


ORIGINAL ARTICLE

Heterozygous loss of epilepsy gene *KCNQ2* alters social, repetitive and exploratory behaviors

Eung Chang Kim¹ | Jaimin Patel¹ | Jiaren Zhang¹ | Heun Soh² |
Justin S. Rhodes^{3,4,5} | Anastasios V. Tzingounis² | Hee Jung Chung^{1,3,5} 

¹Department of Molecular and Integrative Physiology, University of Illinois at Urbana-Champaign, Urbana, Illinois

²Department of Physiology and Neurobiology, University of Connecticut, Storrs, Connecticut

³Beckman Institute for Advanced Science and Technology, University of Illinois at Urbana-Champaign, Urbana, Illinois

⁴Department of Psychology, University of Illinois at Urbana-Champaign, Urbana, Illinois

⁵Neuroscience Program, University of Illinois at Urbana-Champaign, Urbana, Illinois

Correspondence

Hee Jung Chung, Department of Molecular and Integrative Physiology, University of Illinois at Urbana-Champaign, 407 South Goodwin Avenue, 524 Burrill Hall, Urbana, IL 61801.
Email: chunghj@illinois.edu

Funding information

National Heart, Lung, and Blood Institute, Grant/Award Number: HL137094; National Institute of Neurological Disorders and Stroke, Grant/Award Numbers: NS083402, NS101596, NS104293, NS10887

Abstract

KCNQ/K_v7 channels conduct voltage-dependent outward potassium currents that potentially decrease neuronal excitability. Heterozygous inherited mutations in their principle subunits *K_v7.2/KCNQ2* and *K_v7.3/KCNQ3* cause benign familial neonatal epilepsy whereas patients with de novo heterozygous *K_v7.2* mutations are associated with early-onset epileptic encephalopathy and neurodevelopmental disorders characterized by intellectual disability, developmental delay and autism. However, the role of *K_v7.2*-containing *K_v7* channels in behaviors especially autism-associated behaviors has not been described. Because pathogenic *K_v7.2* mutations in patients are typically heterozygous loss-of-function mutations, we investigated the contributions of *K_v7.2* to exploratory, social, repetitive and compulsive-like behaviors by behavioral phenotyping of both male and female *KCNQ2^{+/-}* mice that were heterozygous null for the *KCNQ2* gene. Compared with their wild-type littermates, male and female *KCNQ2^{+/-}* mice displayed increased locomotor activity in their home cage during the light phase but not the dark phase and showed no difference in motor coordination, suggesting hyperactivity during the inactive light phase. In the dark phase, *KCNQ2^{+/-}* group showed enhanced exploratory behaviors, and repetitive grooming but decreased sociability with sex differences in the degree of these behaviors. While male *KCNQ2^{+/-}* mice displayed enhanced compulsive-like behavior and social dominance, female *KCNQ2^{+/-}* mice did not. In addition to elevated seizure susceptibility, our findings together indicate that heterozygous loss of *K_v7.2* induces behavioral abnormalities including autism-associated behaviors such as reduced sociability and enhanced repetitive behaviors. Therefore, our study is the first to provide a tangible link between loss-of-function *K_v7.2* mutations and the behavioral comorbidities of *K_v7.2*-associated epilepsy.

KEYWORDS

anxiety, epilepsy, excitability, *KCNQ2*, *K_v7* channels, locomotion, seizures, social dominance, social interaction

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

© 2019 The Authors. Genes, Brain and Behavior published by International Behavioural and Neural Genetics Society and John Wiley & Sons Ltd.

1 | INTRODUCTION

Neurodevelopmental disorders (NDDs) are a collection of heterogeneous disorders which include but are not limited to autism spectrum disorder (ASD), intellectual disability, developmental delay and epilepsy.^{1,2} These disorders can occur alone or together in the early childhood of NDD patients and persist throughout life.¹ Among NDDs, ASD is estimated to occur in 1 out of 59 children (<https://www.cdc.gov/ncbddd/autism/data.html>). The main symptoms of ASD are behavioral abnormalities including difficulty with social interaction and communication as well as restrictive and repetitive behaviors.³ Additional ASD symptoms include obsessive interests, compulsive behavior, impulsivity, self-harm, aggression, hyperactivity, anxiety, hypersensitivity to auditory and tactile stimulation, sleep problems, learning disability and speech delay.⁴⁻⁹ Current available treatments for children with NDDs have limited efficacy alleviating the behavioral symptoms associated with ASD and seizures that are comorbid with some NDDs.¹⁰ To understand the etiology underlying NDDs, recent efforts have focused on next generation sequencing of whole genome and targeted sequencing of well-defined ASD cohorts.^{11,12} These efforts have dramatically accelerated discovery of genes affected by NDDs, providing key initial insights into the possible signaling pathways and mechanisms underlying NDDs.^{11,12}

Among 35 genes with excess missense de novo mutations, significant numbers of recurrent mutations have been found in *KCNQ2* and *KCNQ3* genes,¹² which encode the principle subunits of neuronal *KCNQ/K_v7* potassium (K^+) channels, *K_v7.2* and *K_v7.3*.¹³ *K_v7* channels are mostly heterotetramers of *K_v7.2* and *K_v7.3* subunits.^{14,15} *K_v7.2* and *K_v7.3* are found throughout the brain, including the hippocampus and cortex with overlapping distribution.^{16,17} Subcellularly, *K_v7.2* and *K_v7.3* are preferentially enriched at the plasma membrane of axonal initial segments (AIS) and distal axons¹⁷⁻¹⁹ where AP initiates and propagates.²⁰ Because these slowly activating and noninactivating K^+ currents are activated at the subthreshold potentials of action potential (AP) generation,¹³ they allow the firing of a single AP but effectively prevent repetitive and burst firing of APs.^{14,21-26} In contrast, inhibition of their currents by muscarinic agonists (hence, named "M-currents") leads to profound increase in AP frequency.¹³ In addition, *K_v7* currents regulate AP threshold and resting membrane potential.^{14,21-25}

The physiological significance of neuronal *K_v7* channels is underscored by the fact that more than 200 heterozygous mutations in *KCNQ2* and *KCNQ3* genes have been associated with early-onset epilepsy, including Benign Familial Neonatal Encephalopathy (BFNE [MIM: 121200]) and Epileptic Encephalopathy (EE [MIM: 613720]) (RIKEE database www.rikee.org). In BFNE, patients have inherited *KCNQ2* and *KCNQ3* mutations and display neonatal seizures that spontaneously remit after several weeks to months with benign psychomotor and intellectual outcomes.²⁷ In EE, patients with de novo *KCNQ2* and *KCNQ3* variants have early-onset seizures, developmental delay and intellectual disability.²⁸⁻³⁸ Although a few gain-of function EE mutations of *K_v7.2* has been reported,³⁹ experimentally-characterized epilepsy mutations mostly decrease current and surface expression of

heteromeric *K_v7.2/K_v7.3* channels by 20% to 75%.^{27,32,40-43} Consistent with the loss-of-function mutations, the heterozygous *K_v7.2* knockout mice and the heterozygous *K_v7.2* knock-in mice for BFNE Y284C mutation display increased seizure propensity.^{44,45} Conditional homozygous deletion of *KCNQ2* from excitatory pyramidal neurons during embryonic development in mice also results in cortical hyperexcitability, spontaneous seizures and early death.²⁴ In contrast, the *K_v7* channel opener ezogabine/retigabine suppresses seizures in rodents and humans.⁴⁶

Despite its well-documented roles in dampening neuronal excitability and seizures,^{14,21-25,46,47} precise roles for *K_v7* channels in behaviors other than seizures remain unclear. Anti-depressant efficacy by *K_v7* channel opener ezogabine/retigabine was recently observed in humans with major depressive disorders⁴⁸ and a social defeat stress model of depression in rodents.⁴⁹ Although some patients with de novo EE mutations in *KCNQ2* have ASD as a behavioral comorbidity,^{36,42,50} accumulating whole-exome or targeted sequencing studies on well-defined ASD cohorts have identified NDD variants in both *KCNQ2* and *KCNQ3* genes in patients who do not have epilepsy.¹² The *KCNQ2* and *KCNQ3* transcripts are expressed early in the neocortex and hippocampus of the human brain prior to birth (<http://hbatlas.org/>) and in the embryonic neocortex of the mouse (<http://hbatlas.org/mouseNCXtranscriptome/>). The neonatal brain largely relies on *K_v7* channels for neuronal inhibition^{24,51} when GABA acts as an excitatory rather than an inhibitory neurotransmitter.⁵² Therefore, disruption of *K_v7* currents during the embryonic development of the brain may contribute to the onset of behaviors implicated in neuropsychiatric developmental disorders such as ASD. However, whether neuronal *K_v7* channels contribute to ASD-associated behaviors is unknown.

Recurrent heterozygous de novo NDD mutations are enriched at the key functional domains in *K_v7.2* and *K_v7.3* important for voltage-dependent activation of *K_v7* channels.¹² Furthermore, pathogenic *K_v7.2* mutations characterized to date are typically heterozygous loss-of-function mutations.^{27,32,40-43} Therefore, we hypothesize that heterozygous loss of *K_v7* currents by genetic knockout of *KCNQ2* gene would lead to the core ASD-associated behaviors in mice including reduced sociability and repetitive behaviors. In this study, we test this hypothesis by behavioral phenotyping both male and female heterozygous *KCNQ2*^{+/-} mice and their wild type *KCNQ2*^{+/+} littermates. Our results demonstrate for the first time a critical role of *K_v7.2* in multiple behaviors in mice including circadian-dependent locomotor activity, as well as exploratory, social approach and repetitive behaviors, thereby offering a genotype-phenotype link between the heterozygous loss of *K_v7* current and ASD-associated behaviors. Importantly, sex differences have been observed in social dominance and compulsive-like behaviors in *KCNQ2*^{+/-} mice.

2 | MATERIALS AND METHODS

2.1 | Experimental animals

All animal procedures were approved by the Institutional Animal Care and Use Committee of the University of Illinois at Urbana Champaign

and University of Connecticut. Heterozygous *KCNQ2* gene knockout (*KCNQ2*^{+/-}) mice on the C57BL/6J background were obtained from the Jackson Laboratory (*Kcnq2*^{tm1Dgen}/*Kcnq2*⁺, Stock Number: 005830²³). *KCNQ2*^{+/-} mice contain a deletion in the *KCNQ2* gene from base 418 to 535 by insertion of a LacO-SA-IRES-lacZ-Neo555G/Kan construct. These mice were originally produced by the Deltagen and were backcrossed at least five generations to C57BL/6J mice at the Jackson Laboratory. Upon arrival at our laboratory, these mice were backcrossed three more generations to C57BL/6J mice (Jackson Laboratory, Stock Number: 000664) before performing behavioral studies. For the tube dominance test and the three-chamber social interaction test, the outbred CD-1 (ICR) mice (Charles River Laboratory, Stock Number: 022) and the inbred C57BL/6J mice (Jackson Laboratory, Stock Number: 000664) were used as novel social targets, respectively. Both strains have been shown to be suitable for performing social behavioral tasks.⁵³ Breeding pairs to generate experimental mice consisted of *KCNQ2*^{+/-} mice crossed with C57BL/6J mice, yielding *KCNQ2*^{+/+} and *KCNQ2*^{+/-}. Mice were bred and housed on a normal 14:10 light:dark cycle with food and water available ad libitum. At weaning, mice were group housed (up to five mice per cage) with littermates of the same sex. Genotyping of these mice was determined by Polymerase Chain Reaction (PCR) on their tail genomic DNA. The primers used in genotyping were the wild-type forward primer (5'-ATC GTG ACT ATC GTG GTA TTC GGT G-3'), the reverse primer common to both target and endogenous genes (5'-GGT GAT AAG AAG GAA CTT CCA GAA G-3') and the Neo forward primer specific for the target gene only (5'-GGG CCA GCT CAT TCC TCC CAC TCA T-3'). The target gene refers to the truncated *KCNQ2* gene that is present only in *KCNQ2*^{+/-} mice. For electrophysiological recordings, we used constitutive *KCNQ2* heterozygous (*Kcnq2*^{+/-}) mice generated by crossing *Kcnq2*^{f/f24} to *Hprt-cre* (Jax stock# 004302) kept in a C57BL/6J background.

2.2 | Whole brain lysate preparation and western blotting

Mice were sacrificed by CO₂ inhalation and rapidly decapitated. Their brains were removed, dissected for specific brain regions (hippocampi, cortexes and cerebellums) and stored in -80°C. The dissected brain regions per mouse were homogenized in ice-cold homogenization buffer (solution A) containing (in mM): 320 sucrose, 1 NaHCO₃, 1 MgCl₂, 0.5 CaCl₂, 0.4 HEPES (pH 7.4) and Halt protease inhibitors (Thermo Fisher Scientific) as previously described.⁵⁴ After centrifuging 1400g for 10 minutes at 4°C, the homogenate supernatant (S1) was separated from insoluble tissue and nuclear pellet (P1). The S1 fraction was then centrifuged at 13800g for 10 min at 4°C. The supernatant (S2) was removed, and the remaining pellet (P2 membrane fraction) was resuspended in ice-cold solution B containing (in mM): 160 sucrose, 6 Tris-HCl, 0.5% Triton-X (pH 8.0) and Halt protease inhibitors. The S2 fraction is enriched with cytosolic soluble proteins. The P2 fraction is enriched with transmembrane proteins and membrane-bound proteins. BCA assay (Pierce) analysis was performed to determine protein concentrations across samples, which

were subsequently normalized to 0.5 mg/mL in Solution A (pH 7.4). The S1, S2 and P2 fractions were stored at -80°C until use. Hippocampal S2 and P2 fractions were immunoblotted with antibodies for K_v7.2 (1:200, Neuromab cat# 73-079), K_v7.3 (1:200, Alomone cat# APC-051) and GAPDH (1:1000, Cell Signaling) as described.⁵⁴ After incubating in HRP-conjugated secondary antibodies, the blots were visualized with enhanced chemifluorescence substrate (ECL, Thermo Fisher Scientific), and developed with a Konica SRX-101A film processor.

2.3 | Slice electrophysiology

Kcnq2 heterozygous knockout and control littermates (P21-P25) were anesthetized with isoflurane and rapidly decapitated. Both male and female mice were used. The brain was quickly removed and placed in ice-cold sucrose based cutting solution consisting of the following: 25 mM NaHCO₃, 200 mM sucrose, 10 mM glucose, 2.5 mM KCl, 1.3 mM NaH₂PO₄, 0.5 mM CaCl₂ and 7 mM MgCl₂. Transverse hippocampus slices were cut at 300 μm using a vibrating microtome (Leica VT1200S). Slices were then transferred to artificial cerebrospinal fluid (ACSF) consisting of the following (in mM): 125 NaCl, 26 NaHCO₃, 2.5 KCl, 1 NaH₂PO₄, 1.3 MgCl₂, 2.5 CaCl₂ and 12 glucose and equilibrated at 35°C for 30 minutes, and then maintained at room temperature for at least 1 hour before electrophysiological recordings. Cutting solution and ACSF were saturated with 95% O₂ and 5% CO₂. All experiments were performed at near normal physiological temperature (32°C). Whole-cell recordings were obtained using borosilicate glass electrodes having resistances of 2 to 4 MΩ. For current-clamp whole cell recordings we used an internal solution consisted of the following (in mM): 130 potassium methylsulfate, 10 KCl, 5 Tris-phosphocreatine, 10 HEPES, 4 NaCl, 4 Mg₂ATP and 0.4 Na₄GTP. The pH was adjusted to 7.2 to 7.3 with KOH. CNQX (4 μM), D-AP5 (10 μM) and picrotoxin (100 μM) were added in all slice experiments to block AMPA-mediated, NMDA-mediated, GABA-mediated synaptic transmission, respectively. CA1 pyramidal neurons were identified using 40x water-immersion objective lens on an upright microscope (BX51W, Olympus). Recordings were performed using a Multiclamp 700B amplifier (Molecular Devices Molecular Devices, Sunnyvale, CA), low pass-filtered at 2 kHz, sampled at 10 kHz, and analyzed off-line using either Prism 7 (Graphpad) or Clamfit 10 (Molecular Devices, Sunnyvale, CA). All recordings were performed blind.

2.4 | Mice for behavioral studies

A total of 23 male mice were used (*KCNQ2*^{+/+} n = 12; *KCNQ2*^{+/-} n = 11) and a total of 11 female mice were used (*KCNQ2*^{+/+} n = 5; *KCNQ2*^{+/-} n = 6). All behavioral tests were performed in a separate room from the colony, which were maintained on a "reverse" light: dark schedule with lights off at 10 AM and lights on at 10 PM Under a "reverse" light:dark schedule, we were able to perform all behavioral tasks during the dark phase when the mice are more active. This behavioral test room contained a separate animal housing area which was far from the behavioral testing area. At 2 weeks before the first

behavioral test, all test mice as well as novel social target mice were moved from the initial housing room to the behavioral test room on a "reverse" 12:12 light:dark cycle where they were subsequently habituated to this new cycle for 2 weeks. All mice were tested on all described tests in the following order: open field, habituated home cage activity, elevated plus maze, rotarod, self-grooming, marble burying, social interaction test, social dominance tube and urine marking test. Each test was performed at least 2 days after the previous test. Between each test, mice were returned to the animal housing area within this behavior test room. The order of these tests was chosen to conduct in general from least invasive to most invasive tests, and to reduce the potential impact of one behavioral assay to the subsequent ones.^{55,56} All apparatus was cleaned with Clidox (Pharmaceutical Research Laboratories) after testing each mouse to eliminate any residual olfactory cues and mouse droppings. Mice were weighed at the time of each behavior study. Except for the habituated home cage activity which allows continuous video monitoring during 12:12 light:dark cycles (see the method description below),⁵⁷ each test was always performed under red light during the dark phase starting at 10 AM as described.⁵⁸ Mice were at 4 months old when behavioral studies began and at 6 months old when the last behavioral studies were conducted. After completing all the behavioral assays, all tested mice were used for scoring behavioral seizures induced by kainic acid except for one female *KCNQ2*^{+/-} mouse because of its death.

2.5 | Open field test

The open field test was performed as described⁵⁷ to evaluate exploratory and anxiety-like behavior in mice. Mice were placed in the center of an open field arena (26 in. × 26 in. × 12 in.) and allowed to explore for 5 minutes. Distance traveled throughout the arena, number of entries into and duration in the central square (14 in. × 14 in.), 6 in. away from the sides, were recorded by TopScan video-tracking software (CleverSystems, Reston, VA). After each trial, the mouse was returned to their home cage and the arena was cleaned before the next test mouse.

2.6 | Habituated home cage activity

Mice were placed individually into custom-made acrylic home cages (18.5 × 33.5 × 16 cm) with clear plastic lids that allow for continuous video tracking by TopScan, as described previously.⁵⁸ Mice were measured continuously for 4 days with the normal light cycle of 12 hours of light and 12 hours of dark. After an extended habituation period of 3 days, the average distance traveled during the 4th day was compared between genotypes. Day-4 was chosen to represent behavioral patterns established in an acclimated environment of the home cage, without any confounding effects of novelty. After the test, the mice were individually housed in the animal facilities, and then left undisturbed for 4 days. The video tracking cages were cleaned prior to the next test mice.

2.7 | Elevated plus maze (EPM) test

The EPM test was performed as described⁵⁸ to monitor open arm avoidance, exploration and general anxiety-like behavior. The maze is constructed of acrylic. There are two open arms (30 × 5 cm) and two enclosed arms (30 × 5 cm). The closed arms contain 20 cm-high walls, whereas the open arms have no walls. Arms emerge from the central zone platform (5 × 5 cm) and are arranged such that two pairs of identical arms are opposite to each other. The entire apparatus is raised to a height of 50 cm above floor. Each mouse was placed in the central zone platform of the maze and monitored for 5 minutes using the TopScan program while the experimenter sat 2 m away directly in line with the closed arms. The number of entries, the duration, the total distance traveled and the average velocity of the mice in the open and closed arms were compared between groups. After the test, the mouse was returned to their home cage and the EPM was cleaned before the next test mouse.

2.8 | Rotarod test

Rotarod testing was performed as described to assess motor coordination.⁵⁹ Mice were placed on the rotarod apparatus starting at 0 rotation per minute (rpm). The start switch was then turned on to rotate the dowel at a constant acceleration rate (60 rpm/m). The latency of the mice to fall off the apparatus was recorded by photobeam counters as well as an experimenter using a stopwatch. Each mouse was subjected to three trials and the average latency from three trials was computed. The dowel was cleaned between each trial and dried with a paper towel.

2.9 | Self-grooming

Self-grooming behavior of the mice was observed to monitor repetitive behaviors in mice as previously described⁶⁰ by video monitoring for 10 minutes when test mice were undisturbed in their home cages without bedding. The duration of grooming events and the total number of grooming events in 10 minutes in each individual mouse was quantified. Based on average grooming times previously exhibited in C57BL/6J mice,⁶¹ repetitive self-grooming was quantified as a period ≥2 seconds spent grooming with no more than a 5-second interval between grooming bouts.

2.10 | Marble burying test

Spontaneous burying of marbles by mice in a novel cage was observed to monitor compulsive-like behaviors in mice as previously described.^{62,63} In brief, 20 marbles were placed on top of the approximately 5 cm-deep bedding and arranged in a rectangular, 4 by 5 array in a cage that is half filled with bedding. The mouse was placed in this cage for 30 minutes. The mouse was then removed and the cage floor photographed. The total number of buried marbles was recorded. A marble that was two-thirds or more under bedding was considered

“buried” experimentally and counted. This procedure was repeated with a new cage with fresh bedding and marbles.

2.11 | The three-chamber social interaction test

The three-chamber social interaction test was performed with modifications as described^{64–66} to measure affiliative behavior and social recognition. The three-chamber social testing rectangular arena was made with clear plexiglas. Each chamber is 20 × 40 × 25 cm in size with clear dividing walls which has rectangular openings (5 × 8 cm) allowing access into each chamber. The test was composed of three sessions: habituation, sociability and social novelty. Each session lasted 10 minutes. Between sessions, mice were removed from the chambers, and the chambers of the arena were thoroughly cleaned. For “habituation,” the test mouse (*KCNQ2*^{+/-} or *KCNQ2*^{+/+}) was placed in the center chamber and allowed to freely explore the empty left and right chambers. After the habituation in the empty three-chamber apparatus for 10 minutes, the test mouse was reintroduced to the center chamber and allowed to explore the left and right chambers each of which contained an empty cylinder wire cage (15 cm diameter and 20 cm height). During this habitation period, the test mouse did not show any baseline side preference for either a left or a right chamber (Figure S2, Tables S6–7). In the “sociability” session, a C57BL/6J mouse (a stranger mouse-1 of the same sex which never encountered the test mouse before) was placed underneath the wire cage in the left chamber. The test mouse was then placed in the center chamber and allowed to freely explore the left chamber containing the novel social target and the right chamber containing an empty cage. The wire cage allows olfactory, visual, auditory and minimal tactile interaction but prevents fighting between the test mouse and the stranger mouse-1. This first session measures affiliative behavior of a test mouse which is defined as the propensity to spend time with a stranger mouse rather than with a familiar object. In the “social novelty” session, another C57BL/6J mouse (stranger mouse-2 of the same sex which never encountered the test mouse before) was placed underneath the wire cage in the right chamber. The test mouse was then placed in the center chamber and allowed to freely explore both the left chamber containing familiar stranger-1 mouse and the right chamber containing a novel stranger mouse-2. The mouse behavior was tracked and recorded by the TopScan “sniffing” program, which automatically detects when the mouse is sniffing an object, defined by the physical contacts between the boundaries of the object and the nose, head and forelimbs.⁶⁵ The amount of time spent in sniffing the cage and the number of entries into the sniffing zone in each chamber were recorded. In addition, the velocity of the mice and the total distance traveled by the mice while in the sniffing zones and throughout the entire arena were also recorded. This test measures social recognition and preference for social novelty, which is defined as the propensity to spend time with a previously unencountered mouse rather than with a familiar mouse.

2.12 | Tube dominance test

The tube dominance test was performed as described^{67,68} to measure social approach-avoidance behavior and measure dominant vs submissive behaviors in mice. The tube apparatus is composed of clear plexiglas 30 cm in length and 4 cm diameter, a size just sufficient to permit one adult mouse to pass through without reversing direction and to restrict aggressive fights. Mice of the same sex but different genotypes (ie, the test mouse [*KCNQ2*^{+/+} or *KCNQ2*^{+/-}] against CD-1 mouse) were released into opposite ends of a tube at the same time. The subject mouse (*KCNQ2*^{+/-} or *KCNQ2*^{+/+}) was tested against an outbred CD-1 (ICR) mouse of the same sex purchased from the Charles River Laboratories. The CD-1 mice were used as social targets in this test because they have been shown to display more aggressive behavior than C57BL/6J mice.⁵³ The test was repeated using a round-robin design such that each *KCNQ2*^{+/-} or *KCNQ2*^{+/+} mouse was matched with every other CD-1 mouse 2 times. The two mice interacted in the center of the tube. The more dominant mouse will show greater aggression and force its opponent out of the tube. When one mouse has all four paws out of the tube, it is declared the loser, ending the match. The dominant mouse remaining inside the tube is declared the winner. The winner receives +1 point, whereas the loser gets -1 point. If the two mice stay or pass each other in the tube, both mice would receive 0. The number of wins is reported as a percentage (%) of total number of matches.

2.13 | Urine marking test

The urine marking assay was performed as described^{69,70} to observe social dominance hierarchy. In this test, dominant mice mark larger territories than subordinate mice. The filter paper was placed in an empty cage. The cage was divided into two compartments of equal size by a wire mesh partition which allows mice in each compartment to see and smell each other but prevents their physical contact. Each pair of mice (ie, the test mouse *KCNQ2*^{+/+} or *KCNQ2*^{+/-} against CD-1 mouse purchased from the Charles River Laboratories) was placed on opposite sides of a partition and allowed to interact through the wire mesh partition and mark on the filter paper for 30 minutes. After the end of each session, the mice were moved to their home cages. The urine-marked filter papers were dried overnight. To analyze the urine marks visualized by a UV light source, a transparent grid (each 1 × 1 cm) was placed over the dried filter paper. The number and the area of scent marks as well as the average distance of urine marking from the wire partition were measured.

2.14 | Kainate-induced seizures

To measure seizure propensity, both male and female mice were subjected to intraperitoneal (i.p.) injection of vehicle control (saline) or kainate (Abcam, 15 mg/kg) as described.⁵⁴ The mice were at 6-month old, and weighed 24 to 33 g when kainate injections were performed in the laboratory under the bright light. Mice were returned to their home cage and monitored for their behavioral seizures every 10 minutes using modified Racine, Pinal and Rovner scale,⁷¹ which consists of eight

stages: (stage-1) facial movements only, (stage-2) facial movements and head nodding, (stage-3) facial movements, head nodding and forelimb clonus, (stage-4) facial movements, head nodding, forelimb clonus and rearing, (stage-5) facial movements, head nodding, forelimb clonus, rearing, loss of balance and falling, (stage-6) a stage-5 terminating with multiple rearing and falling episodes, (stage-7) a stage-6 with a violent jumping and running fit, (stage-8) a stage-7 with periods of tonus and (stage-9) death. At 2 hours post injection, the mice were euthanized and their brains were stored at -80°C until use.

2.15 | Statistical analysis

All analyses are reported as mean \pm SEM. The n values indicate number of mice. Origin Pro 9.5 (Origin Lab) was used to perform statistical analyses. Data were analyzed using two-way ANOVA with genotype as one factor and sex as the other. For social interaction tests, data were analyzed using three-way repeated measures ANOVA with chamber entered as the within-subjects factor, and genotype and sex as between-subjects factors. In cases where the three-way interaction was significant, two-way repeated measures analyses were conducted separately for each sex. Tukey tests were used to establish post hoc pair-wise differences between means of >2 groups. The Student two-tailed *t* test was used to establish pair-wise differences between means of two groups. A priori value (*P*) $< .05$ was used to establish statistical significance.

3 | RESULTS

3.1 | Heterozygous loss of $K_v7.2$ increases exploratory behavior without affecting habituated home cage activity or motor coordination

To examine the effects of the heterozygous loss of $K_v7.2$ in behaviors, both male and female heterozygous $KCNQ2^{+/-}$ mice and their wild-

type $KCNQ2^{+/+}$ littermates at age 16 weeks were subjected to nine behavioral tests and then kainate-induced seizures (Figure 1A-C, Table S1). After confirming the genotype (Figure 1B,C), the mice were tested during the dark phase when they were most active because of their nocturnal nature (Figure 1A).

In the home cage, both male and female $KCNQ2^{+/-}$ mice displayed similar total 24 hours activity as the wild-type mice on day 4 (genotype: $F_{(1, 30)}=0.02$, $P = .889$) (Figure 2A,B). The $KCNQ2^{+/-}$ mice showed similar activity in their home cage during the dark phase compared with wild-type mice (genotype: $F_{(1, 30)}=0.80$, $P = .378$) (Figure 2A,C, Table S2). During the light phase when mice were generally less active, $KCNQ2^{+/-}$ mice displayed significantly greater activity compared with wild-type mice (genotype: $F_{(1, 30)}=10.08$, $P = .003$) (Figure 2A,D, Table S2). Both $KCNQ2^{+/+}$ and $KCNQ2^{+/-}$ mice were similar in their latency to fall from the rotarod (genotype: $F_{(1, 30)}=0.13$, $P = .726$), indicating no gross abnormality in motor coordination (Figure 2E, Table S2). In the open field test, the total distance traveled in the entire arena was not significantly different between $KCNQ2^{+/+}$ and $KCNQ2^{+/-}$ mice (genotype: $F_{(1, 31)}=0.07$, $P = .796$) (Figure 3A,B, Table S3). Taken together these results indicate that $KCNQ2^{+/+}$ and $KCNQ2^{+/-}$ mice show comparable levels of physical activity and motor coordination in tests of exploratory behavior and in the home cage under habituated conditions and motor coordination on the rotarod.

However, the $KCNQ2^{+/-}$ mice displayed a higher number of entries into the center of the open field arena compared with the wild-type littermates (genotype: $F_{(1, 31)}=6.38$, $P = .017$) (Figure 3A,C, Table S3). Furthermore, both male and female $KCNQ2^{+/-}$ mice spent significantly more time within the center of the arena than the wild type mice (genotype: $F_{(1, 31)}=12.57$, $P = .001$) without increasing the total distance traveled within the center (genotype: $F_{(1, 31)}=2.23$, $P = .145$) (Figure 3D,E, Table S3). Interestingly, the male $KCNQ2^{+/-}$ mice traveled faster whereas the female $KCNQ2^{+/-}$ moved slower within the center of the arena than the wild type male mice (sex: $F_{(1, 31)}=5.74$, $P = .026$, interaction:

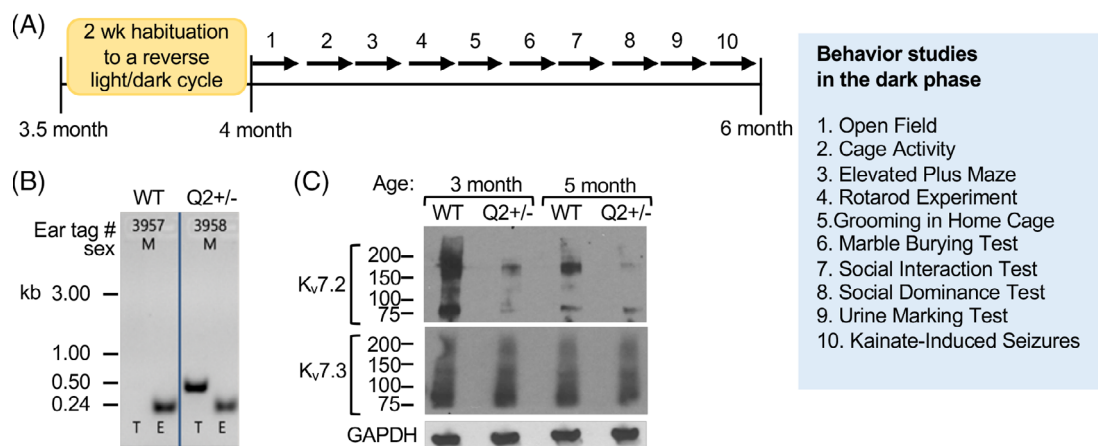


FIGURE 1 An overview of behavioral assays performed with $KCNQ2^{+/+}$ and $KCNQ2^{+/-}$ mice. A, The order of behavioral assays performed on $KCNQ2^{+/+}$ (WT) and $KCNQ2^{+/-}$ ($Q2^{+/-}$) mice. All behavioral assays mice were performed in the dark phase except for the home cage activity during a light:dark cycle (12 hours each) measured over 4 days. B, Sample genotyping results for the male (M) cohorts of WT and $Q2^{+/-}$ mice showing a 240-bp PCR product from the wild-type allele ("E" lanes) and a 427-bp product from the knockout allele ("T" lanes). C, Western blots of $K_v7.2$, $K_v7.3$ and GAPDH in the hippocampal membrane P2 fractions of WT and $Q2^{+/-}$ mice at age 3 and 5 months. The $Q2^{+/-}$ mice displayed reduced protein expression of $K_v7.2$ but not $K_v7.3$ in the hippocampi compared with the WT mice

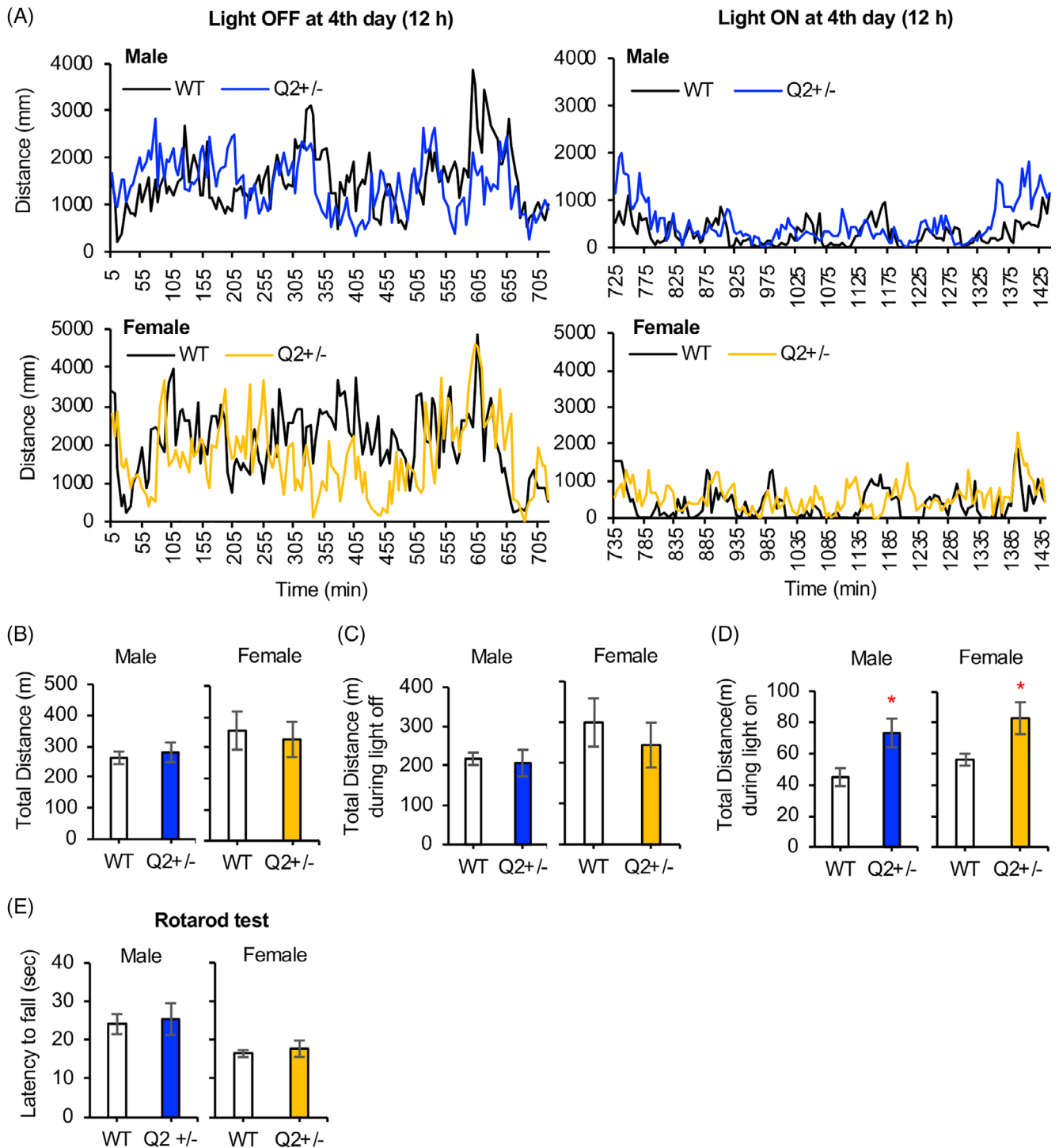


FIGURE 2 The $KCNQ2^{+/-}$ mice show increased home cage activity during the light phase. A–D, Home cage activity of male and female $KCNQ2^{+/+}$ (WT) and $KCNQ2^{+/-}$ ($Q2^{+/-}$) mice was examined using continuous video recording for 4 days. A, Sample traces of home cage activity of $KCNQ2^{+/+}$ (WT) and $KCNQ2^{+/-}$ ($Q2^{+/-}$) mice at the 4th day of recording. The locomotor activities of male mice and female mice were assessed by measuring the distance which they traveled per minute over a 12 hours dark phase (Light OFF) followed by a 12 hours light phase (Light ON). Quantifications of the average total distance traveled during a 24 hours period (B), a 12 hours dark phase (C) and a 12 hours light phase (D). The total distance traveled by $Q2^{+/-}$ mice during the light phase was significantly greater than that of WT mice for both sexes. E, The rotarod tests revealed no difference in latency to fall for WT and $Q2^{+/-}$ mice. Male mice (WT $n = 12$; $Q2^{+/-}$ $n = 11$). Female mice (WT $n = 5$; $Q2^{+/-}$ $n = 6$). Data shown represent the Ave \pm SEM. Student t test results are shown here (* $P < .05$). Table S2 shows two-way ANOVA test results with genotype as one factor and sex as the other

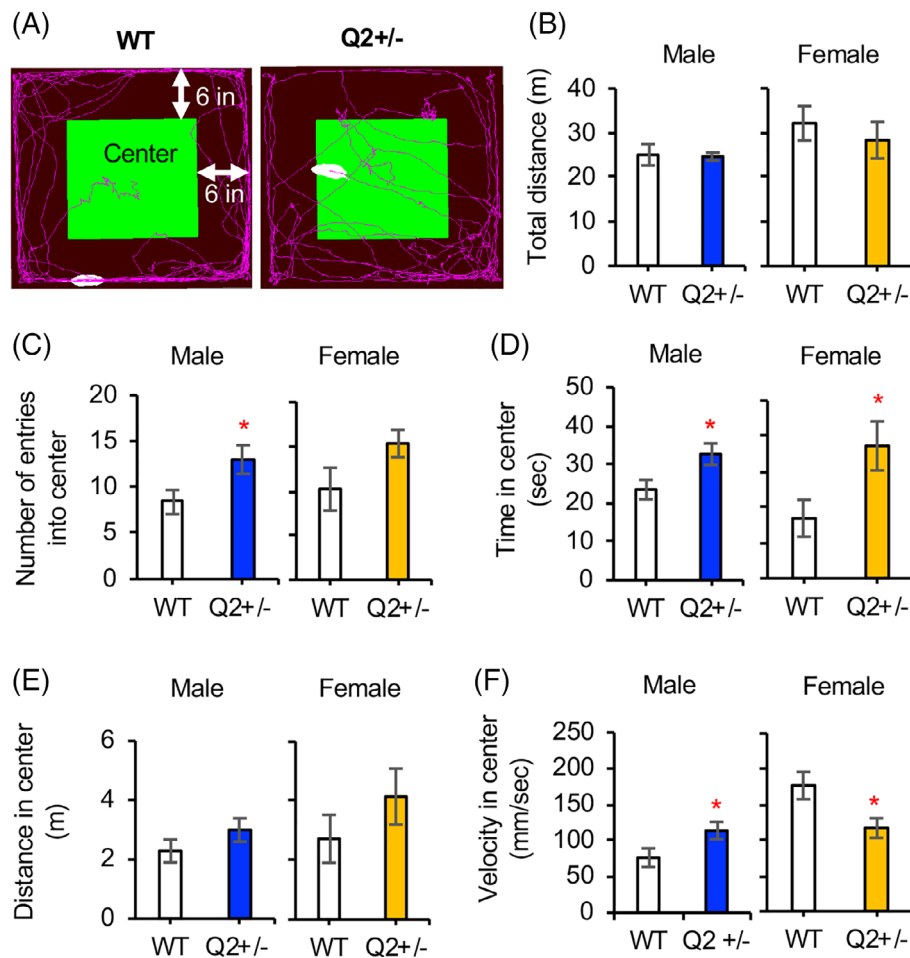


FIGURE 3 The $KCNQ2^{+/-}$ mice display increased exploratory behavior in the center of the open field. A, Sample traces of the open field activity of male $KCNQ2^{+/+}$ (WT) and $KCNQ2^{+/-}$ (Q2+/-) mice for 5 minutes. The traces are shown in red. The mice are shown in white. B, The total distance traveled throughout the open field arena was similar between WT and Q2+/- regardless of sex. B-F, Quantifications of the entries into the center zone (C) as well as the duration (D), distance (E) and velocity (F) within the center zone. The male Q2+/- mice displayed more entry into the center zone and spent more time and traveled with higher velocity in the central zone than WT mice (WT $n = 12$; Q2+/- $n = 11$). The female Q2+/- mice spent more time but traveled slower in the central zone than WT mice (WT $n = 5$; Q2+/- $n = 6$). Data shown represent the Ave \pm SEM. Student t test results are shown here (* $P < .05$). Table S3 shows two-way ANOVA test results with genotype as one factor and sex as the other

$F_{(1, 31)}=4.67, P = .039$) (Figure 3F, Table S3). In the EPM, the total distance traveled throughout the EPM was not different between $KCNQ2^{+/-}$ and wild-type mice (genotype: $F_{(1, 30)}=1.92, P = .968$) (Figure 4A,B, Table S4). However, both male and female $KCNQ2^{+/-}$ groups displayed significantly more entries into the open arms (genotype: $F_{(1, 30)}=10.52, P = .003$) (Figure 4A,C, Table S4). Compared with the wild-type group, the $KCNQ2^{+/-}$ mice traveled longer distances and spent more time in the open arms (genotype: $F_{(1, 30)}=6.99, P = .013$) (Figure 4D-F, Table S4). There was a trend in the distances traveled in the open arms (genotype: $F_{(1, 30)}=4.11, P = .052$) and a significant effect of sex in the latency to enter the open arms (genotype: $F_{(1, 30)}=5.24, P = .029$) (Figure 4E,F Table S4). In contrast, no significant differences in the total number of entries, distance and duration in the closed arms were observed in the $KCNQ2^{+/-}$ mice compared with the wild type mice (Figure S1, Table S4). These results together suggest that the heterozygous loss of $K_v7.2$ leads to enhanced exploratory behavior or decreased anxiety-like behavior, without affecting motor coordination or general activity levels.

3.2 | Heterozygous loss of $K_v7.2$ increases repetitive and compulsive-like behaviors

During the video recording of the home cage activity, we observed that the $KCNQ2^{+/-}$ mice have the tendency to groom more than the

wild-type littermates. To test if this trend is significant, we quantified the grooming behavior of $KCNQ2^{+/+}$ and $KCNQ2^{+/-}$ mice. The $KCNQ2^{+/+}$ mice displayed bouts of self-grooming that lasted between 10 and 25 seconds (Figure 5A), consistent with the reported duration of self-grooming in C57BL/6J strain for both sexes.⁷² Compared with the wild type littermates, the average duration of a grooming event was increased in $KCNQ2^{+/-}$ mice (genotype: $F_{(1, 30)}=8.79, P = .006$) (Figure 5A) and sex differences were observed (sex: $F_{(1, 30)}=13.77, P = .001$) (Table S5). The total number of grooming events in the 10 minutes test period was different between $KCNQ2^{+/+}$ and $KCNQ2^{+/-}$ groups (genotype: $F_{(1, 30)}=5.19, P = .03$) (Figure 5B). Such elevation in the innate self-grooming behavior is suggestive of repetitive behaviors.^{60,61}

To investigate this further, we performed a marble burying test which scores spontaneous burying of marbles in rodents as a measure of their compulsive-like behavior.^{62,63} In this test, the $KCNQ2^{+/-}$ mice buried more marbles than the wild-type mice (genotype: $F_{(1, 30)}=5.82, P = .022$). There was a significant interaction between sex and genotype (interaction: $F_{(1, 30)}=7.06, P = .013$). Post hoc tests indicated that the male $KCNQ2^{+/-}$ mice buried twice as many marbles as the wild-type mice (Tukey test, $P = .001$) (Figure 5C,D, Table S5). This genotype difference in marble burying behavior was not observed in the females (Tukey test, $P = .999$) (Figure 5C,D, Table S5). These findings

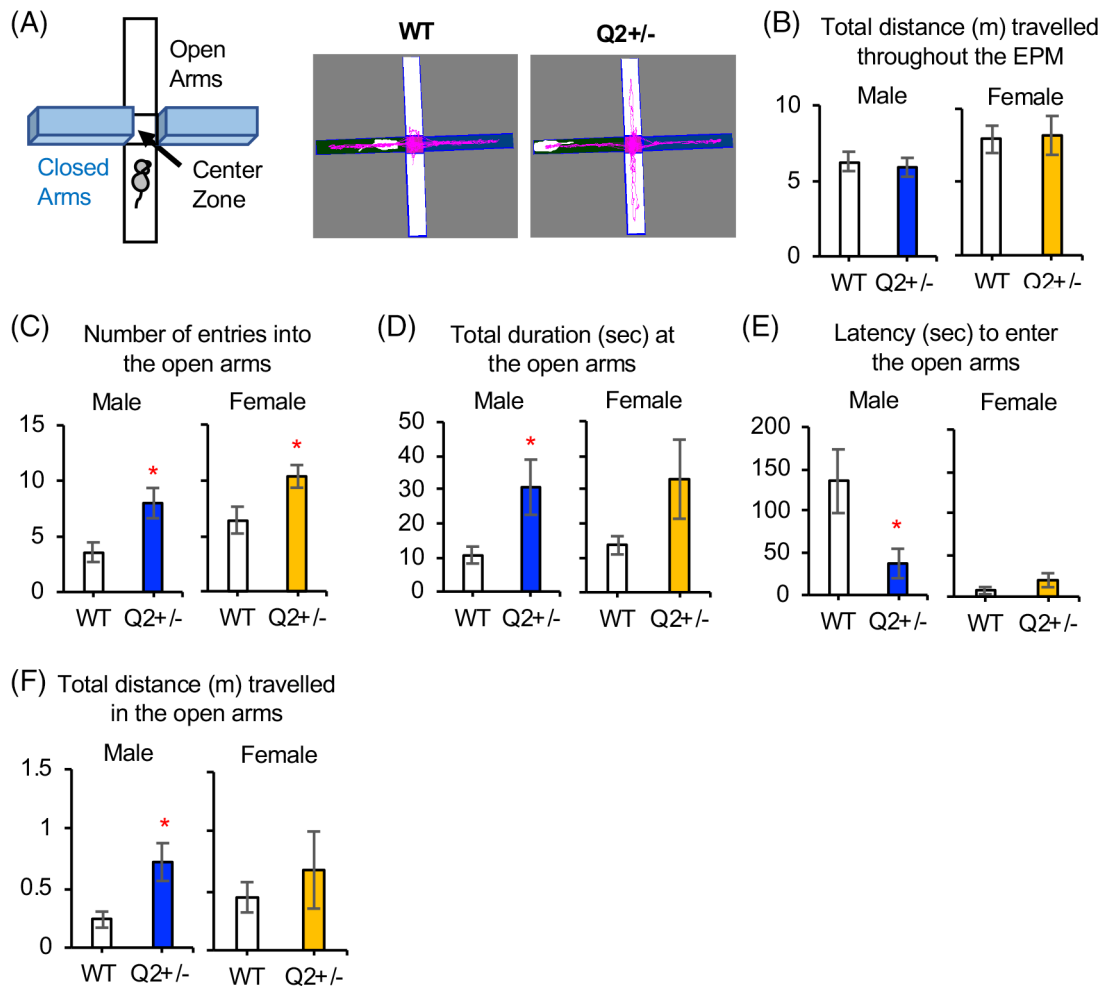


FIGURE 4 The *KCNQ2^{+/-}* mice display increased exploratory behavior in the open arms of the elevated plus maze. **A**, A schematic of an elevated plus maze (EPM) and sample traces of locomotor activity of male *KCNQ2^{+/+}* (WT) and *KCNQ2^{+/-}* (Q2+/-) mice for 5 minutes in the EPM. The traces are shown in red. The mice are shown in white. **B**, The total distance traveled throughout EPM was similar between WT and Q2+/- regardless of sex. **C-F**, Quantifications of the number entries into the open arms (**C**), duration at the open arms (**D**), latency to enter the open arms (**E**) and total distance traveled in the open arms (**F**). The male Q2+/- mice had more entry into the open arms with decreased latency and spent more time and traveled longer distance within the open arms compared with the WT mice (WT $n = 12$; Q2+/- $n = 11$). The female Q2+/- mice had more entry into the open arms compared with the WT mice (WT $n = 5$; Q2+/- $n = 6$). Data shown represent the Ave \pm SEM. Student t test results are shown here ($*P < .05$). Figure S1 shows the quantifications of the number entries into the closed arms, duration at the closed arms, latency to enter the closed arms and total distance traveled in the closed arms. Table S4 shows two-way ANOVA test results with genotype as one factor and sex as the other

indicate that heterozygous loss of $K_{v}7.2$ increases compulsive-like behaviors in male mice but not female mice.

3.3 | Heterozygous loss of $K_{v}7.2$ decreases social interest

To examine social behaviors, we first used the three-chamber social interaction test which measures social motivation and approach (Figure 6A).⁶⁴ After habituation in the empty three-chamber apparatus for 10 minutes, the subject mouse (*KCNQ2^{+/+}* or *KCNQ2^{+/-}*) was reintroduced to the center chamber of the apparatus that contained an empty wire cage in the left and right chambers. During this second habituation time, the subject *KCNQ2^{+/+}* or *KCNQ2^{+/-}* mouse spent

similar amounts of time in the left and right chambers (Figure S2A), as indicated by no significant main effect of chamber ($F_{(1, 30)}=1.89$, $P = .179$) or interaction between chamber and genotype ($F_{(1, 30)}=0.19$, $P = .663$). Both genotypes also displayed similar sniffing duration toward the empty wire cages (chamber: $F_{(1, 30)}=0.52$, $P = .477$; interaction between chamber and genotype: $F_{(1, 30)}=1.73$, $P = .198$), and latency (chamber: $F_{(1, 30)}=0.29$, $P = .594$; interaction between chamber and genotype: $F_{(1, 30)}=0.01$, $P = .920$) (Figure S2B-C, Tables S6-7). In addition, no significant effect of sex or interactions between sex was observed for any of these outcome variables. These results indicate that there is no significant preference for one side or another and no difference between *KCNQ2^{+/+}* and *KCNQ2^{+/-}* mice in their interaction with the inanimate wire cages in the left and right chambers.

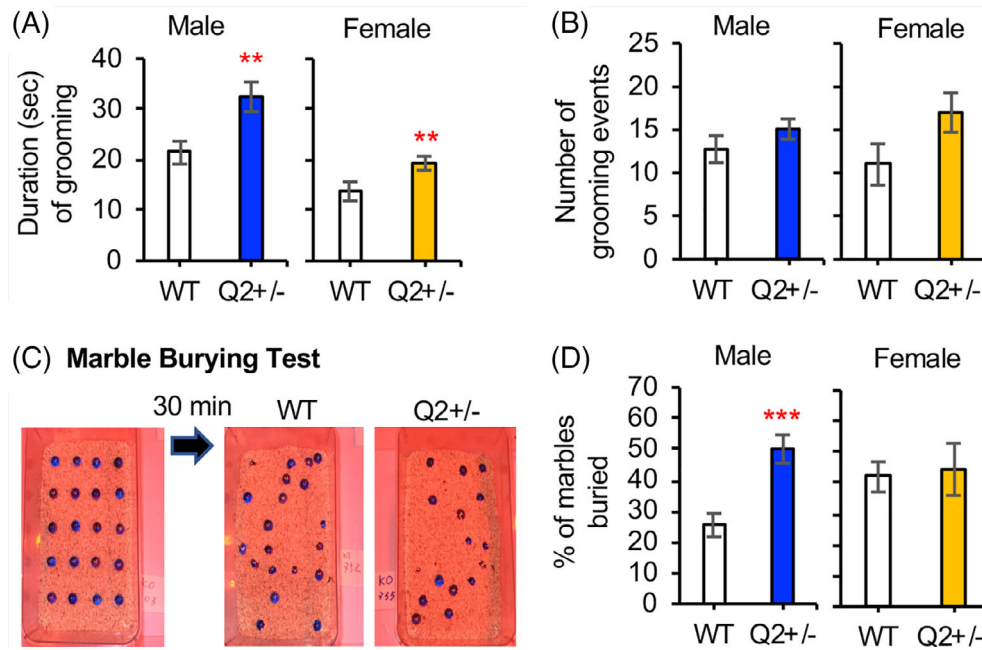


FIGURE 5 The $KCNQ2^{+/-}$ mice display increased repetitive grooming and marble burying behaviors. A-B, The grooming event of $KCNQ2^{+/+}$ (WT) and $KCNQ2^{+/-}$ ($Q2^{+/-}$) mice were recorded for 10 minutes in their home cages without the bedding. A, Both male and female $Q2^{+/-}$ mice groomed themselves for a longer duration of time during their grooming events compared with their WT littermates. B, WT and $Q2^{+/-}$ mice displayed similar number of grooming events regardless of sex. C-D, The WT and $Q2^{+/-}$ mice were subjected to the marble bearing test for 30 minutes. C, Representative results of the marble burying activity of male WT and $Q2^{+/-}$ mice. D, The male $Q2^{+/-}$ mice buried a larger percentage (%) of the marbles than the WT mice (WT $n = 12$; $Q2^{+/-} n = 11$). The female $Q2^{+/-}$ mice buried similar amounts of marbles as the WT mice (WT $n = 5$; $Q2^{+/-} n = 6$). Data shown represent the Ave \pm SEM. Student t test results are shown here (** $P < .01$, *** $P < .005$). Table S5 shows two-way ANOVA test results with genotype as one factor and sex as the other

To test social motivation and approach, a novel social target C57BL/6J mouse (stranger mouse-1) was placed in the wire cage of the left chamber and the subject ($KCNQ2^{+/+}$ or $KCNQ2^{+/-}$) mouse was introduced in the center chamber (Figure 6A). The three-way ANOVA indicated a trend for an interaction between chamber and genotype for duration spent on the left or right sides of the chamber ($F_{(1, 30)} = 4.14$, $P = .051$). Hence, data were analyzed using separate two-way ANOVAs for each chamber. The two-way repeated ANOVA revealed that $KCNQ2^{+/+}$ mice spent significantly more time in the left chamber containing the stranger mouse-1 than $KCNQ2^{+/-}$ mice (genotype: $F_{(1, 30)} = 5.05$, $P = .032$) whereas both genotypes spent similar duration at the right chamber containing an empty cage (genotype: $F_{(1, 30)} = 0.93$, $P = .343$) (Figure 6B, Tables S6-7). Interestingly, sex differences were observed for the duration at the left chamber (sex: $F_{(1, 30)} = 23.87$, $P < .0001$) and duration of sniffing at the stranger mouse-1 (sex: $F_{(1, 30)} = 9.20$, $P = .005$). Females spent longer time in the left chamber (Figure 6B) but sniffed a stranger mouse-1 for shorter time than males did (Figure 6C, Tables S6-7). In addition, $KCNQ2^{+/+}$ males spent significantly more time sniffing at the stranger mouse-1 compared with $KCNQ2^{+/-}$ males (genotype: $F_{(1, 21)} = 5.07$, $P = .035$) (Figure 6C, Tables S6-7). Lastly, the $KCNQ2^{+/-}$ mice also displayed increased latency of sniffing at the novel social target compared with the wild-type mice (genotype: $F_{(1, 30)} = 4.59$, $P = .040$) (Figure 6C,D, Tables S6-7). These results together indicate that the $KCNQ2^{+/-}$ mice

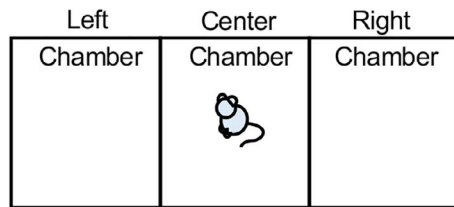
display reduced social approach and motivation than their wild type littermates.

To test the preference for social novelty and social memory, another novel target C57BL/6J mouse was placed in the wire cage of the right chamber (Figure 6A). In the second session of this three-chamber test, a mouse would normally recall its previous contact with the stranger mouse-1 in the left chamber and thus will spend more time with the newly introduced stranger mouse-2 in the right chamber (Figure 6A). A significant three-way interaction between sex, genotype and chamber was observed for duration at the chamber (interaction: $F_{(1, 29)} = 8.60$, $P = .007$) and duration sniffing (interaction: $F_{(1, 29)} = 6.93$, $P = .014$) (Table S6), hence sexes were analyzed separately (Figure 6E,F). Within males, a main effect of chamber was observed (chamber: $F_{(2, 40)} = 28.82$, $P < .0001$), but no significant effect of genotype, or interaction between genotype and chamber was detected. These data indicate that both $KCNQ2^{+/+}$ and $KCNQ2^{+/-}$ males preferred the novel mouse (a stranger mouse-2) in the right chamber.

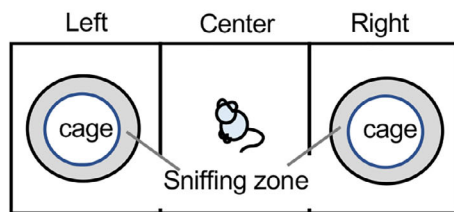
In females, a significant interaction was observed between genotype and chamber for duration (interaction: $F_{(2, 19)} = 4.49$, $P = .026$). Wild-type females spent a longer time in the right chamber containing a stranger mouse-2 than the left chamber containing a familiar mouse (Tukey test, $P = .026$) (Figure 6E, Tables S6-7), indicating that they display preference for social novelty. In contrast, $KCNQ2^{+/-}$ female mice

(A) The 3-Chamber Social Interaction Test

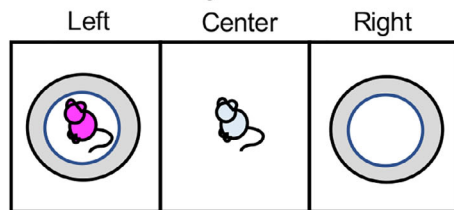
1. Habituation in an empty apparatus



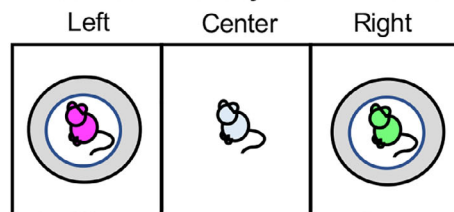
2. Habituation with 2 empty wire cages






3. Test sociability to a novel mouse

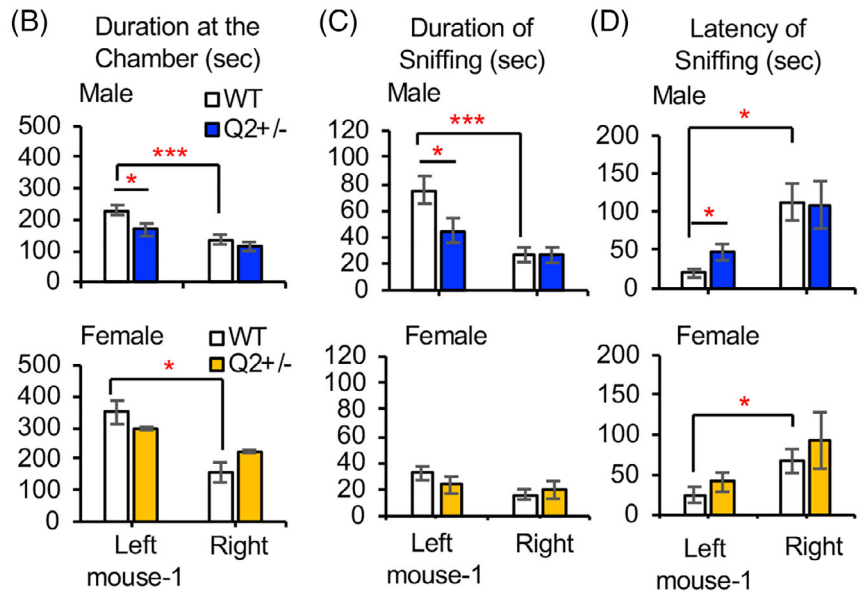


4. Test social novelty to a 2nd mouse



-  A test mouse (WT or Q2+/-)
-  A stranger mouse-1
-  A stranger mouse-2

Sociability Test



Social Novelty Test

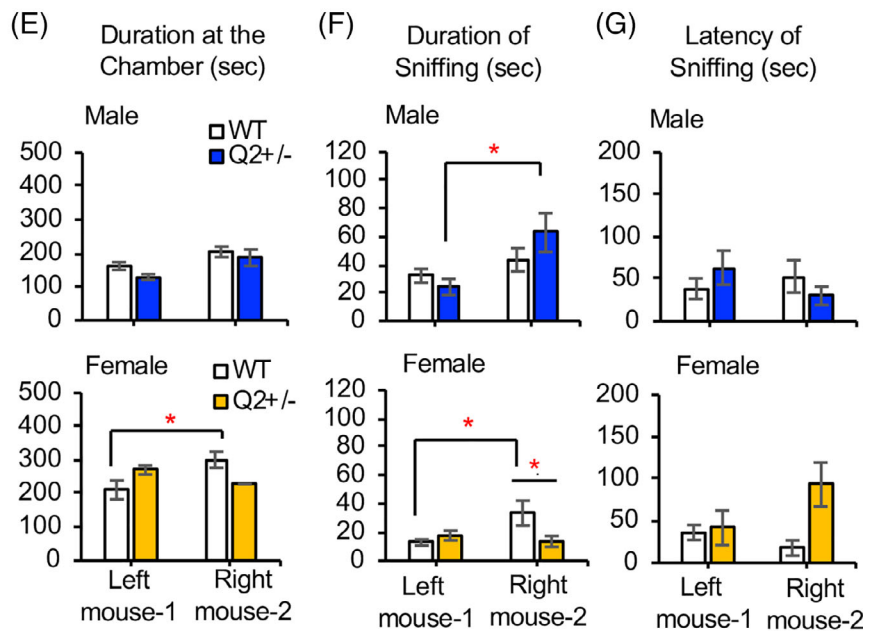


FIGURE 6 The *KCNQ2*^{+/-} mice display reduced sociability. The *KCNQ2*^{+/+} (WT) and *KCNQ2*^{+/-} (Q2+/-) mice were subjected to the three-chamber social interaction test. A, The design of the three-chamber social interaction test. The test mouse was first habituated in the empty apparatus for 10 minutes (step-1) and then in the apparatus containing an empty wire cage each in the left and right chambers for 10 minutes (step-2). During the "sociability" test, a stranger mouse-1 (age-matched C57BL/6J) was placed inside the wire cage of the left chamber, and the test mouse was introduced into the center chamber and allowed to explore for 10 minutes (step-3). During the "social novelty" test, a second stranger mouse-2 (another age-matched C57BL/6) was placed inside the wire cage of the right chamber, and the test mouse was introduced into the center chamber and allowed to explore for 10 minutes (step-4). B-D, Quantifications of the time spent at each chamber (B) as well as the duration (C) and latency (D) of sniffing behavior during the "sociability" test. E-G, Quantifications of the time spent at each chamber (E) as well as the duration (F) and latency (G) of sniffing behavior during the "social novelty" test. Male mice (WT n = 12; Q2+/- n = 11). Female mice (WT n = 5; Q2+/- n = 6). Data shown represent the Ave ± SEM. Post hoc Tukey test results are shown here (*P < .05, ***P < .005). Figure S2 shows the quantification of the time spent at each chamber, as well as the duration and latency of sniffing behavior during the "habituation with 2 empty cages." Table S6 shows three-way ANOVA test results and Table S7 shows two-way ANOVA test results with chamber as one factor and genotype as the other, or with genotype as one factor and sex as the other

spent similar times in left and right chambers (Tukey test, $P = .546$) (Figure 6E, Tables S6-7). Similarly, in females, a significant interaction was observed between genotype and chamber for duration sniffing (interaction: $F_{(1, 9)} = 7.12$, $P = .026$). Wild-type females spent more time sniffing a new stranger mouse-2 compared with a familiar mouse (Tukey test, $P = .049$) whereas $KCNQ2^{+/+}$ females did not (Tukey test, $P = .921$) (Figure 6F). $KCNQ2^{+/-}$ females spent less time sniffing a new stranger mouse-2 compared with their wild-type littermates (Tukey test, $P = .047$) (Figure 6F, Table S7). Finally, a significant interaction between genotype and chamber was observed for latency to visit the novel mouse in females (interaction: $F_{(1, 9)} = 6.54$, $P = .031$). Post hoc tests indicated a trend for the $KCNQ2^{+/-}$ to display a longer latency to sniff the new stranger mouse-2 as compared with $KCNQ2^{+/+}$ (Tukey test, $P = .056$) (Figure 6G, Tables S6-7) whereas no

genotype difference was observed for latency to sniff the familiar mouse. Taken together, these results on duration, sniffing and latency indicate that the $KCNQ2^{+/-}$ female mice display no preference for the novel social target with the familiar social target.

3.4 | Heterozygous loss of $K_v7.2$ increases social dominance

To further examine social behaviors, we next performed the tube dominance test (Figure 7A), which provides a reliable measure of social dominance and social aggression in rodents.⁶⁷⁻⁶⁹ We found main effects of genotype for the average score (genotype: $F_{(1, 30)} = 9.56$, $P = .004$) and % of wins (genotype: $F_{(1, 30)} = 4.72$, $P = .038$). $KCNQ2^{+/-}$ displayed higher total score and greater % of

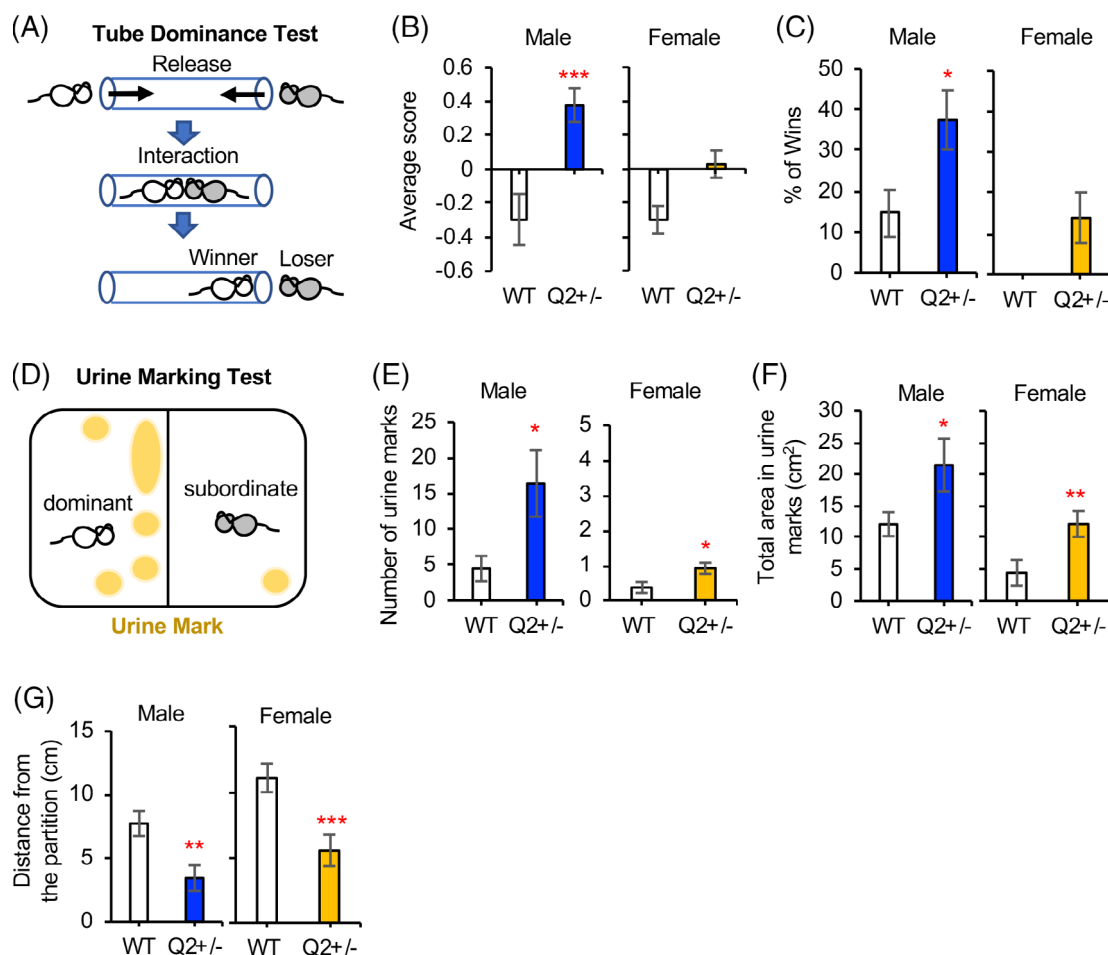


FIGURE 7 The $KCNQ2^{+/-}$ mice display increased social dominance behavior. A-C, The $KCNQ2^{+/+}$ (WT) and $KCNQ2^{+/-}$ (Q2+/-) mice were subjected to the tube dominance test. A, The schematic of the tube dominance test. The test mouse (WT or Q2+/-) is placed at one end of the tube and the age matched control mouse (CD-1) is placed at the other end at the same time. A mouse which forces its opponent to retreat backward and exit the tube is deemed the winner and receives +1 point. The retreating mouse is deemed the loser and receives -1 point. If the mice stay in the middle of the tube or pass each other, they receive 0. B-C, Quantifications of average score (B) and % of win (C). The male and female Q2+/- mice received higher scores and % of win than the WT mice against CD-1 mice. D-G, Male WT and Q2+/- mice were subjected to urine marking test for 30 minutes. D, Schematic of a urine test during which the test mouse and CD-1 mouse were separated by a wire mesh partition. Quantifications of the number of urine marks (E), the total area of urine marks (F) and the distance of urine marks from the partition (G). Both male and female Q2+/- mice displayed significantly more urine marks with larger areas near the partition compared with the WT mice. Male mice (WT $n = 12$; Q2+/- $n = 11$). Female mice (WT $n = 5$; Q2+/- $n = 6$). Data shown represent the Ave \pm SEM. Student t test results are shown here (* $P < .05$, ** $P < .01$, *** $P < .005$). Table S8 shows two-way ANOVA test results with genotype as one factor and sex as the other

wins against male CD-1 mice than *KCNQ2*^{+/+} (Figure 7B,C). We also observed sex differences for % of wins (sex: $F_{(1, 30)}=5.09$, $P = .032$). Compared with males, all tested female *KCNQ2*^{+/+} mice retreated out of the tube whereas most female *KCNQ2*^{+/-} mice stayed in the middle of the tube facing female CD-1 mice during the entire duration of this test (Figure 7B). Only a few female *KCNQ2*^{+/-} mice forced the CD-1 mice out of the tube, resulting in a small % of wins and no genotype differences (Figure 7C, Table S8).

To confirm that the *KCNQ2*^{+/-} mice are more socially dominant than the wild-type mice, we performed the urine marking test (Figure 7D), in which the socially dominant male mice mark larger territories than the subordinate mice for social communication and convey dominance status.^{69,70} We found a significant interaction between sex and genotype for the total number of urine marks (interaction: $F_{(1, 89)}=0.14$, $P = .032$). When novel CD-1 mice were introduced, both male and female *KCNQ2*^{+/-} mice displayed a significantly larger number of urine markings compared with the wild type mice (genotype: $F_{(1, 89)}=0.13$, $P = .019$), although females marked far less than males (sex: $F_{(1, 89)}=0.12$, $P = .0003$) (Figure 7E, Table S8). *KCNQ2*^{+/-} mice also displayed a greater area of urine markings compared with the wild type mice (genotype: $F_{(1, 89)}=9.50$, $P = .003$) and sex differences were observed (sex: $F_{(1, 89)}=9.42$, $P = .003$) (Figure 7F, Table S8). Furthermore, the urine marks of *KCNQ2*^{+/-} mice were much closer to the wire mesh partition compared with the wild type mice (genotype: $F_{(1, 89)}=20.60$, $P < .0001$) (Figure 7G). These results are consistent with the tube dominance test findings that heterozygous loss of *K_v7.2* enhances socially dominant behavior in mice.

3.5 | Heterozygous loss of *K_v7.2* increases seizure propensity and hippocampal excitability

Heterozygous knockout of *KCNQ2* gene has been previously shown to elevate sensitivity to pentylenetetrazole-induced seizures.⁴⁴ To confirm whether *KCNQ2*^{+/-} mice in our studies display heightened seizure propensity, we subjected *KCNQ2*^{+/+} and *KCNQ2*^{+/-} mice to i.p. injections of chemoconvulsant kainate (kainic acid) at a lower dose of 15 mg/kg. Systemic injections of kainate cause epileptiform seizures in the hippocampus which propagate to other limbic structures and induce hippocampus-restricted neuropathology comparable to those seen in patients with temporal lobe epilepsy (TLE).⁷³ The average cumulative behavioral seizure score for *KCNQ2*^{+/-} mice was significantly higher than that of *KCNQ2*^{+/+} sibling mice at 6-month of age (genotype: $F_{(1, 27)}=35.22$, $P < .0001$) (Figure 8A,B) although both groups had similar weight (genotype: $F_{(1, 27)}= 0.16$, $P = .688$) (Figure 8C, Table S9). Sex differences were observed for seizure score (sex: $F_{(1, 27)}=6.05$, $P = .021$) and weight (sex: $F_{(1, 27)}=34.49$, $P < .0001$) (Table S9). These results indicate that heterozygous loss of *K_v7.2* increases seizure propensity at 6 month of age and eliminate the body weight as a confounding factor in our behavioral studies. Consistent with increased seizure susceptibility, deletion of one *Kcnq2* copy significantly increased excitability of CA1 pyramidal neurons (Figure 8D). We assessed CA1 pyramidal neuron excitability by injecting an increasing amount of current for 1 second in cells held at the resting

membrane potential. We found that CA1 pyramidal neuron firing induced by 300 pA injection plateaued at 36 ± 2.1 APs in wild-type mice and at 43 ± 2.2 APs in *KCNQ2*^{+/-} mice ($t = 2.302$, $df = 16$, $P = .0351$). These results indicate that the *KCNQ2*^{+/-} hippocampal CA1 neurons are more excitable than their wild-type counterparts.

4 | DISCUSSION

Recent whole-exome or targeted sequencing studies on well-defined ASD cohorts have implicated heterozygous variants in *KCNQ2* and *KCNQ3* genes,¹² suggesting that disruption of *K_v7* currents may contribute to ASD. In this study, we provide strong evidence that neuronal *K_v7* channels containing *K_v7.2* contribute to multiple behaviors in mice including circadian-dependent activity, exploratory behavior, social behavior, repetitive and compulsive-like behavior, in addition to dampening seizure susceptibility (Figure 9). Our findings also reveal that genetic heterozygous loss of *K_v7.2* in mice leads to behavioral abnormalities reminiscent of clinical symptoms of ASD.^{5,6,8,9,74,75}

4.1 | Role of *K_v7* channels in locomotor activity

We discover that *KCNQ2*^{+/-} mice show increased home cage activity during the light phase compared with their wild-type siblings without grossly affecting their motor activity and coordination, although no differences were detected during the dark phase when mice are most active (Figure 2, Table S2). These results suggest that reduction in *K_v7* current leads to hyperactivity during the light phase when mice are generally less active. These findings are reminiscent of clinical reports that some ASD patients experience sleep problems.⁹ *K_v7.2* is expressed in the reticular thalamic nucleus,¹⁶ a brain area critical in regulating sleep wake cycle transitions.⁷⁶ Therefore, heterozygous loss of *K_v7.2* in reticular thalamic nucleus could also disrupt wake to sleep transition, leading to hyperactivity of mice during the inactive light phase.

Although speculative, hyperactivity of *KCNQ2*^{+/-} mice during the light phase (Figure 2) may also result from altered circadian-dependent activity. *K_v7.2* is highly expressed in the hippocampus^{16,17} which projects directly to suprachiasmatic nucleus (SCN) in the hypothalamus,⁷⁷ the central coordinator of circadian rhythm.⁷⁸ The hippocampus expresses all clock genes necessary for circadian oscillation of neuronal activity that modulates hippocampal-dependent learning and memory.⁷⁹ Interestingly, human patients with TLE and rodent models of TLE display more seizures during the day.⁸⁰ Considering a very strong *K_v7.2* expression in the hippocampus^{16,17} and kainate-induced status epilepticus originates from hippocampal hyperexcitability,⁷³ it is tempting to speculate that increased seizure propensity and home cage activity of *KCNQ2*^{+/-} mice during the inactive light period (Figures 2 and 8A,C) may result from reduced *K_v7* current and a subsequent increase in hippocampal excitability (Figure 8D) that may alter SCN rhythmicity in these mice.

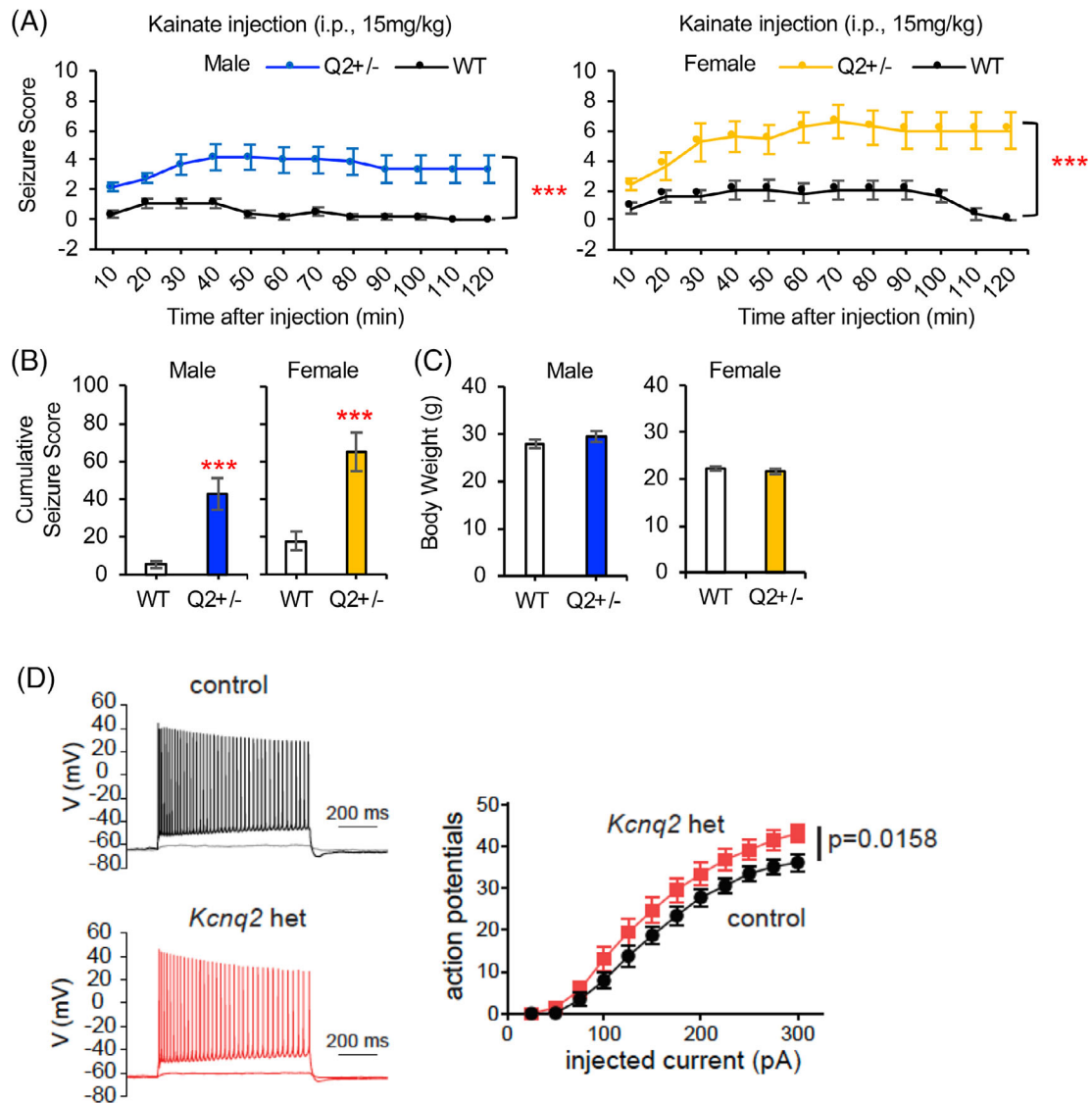


FIGURE 8 The *KCNQ2*^{+/-} mice display increased seizure propensity to chemoconvulsant kainate and enhanced hippocampal excitability. *KCNQ2*^{+/+} (WT) and *KCNQ2*^{+/-} (*Q2*^{+/-}) mice at 6-month of age were intraperitoneally injected with kainate (15 mg/kg) and vehicle control (saline). A, Behavioral seizures were scored based on modified Racine scale and Pinal and Rovner scale for the first 2 hours post injection. B, Quantification of cumulative behavioral seizure scores. C, The average body weights of the WT and *Q2*^{+/-} mice at 6-month of age. Both male and female *Q2*^{+/-} mice displayed significantly increased seizure scores during the first 2 hours post injection compared with the WT mice. D, Left, representative voltage traces to various current injections (1 s, 0 to +300 pA) in CA1 pyramidal neurons from *KCNQ2*^{+/+} (control) or *KCNQ2*^{+/-} (*Kcnq2* het) mice. Right, summary graph showing the effect of deleting one copy of *KCNQ2* on AP number across a range of current injections (two-way repeated measures ANOVA; $n = 9$ neurons from two control mice; $n = 9$ neurons from four *Kcnq2* het mice). Data shown represent the Ave \pm SEM. Student *t* test results for A-C are shown here (***) $P < .005$. Table S9 shows two-way ANOVA test results with genotype as one factor and sex as the other

4.2 | Role of *K_v7* channels in exploratory behavior, anxiety or perception of danger

Mice display aversions to environments that are new, brightly lit, open or large. They perceive these environments as dangerous and show anxiety responses.⁸¹ In our open field and EPM tests performed during the dark phase, *KCNQ2*^{+/-} mice exhibited significant increases in the number of entries and the time spent in the center of the open field and the open arms in the EPM compared with the wild-type mice (Figures 3 and 4, Tables S3, S4). The increased exploratory behavior of

KCNQ2^{+/-} mice in these tests suggests that heterozygous loss of *K_v7.2* reduces anxiety-related behavior in mice. This is somewhat contrary to the well-documented reports that patients with ASD experience greater anxiety than those without ASD.⁷ However, multiple mouse models of autism and Fragile X Syndrome (FXS) which accounts for about 2% to 3% of all ASD cases show reduced nonsocial anxiety measured by the same behavioral tests although they display typical autism-associated behaviors including social avoidance and repetitive behaviors.⁸²⁻⁸⁶ For example, FXS mouse model *Fmr1*

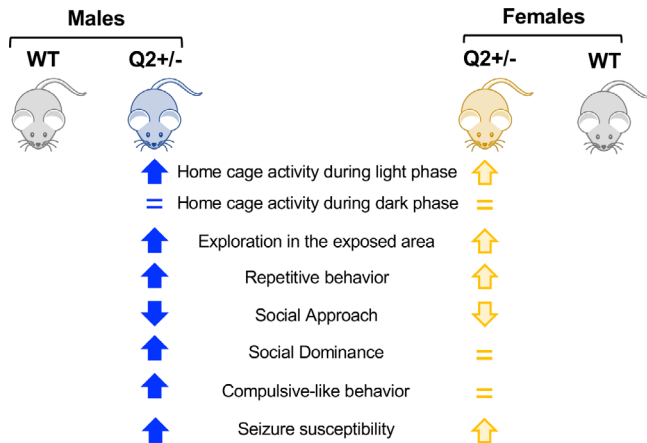


FIGURE 9 A summary of behavioral assays performed with $KCNQ2^{+/+}$ and $KCNQ2^{+/-}$ mice. Compared with the wild type $KCNQ2^{+/+}$ (WT) littermates, $KCNQ2^{+/-}$ mice ($Q2^{+/-}$) display abnormalities in multiple behaviors including circadian-dependent activity, exploratory behavior, social behavior, repetitive and compulsive-like behavior, in addition to enhanced seizure susceptibility

knockout mice have been reported to spend more time in the open arms on the EPM test, and such behavior could be attributed to cognitive impairment.^{82,87}

Although the open field and EPM tests are widely used to examine anxiety in mice,⁸¹ increased entry and duration of $KCNQ2^{+/-}$ mice to the exposed center or open arms could result from their hyperarousal to novel environments, especially considering that these tests were performed in the dark (Figures 3 and 4). K_v7 currents regulate intrinsic excitability of pyramidal neurons in the hippocampus and cortex including prefrontal cortex,^{14,21-25,47} the brain regions implicated in higher cognitive and executive functions.^{88,89} Therefore, our data may also reflect a decrease in the cognitive ability of $KCNQ2^{+/-}$ mice to recognize the potential danger of open spaces and/or perceive fear, similar to *Fmr1* knockout mice.^{82,87} Indeed, patients with ASD were slower to detect and respond to both social and nonsocial hazards in comparison to individuals without ASD.^{74,90} The underlying circuitry affected in $KCNQ2^{+/-}$ mice is unclear. Elevated activity in the amygdala is associated with increased anxiety and perception of fear,^{91,92} whereas the medial prefrontal cortex (PFC) exerts an inhibitory effect on the amygdala.⁹³ Considering the enrichment of $K_v7.2$ in the medial PFC but not the amygdala,^{94,95} reduced anxiety and/or perception of danger and fear could be attributed to inhibition of amygdala by increased medial PFC activity in $KCNQ2^{+/-}$ mice.

4.3 | Role of K_v7 channels in repetitive and compulsive-like behavior

Repetitive self-grooming behaviors in rodents are thought to recapitulate pathological repetitive behaviors seen in humans⁶⁰ and are widely used to identify neural circuits and genes underlying repetitive behaviors in ASD.⁶¹ For example, the knockout mice for ASD-associated *SHANK2-3* genes display increased frequency and/or duration of self-

grooming in addition to social deficits.^{96,97} We discover that $KCNQ2^{+/-}$ mice exhibited significant increases in the duration of self-grooming compared with the wild-type mice (Figure 5A,B, Table S5). Abnormal stereotypic grooming behavior is mediated by GABAergic output from the striatum⁹⁸ and K_v7 channels are potent regulators of excitability in striatal presynaptic dopaminergic activity and GABAergic striatal medium spiny neurons.^{99,100} Therefore, our findings of increased self-grooming duration in $KCNQ2^{+/-}$ mice (Figure 5A,B) may be attributed to the changes in presynaptic and/or postsynaptic striatal activity upon reduction in K_v7 current.

Another behavioral assay which we performed with the $KCNQ2^{+/-}$ mice was marble burying test, which uses the tendency of mice to dig burrows or escape tunnels in nature.^{62,63} Although this test has been considered to assess the repetitive and compulsive-like behaviors of obsessive-compulsive disorder (OCD), this test has also been used to measure for autistic behavior or anxiety.^{61,101} OCD features disruptive thoughts (termed obsessions) that leads to behavioral compulsions such as repetitive behaviors often to reduce anxiety caused by the obsessions.⁶³ In this study, we discover that the male but not female $KCNQ2^{+/-}$ mice buried more marbles than the wild-type mice (Figure 5C,D, Table S5). These sex differences in marble-burying behaviors of $KCNQ2^{+/-}$ mice are intriguing because of the sex differences seen in anxiety disorders and autism. At age 5 and younger, boys with autism have more restricted interests and repetitive behaviors than girls diagnosed with autism.^{102,103} In contrast, multiple anxiety disorders except for social anxiety disorder are more prevalent in women than men.¹⁰⁴ Because the female $KCNQ2^{+/-}$ mice show reduced anxiety in open field and EPM tests to a similar extent as male mice (Figures 3 and 4), increased marble-bearing behaviors in male $KCNQ2^{+/-}$ mice (Figure 5C,D) may most likely reflect repetitive and compulsive-like behavior rather than increased anxiety.

4.4 | Role of K_v7 channels in social interaction and motivation

Impaired social interaction is one of the core symptoms of autism in humans.¹⁰⁵ Social interaction is driven by social motivation and social recognition. Social motivation reflects the interest to approach and interact with another conspecific social target, whereas social recognition reflects the ability to distinguish between different social targets. In the three-chamber social interaction test, $KCNQ2^{+/+}$ spent longer time in the chamber sniffing the stranger mouse-1 than in the chamber containing the empty cage, but $KCNQ2^{+/-}$ mice did not (Figure 6A-D, Tables S6-7). These results suggest deficits in social recognition, motivation or both. The impaired sociability of $KCNQ2^{+/-}$ mice are reminiscent of the core clinical symptoms of ASD and suggest that reduced K_v7 current may contribute to ASD-associated behaviors in patients with de novo $KCNQ2$ mutation.

Furthermore, the female $KCNQ2^{+/-}$ mice displayed reduced preference for novel social target compared with the female wild-type littermates (Figure 6E-G, Tables S6-7). Such behavior of $KCNQ2^{+/-}$ females may reflect defects in the formation or retrieval of the memory of the familiar mice, just as children with autism struggle to form

working memories and autobiographical memories and show defects in social cognition.^{106,107} The male *KCNQ2*^{+/+} mice exhibited preference for novel stranger C57BL/6J mice compared with familiar mice (Figure 6E-G, Tables S6-7) consistent with the previous report of the preference of the C57BL/6J males for novel males.¹⁰⁸ Interestingly, the two-way ANOVA revealed that *KCNQ2*^{+/-} males also exhibited preference for novel stranger C57BL/6J mice, revealing significant sex differences (Figure 6E-G, Tables S6-7).

K_v7 channels are highly expressed in the hippocampus and cortex including medial PFC,^{16,17} brain regions important for social approach and cognition.^{109,110} We speculate that hippocampal and cortical hyperexcitability (Figure 8D)^{24,111} especially in the medial PFC of *KCNQ2*^{+/-} mice may disrupt their social recognition and/or memory, resulting in altered social behaviors in these mice. Recent studies on MRI brain scans in children with autism have revealed that the mesolimbic reward pathway, the key reward circuit in the brain, is impaired in children with ASD and the degree of such structural impairment correlates with greater social impairment of the patients.¹¹² This interesting study supports the notion that social interaction is generally perceived as rewarding in healthy individuals but not in patients with ASD. Therefore, altered *K_v7* activity in the ventral tegmental area and nucleus accumbens within the mesolimbic dopamine system^{99,113-115} may also contribute to decreased sociability in *KCNQ2*^{+/-} mice.

4.5 | Role of *K_v7* channels in social dominance and aggression

As a major aspect of social hierarchy, social dominance can be observed in many social species across the animals, human and non-human primates.¹¹⁶ Socially dominant rodents are more aggressive and display reduced fear and stress responses.¹¹⁶ The increased social dominant behaviors of the *KCNQ2*^{+/-} mice (Figure 7A-C, Table S8) are intriguing in the context of the prevalence of aggressive behaviors in children with ASD.⁷⁵ Aggression positively correlates with the severity of social impairment and repetitive behavior.¹¹⁷ Functional involvement of PFC in social dominance and aggression has also been reported in humans and rodents.^{118,119} Socially, dominant mice display increased excitatory synaptic strength in layer V pyramidal neurons in the medial PFC⁶⁹ where *K_v7.2* is highly expressed^{16,17} (alleninstitute.org/). Because *K_v7* current suppresses intrinsic excitability and postsynaptic transmission of PFC layer V pyramidal neurons,⁹⁴ the heterozygous loss of *K_v7.2* may likely contribute to the increased social dominant behaviors in *KCNQ2*^{+/-} mice by increasing the activity of PFC layer V pyramidal neurons.

Enhanced social dominant behavior can also be viewed as a risk-taking behavior, because such behavior will increase the social challenges in rodents.¹²⁰ Potential risk-taking behaviors of *KCNQ2*^{+/-} mice are reflected in their behaviors during the open field and EPM tests (Figures 3 and 4). In these tests, mice are exposed to two conflicting choices. One choice is their interest to explore the novel environment. The second choice is to avoid exploration especially in the exposed area because such action is too risky and evokes fear.

Interestingly, damages to the PFC are shown to impair decision making involving evaluations about risks and rewards¹²¹ and patients with damaged PFC make risky choices and decisions.¹²² Therefore, it is possible that increased excitability and synaptic strength in the PFC of *KCNQ2*^{+/-} mice may alter their risk assessment and fear response, ultimately leading them to make risky decisions.

4.6 | Role of *K_v7* channels in hippocampal and cortical hyperexcitability

Approximately 30% of autistic children display epilepsy, and conversely, several forms of epilepsy also display ASD as a comorbidity.^{2,123} Concerted effort of genome sequencing has identified de novo heterozygous mutations in *K_v7.2* and *K_v7.3* in patients with NDD and autism¹² as well as epilepsy (rikee.org). Some of these mutations cause severe EE in many patients.^{28-38,42,124,125} These patients have spontaneous seizures after birth and exhibit profound neurodevelopmental delay, intellectual disability, psychiatric disorders and show comorbidities such as myokymia and ASD.²⁸⁻³⁸ Interestingly, two pathogenic heterozygous *KCNQ2* variants (M1T and M1V) that disrupt the start codon ATG have been identified in patients with BFNE and EE.^{31,126,127} These variants essentially lead to heterozygote ablation of *KCNQ2*, supporting the use of the *KCNQ2*^{+/-} mice for investigating how heterozygous loss of *KCNQ2* affects seizure generation and behaviors. Although *KCNQ2*^{+/-} mice did not display spontaneous seizures,⁴⁴ we found that *KCNQ2*^{+/-} mice show increased seizure susceptibility to chemoconvulsant kainate and enhanced hippocampal excitability (Figure 8, Table S9).

Developmental delay and intellectual disability in ASD could result from abnormal hyperactivity in the hippocampus that express *K_v7.2*¹⁶ and mediates rhythmic theta oscillation critical for learning and memory and synaptic plasticity.¹²⁸⁻¹³¹ M-channels have been shown to facilitate hippocampal network resonance at the theta frequency in rat hippocampal neurons.¹³² Furthermore, pharmacological activation of *K_v7* channels dampened stress-induced impairments in memory retrieval and hippocampal long-term potentiation (LTP).¹³³ Conversely, *K_v7* channel blocker XE991 enhanced learning and memory in healthy juvenile mice and alleviated kainite-induced cognitive impairment.¹³⁴ Considering the early developmental expression of *K_v7* subunits (<http://hbatlas.org/>), disruption of *K_v7* function early in development may negatively impact the formation of circuitry and synaptic plasticity that underlie learning and memory in ASD.

5 | CONCLUSION

In this study, we subjected *KCNQ2*^{+/+} and *KCNQ2*^{+/-} mice to nine behavioral assays in the test battery. Although the order of these tests was chosen to conduct in general from least invasive to most invasive tests, it is possible that the behavioral responses of these mice in the social novelty test might have been affected by six prior tests.^{55,56} The potential impact of one behavioral assay to the subsequent ones

is an important limitation to acknowledge in the behavioral test battery.^{55,56}

In summary, our findings provide original evidence that neuronal K_v7 channels containing $K_v7.2$ is involved in the modulation of multiple behaviors in mice including locomotor activity during the light phase, exploratory behavior, social behavior, repetitive and compulsive-like behavior (Figures 1–7 and 9). In addition, these mice show increased hippocampal excitability and seizure propensity (Figures 8 and 9). Some of the behavioral abnormalities observed in heterozygous $K_v7.2$ knockout mice are reminiscent of clinical symptoms of ASD, suggesting that reduced K_v7 current may contribute to ASD-associated behaviors. Furthermore, our data provides the pharmacological enhancement of K_v7 current^{135–137} as a potential therapeutic means for treating ASD-associated behaviors.

ACKNOWLEDGMENTS

This research was supported by the National Institute of Neurological Disorders and Stroke (NINDS) Research Project Grant NS083402 (H.J.C.), NINDS NS101596, NINDS NS10887, NHLBI HL137094 (A.T.) and NINDS NS104293 (J.S.R.). The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

AUTHOR CONTRIBUTIONS

H.J.C. conceived of the study and participated in its design and coordination. E.C.K., J.P. and A.T. carried out the experiments and statistical analyses. J.S.R. provided critical expertise for behavior experiments and statistical analyses. H.J.C., E.C.K., J.P. and J.Z. drafted the manuscript. All authors read and approved the final manuscript.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

ORCID

Hee Jung Chung  <https://orcid.org/0000-0002-5555-9907>

REFERENCES

- Mullin AP, Gokhale A, Moreno-De-Luca A, Sanyal S, Waddington JL, Faundez V. Neurodevelopmental disorders: mechanisms and boundary definitions from genomes, interactomes and proteomes. *Transl Psychiatry*. 2013;3:e329.
- Tuchman R, Cuccaro M. Epilepsy and autism: neurodevelopmental perspective. *Curr Neurol Neurosci Rep*. 2011;11(4):428–434.
- Lyall K, Croen L, Daniels J, et al. The changing epidemiology of autism spectrum disorders. *Annu Rev Public Health*. 2017;38:81–102.
- Tye C, Runicles AK, Whitehouse AJO, Alvares GA. Characterizing the interplay between autism spectrum disorder and comorbid medical conditions: an integrative review. *Front Psychiatry*. 2018;9:751.
- Fitzpatrick SE, Srivorakiat L, Wink LK, Pedapati EV, Erickson CA. Aggression in autism spectrum disorder: presentation and treatment options. *Neuropsychiatr Dis Treat*. 2016;12:1525–1538.
- Anagnostou E. Clinical trials in autism spectrum disorder: evidence, challenges and future directions. *Curr Opin Neurol*. 2018;31(2):119–125.
- McVey AJ. The neurobiological presentation of anxiety in autism spectrum disorder: a systematic review. *Autism Res*. 2019;12(3):346–369.
- Hitoglou M, Ververi A, Antoniadis A, Zafeiriou DI. Childhood autism and auditory system abnormalities. *Pediatr Neurol*. 2010;42(5):309–314.
- Kotagal S, Broomall E. Sleep in children with autism spectrum disorder. *Pediatr Neurol*. 2012;47(4):242–251.
- Doyle CA, McDougle CJ. Pharmacotherapy to control behavioral symptoms in children with autism. *Expert Opin Pharmacother*. 2012;13(11):1615–1629.
- Wilfert AB, Sulovari A, Turner TN, Coe BP, Eichler EE. Recurrent de novo mutations in neurodevelopmental disorders: properties and clinical implications. *Genome Med*. 2017;9(1):101.
- Geisheker MR, Heymann G, Wang T, et al. Hotspots of missense mutation identify neurodevelopmental disorder genes and functional domains. *Nat Neurosci*. 2017;20(8):1043–1051.
- Brown DA, Passmore GM. Neural KCNQ (Kv7) channels. *Br J Pharmacol*. 2009;156(8):1185–1195.
- Shah M, Mistry M, Marsh SJ, Brown DA, Delmas P. Molecular correlates of the M-current in cultured rat hippocampal neurons. *J Physiol*. 2002;544(Pt 1):29–37.
- Wang HS, Pan Z, Shi W, et al. KCNQ2 and KCNQ3 potassium channel subunits: molecular correlates of the M-channel. *Science*. 1998;282(5395):1890–1893.
- Cooper EC, Harrington E, Jan YN, Jan LY. M channel KCNQ2 subunits are localized to key sites for control of neuronal network oscillations and synchronization in mouse brain. *J Neurosci*. 2001;21(24):9529–9540.
- Pan Z, Kao T, Horvath Z, et al. A common ankyrin-G-based mechanism retains KCNQ and NaV channels at electrically active domains of the axon. *J Neurosci*. 2006;26(10):2599–2613.
- Chung HJ, Jan YN, Jan LY. Polarized axonal surface expression of neuronal KCNQ channels is mediated by multiple signals in the KCNQ2 and KCNQ3 C-terminal domains. *Proc Natl Acad Sci U S A*. 2006;103(23):8870–8875.
- Rasmussen HB, Frokjaer-Jensen C, Jensen CS, et al. Requirement of subunit co-assembly and ankyrin-G for M-channel localization at the axon initial segment. *J Cell Sci*. 2007;120(Pt 6):953–963.
- Bennett V, Lorenzo DN. An adaptable Spectrin/Ankyrin-based mechanism for long-range organization of plasma membranes in vertebrate tissues. *Curr Top Membr*. 2016;77:143–184.
- Shah MM, Migliore M, Valencia I, Cooper EC, Brown DA. Functional significance of axonal Kv7 channels in hippocampal pyramidal neurons. *Proc Natl Acad Sci U S A*. 2008;105(22):7869–7874.
- Yue C, Yaari Y. Axo-somatic and apical dendritic Kv7/M channels differentially regulate the intrinsic excitability of adult rat CA1 pyramidal cells. *J Neurophysiol*. 2006;95(6):3480–3495.
- Tzingounis AV, Nicoll RA. Contribution of KCNQ2 and KCNQ3 to the medium and slow after hyperpolarization currents. *Proc Natl Acad Sci U S A*. 2008;105(50):19974–19979.
- Soh H, Pant R, LoTurco JJ, Tzingounis AV. Conditional deletions of epilepsy-associated KCNQ2 and KCNQ3 channels from cerebral cortex cause differential effects on neuronal excitability. *J Neurosci*. 2014;34(15):5311–5321.
- Gu N, Vervaeke K, Hu H, Storm JF. Kv7/KCNQ/M and HCN/h, but not KCa2/SK channels, contribute to the somatic medium after-hyperpolarization and excitability control in CA1 hippocampal pyramidal cells. *J Physiol*. 2005;566(Pt 3):689–715.

26. Battfeld A, Tran BT, Gavrili J, Cooper EC, Kole MH. Heteromeric Kv7.2/7.3 channels differentially regulate action potential initiation and conduction in neocortical myelinated axons. *J Neurosci*. 2014;34(10):3719-3732.
27. Maljevic S, Wuttke TV, Seebom G, Lerche H. KV7 channelopathies. *Pflugers Arch*. 2010;460(2):277-288.
28. Abidi A, Devaux JJ, Molinari F, et al. A recurrent KCNQ2 pore mutation causing early onset epileptic encephalopathy has a moderate effect on M current but alters subcellular localization of Kv7 channels. *Neurobiol Dis*. 2015;80:80-92.
29. Kato M, Yamagata T, Kubota M, et al. Clinical spectrum of early onset epileptic encephalopathies caused by KCNQ2 mutation. *Epilepsia*. 2013;54(7):1282-1287.
30. Kwong AK, Ho AC, Fung CW, Wong VC. Analysis of mutations in 7 genes associated with neuronal excitability and synaptic transmission in a cohort of children with non-syndromic infantile epileptic encephalopathy. *PLoS One*. 2015;10(5):e0126446.
31. Milh M, Boutry-Kryza N, Sutera-Sardo J, et al. Similar early characteristics but variable neurological outcome of patients with a de novo mutation of KCNQ2. *Orphanet J Rare Dis*. 2013;8:80.
32. Millichap JJ, Park KL, Tsuchida T, et al. KCNQ2 encephalopathy: features, mutational hot spots, and ezogabine treatment of 11 patients. *Neurol Genet*. 2016;2(5):e96.
33. Numis AL, Angriman M, Sullivan JE, et al. KCNQ2 encephalopathy: delineation of the electroclinical phenotype and treatment response. *Neurology*. 2014;82(4):368-370.
34. Saitsu H, Kato M, Koide A, et al. Whole exome sequencing identifies KCNQ2 mutations in Ohtahara syndrome. *Ann Neurol*. 2012;72(2):298-300.
35. Weckhuysen S, Mandelstam S, Suls A, et al. KCNQ2 encephalopathy: emerging phenotype of a neonatal epileptic encephalopathy. *Ann Neurol*. 2012;71(1):15-25.
36. Weckhuysen S, Ivanovic V, Hendrickx R, et al. Extending the KCNQ2 encephalopathy spectrum: clinical and neuroimaging findings in 17 patients. *Neurology*. 2013;81(19):1697-1703.
37. Zhang Q, Li J, Zhao Y, Bao X, Wei L, Wang J. Gene mutation analysis of 175 Chinese patients with early-onset epileptic encephalopathy. *Clin Genet*. 2017;91(5):717-724.
38. Samanta D, Ramakrishnaiah R, Willis E, Frye RE. Myoclonic epilepsy evolved into west syndrome: a patient with a novel de novo KCNQ2 mutation. *Acta Neurol Belg*. 2015;115(3):475-478.
39. Niday Z, Tzingounis AV. Potassium channel gain of function in epilepsies: an unresolved paradox. *Neuroscientist*. 2018;24(4):368-380.
40. Soldovieri MV, Cilio MR, Miceli F, et al. Atypical gating of M-type potassium channels conferred by mutations in uncharged residues in the S4 region of KCNQ2 causing benign familial neonatal convulsions. *J Neurosci*. 2007;27(18):4919-4928.
41. Orhan G, Bock M, Schepers D, et al. Dominant-negative effects of KCNQ2 mutations are associated with epileptic encephalopathy. *Ann Neurol*. 2014;75(3):382-394.
42. Miceli F, Soldovieri MV, Ambrosino P, et al. Early-onset epileptic encephalopathy caused by gain-of-function mutations in the voltage sensor of Kv7.2 and Kv7.3 potassium channel subunits. *J Neurosci*. 2015;35(9):3782-3793.
43. Wuttke TV, Jurkat-Rott K, Paulus W, Garncarek M, Lehmann-Horn F, Lerche H. Peripheral nerve hyperexcitability due to dominant-negative KCNQ2 mutations. *Neurology*. 2007;69(22):2045-2053.
44. Watanabe H, Nagata E, Kosakai A, et al. Disruption of the epilepsy KCNQ2 gene results in neural hyperexcitability. *J Neurochem*. 2000;75(1):28-33.
45. Singh NA, Otto JF, Dahle EJ, et al. Mouse models of human KCNQ2 and KCNQ3 mutations for benign familial neonatal convulsions show seizures and neuronal plasticity without synaptic reorganization. *J Physiol*. 2008;586(14):3405-3423.
46. Blackburn-Munro G, Dalby-Brown W, Mirza NR, Mikkelsen JD, Blackburn-Munro RE. Retigabine: chemical synthesis to clinical application. *CNS Drug Rev*. 2005;11(1):1-20.
47. Yue C, Yaari Y. KCNQ/M channels control spike afterdepolarization and burst generation in hippocampal neurons. *J Neurosci*. 2004;24(19):4614-4624.
48. Tan A, Costi S, Morris LS, et al. Effects of the KCNQ channel opener ezogabine on functional connectivity of the ventral striatum and clinical symptoms in patients with major depressive disorder. *Mol Psychiatry*. 2018. <https://doi.org/10.1038/s41380-018-0283-2>.
49. Friedman AK, Juarez B, Ku SM, et al. KCNQ channel openers reverse depressive symptoms via an active resilience mechanism. *Nat Commun*. 2016;7:11671.
50. Epi KC, Epilepsy Phenome/Genome Project, Allen AS, et al. De novo mutations in epileptic encephalopathies. *Nature*. 2013;501(7466):217-221.
51. Peters HC, Hu H, Pongs O, Storm JF, Isbrandt D. Conditional transgenic suppression of M channels in mouse brain reveals functions in neuronal excitability, resonance and behavior. *Nat Neurosci*. 2005;8(1):51-60.
52. Ben-Ari Y. The GABA excitatory/inhibitory developmental sequence: a personal journey. *Neuroscience*. 2014;279:187-219.
53. Hsieh LS, Wen JH, Miyares L, Lombroso PJ, Bordey A. Outbred CD1 mice are as suitable as inbred C57BL/6J mice in performing social tasks. *Neurosci Lett*. 2017;637:142-147.
54. Baculis BC, Weiss AC, Pang W, et al. Prolonged seizure activity causes caspase dependent cleavage and dysfunction of G-protein activated inwardly rectifying potassium channels. *Sci Rep*. 2017;7(1):12313.
55. McIlwain KL, Merriweather MY, Yuva-Paylor LA, Paylor R. The use of behavioral test batteries: effects of training history. *Physiol Behav*. 2001;73(5):705-717.
56. Paylor R, Spencer CM, Yuva-Paylor LA, Pieke-Dahl S. The use of behavioral test batteries, II: effect of test interval. *Physiol Behav*. 2006;87(1):95-102.
57. Zombeck JA, Deyoung EK, Brzezinska WJ, Rhodes JS. Selective breeding for increased home cage physical activity in collaborative cross and Hsd:ICR mice. *Behav Genet*. 2011;41(4):571-582.
58. Majdak P, Bucko PJ, Holloway AL, et al. Behavioral and pharmacological evaluation of a selectively bred mouse model of home cage hyperactivity. *Behav Genet*. 2014;44(5):516-534.
59. Clark PJ, Brzezinska WJ, Thomas MW, Ryzhenko NA, Toshkov SA, Rhodes JS. Intact neurogenesis is required for benefits of exercise on spatial memory but not motor performance or contextual fear conditioning in C57BL/6J mice. *Neuroscience*. 2008;155(4):1048-1058.
60. Kalueff AV, Stewart AM, Song C, Berridge KC, Graybiel AM, Fentress JC. Neurobiology of rodent self-grooming and its value for translational neuroscience. *Nat Rev Neurosci*. 2016;17(1):45-59.
61. Silverman JL, Yang M, Lord C, Crawley JN. Behavioural phenotyping assays for mouse models of autism. *Nat Rev Neurosci*. 2010;11(7):490-502.
62. Angoa-Perez M, Kane MJ, Briggs DI, Francescutti DM, Kuhn DM. Marble burying and nestlet shredding as tests of repetitive, compulsive-like behaviors in mice. *J Vis Exp*. 2013;82:50978.
63. 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 (Berl)*. 2009;204(2):361-373.
64. Moy SS, Nadler JJ, Perez A, et al. Sociability and preference for social novelty in five inbred strains: an approach to assess autistic-like behavior in mice. *Genes Brain Behav*. 2004;3(5):287-302.
65. Yang M, Silverman JL, Crawley JN. Automated three-chambered social approach task for mice. *Curr Protoc Neurosci*. 2011; Chapter 8: Unit 8. 26, 56:8.26.1-8.26.16.

66. Kaidanovich-Beilin O, Lipina T, Vukobradovic I, Roder J, Woodgett JR. Assessment of social interaction behaviors. *J Vis Exp*. 2011;(48):e2473.
67. Lindzey G, Winston H, Manosevitz M. Social dominance in inbred mouse strains. *Nature*. 1961;191:474-476.
68. Messeri P, Eleftheriou BE, Oliverio A. Dominance behavior: a phylogenetic analysis in the mouse. *Physiol Behav*. 1975;14(1):53-58.
69. Wang F, Zhu J, Zhu H, Zhang Q, Lin Z, Hu H. Bidirectional control of social hierarchy by synaptic efficacy in medial prefrontal cortex. *Science*. 2011;334(6056):693-697.
70. Arakawa H, Arakawa K, Blanchard DC, Blanchard RJ. Scent marking behavior in male C57BL/6J mice: sexual and developmental determination. *Behav Brain Res*. 2007;182(1):73-79.
71. Pinel JP, Rovner LI. Electrode placement and kindling-induced experimental epilepsy. *Exp Neurol*. 1978;58(2):335-346.
72. Yang M, Clarke AM, Crawley JN. Postnatal lesion evidence against a primary role for the corpus callosum in mouse sociability. *Eur J Neurosci*. 2009;29(8):1663-1677.
73. Ben-Ari Y, Cossart R. Kainate, a double agent that generates seizures: two decades of progress. *Trends Neurosci*. 2000;23(11):580-587.
74. Sheppard E, van Loon E, Underwood G, Ropar D. Attentional differences in a driving hazard perception task in adults with autism spectrum disorders. *J Autism Dev Disord*. 2017;47(2):405-414.
75. Kanne SM, Mazurek MO. Aggression in children and adolescents with ASD: prevalence and risk factors. *J Autism Dev Disord*. 2011;41(7):926-937.
76. Steriade M. Sleep, epilepsy and thalamic reticular inhibitory neurons. *Trends Neurosci*. 2005;28(6):317-324.
77. Paul JR, Davis JA, Goode LK, et al. Circadian regulation of membrane physiology in neural oscillators throughout the brain. *Eur J Neurosci*. 2019;1-30. <https://doi.org/10.1111/ejn.14343>.
78. Welsh DK, Takahashi JS, Kay SA. Suprachiasmatic nucleus: cell autonomy and network properties. *Annu Rev Physiol*. 2010;72:551-577.
79. Snider KH, Sullivan KA, Obrietan K. Circadian regulation of hippocampal-dependent memory: circuits, synapses, and molecular mechanisms. *Neural Plast*. 2018;2018:7292540.
80. Quigg M, Straume M, Menaker M, Bertram EH 3rd. Temporal distribution of partial seizures: comparison of an animal model with human partial epilepsy. *Ann Neurol*. 1998;43(6):748-755.
81. Bailey KR, Crawley JN. Anxiety-related behaviors in mice. In: Buccafusco JJ, ed. *Methods of Behavior Analysis in Neuroscience*. 2nd ed. Boca Raton, FL: CRC Press/Taylor & Francis; 2009. Chapter 5.
82. Arsénault J, Gholizadeh S, Niibori Y, et al. FMRP expression levels in mouse central nervous system neurons determine behavioral phenotype. *Hum Gene Ther*. 2016;27(12):982-996.
83. Qin M, Xia Z, Huang T, Smith CB. Effects of chronic immobilization stress on anxiety-like behavior and basolateral amygdala morphology in *Fmr1* knockout mice. *Neuroscience*. 2011;194:282-290.
84. Becker JA, Clesse D, Spiegelhalter C, Schwab Y, Le Merrer J, Kieffer BL. Autistic-like syndrome in mu opioid receptor null mice is relieved by facilitated mGluR4 activity. *Neuropsychopharmacology*. 2014;39(9):2049-2060.
85. Filliol D, Ghazizadeh S, Chluba J, et al. Mice deficient for delta- and mu-opioid receptors exhibit opposing alterations of emotional responses. *Nat Genet*. 2000;25(2):195-200.
86. Spencer CM, Alekseyenko O, Serysheva E, Yuva-Paylor LA, Paylor R. Altered anxiety-related and social behaviors in the *Fmr1* knockout mouse model of fragile X syndrome. *Genes Brain Behav*. 2005;4(7):420-430.
87. Hampson DR, Hooper AWM, Niibori Y. The application of adeno-associated viral vector gene therapy to the treatment of fragile X syndrome. *Brain Sci*. 2019;9(2):2019.
88. Rubin RD, Watson PD, Duff MC, Cohen NJ. The role of the hippocampus in flexible cognition and social behavior. *Front Hum Neurosci*. 2014;8:742.
89. Alvarez JA, Emory E. Executive function and the frontal lobes: a meta-analytic review. *Neuropsychol Rev*. 2006;16(1):17-42.
90. Zurcher NR, Rogier O, Boshyan J, et al. Perception of social cues of danger in autism spectrum disorders. *PLoS One*. 2013;8(12):e81206.
91. Etkin A, Wager TD. Functional neuroimaging of anxiety: a meta-analysis of emotional processing in PTSD, social anxiety disorder, and specific phobia. *Am J Psychiatry*. 2007;164(10):1476-1488.
92. Robinson OJ, Charney DR, Overstreet C, Vytal K, Grillon C. The adaptive threat bias in anxiety: amygdala-dorsomedial prefrontal cortex coupling and aversive amplification. *Neuroimage*. 2012;60(1):523-529.
93. Quirk GJ, Likhtik E, Pelletier JG, Pare D. Stimulation of medial prefrontal cortex decreases the responsiveness of central amygdala output neurons. *J Neurosci*. 2003;23(25):8800-8807.
94. Peng H, Bian XL, Ma FC, Wang KW. Pharmacological modulation of the voltage-gated neuronal Kv7/KCNQ/M-channel alters the intrinsic excitability and synaptic responses of pyramidal neurons in rat prefrontal cortex slices. *Acta Pharmacol Sin*. 2017;38(9):1248-1256.
95. Kapfhamer D, Berger KH, Hopf FW, et al. Protein phosphatase 2a and glycogen synthase kinase 3 signaling modulate prepulse inhibition of the acoustic startle response by altering cortical M-type potassium channel activity. *J Neurosci*. 2010;30(26):8830-8840.
96. Schmeisser MJ, Ey E, Wegener S, et al. Autistic-like behaviours and hyperactivity in mice lacking *ProSAP1/Shank2*. *Nature*. 2012;486(7402):256-260.
97. Peca J, Feliciano C, Ting JT, et al. *Shank3* mutant mice display autistic-like behaviours and striatal dysfunction. *Nature*. 2011;472(7344):437-442.
98. Zhang K, Hill K, Labak S, Blatt GJ, Soghomonian JJ. Loss of glutamic acid decarboxylase (*Gad67*) in *Gpr88*-expressing neurons induces learning and social behavior deficits in mice. *Neuroscience*. 2014;275:238-247.
99. Hansen HH, Andreasen JT, Weikop P, Mirza N, Scheel-Kruger J, Mikkelsen JD. The neuronal KCNQ channel opener retigabine inhibits locomotor activity and reduces forebrain excitatory responses to the psychostimulants cocaine, methylphenidate and phencyclidine. *Eur J Pharmacol*. 2007;570(1-3):77-88.
100. Hansen HH, Weikop P, Mikkelsen MD, Rode F, Mikkelsen JD. The pan-Kv7 (KCNQ) channel opener Retigabine inhibits striatal excitability by direct action on striatal neurons in vivo. *Basic Clin Pharmacol Toxicol*. 2017;120(1):46-51.
101. Albelda N, Joel D. Current animal models of obsessive compulsive disorder: an update. *Neuroscience*. 2012;211:83-106.
102. Tillmann J, Ashwood K, Absoud M, et al. Evaluating sex and age differences in ADI-R and ADOS scores in a large European multi-site sample of individuals with autism spectrum disorder. *J Autism Dev Disord*. 2018;48(7):2490-2505.
103. Frazier TW, Georgiades S, Bishop SL, Hardan AY. Behavioral and cognitive characteristics of females and males with autism in the Simons simplex collection. *J Am Acad Child Adolesc Psychiatry*. 2014;53(3):329-340.e1-3.
104. McLean CP, Asnaani A, Litz BT, Hofmann SG. Gender differences in anxiety disorders: prevalence, course of illness, comorbidity and burden of illness. *J Psychiatr Res*. 2011;45(8):1027-1035.
105. Rapin I, Tuchman RF. Autism: definition, neurobiology, screening, diagnosis. *Pediatr Clin North Am*. 2008;55(5):1129-1146. viii.
106. Millward C, Powell S, Messer D, Jordan R. Recall for self and other in autism: children's memory for events experienced by themselves and their peers. *J Autism Dev Disord*. 2000;30(1):15-28.
107. Brezis RS. Memory integration in the autobiographical narratives of individuals with autism. *Front Hum Neurosci*. 2015;9:76.

108. Pearson BL, Defensor EB, Blanchard DC, Blanchard RJ. C57BL/6J mice fail to exhibit preference for social novelty in the three-chamber apparatus. *Behav Brain Res*. 2010;213(2):189-194.
109. Kennedy DP, Courchesne E. The intrinsic functional organization of the brain is altered in autism. *Neuroimage*. 2008;39(4):1877-1885.
110. Lee E, Rhim I, Lee JW, et al. Enhanced neuronal activity in the medial prefrontal cortex during social approach behavior. *J Neurosci*. 2016;36(26):6926-6936.
111. Soh H, Park S, Ryan K, Springer K, Maheshwari A, Tzingounis AV. Deletion of KCNQ2/3 potassium channels from PV+ interneurons leads to homeostatic potentiation of excitatory transmission. *Elife*. 2018;7:e38617.
112. Supekar K, Kochalka J, Schaer M, et al. Deficits in mesolimbic reward pathway underlie social interaction impairments in children with autism. *Brain*. 2018;141(9):2795-2805.
113. Drion G, Bonjean M, Waroux O, et al. M-type channels selectively control bursting in rat dopaminergic neurons. *Eur J Neurosci*. 2010;31(5):827-835.
114. Koyama S, Appel SB. Characterization of M-current in ventral tegmental area dopamine neurons. *J Neurophysiol*. 2006;96(2):535-543.
115. Sotty F, Damgaard T, Montezinho LP, et al. Antipsychotic-like effect of retigabine [N-(2-amino-4-[fluorobenzylamino]-phenyl)carbamic acid ester], a KCNQ potassium channel opener, via modulation of mesolimbic dopaminergic neurotransmission. *J Pharmacol Exp Ther*. 2009;328(3):951-962.
116. Sapolsky RM. The influence of social hierarchy on primate health. *Science*. 2005;308(5722):648-652.
117. Reese RM, Richman DM, Belmont JM, Morse P. Functional characteristics of disruptive behavior in developmentally disabled children with and without autism. *J Autism Dev Disord*. 2005;35(4):419-428.
118. Bicks LK, Koike H, Akbarian S, Morishita H. Prefrontal cortex and social cognition in mouse and man. *Front Psychol*. 2015;6:1805.
119. Aleyasin H, Flanigan ME, Russo SJ. Neurocircuitry of aggression and aggression seeking behavior: nose poking into brain circuitry controlling aggression. *Curr Opin Neurobiol*. 2018;49:184-191.
120. Davis JF, Krause EG, Melhorn SJ, Sakai RR, Benoit SC. Dominant rats are natural risk takers and display increased motivation for food reward. *Neuroscience*. 2009;162(1):23-30.
121. St Onge JR, Floresco SB. Prefrontal cortical contribution to risk-based decision making. *Cereb Cortex*. 2010;20(8):1816-1828.
122. Fellows LK, Farah MJ. The role of ventromedial prefrontal cortex in decision making: judgment under uncertainty or judgment per se? *Cereb Cortex*. 2007;17(11):2669-2674.
123. Spence SJ, Schneider MT. The role of epilepsy and epileptiform EEGs in autism spectrum disorders. *Pediatr Res*. 2009;65(6):599-606.
124. Millichap JJ, Miceli F, De Maria M, et al. Infantile spasms and encephalopathy without preceding neonatal seizures caused by KCNQ2 R198Q, a gain-of-function variant. *Epilepsia*. 2017;58(1):e10-e15.
125. Mulkey SB, Ben-Zeev B, Nicolai J, et al. Neonatal nonepileptic myoclonus is a prominent clinical feature of KCNQ2 gain-of-function variants R201C and R201H. *Epilepsia*. 2017;58(3):436-445.
126. Richards MC, Heron SE, Spendlove HE, et al. Novel mutations in the KCNQ2 gene link epilepsy to a dysfunction of the KCNQ2-calmodulin interaction. *J Med Genet*. 2004;41(3):e35.
127. Grinton BE, Heron SE, Pelekanos JT, et al. Familial neonatal seizures in 36 families: clinical and genetic features correlate with outcome. *Epilepsia*. 2015;56(7):1071-1080.
128. Buzsaki G. Theta oscillations in the hippocampus. *Neuron*. 2002;33(3):325-340.
129. Seager MA, Johnson LD, Chabot ES, Asaka Y, Berry SD. Oscillatory brain states and learning: impact of hippocampal theta-contingent training. *Proc Natl Acad Sci U S A*. 2002;99(3):1616-1620.
130. Winson J. Loss of hippocampal theta rhythm results in spatial memory deficit in the rat. *Science*. 1978;201(4351):160-163.
131. Huerta PT, Lisman JE. Heightened synaptic plasticity of hippocampal CA1 neurons during a cholinergically induced rhythmic state. *Nature*. 1993;364(6439):723-725.
132. Hu H, Vervaeke K, Storm JF. Two forms of electrical resonance at theta frequencies, generated by M-current, h-current and persistent Na+ current in rat hippocampal pyramidal cells. *J Physiol*. 2002;545(3):783-805.
133. Li C, Huang P, Lu Q, Zhou M, Guo L, Xu X. KCNQ/Kv7 channel activator flupirtine protects against acute stress-induced impairments of spatial memory retrieval and hippocampal LTP in rats. *Neuroscience*. 2014;280:19-30.
134. Fontan-Lozano A, Suarez-Pereira I, Delgado-Garcia JM, Carrion AM. The M-current inhibitor XE991 decreases the stimulation threshold for long-term synaptic plasticity in healthy mice and in models of cognitive disease. *Hippocampus*. 2011;21(1):22-32.
135. Deeks ED. Retigabine (ezogabine): in partial-onset seizures in adults with epilepsy. *CNS Drugs*. 2011;25(10):887-900.
136. Xiong Q, Sun H, Li M. Zinc pyrithione-mediated activation of voltage-gated KCNQ potassium channels rescues epileptogenic mutants. *Nat Chem Biol*. 2007;3(5):287-296.
137. Wickenden AD, Krajewski JL, London B, et al. N-(6-chloro-pyridin-3-yl)-3,4-difluoro-benzamide (ICA-27243): a novel, selective KCNQ2/Q3 potassium channel activator. *Mol Pharmacol*. 2008;73(3):977-986.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

How to cite this article: Kim EC, Patel J, Zhang J, et al. Heterozygous loss of epilepsy gene *KCNQ2* alters social, repetitive and exploratory behaviors. *Genes, Brain and Behavior*. 2020;19:e12599. <https://doi.org/10.1111/gbb.12599>