Supplemental Methods

In situ hybridization (ISH)

Due to a limitation of the maximal number of sections that could be processed together for ISH, the 22 subject pairs were divided in two groups, each containing 11 subject pairs. One pair of sections from each of 11 subject pairs within a group was processed side by side in a single ISH run and subsequent signal detection. Total of 6 ISH runs were performed. After fixation with 4% paraformaldehyde in PBS, the sections were acetylated with 0.25% acetic anhydrate in 0.1 M triethanolamine/0.9% NaCl for 10 min, and dehydrated through a graded ethanol series. The sections were then hybridized with ^{35}S-labeled riboprobes (2×10^7 dpm/ml) in hybridization buffer containing 50% formamide, 0.75 M NaCl, 20 mM 1,4-piperazine diethane sulfonic acid, pH 6.8, 10 mM EDTA, 10% dextran sulfate, 5× 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 at 56°C for 16 hr. The sections were washed in a solution containing 0.3 M NaCl, 20 mM Tris-HCl, pH 8.0, 1mM EDTA, pH 8.0, and 50% formamide at 63°C, treated with 20 µg/ml RNase A (Sigma-Aldrich, St Louis, MO) at 37°C, and washed in 0.1× SSC (150 mM NaCl, 15 mM sodium citrate) at 66°C. Sections were then dehydrated through a graded ethanol series, air dried, and exposed to BioMax MR film (Kodak, Rochester, NY) for 10 days. Sections were then coated with NTB emulsion (Kodak) diluted 1:1 with distilled water by using a mechanical dipper (Aiden, Kobe, Japan), at a constant withdrawal speed of 15 mm/s and temperature (43°C). Sections were exposed for four weeks at 4°C, developed with D-19 (Eastman Kodak, Rochester, NY), and counterstained with 0.5% cresyl violet.

Film analysis of KCNS3 mRNA expression

Quantification was performed without knowledge of subject diagnosis by random coding of the sections. Film autoradiograms of sections processed for ISH of KNCS3 mRNA were captured, digitized, and analyzed using a Microcomputer Imaging Device (MCID) system (InterFocus Imaging Ltd, Cambridge, UK). Optical density was measured in the gray matter of PFC area 9 and expressed as nanocuries per gram of tissue (nCi/g) by reference to Carbon-14 radioactive standards (ARC Inc., St. Louis, MO) exposed on the same film. All cortical optical density measures were corrected by subtracting background measures in the white matter of each section. The mean (±SD) total area of gray matter sampled in each subject was 635±119 mm^2 for schizophrenia subjects and 689±124 mm^2 for comparison subjects.

Grain counting analysis of KCNS3 mRNA expression at the cellular level

Using the MCID system equipped with a motor-driven stage, sampling frames with a size of 220×220 μm were systematically and randomly placed within the gray matter of area 9 for each emulsion-coated, Nissl-counterstained section (Figure S1). As RNase A treatment during the ISH procedures degrades Nissl-stainable substances within the cytoplasm, it is not possible to draw contours of the neuronal soma. 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 GABA neurons (≈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 S1). In a dark-field image of the same sampling frame, the number of grains within each circle was determined (Figure S1). Using the same sampling frames, background grain density was determined for each

section by counting the number of grains within 22-μm-diameter circles centered on glial nuclei. Small glial nuclei, stained darkly with cresyl violet, were easily discriminated from large and faintly stained neuronal nuclei (Figure S1). Totals of 39,373 and 37,025 neurons were sampled for schizophrenia and comparison subjects, respectively. Histograms of grain number per neuron (log_{10}-transformed) for all sampled neurons revealed a distribution that appeared bimodal in both subject groups, representing nonspecifically and specifically labeled neuron populations (2, 3). Similar histograms including only neurons with a grain number ≥5×background showed a distribution that appeared normal and unimodal in both the schizophrenia and comparison groups. Therefore, a threshold of 5×background provided a cutoff at the point of rarity in the distribution of all cells that permitted the identification of specifically labeled neurons, referred to as KCNS3 mRNA-expressing neurons. Mean numbers of KCNS3 mRNA-expressing neurons sampled per subject were 56±26 and 86±26 for schizophrenia and comparison subjects, respectively.

Laser microdissection (LMD) of individual neurons

To collect neurons by LMD, the sections (12 μm) of PFC area 9 were cut and thaw-mounted onto room temperature glass PEN membrane slides (LEICA-Microsystems, Bannockburn, IL) previously UV-treated at 254 nm for 30 min. Sections were then dried for 20 s on a plate warmer at 50°C, placed in the cryostat to equilibrate at -20°C for 30 min and then stored at -80°C. On the day of the microdissection, slides were fixed in acetone, and incubated in a solution containing biotinylated lectin *Vicia villosa* agglutinin (VVA) (B-1235, 1:400, Vector Labs, Burlingame CA) and mouse anti-NeuN antibody (MAB377, 1:400, Millipore, Billerica MA) for the dual-staining of perineuronal nets and all neurons, respectively. Slides were then incubated with the mixture of Streptavidin-Alexa488 (S11223, 1:250, Invitrogen) and Donkey Alexa568 anti-mouse IgG

(A100037, 1:250, Invitrogen), and briefly dehydrated in 50% ethanol. Using a LEICA microdissection system (LMD 6500), neurons dual-labeled with VVA and NeuN as well as neurons single-labeled with NeuN were individually dissected, using the 40× objective with the following settings: Power 15, Aperture 9, Speed 12, Balance 14, and Offset 150. The majority of the dual-labeled neurons had a multipolar morphology typical of interneurons. The boundary of the cell body was determined by the location of the VVA labeling which frequently extended to outline proximal dendrites. Only the cell body outlined by VVA-positive pericellular nets was dissected.

Microarray procedures

Cell samples collected by LMD were lysed in RLT Buffer Plus (Qiagen Sciences, Germantown, MD) and RNA was extracted using the QIAGEN Micro RNeasy Plus kit following the manufacturer's instruction without addition of poly-A carrier. Extracted RNA from single cell samples was reverse transcribed into cDNA and amplified with one single round of amplification using the Ovation_Pico WTA System (Nugen Technologies, San Carlos, CA). Following amplification, 2.5 μg were labeled using the Encore Biotin module (Nugen) and the resulting labeled cDNA was loaded on an Affymetrix GeneChip® HT HG-U133^{+} PM Array Plate (Affymetrix, Santa Clara, CA).

Assessments of the relationships between clinical factors and the lower KCNS3 mRNA levels in schizophrenia

Our psychological autopsy provided information regarding clinical factors in schizophrenia subjects such as sex; family history of first degree relatives with schizophrenia; age at onset of

schizophrenia; suicide as the manner of death; history of marriage; socioeconomic status; independency of living at time of death; any substance abuse or dependency at time of death; and use of antidepressants, benzodiazepines/anticonvulsants, or antipsychotics at time of death. For ISH data, within-subject-pair percent differences in film optical density were calculated for each pair. We then divided the 22 pairs into two groups based on each clinical factor and compared the within-subject-pair percent differences between these two groups (3). For microarray data, within-subject-pair $\log_2$-transformed schizophrenia-to-comparison signal ratios were calculated for each pair. The 14 subject pairs were divided into two groups based on each clinical factor and the within-subject-pair ratios were compared between these two groups. For the effect of age at onset of schizophrenia, subject pairs were divided into earlier and later groups with the age of onset being ≤18 and ≥19, respectively (4). For the effect of socioeconomic status, the subject pairs were divided into lower and higher groups based on the Hollingshead Two Factor Index of Social Position of schizophrenia subjects being ≤24 and ≥25, respectively (4). The effects of family history and antipsychotic medication could not be assessed in microarray data because, among the 14 subjects with schizophrenia, only one subject had a family history of first-degree relatives with schizophrenia and two subjects were off antipsychotic medication at time of death. The comparisons were done by two-sample t-tests.

Supplemental References

1. Volk DW, Austin MC, Pierri JN, Sampson AR, Lewis DA: Decreased glutamic acid decarboxylase$_{67}$ messenger RNA expression in a subset of prefrontal cortical gamma-aminobutyric acid neurons in subjects with schizophrenia. Arch Gen Psychiatry 2000; 57:237-45.

2. Gerfen CR, McGinty JF, Young WS, 3rd: Dopamine differentially regulates dynorphin, substance P, and enkephalin expression in striatal neurons: in situ hybridization histochemical analysis. J Neurosci 1991; 11:1016-31.

3. Hashimoto T, Volk DW, Eggan SM, Mirnics K, Pierri JN, Sun Z, Sampson AR, Lewis DA: Gene expression deficits in a subclass of GABA neurons in the prefrontal cortex of subjects with schizophrenia. J Neurosci 2003; 23:6315-26.

4. Volk DW, Radchenkova PV, Walker EM, Sengupta EJ, Lewis DA: Cortical opioid markers in schizophrenia and across postnatal development. Cerebral Cortex 2012; 22:1215-23.

TABLE S1. Demographic and postmortem characteristics of human subjects for in situ hybridization study.

	Comparison subjects									Schizophrenia subjects										
Pair	Case	Sex	Race	Age	PMI	pH	RIN	ST	Cause of death	Case	DSM-IV diagnosis	Sex	Race	Age	PMI	pH	RIN	ST	Cause of death	Medications at time of death
1	592	M	B	41	22.1	6.7	9.0	189	ASCVD	533	Chronic undifferentiated schizophrenia	M	W	40	29.1	6.8	8.4	199	Accidental asphyxiation	P
2	567	F	W	46	15.0	6.7	8.9	193	Mitral valve prolapse	537	Schizoaffective disorder	F	W	37	14.5	6.7	8.6	198	Suicide by hanging	O
3	1406	M	B	27	14.6	6.3	8.3	56	Peritonitis due to ruptured appendix	547	Schizoaffective disorder	M	B	27	16.5	7.0	7.4	197	Heat stroke	B C D L O P
4	630	M	W	65	21.2	7.0	9.0	183	ASCVD	566	Chronic undifferentiated schizophrenia; AAR	M	W	63	18.3	6.8	8.0	193	ASCVD	B D O P
5	604	M	W	39	19.3	7.1	8.6	187	Hypoplastic coronary artery	581	Chronic paranoid schizophrenia; ADC; OAC	M	W	46	28.1	7.2	7.9	191	Accidental combined drug overdose	B C O P
6	546	F	W	37	23.5	6.7	8.6	197	ASCVD	587	Chronic undifferentiated schizophrenia; AAR	F	B	38	17.8	7.0	9.0	190	Myocardial hypertrophy	B L P
7	551	M	W	61	16.4	6.6	8.3	196	Cardiac tamponade	625	Chronic disorganized schizophrenia; AAC	M	B	49	23.5	7.3	7.6	183	ASCVD	D O P
8	685	M	W	56	14.5	6.6	8.1	176	Hypoplastic coronary artery	622	Chronic undifferentiated schizophrenia	M	W	58	18.9	6.8	7.4	184	Right middle cerebral artery infarction	O
9	681	M	W	51	11.6	7.2	8.9	176	Hypertrophic cardiomyopathy	640	Chronic paranoid schizophrenia	M	W	49	5.2	6.9	8.4	182	Pulmonary embolism	D P
10	806	M	W	57	24.0	6.9	7.8	155	Pulmonary tromboembolism	665	Chronic paranoid schizophrenia; ADC	M	B	59	28.1	6.9	9.2	179	Intestinal hemorrhage	D O P
11	822	M	B	28	25.3	7.0	8.5	152	ASCVD	787	Schizoaffective disorder; ODC	M	B	27	19.2	6.7	8.4	158	Suicide by gun shot	O P
12	727	M	B	19	7.0	7.2	9.2	169	Trauma	829	Schizoaffective disorder; ADC; OAR	M	W	25	5.0	6.8	9.3	150	Suicide by drug overdose	B C O
13	871	M	W	28	16.5	7.1	8.5	141	Trauma	878	Disorganized schizophrenia; ADC	M	W	33	10.8	6.7	8.9	140	Myocardial fibrosis	C D P
14	700	M	W	42	26.1	7.0	8.7	173	ASCVD	539	Schizoaffective disorder; ADR	M	W	50	40.5	7.1	8.1	198	Suicide by combined drug overdose	C D P
15	988	M	W	82	22.5	6.2	8.4	119	Trauma	621	Chronic undifferentiated schizophrenia	M	W	83	16.0	7.3	8.7	184	Accidental asphyxiation	N
16	686	F	W	52	22.6	7.0	8.5	175	ASCVD	656	Schizoaffective disorder; ADC	F	B	47	20.1	7.3	9.2	180	Suicide by gun shot	P
17	634	M	W	52	16.2	7.0	8.5	182	ASCVD	722	Chronic undifferentiated schizophrenia; OAR; ODR	M	B	45	9.1	6.7	9.2	170	Upper gastrointestinal bleeding	P
18	852	M	W	54	8.0	6.8	9.1	144	Cardiac tamponade	781	Schizoaffective disorder; ADR	M	B	52	8.0	6.7	7.7	159	Peritonitis	D O P
19	987[†]	F	W	65	21.5	6.8	9.1	119	ASCVD	802	Schizoaffective disorder; ADC; ODR	F	W	63	29.0	6.4	9.2	155	Right ventricular dysplasia	C P
20	818	F	W	67	24.0	7.1	8.4	153	Anaphylactic reaction	917	Chronic undifferentiated schizophrenia	F	W	71	23.8	6.8	7.0	133	ASCVD	P
21	857	M	W	48	16.6	6.7	8.9	143	ASCVD	930	Disorganized schizophrenia; OAR; ADR	M	W	47	15.3	6.2	8.2	129	ASCVD	C P
22	739	M	W	40	15.8	6.9	8.4	168	ASCVD	933	Disorganized schizophrenia	M	W	44	8.3	5.9	8.1	129	Myocarditis	C D P
	Mean			48.0	18.4	6.8	8.6	161.2						47.9	18.4	6.8	8.4	171.9		
	SD			15.3	5.4	0.1	0.4	32.8						14.5	9.0	0.1	0.7	23.7		

Abbreviations: AAC, alcohol abuse, current at time of death; AAR, alcohol abuse, in remission at time of death; ADC, alcohol dependence, current at time of death; ADR, alcohol dependence, in remission at time of death; ASCVD, arteriosclerotic cardiovascular disease; B, benzodiazepines; C, anticonvulsants; D, antidepressants; L, lithium; N, no medications; O, other medication(s); OAC, other substance abuse, current at time of death; OAR, other substance 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; P, antipsychotics; PMI, postmortem interval in hours; RIN, RNA integrity number; ST, Storage time in months at -80°C.

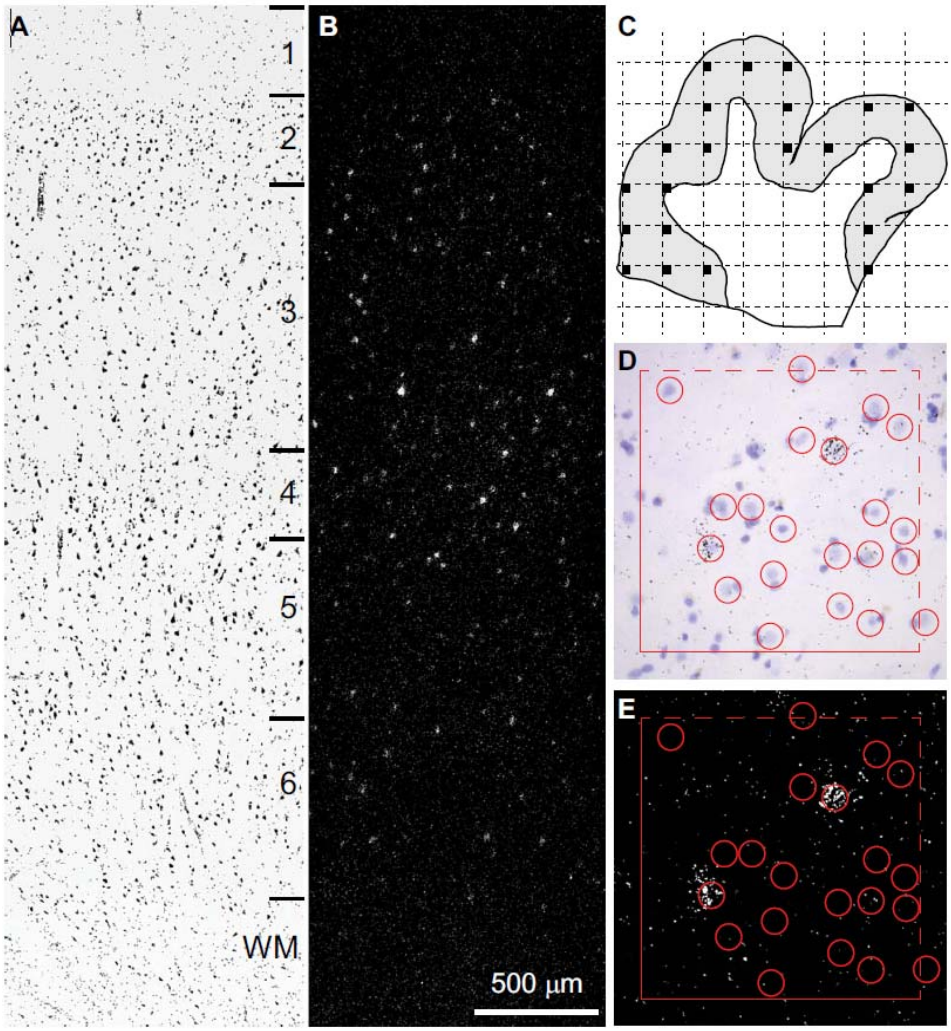
[†] History of posttraumatic stress disorder, in remission 39 years at time of death.

TABLE S2. Demographic and postmortem characteristics of human subjects for DNA microarray study.

	Comparison subjects									Schizophrenia subjects									
Pair	Case	Sex	Race	Age	PMI	pH	RIN	ST	Cause of death	Case	DSM-IV diagnosis	Sex	Race	Age	PMI	pH	RIN	ST	Cause of death	Medications at time of death
1	1086	M	W	51	24.2	6.8	8.1	103.2	ASCVD	10025	Disorganized schizophrenia; OAR	M	B	52	27.1	6.7	7.8	82.3	ASCVD	N
2	1336	M	W	65	18.4	6.8	8.0	67.5	Cardiac tamponade	1173	Disorganized schizophrenia; ADR	M	W	62	22.9	6.4	7.7	94.2	ASCVD	O P
3	1122	M	W	55	15.4	6.7	7.9	99.0	Cardiac tamponade	1105	Schizoaffective disorder	M	W	53	7.9	6.2	8.9	101.1	ASCVD	P
4	970	M	W	42	25.9	6.4	7.2	120.5	ASCVD	1222	Undifferentiated schizophrenia; AAC	M	W	32	30.8	6.4	7.5	87.6	Suicide by combined drug overdose	D P
5	1324	M	W	43	22.3	7.0	7.3	70.1	Aortic dissection	10020	Paranoid schizophrenia; AAC; OAC	M	W	38	28.8	6.6	7.4	83.8	Suicide by salicylate overdose	C D P
6	1307	M	B	32	4.8	6.7	7.6	73.1	ASCVD	10024	Paranoid schizophrenia	M	B	37	6.0	6.1	7.5	82.5	ASCVD	O
7	1159	M	W	51	16.7	6.5	7.6	95.5	ASCVD	1296	Undifferentiated schizophrenia	M	W	48	7.8	6.5	7.3	75.8	Pneumonia	D O P
8	1326	M	W	58	16.4	6.7	8.0	69.7	ASCVD	1314	Undifferentiated schizophrenia	M	W	50	11.0	6.2	7.2	72.3	ASCVD	C D O P
9	1099	F	W	24	9.1	6.5	8.6	101.6	Cardiomyopathy	10023	Disorganized schizophrenia	F	B	25	20.1	6.7	7.4	83.0	Suicide by drowning	B D P
10	1047	M	W	43	13.8	6.6	9.0	109.2	ASCVD	1209	Schizoaffective disorder	M	W	35	9.1	6.5	8.7	89.9	Suicide by diphenhydramine overdose	L O P
11	1092	F	B	40	16.6	6.8	8.0	102.6	Mitral valve prolapse	1178	Schizoaffective disorder	F	B	37	18.9	6.1	8.4	94.0	Pulmonary embolism	B P
12	1391	F	W	51	7.8	6.6	7.1	59.0	ASCVD	1189	Schizoaffective disorder; AAR	F	W	47	14.4	6.4	8.3	92.2	Suicide by combined drug overdose	B C D O P
13	1282	F	W	39	24.5	6.8	7.5	78.3	ASCVD	1211	Schizoaffective disorder	F	W	41	20.1	6.3	7.8	89.7	Sudden unexpected death	D O P
14	902	M	W	60	23.6	6.7	7.7	134.8	ASCVD	1361	Schizoaffective disorder; ODC	M	W	63	23.2	6.4	7.7	65.0	Cardiomyopathy	C O P
	Mean			46.7	17.1	6.7	7.8	91.7						44.3	17.7	6.4	7.8	85.2		
	SD			11.3	6.6	0.2	0.5	22.4						11.2	8.4	0.2	0.5	9.6		

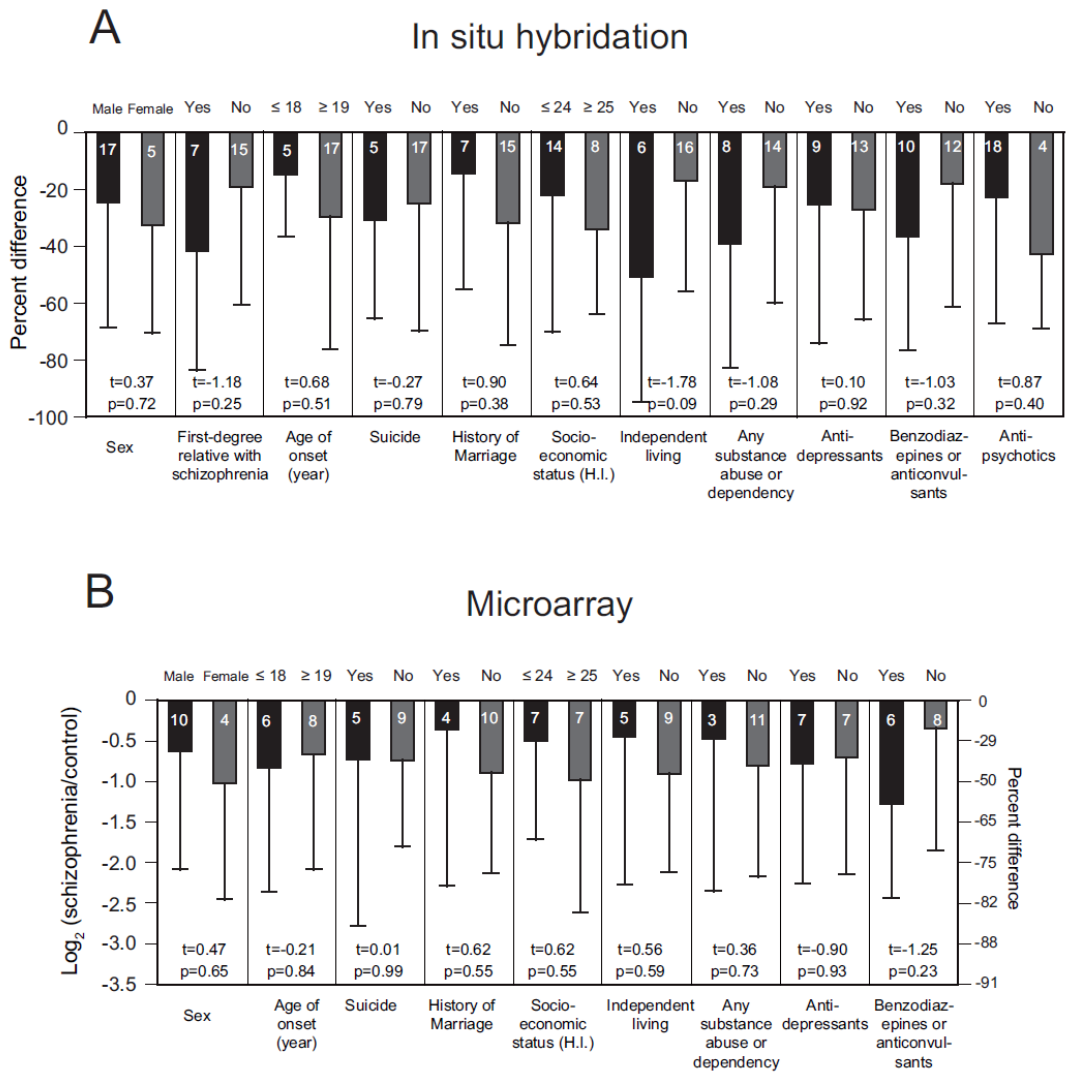
Abbreviations: AAC, alcohol abuse, current at time of death; AAR, alcohol abuse, in remission at time of death; ADR, alcohol dependence, in remission at time of death; ASCVD, arteriosclerotic cardiovascular disease; B, benzodiazepines; C, anticonvulsants; D, antidepressants; L, lithium; N, no medications; O, other medication(s); OAC, other substance abuse, current at time of death; OAR, other substance abuse, in remission at time of death; ODC, other substance dependence, current at time of death; P, antipsychotics; PMI, postmortem interval in hours; RIN, RNA integrity number; ST, Storage time in months at -80°C.

FIGURE S1. KCNS3 mRNA expression visualized by emulsion autoradiography in area 9 of a comparison subject and sampling for grain counting analysis.



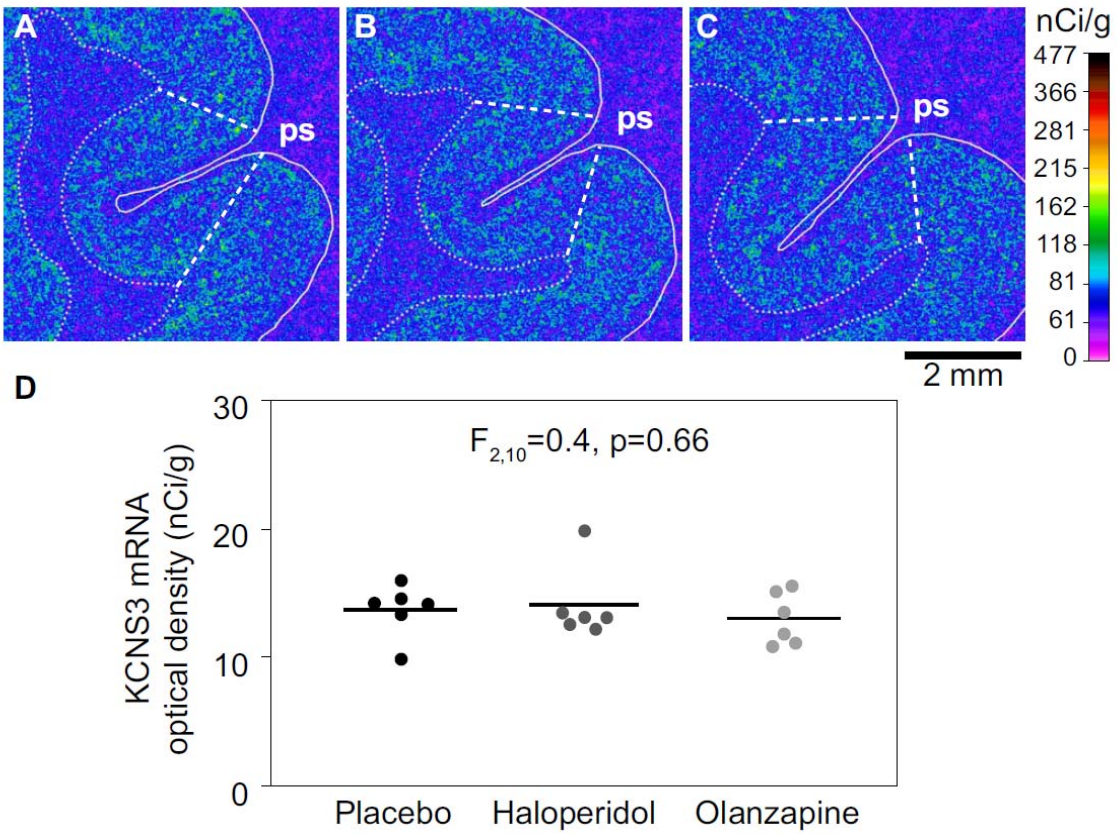
(A) Brightfield photomicrograph of a representative traverse from a Nissl-stained section. The six cortical layers and white matter (WM) are identified on the right. (B) Darkfield photomicrograph of the corresponding traverse in an adjacent emulsion-dipped section illustrating the distribution of silver grain clusters that represent KCNS3 mRNA-expressing neurons. (C) A typical contour of area 9 for the sampling is indicated by gray color in a schematic drawing of a dorsal PFC section. A sampling grid is randomly superimposed on this contour to designate sampling frames (small black squares). (D) Representative brightfield image of a 220×220 µm sampling frame in which Nissl-stained neuronal nuclei were identified and sampled within inclusion and exclusion boundaries, indicated by broken and solid lines, respectively. Note that grain clusters identified in the dark-field image in panel (E) are located over some of the lightly Nissl-stained neuronal nuclei in the brightfield 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 darkfield image.

FIGURE S2. The effects of clinical factors, such as sex; family history; age of onset; suicide as the manner of death; history of marriage; socioeconomic status; independency of living at time of death; substance abuse or dependency at time of death; and treatment with antidepressants, benzodiazepines/anticonvulsants, or antipsychotics at time of death, on the expression changes in KCNS3 mRNA in subjects with schizophrenia.



(A) The mean (±SD) within-subject-pair percent differences in the film optical densities from the matched comparison subject are compared between subject pairs grouped by clinical factors. (B) The mean (±SD) within-subject-pair $\log_2$-transformed schizophrenia-to-comparison signal ratios in microarray analyses of individually dissected VVA-labeled neurons are compared between subject pairs grouped by clinical factors. The corresponding percentage differences are shown on the right axis. (A and B) The numbers within each bar indicate the number of subject pairs. Socioeconomic status was evaluated by using the Hollingshead Two Factor Index of Social Position (H.I.). None of these clinical factors had a significant effect on the expression changes in KCNS3 mRNA detected by in situ hybridization or microarray.

FIGURE S3. In situ hybridization film analysis for KCNS3 mRNA in monkeys exposed to antipsychotics



(A-C) Representative pseudocolored film autoradiograms illustrate the expression of KCNS3 mRNA in the PFC of placebo-exposed (A), haloperidol-exposed (B), and olanzapine-exposed (C) monkeys. Antipsychotics were administered to mimic the clinical treatment of individuals with schizophrenia. KCNS3 mRNA expression was measured in the gray matter regions indicated by dashed lines that correspond to area 46 around the principal sulcus (ps). Solid lines indicate the pial surface and dotted lines the gray matter-white matter border. (D) KCNS3 mRNA film optical densities in the PFC of placebo-exposed, haloperidol-exposed, and olanzapine-exposed monkeys are indicated individually by black, dark gray and light gray circles, respectively. Horizontal bars indicate group means.