Supporting Information

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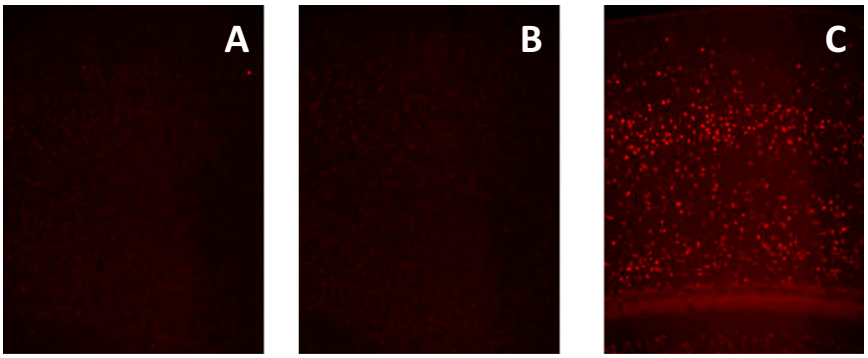

Fig. S1. Isofluorane anesthesia blocks 2,5-dimethoxy-4-iodoamphetamine–induced immediate early gene early growth response protein 1 (egr-1) response in the somatosensory cortex: saline i.p. and 2% isoflurane amesthesia (*A*), 2 mg/kg DOI i.p. and 2% isoflurane anesthesia (*B*), and 2 mg/kg DOI i.p. without anesthesia (*C*). To minimize the effect of external stimuli on the sensory cortex, mice were habituated to red light and silence 24 h before the experiment. On the day of the experiment, an awake animal was injected with 2 mg/kg DOI i.p. Two other mice were anesthetized with 2% isoflurane. Five minutes after anesthesia was applied, animals got 2 mg/kg DOI or saline i.p. Thirty minutes after injections, the animals were waken up, and 90 min after the injections, all animals were anesthetized using euthasol and perfused with 4% (vol/vol) paraformaldehyde (PFA). The brains were collected, fixed with 4% (vol/vol) PFA, treated with sucrose gradient [10–20% (vol/vol)], and frozen in Tissue-Tek O.C.T. compound (Sakura Finetek USA, Inc.). Immunohistochemical analysis of brain tissues showing nuclear localization, using egr-1 rabbit monoclonal antibodies (Cell Signaling Technology, USA, #4153), was performed.

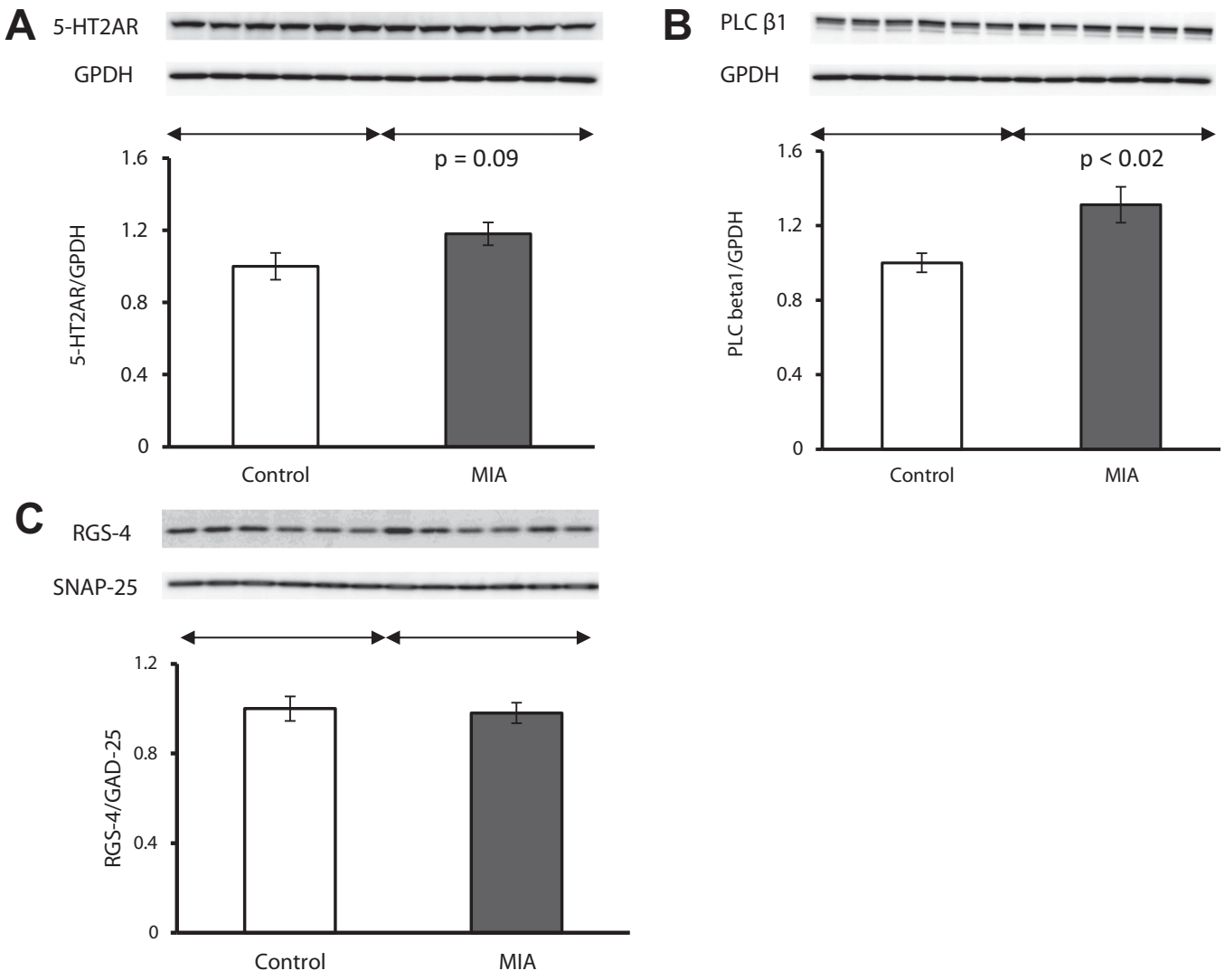

Fig. S2. MIA offspring display a trending increase at the protein level for serotonin receptor 2A (5-HT2AR) and phospholipase C β1 (PLC β1), but not regulator of G protein signaling 4 (RGS4) in the prefrontal cortex (PFC). Representative Western blots and corresponding densitometric analysis (normalized to GPDH or SNAP-25) for 5-HT2AR (*A*), PLC β1 (*B*), and RGS-4 (*C*) are shown. PFC was isolated (six animals per group), then frozen in dry ice, and stored at −80 °C. Brain samples were sonicated in radioimmunoprecipitation assay buffer (150 mM sodium chloride, 1.0% Triton X-100, 0.5% sodium deoxycholate, 0.1% SDS, 50 mM Tris, pH 8.0) with protease inhibitor mixture. A BCA protein assay was conducted to quantify protein and ensure equal loading. Protein samples were heated in a sample buffer at 95 °C for 5 min and subjected to SDS/PAGE on 4–20% acrylamide gels, followed by transfer to polyvinylidine difluoride membranes for overnight. Blocking of membranes occurred in Tris-buffered saline and Tween 20 buffer with 5% milk for an hour at room temperature. The membranes were then rinsed and incubated with the primary antibody overnight at 4 °C, followed by secondary antibody incubation for 1 h. Data are mean ± SEM.

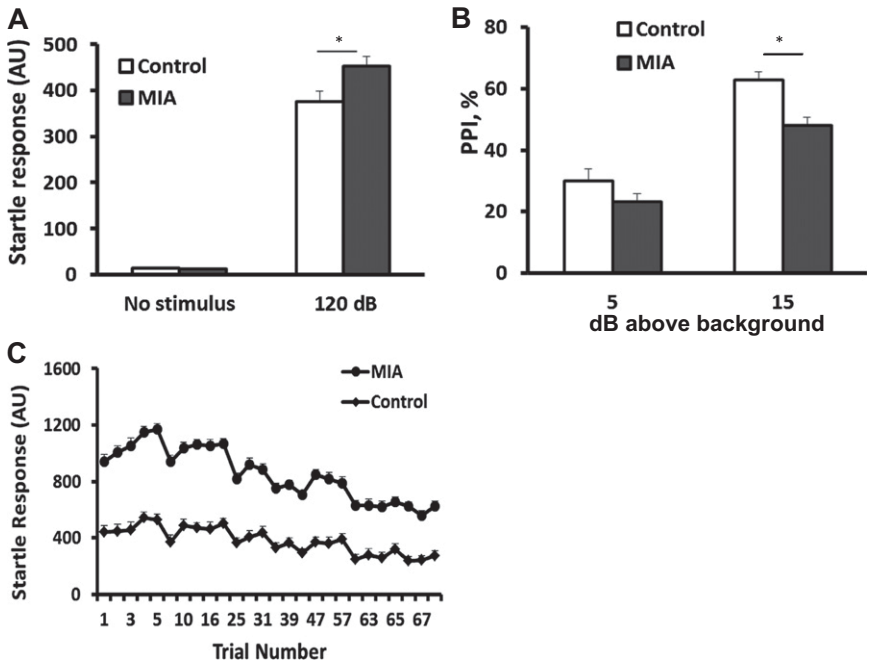

Fig. S3. Maternal immune activation (MIA) offspring display an increased acoustic startle response (ASR) and deficit in prepulse inhibition (PPI) of ASR but exhibit a similar pattern of habituation to a repeated 120 dB stimulus. (*A*) Baseline startle response and the response to auditory-evoked startle stimulus (120 dB) were measured in MIA and control offspring. AUs, arbitrary units [$F_{(1,\ 118)} = 5.858$, $P < 0.02$]. The Bonferroni post hoc reveals a significantly increased response to the stimulus sound in MIA offspring (*$P < 0.01$). (*B*) PPI was measured with prepulse stimuli 5 and 15 dB above the background [$F_{(1,\ 118)} = 11.84$, $P < 0.0008$]. The Bonferroni post hoc test for the response to prepulse 15 dB above the background is $P < 0.01$. (*C*) Mice were exposed to 26 sound stimuli with an intensity of 120 dB. Although MIA offspring display increased ASR, they show no difference in the amount of habituation. Indexes of habituation were 0.96 ± 0.18 and 0.89 ± 0.14 for MIA and control offspring, respectively [$t_{(59)} = 0.315$, $P = 0.753$]. Data are mean $\pm$ SEM.

Table S1. Active volume of DOI-induced brain structures, which are revealed by 2 × 2 mulifactorial analysis ($P < 0.0001$)

Brain structure	% coverage	Active volume, no. voxels
Olfactory areas		
Anterior olfactory nucleus	9.2	431
Accessory olfactory bulb	3.7	45
Taenia tecta dorsal part	17.1	296
Subependymal zone	14.6	163
Main olfactory bulb granule layer	9.4	810
Cerebral cortex	9.2	22,221
Endopiriform nucleus dorsal part	11.4	199
Midbrain	8.4	3,879
Hippocampus		
Hippocampal formation	14.3	2,795
Ventral hippocampal commisure	22.7	44
Striatum		
Caudoputamen	10.5	4,750
Lateral septal nucleus	6.5	57
Thalamus		
Ventral posteromedial nucleus	15.9	315
Reticular nucleus	10.1	540
Ventral posterolateral nucleus	14.6	396
Medial geniculate complex	3.6	86
Lateral posterior nucleus	19.3	555
Posterior complex	28.0	743
External medullary lamina	4.8	21
Mediodorsal nucleus	14.2	232
Anteromedial nucleus	32.5	319
Ventral anterior lateral complex	32.9	280
Anteroventral nucleus	7.1	28
Paraventricular nucleus	24.3	712
Parataenial nucleus	15.9	101
Intermediodorsal nucleus	13.6	22
Parafascicular nucleus	94.4	1,181
Stria medullaris	9.3	56
Cerebellum	3.9	4,992
Fiber tract		
Corpus callosum	14.8	3,556
Stria terminalis	17.6	164

Areas that showed more than 2.5% coverage are presented.

Table S2. Manganese-enhanced magnetic resonance imaging signal increase in MIA versus control offspring ($P < 0.05$)

Brain structure	% coverage	Active volume, no. voxels
Olfactory areas		
Taenia tecta dorsal part	2.5	43
Subependymal zone	3.1	35
Main olfactory bulb granule layer	1.9	167
Cerebral cortex	3.2	7,749
Endopiriform nucleus dorsal part	3.0	53
Midbrain	2.0	915
Hippocampus		
Hippocampal formation	2.7	534
Ventral hippocampal commisure	14.9	29
Striatum		
Caudoputamen	3.3	1,509
Thalamus		
Medial geniculate complex	1.9	45
Lateral posterior nucleus	11.8	339
Mediodorsal nucleus	0.9	14
Anteromedial nucleus	10.7	105
Ventral anterior lateral complex	10.0	85
Paraventricular nucleus	10.6	310
Parafascicular nucleus	94.4	1,181
Stria medullaris	6.0	36
Cerebellum	1.3	1,651
Fiber tract		
Corpus callosum	3.0	714
Stria terminalis	5.8	54

Table S3. The primers used in quantitative RT-PCR

Primer name	Sequences
Egr-1	5′-GAGATGCAATTGATGTCTCCGCTG-3′
	5′-GCGATGTCAGAAAAGGACTCTGTGG-3′
Pan–brain-derived neurotrophic factor	5′-CAGCAAAGCCACAATGTTCC-3′
	5′-TGTCGTCGTCAGACCTCTCG-3′
COX-2	5′-GGATGGAAAATTGAAATATCAGGTC-3′
	5′-GCTCAGGTGTTGCACGTAGTC-3′
PLC β1	5′-CATGGCAGGCTGCTGTACCC-3′
	5′-AATAATTGGAGTAACAATAGTGGAGTCATCA-3′
RGS4	5′-GCTTCCTGCCTGAGGAGTGCA-3′
	5′-ATCCAGAAGTCAATGTTCTCCTCGC-3′
Cytoplasmic phospholipase A_2	5′-AGTGATGATGAGGCTCAAGGAC-3′
	5′-TCTTACTTTTGACATCCAGTGGTTC-3′
Serotonin transporter	5′-GCACCAACTACTTCGCCCAGGAC-3′
	5′-GCTGTCACCCACACCACCTTGC-3′
Integrin β 3	5′-GTGTGCCGCTGTGACCAGGG-3′
	5′-ACATTGCCCATGGCCGGAA-3′
β-actin	5′-CACACCCGCCACCAGTTCG-3′
	5′-CGCAGCTCATTGTAGAAGGTGTGG-3′