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The involvement of potassium channel ORK1 in short-term memory and sleep in *Drosophila*

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Abstract

Background: The sleep and cognitive dysfunction are common in major depressive disorders (MDDs). Recently, the 2-pore domain potassium channel twik-related K(+) channel 1 (TREK-1) has been identified to be closely related to the etiology of MDD. However, whether TREK-1 is involved in the regulation of sleep and cognition is still unknown.

Methods: The present study tried to dissect the role of outwardly rectifying K+ channel-1 (ORK1) (TREK-1 homolog in *Drosophila*) in sleep and cognition in *Drosophila*. The mutant and over-expressed lines of *ork1* were generated using *Drosophila* genetics. Sleep analysis and short-term memory experiments were used to test sleep time and short-term memory of the mutant and over-expressed ORK1 lines, respectively.

Results: Our results showed that the learning index of *ork1* mutant lines was increased compared with the wild type. However, *ork1* mutant could obviously decrease sleep time in *Drosophila*. Contrary to the *ork1* mutant lines, we also found that ORK1 over-expression could increase sleep time and decreased learning index in *Drosophila*.

Conclusion: Results from this study suggest that ORK1 might play an important role in the regulation of sleep time and short-term memory in *Drosophila*.

Abbreviations: cDNA = complementary DNA, K2P = the 2-pore domain K+, MDD = major depressive disorder, ORK1 = outwardly rectifying K+ channel-1, pUAST = P element-based vector for Gal4-regulated expression of genes in *Drosophila*, RT-PCR = reverse-transcriptase polymerase chain reaction, TREK-1 = twik-related K(+) channel 1.

Keywords: cognition, depression, *Drosophila*, learning and memory, ORK1, sleep behavior

1. Introduction

Major depressive disorder (MDD) is one of the most common mental disorders in humans, characterized by often recurrent, high disability rate, and high mortality rate.[1] Counted in lives lost and socioeconomic costs, MDD is a significant contributor to the global burden of disease and affects people in all communities across the world.[2] The World Health Organization estimates that 350 million people around the world are affected by depression. On average about 1 in 20 people reported having an episode of depression in the previous year.[3,4]

Cognitive dysfunction in the major depression has been frequently reported.[5–8] Previous studies showed that the depression patients have the defects in attention, memory, and executive function.[9] The animal model of depression can mimic the symptoms of depression patients in information processing (including memory, attention, and understanding).[10,11] Lesions in temporal lobe typically disrupt the episodic memory.[12] Cognitive impairment is also likely to be a key contributor affecting the subject's ability function occupationally.[13]

Very close relationships between MDD and sleep disorders have also been observed.[14,15] More than 3 quarters of depressed patients have sleep disturbance, and about 40% of young depressed adults and 10% of older patients have hypersomnia symptoms.[16] Sleep disturbance is one of the key symptoms of MDD, and one of the few proven risk factors for suicide.[3,17] Meanwhile the sleep problem is the main risk of relapse and recurrence of MDD.

Recent animal model studies have demonstrated that the twik-related K(+) channel 1 (TREK-1) protein, one of the 17 members of the 2-pore domain K+ (K2P) potassium channel family, is closely related with depression and inhibited by the antidepressant fluoxetine and paroxetine.[18,19] Furthermore, TREK-1 is reported to have an important functional role in mood regulations. Deletion of TREK-1 in mice caused a substantially reduced elevation of corticosterone levels under stress, and produced behavior similar to that of naive animals treated with fluoxetine in various behavioral tests. These findings suggested that the blocker of the TREK-1 channel might potentially be a new type of antidepressant.[11,20] TREK-1 has been found to be potential therapeutic target of antidepressant drugs.[21] However,

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further animal model research on TREK-1 function in MDD is needed. Especially whether TREK-1 is involved in the regulation of sleep and cognition is still unknown.

Drosophila is an excellent cognitive animal model.[22] The *Drosophila* brain has approximately 100,000 neurons, so it is relatively small yet sufficiently complex to provide a suitable model.[23] Despite the evolutionary distance between flies and humans, a strong conservation of genes, pathways, and regulatory molecular networks has been demonstrated. About 75% of human disease genes have related sequences in *Drosophila*.[24] In the present study, we used *Drosophila* as an animal model to analyze the function outwardly rectifying K+ channel-1 (ORK1), a *Drosophila* homolog of the potassium channel TREK-1, on learning and memory and sleep. We found that decreased expression of *ork1* could increase learning ability and decreased sleep time in *Drosophila*. Contrary to the *ork1* mutant lines, *ork1* over-expression could increase the sleep time and decrease learning ability in *Drosophila*.

2. Methods and materials

2.1. Fly stocks

Flies were maintained at 25°C on a standard medium with 60% to 80% relative humidity. W^{1118} flies were used as a wild-type strain. d01340 was obtained from the Exelixis collection at Harvard Medical School. This transgenic insertion stock derived by transposable elements mobilization using the P-element construct, its insertion position is X chromosome (X:10,890,947..10,890,947). C155-Gal4 was obtained from the Bloomington Stock Center at Indiana University (Bloomington, IN). To remove possible modifiers and allow comparisons in a common genetic background, d01340 line was outcrossed into W^{1118} background.

2.2. UAS-ork1 FL constructs

The full-length *ork1* complementary DNA (cDNA) amplified using the primers by *Eco*RI and *Xba*I digestion and inserted into the P element-based vector for Gal4-regulated expression of genes in *Drosophila* (pUAST)-attB transformation vector. The transgenic flies were generated using established protocols to target the second chromosome. All generated stocks were outcrossed to W^{1118} for at least 3 generations before performing experiments.

2.3. RT-PCR experiments

Total ribonucleic acid was extracted using Trizol (Invitrogen, Carlsbad, CA) and cDNA was reverse transcribed using the cDNA Synthesis Kit (Thermo Scientific, MD, K1622). The following primers were used to analyze the expression of *ork1* and Actin5C (control) in the third instar larvae body, larvae brain, and adult brain.

ork1-Forward: 5′-GATGTTCGGGGCGGCAATCT-3′
ork1-Reverse: 5′-GCGAAGGTGGTTGGCGATAT-3′
Actin5C-Forward: 5′-GTCGCTTAGCTCAGCCTCG-3′
Actin5C-Reverse: 5′-TAACCCTCGTAGATGGGCAC-3′
pUAST-Forward: 5′-AACAAGCGCAGCTGAACAAGC-3′
pUAST-Reverse: 5′-AGCAGTAGCCTCATCATCACTAG-3′

2.4. Sleep analysis

The sleep assays were performed as described by Li.[25] Briefly, 3- to 5-day-old male flies raised in light/dark (LD)-entrained cultures were selected for sleep assays. Then flies were put into the 65×5 mm glass tubes which were filled with 5% sucrose and 2% agarose. DAM5 monitors (Trikinetics, waltham, MA) were placed in a DigiTherm CircKinetics incubator (Tritech, Research, waltham, MA) during LD cycles at 25°C. The measurement of locomotor activity levels lasted for 5 to 7 days. Data were collected in 1 min bins, and a sliding window was applied. Sleep was identified as 5 consecutive minutes of inactivity as previously described.[25,26] Data were analyzed using ClockLab software (Actimetrics, wilmette, IL).

2.5. Short-term memory experiments

The learning experiments were performed as described by Sun and Tully[27,28] Briefly, 3- to 5-day-old male flies were used in learning experiments, they were sequentially exposed to 2 odors (Benzaldehyde, BEN, Sigma, hong kong, China #B1334 and 3-Octanol, OCT, Sigma #218405) for 60 s each. The concentrations for 2 different odors (BEN and OCT) were used when tested flies show an equal preference for both odors. In this study, BEN (0.1%) and OCT (1.5%) diluted in mineral oil (Sigma, #330760). The first odor was paired with an electric shock (60 V, 12×1.5 s pulses), and the second odor was not paired. The associative learning was tested within 3 min of training completion. During the testing, flies were exposed to both odors simultaneously in a T-Maze (Made in Institute of Neuroscience, Shanghai). All training and testing were performed in a climate-controlled room with 75% humidity at 25°C under a dim red light. The associative learning was tested within 3 min of training completion. Sensorimotor responses were tested in untrained flies of the same age. Flies were placed in a T-Maze and given a choice between an odor and clean air or between 2 electric grids (with only one of the grids connected to the stimulator). The avoidance values represent the percentage of flies that avoided the odor or electric shock minus the percentage of flies that did not. All experiments were performed blind to avoid any subconscious bias.

2.6. Statistical analysis

In all experiments, analyses, and whenever possible, experiments were performed blind with respect to the genotypes used. All the averaged data in this study were reported as mean ± standard error of the mean. $P \leq .05$ was considered to be significant. All statistical analyses were done with GraphPad Prism5 GraphPad Prism5 (GraphPad software Inc, la jolla, CA). Two groups were analyzed by using nonparametric Mann–Whitney test and multiple groups were made with Kruskal–Wallis test of 1-way analysis of variance. *P* values and the test used are indicated in figure legends.

3. Results

3.1. Expression of ork1 in the brain

Based on the bioinformatics, we identified the *Drosophila* homolog of human *Trek1* gene (Fig. 1A). Human TREK1 and *Drosophila* ORK1 have the same domain architecture. ORK1 (KCNKO) is one of the first members of the K2P channel superfamily identified in a pluricellular organism and has been biophysically characterized.[29,30] To gain insight into the function of ORK1, we ordered the mutant line carrying a P-element insertion in close vicinity to the *ork1* gene (Fig. 1B). *ork1* expression, determined by reverse-transcriptase polymerase chain reaction (RT-PCR), was dramatically reduced in line d01340, which displayed a 50% reduction in *ork1* expression level. It revealed that this line is to be partial loss-of-function

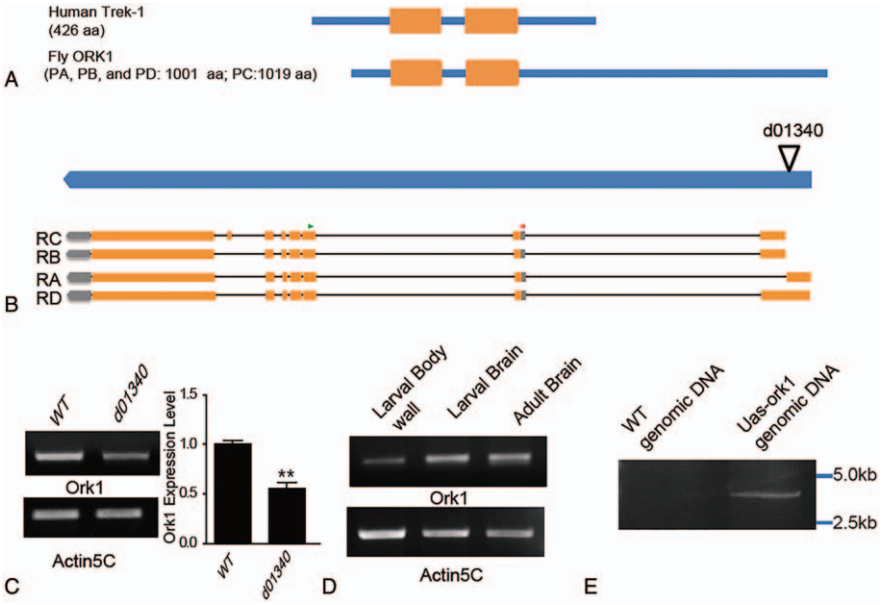

Figure 1. Schematic diagram of *ork1* gene and protein structure. (A) ORK1 protein domain structure alignment with human Trek1. (B) Genomic organization of *ork1* gene, and the position of the d01340 P-element insertion. ▽ indicates PCR primers position. (C) Identification of knock down mutants of *ork1* by RT-PCR. (D) RT-PCR analyses of *ork1* mRNA expression in larval and adult brain tissues. n = 5, $^{**}P < .01$. (E) Identification of pUAS-*ork1* by PCR. ORK1 = outwardly rectifying K+ channel-1, PCR = polymerase chain reaction, RT-PCR = reverse-transcriptase polymerase chain reaction.

mutants for ORK1 (Fig. 1C). To address whether ORK1 is involved in higher nervous activity, such as learning and automatic cardiac activity (sleep), we firstly analyzed the expression of *ork1* in the brain by RT-PCR. We found that *ork1* mRNA was expressed in larval brain, larval body wall, and adult brain (Fig. 1D). We also generated p[UAS-*ork1*] transgenic flies and identified this over-expression fly is working (Fig. 1E).

3.2. Night sleep behavior of ork1 mutant and over-expression flies

To examine the role of ORK1 in sleep, we assessed sleep behavior in *ork1* mutant flies and over-expression flies during 12 h LD cycles. The $ork1^{d01340}$ line was outcrossed for more than 6 generations with the W^{1118} before behaviors test. Meanwhile, C155-Gal4 transgenic flies were crossed with the UAS-ORK1 on wild-type background and with the ork1 over-expressed allele. We found that *ork1* mutant flies showed significantly less total night sleep time (624 ± 39.9 min vs. 558 ± 64.5 min, n = 64; $P < .05$, Fig. 2A–C), an increased fragmented sleep, and longer total sleep episode (23 ± 3.5 min vs. 54 ± 7.2 min; $P < .05$, Fig. 2D) compared with wild-type flies. Further analysis revealed that flies lacking *ork1* exhibited more night sleep episodes with shorter durations compared with wild-type flies. Sleep time of *ork1* mutant was mainly due to a reduction in night sleep (624 ± 39.9 min vs. 558 ± 64.5 min, n = 64; $P < .01$, Fig. 2C and E). Conversely, ORK1 over-expression flies exhibit importantly more total night sleep time mean C155-Gal4, UAS-ork1, vs. ORK1OE (532 ± 64.9 min, 541 ± 59.9 min vs. 577 ± 69.4 min, n = 64; $P < .05$, Fig. 3A–C), a decreased fragmented sleep, and shorter total sleep episode (23 ± 4.6 min, 22 ± 3.9 min vs. 15 ± 3.7 min; $P < .01$, Fig. 3D) compared with control flies. Further analysis revealed that over-expressed ORK1 flies exhibited less night sleep episodes with longer duration compared with control flies ($P < .01$, Fig. 3E).

3.3. Short-term memory of ork1 mutant and over-expressed flies

Here, we used the T-Maze to test the learning and memory ability of the *ork1* mutant and over-expression flies. Interestingly, not only the *ork1* mutant but also ORK1 over-expression all exhibited normal responses to odors and electric shocks (Fig. 4A–C). However, the *ork1* mutant had a higher learning and memory index compared with the wild-type adults, conversely, ORK1 over-expression had a lower learning and memory index compared with control (WT, LI = 52.6 ± 1.077, n = 9 vs. $ork1^{d01340}$, LI = 65.9 ± 1.053, n = 9, $P < .05$; C155-Gal4, LI = 40.3 ± 1.076, n = 9 vs. ORK1 OE, LI = 52.1 ± 1.039, n = 9, $P < .05$) (Fig. 4D).

4. Discussion

Depression is a common but serious mood disorder with complex etiology. The sleep and cognitive dysfunction are common in MDD. Recently, the 2-pore domain potassium channel TREK-1 has been identified to be closely related to the etiology of MDD. However, whether TREK-1 is involved in the regulation of sleep and cognition is elusive. In the present study, we have investigated ORK1 (human TREK1 homolog) function in sleep and cognition in *Drosophila*. We found that decreased expression of *ork1* could increase learning ability and decreased sleep time in *Drosophila*. Contrary to the *ork1* mutant lines, *ork1* over-expression could increase the sleep time and decrease learning ability in *Drosophila*. Our results indicated that ORK1 could affect sleep time, the ability of learning, and memory in *Drosophila*.

In order to identify and study the molecular and physiological mechanisms that such genes participate in, numerous animal models have been established in a variety of species.[31] Exactly, *Drosophila melanogaster* is an excellent animal model to study the molecular and physiological mechanisms of MDD. The history of the use of *Drosophila* in modern biological sciences is a

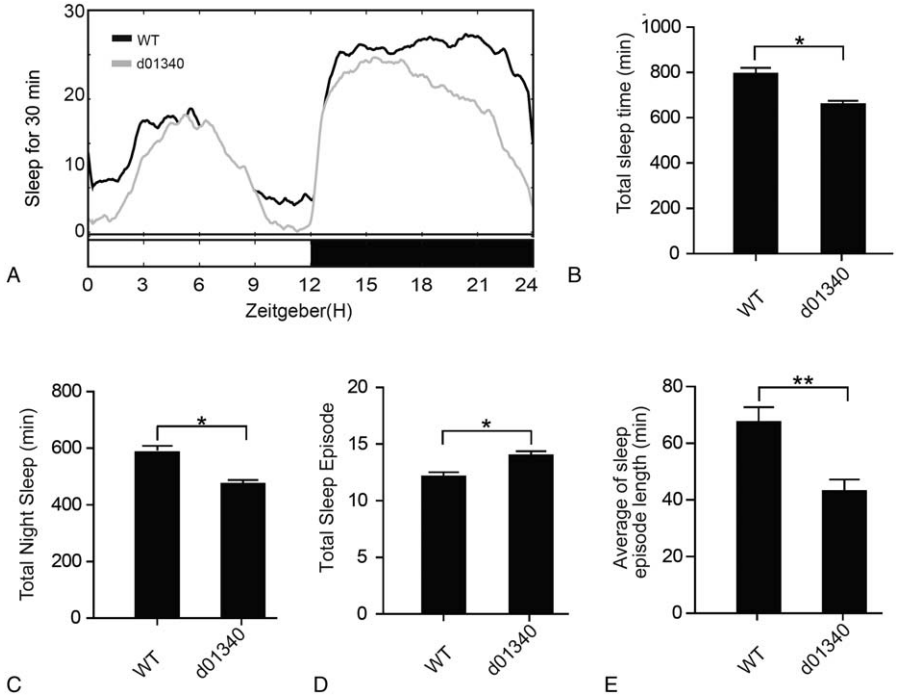

Figure 2. Abnormal night sleep behavior in *ork1* mutant flies. (A) Average sleep traces for *ork1* mutant and WT control flies, plotted as a 30 min moving average; n = 64. (B, C) Quantification of total sleep time of *ork1* mutant and WT control flies per night and total night sleep after lights off; n = 64, $*P < .05$. (D) Quantification of total sleep episodes per night of *ork1* mutant and WT control flies; n = 64, $*P < .05$. (E) Quantification of average sleep episode length for each genotype; n = 64, $**P < .01$.

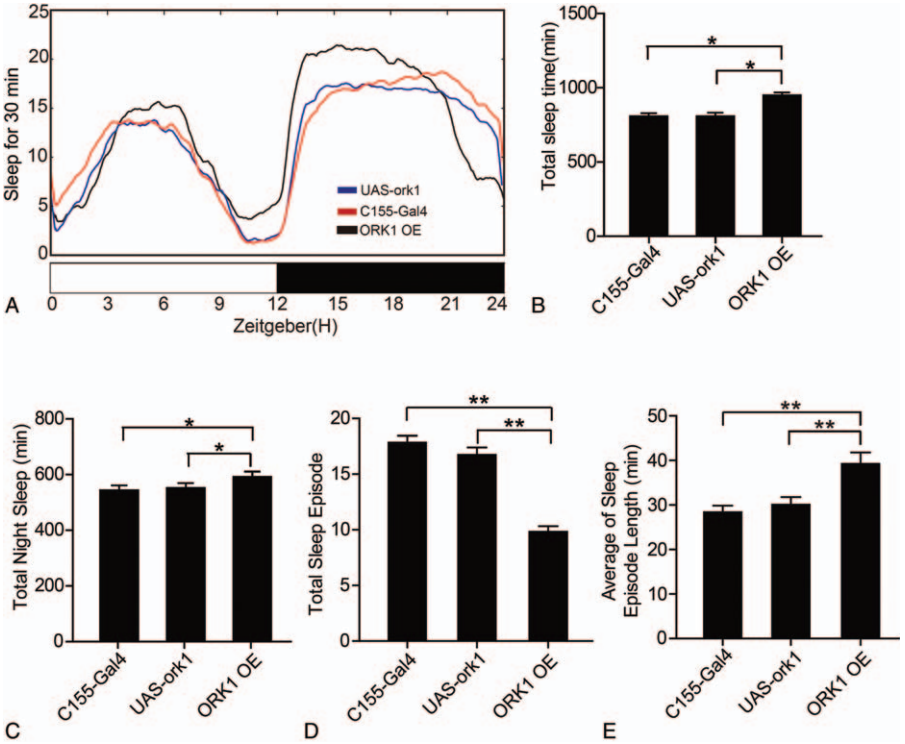

Figure 3. Abnormal night sleep behavior in *ork1* over-expression flies. Average sleep traces for *ork1* over-expression and C155-Gal4, UAS-*ork1* control flies, plotted as a 30 min moving average; n = 64. (B, C) Quantification of total sleep time of ORK1 over-expression and C155-Gal4, UAS-*ork1* control flies per night and total night sleep after lights off; n = 64, $*P < .05$. (D) Quantification of total sleep episode per night of ORK1 over-expression and C155-Gal4, UAS-*ork1* control flies; n = 64, $**P < .01$ (E). Quantification of average sleep episode length for each genotype; n = 64, $**P < .01$. ORK1 = outwardly rectifying K+ channel-1.

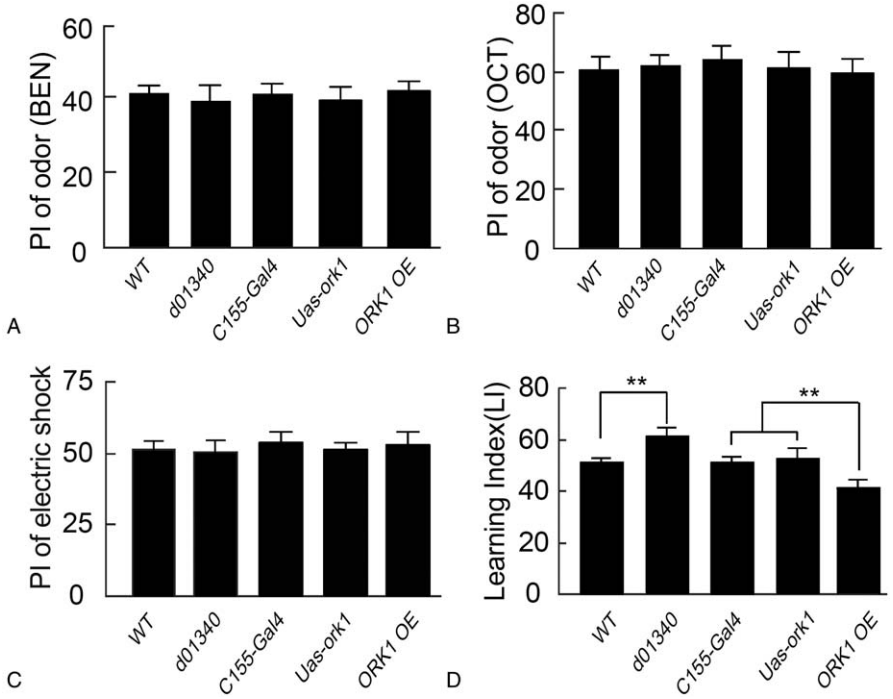

Figure 4. ORK1 involves in the learning and memory formation. (A, B) There is no difference among the genotypes (WT, d01340, C155-Gal4, UAS-*ork1*, and ORK1 OE) with respect to responses to the odors (BEN and OCT); n = 64. (C) No significant difference among the genotypes (WT, d01340, C155-Gal4, UAS-*ork1*, and ORK1 OE) with respect to responses to the electric shock. (D) Comparing to WT flies, the *ork1* hypomorphic mutants (d01340) have the higher LI value ($P < .01$); and the ORK1 over-expression flies driven by C155-Gal4 show the lower L1 value ($P < .01$) compared with C155-Gal4, UAS-*ork1* control. BEN = benzaldehyde, OCT = 3-octanol, ORK1 = outwardly rectifying K+ channel-1. LI = learning index.

rich one, spanning more than a century.[23] Even though the morphology of the fly differs substantially from mammalian ones, the genes involved in these processes are highly conserved.[32] And many basic biological, physiological, and neurological properties are conserved between mammals and *Drosophila*.[33]

The cognition alteration in the major depression was very complicated. On the one hand, the depression patients have the defects in attention, memory, and executive function. On the other hand, the depression patients can often pay continued priority attentions to the negative information (sad faces and sad voices).[34] Until now, the underlying mechanism of cognitive dysfunction in MDD is still elusive. TREK1 has been implicated in mood regulation. TREK1 knockout mice (TREK1−/−) display a depression-resistant phenotype.[11] Recent work has also shown that Trek channels mediate, in part, the inhibitory effect of $GABA_B$ receptor stimulation on neurons in the hippocampus and entorhinal cortex,[35,36] and have suggested a role for Trek channels in learning and memory. However, the results were inconsistent. Another group observed no influence of Trek ablation on learning and memory in mice. In the present study, we found that *ork1* (TREK-1 homolog in *Drosophila*) mutant could obviously enhance the short-term memory in *Drosophila*. And contrary to the mutant, *ork1* over-expression weaken the short-term memory in *Drosophila*, suggested that ORK1 could negatively regulate cognition in *Drosophila*.

Although sleep disorders are also very common in MDD,[8,14,15] the relationship between sleep disturbances and depression is still unclear. Sleep deprivation and hypersomnia symptoms could both found in MDD.[16] Studies found that sleep deprivation could obviously ameliorate depressive symptom in depressive patients.[37] However, in normal Sprague Dawley rats, sleep deprivation induced anxiety-like behavior, lead to significant depression-like behavior and short-term memory impairment.[38] In the present study, we found that *ork1* mutant could obviously decrease sleep time and *ork1* over-expression could increase sleep time in *Drosophila*. Our results suggest that ORK1 could regulate sleep time in *Drosophila*. To our knowledge, this is first report about relationship between ORK1 and sleep time. However, we did not detect the effect of ORK1 on sleep quality, which is even more important than sleep time.

In this study, we preliminarily explored the effects of ORK1 on the cognitive function and sleep. Our results suggest that ORK1 might play an important role in the regulation of sleep time and short-term memory in *Drosophila*. We acknowledge that there are many limitations in the present study. Firstly, we used the P {XP}d01340 as a mutant of *ork1* gene. However, this line is a partial loss-of-function mutant for *ork1*. Secondly, we did not produce the specific anti-ORK1 antibody. The expression pattern of ORK1 is best illustrated by immunostaining using the specific anti-ORK1 antibody. Third, here we used 1 Gal4 line (C155-Gal4) to analyze the gain of function of ORK1. So we will use more Gal4 lines in the next study.

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