Data Supplement for Kimoto et al., Lower Expression of Glutamic Acid Decarboxylase 67 in the Prefrontal Cortex in Schizophrenia: Contribution of Altered Regulation by Zif268. Am J Psychiatry (doi: 10.1176/appi.ajp.2014.14010004)

Supplemental Methods

Tissue preparation

Frozen tissue blocks containing the middle portion of the right superior frontal sulcus were confirmed to contain prefrontal cortical area 9 using Nissl-stained, cryostat tissue sections for each subject (1). For quantitative polymerase chain reaction (qPCR) studies, gray matter from adjacent sections was separately collected into a tube containing TRIzol reagent (Invitrogen, Grand Island, NY) in a manner that ensured minimal white matter contamination and excellent RNA preservation (2). Total RNA for each subject was extracted and purified with RNeasy Mini Kit (Qiagen, Valencia, CA). For *in situ* hybridization, coronal cryostat sections (20 μm) from each subject were mounted on Superfrost Plus glass slides (Fisher Scientific, Hampton, NH) and stored at -80°C until processed. Three sections from each subject, separated at anterior-posterior intervals of approximately 300 μm, were matched within subject pairs and then used to assess Zif268 mRNA expression as described previously (3).

Quantitative polymerase chain reaction (qPCR)

The difference in cycle threshold for each target transcript was calculated by subtracting the mean cycle threshold for the three internal reference transcripts (β-actin, cyclophilin A, and glyceraldehyde-3-phosphate dehydrogenase) from the mean cycle threshold of the target transcript. Because this difference in cycle threshold (ΔCT) represents the $\log_2$-transformed expression ratio of each target transcript to the reference transcripts, the relative expression level of the target transcript is determined as $2^{-\Delta CT}$ (4).

In situ hybridization

Tissue sections from each pair were processed side by side, and the location of the slides in the hybridization container was counterbalanced between diagnostic groups during

each run. After fixation with 4% paraformaldehyde in PBS solution, the sections were acetylated with 0.25% acetic anhydrate in 0.1 M triethanolamine/0.9% NaCl for 10 min and dehydrated with a graded alcohol series. The sections were then hybridized with ^{35}S-labeled riboprobes ($1.0x10^7$ cpm/ml) in hybridization buffer at 58°C for 16 h. The hybridization buffer contained 50% formamide, 0.75 M NaCl, 20 mM 1,4-piperazine diethane sulfonic acid, pH 6.8, 10 mM EDTA, 10% dextran sulfate, 5x Denhardt's solution (0.2 mg/ml Ficoll, 0.2 mg/ml polyvinylpyrrolidone, 0.2 mg/ml BSA), 50 mM dithiothreitol, 0.2% SDS, and 100 µg/ml yeast tRNA. The sections were then washed in a solution of 0.3 M NaCl, 20 mM Tris-HCl, pH 8.0, 1 mM EDTA, pH 8.0, and 50% formamide at 65°C, treated with 20 µg/ml RNase A (Sigma-Aldrich, St Louis, MO) at 37°C, washed in 0.1x SSC (150 mM NaCl, 15 mM sodium citrate) at 68°C, dehydrated through a graded ethanol series, and air dried. Sections from both subjects in a pair, as well as ^{14}C radioactive standards (ARC Inc., St. Louis, MO), were exposed on the same BioMax MR film (Eastman Kodak, Rochester, NY) for 4 days. After that, sections were coated with NTB2 emulsion (Eastman Kodak) diluted 3:1 with water, exposed for 35 days, developed with D-19 (Eastman Kodak) and counterstained with cresyl violet.

Film analysis of Zif268 mRNA expression

Each section was randomly coded, so that subject number and diagnosis were unknown to the rater. Autoradiographic film images were captured, digitized and analyzed using a Microcomputer Imaging Device (MCID) system (InterFocus Imaging Ltd, Cambridge, UK). Optical density (OD) was measured in the gray matter of dorsolateral prefrontal cortex (DLPFC) area 9 and expressed as nanocuries per gram of tissue (nCi/g) by reference to radioactive ^{14}C radioactive standards exposed on the same autoradiographic film. The mean (SD) total area of gray matter sampled in each subject was 364 (169) mm^2 for schizophrenia subjects and 321 (158) mm^2 for comparison subjects.

Zif268 mRNA expression as a function of cortical layer was quantified in approximately 1-mm-wide cortical traverses extending from the pial surface to the white matter. Three cortical

traverses were sampled for each section (9 traverses per subject). Each traverse was divided into 50 equal bins parallel to the pial surface and the OD was determined for each bin. These bins were then combined into six zones that approximated the laminar boundaries in the DLPFC determined in previous studies (1,5). These zones (i.e., bins 1-5, 6-10, 11-25, 26-30, 31-40, and 41-50) correspond to cortical layers 1-6, respectively. Background measures in each section were quantified within deep white matter where no specific expression of Zif268 mRNA was observed. All sampled areas for both total gray and laminar film analyses were corrected by subtracting the corresponding background measure from the same slide.

Grain counting analysis of Zif268 mRNA expression at the cellular level

Evaluation of Zif268 mRNA expression at the cellular level was performed by measuring silver grain accumulation in emulsion-dipped, Nissl-counterstained sections. Using the MCID imaging software coupled to a microscope equipped with a motor-driven stage, three 1 mm-wide cortical traverses extending from the pial surface to the white matter were placed on each tissue section (9 traverses per subject). In each cortical traverse, four sampling frames (100x150 μm) were placed in layers deep 3-4, defined as 40%-60% of the distance from the pial surface to the white matter border (Figures S1A and B). The film analysis had indicated that a large difference in Zif268 mRNA expression between subjects groups was present in this location. The edges of the frame were equidistant from the border of the traverse and the edge of the next sampling frame, and the top and bottom of the frames were equidistant from the upper or lower borders of layers deep 3-4. Because RNase A treatment during the *in situ* hybridization procedure degrades Nissl-stainable substances within the cytoplasm, it was not possible to draw contours around the soma of neurons. Thus, the number of grains per neuron was counted within circles with a fixed size of 22 μm diameter that cover the largest cross-sectional area of interneurons (~400 μm^2) observed in previous studies (1). In a bright-field image of the sampling frame, the circles were centered over every Nissl-stained neuronal nucleus (Figure S1C). In a dark-field image of the same sampling frame, the number of grains

within each circle was counted (Figure S1D). Background grain density was measured in each sampling frame by using the same sampling circle to count grains over 2 glial nuclei that did not overlap with neuronal nuclei. The smaller size and intense cresyl violet staining of glial nuclei distinguished them from the larger, more faintly stained neuronal nuclei. Total neuron numbers sampled in layers deep 3-4 were 4053 and 4050 for schizophrenia and comparison subjects, respectively.

Grain density per neuron (i.e., number of grains within the 22 μm diameter circle) was calculated for all neurons and a threshold of grain density per neuron was established to identify specifically labeled neurons. For both schizophrenia and comparison groups, histograms of the grain number per neuron ($\log_{10}$ transformed) of all sampled neurons revealed a distribution that appeared bimodal, presumably representing the modes of unlabeled neurons and specifically labeled neurons (6,7). Similar histograms of only neurons with grain density ≥5× background showed a distribution that appeared normal and unimodal in both subject groups. Therefore, the threshold of ≥5× background provided a cutoff at the point of rarity in the distribution of sampled neurons and permitted the identification of specifically-labeled neurons, referred to as Zif268 mRNA positive neurons.

References

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

Subject Group[a]		Case No.	S/R/A[b]	PMI[c]	pH	RIN	Storage time[d]	Cause of death[e]	*DSM IV* Diagnoses[f] Primary Substance[g]		Anti-psychotics ATOD	Anti-depressants ATOD	BZ/VPA ATOD[h]
1*	C	592	M/B/41	22.1	6.7	9.0	203	ASCVD	N				
	S	533	M/W/40	29.1	6.8	8.4	213	Accidental Asphyxiation	US		Y	N	N
2*	C	567	F/W/46	15.0	6.7	8.9	208	Mitral valve prolapse	N				
	S	537	F/W/37	14.5	6.7	8.6	213	Suicide by hanging	SA		N	N	N
3*	C	516	M/B/20	14.0	6.9	8.4	215	Homicide by gun shot	N				
	C	1406[#]	M/B/27	14.6	6.4	8.3	60	Peritonitis	N				
	S	547	M/B/27	16.5	7.0	7.4	211	Heat stroke	SA		Y	Y	Y
4*[†]	C	630	M/W/65	21.2	7.0	9.0	198	ASCVD	N				
	S	566	M/W/63	18.3	6.8	8.0	208	ASCVD	US	AAR	Y	Y	Y
5*[†]	C	604	M/W/39	19.3	7.1	8.6	201	Hypoplastic coronary artery	N				
	S	581	M/W/46	28.1	7.2	7.9	206	Accidental combined drug overdose	PS	ADC; OAC	Y	N	Y
6*[†]	C	546	F/W/37	23.5	6.7	8.6	211	ASCVD	N				
	S	587	F/B/38	17.8	7.0	9.0	204	Myocardial hypertrophy	US	AAR	Y	N	Y
7*	C	551	M/W/61	16.4	6.6	8.3	210	Cardiac tamponade	N				
	S	625	M/B/49	23.5	7.3	7.6	198	ASCVD	DS	AAC	Y	Y	N
8*	C	685	M/W/56	14.5	6.6	8.1	191	Hypoplastic coronary artery	N				
	S	622	M/W/58	18.9	6.8	7.4	198	Right MCA infarction	US		N	N	N
9*[†]	C	681	M/W/51	11.6	7.2	8.9	191	Hypertrophic cardiomyopathy	N				
	S	640	M/W/49	5.2	6.9	8.4	196	Pulmonary embolism	PS		Y	Y	N
10*[†]	C	806	M/W/57	24.0	6.9	7.8	170	Pulmonary embolism	N				
	S	665	M/B/59	28.1	6.9	9.2	194	Intestinal hemorrhage	PS	ADC	Y	Y	N
11*	C	822	M/B/28	25.3	7.0	8.5	167	ASCVD	N				
	S	787	M/B/27	19.2	6.7	8.4	173	Suicide by gun shot	SA	ODC	Y	N	N
12*[†]	C	727	M/B/19	7.0	7.2	9.2	184	Trauma	N				
	S	829	M/W/25	5.0	6.8	9.3	165	Suicide by drug overdose	SA	ADC; OAR	N	N	Y
13*[†]	C	871	M/W/28	16.5	7.1	8.5	156	Trauma	N				
	S	878	M/W/33	10.8	6.7	8.9	156	Myocardial fibrosis	DS	ADC	Y	Y	Y
14*	C	575	F/B/55	11.3	6.8	9.6	206	ASCVD	N				
	S	517	F/W/48	3.7	6.7	9.3	215	Intracerebral hemorrhage	DS	ADC	Y	N	N
15*[†]	C	700	M/W/42	26.1	7.0	8.7	188	ASCVD	N				
	S	539	M/W/50	40.5	7.1	8.1	212	Suicide by combined drug overdose	SA	ADR	Y	Y	Y

Subject Group[a]		Case No.	S/R/A[b]	PMI[c]	pH	RIN	Storage time[d]	Cause of death[e]	*DSM IV* Diagnoses[f] Primary Substance[g]		Anti-psychotics ATOD	Anti-depressants ATOD	BZ/VPA ATOD[h]
16*	C	988	M/W/82	22.5	6.2	8.4	135	Trauma	N				
	S	621	M/W/83	16.0	7.3	8.7	199	Accidental asphyxiation	US		N	N	N
17*	C	686	F/W/52	22.6	7.0	8.5	190	ASCVD	N				
	S	656	F/B/47	20.1	7.3	9.2	195	Suicide by gun shot	SA	ADC	Y	N	N
18*	C	634	M/W/52	16.2	7.0	8.5	197	ASCVD	N				
	S	722	M/B/45	9.1	6.7	9.2	185	Upper GI bleeding	US	ODR; OAR	Y	N	N
19*†	C	852	M/W/54	8.0	6.8	9.1	159	Cardiac tamponade	N				
	S	781	M/B/52	8.0	6.7	7.7	174	Peritonitis	SA	ADR	Y	Y	N
20*	C	987	F/W/65	21.5	6.8	9.1	135	ASCVD	N				
	S	802	F/W/63	29.0	6.4	9.2	170	Right ventricular dysplasia	SA	ADC; ODR	Y	N	Y
21*†	C	818	F/W/67	24.0	7.1	8.4	168	Anaphylactic reaction	N				
	S	917	F/W/71	23.8	6.8	7.0	148	ASCVD	US		Y	N	N
22*†	C	857	M/W/48	16.6	6.7	8.9	158	ASCVD	N				
	S	930	M/W/47	15.3	6.2	8.2	145	ASCVD	DS	ADR; OAR	Y	N	Y
23*†	C	739	M/W/40	15.8	6.9	8.4	183	ASCVD	N				
	S	933	M/W/44	8.3	5.9	8.1	144	Myocarditis	DS		Y	Y	Y
24*	C	1047	M/W/43	13.8	6.6	9.0	126	ASCVD	N				
	S	1209	M/W/35	9.1	6.5	8.7	107	Diphenhydramine overdose	SA		Y	N	N
25*†	C	1086	MW/51	24.2	6.8	8.1	120	ASCVD	N				
	S	10025	MB/52	27.1	6.7	7.8	99	ASCVD	DS	OAR	N	N	N
26*	C	1092	F/B/40	16.6	6.8	8.0	120	Mitral valve prolapse	N				
	S	1178	F/B/37	18.9	6.1	8.4	111	Pulmonary embolism	SA		Y	N	Y
27*	C	10005	M/W/42	23.5	6.7	7.4	107	Trauma	N				
	S	1256	M/W/34	27.4	6.4	7.9	99	Hanging	US		Y	N	N
28*	C	1336	M/W/65	18.4	6.8	8.0	85	Cardiac tamponade	N				
	S	1173	M/W/62	22.9	6.4	7.7	111	ASCVD	DS	ADR	Y	N	N
29*†	C	1122	M/W/55	15.4	6.7	7.9	116	Cardiac tamponade	N				
	S	1105	M/W/53	7.9	6.2	8.9	118	ASCVD	SA		Y	N	N
30*†	C	1284	M/W/55	6.4	6.8	8.7	95	ASCVD	N				
	S	1188	M/W/58	7.7	6.2	8.4	109	ASCVD	US	AAR; OAR	Y	N	Y
31*†	C	1191	M/B/59	19.4	6.2	8.4	109	ASCVD	N				
	S	1263	M/W/62	22.7	7.1	8.5	98	Asphyxiation	US	ADR	Y	Y	N
32*	C	970	M/W/42	25.9	6.4	7.2	137	ASCVD	N				
	S	1222	M/W/32	30.8	6.4	7.5	105	Combined drug overdose	US	AAC	Y	Y	N

Subject Group[a]		Case No.	S/R/A[b]	PMI[c]	pH	RIN	Storage time[d]	Cause of death[e]	*DSM IV* Diagnoses[f] Primary	Substance[g]	Anti-psychotics ATOD	Anti-depressants ATOD	BZ/VPA ATOD[h]
33*	C	10003	M/W/49	21.2	6.5	8.4	109	Trauma	N				
	S	1088	M/W/49	21.5	6.5	8.1	120	Combined drug overdose	US	ADC; OAC	Y	Y	N
34*†	C	1247	F/W/58	22.7	6.4	8.4	101	ASCVD	N				
	S	1240	F/B/50	22.9	6.3	7.7	101	ASCVD	US	ADR	Y	N	N
35*†	C	1324	M/W/43	22.3	7.0	7.3	87	Aortic dissection	N				
	S	10020	M/W/38	28.8	6.6	7.4	101	Salicylate overdose	PS	AAC; OAC	Y	Y	Y
36*	C	1099	F/W/24	9.1	6.5	8.6	119	Cardiomyopathy	N				
	C	1196[#]	F/W/36	14.5	6.4	8.2	92	Positional asphyxia	N				
	S	10023	F/B/25	20.1	6.7	7.4	100	Suicide by drowning	DS		Y	Y	Y
37*	C	1307	M/B/32	4.8	6.7	7.6	90	ASCVD	N				
	S	10024	M/B/37	6.0	6.1	7.5	99	ASCVD	PS		N	N	N
38*	C	1391	F/W/51	7.8	6.6	7.1	76	ASCVD	N				
	S	1189	F/W/47	14.4	6.4	8.3	109	Combined drug overdose	SA	AAR	Y	Y	Y
39*	C	1282	F/W/39	24.5	6.8	7.5	95	ASCVD	N				
	S	1211	F/W/41	20.1	6.3	7.8	107	Sudden unexpected death	SA		Y	Y	N
40*†	C	1159	M/W/51	16.7	6.5	7.6	113	ASCVD	N				
	S	1296	M/W/48	7.8	6.5	7.3	93	Pneumonia	US		Y	Y	N
41*	C	1326	M/W/58	16.4	6.7	8.0	87	ASCVD	N				
	S	1314	M/W/50	11.0	6.2	7.2	89	ASCVD	US		Y	Y	Y
42*†	C	902	M/W/60	23.6	6.7	7.7	152	ASCVD	N				
	S	1361	M/W/63	23.2	6.4	7.7	82	Cardiomyopathy	SA	ODC	Y	N	Y
43●	C	1374	M/W/43	21.7	6.6	7.2	79	ASCVD	N				
	S	904	M/W/33	28.0	6.2	7.1	150	Pneumonia	SA		Y	N	Y
44●	C	1555	M/W/17	15.1	6.9	7.9	44	Trauma	N				
	S	1649	M/B/17	21.4	6.9	8.1	29	Hanging	US		Y	Y	N
45●	C	1268	M/B/49	19.9	7.1	7.9	96	ASCVD	N				
	S	1230	M/W/50	16.9	6.6	8.2	102	Doxepin overdose	US		Y	Y	N
46●	C	1466	F/B/64	20.0	6.7	8.8	61	Trauma	N				
	S	1341	F/W/44	24.5	6.6	8.8	83	Trauma	SA	ODC	Y	N	Y
47●	C	1518	M/W/50	20.7	6.4	7.7	50	ASCVD	N				
	S	1367	M/W/47	28.9	6.6	7.2	80	Combined drug overdose	SA	ADC; ODR	N	N	N
48●	C	1386	M/W/46	21.2	6.7	8.3	75	ASCVD	N				
	S	1420	M/W/47	23.4	6.8	8.2	69	Jump	SA	AAR; ODC; OAR	Y	Y	N

Subject Group[a]		Case No.	S/R/A[b]	PMI[c]	pH	RIN	Storage time[d]	Cause of death[e]	*DSM IV* Diagnoses[f] Primary	Substance[g]	Anti-psychotics ATOD	Anti-depressants ATOD	BZ/VPA ATOD[h]
49●	C	1472	M/W/61	23.8	6.5	8.0	60	Pulmonary embolism	N				
	S	1453	M/W/62	11.1	6.4	8.2	63	Trauma	PS	ADR	N	N	Y
50●	C	1026	M/W/59	19.8	6.3	7.4	128	ASCVD	N				
	S	1454	M/W/59	24.1	6.1	7.6	62	Trauma	PS	AAR; ODC	Y	Y	N
51●	C	694	M/W/38	20.7	7.0	7.7	189	Subarachnoid hemorrhage	N				
	S	1455	M/W/42	8.2	6.4	7.7	62	Peritonitis	PS	AAR; OAC	Y	N	Y
52●	C	1350	M/W/21	24.2	6.4	7.3	82	Trauma	N				
	S	1474	M/W/37	39.9	6.7	7.0	60	Hanging	SA	ADR	N	N	N
53●	C	1792	F/W/36	28.1	6.5	7.5	5	Pulmonary embolism	N				
	S	1506	F/W/47	14.1	6.6	7.5	55	Combined drug overdose	SA	ADC	Y	Y	N
54●	C	1524	M/W/66	9.4	6.4	8.1	48	Intestinal infarction	N				
	S	1542	M/W/65	17.4	6.7	7.8	45	Combined drug overdose	PS		Y	Y	Y
55●	C	1270	F/W/73	19.7	6.7	7.7	96	Trauma	N				
	S	1579	F/W/69	16.1	6.7	7.7	39	ASCVD	SA	ADR; ODC	Y	N	Y
56●	C	1372	M/W/37	20.5	6.6	9.0	79	Asphyxiation	N				
	S	1581	M/W/32	18.4	6.8	9.0	39	ASCVD	PS	ODC; OAC	Y	Y	N
57●	C	1543	F/W/45	17.9	6.8	7.4	45	Subarachnoid hemorrhage	N				
	S	10026	F/W/46	23.8	6.6	7.6	98	Thermal injuries	US		Y	Y	N
58●	C	1583	M/W/58	19.1	6.8	8.2	39	Trauma	N				
	S	1686	M/B/56	14.1	6.2	8.3	22	ASCVD	PS	AAR	Y	Y	Y
59●	C	1554	M/W/50	23.2	6.5	7.6	44	ASCVD	N				
	S	1691	M/W/51	31.9	6.6	7.7	20	Combined drug overdose	PS	ADR; ODC	Y	N	Y
60●	C	1635	M/W/66	25.3	6.8	8.2	31	Cardiac tamponade	N				
	S	1706	M/B/60	28.1	6.8	8.4	17	Sepsis	SA	AAR; ODC; OAR	Y	N	N
61●	C	1384	M/W/67	21.9	6.6	7.0	77	ASCVD	N				
	S	1712	M/W/63	15.1	6.2	7.1	15	ASCVD	SA	ADR; ODC	Y	Y	Y
62●	C	1558	M/W/54	24.4	6.9	7.7	43	ASCVD	N				
	S	1734	M/W/54	28.6	6.1	7.7	12	Pneumonia	US	AAR; ODC; OAR	Y	N	N

*** Subject** pairs used for gray matter *in situ* hybridization study. **† Subject** pairs used for grain counting analysis *in situ* hybridization study. **● Subject** pairs newly analyzed for GAD67 mRNA by qPCR. # Due to limited availability of fresh frozen tissue sections, comparison subjects 1406 and 1196 were substituted for subjects 516 and 1099 in pair 3 and pair 36, respectively for the in situ hybridization study only. a: C, normal comparison; S, schizophrenia; b: A, age in years; B, black; F, female; M, male; R, race; S, sex; W, white; c: PMI, postmortem interval (hours); d: Storage time (months) at -80C; e: ASCVD, arteriosclerotic cardiovascular disease; MCA, middle coronary artery; f: DS, disorganized schizophrenia; PS, paranoid schizophrenia; SA, schizoaffective disorder; US, undifferentiated schizophrenia; g: 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; h: BZ/VPA ATOD; BZ, benzodiazepines; VPA, Sodium valproate; ATOD, at time of death; Y, yes; N, no.

Table S2: Primer design for qPCR (A) and *in situ* hybridization (B)

A

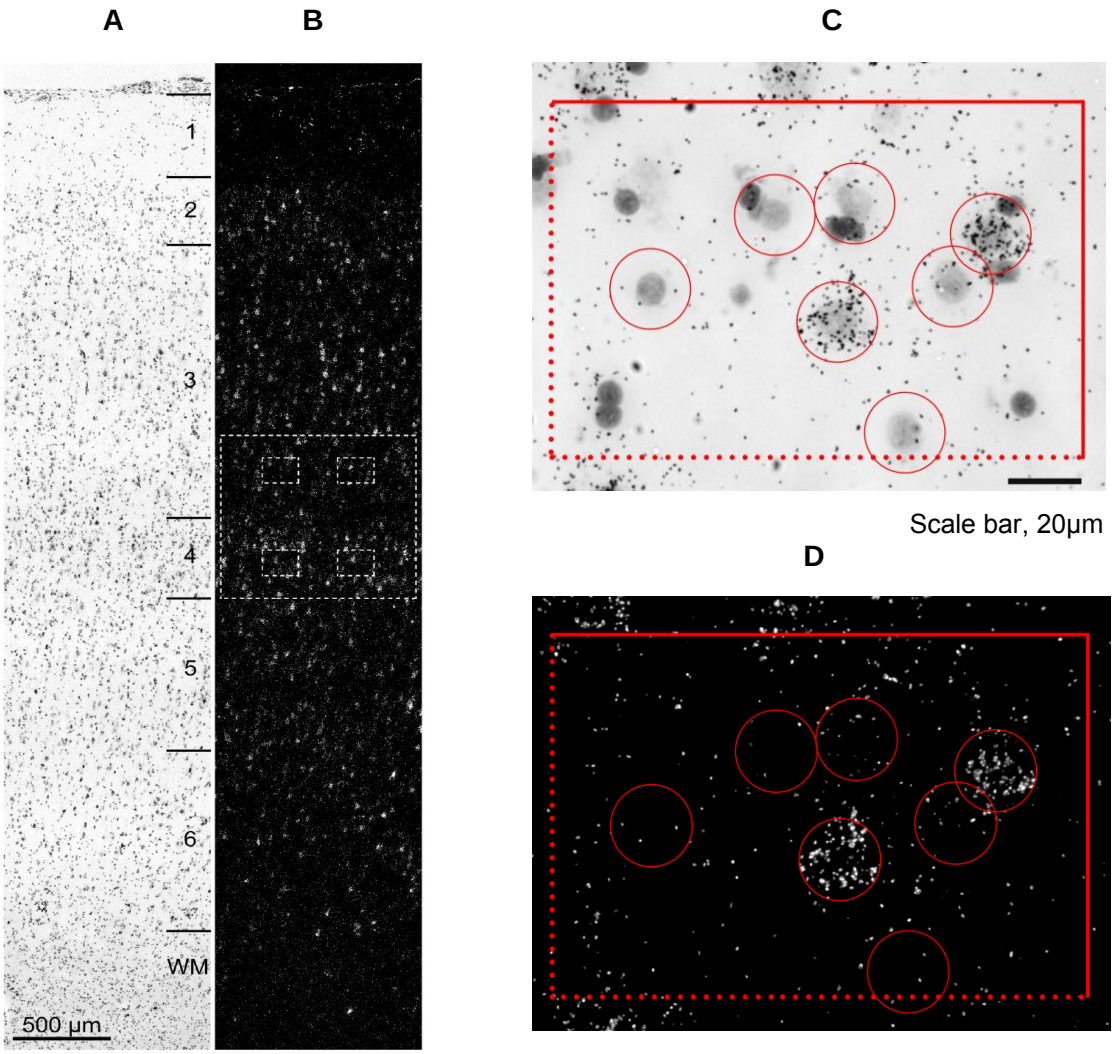
Genes	Accession #	Amplicon size (bp[a])	Position	Forward Primer (F) Reverse Primer (R)
Beta actin	NM_001101	101	1146-1246	(F) GATGTGGATCAGCAAGCA (R) AGAAAGGGTGTAACGCAACTA
Cyclophilin A	NM_021130	126	159-284	(F) GCAGACAAGGTCCCAAAG (R) GAAGTCACCACCCTGACAAC
Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)	NM_002046	87	556-642	(F) TGCACCACCAACTGCTTAGC (R) GGCATGGACTGTGGTCATGAG
Zif268	NM_001964	85	594-678	(F) CTCTCTGAACAACGAGAAGGTG (R) GCGGCCAGTATAGGTGATG
Glutamate decarboxylase 67 kDa (GAD67)	NM_000817	86	2495-2580	(F) GTTTCCCGCTCCAAGAGAAT (R) TGGAGTTGTTGGACAAGCTG
c-fos	NM_005252	81	227-307	(F) GCAGACTACGAGGCGTCA (R) TGCGGGTGAGTGGTAGTAAG
c-jun	NM_002228	99	1731-1829	(F) CAGACAGTGCCCGAGATG (R) GTTCCTCATGCGCTTCCT
EGR-2	NM_001136177	90	347-436	(F) ACTGGAGAGAAGAGGTCGTTG (R) GCCCATGTAAGTGAAGGTCTG

B

Genes	Accession #	Amplicon size (bp[a])	Position	Forward Primer (F) Reverse Primer (R)
Zif268	NM_001964	427	1461-1887	(F) CTGCGACATCTGTGGAAGAA (R) TGTCCTGGGAGAAAAGGTTG

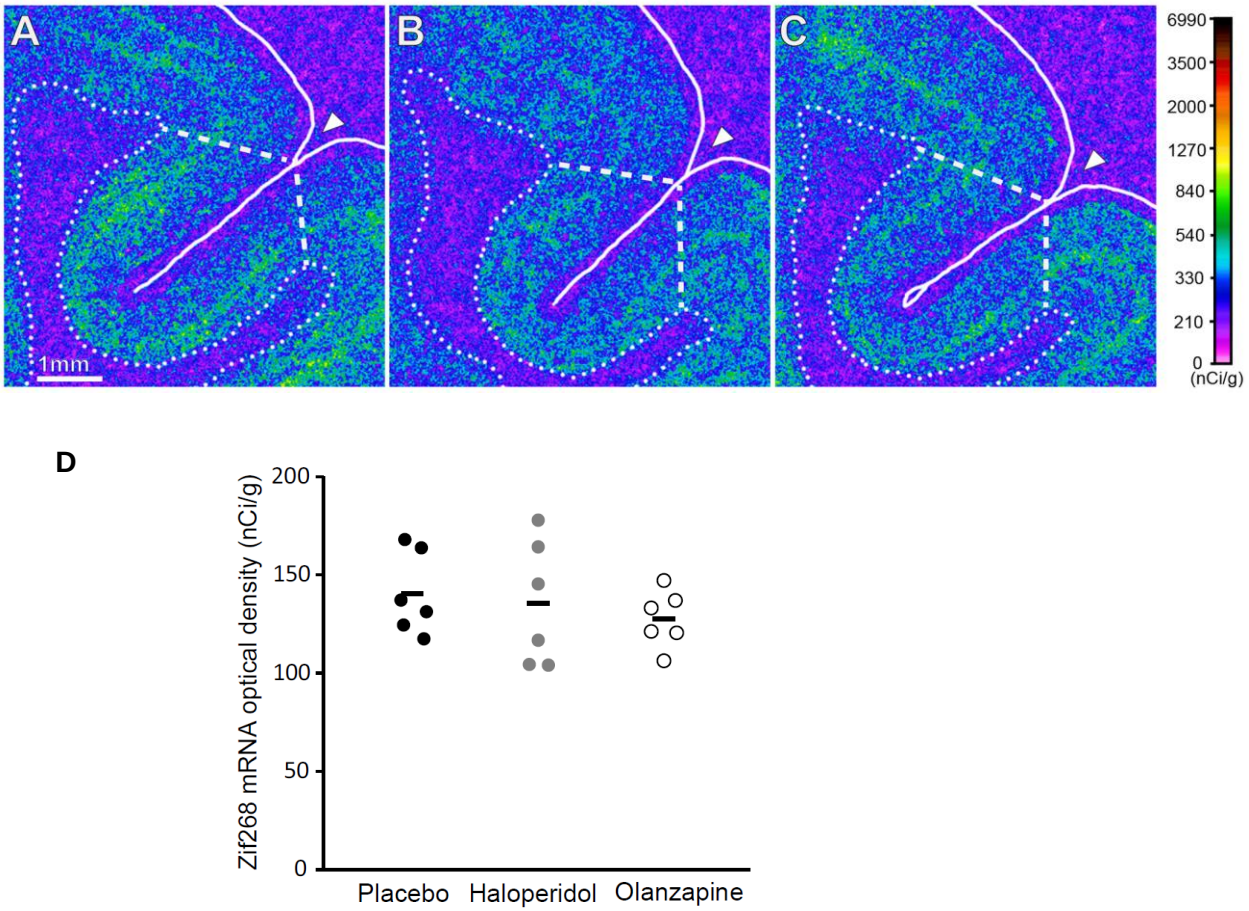
[a]bp=base pairs

Figure S1: Sampling strategy for grain counting analysis of Zif268 mRNA expression



(A) Bright-field photomicrograph of a representative cortical traverse from a prefrontal cortical Nissl-stained tissue section. (B) Dark-field photomicrograph of an emulsion-dipped section hybridized with an antisense ^{35}S-labeled probe for Zif268 mRNA, illustrated as accumulations of silver grains. One region of interest (large dashed rectangle) was placed across layers deep 3-4. Four 100×150 µm sampling frames (smaller dashed rectangles) were placed in each region of interest such that the top or bottom edges of each frame were equidistant to each other and to the borders of the region of interest. (C) A representative bright-field image of a 100x150 µm sampling frame placed in layers deep 3-4 where Nissl-stained neuronal nuclei were identified and sampled within inclusion and exclusion boundaries, indicated by dotted and solid lines, respectively. Note that grain clusters identified in the dark-field image (D) are located over some of the lightly Nissl-stained neuronal nuclei in the bright-field image but not over the darkly stained glial nuclei. Circles with a diameter of 22 µm were centered over all neuronal nuclei in every counting frame, and the number of grains in each circle was counted in the corresponding dark-field image.

Figure S2: *In situ* hybridization film analysis for Zif268 mRNA expression levels in antipsychotic medication-exposed monkeys



Representative pseudocolored film autoradiographs illustrated Zif268 mRNA expression levels in prefrontal cortex area 46 of control monkey (A), and age-, sex-, and body weight-matched monkeys chronically exposed to haloperidol (B) or olanzapine (C). Zif268 mRNA expression was measured in the gray matter regions indicated by dashed lines that correspond to area 46 around the principal sulcus (arrowheads). Solid and dotted lines indicate the pial surface and the gray/white matter border. (D) No statistically significant differences were found in Zif268 mRNA levels in monkeys chronically exposed to haloperidol or olanzapine relative to placebo ($F_{2,10}$=0.66, p=0.54). Horizontal bars indicate group means.