Yes	Yes	None	No	Yes	No
13	871	M/W	28	16.5	178	8.5	7.1	Trauma	878	Disorganized schizophrenia; ADC	M/W	33	10.8	177	8.9	6.7	Myocardial fibrosis	No	Yes	Both	Yes	Yes	No
14	575	F/B	55	11.3	228	9.6	6.8	ASCVD	517	Disorganized schizophrenia; ADC	F/W	48	3.7	237	9.3	6.7	Intracerebral hemorrhage	No	Yes	Atypical	No	No	No
15	700	M/W	42	26.1	210	8.7	7.0	ASCVD	539	Schizoaffective disorder; ADR	M/W	50	40.5	234	8.1	7.1	Suicide by combined drug overdose	Yes	Unknown	Atypical	Yes	Yes	Yes
16	988	M/W	82	22.5	157	8.4	6.2	Trauma	621	Chronic undifferentiated schizophrenia	M/W	83	16.0	220	8.7	7.3	Accidental asphyxiation	No	Unknown	None	No	No	No
17	686	F/W	52	22.6	212	8.5	7.0	ASCVD	656	Schizoaffective disorder; ADC	F/B	47	20.1	216	9.2	7.3	Suicide by gun shot	Yes	Yes	Atypical	No	No	Yes
18	634	M/W	52	16.2	219	8.5	7.0	ASCVD	722	Chronic undifferentiated schizophrenia; ODR; OAR	M/B	45	9.1	206	9.2	6.7	Upper GI bleeding	No	Yes	Typical	No	No	No
19	852	M/W	54	8.0	181	9.1	6.8	Cardiac tamponade	781	Schizoaffective disorder; ADR	M/B	52	8.0	196	7.7	6.7	Peritonitis	No	Yes	Typical	Yes	No	No
20	987	F/W	65	21.5	157	9.1	6.8	ASCVD	802	Schizoaffective disorder; ADC; ODR	F/W	63	29.0	192	9.2	6.4	Right ventricular dysplasia	No	Yes	Both	No	Yes	Yes
21	818	F/W	67	24.0	190	8.4	7.1	Anaphylactic reaction	917	Chronic undifferentiated schizophrenia	F/W	71	23.8	170	7.0	6.8	ASCVD	No	Yes	Typical	No	No	No
22	857	M/W	48	16.6	180	8.9	6.7	ASCVD	930	Disorganized schizophrenia; ADR; OAR	M/W	47	15.3	167	8.2	6.2	ASCVD	No	Yes	None	No	Yes	No
23	739	M/W	40	15.8	205	8.4	6.9	ASCVD	933	Disorganized schizophrenia	M/W	44	8.3	166	8.1	5.9	Myocarditis	No	No	Atypical	Yes	Yes	No
24	1047	M/W	43	13.8	148	9.0	6.6	ASCVD	1209	Schizoaffective disorder	M/W	35	9.1	129	8.7	6.5	Suicide by diphenhydramine overdose	Yes	No	Atypical	No	No	Yes
25	1086	M/W	51	24.2	142	8.1	6.8	ASCVD	10025	Disorganized schizophrenia; OAR	M/B	52	27.1	121	7.8	6.7	ASCVD	No	Yes	None	No	No	No
26	1092	F/B	40	16.6	141	8.0	6.8	Mitral valve prolapse	1178	Schizoaffective disorder	F/B	37	18.9	133	8.4	6.1	Pulmonary embolism	No	Yes	Atypical	No	Yes	No
27	10005	M/W	42	23.5	129	7.4	6.7	Trauma	1256	Undifferentiated schizophrenia	M/W	34	27.4	121	7.9	6.4	Suicide by hanging	No	No	Atypical	No	No	No
28	1336	M/W	65	18.4	106	8.0	6.8	Cardiac tamponade	1173	Disorganized schizophrenia; ADR	M/W	62	22.9	133	7.7	6.4	ASCVD	No	Yes	Atypical	No	No	Yes
29	1122	M/W	55	15.4	138	7.9	6.7	Cardiac tamponade	1105	Schizoaffective disorder	M/W	53	7.9	140	8.9	6.2	ASCVD	No	Yes	Atypical	No	No	No

Unaffected Comparison Subjects									Subjects with Schizophrenia														
Pair	**Case**	**Sex/ Race**	**Age**	**PMI[a]**	**Storage Time[b]**	**RIN**	**pH**	**Cause of Death**	**Case**	**DSM IV diagnosis**	**Sex/ Race**	**Age**	**PMI[a]**	**Storage Time[b]**	**RIN**	**pH**	**Cause of Death**	**NSAID ATOD**	**Tobacco ATOD**	**Anti-psychotic ATOD**	**Anti-depressant ATOD**	**Benzodiazepine/ Anticonvulsant ATOD**	**Living Independently ATOD**
30	1284	M/W	55	6.4	117	8.7	6.8	ASCVD	1188	Undifferentiated schizophrenia; AAR; OAR	M/W	58	7.7	131	8.4	6.2	ASCVD	No	Yes	Atypical	No	No	No
31	1191	M/B	59	19.4	131	8.4	6.2	ASCVD	1263	Undifferentiated schizophrenia; ADR	M/W	62	22.7	120	8.5	7.1	Accidental asphyxiation	Yes	Yes	Both	Yes	No	No
32	970	M/W	42	25.9	159	7.2	6.4	ASCVD	1222	Undifferentiated schizophrenia; AAC	M/W	32	30.8	126	7.5	6.4	Suicide by combined drug overdose	No	No	Atypical	Yes	No	Yes
33	10003	M/W	49	21.2	130	8.4	6.5	Trauma	1088	Undifferentiated schizophrenia; ADC; OAC	M/W	49	21.5	142	8.1	6.5	Accidental combined drug overdose	No	Yes	Unknown	Yes	No	No
34	1247	F/W	58	22.7	122	8.4	6.4	ASCVD	1240	Undifferentiated schizophrenia; ADR	F/B	50	22.9	123	7.7	6.3	ASCVD	Yes	Yes	Atypical	No	No	No
35	1324	M/W	43	22.3	109	7.3	7	Aortic dissection	10020	Schizophrenia, paranoid type; AAC; OAC	M/W	38	28.8	123	7.4	6.6	Suicide by salicylate overdose	Yes	Yes	Atypical	Yes	Yes	No
36	1099	F/W	24	9.1	140	8.6	6.5	Cardiomyopathy	10023	Disorganized schizophrenia	F/B	25	20.1	122	7.4	6.7	Suicide by drowning	No	No	Atypical	No	Yes	Yes
37	1307	M/B	32	4.8	112	7.6	6.7	ASCVD	10024	Paranoid schizophrenia	M/B	37	6.0	121	7.5	6.1	ASCVD	Yes	No	None	No	No	No
38	1391	F/W	51	7.8	98	7.1	6.6	ASCVD	1189	Schizoaffective disorder; AAR	F/W	47	14.4	131	8.3	6.4	Suicide by combined drug overdose	Yes	Yes	Atypical	Yes	Yes	Yes
39	1282	F/W	39	24.5	117	7.5	6.8	ASCVD	1211	Schizoaffective disorder	F/W	41	20.1	129	7.8	6.3	Sudden unexplained death	Yes	Yes	Both	Yes	No	No
40	1159	M/W	51	16.7	134	7.6	6.5	ASCVD	1296	Undifferentiated schizophrenia	M/W	48	7.8	115	7.3	6.5	Pneumonia	No	Yes	Both	Yes	No	No
41	1326	M/W	58	16.4	109	8.0	6.7	ASCVD	1314	Undifferentiated schizophrenia	M/W	50	11.0	111	7.2	6.2	ASCVD	Yes	No	Typical	Yes	No	No
42	902	M/W	60	23.6	174	7.7	6.7	ASCVD	1361	Schizoaffective disorder; ODC	M/W	63	23.2	104	7.7	6.4	Cardiomyopathy	No	Yes	Atypical	No	Yes	No
43	1374	M/W	43	21.7	102	7.2	6.6	ASCVD	904	Schizoaffective disorder	M/W	33	28.0	174	7.1	6.2	Pneumonia	No	Yes	Atypical	No	Yes	No
44	1555	M/W	17	15.1	67	7.9	6.9	Trauma	1649	Undifferentiated schizophrenia	M/B	17	21.4	52	8.1	6.9	Suicide by hanging	No	No	Atypical	Yes	No	No
45	1268	M/B	49	19.9	119	7.9	7.1	ASCVD	1230	Undifferentiated schizophrenia	M/W	50	16.9	125	8.2	6.6	Suicide by doxepin overdose	No	Yes	Typical	Yes	No	No
46	1466	F/B	64	20.0	84	8.8	6.7	Trauma	1341	Schizoaffective disorder; ODC	F/W	44	24.5	106	8.8	6.6	Trauma	No	No	Atypical	No	Yes	No
47	1518	M/W	50	20.7	73	7.7	6.4	ASCVD	1367	Schizoaffective disorder; ADC; ODR	M/W	47	28.9	103	7.2	6.6	Combined drug overdose	No	No	None	No	No	Yes
48	1386	M/W	46	21.2	99	8.3	6.7	ASCVD	1420	Schizoaffective disorder; AAR; ODC; OAR	M/W	47	23.4	92	8.2	6.8	Suicide by jump	No	Yes	Atypical	Yes	No	No
49	1472	M/W	61	23.8	84	8.0	6.5	Pulmonary embolism	1453	Paranoid schizophrenia; ADR	M/W	62	11.1	87	8.2	6.4	Trauma	No	Yes	Typical	No	Yes	No
50	1026	M/W	59	19.8	151	7.4	6.3	ASCVD	1454	Paranoid schizophrenia; AAR; ODC	M/W	59	24.1	86	7.6	6.1	Trauma	Yes	Yes	Typical	Yes	No	Yes
51	694	M/W	38	20.7	212	7.7	7.0	Subarachnoid hemorrhage	1455	Paranoid schizophrenia; AAR; OAC	M/W	42	8.2	86	7.7	6.4	Peritonitis	Yes	Yes	Atypical	No	Yes	Yes
52	1350	M/W	21	24.2	105	7.3	6.4	Trauma	1474	Schizoaffective disorder; ADR	M/W	37	39.9	83	7.0	6.7	Suicide by hanging	No	No	None	No	No	Yes
53	1792	F/W	36	28.1	27	7.5	6.5	Pulmonary embolism	1506	Schizoaffective disorder; ADC	F/W	47	14.1	77	7.5	6.6	Combined drug overdose	No	Yes	Both	Yes	No	Yes
54	1524	M/W	66	9.4	72	8.1	6.4	Intestinal infarction	1542	Paranoid schizophrenia	M/W	65	17.4	69	7.8	6.7	Combined drug overdose	No	Yes	Both	Yes	Yes	No
55	1270	F/W	73	19.7	119	7.7	6.7	Trauma	1579	Schizoaffective disorder; ADR; ODC	F/W	69	16.1	63	7.7	6.7	ASCVD	No	Yes	Typical	No	Yes	Yes
56	1372	M/W	37	20.5	102	9.0	6.6	Asphyxiation	1581	Paranoid schizophrenia; ODC; OAC	M/W	32	18.4	62	9.0	6.8	ASCVD	No	Yes	Atypical	Yes	No	No
57	1543	F/W	45	17.9	69	7.4	6.8	Subarachnoid hemorrhage	10026	Undifferentiated schizophrenia	F/W	46	23.8	121	7.6	6.6	Suicide by thermal injuries	No	Yes	Atypical	Yes	No	No
58	1583	M/W	58	19.1	62	8.2	6.8	Trauma	1686	Schizophrenia, paranoid type; AAR	M/B	56	14.1	45	8.3	6.2	ASCVD	No	Yes	Both	Yes	Yes	No
59	1554	M/W	50	23.2	67	7.6	6.5	ASCVD	1691	Schizophrenia, paranoid type; ADR; ODC	M/W	51	31.9	44	7.7	6.6	Combined drug overdose	Yes	Yes	Typical	No	Yes	No

Unaffected Comparison Subjects									Subjects with Schizophrenia															
Pair	**Case**	**Sex/ Race**	**Age**	**PMI[a]**	**Storage Time[b]**	**RIN**	**pH**	**Cause of Death**	**Case**	**DSM IV diagnosis**	**Sex/ Race**	**Age**	**PMI[a]**	**Storage Time[b]**	**RIN**	**pH**	**Cause of Death**	**NSAID ATOD**	**Tobacco ATOD**	**Anti-psychotic ATOD**	**Anti-depressant ATOD**	**Benzodiazepine/ Anticonvulsant ATOD**	**Living Independently ATOD**
60	1635	M/W	66	25.3	54	8.2	6.8	Cardiac tamponade	1706	Schizoaffective disorder; AAR; ODC; OAR	M/B	60	28.1	40	8.4	6.8	Sepsis	No	Yes	Atypical	No	No	No
61	1384	M/W	67	21.9	100	7.0	6.6	ASCVD	1712	Schizoaffective disorder; ADR; ODC	M/W	63	15.1	39	7.1	6.2	ASCVD	Yes	No	Atypical	Yes	Yes	No
62	1558	M/W	54	24.4	67	7.7	6.9	ASCVD	1734	Schizophrenia, undifferentiated type; AAR; ODC; OAR	M/W	54	28.6	35	7.7	6.1	Pneumonia	Yes	Yes	Typical	No	No	No
		Mean	48.7	18.8	145.4	8.2	6.7					47.7	19.2	141.7	8.1	6.6		16Y/46N	41Y/13N/ 8Unk	Typical16/ Atypical 28/ Both8/ None9/ Unknown 1	27Y/35N	24Y/38N	17Y/45N
		SD	13.8	5.5	56.4	0.6	0.2					12.7	8.5	60.9	0.6	0.3							

[a] PMI, postmortem interval (hours);

[b] Storage time (months) at -80C;

Other abbreviations: ASCVD, arteriosclerotic cardiovascular disease; MCA, middle cerebral artery; ATOD, at time of death; ADC, alcohol dependence, current at time of death; ADR, alcohol dependence, in remission at time of death; AAC, alcohol abuse, current at time of death; AAR, alcohol abuse, in remission at time of death; ODC, other substance dependence, current at time of death; ODR, other substance dependence, in remission at time of death; OAC, other substance abuse, current at time of death; OAR, other substance abuse, in remission at time of death; U, unknown; M, male; F, female; W, white; B, black.

Supplemental Table S2: qPCR primer design

Gene	Species	Accession #	Amplicon Size (bp)	Position	Forward Primer (F) Reverse Primer (R)
Interleukin-1 receptor (IL-1R)	Human	NM_000877	102	1789-1890	(F) GCTTGAGCTGGAGAAAATCC (R) TGTGTAAAGTCCCCTGACCA
Toll-like receptor 4 (TLR4)	Human	NM_138554.4	58	2073-2130	(F) AATGGATCAAGGACCAGAGG (R) GTTGCACATTCCATTCGTTC
Tumor necrosis factor receptor superfamily member 1A (TNFR)	Human	NM_001065.3	117	625-741	(F) GAAATGGGTCAGGTGGAGAT (R) GCAATTGAAGCACTGGAAAA
TNF receptor superfamily member 1B (TNFR2)	Human	NM_001066.2	119	631-749	(F) CCCACCAGATCTGTAACGTG (R) CACTGGCTGGGGTAAGTGTA
TNF receptor superfamily member 5 (CD40)	Human	NM_001250.	64	486-549	(F) GCAGATTGCTACAGGGGTTT (R) ATTGGAGAAGAAGCCGACTG
TNF receptor superfamily member 3 (lymphotoxin beta receptor; LTβR)	Human	NM_002342.2	60	1525-1584	(F) CTACACCCCACCAGGAAGAT (R) CACCACAGTGCTCTGTCTCC
IκB kinase alpha subunit (IKKα)	Human	NM_001278.3	51	1856-1906	(F) CTCAAGGAGCTGTTTGGTCA (R) CTTCTGCTTACAGCCCAACA
IKKβ	Human	NM_001556.2	112	1570-1681	(F) AACAGCTGCCTCTCCAAAAT (R) CGCTGTACTTCTCCAGGTCA
NF-κB-inducing kinase (NIK)	Human	NM_003954.4	164	2161-2324	(F) AAGCCAATTACCACCAGACC (R) GCTCAAAGTCAAGGGAGGAG
NFκB inhibitor alpha (IκBα)	Human	NM_020529.2	65	949-1013	(F) TGCCAGAGAGTGAGGATGAG (R) GTCCTCTGTGAACTCCGTGA
IκBβ	Human	NM_002503.4	100	710-809	(F) GAAAACTACGAGGGCCACAC (R) GTTTGTCAAGGTCAGCTCCA
IκBε	Human	NM_004556.2	82	1444-1525	(F) CCAGGACCTGACTGAGGAAT (R) GTCGGTACACAGCAGCAGTT
RelA	Human	NM_021975.3	50	514-563	(F) ACCTGGAGCAGGCTATCAGT (R) GAAGGGGTTGTTGTTGGTCT
RelB	Human	NM_006509.3	81	1048-1128	(F) GCCGAATTAACAAGGAAAGC (R) TGTCCTCTTTCTGCACCTTG
cRel	Human	NM_002908.3	83	1472-1554	(F) ATGCCTACAGGGGTTTCAAG (R) GAGGCATGATGTGACAATCC
Beta actin	Human	NM_001101	101	1146-1246	(F) GATGTGGATCAGCAAGCA (R) AGAAAGGGTGTAACGCAACTA
Cyclophilin	Human	NM_021130	126	159-284	(F) GCAGACAAGGTCCCAAAG (R) GAAGTCACCACCCTGACAAC
Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)	Human	NM_002046	87	556-642	(F) TGCACCACCAACTGCTTAGC (R) GGCATGGACTGTGGTCATGAG

Gene	**Species**	**Accession #**	**Amplicon Size (bp)**	**Position**	**Forward Primer (F) Reverse Primer (R)**
IL-1R	Macaca mulatta	XM_001107510	102	1778-1880	(F) GCTTGAGCTGGAGAAAATCC (R) TGTGTAAAGTCCCCTGACCA
TLR4	Macaca mulatta	NM_001037092	58	1774-1832	(F) AGTGGATCAAGGACCAGAGG (R) GTTGCACATTCCATTCGTTC
TNFR	Macaca mulatta	XM_001118232	117	165-281	(F) GAAATGGGCCAGGTGGAGAT (R) GCAATTGAAGCACTGGAAAA
TNFR2	Macaca mulatta	NM_001266205	119	624-742	(F) CCCACCAGATCTGTCACGTG (R) CACTGGCTGGGGTAAGTGTA
CD40	Macaca mulatta	NM_001265862	64	468-531	(F) GCAGATTGCTACAGGGGTTT (R) ATTGGAGAAGAAGCCGACCG
LTβR	Macaca mulatta	NM_001265736	60	1301-1360	(F) CTACTCCCCACCAGGAAGAT (R) CACCACAGTGCTCTGTCTCC
IKKα	Macaca mulatta	XM_001107171	51	1888-1938	(F) CTCAAGGAGCTGTTTGGTCA (R) CTTCTGCTTACAGCCCAACA
IKKβ	Macaca mulatta	NM_001265946	112	1440-1551	(F) AACAGCTGCCTCTCCAAGAT (R) CGCTGTACTTCTCCAGGTCA
NIK	Macaca mulatta	NM_001266698	164	2140-2303	(F) AAGCCAATTATCACCAGACC (R) GCTCAAAGTCAAGGGAGGAG
IκBα	Macaca mulatta	NM_001257750	65	929-993	(F) TGCCGGAGAGTGAGGATGAG (R) ATCCTCCGTGAACTCCGTGA
IκBβ	Macaca mulatta	NM_001258157	100	656-755	(F) GAAAACTACGAGGGCCACAC (R) GTTTGTCAAGGTCAGCTCCA
IκBε	Macaca mulatta	XM_002808387.1	82	1419-1500	(F) CCAGGACCTCACTGAGGAAT (R) GTCGGTACACAGCAGCAGTT
RelA	Macaca mulatta	XM_001113258	50	638-686	(F) ACCTGGAGCAGGCTATCACT (R) GAAGGGATTGTTGTTCGTCT
RelB	Macaca mulatta	XM_001104559	81	806-886	(F) GCCGAATTAACAAGGAAAGC (R) TGTCCTCTTTCTGCACCTTG
cRel	Macaca mulatta	XM_001115312	83	1323-1405	(F) ATGCCTACAGGGGTTTCAAG (R) GAGGCATGATGTGACAATCC
Beta actin	Macaca mulatta	NM_001033084	101	1087-1187	(F) GATGTGGATCAGCAAGCA (R) GAAAGGGTGTAACGCAACTA
Cyclophilin	Macaca mulatta	NM_001032809	126	76-201	(F) GCAGACAAGGTTCCAAAG (R) GAAGTCACCACCCTGACAC
GAPDH	Macaca mulatta	XM_001105471	93	527-619	(F) TGCACCACCAACTGCTTAGC (R) AGTGATGGCGTGGACTGTG
IL-1R	Mus musculus	NM_001123382.1	115	760-874	(F) ATGAGTTACCCGAGGTCCAG (R) CTGTGCTCTTCAGCCACATT
TNFR	Mus musculus	NM_011609.4	148	568-715	(F) TACGGCTTCCCAGAATTACC (R) CTCACTCAGGTAGCGTTGGA

Gene	Species	Accession #	Amplicon Size (bp)	Position	Forward Primer (F) Reverse Primer (R)
LTβR	Mus musculus	NM_010736.3	64	747-810	(F) CAACCCCATACCAGATGTGA (R) CCGAGTAGGAGGTACCTGGA
IKKα	Mus musculus	NM_001162410.1	79	1886-1964	(F) CTCAAGGAGCTGTTTGGTCA (R) CTTCCACCTTGGGGAGTAGA
IKKβ	Mus musculus	NM_001159774.1	51	1808-1858	(F) CCCTGGATGACCTAGAGGAA (R) CCCTGAGTCTTCGGTAGAGC
NIK	Mus musculus	NM_016896.3	136	1784-1919	(F) AGAAGTGGTGATGGGAAAGC (R) GCTGGCAATCTTGAGACAAA
IκBα	Mus musculus	NM_010907.2	64	932-995	(F) CAGCTGACCCTGGAAAATCT (R) CCGTGTCATAGCTCTCCTCA
IκBβ	Mus musculus	NM_010908.4	132	242- 373	(F) CGCCCTTAGTCTTTGGCTAC (R) TCTGCAGGTCAAGGTACTCG
RelA	Mus musculus	NM_009045.4	120	597-716	(F) GCCTCATCCACATGAACTTG (R) CGCTTCTTCACACACTGGAT
RelB	Mus musculus	NM_009046.2	52	865-916	(F) GGGAATTGACCCCTACAATG (R) GTCGACCTCCTGATGGTTCT
cRel	Mus musculus	NM_009044.2	133	1238-1370	(F) GGGATCAACTGGAGAAGGAA (R) GGACCCGCATGAAGAATAGT
Beta actin	Mus musculus	NM_007393	95	955-1049	(F) CCTCTATGCCAACACAGTGC (R) TGCTAGGAGCCAGAGCAGTA
Cyclophilin	Mus musculus	NM_008907	63	383-445	(F) CTGCACTGCCAAGACTGAAT (R) CCTTCTTTCACCTTCCCAAA
GAPDH	Mus musculus	NM_008084	64	431-494	(F) CATGTTTGTGATGGGTGTGA (R) TGCATTGCTGACAATCTTGA

Table S3. Table of poly(I:C)-exposed pregnant mice and outcomes.

Gestational Exposure Time Frame	Injection	Injected Pregnant Mice (n)	Not pregnant or no pups (n)	Mothers that cannabalized pups (n)	Mothers with failure to thrive pups (n)	Mothers with healthy pups (n)	Mean offspring per mother (SD)[a]	Total male offspring studied (n)[b]	Total female offspring studied (n)[b]
E11-13	Poly(I:C)	12	2	1	2	7	6.9 (2.0)	7	7
E11-13	Normal Saline	12	3	0	1	8	6.5 (1.1)	7	7
E15-17	Poly(I:C)	12	2	0	0	10	5.3 (1.8)	8	8
E15-17	Normal Saline	12	3	1	0	8	5.4 (1.7)	8	8

[a] The mean number of offspring per mother with healthy pups did not differ between poly(I:C)-injected mothers and normal saline-injected mothers at E11-13 (t_{13}=0.43, p=.67) or E15-17 (t_{14}=0.15, p=.89).

[b] Only one male and/or female offspring per injected mother was included in the study.

Supplemental Table S4. Effects of maternal immune activation on NF-κB-related mRNA levels in the frontal cortex of male and female adult offspring

Immune Marker	*E11-13 Maternal Injections*			*E15-17 Maternal Injections*		
	Normal Saline	**Poly(I:C)**	**T test**	**Normal Saline**	**Poly(I:C)**	**T test**
IL-1R						
Male	0.00154 ± 0.00016	0.00150 ± 0.00018	p=.73	0.00158 ± 0.00029	0.00162 ± 0.00031	p=.79
Female	0.00140 ± 0.00023	0.00142 ± 0.00017	p=.83	0.00132 ± 0.00018	0.00133 ± 0.00014	p=.84
All	0.00147 ± 0.00020	0.00146 ± 0.00017	p=.95	0.00145 ± 0.00027	0.00148 ± 0.00028	p=.77
TNFR						
Male	0.00683 ± 0.00071	0.00672 ± 0.00084	p=.80	0.00672 ± 0.00067	0.00680 ± 0.00114	p=.87
Female	0.00682 ± 0.00065	0.00696 ± 0.00047	p=.65	0.00607 ± 0.00074	0.00644 ± 0.00061	p=.29
All	0.00682 ± 0.00065	0.00684 ± 0.00067	p=.94	0.00640 ± 0.00076	0.00662 ± 0.00090	p=.45
LTβR						
Male	0.00271 ± 0.00037	0.00266 ± 0.00026	p=.77	0.00257 ± 0.00012	0.00258 ± 0.00023	p=.96
Female	0.00263 ± 0.00009	0.00265 ± 0.00017	p=.83	0.00257 ± 0.00023	0.00269 ± 0.00030	p=.38
All	0.00267 ± 0.00026	0.00265 ± 0.00021	p=.85	0.00257 ± 0.00018	0.00263 ± 0.00026	p=.43
IKKα						
Male	0.0126 ± 0.0031	0.0129 ± 0.0022	p=.84	0.0121 ± 0.0014	0.0125 ± 0.0016	p=.61
Female	0.0125 ± 0.0011	0.0126 ± 0.0016	p=.91	0.0111 ± 0.0016	0.0113 ± 0.0011	p=.75
All	0.0126 ± 0.0023	0.0128 ± 0.0018	p=.81	0.0116 ± 0.0015	0.0119 ± 0.0015	p=.57
IKKβ						
Male	0.00932 ± 0.00089	0.00907 ± 0.00088	p=.61	0.00911 ± 0.00085	0.00920 ± 0.00111	p=.87
Female	0.00925 ± 0.00085	0.00925 ± 0.00093	p=.99	0.00888 ± 0.00105	0.00893 ± 0.00075	p=.92
All	0.00928 ± 0.00084	0.00916 ± 0.00088	p=.71	0.00900 ± 0.00093	0.00906 ± 0.00092	p=.85
NIK						
Male	0.00204 ± 0.00023	0.00178 ± 0.00035	p=.13	0.00176 ± 0.00034	0.00161 ± 0.00038	p=.43
Female	0.00151 ± 0.00021	0.00152 ± 0.00039	p=.94	0.00137 ± 0.00041	0.00133 ± 0.00034	p=.82
All	0.00177 ± 0.00035	0.00165 ± 0.00038	p=.39	0.00157 ± 0.00041	0.00147 ± 0.00038	p=.50
IκBα						
Male	0.01055 ± 0.00104	0.00952 ± 0.00119	p=.11	0.00943 ± 0.00086	0.00929 ± 0.00152	p=.82
Female	0.00812 ± 0.00062	0.00830 ± 0.00080	p=.65	0.00811 ± 0.00096	0.00839 ± 0.00095	p=.56
All	0.00933 ± 0.00151	0.00891 ± 0.00116	p=.42	0.00877 ± 0.00111	0.00884 ± 0.00131	p=.88
IκBβ						
Male	0.0185 ± 0.0010	0.0185 ± 0.0008	p=.91	0.0183 ± 0.0009	0.0179 ± 0.0010	p=.42
Female	0.0178 ± 0.0004	0.0177 ± 0.0004	p=.55	0.0169 ± 0.0009	0.0168 ± 0.0006	p=.91
All	0.0182 ± 0.0008	0.0181 ± 0.0007	p=.90	0.0176 ± 0.0011	0.0174 ± 0.0010	p=.58
RelA						
Male	0.00684 ± 0.00041	0.00671 ± 0.00041	p=.57	0.00685 ± 0.00061	0.00669 ± 0.00059	p=.60
Female	0.00610 ± 0.00051	0.00621 ± 0.00056	p=.72	0.00607 ± 0.00075	0.00623 ± 0.00031	p=.59
All	0.00647 ± 0.00059	0.00646 ± 0.00054	p=.96	0.00646 ± 0.00077	0.00646 ± 0.00051	p=.99
RelB						
Male	0.00243 ± 0.00053	0.00239 ± 0.00040	p=.87	0.00233 ± 0.00300	0.00244 ± 0.00057	p=.64
Female	0.00233 ± 0.00050	0.00231 ± 0.00058	p=.93	0.00235 ± 0.00051	0.00227 ± 0.00045	p=.75
All	0.00238 ± 0.00050	0.00235 ± 0.00048	p=.86	0.00234 ± 0.00040	0.00235 ± 0.00051	p=.93

Immune Marker	*E11-13 Maternal Injections*			*E15-17 Maternal Injections*		
	Normal Saline	**Poly(I:C)**	**T test**	**Normal Saline**	**Poly(I:C)**	**T test**
cRel						
Male	0.00240 ± 0.00020	0.00221 ± 0.00029	p=.23	0.00220 ± 0.00017	0.00215 ± 0.00042	p=.72
Female	0.00204 ± 0.00028	0.00205 ± 0.00027	p=.94	0.00196 ± 0.00045	0.00178 ± 0.00021	p=.35
All	0.00222 ± 0.00030	0.00213 ± 0.00028	p=.45	0.00208 ± 0.00035	0.00197 ± 0.00037	p=.38

Values are mean ± standard deviation. Seven male and seven female offspring from the E11-13 maternal injections and eight male and eight female offspring from the E15-17 maternal injections were included in the study.