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RESEARCH PAPER

Prefrontal inhibition of neuronal K_v7 channels enhances prepulse inhibition of acoustic startle reflex and resistance to hypofrontality

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Background and Purpose: Dysfunction of the prefrontal cortex (PFC) is involved in the cognitive deficits in neuropsychiatric diseases, such as schizophrenia, characterized by deficient neurotransmission known as NMDA receptor hypofrontality. Thus, enhancing prefrontal activity may alleviate hypofrontality-induced cognitive deficits. To test this hypothesis, we investigated the effect of forebrain-specific suppression or pharmacological inhibition of native K_v7/KCNQ/M-current on glutamatergic hypofrontality induced by the NMDA receptor antagonist MK-801.

Experimental Approach: The forebrain-specific inhibition of native M-current was generated by transgenic expression, in mice, of a dominant-negative pore mutant G279S of $K_v7.2$/KCNQ2 channels that suppresses channel function. A mouse model of cognitive impairment was established by single i.p. injection of 0.1 $mg{\cdot}kg^{-1}$ MK-801. Mouse models of prepulse inhibition (PPI) of acoustic startle reflex and Y-maze spontaneous alternation test were used for evaluation of cognitive behaviour. Hippocampal brain slice recordings of LTP were used to assess synaptic plasticity. Hippocampus and cortex were dissected for detecting protein expression using western blot analysis.

Key Results: Genetic suppression of K_v7 channel function in the forebrain or pharmacological inhibition of K_v7 channels by the specific blocker XE991 enhanced PPI and also alleviated MK-801 induced cognitive decline. XE991 also attenuated MK-801-induced LTP deficits and increased basal synaptic transmissions. Western blot analysis revealed that inhibiting K_v7 channels resulted in elevation of pAkt1 and pGSK-3β expressions in both hippocampus and cortex.

Conclusions and Implications: Both genetic and pharmacological inhibition of K_v7 channels alleviated PPI and cognitive deficits. Mechanistically, inhibition of K_v7 channels promotes synaptic transmission and activates Akt1/GSK-3β signalling.

Abbreviations: fEPSPs, field excitatory postsynaptic potentials; HFS, high-frequency stimulation; I/O, input–output; PFC, prefrontal cortex; PPF, paired-pulse facilitation; PPI, prepulse inhibition; Tg, transgenic; WT, wild-type.

Jing Wang and Wenwen Yu contributed equally to this work.

KEYWORDS

glutamatergic hypofrontality, KCNQ2, Kv7, M-current, prefrontal cortex, prepulse inhibition, XE991

1 | INTRODUCTION

The prefrontal cortex (PFC) is profusely connected with brain regions critical for sensory perception, cognition, and emotion (Kolb et al., 2012). Dysfunction of the PFC is involved in the pathophysiology of several neuropsychiatric disorders, including schizophrenia, anxiety, and attention deficit (Goto, Yang, & Otani, 2010; Senkowski & Gallinat, 2015). For instance, the hippocampal–PFC or thalamic–PFC connectivity in patients with schizophrenia is significantly reduced, resulting in impairment of executive cognitive function (Bähner & Meyer-Lindenberg, 2017; Giraldo-Chica, Rogers, Damon, Landman, & Woodward, 2018). In contrast, the enhancement of the dorsolateral PFC function induced by repetitive transcranial magnetic stimulation promotes the improvement of cognitive abilities in healthy individuals (Bagherzadeh, Khorrami, Zarrindast, Shariat, & Pantazis, 2016), and pharmacological enhancement of PFC activity also improves the inhibition of emotional responses in schizophrenics (Kindler, Weickert, Schofield, Lenroot, & Weickert, 2016).

Functional brain imaging has shown that the PFC is hypofunctional in schizophrenia, a debilitating mental disorder characterized by psychosis, including impaired prepulse inhibition (PPI) of acoustic startle response and cognitive deficits (Bedford, Surguladze, Giampietro, Brammer, & David, 2012; Dreher et al., 2012). The PPI measurement is a clinical evaluation of the sensorimotor gating function in schizophrenics (Mena et al., 2016). Sensorimotor gating underlies the neural control mechanisms that filter out irrelevant sensory stimuli to ensure the process of meaningful sensory information is closely related to cognitive function (Dissanayake, Mason, & Marsden, 2013; Tóth et al., 2017). Clinical and preclinical studies indicate that disruptions in PFC function lead to impairment of the PPI (Alam et al., 2015; Day-Wilson, Jones, Southam, Cilia, & Totterdell, 2006; Hazlett & Buchsbaum, 2001; Zavitsanou, Cranney, & Richardson, 1999). Conversely, the recovery of PFC function by pharmacological means can alleviate PPI damage (Fejgin et al., 2009).

Hypofrontality is manifested by decreased utilization of glucose and blood flow in the PFC, accounting for impairment in neurotransmission, synaptic connectivity and cognition (Pratt, Winchester, Egerton, Cochran, & Morris, 2008). Hypofunction of the **NMDA** receptor system in the PFC has been causally linked to hypofrontality (Pratt et al., 2008), which is thought to be responsible for the pathophysiology of schizophrenia (Olney & Farber, 1995). NMDA receptor antagonists, such as **ketamine**, **phencyclidine**, and **dizocilpine** (MK-801), can produce schizophrenia-like symptoms, such as locomotor hyperactivity, social avoidance, PPI disruption of the startle response (Adell, Jimenez-Sanchez, Lopez-Gil, & Romon, 2012), decreased PFC function, and cognitive impairment, in monkeys (Jentsch et al., 1997). Systemic administration of NMDA receptor antagonists also leads to deficits in PFC-dependent working memory (Auger & Floresco, 2017). All these studies suggest that enhancing PFC function may alleviate PPI impairment and the cognitive deficits common in schizophrenia.

What is already known

- Dysfunction of the prefrontal cortex contributes to cognitive deficits in neuropsychiatric diseases, such as schizophrenia.
- Inhibition of native M-current increases neuronal excitation in the PFC

What this study adds

- Inhibiting K_v7 channels alleviates prepulse inhibition impairment and cognitive deficits induced by NMDA receptor blockade.
- Inhibiting K_v7 channels improves synaptic transmission and activates the Akt1/GSK-3β signalling pathway.

What is the clinical significance

- Pharmacological inhibition of K_v7 channels may have therapeutic potential in neuropsychiatric disorders such as schizophrenia.

The K_v7/KCNQ/M channels encoded by the genes *KCNQ1–5* are a subfamily of **voltage-gated K^+ channel** superfamily (Brown & Passmore, 2009). Whereas $K_v7.1$/KCNQ1 channels are primarily expressed in the heart, the $K_v7.2–7.5$/KCNQ2–5 channels are mostly found in the CNS (Delmas & Brown, 2005), and mutations in these channels cause neonatal epilepsy (Biervert et al., 1998; Charlier et al., 1998). The neuronal K_v7/KCNQ/M-currents are characteristic of slowly activating and non-inactivating voltage-gated K^+ currents that regulate neuronal excitability by suppressing repetitive firing, action potential threshold, and controlling spontaneous firings (Brown & Passmore, 2009; Wang et al., 1998). Our previous investigations have shown that inhibition of M-current (carried by K_v7 channels) increased the intrinsic excitability of neurons and excitatory synaptic response in the PFC, suggesting a beneficial effect on cognition (Peng, Bian, Ma, & Wang, 2017). However, whether regulating K_v7 channel function in the PFC can alleviate PPI impairment or cognitive deficits induced by glutamatergic hypofrontality, remains largely unknown.

In this study, we investigated the effect of enhancing prefrontal activity by genetic suppression of native M-current or pharmacological inhibition of K_v7 channels on PPI of acoustic startle response and working memory in mice treated with the NMDA receptor antagonist, MK-801. Our findings show that genetic or pharmacological inhibition of neuronal K_v7 channels can alleviate PPI and cognitive deficits induced by NMDA antagonists, thus suggesting a therapeutic potential for such inhibition of K_v7 channels in the treatment of schizophrenia or cognitive deficit disorders.

2 | METHODS

2.1 | Generation of transgenic mice

The generation of our transgenic (Tg) mice expressing a dominant-negative pore mutation of rat $K_v7.2$/ **KCNQ2** channel (rQ2-G279S) was made using a forebrain-specific αCaMKII promoter that drives specific expression in the forebrain (Bi et al., 2011). Briefly, the pore-lining residue Gly279 of rat KCNQ2 channel was mutated into Ser, mutant rQ2-G279S, that exerts dominant-negative effect upon co-assembly with wild-type (WT) KCNQ channel. rQ2-G279S cDNA was subcloned into 265-plasmid with forebrain-specific αCaMKII promoterv that is widely used for gene expression (Tsien et al., 1996). The αCaMKII expression is very low at birth, and only reaches the adult level until 16 days after birth (Burgin et al., 1990). B6D2F1/Crl (RRID:IMSR_CRL:099) strain Tg mice were obtained by microinjection of linear 265-plasmid with rQ2-G279S cDNA into mouse fertilized eggs. Tg mice were selected by genomic typing with transgene-specific PCR primers (upstream 5′-GCT AGA GGA TCT TTG TGT AAG GAA C-3′, downstream 5′GGA AAG TCC TTG GGG TCT TCT ACC-3′). The genotypic identification of Tg mice was carried out by genomic PCR. Expression and distribution of mutant genes encoding rQ2-G279S were identified by real-time quantitative PCR and in situ hybridization. In situ hybridization showed that rQ2-G279S mutant mRNAs were highly expressed in the cortex, striatum, hippocampus, thalamus, and other forebrain regions, and they were rarely expressed in the cerebellum, almost not in the midbrain, pons, and medulla oblongata (Bi et al., 2011).

2.2 | Animals

All animal care and experimental procedures complied with the ethical guidelines of the International Association for Animal Welfare and were approved by the Animal Use and Care Committee of Qingdao University. Animal studies are reported in compliance with the ARRIVE guidelines (Percie du Sert et al., 2020) and with the recommendations made by the *British Journal of Pharmacology* (Lilley et al., 2020). The rQ2-G279S Tg and WT mice at age of 8–12 weeks were housed in the Animal Management Center of Qingdao University. Adult (8 to 10 weeks old) C57BL/6J (RRID:IMSR_JAX:000664) male mice were provided by Beijing Vital River Laboratory Animal Technology Co. Ltd. Mice were housed in plastic cages (three to five per cage) with natural poplar wood bedding material, in controlled room temperature (23 ± 2°C) and humidity (50 ± 5%) , with 12-h light/dark cycle (lights on at 7:00 a.m.) and free access to food and water. Behavioural experiments were conducted between 9:00 a.m. and 5:00 p.m. Mice were acclimated in the laboratory for 1 week before the experiments. After behavioural experiments, mice were anaesthetized with isoflurane and quickly decapitated and removed the whole brain for further study.

2.3 | Drug administration

To establish the model of cognitive impairment, the NMDA receptor antagonist MK-801 (0.1 $mg{\cdot}kg^{-1}$, i.p.) was administered 30 min before testing. Other compounds, such as **XE991** (1 $mg{\cdot}kg^{-1}$, i.p.), **retigabine** (10 $mg{\cdot}kg^{-1}$, i.p.) or **clozapine** (1 $mg{\cdot}kg^{-1}$, i.p.), were administered to mice 60 min prior to behavioural or electrophysiological tests. The dose and time for administration of XE991 or retigabine were based on previous publications (Fontán-Lozano, Suárez-Pereira, Delgado-García, & Carrión, 2011; Nielsen, Mathiesen, & Blackburn-Munro, 2004). All drugs were prepared freshly on the test day.

2.4 | Test for PPI of acoustic startle response

The PPI protocol was designed as previously described (Yee, Chang, & Feldon, 2004). The PPI test was carried out within four sound-attenuated startle chambers and the mouse whole-body startle was detected by a piezoelectric motion sensor on the platform (Pan Lab Harvard Apparatus). One day before the experiment, mice were habituated to the test apparatus for 15 min with 68 dB background white noise. The PPI test session consisted of 5-min acclimation period with background noise (68 dB), followed by seven trial types presented 10 times (about 15 min). Each trial consisted of 68 dB background noise, pulse-alone stimulus (120 dB), and five types of prepulse (72, 76 80, 84, and 88 dB) + pulse trials. The background noise was present during the test, and the pulse-alone trials were lasted for 40 ms, and the prepulse sounds were presented for 20 ms and applied 80 ms prior to the presentation of the pulse-alone stimulus. The intervals between trials were ranging from 8 to 22 s with an average of 15 s.

The per cent of PPI was computed by applying the following formula: %PPI = [1 − (startle response for prepulse + startle trial)/(startle response for startle stimulus alone trial)] × 100%. When the value is 0, there is no PPI, and the greater the value of PPI, the deeper the degree of inhibition.

2.5 | Y-maze test

The Y-maze test is a spontaneous alternating behavioural assay that evaluates short-term spatial working memory and examines the

function of the basal forebrain, hippocampus, PFC, and septum. The Y-maze apparatus is made up with three arms of equal size, which are termed respectively: start arm, familiar arm, and novel arm. The protocol consisted of 10-min habituation and 4-min test. For the 10-min habituation, the novel arm was blocked, and other arms were open. Each mouse was allowed to move only in the start arm and the familiar arm. During the 4-min test, each mouse was allowed to freely explore three arms. The habituation and testing are separated by 2 min of intertrial interval (Cortes-Canteli et al., 2010). Mouse activities were recorded with a video camera mounted above the maze and attached with an interface to Smart 3.0 video tracking software.

After completion of Y-maze or PPI tests, the hippocampus and cortex were isolated and extracted for measurement of gene and protein expression.

2.6 | Electrophysiology

2.6.1 | Brain slice preparations

Animals were anaesthetized with isoflurane before decapitation and removal of the brain. Coronal brain slices (400 μm thickness) were prepared by Vibratome (Leica VT1200S) and incubated in oxygenated artificial CSF for 1 h at room temperature (Winder, Mansuy, Osman, Moallem, & Kandel, 1998).

2.6.2 | Field potential recordings

The field excitatory postsynaptic potentials (fEPSPs) were recorded from the stratum radiatum of the hippocampal CA1 region through stimulation of the Schaffer collateral pathway. By increasing stimulation intensity (0.02 to 0.12 mA at intervals of 0.02 mA), the input–output (I/O) curves between stimulation intensity and evoked field potentials were established for evaluation of synaptic transmissions. The stimulus intensities were adjusted to evoke the fEPSP slope of 50% of the maximum fEPSP slope. Paired-pulse facilitation (PPF), a short-term synaptic plasticity, was tested at inter-stimulus intervals of 20, 40, 60, 80, 100, 300, 500, 700, and 900 ms (Lee et al., 2003). The paired-pulse ratio was calculated by the second pulse-evoked fEPSP divided by the first one. LTP was induced through a high-frequency stimulation (HFS; 100 Hz for 1 s) after 20 min of stable baseline recordings (Bozdagi, Nagy, Kwei, & Huntley, 2007).

2.7 | Western blot

The immuno-related procedures used comply with the recommendations made by the *British Journal of Pharmacology* (Alexander et al., 2018). Briefly, total proteins were extracted from the whole hippocampus and forebrain cortex tissues by RIPA lysis buffer with 1% protease and phosphatase inhibitor cocktail (Thermo Fisher Scientific). Protein concentrations were measured by the BCA Protein Assay Kit (Thermo Fisher Scientific). Primary antibodies included rabbit anti-**Akt1** (1:1000, CST Cat# 2938, RRID: AB_915788), rabbit anti-pAkt1 (Ser473) (1:1000, CST Cat# 9018, RRID:AB_2629283), rabbit anti-**GSK-3β** (1:1000, CST Cat# 9315, RRID:AB_490890), rabbit anti-pGSK-3β (Ser9) (1:1000, CST Cat# 9323, RRID:AB_2115201), rabbit anti-**β-catenin** (1:1000, CST Cat# 8480, RRID:AB_11127855), and rabbit anti-GAPDH (1:10000). The protein bands were detected on the Bio-Rad ChemiDoc XRS + system using the enhanced chemiluminescence kit. The relative expression of proteins was measured by Image Lab software (Bio-Rad Laboratories, RRID:SCR_008426).

2.8 | Data and statistical analysis

We have complied with the recommendations of the *British Journal of Pharmacology* on experimental design and analysis in pharmacology (Curtis et al., 2018). Studies were designed to generate groups in equal size. All mice were randomized for treatment, and the experimenters were blind to the treatment of the animals while conducting the tests and analysing the data. Statistical analyses for group comparisons were undertaken only if group size (n) ≥ 5. Data from group sizes (n) < 5 were not subjected to statistical analysis. Five independent repeats at least were performed for each experiment. The group size was based on the expected difference from our pilot studies using similar protocols in mice without carrying out a formal power analysis. The group size was the number of independent values, and statistical analysis was performed using independent values corresponding to the data. Data were used for statistical analysis without normalization. Potential outliers were included in data analysis and presentation. Statistical analysis was performed using the GraphPad Prism 5.0 software (RRID: SCR_002798). All data were expressed as the mean ± SEM. Statistical significance was analysed by one-way ANOVA or two-way ANOVA. Data for normal distribution were analysed by Kolmogorov–Smirnov test, and the homogeneity of variance was confirmed by Bartlett's test. The Bonferroni post hoc tests were conducted only if the F in ANOVA achieved $P < 0.05$, and there was no significant variance inhomogeneity; otherwise, the data were converted into logarithms for correction. The P value < 0.05 was considered to be statistically significant.

2.9 | Materials

MK-801 was purchased from MedChemExpress (Shanghai, China). XE991 was purchased from Abcam (Shanghai, China). Retigabine was from Simcere (Shanghai, China). Clozapine was purchased from Sigma (St. Louis, MO, USA). The GAPDH antibody (Abcam Cat# ab181602, RRID:AB_2630358) was purchased from Abcam (Shanghai, China) and all other antibodies were purchased from Cell Signaling Technology (USA).

2.10 | Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in the IUPHAR/BPS Guide to PHARMACOLOGY (http://www.guidetopharmacology.org) and are permanently archived in the Concise Guide to PHARMACOLOGY 2019/20 (Alexander, Fabbro et al., 2019; Alexander, Mathie et al., 2019).

3 | RESULTS

3.1 | Tg mice with forebrain-specific suppression of K_v7 channel function were resistant to impairment of PPI induced by the NMDA receptor antagonist MK-801

We started by examining the phenotype of sensorimotor gating in Tg mice previously generated using a forebrain-specific αCaMKII promoter that drives the expression of a dominant-negative rKCNQ2 G279S pore mutant suppressing native KCNQ/M-current and enhancing forebrain excitability (Bi et al., 2011). Using the PPI test (Figure 1a), we surprisingly found that the Tg mice, both male and female, exhibited a stronger attenuation of startle response with an increased PPI at different prepulse intensities of 72, 76, 80, 84, and 88 dB, compared with WT mice (Figure 1b). To further confirm this observation, we injected the NMDA receptor antagonist MK-801 (i.p., 0.1 mg·kg^{-1}) and observed that WT mice treated with MK-801 showed weak inhibition of startle response with significant reduction of PPI at prepulse intensities of 72, 76, 80, 84, and 88 dB, compared to vehicle-treated WT mice (Figure 1c). In contrast, the Tg mice exhibited a greater inhibition of startle response and resilience to PPI impairment induced by MK-801 (Figure 1c). These results demonstrate Tg mice with suppression of native M-current function in the forebrain were resistant to impairment of sensorimotor gating.

To further test the modulatory effect of K_v7 channels on PPI, we administered (i.p.) two specific modulators of the M-current, a channel blocker XE991 developed by DuPont in the 1980s as a memory enhancer, and a channel opener retigabine, a FDA-approved antiepileptic agent, and evaluated their effects on MK-801-mediated PPI impairment. As shown in Figure 1d, MK-801 (0.1 mg·kg^{-1}) significantly reduced the magnitude of PPI at 80, 84, and 88 dB prepulses, as compared with the vehicle group. In contrast, the specific K_v7 channel blocker XE991 (1 mg·kg^{-1}, i.p.) or the antipsychotic clozapine (1 mg·kg^{-1}, i.p.) as positive control significantly reversed MK-801-induced PPI deficits at 84 and 88 dB prepulses. Conversely, co-administration of XE991 with the K_v7 channel opener retigabine (10 mg·kg^{-1}, i.p.) reversed the PPI enhancement caused by XE991 (Figure 1d). These results are consistent with the observation of increased sensorimotor gating in rQ2-G279S Tg mice, further demonstrating that inhibiting K_v7 channels leads to improvements in sensorimotor gating.

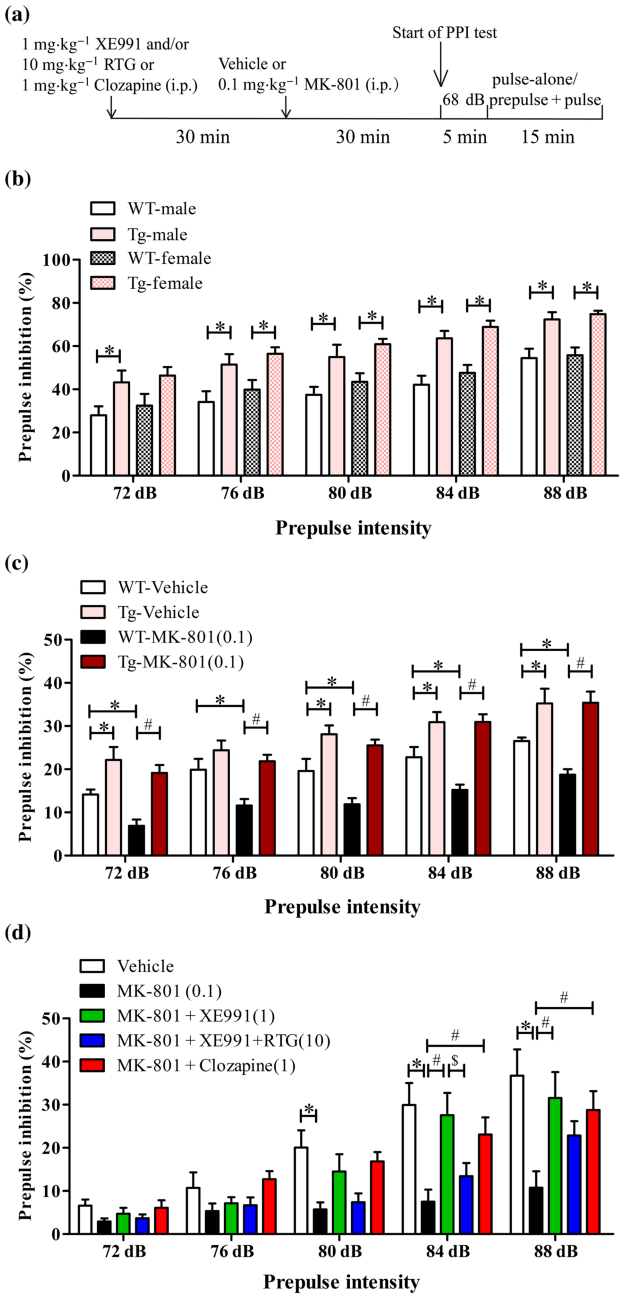

FIGURE 1 Genetic and pharmacological inhibition of K_v7 channels increased resistance to PPI impairment induced by the NMDA receptor antagonist MK-801. (a) The time-course of protocols to assay PPI of acoustic startle response in mice. (b) Enhancement of PPI in rQ2-G279S Tg mice, compared to WT controls. $^{*}P < 0.05$, significantly different as indicated. (c) Resistance to PPI deficits induced by MK-801 (0.1 mg·kg^{-1}) in Tg mice compared with WT mice. $^{*}P < 0.05$, significantly different from WT-Vehicle, $^{\#}P < 0.05$, significantly different fromWT-MK-801 (0.1 mg·kg^{-1}). (d) Amelioration of MK-801-induced PPI deficits by administration of XE991 (1 mg·kg^{-1}, i.p.) or clozapine (1 mg·kg^{-1}, i.p.) as positive control. Retigabine (RTG; 10 mg·kg^{-1}, i.p.) was used to antagonize XE991-mediated effect. Data shown are means ± SEM; n = 8–12. $^{*}P < 0.05$, significantly different from Vehicle, $^{\#}P < 0.05$, significantly different from MK-801 (0.1 mg·kg^{-1}), $^{\$}P < 0.05$, significantly different from MK-801 (0.1 mg·kg^{-1}) + XE991 (1 mg·kg^{-1}); two-way ANOVA, followed by post hoc Bonferroni's test

3.2 | Attenuation of MK-801-induced memory deficit by inhibition of K_v7 channel activity in the Y-maze test

To assess the effect of K_v7 channel inhibition on spatial working memory, we utilized the mouse model of the Y-maze test and recorded the time spent in the novel arm (Figure 2a). As shown in Figure 2b, the Tg mice, both male and female, spent more time in the novel arm than WT mice with no difference in their total travel distance. To further confirm the effect of Kv7 suppression on attenuation of cognitive deficit induced by NMDA antagonism, we used the two specific K_v7 channel modulators, the blocker XE991 and the opener retigabine, and tested their effects on memory impairments induced by MK-801. The i.p. injection of MK-801 (0.1 mg·kg^{-1}) resulted in a significant decrease of the time spent in the novel arm and an increase of total distance travelled, compared with data from the vehicle control group (Figure 2c). In contrast, i.p. administration of the specific K_v7 channel blocker XE991 (1 mg·kg^{-1}) caused a significant increase of the time spent and decrease of the total distance in mice treated with MK-801 (Figure 2c). The XE991-mediated effect was abolished by co-injection of the channel opener retigabine (10 mg·kg^{-1}, i.p.) (Figure 2c). In another control, we also tested the effect of the antipsychotic drug clozapine on MK-801-induced memory deficiency. Similarly, clozapine at 1 mg·kg^{-1} (i.p.) increased the time spent in the novel arm (Figure 2c). There was no impairment of locomotion between the groups of vehicle control mice and mice treated with XE991 (1 mg·kg^{-1}) (Figure 2c). These results indicate that inhibition of K_v7 channels attenuated the spatial working memory deficiency.

3.3 | Improvement of impaired synaptic transmission induced by MK-801, after inhibition of K_v7 channels

To assess the effect of K_v7 channel blockade on synaptic transmission, we carried out recordings of LTP induced by high-frequency tetanic stimulation in acute mouse brain slices from the hippocampal CA1 region. As shown in Figure 3a, hippocampal LTP, with increased fEPSP slope, was induced by HFS in brain slices from WT mice, compared with a significant reduction of LTP magnitude in mice treated with MK-801 (0.1 mg·kg^{-1}, i.p.). In contrast, co-administration of

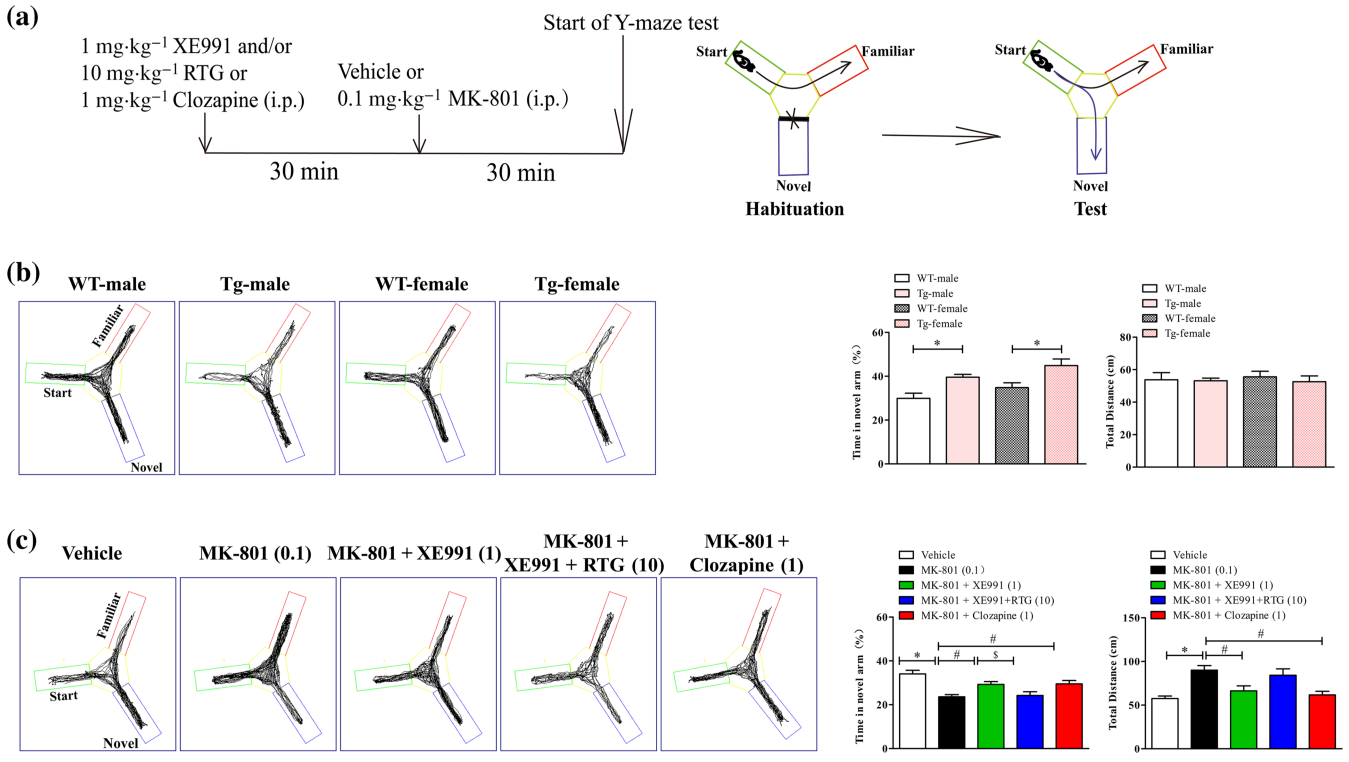

FIGURE 2 Attenuation of MK-801-induced memory impairment by inhibition of K_v7 channel activity in Y-maze test. (a) Time-course of protocols for Y-maze test in mice. (b) Left panels: Representative motion trajectories of mice in different groups during Y-maze test. Right panels: A summary from left panels for the time spent in the novel arm and total distance travelled in three arms between the rQ2-G279S Tg mice and WT mice. $^{*}P < 0.05$, significantly different as indicated. (c) Left panels: Representative motion trajectories of mice in different groups of mice treated with MK-801 (0.1 mg·kg^{-1}, i.p.). Clozapine (1 mg·kg^{-1}) and RTG (10 mg·kg^{-1}) were i.p. administered as controls. Right panels: A summary from left panels for the time spent in the novel arm and the total distance in three arms in different groups. $^{*}P < 0.05$, significantly different from Vehicle, $^{\#}P < 0.05$, significantly different from MK-801 (0.1 mg·g^{-1}), $^{\$}P < 0.05$, significantly different from MK-801 (0.1 mg·kg^{-1}) + XE991 (1 mg·kg^{-1}); one-way ANOVA, followed by post hoc Bonferroni's test. Data shown are means ± SEM; n = 6–10

XE991 partly reversed MK-801-induced decrease of LTP magnitude with fEPSP slope (Figure 3a), whereas co-injection of retigabine (10 mg·kg^{-1}, i.p.) with XE991 reduced the fEPSP slope further (Figure 3a). Similarly, co-treatment with the antipsychotic drug clozapine as a positive control also partly reversed MK-801-induced decrease of LTP (Figure 3a). Further analysis of the I/O relationship revealed a significant increase of fEPSP slope after tetanization (0.02–0.2 mA) in both XE991 and clozapine groups as compared with MK-801 (0.1 mg·kg^{-1}, i.p.) alone group (Figure 3b). These results show that pharmacological inhibition of K_v7 channels by the blocker XE991 can enhance synaptic strength and plasticity, thus facilitating hippocampal synaptic neurotransmissions.

To test the effect of K_v7 channel inhibition on presynaptic function, we examined the short-term plasticity by recording the PPF in hippocampal slices. The analysis of PPF index by applying a short interpulse interval (20–900 ms) was not affected by either XE991 (1 mg·kg^{-1}, i.p.) or clozapine (1 mg·kg^{-1}, i.p.), compared with the effects of MK-801 (0.1 mg·kg^{-1}, i.p.), but there was a significant increase of the PPF ratio in WT mice (Figure 3c), suggesting that the effect of K_v7 channel inhibition on cognition may not be involved in the presynaptic transmission.

3.4 | Up-regulation of Akt1/GSK-3β signalling by genetic or pharmacological inhibition of K_v7 channel function

Activation or phosphorylation of the Akt1/GSK-3β pathway has been shown to be involved in either sensorimotor gating (Chen & Lai, 2011; Lovestone, Killick, Di Forti, & Murray, 2007) or Kv7.2 channel function (Borsotto et al., 2007; Kapfhamer et al., 2010). To examine any effect of K_v7 channel inhibition on Akt1/GSK-3β signalling in hippocampus and cortex tissues from Tg mice, we measured the expression and phosphorylation of Akt1 and GSK-3β proteins. As shown in Figure 4, the phosphorylated forms of Akt1 (Ser473) and of GSK-3β (Ser9) in the hippocampus and cortex of Tg mice were higher than those in WT mice (Figure 4a,b). Administration of MK-801 (0.1 mg·kg^{-1}, i.p.) significantly decreased the phosphorylated levels of Akt1 and GSK-3β in

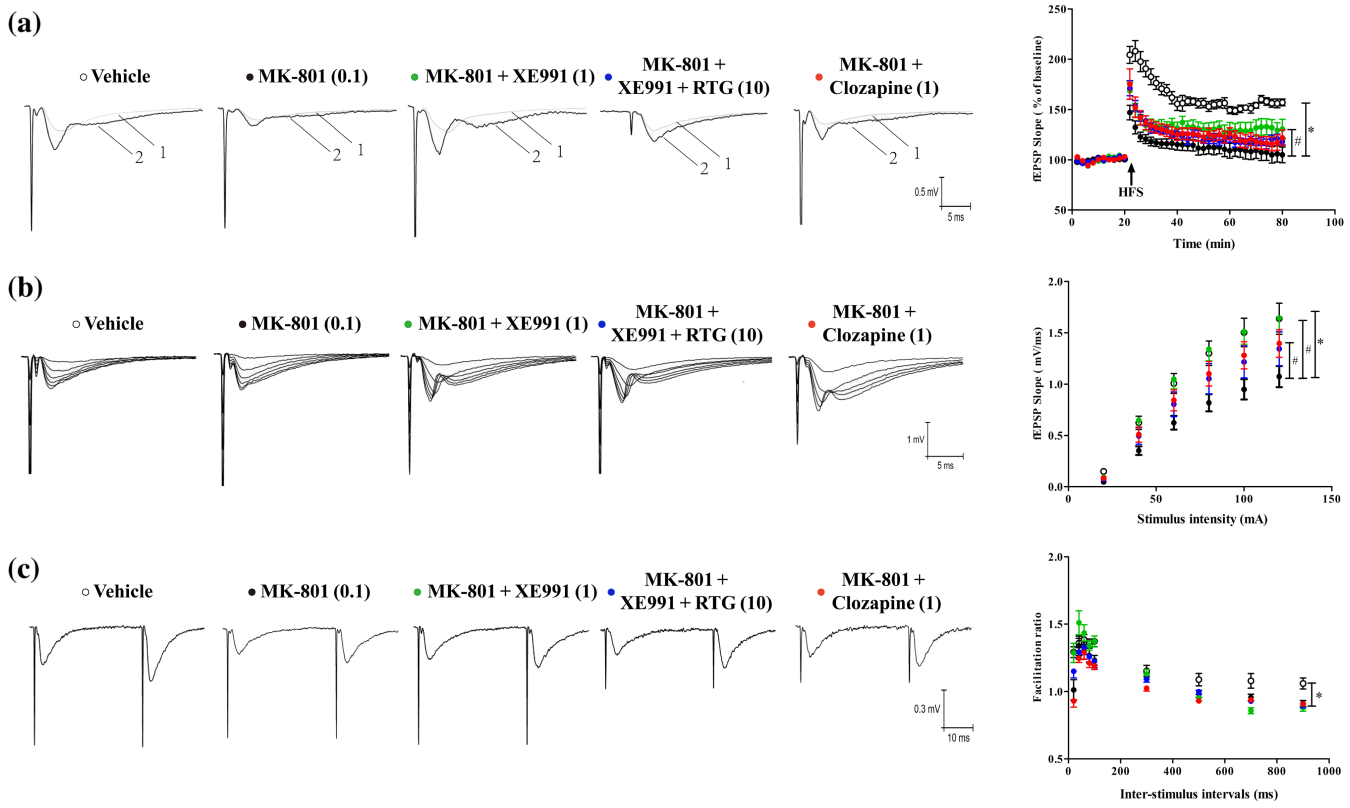

FIGURE 3 Amelioration of MK-801-induced deficits of hippocampal synaptic transmission by pharmacological inhibition of K_v7 channels. (a) Left panels: Representative traces of fEPSPs before (line 1) and after (line 2) LTP induction in mice treated i.p. with MK-801 (0.1 mg·kg^{-1}) or in combination with XE991 (0.1 mg·kg^{-1}) or RTG (10 mg·kg^{-1}) and clozapine (1 mg·kg^{-1}). Right panels: The fEPSP slope for HFS-induced LTP in CA1 area of the hippocampus and amelioration of 0.1 mg·kg^{-1} MK-801-induced LTP impairment in response to XE991 (1 mg·kg^{-1}, i.p.). (b) Left panels: Representative traces of fEPSPs elicited a series of stimulus from 0.02 to 0.12 mA in different groups. Right panels: A plot of I/O curve for the fEPSP slope against the stimulus intensity. (c) Left panels: Representative traces of PPF by giving an interpulse interval of 40 ms. Right panels: The PPF in the hippocampal CA1 area in vehicle control mice and drug-treated mice. Facilitation ratios were plotted against interpulse intervals. Data shown are means ± SEM; n = 10–12. $^{*}P < 0.05$, significantly different from Vehicle, $^{\#}P < 0.05$, significantly different from MK-801 (0.1 mg·kg^{-1}); two-way ANOVA, followed by post hoc Bonferroni's test

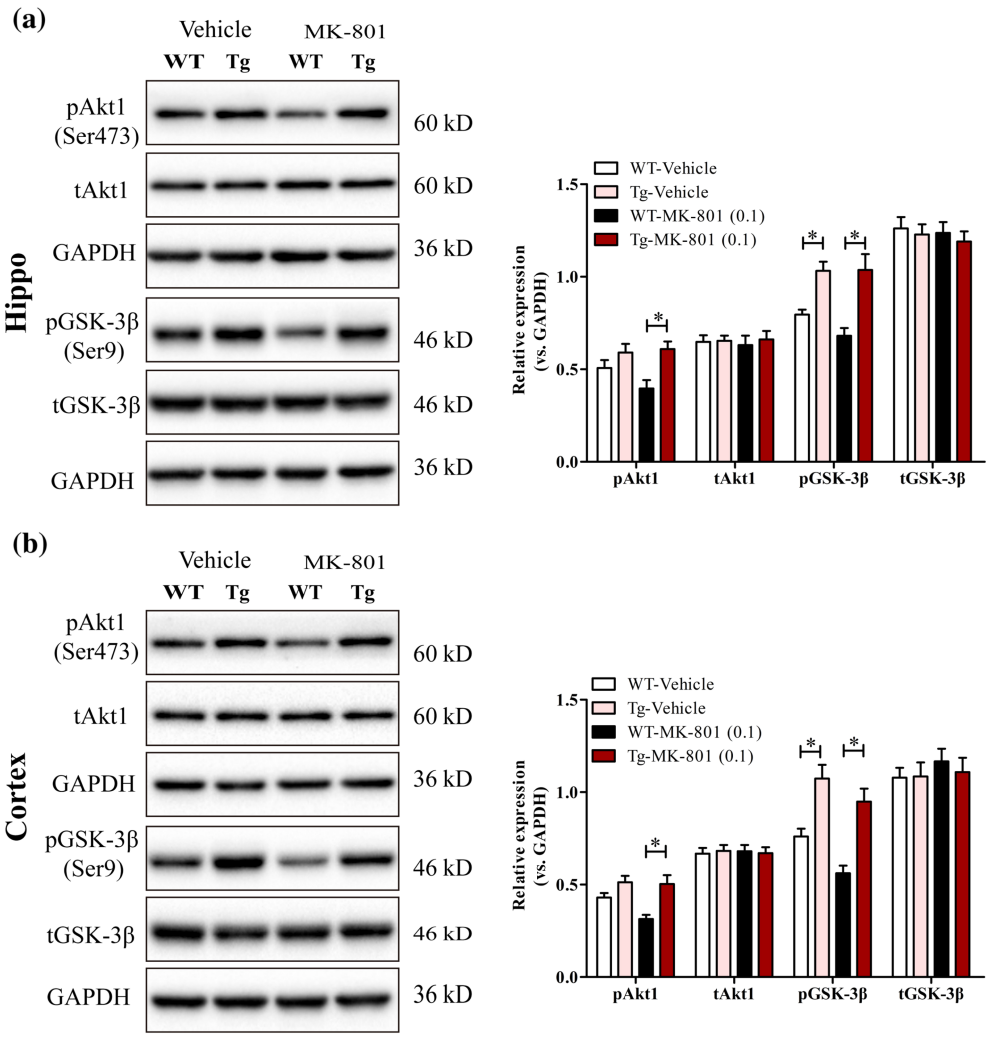

FIGURE 4 Genetic suppression of K_v7 channels prevents MK-801-induced decrease of pAkt1 (Ser473) and pGSK-3β (Ser9) in the hippocampus and cortex. (a) Left panels: Representative western blots of pAkt1 (Ser473), tAkt1, pGSK-3β (Ser9), tGSK-3β, and GAPDH levels from the hippocampi of WT and Tg mice treated i.p. with or without MK-801 (0.1 mg·kg^{-1}). Right panels: Quantification of the relative changes in pAkt1 (Ser473), tAkt1, pGSK-3β (Ser9), and tGSK-3β expression from left panels. (b) Left panels: Representative western blots of pAkt1 (Ser473), tAkt1, pGSK-3β (Ser9), tGSK-3β, and GAPDH levels from the cortices. Right panels: Quantification of the relative changes in pAkt1 (Ser473), tAkt1, pGSK-3β (Ser9), and tGSK-3β expression from left panels. Data shown are means ± SEM; n = 6–8. $^*P < 0.05$, significantly different as indicated; one-way ANOVA, followed by post hoc Bonferroni's test

WT mice but not Tg mice (Figure 4a,b), indicating that Tg mice are resistant to MK-801-induced reduction of phosphorylated Akt1 and GSK-3β. In contrast, there was no significant alteration in total protein expression levels of Akt1 (tAkt1) and GSK-3β (tGSK-3β) in the hippocampus or cortex, either in the vehicle-treated groups or the MK-801-treated groups (Figure 4a,b).

To further confirm the observations from the Tg mice, we also examined the effect of pharmacological inhibition of K_v7 channels on phosphorylated Akt1 and GSK-3β proteins. Treatment of the mice with MK-801 (0.1 mg·kg^{-1}, i.p.) significantly reduced the phosphorylated pAkt1 (Ser473) and pGSK-3β (Ser9) in the hippocampus and cortex from WT mice (Figure 5a–d). In contrast, pretreatment with XE991 (1 mg·kg^{-1}, i.p.) or clozapine (1 mg·kg^{-1}, i.p.), as control, resulted in a significant increase of phosphorylated pAkt1 (Ser473) and pGSK-3β (Ser9), compared with the MK-801 group (Figure 5a–d). Co-injection of XE991 with retigabine (10 mg·kg^{-1}, i.p.) partly reversed the enhanced phosphorylation of Akt1 and GSK-3β (Figure 5a–d). There was also no significant difference in tAkt1 and tGSK-3β proteins among all treatment groups (Figure 5a–d). These results further confirm that inhibition of K_v7 channel function can lead to activation of Akt1/GSK-3β signalling.

4 | DISCUSSION

In this study, we tested the hypothesis that enhancing prefrontal excitability by inhibition of neuronal K_v7 channel activity may alleviate glutamatergic hypofrontality that has been linked to the pathogenesis of neuropsychiatric disorders such as schizophrenia (Arnsten et al., 2019; Arnsten, Paspalas, Gamo, Yang, & Wang, 2010). Using the PPI assay, we examined the effect of genetic suppression and pharmacological inhibition of neuronal K_v7 channels on the acoustic startle response, a response that is deficient in patients with schizophrenia (Mena et al., 2016) or schizophrenia-like models (Takahashi & Kamio, 2018). Our findings showed that genetic or pharmacological inhibition of K_v7 channel function in the forebrain enhanced acoustic startle response and alleviated PPI impairment induced by NMDA receptor antagonists. Mechanistically, inhibition of K_v7 channels leads to increases of synaptic transmission and plasticity, and activation of Akt1/GSK-3β signalling. Therefore, enhancing prefrontal activity by pharmacological inhibition of native K_v7 channels/M-current may be a promising strategy for therapy of cognitive deficits in schizophrenia or similar neuropsychiatric disorders.

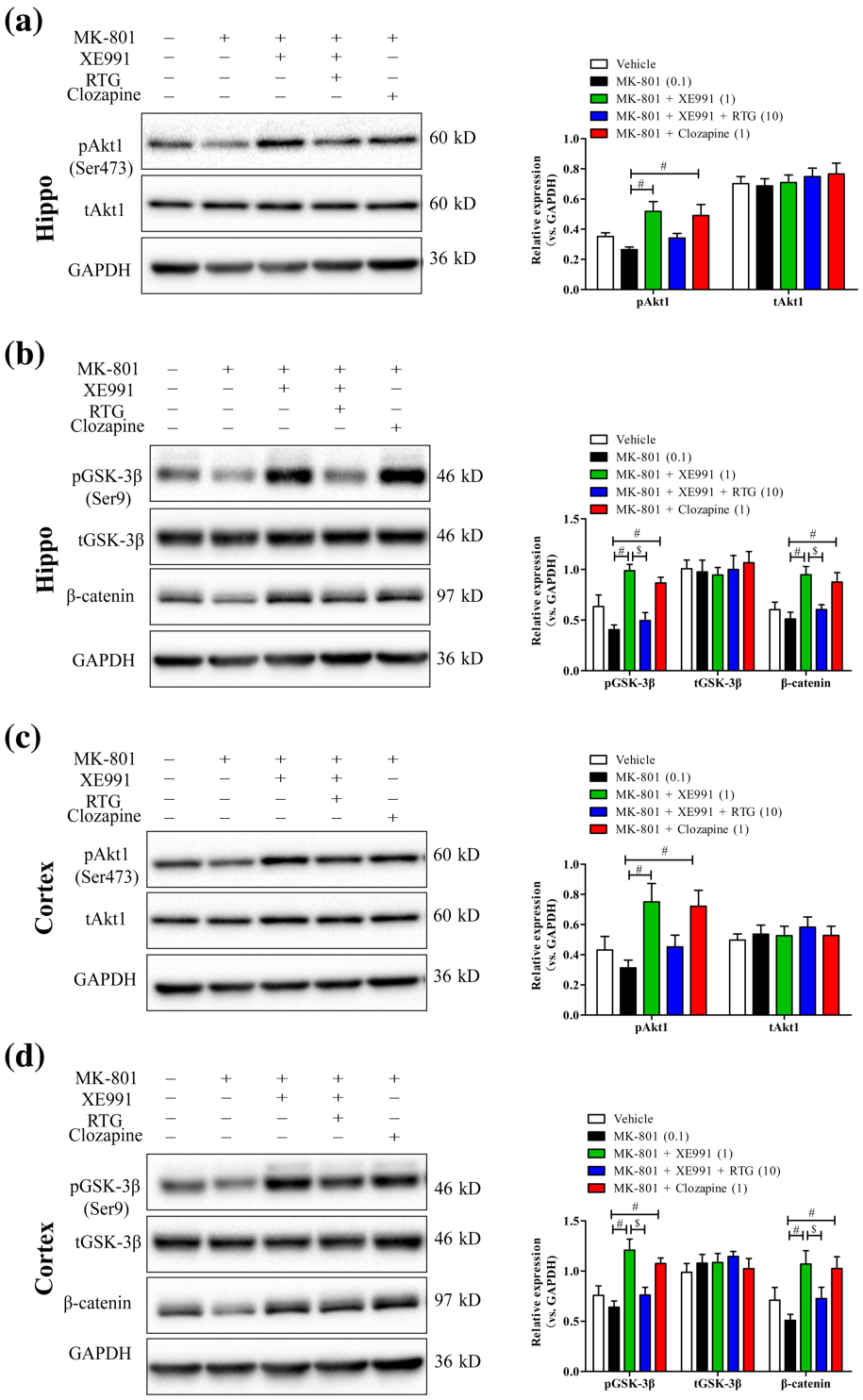

FIGURE 5 Pharmacological inhibition of K_v7 channels increases the phosphorylation of pAkt1 (Ser473) and pGSK-3β (Ser9) in the hippocampus and cortex. (a) Left panels: Representative western blots of pAkt1 (Ser473), tAkt1, and GAPDH levels from the hippocampi (Hippo) of mice treated i.p. with MK-801 (0.1 mg·kg^{-1}) in combination with XE991 (1 mg·kg^{-1}) or RTG (10 mg·kg^{-1}) or clozapine (1 mg·kg^{-1}). Right panels: Quantification of the relative changes in pAkt1 (Ser473) and tAkt1 expressions from left panels. (b) Left panels: Representative western blots of pGSK-3β (Ser9), tGSK-3β, β-catenin, and GAPDH levels from the cortices in different groups. Right panels: Quantification of the relative changes in pGSK-3β (Ser9), tGSK-3β, and β-catenin expressions from left panels. (c) Left panels: Representative western blots of pAkt1 (Ser473), tAkt1, and GAPDH levels from the hippocampi. Right panels: Quantification of the relative changes in pAkt1 (Ser473) and tAkt1 expression from left panels. (d) Left panels: Representative western blots of pGSK-3β (Ser9), tGSK-3β, β-catenin, and GAPDH levels from the cortices. Right panels: Quantification of the relative changes in pGSK-3β (Ser9), tGSK-3β, and β-catenin expressions from their left panels. Data shown are means ± SEM; n = 6-8. $^{\#}P < 0.05$, significantly different from MK-801, $^{\$}P < 0.05$, significantly different from MK-801 (0.1 mg·kg^{-1}) + XE991 (1 mg·kg^{-1}); one-way ANOVA, followed by post hoc Bonferroni's test

However, a very significant outcome of loss-of-function mutations in either KCNQ2 or KCNQ3 genes is that they can cause neonatal epilepsy (Biervert et al., 1998; Charlier et al., 1998; Singh et al., 1998), and Tg suppression of KCNQ2 induces seizures and memory deficits in mice (Peters, Hu, Pongs, Storm, & Isbrandt, 2005). Nevertheless, there is evidence that most patients with benign familial neonatal convulsions (BFNC) caused by mutations in either $K_v7.2$ or $K_v7.3$ channel gene can develop normally in psychomotor function, and only about 15% of individuals suffer from seizures later in life (Maljevic, Wuttke, & Lerche, 2008; Ronen, Rosales, Connolly, Anderson, & Leppert, 1993). KCNQ2 and KCNQ3 mutations identified in BFNC exert no dominant-negative effect on heteromeric KCNQ2 and KCNQ3 channels (Schroeder, Kubisch, Stein, & Jentsch, 1998). Among the KCNQ2 mutations that have been tested, none of them exert complete dominant-negative effects on WT KCNQ2 (Richards et al., 2004; Schroeder et al., 1998), which is known as haploinsufficiency. Previous studies have reported that Tg mice with systemic inhibition of K_v7 channels show an increased excitability and

spontaneous seizure activity in the male mutants but not in females, and the occurrence of spontaneous seizures varies greatly or is less likely to occur due to the fact that the mosaic expression of the G279S mutant that can only cause partial loss or inhibition of M-currents (Peters et al., 2005). In this study, we adopted a regional Tg approach using a forebrain-specific αCaMKII promoter that drives the mutant expression and specifically suppresses the function of forebrain $K_v7.2$ channels. We previously investigated the effect of regional suppression of KCNQ2 on development and spontaneous seizures. The analysis of Nissl-stained coronal sections of hippocampus revealed that there was no obvious structural change in adult Tg mice, compared with WT mice. In addition, we find that there is no significant difference between Tg and WT mice in spontaneous activity, and the Tg mice do not exhibit spontaneous epileptic behaviours (Bi et al., 2011). Therefore, genetic suppression of M-currents does not necessarily cause development of epilepsy.

The PFC is an important region involved in regulating a variety of cognitive and emotional functions, and glutamatergic dysfunction in the PFC has been proposed to underlie the pathophysiology of schizophrenia (Corti et al., 2011; Goldman-Rakic & Selemon, 1997). In the present study, our findings show that the Tg mice with genetic suppression of K_v7/M-current or the WT mice treated with the K_v7 channel inhibitor XE991 exhibited a stronger PPI of acoustic startle reflex and resistance to PPI impairment induced by MK-801, which is consistent with an observation that inhibition of K_v7 channels may enhance PFC excitability and improve cognitive deficits (Peng et al., 2017). Collectively, these results indicate that genetic or pharmacological inhibition of K_v7 channels alleviates the PPI deficit of acoustic startle response induced by glutamatergic hypofunction.

A previous report shows that a systemic knockout of KCNQ2 or pharmacological disruption of M-current with the K_v7 channel inhibitor **linopirdine** attenuated PPI (Kapfhamer et al., 2010), which is inconsistent with our findings. Their systemic KCNQ2 knockout in the whole brain regions and neurons involved in PPI was generated by gene trapping technique, while our forebrain-specific suppression of K_v7 channels was achieved by αCaMKII promoter that drives region-specific expression of a dominant-negative G279S pore mutant. In comparison to linopirdine, XE991 is more selective in blocking K_v7 channels/M-current with better penetration of blood–brain barrier and higher efficacy in vivo (Song et al., 2009). Also, higher doses of linopirdine (5 $mg{\cdot}kg^{-1}$) may have side effects or activate molecular targets other than K_v7 channels. These may partly explain the discrepancy in the results.

Clinical studies have shown that glutamatergic hypofrontality underlies the pathophysiology and treatment of schizophrenia (Krystal et al., 1999; Tamminga, 1998). Dysfunction of NMDA receptors results in a loss of synaptic plasticity in the hippocampus (Wiescholleck & Manahan-Vaughan, 2013), and regulation of K_v7 channels can affect postsynaptic plasticity in the hippocampus (Petrovic et al., 2012). Also, XE991 decreased the induction threshold of LTP in hippocampal CA1 region and promoted the induction of LTP (Song et al., 2009), and the K_v7 channel activator **flupirtine** improved the spatial learning and memory and hippocampal LTP injury induced by acute stress (Li et al., 2014). All these observations are consistent with our results that inhibition of K_v7 channels by XE991 can alleviate LTP impairment or the slope decrease of I/O curve induced by the NMDA receptor antagonist MK-801.

Earlier work showed that MK-801 was washed out of the membrane in 10 min (Moring, Niego, Ganley, Trumbore, & Herbette, 1994), whereas MK-801 is a lipophilic compound (Elhallaoui, Laguerre, Carpy, & Ouazzani, 2002), and it also binds to the hydrophobic pocket in the NMDA receptor–channel complex (Long, Mastropaolo, Rosse, & Deutsch, 2007), indicating a tight binding of MK-801 to the membranes. The residual effects of MK-801-induced LTP deficits can persist for a longer period of time as MK-801-mediated effects on impairment of hippocampal LTP can be observed even 1 week after a single injection (i.p.) of MK-801 (Grüter, Wiescholleck, Dubovyk, Aliane, & Manahan-Vaughan, 2015; Kalweit, Amanpour-Gharaei, Colitti-Klausnitzer, & Manahan-Vaughan, 2017; Wiescholleck & Manahan-Vaughan, 2012). Thus, it is less likely that washing of brain slices in drug-free condition for a while has any significant effects on recordings.

The Akt/GSK-3β signalling pathway is an important component of the regulation of learning and memory and synaptic plasticity. Akt, also known as protein kinase B, regulates synaptic plasticity by affecting LTP and working memory (Freyberg, Ferrando, & Javitch, 2010). Akt expression is significantly reduced in the PFC of schizophrenic patients, and Akt1 deficiency leads to abnormal PFC function (Li, Yang, & Gao, 2016). GSK-3β is a serine/threonine protein kinase that can be inactivated by phosphorylated Akt1 (Mao et al., 2017). Inactivation of GSK-3β can promote the induction of LTP and the formation of hippocampal-dependent memory (Li et al., 2014). The dopaminergic and glutamatergic signalling can converge on the Akt/GSK-3β pathway (Freyberg et al., 2010) as the dysfunction of these signalling pathways plays an important role in the pathophysiology of schizophrenia (Seeman, 1987; Carlsson et al., 2001). Clinical evidence shows that the protein levels of Akt1, GSK-3β, and its downstream component, **β-catenin**, are significantly reduced in the brain and lymphocytes of patients with schizophrenia, while antipsychotic drugs and antipsychotic treatment of lithium and electric shock have been shown to activate Akt and inhibit the effect of GSK-3β in rats (Emamian, 2012). Our western blot analysis reveals that both genetic and pharmacological inhibition of K_v7 channels reversed the reduction of pAkt1 and pGSK-3β levels in hippocampus and cortex induced by MK-801, indicating that the effect of K_v7 channels in improving cognitive deficits involved activation of the Akt1/GSK-3β signalling pathway.

Mechanistically, inhibition of K_v7 channels causes membrane depolarization and increases the release of neurotransmitters such as **glutamate**, **ACh** and **dopamine**, thus activating the Akt1/GSK-3β signalling pathway (Beaulieu et al., 2004; Jensen, Lange, Thomsen, Hansen, & Mikkelsen, 2011; Zaczek et al., 1998). In addition, inhibiting K_v7 channels also promotes the LTP at Schaffer collateral synapses through membrane depolarization during and after the postsynaptic action potentials (Petrovic et al., 2012), and the LTP induction of hippocampal CA1 area also causes an increase of phosphorylated GSK-3β (Hooper et al., 2007). Based on published reports and our

observations, we propose that inhibition of K_v7 channels can lead to LTP induction and GSK-3β phosphorylation, which may help explain the observed behavioural changes in this study.

In conclusion, the present study demonstrates that both genetic and pharmacological inhibitions of neuronal K_v7 channels can improve PPI and cognitive deficits induced by glutamatergic hypofunction. The improvement on glutamatergic hypofrontality by inhibition of neuronal K_v7 channels is mechanistically involved in enhancement of synaptic transmission and activation of Akt1/GSK-3β signalling. Therefore, pharmacological inhibition of neuronal K_v7 channels may be an effective strategy for improvement of cognitive deficits in neuropsychiatric disorders such as schizophrenia.

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AUTHOR CONTRIBUTIONS

J.W. performed behavioural experiments and molecular biology experiments. W.Y. performed electrophysiological experiments. Q.G. performed some behavioural experiments. J.W. and W.Y. analysed the data and drafted the manuscript. C.J. and K.W. designed and supervised the project and finalized the manuscript.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

DECLARATION OF TRANSPARENCY AND SCIENTIFIC RIGOUR

This Declaration acknowledges that this paper adheres to the principles for transparent reporting and scientific rigour of preclinical research as stated in the *BJP* guidelines for Design and Analysis, Immunoblotting and Animal Experimentation, and as recommended by funding agencies, publishers and other organisations engaged with supporting research.

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