An autism-associated mutation in salt inducible kinase 1 causes repetitive behavior and social deficits due to enhanced excitatory neuronal functions in mice.

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Research

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Abstract

Background:

Six mutations in the salt inducible kinase 1 (SIK1) coding gene have been identified in the early infantile epileptic encephalopathy (EIEE-30) patients accompanied by autistic symptoms, such as repetitive behavior and social behavioral deficits. Among these mutations, two are nonsense mutations that truncate the C-terminal region. It has been shown that the C-terminal truncated form of SIK1 protein affects the subcellular distribution of SIK1 protein, tempting to speculate the relevance to the pathophysiology of the disorders.

Methods:

We generated SIK1 mutant (SIK1-MT) mice recapitulating the C-terminal truncated mutations using CRISPR/Cas9-mediated genome editing. We performed cellular assays to examine the subcellular localization of SIK1-MT. We also performed patch clamp electrophysiological recording and behavioral tests to evaluate the neuronal functions and behaviors in SIK1-MT mice. Pharmacological experiments using risperidone were also performed to examine the potential therapeutics of the disorder.

Results:

SIK1-MT protein was distributed in the nucleus and cytoplasm, whereas the distribution of wild-type SIK1 was restricted to the nucleus. We found the disruption of excitatory and inhibitory (E/I) synaptic balance due to an increase in excitatory synaptic transmission and enhancement of neural excitability in the pyramidal neurons in layer 5 of the medial prefrontal cortex in SIK1-MT mice. We also found the increased repetitive behavior and social behavioral deficits in SIK1-MT mice. The risperidone administration attenuated the neural excitability and excitatory synaptic transmission, but the disrupted E/I synaptic balance was unchanged because it also reduced the inhibitory synaptic transmission. Risperidone also eliminated the repetitive behavior, but not social behavioral deficits.

Limitations:

We failed to identify drugs that can cure the social behavioral deficits in this mouse model.

Conclusions:

In the present study, we generated model mice for EIEE-30 recapitulating C-terminal truncated SIK1 mutation discovered in human patients. We found that the C-terminal deletion of SIK1 affects the subcellular distribution of SIK1, resulting in the elevated excitability of neuronal networks and autistic behaviors in the mutant mice. Repetitive behavior, but not social deficits, was restored by risperidone, probably due to the decrease of both excitatory and inhibitory synaptic functions by the drug.

Background

Salt-inducible kinases (SIKs) are a family of AMP-activated protein kinase (AMPK) consisting of SIK1, SIK2, and SIK3 [1-3]. SIK proteins distribute all over the body and the expression of SIK1 is shown to be induced by intrinsic simulations including high dietary salt intake, adrenocorticotrophic hormone, neurotrophic factors, and neuronal depolarization [4-7]. SIK1 protein has an N-terminal serine/threonine kinase domain, ubiquitin associated area (UBA), proline-glutamate-serine-threonine (PEST) domain, and C-terminal nuclear localization regulatory domain (NLD). The kinase activity of SIK1 is conferred by phosphorylation of threonine at position 182 within the serine/threonine kinase domain by liver kinase B1 (LKB1) [8, 9]. It has been shown that the translocation of SIK1 protein differentiates the activation of downstream signaling pathways in some organs, such as liver and adrenal gland [10, 11]. Substrates of SIK1, such as cAMP-regulated transcriptional coactivators (CRTC) and the class IIa histone deacetylases (HDAC) are distributed both inside and outside of the nucleus of the cell. Thus, the cellular localization of SIK1 protein is an important determinant of SIK1-dependent signaling pathways. The NLD of SIK1 regulates the cellular localization of SIK1 protein without altering the intrinsic kinase activity.

Six mutations in the SIK1 gene were identified in the patients with early infantile epileptic encephalopathy (EIEE-30) [12]. Two patients died before one year old. The remaining four showed autistic symptoms including absent language, impaired socialization, and repetitive behavior. Out of these patients with autism, two had missense mutations and the other two had nonsense mutations within NLD coding exon resulting in the C-terminal truncation of the SIK1 protein [12]. This report demonstrated that the C-terminal truncated mutations seem to affect the localization of SIK1 protein. Considering the symptoms of human cases, the mutations alter the neuronal functions related to the symptoms of autism spectrum disorder (ASD).

In this study, we generated SIK1 mutant (SIK1-MT) mice lacking NLD using CRISPR/Cas9-mediated genome editing, as disease models of the human cases. We studied these mice by focusing on the behaviors and the synaptic function of pyramidal neurons in the deep layer of the medial prefrontal cortex (mPFC), where the relevance to autistic symptoms is suggested [13-15]. We identified that the SIK1-MT mice showed an imbalance between excitatory and inhibitory synaptic function by increased excitatory synaptic transmission level. We also found that the excitability was increased in the pyramidal neurons of the mPFC. The SIK1-MT mice showed an increase in the repetitive behavior and deficits in social novelty preference that are compatible with the core symptoms of ASD. We also investigated the effect of risperidone as candidate therapeutics of this disorder [16, 17] and found that risperidone rescued those synaptic abnormalities and repetitive behavior, whereas it did not change the social behavior in the SIK1-MT mice.

Methods

Generation of SIK1-MT mice and animal usage

We produced single guide RNA (sgRNA) targeting the SIK1 gene and mRNA of Cas9 using the MEGAscript T3 Transcription Kit (Thermo Scientific) by following the manufacturer's protocol. For the sgRNA vector

construction, annealing oligo DNAs (Oligo#1, Table S1) were cloned into pCG-SapI [18]. sgRNA was prepared by in vitro transcription using PCR amplified fragment prepared by PCR with primers (Oligo#2) using pCG-SapI-gRNA as a template. Cas9 mRNA was prepared by in vitro transcription using pFNLCas9A95.

Electroporation of mRNAs to the fertilized eggs from C57BL/6JmsSlc mice (Japan SLC inc) was performed by following the TAKE method [19]. The poring pulse was set to voltage: 40 V, pulse width: 3 ms, pulse interval: 50 ms, and the number of pulses: 4. The transfer pulse was set to voltage: 5 V, pulse width: 50 ms, pulse interval: 50 ms, and the number of pulses: ± 5 . After the electroporation, embryos were transferred to the oviducts of pseudo pregnant mothers and kept until natural delivery. Fourteen pups were obtained and the indel mutations were screened by genomic PCR and Sanger sequencing with a primer set (Oligo#3). A male mouse having 8 bp deletion that caused a frameshift in the C-terminal region of the SIK1 protein was isolated and backcrossed with C57Bl/6J mice for more than six generations. The genotyping of the mice was performed by genomic PCR using primer pairs (Oligo#3), followed by the digestion of unmatched PCR products by T7 endonuclease I (New England Biolabs).

Risperidone treatments

To investigate the pharmacological effects of risperidone on the physiological characteristics of neurons and behavior of the SIK1-MT mice, we injected risperidone (0.05mg/kg BW in saline, i.p.) to mice one hour before the experiments [20]. Injected mice were returned to their home cage until further experimental procedures. Saline-injected (n=9) and risperidone-injected (n=9) two-week-old male were used for electrophysiological experiments. Saline-injected (n=10) and risperidone-injected (n=9) two-month-old male were used for behavioral tests.

Construction of plasmids

Wild-type mouse brain was lysed with TRIzol RNA Isolation Reagents (Thermo Fisher) and RNA was purified by the standard phenol/chloroform extraction or with an RNA purification column, RNA Nucleospin RNA plus (Thermo Fisher). cDNA of SIK1 was obtained by standard RT-PCR using a random hexamer mixture and a primer set for SIK1 (Oligo#4, Table S1). PCR amplicon was subcloned into pCR-Blunt TOPO vector (pCR-SIK1) and confirmed the sequence of SIK1 cDNA. pCR-SIK1 was subjected to the mutagenesis and introduced the 8-bp deletion using a primer pair (Oligo #5) to construct pCR-SIK1mt. cDNAs of SIK1 and SIK1-MT were cloned into pCAGGS vector (pCAGGS-SIK1 and pCAGGS-SIK1mt, respectively). For the overexpression of SIK1 variants, we made cDNAs of SIK1 fused with Venus YFP [21] at N-terminal of SIK1. Venus YFP was amplified by PCR using a set of primers (Oligo #6) and inserted Venus cDNA amplicon into pCR-SIK1 using In-Fusion HD Cloning Kit (Takara).

Cell Culture

HEK-293T cells (8×10^4) were plated on 24-well dishes a day before the transfection. They were transfected with 2 μ g of pCAGGS-Venus-SIK1-WT, SIK1-MT, or -Q614X using polyethylenimine. Forty-eight

hours after transfection, they were incubated with Forskolin (10 mg/mL) and IBMX (3-isobutyl-1-methylxanthine, 18 mg/mL) or with control vehicle (dimethyl sulfoxide). After three hours the cells were fixed with 4% paraformaldehyde and 4% sucrose in PBS followed by three times washing with PBS. Cells were counterstained with DAPI (4',6-diamidino-2-phenylindole) and washed three times with PBS. The cells were mounted on a slide glass with slow fade gold antifade reagent (Thermo Fisher). Fluorescence images were taken by a confocal laser-scanning microscope (TCS SP8; Leica Microsystems).

Quantification of SIK1-MT mRNA levels

To quantify the expression levels of mRNA of the SIK1-MT, we utilized a sequencing-based quantification method [22]. First, we amplified PCR products using cDNA from wild-type or SIK1-MT mice using a primer set (Oligo #7). PCR products were also prepared using pCAGGS-SIK1 plasmid or a mixture of pCAGGS-SIK1 and pCAGGS-SIK1mt at 1:1 or 1:0.5 ratio as templates. PCR amplicons from plasmids were used as standards for quantitative estimation. The PCR amplicons were subjected to Sanger sequencing and the fluorescent data from the sequences around the sgRNA target site were analyzed by the EditR program [23]. The ratio between wild-type and mutant sequences was calculated in each nucleotide and converted into a vector. The expression level (EL) was calculated by the following formula.

$$EL = \|\vec{s} - \vec{s}_{wt}\| / \|\vec{s}_{wt} - \vec{s}_{mt}\|$$

Here, $\vec{s}$, $\vec{s}_{wt}$, and $\vec{s}_{mt}$ represent vectors obtained by the calculation using data from samples from brain cDNA, pCAGGS-SIK1, and a mixture pCAGGS-SIK1 and pCAGGS-SIK1mt at 1:1 ratio (100% MT). We also calculated the expression level using the PCR product from another mixture of pCAGGS-SIK1 and pCAGGS-SIK1mt at 1:0.5 ratio (50% MT). Most of the EL distributed zero to one and a value close to one indicate that the expression level of the SIK1mt is similar to that of the wild-type SIK1.

Histology

Histological analysis was followed by previous procedures [24, 25]. Briefly, under deep anesthesia, two months old mice were perfused transcardially with ice-cold phosphate-buffered saline (PBS, pH 7.4), followed by 4% paraformaldehyde in PBS. Fifty- μ m-thick coronal sections were prepared with a sliding microtome (REM-700, Yamato Kohki Industrial). The sections were washed with PBS, blocked with PBS containing 1% bovine serum albumin, 0.1% Triton-X100 and 10% of normal donkey serum and incubated with mouse anti-parvalbumin (1:2000, Sigma), rat anti-somatostatin (1:200, Merck Millipore), or rabbit anti-Satb2 antibody (1:200, Abcam). After overnight incubation with primary antibodies, the brain sections were washed with PBS containing 0.1% Triton-X100 and incubated with Alexa 488-conjugated donkey antibody against rabbit IgG or Cy2-conjugated donkey antibody against mouse or rat IgG (Jackson immunoresearch), respectively, for 2-3 hours at room temperature. After further washing with PBS, brain sections were mounted on a slide glass, counterstained with DAPI, and coverslipped.

Fluorescence images were taken with an all-in-one fluorescent microscope (BZ-X710, Keyence) and a confocal laser-scanning microscope (TCS SP8; Leica Microsystems).

Electrophysiology

Patch clamp recordings in acute brain slices were done as described previously [24]. Briefly, postnatal 14-19 day-old mouse brains were removed and placed immediately in ice-cold slicing artificial corticospinal fluid (ACSF, in mM: 85 NaCl, 75 sucrose, 2.5 KCl, 1.25 NaH₂PO₄, 24 NaHCO₃, 25 glucose, 0.5 CaCl₂, and 4 MgCl₂) saturated with 95% O₂/5% CO₂ for 2 min. The chilled brains were trimmed coronally with razor blades and placed in a vibratome chamber (Campden 7000smz). Three hundred fifty- μ m-thick coronal sections were transferred to a recovery chamber filled with recording ACSF (in mM: 126 NaCl, 2.5 KCl, 1.25 NaH₂PO₄, 26 NaHCO₃, 10 glucose, 2 CaCl₂, and 2 MgCl₂), followed by incubating at 32°C for 30 min, and then at room temperature for 30 min. In current-clamp experiments, pyramidal neurons were patched with glass pipettes (4-8 M Ω) filled with a potassium-based intra-cellular solution (ICS, in mM: 130 K Gluconate, 6 KCl, 10 HEPES, 1 EGTA, 2.5 MgCl₂, 2 magnesium ATP, 0.5 sodium GTP, 10 phosphocreatine sodium, 290 mOsm) under microscopy (BX50WI, Olympus). Resting membrane potential was measured immediately after establishing whole-cell recording. Hyperpolarizing and depolarizing step pulses (700 ms) were applied to characterize neuronal firing property. Membrane potential at which the temporal rate of the potential reached to 10 mV/ms was defined as the action potential threshold. Postsynaptic responses were measured in voltage-clamp mode using cesium based ICS (in mM: 130 CsOH, 130 Gluconic acid, 6 CsCl, 10 HEPES, 1 EGTA, 2.5 MgCl₂, 2 magnesium ATP, 0.5 sodium GTP, 10 phosphocreatine sodium, 290 mOsm). Miniature postsynaptic currents were recorded in the presence of 1 mM tetrodotoxin (Abcam). To record mEPSCs (Glutamatergic), cell membrane potential was held at -60 mV for three-minutes' recording and then cell membrane potential was shifted to 0 mV to record mIPSCs (GABAergic) from the same cell, for three minutes as well. E/I balance index was calculated by averaging $(\text{Freq}^{\text{mEPSC}})/(\text{Freq}^{\text{mEPSC}} + \text{Freq}^{\text{mIPSC}})$, where $\text{Freq}^{\text{mEPSC}}$ and $\text{Freq}^{\text{mIPSC}}$ represent the frequency of mEPSC and mIPSC, respectively. Evoked postsynaptic currents were triggered with 0.1 msec current injections by a nichrome-wire electrode placed at position 100-150 μ m from the soma of neurons recorded. For recording evoked AMPA and NMDA-EPSC, 100 μ M picrotoxin was added in bath solution with the holding potentials at -70 mV and 40 mV. We used 5 mM of QX-314 in the pipette solution to block sodium channel-mediated currents. To calculate the NMDA to AMPA ratio, the amplitude of NMDA current at 50 ms after the onset was divided by peak amplitude of AMPA current. Paired-Pulse ratio (PPR) was done holding the cell at -70 mV for excitatory PPR (ePPR) in presence of 100 μ M picrotoxin. For inhibitory PPR (iPPR) the cells were held at 0mV in presence of 20 μ M DNQX. All PPR experiments were done with (30, 50, 100, 200 ms) stimulation intervals. Access resistance was monitored throughout the recording, and cells with access resistance over 25 M Ω were rejected. All data were acquired at 10kHz with an EPC10 double amplifier (HEKA) operated by Patch Master software (HEKA). Data analysis was performed with the Mini Analysis Program (Synaptosoft) and custom made programs of Igor Pro (WaveMetrics).

Behavioral studies

Two to three-month-old male heterozygote SIK1-MT (n=8) and the littermate wild-type mice (n=9) were used in all behavioral experiments except for . All experiments and analyses were performed blindly to the genotype and pharmacological treatment.

Open Field Tests

Each mouse was placed in the corner of the open field apparatus (50 × 50 × 40 cm). The apparatus was surrounded by a sound-attenuating white chest and illuminated at approximately 100 lux. Subject behaviors were recorded from the above of the apparatus using a CCD camera (WAT-902B; Watec, Yamagata, Japan). Analog images were converted to digital images (720 × 480 pixels) using Monster HD264 (SKNET, Yokohama, Japan). The video frame rate was 30 frames per second (fps). The test lasted 30 min. We measured the travel distance, time spent in the center area (25 × 25 cm), vertical activity (rearing and leaning), and grooming. Part of the behavioral parameters, such as the travel distance and time spent in the center area, were analyzed using idTracker [26] and custom made programs run on MATLAB. Other parameters were analyzed by a trained observer.

Elevated plus-maze test

The elevated plus maze consisted of two open arms (25 × 5 cm) and two closed arms of the same size with 15-cm high walls made of transparent plastic. The maze was arranged in a manner such that arms of the same type were opposite each other, connected by a central area (5 × 5 cm), and the entire maze was elevated to a height of 50 cm above the floor. To keep the mice from falling over, the open arms were surrounded by a 3-mm high edge. The animals were placed individually in the center of the maze, facing a closed arm. Mouse behaviors were recorded during a 5-min test period using a web camera (HD Webcam C615; Logitech, Tokyo, Japan). The video images (640 × 480 pixels) were recorded at 30 fps and analyzed using idTracker. The number of entries into the open arms was analyzed.

Marble burying test

Each mouse was placed into an arena (25 × 25 × 31 cm) filled with 5-cm-deep wood chip bedding (CLEA Japan, Tokyo, Japan) and habituated to the test arena and bedding for 10 min. After the habituation period, mice were returned to the transfer cage, and 16 small blue glass marbles (12.5 mm diameter) were placed evenly spaced in five rows of four. The subject mouse was again placed into the arena containing the sixteen marbles. After 10 min, the subject was removed from the arena, and the number of buried marbles (defined as at least two-thirds covered) was counted.

Sociability and Social Novelty Tests

The apparatus was a rectangular, three-chambered box. The chamber was 20 × 40 × 25 cm and the dividing walls were made from transparent Plexiglas, with small openings (5 × 3 cm) allowing access into each chamber. The mouse was placed in the central chamber and allowed to explore the whole chamber for 10 min (the doorways into the two side chambers were opened). The two side chambers contained an inverted empty small black wire cup. A clear glass cylinder was placed on top of the inverted cup to

prevent lifting or climbing on top. Following the habituation period, mice were placed back into the central chamber, and the doorways into the two side chambers were closed. In the sociability test, an unfamiliar male mouse (stranger 1, S1) that had no prior contact to the subject mouse was placed in one of the two cups, and then the doorways were unblocked. The location of S1 in the left or right side chambers was systematically alternated between trials. The subject behaviors were recorded for 10 min using a CCD camera. After the sociability test, the subject mouse was again confined in the central chamber. In the social novelty preference test, a second unfamiliar male mouse (stranger 2, S2) was enclosed in the cup that had been empty (E) during the sociability test, and the doorways were again unblocked. The stranger mice were at least two weeks younger than the subject mice and had previously been habituated to placement in the small wire cup. In both tests, the amount of time that the subject head was within a 2-cm distance of the wire cup was measured as “time spent around the cup”.

Recording ultrasonic vocalization

Ultrasonic vocalization (USV) was recorded using a USB-microphone (Ultramic 250, Dodotronic) with a recording software (SEA, Sound emission analyzer, The Centro Interdisciplinare di Bioacustica e Ricerche Ambientali). P5-P14 mouse was separated from the mother and placed in a recording styrofoam box containing beddings. The number of USV in 10 minutes was counted by an observer, who has no information on the genotype of the subjects.

Sample size and Statistical analysis

Samples sizes were determined based on established practice and our previous experience in respective assays. The number of independent samples (e.g., neurons) is indicated on the graphs and the numbers of animals indicated in the figure legends. All values represent the average of independent experiments $\pm$ SEM. The variance among the analyzed samples was similar. Statistical significance was determined by Student's t-test (for two groups) or one-way ANOVA followed by Bonferroni's post-hoc test (for multiple groups). Statistical analysis was performed by custom-written R scripts, MATLAB (Mathworks), or Prism 6.0 (Graphpad Software Inc.). Statistical significance is indicated by asterisks (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). All data are expressed as means $\pm$ SEM. All the values were described in Supplementary information (Table S2).

Results

Generation of SIK1-MT mice using CRISPR/Cas9-mediated genome editing.

Truncations of the SIK1 gene in the two patients with EIEE-30 occur within an NLD in C-terminal region [12]. To generate mutant mice recapitulating the human cases as disease models, we employed CRISPR/Cas9-mediated genome editing technology. We designed an sgRNA targeting a location near one of the mutation sites found in the patients. We electroporated the sgRNA and Cas9 mRNA in fertilized eggs at 1-cell stage and transferred to the oviducts of pseudo pregnant mothers. We obtained fourteen offsprings; three had in/del mutations and eleven had no mutation. One of the mutant mice had a

deletion of eight nucleotides resulting in the production of the C-terminal truncated form of SIK1 protein, resembling that in human patients (SIK1-MT, Figure 1a). SIK1 mutations found in patients are hemizygotic. We crossed these mutant mice with C57Bl/6J mice for more than six generations and used heterozygote mutant mice for all the experiments as disease models.

The previous report by Hansen et al. demonstrated that wild type and truncated mutant SIK1 proteins were differently distributed in the cells [12, 27]. To examine the subcellular distribution of the SIK1-MT, we transfected HEK293T cells with plasmids expressing wild-type (SIK1-WT) or mutant SIK1 proteins fused with yellow fluorescent protein (Venus). As expected, SIK1-WT was restricted to the nucleus of HEK293T cells in a punctate fashion (Figure 1b, Figure S1). In contrast, SIK1-MT and SIK1-Q614X, a nonsense mutation found in the human patient, were diffusely distributed in the nucleus and cytoplasm (Figure 1b). It is known that SIK1 protein is translocated from the nucleus to the cytoplasm when phosphorylated at S577 by PKA [28]. To examine whether the cellular distribution of S577 phosphorylated SIK1 mutants was altered, we incubated these transfected HEK293T cells with an adenylate cyclase activator, forskolin and a phosphodiesterase inhibitor, IBMX to activate PKA. SIK1-WT was translocated from the nucleus to the cytosol by PKA activation (Figure S1) mimicking the localization of SIK1-MT and SIK1-Q614X. The activation of PKA did not alter the localization of SIK1-MT and SIK1-Q614X. These results suggest that SIK1-MT replicates the characteristics of the C-terminal truncated mutants found in EIEE30 patients and S577 phosphorylated form of SIK1-WT.

In the heterozygote SIK1-MT mice, the expression level of SIK1-MT mRNA was comparable to that of SIK1-WT mRNA (Figure S2). SIK1-MT mice grow normally without showing early lethality or epileptic seizures (Figure 1c). No gross morphological abnormality was observed in the SIK1-MT brain (Figure 1d). Distribution of neuronal markers, including parvalbumin, somatostatin and Satb2, was also unaltered in the SIK1-MT brain (Figure S3).

Excitatory synaptic function is increased in pyramidal neurons in layer 5 of the medial prefrontal cortex in SIK1-MT mice.

The patients with SIK1 truncation have been reported to exhibit autistic behavioral phenotypes, such as social deficits and repetitive behavior [12]. Recent studies indicate that the deep layer of the mPFC is responsible for these behavioral abnormalities both in humans and rodents [15, 29, 30]. Thus, we studied the neuronal function in layer 5 of the mPFC in SIK1-MT mice. First, we measured miniature excitatory postsynaptic currents (mEPSCs) by whole cell recording in a voltage clamp mode (Figure 1e). We observed that the frequency, but not amplitude, of mEPSCs was significantly increased in SIK1-MT mice (Figure 1e-g). The rise and decay times of mEPSCs were not changed in SIK1-MT mice (Figure S4). We did not observe changes in frequency, amplitude, rise time, and decay time of miniature inhibitory postsynaptic currents (mIPSCs) in SIK1-MT mice (Figure 1h-j, see Figure S4), resulting in the excitatory shift of synaptic function (Figure 1k and l). The increase in the frequency of mEPSCs is considered to be due to either the increase of the excitatory synapse number or release probability of synaptic vesicles. To discriminate these possibilities, we measured the paired-pulse ratio (PPR). We found the AMPA receptor-

mediated PPR, as well as GABA receptor-mediated, were unchanged in all stimulation intervals we analyzed (Figure S5), indicating that the number of the functional excitatory synapse was increased in SIK1-MT mice. We also examined the composition of postsynaptic glutamatergic receptors as a ratio of the action potential dependent NMDA and AMPA receptor-mediated postsynaptic currents, but no change was detected in this ratio (Figure S6).

Excitability of pyramidal neurons in layer 5 of the mPFC is increased in the SIK1-MT mice.

Next, we examined the membrane property and excitability of layer 5 pyramidal neurons in the mPFC of the SIK1-WT and -MT mice using whole cell recording in a current clamp mode. The resting potential was not changed, but input resistance was increased and membrane capacitance was decreased in the SIK1-MT mice compared to the wild-type control mice (Figure 2a-c). The threshold, half-peak width, and rise time of action potential were unchanged (Figure 2d-f), but the decay time was decreased and the spike frequency was increased in SIK1-MT mice compared to the wild-type control mice (Figure 2 g-i).

Repetitive behavior and social novelty preference are distorted in SIK1-MT mice.

To evaluate the effect of the mutation on the human cases, we examined the behaviors of the SIK1-MT mice in relevance to autistic symptoms. We first observed the general movement in the open field arena. The travel distance was unchanged, indicating the locomotor activity was normal in the SIK1-MT mice. The time spent in the center and the vertical activity that indicates the anxiety level was also unchanged in the SIK1-MT mice (Figure 3a-d), but the SIK1-MT mice showed an increased level of grooming (Figure 3e). There was no change in the movement in elevated plus maze also suggests that the anxiety level was unchanged in SIK1-MT mice (Figure 3f and g). Therefore, overgrooming is suggested to reflect increased repetitive behavior. We further examined the repetitive behavior using the marble burying test [31-33]. The number of marbles buried under the woodchip was higher in the SIK1-MT mice compared to the wild-type mice (Figure 3h and i), suggesting that the repetitive behavior was increased in SIK1-MT mice in consistent with the overgrooming behavior observed in the open field test.

Social deficits and language problems are the common symptoms of autism and also reported in the human cases with SIK1 mutation [12, 34]. To evaluate the vocal communication in SIK1-MT mice, we examined the ultrasonic vocalization (USV) of P5-P14 mice after separation from mother [35]. The frequency and variability of USV were unaltered in SIK1-MT compared to SIK1-WT mice (Figure S7). We examined social behaviors using the three-chamber test. In the first round test, both SIK1-MT and wild-type mice showed higher interaction with the stranger mouse (S1) compared to the empty cage (E) at similar levels (Figure 3j and k). In the second round test, we placed a new stranger mouse (S2) in the empty cage and compared the interaction between S2 and the previously exposed stranger mouse in the first round test (S1) to examine the level of social novelty preference. While the interaction with S2 was higher than that with S1 in the wild-type mice, no significant difference in the interaction between S1 and S2 was observed in SIK1-MT mice (Figure 3l and m), suggesting that the social novelty preference was impaired in the SIK1-MT mice.

Risperidone restores the elevated level of excitatory synaptic transmission and excitability of pyramidal neurons in layer 5 of mPFC.

Next, to examine a drug used to ameliorate ASD symptoms, we focused on risperidone, which is the first FDA approved medicine for some autistic symptoms to alleviate aggression and repetitive behaviors [36, 37]. We treated SIK1-MT mice with risperidone by intraperitoneal injection and waited for 1 hour before patch clamp recording from acute brain slices. Acute administration of risperidone significantly attenuated the frequency, but not amplitude, of mEPSCs in pyramidal neurons in layer 5 of the mPFC in SIK1-MT mice compared to the saline injected condition (Figure 4a-c). But the E/I balance was unchanged because risperidone also reduced the frequency of mIPSCs in SIK1-MT mice (Figure 4d-h). The rise time and decay time constant of mEPSC and mIPSC was not altered by the application of risperidone (Figure S8). We further examined the effect of risperidone on the excitability of pyramidal neurons in layer 5 of mPFC. Risperidone increased the membrane capacitance and decreased the frequency of action potential of the pyramidal neurons in layer 5 of the mPFC in SIK1-MT mice without altering resting potential, input resistance, and kinetics of action potential (Figure 4i-p).

Risperidone ameliorated repetitive, but not social, behavior in SIK1-MT mice.

We next examined the effect of risperidone on the behavioral deficits observed in SIK1-MT mice. In the open field test, risperidone reduced the number of grooming in SIK1-MT mice, without affecting the travel distance, the time spent in the center, and the vertical activity (Figure 5a-d). The number of buried marble in the marble burying test was also reduced by risperidone treatment in SIK1-MT mice (Figure 5e). However, risperidone did not show any changes in social interactions observed in the three-chamber test compared to the saline treatment (Figure 5f and g).

Discussion

SIK1 C-terminal truncated mutations were identified in patients with EIEE-30 [12]. To study the effect of these mutations on the etiology of EIEE-30, we generated C-terminal truncated SIK1-MT mice using CRISPR/Cas9-mediated genome editing as disease models. We studied these mice by focusing on the synaptic function and behaviors and found the followings. (1) The frequency of mEPSCs and the neuronal excitability were increased in pyramidal neurons in layer 5 of the mPFC of SIK1-MT mice. (2) Repetitive behavior was increased and the social behavior was impaired in the SIK1-MT mice. (3) Elevated excitatory synaptic transmission and neural excitability in the SIK1-MT mice were restored by risperidone treatment. (4) Increased repetitive behavior, but not social behaviors, in SIK1-MT mice was ameliorated by risperidone treatment.

The mechanism by which the synaptic function of the SIK1-MT mice shifts to excitatory dominant remains unknown. SIK has been shown to regulate the subcellular localization and the biological activities of the class IIa histone deacetylases (HDAC4/5/7/9) [38-40] and cAMP-regulated transcriptional coactivators (CRTCs 1, 2, and 3) [10, 41, 42] through the phosphorylation. SIK1-WT was distributed in the nucleus in a punctate pattern, whereas the SIK1-MT was diffused to the cytoplasm (Figure 1b). The

altered subcellular distribution of SIK1 mutants may affect the expressions of genes related to ion channels or synaptic proteins [27], resulting in the increased excitability and excitatory synaptic inputs of neurons in SIK1-MT mice.

Application of risperidone attenuated the enhanced neural excitability and excitatory synaptic function in mPFC of SIK1-MT mice. We also found that the risperidone rescued increased repetitive behavior. The neuronal activity in mPFC has been linked to the repetitive behavior in rodent models [43-45], which is supported by our findings in this study. Risperidone is the first FDA approved drug for ASD used for ameliorating repetitive and aggressive symptoms of the disorder [36, 37]. It antagonizes serotonin (5-HT_{2A}, 5-HT_{1B}, and 5-HT₇) and dopamine D₂ receptors [46]. It would be interesting to investigate whether the attenuation of the synaptic phenotype in mPFC in SIK1-MT mice by risperidone is due to the inhibition of these receptors.

Risperidone did not rescue deficits in social behavior in SIK1-MT mice, which is commonly observed in other animal models of neurodevelopmental disorders [47-51]. It has been shown that the social behavioral deficit is attributable to the disruption of E/I synaptic balance in various animal models [52-55]. We observed that risperidone also attenuated inhibitory synaptic function as well as excitatory synaptic function in mPFC, retaining disrupted E/I synaptic balance in SIK1-MT mice. The disrupted E/I synaptic balance after administration of risperidone may be a possible mechanism that underlies the social behavioral deficits in SIK1-MT mice (Figure S9). In order to address this hypothesis, future studies to examine whether selective attenuation of excitatory synaptic function by opto- or pharmacogenetics may rescue social deficits in this model are required.

Limitations

In this study, risperidone failed to restore the aberrant synaptic function completely. It would be important to search methods to normalize all the synaptic phenotypes to further investigate the pathophysiological consequences in this model mouse. We only tested the acute effect of risperidone this time. Long-term administration of risperidone may ameliorate disrupted synaptic E/I balance and social behavioral deficits. Further studies will give us a deeper insight on the pharmacological effects of risperidone on the social behavioral deficits.

Conclusion

We generated and analyzed C-terminal truncated SIK1 mutant mice (SIK1-MT) as models for EIEE-30. SIK1-MT mice exhibited social deficits and repetitive behavior consistent with the autistic phenotypes of EIEE-30. Elevated excitability of neurons and excitatory synaptic function were observed in the mPFC of SIK1-MT mice. Acute treatment with risperidone selectively restored the repetitive behavior, but not social deficits, likely due to the attenuation of elevated excitatory synaptic function. Reduction of inhibitory synaptic function occurred by risperidone treatment causing further E/I imbalance. Social deficits may be caused by the disrupted E/I balance in the model mice.

List Of Abbreviations

SIK: Salt inducible kinase

AMPK: AMP-activated protein kinase

EIEE: early infantile epileptic encephalopathy

E/I: excitatory/inhibitory

ANOVA: Analysis of variance

mPFC: medial prefrontal cortex

UBA: ubiquitin associated area

PST: proline-glutamate-serine-threonine

NLD: nuclear localization regulatory domain

CRTC: cAMP-regulated transcriptional coactivators

HDAC: histone deacetylases

sgRNA: single guide RNA

IBMX: 3-isobutyl-1-methylxanthine

DMSO: dimethyl sulfoxide

ACSF: artificial corticospinal fluid

mEPSC: miniature excitatory postsynaptic current

mIPSC: miniature inhibitory postsynaptic current

GABA: gamma-aminobutyric acid

AMPA: α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid

NMDA: N-Methyl-d-aspartate

ePPR: excitatory paired pulse ratio

iPPR: inhibitory paired pulse ratio

USV: ultrasonic vocalization

Declarations

Ethics approval and consent to participate

All procedures on animals in this study were approved by the Animal Care and the Use Committee of Shinshu University School of Medicine.

Consent for publication

Not applicable.

Availability of data and materials

Most of the data generated and analyzed during this study are included in this published article. All datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors have no competing interests.

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Authors' contributions

TM, TY, and KT designed experiments. TM, TY, and KT generated SIK1-MT mice. MB and TM performed electrophysiology. TK, TM and KN performed behavioral experiments. MB, TM, EKS, and YS performed molecular experiments. TM and KT wrote manuscripts.

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References

1. Sun Z, Jiang Q, Li J, Guo J: The potent roles of salt-inducible kinases (SIKs) in metabolic homeostasis and tumorigenesis. *Signal transduction and targeted therapy* 2020, 5(1):150.
2. Wein MN, Foretz M, Fisher DE, Xavier RJ, Kronenberg HM: Salt-Inducible Kinases: Physiology, Regulation by cAMP, and Therapeutic Potential. *Trends in endocrinology and metabolism: TEM* 2018, 29(10):723-735.
3. Sakamoto K, Bultot L, Goransson O: The Salt-Inducible Kinases: Emerging Metabolic Regulators. *Trends in endocrinology and metabolism: TEM* 2018, 29(12):827-840.
4. Wang Z, Takemori H, Halder SK, Nonaka Y, Okamoto M: Cloning of a novel kinase (SIK) of the SNF1/AMPK family from high salt diet-treated rat adrenal. *FEBS letters* 1999, 453(1-2):135-139.
5. Takemori H, Katoh Y, Horike N, Doi J, Okamoto M: ACTH-induced nucleocytoplasmic translocation of salt-inducible kinase. Implication in the protein kinase A-activated gene transcription in mouse adrenocortical tumor cells. *The Journal of biological chemistry* 2002, 277(44):42334-42343.
6. Feldman JD, Vician L, Crispino M, Hoe W, Baudry M, Herschman HR: The salt-inducible kinase, SIK, is induced by depolarization in brain. *Journal of neurochemistry* 2000, 74(6):2227-2238.
7. Finsterwald C, Carrard A, Martin JL: Role of salt-inducible kinase 1 in the activation of MEF2-dependent transcription by BDNF. *PloS one* 2013, 8(1):e54545.
8. Lizcano JM, Goransson O, Toth R, Deak M, Morrice NA, Boudeau J, Hawley SA, Udd L, Makela TP, Hardie DG *et al*: LKB1 is a master kinase that activates 13 kinases of the AMPK subfamily, including MARK/PAR-1. *The EMBO journal* 2004, 23(4):833-843.
9. Jaleel M, McBride A, Lizcano JM, Deak M, Toth R, Morrice NA, Alessi DR: Identification of the sucrose non-fermenting related kinase SNRK, as a novel LKB1 substrate. *FEBS letters* 2005, 579(6):1417-1423.
10. Koo SH, Flechner L, Qi L, Zhang X, Screatton RA, Jeffries S, Hedrick S, Xu W, Boussouar F, Brindle P *et al*: The CREB coactivator TORC2 is a key regulator of fasting glucose metabolism. *Nature* 2005, 437(7062):1109-1111.
11. Yoon YS, Seo WY, Lee MW, Kim ST, Koo SH: Salt-inducible kinase regulates hepatic lipogenesis by controlling SREBP-1c phosphorylation. *The Journal of biological chemistry* 2009, 284(16):10446-10452.
12. Hansen J, Snow C, Tuttle E, Ghoneim DH, Yang CS, Spencer A, Gunter SA, Smyser CD, Gurnett CA, Shinawi M *et al*: De novo mutations in SIK1 cause a spectrum of developmental epilepsies. *American journal of human genetics* 2015, 96(4):682-690.
13. Brumback AC, Ellwood IT, Kjaerby C, lafrati J, Robinson S, Lee AT, Patel T, Nagaraj S, Davatolhagh F, Sohal VS: Identifying specific prefrontal neurons that contribute to autism-associated abnormalities in physiology and social behavior. *Molecular psychiatry* 2018, 23(10):2078-2089.
14. Rinaldi T, Perrodin C, Markram H: Hyper-connectivity and hyper-plasticity in the medial prefrontal cortex in the valproic Acid animal model of autism. *Frontiers in neural circuits* 2008, 2:4.
15. Yizhar O, Fenno LE, Prigge M, Schneider F, Davidson TJ, O'Shea DJ, Sohal VS, Goshen I, Finkelstein J, Paz JT *et al*: Neocortical excitation/inhibition balance in information processing and social

dysfunction. *Nature* 2011, 477(7363):171-178.

16. Shea S, Turgay A, Carroll A, Schulz M, Orlik H, Smith I, Dunbar F: Risperidone in the treatment of disruptive behavioral symptoms in children with autistic and other pervasive developmental disorders. *Pediatrics* 2004, 114(5):e634-641.
17. Aman MG, Arnold LE, McDougle CJ, Vitiello B, Scahill L, Davies M, McCracken JT, Tierney E, Nash PL, Posey DJ *et al*: Acute and long-term safety and tolerability of risperidone in children with autism. *Journal of child and adolescent psychopharmacology* 2005, 15(6):869-884.
18. Uemura T, Mori T, Kurihara T, Kawase S, Koike R, Satoga M, Cao X, Li X, Yanagawa T, Sakurai T *et al*: Fluorescent protein tagging of endogenous protein in brain neurons using CRISPR/Cas9-mediated knock-in and in utero electroporation techniques. *Scientific reports* 2016, 6:35861.
19. Kaneko T, Mashimo T: Simple Genome Editing of Rodent Intact Embryos by Electroporation. *PloS one* 2015, 10(11):e0142755.
20. Burrows EL, Laskaris L, Koyama L, Churilov L, Bornstein JC, Hill-Yardin EL, Hannan AJ: A neuroligin-3 mutation implicated in autism causes abnormal aggression and increases repetitive behavior in mice. *Molecular autism* 2015, 6:62.
21. Nagai T, Ibata K, Park ES, Kubota M, Mikoshiba K, Miyawaki A: A variant of yellow fluorescent protein with fast and efficient maturation for cell-biological applications. *Nature biotechnology* 2002, 20(1):87-90.
22. Carr IM, Robinson JI, Dimitriou R, Markham AF, Morgan AW, Bonthron DT: Inferring relative proportions of DNA variants from sequencing electropherograms. *Bioinformatics* 2009, 25(24):3244-3250.
23. Kluesner MG, Nedveck DA, Lahr WS, Garbe JR, Abrahante JE, Webber BR, Moriarity BS: EditR: A Method to Quantify Base Editing from Sanger Sequencing. *The CRISPR journal* 2018, 1:239-250.
24. Mori T, Kasem EA, Suzuki-Kouyama E, Cao X, Li X, Kurihara T, Uemura T, Yanagawa T, Tabuchi K: Deficiency of calcium/calmodulin-dependent serine protein kinase disrupts the excitatory-inhibitory balance of synapses by down-regulating GluN2B. *Molecular psychiatry* 2019, 24(7):1079-1092.
25. Mori T, Morimoto K: Rabies virus glycoprotein variants display different patterns in rabies monosynaptic tracing. *Frontiers in neuroanatomy* 2014, 7:47.
26. Perez-Escudero A, Vicente-Page J, Hinz RC, Arganda S, de Polavieja GG: idTracker: tracking individuals in a group by automatic identification of unmarked animals. *Nature methods* 2014, 11(7):743-748.
27. Pröschel C, Hansen JN, Ali A, Tuttle E, Lacagnina M, Buscaglia G, Halterman MW, Paciorkowski AR: Epilepsy-causing sequence variations in SIK1 disrupt synaptic activity response gene expression and affect neuronal morphology. *European journal of human genetics : EJHG* 2017, 25(2):216-221.
28. Katoh Y, Takemori H, Doi J, Okamoto M: Identification of the nuclear localization domain of salt-inducible kinase. *Endocrine research* 2002, 28(4):315-318.
29. Baron-Cohen S, Ring H, Moriarty J, Schmitz B, Costa D, Ell P: Recognition of mental state terms. Clinical findings in children with autism and a functional neuroimaging study of normal adults. *The*

British journal of psychiatry : the journal of mental science 1994, 165(5):640-649.

30. Castelli F, Frith C, Happe F, Frith U: Autism, Asperger syndrome and brain mechanisms for the attribution of mental states to animated shapes. *Brain : a journal of neurology* 2002, 125(Pt 8):1839-1849.
31. Gyertyan I: Analysis of the marble burying response: marbles serve to measure digging rather than evoke burying. *Behavioural pharmacology* 1995, 6(1):24-31.
32. Hayashi E, Kuratani K, Kinoshita M, Hara H: Pharmacologically distinctive behaviors other than burying marbles during the marble burying test in mice. *Pharmacology* 2010, 86(5-6):293-296.
33. Thomas A, Burant A, Bui N, Graham D, Yuva-Paylor LA, Paylor R: Marble burying reflects a repetitive and perseverative behavior more than novelty-induced anxiety. *Psychopharmacology* 2009, 204(2):361-373.
34. American Psychiatry Association: Diagnostic and Statistical Manual of Mental Disorders, 5th edn. Washington, DC; 2013.
35. Liu HX, Lopatina O, Higashida C, Fujimoto H, Akther S, Inzhutova A, Liang M, Zhong J, Tsuji T, Yoshihara T *et al*: Displays of paternal mouse pup retrieval following communicative interaction with maternal mates. *Nature communications* 2013, 4:1346.
36. McDougale CJ, Scahill L, McCracken JT, Aman MG, Tierney E, Arnold LE, Freeman BJ, Martin A, McGough JJ, Cronin P *et al*: Research Units on Pediatric Psychopharmacology (RUPP) Autism Network. Background and rationale for an initial controlled study of risperidone. *Child and adolescent psychiatric clinics of North America* 2000, 9(1):201-224.
37. McDougale CJ, Stigler KA, Erickson CA, Posey DJ: Atypical antipsychotics in children and adolescents with autistic and other pervasive developmental disorders. *The Journal of clinical psychiatry* 2008, 69 Suppl 4:15-20.
38. Hsu A, Duan Q, McMahon S, Huang Y, Wood SA, Gray NS, Wang B, Bruneau BG, Haldar SM: Salt-inducible kinase 1 maintains HDAC7 stability to promote pathologic cardiac remodeling. *The Journal of clinical investigation* 2020, 130(6):2966-2977.
39. Berdeaux R, Goebel N, Banaszynski L, Takemori H, Wandless T, Shelton GD, Montminy M: SIK1 is a class II HDAC kinase that promotes survival of skeletal myocytes. *Nature medicine* 2007, 13(5):597-603.
40. Abend A, Shkedi O, Fertouk M, Caspi LH, Kehat I: Salt-inducible kinase induces cytoplasmic histone deacetylase 4 to promote vascular calcification. *EMBO reports* 2017, 18(7):1166-1185.
41. Jagannath A, Butler R, Godinho SIH, Couch Y, Brown LA, Vasudevan SR, Flanagan KC, Anthony D, Churchill GC, Wood MJA *et al*: The CRTC1-SIK1 pathway regulates entrainment of the circadian clock. *Cell* 2013, 154(5):1100-1111.
42. Nixon M, Stewart-Fitzgibbon R, Fu J, Akhmedov D, Rajendran K, Mendoza-Rodriguez MG, Rivera-Molina YA, Gibson M, Berglund ED, Justice NJ *et al*: Skeletal muscle salt inducible kinase 1 promotes insulin resistance in obesity. *Molecular metabolism* 2016, 5(1):34-46.

43. Kim H, Lim CS, Kaang BK: Neuronal mechanisms and circuits underlying repetitive behaviors in mouse models of autism spectrum disorder. *Behavioral and brain functions : BBF* 2016, 12(1):3.
44. Takahata R, Moghaddam B: Activation of glutamate neurotransmission in the prefrontal cortex sustains the motoric and dopaminergic effects of phencyclidine. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology* 2003, 28(6):1117-1124.
45. Aliane V, Perez S, Nieoullon A, Deniau JM, Kemel ML: Cocaine-induced stereotypy is linked to an imbalance between the medial prefrontal and sensorimotor circuits of the basal ganglia. *The European journal of neuroscience* 2009, 30(7):1269-1279.
46. Nasrallah HA: Atypical antipsychotic-induced metabolic side effects: insights from receptor-binding profiles. *Molecular psychiatry* 2008, 13(1):27-35.
47. Peñagarikano O, Abrahams BS, Herman EI, Winden KD, Gdalyahu A, Dong H, Sonnenblick LI, Gruver R, Almajano J, Bragin A *et al*: Absence of CNTNAP2 leads to epilepsy, neuronal migration abnormalities, and core autism-related deficits. *Cell* 2011, 147(1):235-246.
48. Bonini SA, Mastinu A, Maccarinelli G, Mitola S, Premoli M, La Rosa LR, Ferrari-Toninelli G, Grilli M, Memo M: Cortical Structure Alterations and Social Behavior Impairment in p50-Deficient Mice. *Cerebral cortex* 2016, 26(6):2832-2849.
49. Chadman KK: Fluoxetine but not risperidone increases sociability in the BTBR mouse model of autism. *Pharmacology, biochemistry, and behavior* 2011, 97(3):586-594.
50. Gould GG, Hensler JG, Burke TF, Benno RH, Onaivi ES, Daws LC: Density and function of central serotonin (5-HT) transporters, 5-HT1A and 5-HT2A receptors, and effects of their targeting on BTBR T+tf/J mouse social behavior. *Journal of neurochemistry* 2011, 116(2):291-303.
51. Teng BL, Nikolova VD, Riddick NV, Agster KL, Crowley JJ, Baker LK, Koller BH, Pedersen CA, Jarstfer MB, Moy SS: Reversal of social deficits by subchronic oxytocin in two autism mouse models. *Neuropharmacology* 2016, 105:61-71.
52. Rubenstein JL, Merzenich MM: Model of autism: increased ratio of excitation/inhibition in key neural systems. *Genes, brain, and behavior* 2003, 2(5):255-267.
53. Sohal VS, Rubenstein JLR: Excitation-inhibition balance as a framework for investigating mechanisms in neuropsychiatric disorders. *Molecular psychiatry* 2019, 24(9):1248-1257.
54. Lee E, Lee J, Kim E: Excitation/Inhibition Imbalance in Animal Models of Autism Spectrum Disorders. *Biological psychiatry* 2017, 81(10):838-847.
55. Tabuchi K, Blundell J, Etherton MR, Hammer RE, Liu X, Powell CM, Sudhof TC: A neuroligin-3 mutation implicated in autism increases inhibitory synaptic transmission in mice. *Science* 2007, 318(5847):71-76.

Figures

Figure 1

Generation and basic characterization of the SIK1 MT mice. a), Genomic organization of SIK1 gene (top), domain structure of wild-type (middle), and mutant SIK1 (bottom) protein. The mutations identified in human patients are indicated on the domain structure of SIK1-WT. The target sequence of sgRNA is underlined. Converted amino acids after the mutated site are indicated in red. b), Subcellular localization of SIK1 mutants in HEK293T cells, which are counterstained with DAPI. Scale bar indicates 15 μ m. c),

Body weights of males and females of wild-type and mutant are shown in the graph. d), The brain structure is unchanged in the SIK1-MT mice. Sagittal (left) and coronal (right) brain sections were stained with DAPI. Scale bars indicate 1 mm. e), Representative traces of mEPSC recorded from the layer 5 pyramidal neurons in mPFC of wild-type and SIK1-MT mice. f), Cumulative distribution of inter-event intervals of and the mean values of the frequency of mEPSCs were shown in the graph. g), Cumulative distribution and the mean values of the amplitude of mEPSCs were shown in the graph. h), Representative traces of mIPSC recorded from the layer 5 pyramidal neurons in mPFC of wild-type and SIK1-MT mice were shown. i), Cumulative distribution of the inter-event intervals and the mean values of the frequency of mIPSCs were shown in the graph. j), Cumulative distributions and mean values of the amplitude of mIPSCs were shown in the graph. k), Scatter plot between the frequencies of mIPSC (x-axis) and mEPSC (y-axis). Each dot represents a single neuron from WT (black) or MT (red). l), E/I balance index of WT and SIK1-MT mice is shown. The numbers of neurons and mice used in each analysis are shown on the bar (neurons/mice) in the graphs. Statistical analysis was made by Student's t-test (for the mean of the synaptic parameters). Statistical significance was indicated by asterisks (* $p < 0.05$ and *** $p < 0.001$).

Figure 2

Membrane and firing properties were changed in pyramidal neurons in layer 5 of the mPFC. a-c), Resting membrane potential (a), input resistance (b), membrane capacitance (c) of pyramidal neurons in layer 5 of mPFC of SIK1-WT or -MT mice. d-g), threshold (d), half-peak width (e), rise time (f), and decay time (g) of the action potential of the pyramidal neurons in layer 5 of the mPFC. h), Representative traces of induced action potentials responded to 240 pA injected currents. i), Graph for the relationship between spike frequency and injected current. Input resistance and spike frequency of action potential were increased and membrane capacitance and decay time of action potential were decreased in SIK1-MT mice. The numbers of neurons and mice used in each analysis are shown on the bar (neurons/mice) in

the graphs. Statistical analysis was made by Student's t-test. Statistical significance was indicated by asterisks (* $p < 0.05$ and ** $p < 0.01$).

Figure 3

Repetitive and social behaviors were altered in SIK1-MT mice. a), Trajectories of movement of SIK1-WT and -MT mice in the open field arena. b), Total travel distance of SIK1-WT (n=9) and -MT (n=7) mice. c), The percentage of the time spent in the center of the arena. d), The total time of vertical activities in the

open field. e), The number of grooming during the observation period. The number of grooming was increased in SIK1-MT mice. f), Moving trajectories of mice on the elevated plus maze. g), The number of entries into open arms of the elevated plus maze. The number of the entry was unchanged in SIK1-MT mice. h), The top views of the marble burying test field. 16 marbles were placed at the start of the test (top). Buried marbles were counted after 10 minutes' test (bottom). i), The numbers of marbles buried under the woodchip after the test. SIK1-MT mice buried more marbles in this test. j), The sociability test evaluating the sociability by the time spent with the empty cage (E) versus the cage with a stranger mouse (S1). Heatmaps represent the stay time of the test mouse. k), Both WT and SIK1-MT mice interacted more with S1 than E and the preference to E versus S1 was similar between these mice (graph). l), Social novelty test evaluating social memory by the time spent with the stranger mouse (S1) versus novel stranger mouse (S2). Heatmaps represent the stay time of the test mouse. m), WT mice interacted more with S2 than S1, whereas the preference to S2 was significantly decreased in SIK1-MT mice (graph). Statistical analysis was made by Student's t-test. Statistical significance was indicated by asterisks (* $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$).

Figure 4

Risperidone reduces frequencies of mEPSCs and action potentials in the SIK1-MT mice. a), Representative traces of mEPSC recorded from pyramidal neurons in layer 5 of the mPFC in saline (Sal) or risperidone (Ris) injected SIK1-MT mice. b), Cumulative distribution of inter-event intervals and the mean values of the frequency of mEPSCs were shown in the graph. c), Cumulative distribution and the mean values of the amplitude of mEPSCs were shown in the graph. d), Representative traces of mIPSC

recorded from the layer 5 pyramidal neurons in mPFC of saline (Sal) and risperidone (Ris) injected SIK1-MT mice were shown. e), Cumulative distribution of the inter-event intervals and the mean values of the frequency of mIPSCs were shown in the graph. f), Cumulative distributions and mean values of the amplitude of mIPSCs were shown in the graph. g), Scatter plot shows the relationship between the frequencies of mIPSC (x-axis) and mEPSC (y-axis). Each dot represents a single neuron from saline or risperidone injected SIK1-MT mice. h), E/I balance index of saline (Sal) and risperidone (Ris) injected SIK1-MT mice is shown. i-k), Resting membrane potential (i), input resistance (j), membrane capacitance (k) of pyramidal neurons in layer 5 of mPFC of saline (Sal) and risperidone (Ris) injected SIK1-MT mice. l-o), half-peak width (l), rise time (m), and decay time (n) of the action potential of the pyramidal neurons in layer 5 of the mPFC of saline (Sal) and risperidone (Ris) injected SIK1-MT mice. o), Representative traces of induced action potentials responded to 240 pA injected currents. p), Graph for the relationship between spike frequency and injected current. Spike frequency of the action potential was decreased in risperidone-injected SIK1-MT mice. The numbers of neurons and mice used in each analysis are shown on the bar (neurons/mice) in the graphs. Statistical analysis was made by student's t-test. Statistical significance was indicated by asterisks (* $p < 0.05$).

Figure 5

Figure 5

Administration of risperidone ameliorated increased repetitive behaviors but not social deficits in SIK1-MT mice. a), Total travel distance of saline (Sal) and risperidone (Ris) injected SIK1-MT mice. b), The percentage of the time spent in the center of the open field arena. c), Total time of the vertical activities of saline (Sal, n=10) and risperidone (Ris, n=9) injected SIK1-MT mice. d), The number of groomings during the observation period. e), The number of marbles buried in the marble burying test. The number of buried marbles was less in risperidone injected SIK1-MT mice compared to that of saline injected. f-g), Sociability and social novelty test between saline and risperidone injected SIK1-MT mice. Risperidone did not affect the social behavior in SIK1-MT mice. The numbers of neurons and mice used in each analysis are shown on the bar (neurons/mice) in the graphs. Statistical analysis was made by Student's t-test. Statistical significance was indicated by asterisks (* $p < 0.05$ and ** $p < 0.01$).

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