Appendix 1 to Lei H, Lai J, Sun X, et al. Lateral orbitofrontal dysfunction in the *Sapap3* knockout mouse model of obsessive–compulsive disorder. *J Psychiatry Neurosci* 2018.

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Supplementary Methods

Cell type classification

Isolated single units were classified into putative pyramidal neurons and putative inhibitory interneurons based on the shape of their action potential waveforms. By measuring peak half-width, trough half-width and the trough/peak ratio, the single units recorded in our study formed two well-separated clusters when plotted against these three parameters (Fig 2B in the manuscript). The cluster with broader peak half-width (467-700μs) and trough half-width (100-200μs), and low peaks relative to their troughs was deemed as putative pyramidal neurons (294 out of 362). Whereas the cluster with narrower peak half-width (100-300μs) and trough half-width (67-167μs), and low peaks relative to their troughs was deemed as putative inhibitory interneurons (51 out of 362). Single units not in these two clusters were deemed as other types (17 out of 362) and were excluded from further data analysis.

This cell type classification criterion has been validated by many studies combining intra- and extracellular recordings, or using antidromic stimulation or other methods (1). Slice recording and *in vivo* intracellular recording in anesthetized animals in various species, where different types of neurons can be distinguished on the basis of morphology and protein expression, all reviewed that the shape of action potential waveforms were very different between excitatory pyramidal neurons and GABAergic interneurons. The excitatory pyramidal neurons have longer-duration action potentials, whereas GABAergic interneurons have shorter-duration action potentials (2-4). This difference is partially due to the expression of different classes of K^+ and Na^+ channels that differ from one another in their kinetics (5-7). The time course of extracellularly recorded spike waveforms resembles the first derivative of intracellularly recorded action potentials. The distinct shape of action potential waveforms makes it possible to distinguish inhibitory interneurons from pyramidal neurons in extracellular recordings in various brain regions including neocortex (8). In extracellular recording, pyramidal neurons are characterized by broader action potentials and low peaks relative to their troughs (indicating slow membrane repolarization), whereas inhibitory interneurons are characterized by narrower action potentials and high peaks relative to their troughs (indicating rapid membrane repolarization).

Our cell type classification was further supported by the different firing rate and different population size of the two cell types. The mean spontaneous firing rate of putative pyramidal neurons in our study was 2.75 ± 0.30 Hz in WT mice, whereas of putative inhibitory interneurons was 18.07 ± 1.50 Hz. These values are well in the

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ranges of the spontaneous firing rate of pyramidal neurons and inhibitory interneurons in the prefrontal cortex as reported by previous studies, and are consistent with previous findings that the spontaneous firing rate of pyramidal neurons are much lower than that of the inhibitory interneurons. In our study, the putative pyramidal neurons constituted 81.2% (294/362) and the putative inhibitory interneurons constituted 14.1% (51/362) of the total single unit population we recorded. This population proportion is also consistent with studies using morphology and protein expression patterns to classify cell types. They found that pyramidal neurons made up 70-80% of cortical neurons, and interneurons made up 10-15% of cortical neurons. However, extracellular recording has limitations on unambiguously distinguishing cell types. Cortical neurons can be divided into many subcategories on the basis of the protein expression patterns, neurotransmitters, neuropeptides, ionic channels and morphology. These factors are all invisible to extracellular electrode. Given the diversity of cell types that have been identified in the cortex, it is likely that the two classes of neurons in our study are composed of multiple subclasses of neurons.

Data analysis

Since both male and female mice were used in this study, two-way ANOVA was used to examine whether there is a gender effect in LFP oscillation power, mean firing rate of putative pyramidal neurons, mean firing rate of putative inhibitory interneurons, the number of bursts per minute per neuron of pyramidal cells, the percentage of spikes per neuron in the bursting mode of pyramidal cells, intra-burst ISI of pyramidal cells. Wilcoxon rank sum test was also used to examine whether data from male and female mice with the same genetic background are different.

Supplementary Results

Among the 21 WT mice, 19 were male, 2 were female. Among the 24 *Sapap3* KO mice, 20 were male, 4 were female. Two-way ANOVA analysis showed significant effect of genotype, but no gender effect.

Supplementary Figure 1 shows the averaged LFP power spectrogram of male and female mice in WT and KO group respectively. There is no significant difference of LFP oscillation power of all frequency bands between male and female mice within either WT or KO group (Supplementary Table 1). Two-way ANOVA analysis showed significant effect of genotype on LFP oscillation power of all frequency bands, but no gender effect (Supplementary Table 2).

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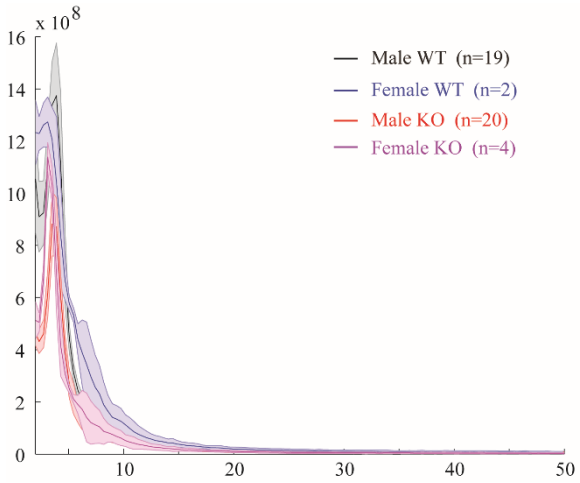

Supplementary Figure1. Averaged LFP power spectrogram of male and female mice in WT and KO group respectively. Shading, SEM.

	WT			KO		
	male	female	*p* value	male	female	*p* value
delta	1.01 ± 0.11	0.95 ± 0.04	0.84	0.57 ± 0.05	0.56 ± 0.18	0.92
theta	0.98 ± 0.10	1.18 ± 0.38	0.50	0.60 ± 0.08	0.53 ± 0.12	0.68
beta	0.99 ± 0.13	1.08 ± 0.41	0.81	0.54 ± 0.07	0.53 ± 0.14	0.93
gamma	0.97 ± 0.22	1.28 ± 0.79	0.63	0.54 ± 0.08	0.63 ± 0.16	0.63

Supplementary Table 1. Normalized LFP oscillation power of different frequency bands of male and female mice in WT and KO group (normalized to the mean of the WT group). Wilcoxon rank sum test between male and female mice within WT or KO group.

	F (genotype)	*p* (genotype)	F (gender)	*p* (gender)
delta	19.36	**0.00008**	0.05	0.83
theta	13.21	**0.0008**	0.02	0.90
beta	14.69	**0.0005**	0.02	0.90
gamma	5.83	**0.02**	0.40	0.54

Supplementary Table 2. Two-way ANOVA analysis of the LFP oscillation power of different frequency bands of male and female mice in WT and KO group.

The number of putative pyramidal neurons and inhibitory interneurons recorded from

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male and female mice in WT and KO group is summarized in Supplementary Table 3. There is no significant difference of the mean firing rate of putative inhibitory interneurons, the number of bursts per minute per neuron of pyramidal cells, the percentage of spikes per neuron in the bursting mode of pyramidal cells, intra-burst ISI of pyramidal cells between male and female mice within either WT or KO group (Supplementary Table 4 and 5). Only the mean firing rate of pyramidal neurons in the WT group showed significant difference between male and female mice (Supplementary Table 4). Two-way ANOVA analysis of the mean firing rate of putative pyramidal neurons showed no significant effect of genotype or gender. Two-way ANOVA analysis of the mean firing rate of putative inhibitory interneurons, the number of bursts per minute per neuron of pyramidal cells, the percentage of spikes per neuron in the bursting mode of pyramidal cells, and the intra-burst ISI of pyramidal cells all showed significant effect of genotype, but no effect of gender (Supplementary Table 6).

WT				KO			
	total Single unit	pyramidal neuron	inter-neuron		total Single unit	pyramidal neuron	inter-neuron
male & female	147	112	21	male & female	215	182	30
male	135	106	20	male	164	142	20
female	12	6	1	female	51	40	10

Supplementary Table 3. The number of putative pyramidal neurons and inhibitory interneurons recorded from male and female mice in WT and KO group.

WT	male				female				
	n	median	mean	SEM	n	median	mean	SEM	*p* value
Firing rate of putative pyramidal neurons (Hz)	106	1.57	2.41	0.24	6	3.54	5.62	1.97	**0.01**
Firing rate of putative inhibitory interneurons (Hz)	20	17.73	18.54	1.50	1	8.75	--	--	--
Number of bursts	106	5.77	11.77	1.77	6	6.05	24.43	17.79	0.38

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per minute									
Percentage of spikes per neuron in bursts (%)	106	16.65	23.48	2.24	6	9.00	11.85	4.77	0.41
Intra-burst ISI (ms)	106	6.75	6.61	0.14	6	6.25	6.41	0.23	0.64

Supplementary Table 4. Comparison of the firing pattern of putative pyramidal neurons and interneurons between male and female WT mice. Wilcoxon rank sum test.

KO	male				female				
	n	median	mean	SEM	n	median	mean	SEM	*p* value
Firing rate of putative pyramidal neurons (Hz)	142	1.59	2.85	0.26	40	1.27	2.19	0.40	0.46
Firing rate of putative inhibitory interneurons (Hz)	20	23.30	21.98	1.67	10	23.61	23.74	2.31	0.71
Number of bursts per minute	142	11.34	19.62	2.40	40	8.86	13.38	3.25	0.26
Percentage of spikes per neuron in bursts (%)	142	29.84	33.09	2.10	40	23.73	29.60	3.38	0.65
Intra-burst ISI (ms)	142	6.29	6.29	0.10	40	5.93	6.03	0.33	0.40

Supplementary Table 5. Comparison of the firing pattern of putative pyramidal neurons and interneurons between male and female KO mice. Wilcoxon rank sum test.

	F (genotype)	*p* (genotype)	F (gender)	p (gender)
Firing rate of putative pyramidal neurons	0.15	0.70	0.02	0.89
Firing rate of	4.31	**0.04**	0.01	0.9

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putative inhibitory interneurons				
Number of bursts per minute	4.44	**0.03**	0.68	0.41
Percentage of spikes per neuron in bursts	12.53	**0.0005**	1.50	0.22
Intra-burst ISI	4.23	**0.04**	1.50	0.22

Supplementary Table 6. Two-way ANOVA analysis of the firing pattern of putative pyramidal neurons and interneurons of male and female mice in WT and KO group.

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