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Supplementary Materials for

Histamine H_2 receptor deficit in glutamatergic neurons contributes to the pathogenesis of schizophrenia

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Fig. S1. Decreased *Hrh2 mRNA* level was found in schizophrenia patients. (A) Representative photographs of RNAscope assay of *Hrh2* mRNA and immunostaining of glutamate in the frontal cortex of schizophrenia patients and controls. Scale bar, 15 μm. **(B)** Quantitative statistics of *Hrh2* mRNA expression in glutamatergic neurons, glutamate intensity and soma size of glutamate$^+$ neurons in schizophrenia patients and controls. n=10 for each group. $^{**}P \leqslant 0.01$. See also Table. S2 for further statistical information.

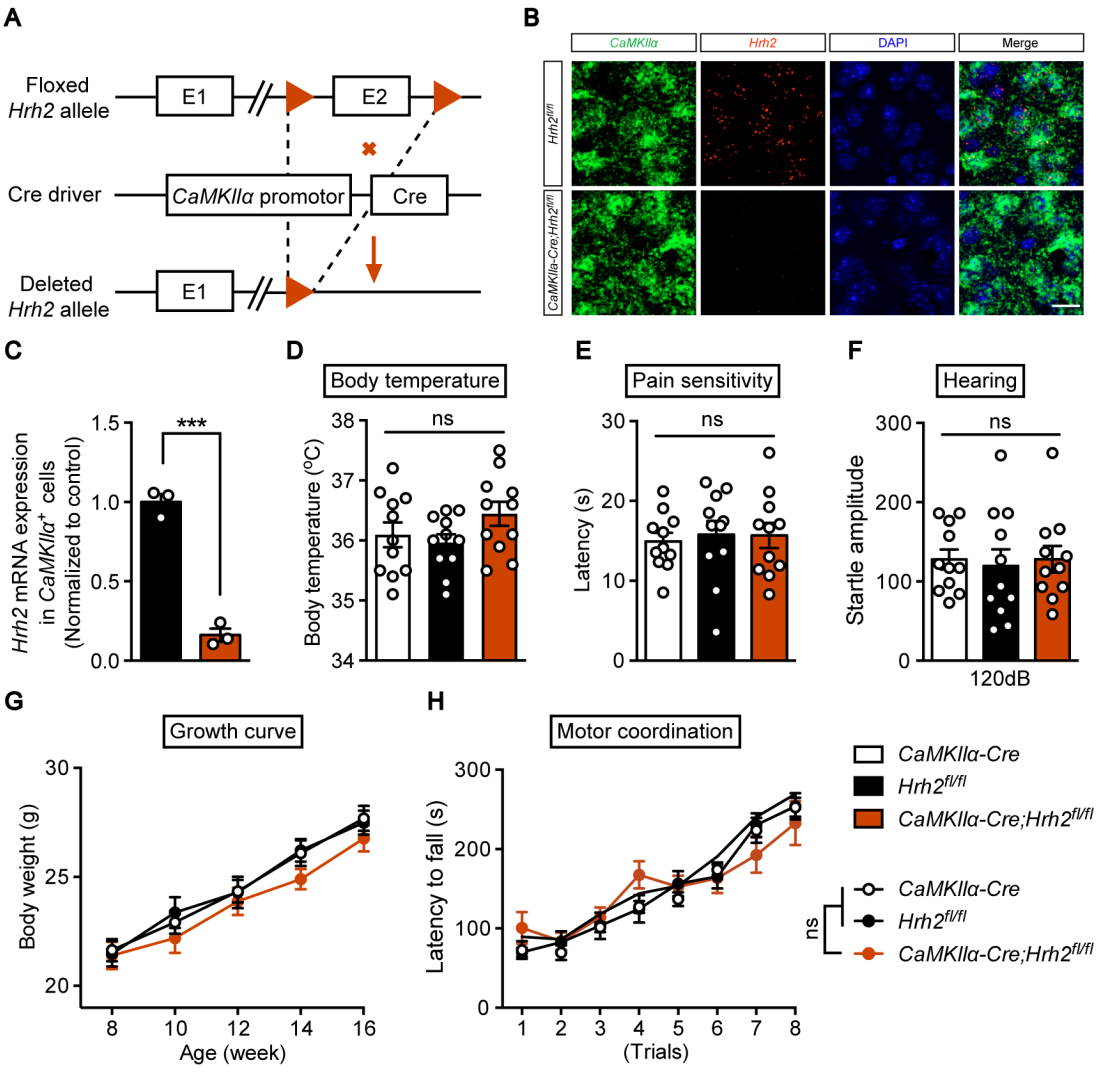

Fig. S2. Decreased histamine H2R expression in glutamatergic neurons does not impair the physiological functions of mice. (A) Schematic diagram of the generation of *CaMKIIα-Cre; Hrh2*$^{fl/fl}$ mice. **(B)** Representative images of RNAscope *in situ* hybridization of *Hrh2* and *CaMKIIα* mRNA together with staining of nuclei with DAPI in mPFC. Scale bar, 20 μm. **(C)** *Hrh2* mRNA expression in *CaMKIIα*$^{+}$ neurons. (D-H) Body temperature, pain sensitivity, hearing, weight, and motor coordination function in *CaMKIIα-Cre*, *Hrh2*$^{fl/fl}$, and *CaMKIIα-Cre; Hrh2*$^{fl/fl}$ mice. **(D)** The body temperature of each mouse assessed by a rectal thermometer. **(E)** Pain sensitivity measured by a hot plate test. **(F)** Analysis of the auditory startle reflex under 120 dB to test the hearing of mice. **(G)** Growth curve from 8 to 16 weeks of age. **(H)** Motor coordination function assessed by the accelerated rotarod test. n=11 mice per group. ***P≤0.001, ns, not significant. See also Table. S2 for further statistical information.

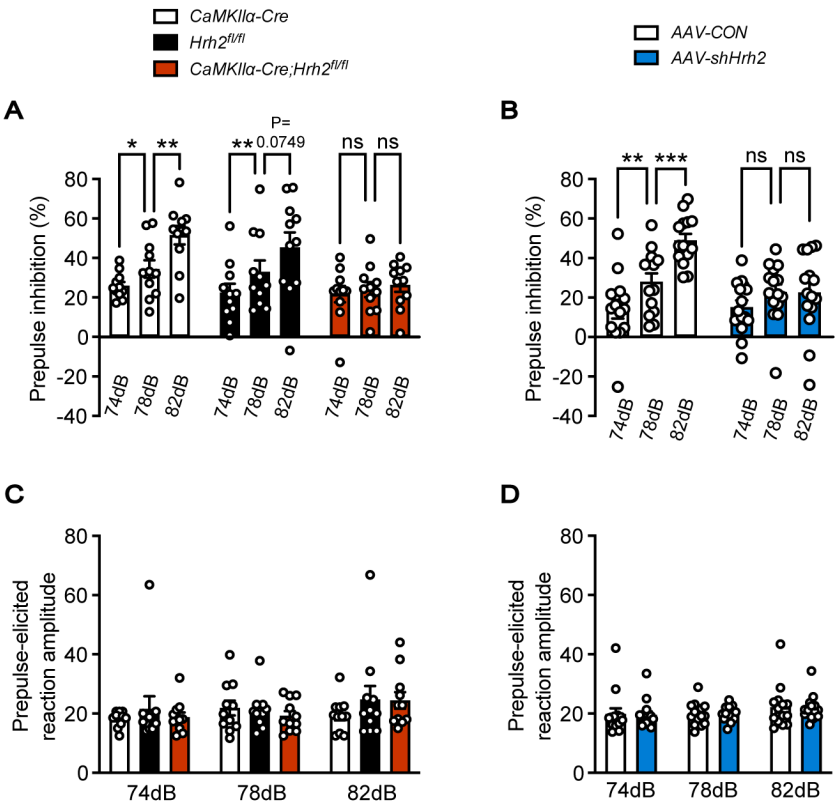

Fig. S3. Sensorimotor gating ability and prepulse-elicited reaction in H2R knockout or knockdown mice. (A-B) Sensorimotor gating ability of mice re-analysed from fig. 1A and fig. 2B. **(C-D)** Prepulse-elicited reaction in H_2R knockout or knockdown mice. n=11 *Hrh2*$^{fl/fl}$, *CaMKIIα-Cre* and *CaMKIIα-Cre; Hrh2*$^{fl/fl}$ mice; 13 *CaMKIIα-Cre+AAV-CON* mice and 14 *CaMKIIα-Cre+AAV-shHrh2* mice. *$P \leqslant 0.05$, **$P \leqslant 0.01$, ***$P \leqslant 0.001$. ns, not significant. See also Table. S2 for further statistical information.

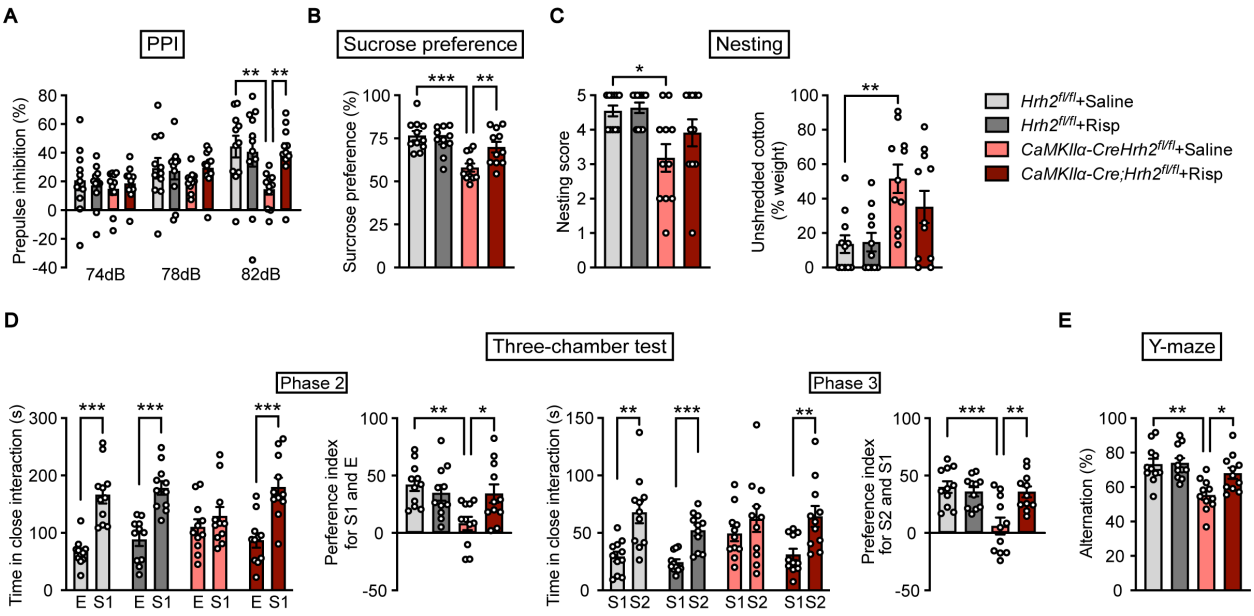

Fig. S4. Antipsychotic agent risperidone rescues sensorimotor gating ability deficit, anhedonia-like behavior and social indifference manifested in *CaMKIIα-Cre; Hrh2*$^{fl/fl}$ mice. (A-E) Sensorimotor gating, hedonic function, social behavior and cognitive behavior in *Hrh2*$^{fl/fl}$ and *CaMKIIα-Cre; Hrh2*$^{fl/fl}$ mice treated with saline or risperidone. **(A)** Percentage of PPI of the auditory startle reflex under 74, 78, 82 dB prepulse intensities. **(B)** Test for sucrose preference within a 48 h period. **(C)** Nesting score and percentage of unshredded cotton in nest-building test. **(D)** Time in close interaction and preference index during phase 2 and phase 3 in three-chamber test. **(E)** Percentage of correct choice in Y-maze test. n=11 mice per group. *$P \leqslant 0.05$, **$P \leqslant 0.01$, ***$P \leqslant 0.001$. See also Table. S2 for further statistical information.

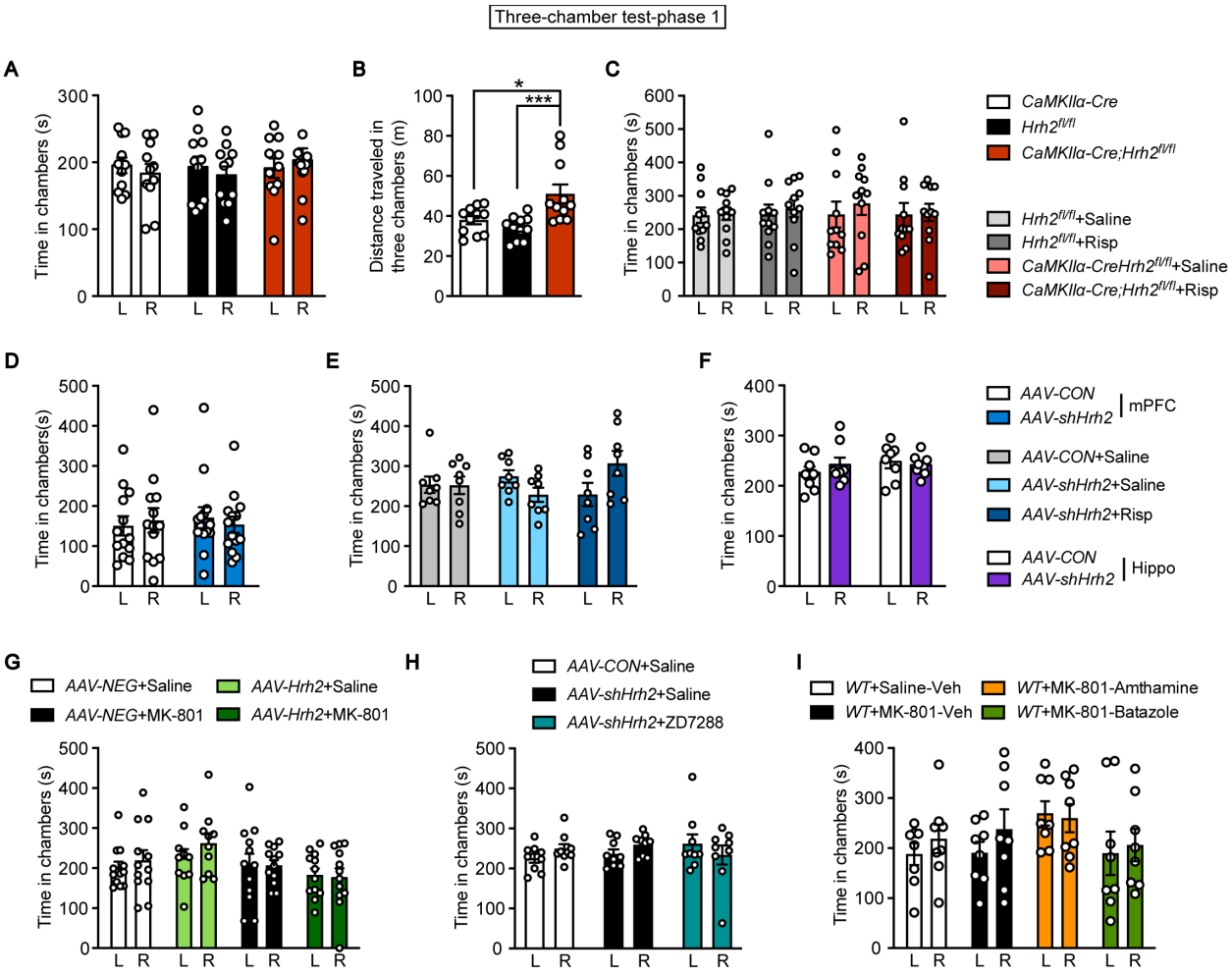

Fig. S5. Behavioral data for phase 1 in the three-chamber test. (A) Time in the left or right chamber and **(B)** total distance traveled during phase 1 for *Hrh2*$^{fl/fl}$, *CaMKII*α*-Cre*, and *CaMKII*α*-Cre; Hrh2*$^{fl/fl}$ mice. **(C-I)** Time in the left or right chamber during phase 1. **(C)** *Hrh2*$^{fl/fl}$ and *CaMKII*α*-Cre; Hrh2*$^{fl/fl}$ mice treated with risperidone, **(D)** *CaMKII*α*-Cre* mice injected in the mPFC with *AAV-FLEX-scramble shRNA-GFP* (*AAV-CON*) or *AAV-FLEX-shHrh2-GFP* (*AAV-shHrh2*) and **(E)** treated with risperidone, **(F)** *CaMKII*α*-Cre* mice injected in the hippocampus with *AAV-CON* or *AAV-shHrh2*, **(G)** *CaMKII*α*-Cre* mice injected in the mPFC with *AAV-NEG* or *AAV-Hrh2*, **(H)** *CaMKII*α*-Cre* mice injected in the mPFC with *AAV-CON* or *AAV-shHrh2* treated with ZD7288 and **(I)** wild-type (WT) mice treated with saline, amthamine, or batazole after chronic exposure to MK-801. See also Table. S2 for further statistical information.

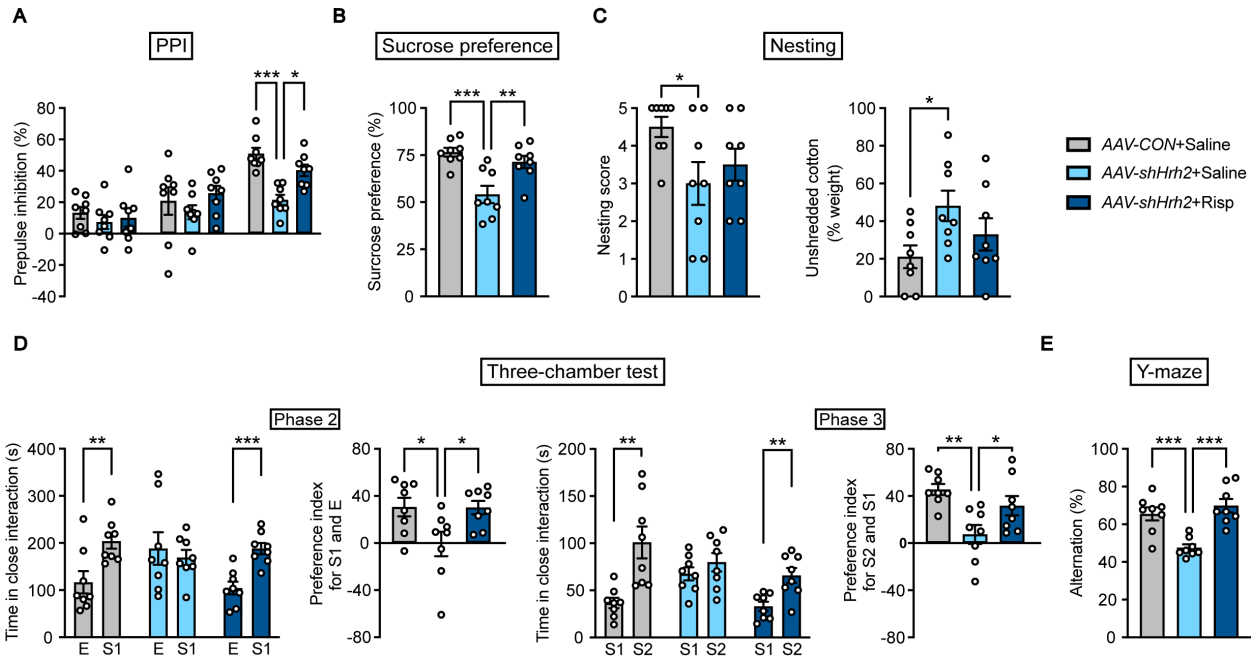

Fig. S6. Antipsychotic agent risperidone rescues sensorimotor gating ability deficit, anhedonia-like behavior and social indifference manifested in *CaMKIIα-Cre* mice injected in the mPFC with *AAV-FLEX-shHrh2-GFP* (*AAV-shHrh2*). (A-E) Sensorimotor gating, hedonic function, social behavior and cognitive behavior in *CaMKIIα-Cre* mice injected in the mPFC with *AAV-FLEX-scramble shRNA-GFP* (*AAV-CON*) and *AAV-FLEX-shHrh2-GFP* (*AAV-shHrh2*) treated with risperidone. **(A)** Percentage of PPI of the auditory startle reflex under 74, 78, 82 dB prepulse intensities. **(B)** Test for sucrose preference within a 48 h period. **(C)** Nesting score and percentage of unshredded cotton in nest-building test. **(D)** Time in close interaction and preference index during phase 2 and phase 3 in three-chamber test. **(E)** Percentage of correct choice in Y-maze test. n=8 mice per group. *$P \leqslant 0.05$, **$P \leqslant 0.01$, ***$P \leqslant 0.001$. See also Table. S2 for further statistical information.

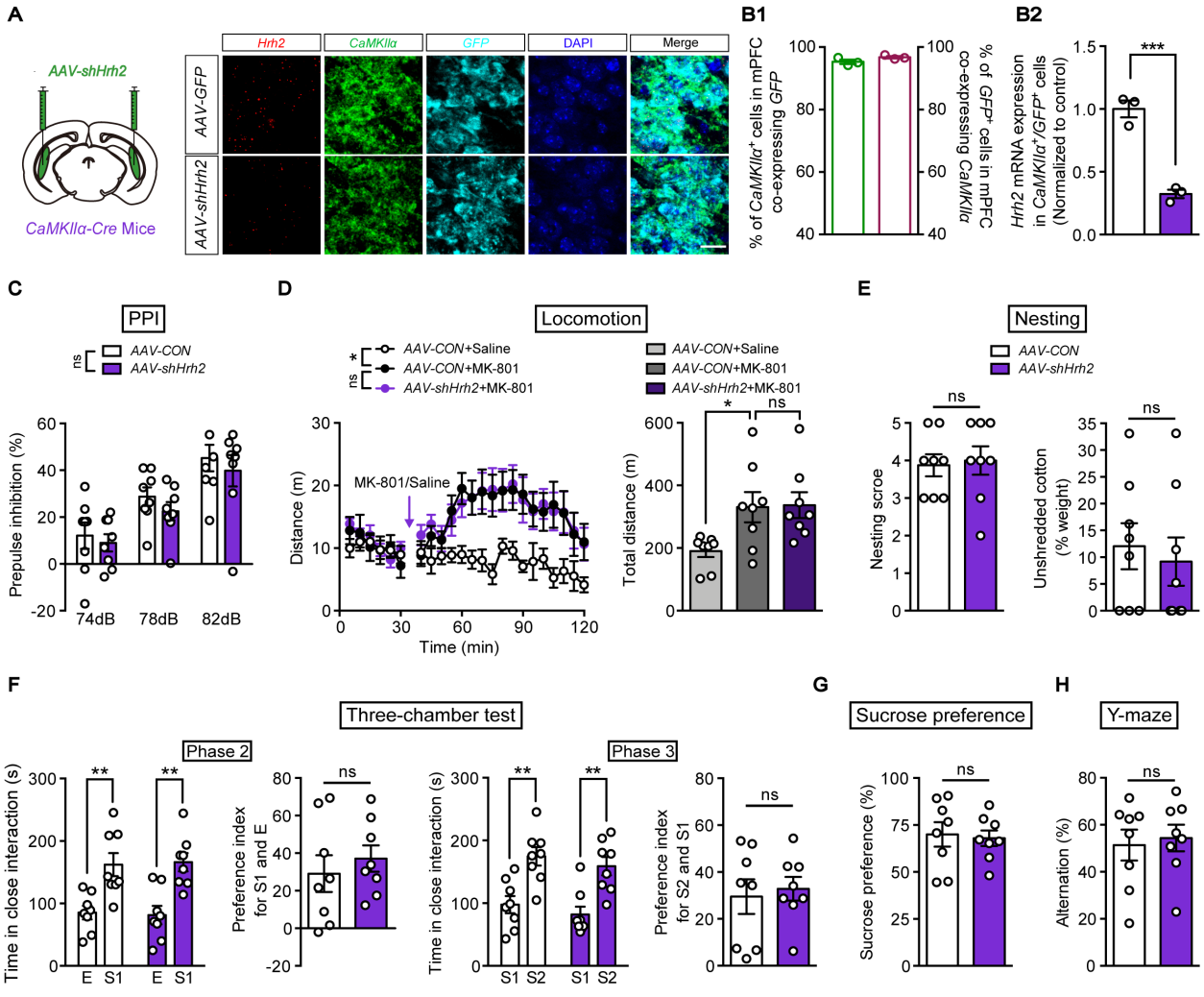

Fig. S7. Knockdown of histamine H_2R in hippocampus glutamatergic neurons does not induce schizophrenia-like behavior. (A) Schematic diagram of the location of the injected virus and representative images of RNAscope *in situ* hybridization of *Hrh2*, *CaMKIIα*, and *GFP* mRNA. **(B1)** The percentage of *CaMKIIα*$^+$ glutamatergic neurons co-expressing *GFP* (left), and the percentage of *GFP*$^+$ cells co-expressing *CaMKIIα* (right). Scale bar, 20 μm. n=3 mice. **(B2)** *Hrh2* expression in *CaMKIIα*$^+$/*GFP*$^+$ cells of *CaMKIIα-Cre* mice injected in the hippocampus with *AAV-FLEX-scramble shRNA-GFP* (*AAV-CON*) *or AAV-FLEX-shHrh2-GFP* (*AAV-shHrh2*) to knock down H_2R. **(C-H)** Sensorimotor gating, susceptibility to MK-801-induced hyperactivity, social behavior, hedonic function, and cognitive behavior in *CaMKIIα-Cre* mice injected in the hippocampus with *AAV-FLEX-scramble shRNA-GFP* or *AAV-FLEX-shHrh2-GFP*. **(C)** Percentage of prepulse inhibition (PPI) of the auditory startle reflex across different prepulse intensities. **(D)** Locomotor activity assessed by the distance traveled in the open field every 5 min and the total distance traveled in the open field after administration of MK-801 or saline. **(E)** Nesting score and the percentage of the weight of cotton left unshredded in a nest-building test. **(F)** Time in close interaction and preference index during sociability testing (phase 2) when exposed to a stranger mouse S1 and during subsequent social novelty recognition testing (phase 3) when exposed to a new stranger mouse S2 together with S1. **(G)** Total consumption of water or sucrose and sucrose preference within a 48 h period. **(H)** Percentage of correct alternation in a Y-maze. n=8 mice for each group. *$P \leqslant 0.05$, **$P \leqslant 0.01$, ***$P \leqslant 0.001$, ns, not significant. See also the Table. S2 for further statistical information.

Table. S1. Clinico-pathological information of schizophrenia patients and control subjects.

	Number	Gender	Age	Braak stage	PMD (min)	BW (g)	CSF pH	Clinical diagnosis	Sample source
Schizo-phrenia	1	M	61	ND	165	1515	ND	Schizophrenia	NBB
	2	M	48	ND	2460	1577	ND	Schizophrenia, hypochondriac and paranoid delusions, nervous, auditory hallucinations, paranoid hallucinative psychosis, paranoid and hypochondric delusions, anxious, isolated, selfneglect	NBB
	3	F	92	3	455	877	6.30	Schizophrenia, retarded, bizarre moodswings, unpredictable behavior disturbances, paranoia, manic-depressive episodes,comprise paranoia, bagatellisation, and negativism	NBB
	4	M	67	1	345	1315	6.29	Paranoid, anxious, passive, odorous hallucinations, suicide attempt, inactivity, diagnosed with paranoid anxious psychosis	NBB
	5	F	55	1	335	1180	6.62	Paranoid, compulsive, depressed, insecure, diagnosed with catatonic schizophrenia	NBB
	6	F	58	1	150	1408	7.0	Schizophrenia, hypertension, right breast cancer	CBB
	7	M	79	4	540	1164	7.0	Schizophrenia, brain stem hemorrhage, herniation,	CBB
	8	F	57	1	540	1100	6.47	Schizophrenia, hypertension, type 2 diabetes	CBB
	9	M	27	ND	455	1410	7.33	Schizophrenia	CBB
	10	M	81	ND	603	1280	6.41	Schizophrenia	CBB
Mean			62.5		604.8	1283	6.678		
SEM			5.85		211.8	66.34	0.14		
Control	1	M	62	0	395	1350	7.33	Delirium, depressed mood, confused, visual hallucinations, delirious	NBB
	2	M	69	1	1155	1337	6.40	None	NBB
	3	F	93	2	350	1145	?	None	NBB
	4	F	60	0	330	1215	7.07	None	NBB
	5	M	72	2	260	1385	6.45	None	NBB
	6	F	58	0		1310	ND	Multiple organ failure, cachexia	CBB
	7	M	72	1	1020	1130	ND	none	CBB
	8	F	57	0	519	1230	6.05	Gastric malignancy, transient ischemic attack, meningeal malignancy (metastasis), hypertension	CBB
	9	M	31	ND	1190	1510	7.0	Muscular dystrophy	CBB
	10	M	83	ND	1480	1370	6.7	Gum cancer, prostate cancer, chronic obstructive pulmonary disease	CBB
Mean			65.7		744.3	1298	6.71		
SEM			5.29		154.5	37.3	0.17		
Signifi-cance (*P*)			0.77		0.31	0.1	0.71		

Abbreviations: BW, brain weight; BZD, benzodiazepine; CSF, cerebrospinal fluid; F, female; Hal, haloperidol; Li, lithium; M, male; Mo, morphine; NBB, Netherlands Brain Bank; CBB, Chinese Brain Bank, ND, no data; None, no medication or no psychiatric symptoms; PMD, postmortem delay.