HHS Public Access

Author manuscript

Behav Brain Res. Author manuscript; available in PMC 2017 April 01.

Published in final edited form as:

Behav Brain Res. 2016 April 1; 302: 29–34. doi:10.1016/j.bbr.2016.01.019.

GRK5 deficiency leads to susceptibility to intermittent hypoxia-induced cognitive impairment

Prabhakar Singh[a], **Wei Peng**[a], **Qiang Zhang**[a], **XueFeng Ding**[a,e], and **William Z. Suo**[a,b,c,d,*]

[a]Laboratory for Alzheimer's Disease and Aging Research, Kansas City Veterans Affairs Medical Center, Kansas City, MO 64128, USA.

[b]Department of Neurology, University of Kansas Medical College, Kansas City, KS 66170, USA.

[c]Department of Physiology, University of Kansas Medical College, Kansas City, KS 66170, USA.

[d]The University of Kansas Alzheimer's Disease Center, Kansas City, KS 66160, USA

[e]Department of Cognitive Sciences, Beijing Institute of Basic Medical Sciences, Beijing, 100850, P.R. China.

Abstract

Obstructive sleep apnea (OSA) leads to cognitive impairment in about 25% patients, though it remains elusive what makes one more susceptible than the other to be cognitively impaired. G protein-coupled receptor kinase-5 (GRK5) deficiency is recently found to render subjects more susceptible to cognitive impairment triggered by over-expression of Swedish mutant ß-amyloid precursor protein. This study is to determine whether GRK5 deficiency also renders subjects more susceptible to the OSA-triggered cognitive impairment. Both wild type (WT) and GRK5 knockout (KO) mice were placed in conditions absence and presence of intermittent hypoxia (IH) with 8%/21% O_2 90-second cycle for 8 hours a day for a month, and then followed by behavioral assessments with battery of tasks. We found that the selected IH condition only induced marginally abnormal behavior (slightly elevated anxiety with most others unchanged) in the WT mice but it caused significantly more behavioral deficits in the KO mice, ranging from elevated anxiety, impaired balancing coordination, and impaired short-term spatial memory. These results suggest that GRK5 deficiency indeed makes the mice more susceptible to wide range of behavioral impairments, including cognitive impairments.

Keywords

sleep apnea; cognitive impairment; intermittent hypoxia; memory; GRK5 deficiency; susceptibility

*Correspondence to: William Z. Suo, Laboratory for Alzheimer's Disease and Aging Research, Veterans Affairs Medical Center, 4801 E. Linwood Blvd., Kansas City, MO 64128. Tel.: 816-861-4700, Ext. 57084; Fax: 816-922-4667; William.Suo@va.gov.

Disclosure: The authors declare that there are no conflicts of interest.

Introduction

Obstructive sleep apnea (OSA) is a common sleep disorder characterized by repeated upper airway obstruction during sleep, which results in fragmented sleep, intermittent hypoxemia and daytime sleepiness [1-3]. OSA is highly prevalent in middle-aged and elderly men (53%) and women (26%) and can have severe consequences, including heart attack and cognitive impairment [4]. Among OSA patients, it was estimated that at least a quarter may have cognitive impairments [3].

Cognitive impairment is a significant health challenge for the society, because it is often irreversible, lack of effective treatment, and requiring disproportionally high cost of care [5]. Therefore, identification of susceptible population helps for early vigilance and prevention. The severity of OSA obviously accounts for part of the variance in cognitive performance [6]. Genetic variance, such as apolipoprotein E (ApoE) epsilon4 allele, increases the risk of OSA-triggered cognitive impairment [6, 7]. Other factors such as concurrent traumatic brain injury (TBI) also increases the risk [8]. Moreover, OSA-triggered cognitive impairment shares many risk factors with Alzheimer's disease (AD), such as hypoxia, hypoxemia, and cerebral hypometabolism, in addition to ApoE4 and TBI. In fact, OSA was reported to promote AD onset[2, 4], and some have even proposed OSA as a possible cause of AD [9, 10]; alternatively, they could be mutual causes and interact in a vicious cycle to make each other worse[2, 11]. Regardless of the actual causal relations, these two diseases are closely related.

Anatomic basis of cognitive impairment is neurodegeneration. Compelling evidence suggests that profound basal forebrain cholinergic (BFC) neurodegeneration is associated with cognitive impairment in AD and other neurodegenerative disorders associated with memory dysfunction, such as Lewy body dementia (LBD) [12-15]. We recently found that G protein-coupled receptor kinase-5 (GRK5) deficiency leads to selective basal forebrain cholinergic (BFC) neuronal vulnerability, which renders the subjects more susceptible to cognitive impairment induced by Swedish mutant ß-amyloid precursor protein (APP) [16]. This finding has let us to speculate whether the same mechanism may also mediate OSA-triggered cognitive impairment.

Intermittent hypoxia has been used to mimic sleep apnea to study OSA-related morbidity in animals. Chronic intermittent hypoxia (CIH) treatment causes cognitive dysfunction in rats and mice and considered a good model to understand OSA-triggered morbidities. Therefore, in this study, we treated GRK5-deficient and normal control mice with CIH and behaviorally assessed them to determine the impact of GRK5 deficiency on the susceptibility to CIH-triggered cognitive impairment.

Material and methods

Animals

GRK5 knockout (KO) mice were originally obtained as a generous gift from Drs. Robert Lefkowitz and Richard Premont at the Duke University [17], and had been bred to a C57/BL6 background for more than 10 generations and maintained in our laboratory for

more than a decade. The particular strain of GRK5KO mice used in this project were further bred with green cholinergic transgenic mice (GCh) that express enhanced green fluorescent protein (EGFP) in their cholinergic neurons under the control of choline acetyltransferase (ChAT) promoter (Jackson Laboratory, Bar Harbor, ME). Homozygote GRK5KO (GCh^{+}/$GRK5^{-/-}$, KO) and wild type control (GCh^{+}/$GRK5^{+/+}$, WT) at 4-month old with mixed genders were randomly divided within genotype into two groups for CIH (WT=14, KO=10) and normoxic (WT=15, KO=13) treatment, respectively. The animals were maintained under a 12 h light/dark daily cycle (lights on from 6:00AM to 6:00PM). Food and water were available *ad libitum*. All animal procedures and use were approved by the Kansas City Veterans Affairs Medical Center Institutional Animal Care and Use Committee.

CIH treatment

The CIH treatments were executed in the ProOx P110 OxyCycler hypoxia chambers (BioSpherix, Lacona, NY, USA). This OxyCycler controls four parallel hypoxia chambers, and each chamber (76×50×50 cm^3) is large enough to house four standard mouse cages (Fig. 1). Therefore, this setup enables a maximum of 80 mice to be treated at a time. For this study, 24 blindly coded mice (WT=14, KO=10) were placed in the OxyCycler hypoxia chambers under condition set to 90-second cycles of 8% and 21% O2 continuously for 8 h/day (10 AM to 6 PM) during the normal sleeping period of mice for 4 weeks. Normoxic controls were subjected to ambient 21% O2 in separate chambers.

Behavioral Assessments

All behavioral tests were performed in our behavioral lab division, which is equipped with the ANY-maze Video Tracking System along with Stoelting mouse behavioral battery devices (Wood Dale, IL). After being transferred to the behavioral division, the mice were habituated to the operator and the room environments for two weeks before testing. The behavioral tasks comprise a battery of tests that provide an extensive analysis of sensorimotor function, anxiogenic tendencies, and mnemonic performance. The procedures were adapted from our previously published protocol [18] with minor modifications. For all behavioral testing, the operator was blind to the experimental variables of genotype and treatments. The following tasks were evaluated in the indicated order with details provided only for the modifications to previously published protocols. (1) Swimming screening: consists of a 15-sec rest on a platform followed by 75 sec of free swimming and exploration. Free-floating, idling, or involuntarily circling mice were removed from subsequent tests. (2) Open field (OF): Evaluates activity/exploratory behavior. The total travel time and distance over a 3-minute period were recorded. (3) Balance beam: Evaluates vestibular and general motor balance. Latency to fall from a beam was recorded for 3 successive trials (60 sec). (4) String agility: Evaluates agility and grip capacity. Animals were permitted to grasp a suspended string only by their forepaws and then released. Within 60 sec, each animal was assessed using a 0-5 rating system. (5) Elevated plus maze (EPM): Evaluates level of anxiety. Closed and open arm entries over a 5-minute period were recorded. (6) Elevated platform (EP): evaluates anxiety levels. Animals were allowed to freely explore the center, middle and outer zones of an elevated platform for a 5-min period. The time spent and distance traveled in the middle and outer zones were used to estimate the animals' anxiogenic tendencies. EP is a novel task that we developed that has better sensitivity for

detecting anxiogenic changes than the EPM [19]. (7) Y Maze: Evaluates spontaneous alternation behavior and spatial working memory. Mice were allowed 5 minutes to explore a non-transparent wall Y-maze with 3 arms. Each arm measured 35 × 5 cm with 10 cm high walls. Mice were placed in the center of the maze facing the center area and allowed to explore for 5 minutes, with the number and sequence of arm choices being recorded. General activity was measured as the total number of arm entries, while basic mnemonic function was measured as a percent spontaneous alternation (the ratio of arm choices differing from the previous two choices divided by the total number of entries). For example, the sequence of arm entries (2,1,3,2,3,1,3,2) has six alternation opportunities (total entries minus two) and the percent alternation would be 67%. (8) MWM: Evaluates reference (spatial) learning over 5 days and reference memory in two intermediate and one final probe trial on day 6. Average latency to find the submerged platform was obtained and averaged for each day of acquisition. On the day following acquisition testing, memory retention was evaluated in a single 60-sec probe trial in which the submerged platform was removed and the animal released from the quadrant opposite the former platform-containing quadrant (Quadrant 2; Q2). Percent of time spent in each quadrant and number of annulus crossings were determined from video tracking records. (9) RAWM: the maze contained 8 radial swim arms extending out of an open central area, with an escape platform located at the end of the goal arm. The RAWM can be used to measure spatial reference memory in a 2-day session, as detailed previously [20]. This procedure involves training mice to use visual and spatial cues to memorize (and swim toward/escape from) the submerged platform in the goal arm, which remains constant for a given mouse. On day 1, the mice are trained for 15 trials (6 trials/block 1; 6 trials/block 2; 3 trials/block 3; each mouse participates in 3 blocks over 3 h to consolidate short-term memory), and the trials alternate between a visible and a hidden platform to facilitate the learning process. On day 2, the mice are trained for 15 more trials, all with a hidden platform. Entry into an incorrect arm or failure to enter any arms for 10 sec is scored as an error. The average number of errors and the average latency over the 60-sec sessions of all 3 blocks each day are used as indices of spatial reference memory.

Statistical Analysis

Statistical analysis was performed using SPSS 11.0 software (IBM Corporation, NY, USA) and GraphPad Prism software. The quantitative data are expressed as the means ± S.E. and were analyzed by ANOVA. Post-hoc comparisons of means were made using Scheffe's or Tukey's methods, where appropriate.

Results

GRK5 deficiency leads to increased anxiogenic tendency induced by CIH

The 4-week CIH treatment began at 4-month old mice, which was followed by 2-week environmental habituation. The actual behavioral assessment was performed at least two weeks after the CIH treatment. The first abnormal behavior was revealed by the open field (OF) task. We found that total travel distance for GRK5 deficient (KO) mice housed in normoxic control (Cont) condition was not significantly different from WT mice; However, the CIH treatment resulted in significant ($p<0.01$) reduction in the total travel distance for the KO mice, but not for the WT mice (Fig. 2A). In consistent with the decreased travel

distance, the CIH treatment also led to significant increase in grooming number for the KO mice without affecting the WT mice, whereas the genotype (GRK5 deficiency) itself did not significantly affect the grooming behavior at the normoxic condition (Fig. 2B and C). The decreased travel distance along with the increased grooming number implies a possibly increased anxiogenic tendency for the CIH-treated KO mice. This possibility was further supported by the elevated platform (EP) task. The EP task is specifically designed to measure anxiogenic tendency with improved sensitivity than the elevated plus maze (EPM) [19, 21]. Due to large variations, the EPM failed to reveal any significant changes between any of the groups. Nevertheless, in the EP task, we found that GRK5 deficiency did not alter the animal's anxiogenic tendencies in the normoxic condition, but the CIH treatment significantly decreased the number of outer zone entries ($p<0.05$) and the time spent in the outer zone ($p<0.001$) for both the WT and KO mice (Fig. 3). Moreover, the CIH-induced decrease of the time in the outer zone was significantly ($p<0.001$) worse for the KO mice as compared to the WT mice (Fig. 3B). Therefore, these results together suggest that although GRK5 deficiency alone is insufficient to cause any significant change in anxiogenic tendencies for the 5.5-month old mice at normoxic condition, it makes the animals more susceptible to the CIH-induced increase of anxiogenic tendencies.

GRK5 deficiency interacts with CIH to impair fine motor coordination of balance

In additional to the anxiogenic tendency change, the balance beam task revealed a significant decrease of the latency to fall only for the CIH-treated KO mice whereas all other three groups did not differ from each other (Fig. 4A). It was clear that neither the GRK5 deficiency nor the CIH treatment alone was sufficient to induce such an effect, rather it took a synergistic interaction of both factors ($p=0.006$). Meanwhile, we analyzed forepaw grip capacity and agility to access muscular function to perform balancing and movement activities. Neither of the two factors or their interaction affected the semi-quantitative grip agility performance (Fig. 4B). Therefore, these results together suggest that there was no significant sensorimotor dysfunction except that the fine motor coordination of balance was impaired by synergistic interaction between the GRK5 deficiency and the CIH treatment.

GRK5 deficiency renders susceptibility to CIH-induced cognitive impairments

We assessed cognitive performance of these mice using Y-maze spontaneous alternation, Morris water maze (MWM), and radial arm water maze (RAWM) tasks. For the Y-maze spontaneous alternation rate (SAR), the WT and KO mice at normoxic condition were not different from each other. The CIH treatment caused reduction of SAR for both WT and KO mice (Fig. 5A), though the reduction in the WT mice was not statistically significant whereas the reduction in the KO mice was significant ($p=0.02$). In the MWM task, the CIH-treated KO mice differentiated themselves from the other three groups during the day 4 and day 5 learning course by significantly longer escape latency (Fig. 5B), though the later probe test of the memory retention did not reveal significant difference between any of the four groups (not shown). For the RAWM task, there was a similarly retarded escape for the CIH-treated KO mice as compared to the other three groups during the second day training (Fig. 5C), though neither the GRK5 deficiency nor the CIH alone was sufficient to induce significant impairment. Taken together, all three tasks revealed the same cognitive deficit on short-term spatial memory.

Discussion

Cognitive impairment does not affect people with equal chances, some is resilient, and some is susceptible. Except that ApoE4 carriers are susceptible [6, 7], whether or not GRK5 deficiency also renders the susceptibility to cognitive impairment triggered by OSA is what we try to determine in this study.

We used a CIH condition that was so mild to induce barely any impairment in the WT mice, except that the EP revealed a mild increase of anxiogenic tendency and the Y-maze showed a non-significant (p=0.06) trend of decrease in SAR. Meanwhile, the GRK5 deficiency alone in the young adult (5.5-month old) mice was insufficient to induce any significant impairment under the normoxic condition, either. Nevertheless, when these two factors were combined, they induced wide range of behavioral deficits, from increase anxiogenic tendency to impaired balance coordination, and to short-term spatial memory deficit.

It is worth noting that the impact of GRK5 deficiency is gene-dose-dependent [22, 23]. For example, aged homozygote, but not heterozygote, GRK5KO mice are cognitively impaired [18], indicating that it takes the maximal GRK5 deficiency and aging together to cause a mild cognitive impairment whereas the partial GRK5 deficiency in the heterozygote plus the same level of aging are insufficient. For the present study, we used homozygote GRK5KO mice, but instead of aged mice, we used young adult (started at 4-month old and ended at 6-month old), which removed the aging factor. As demonstrated by the results in this study, even the maximal GRK5 deficiency is insufficient to cause any significant behavioral impairment, at least by it alone under normoxic condition. This finding suggests that GRK5 deficiency itself is not directly destructive. However, although it alone does not directly cause any impairment, it does worsen the damages induced or triggered by other insults. For example, in addition to the abovementioned study in which the homozygote GRK5KO worsened aging-induced cognitive impairment [18], we recently found that the heterozygote GRK5KO also worsened the cognitive impairment induced by over-expression of Swedish mutant APP in the GAP mice [16]. Therefore, although GRK5 deficiency alone does not directly cause detectable damage, it alters the subject's sensitivity and renders it more susceptible to the damages induced or triggered by other insults.

In contrast to the maximal GRK5 deficiency, we deliberately selected a rather mild CIH condition to study the interactions between the GRK5 deficiency and CIH. Although CIH has been commonly used to experimentally mimic OSA, the CIH condition was in fact executed rather inconsistently across different laboratories [24]. Some studies used long hypoxic intervals of 30 min to an hour, which are difficult to be translated into a CIH condition that uses a hypoxic interval of seconds. The OxyCycler is capable of mimicking OSA more closely with a programmable controller that provides high precision and resolution of the hypoxic interval (0-999 minutes) and hypoxia level (0.1-99.9% O_2) as necessary. Therefore, only those studies that have used this similar equipment were analyzed when we were trying to select a CIH condition. Moreover, we excluded those studies that were performed on rats, because it appeared that rats are more sensitive than mice to the CIH-induced cognitive impairments [24-26]. For mouse, lower oxygen levels (90-second cycles of 5.7%/21% O_2, 8-hour a day for 2-4 weeks) was required to cause significant

oxidative stress and neuronal apoptosis in cortex [27] and memory deficits [28]. We therefore selected 90-second cycles of 8%/21% O_2, 8-hour a day for 4 weeks for our study. It turned out that our CIH condition was very mild, and perhaps bit too mild because it barely caused any significant damage to the WT mice except for the slightly increased anxiogenic tendency. From the perspective of differentiating the GRK5KO mice from the WT mice in terms of their susceptibility to the CIH-induced damages, it was adequate though. Nevertheless, a slightly stronger CIH condition may be preferential for eliciting more severe neurodegeneration at the pathological level.

As such, when the maximal level of GRK5 deficiency was combined with the mildest CIH condition, the wide range of the behavioral deficits displayed by the KO mice as compared with the WT mice clearly indicate that the GRK5 deficiency indeed sets up the susceptibility for these mice to even the mildest hypoxic challenge. As aforementioned, the GRK5 deficiency also renders the mice more susceptible to Swedish APP-induced cognitive impairment [16]. In that study, the relevant mechanistic exploration revealed that the GRK5 deficiency causes selective cholinergic neuronal vulnerability and therefore renders the subjects more susceptible to damages induced by a variety of secondary insults, including excess Aß, free radicals, and others. Therefore, more studies are warranted to investigate whether the similar mechanisms also underlie the susceptibility of GRK5KO mice to the CIH/OSA-triggered behavioral deficits, including the cognitive impairment.

Overall, the results in this study strongly suggest that GRK5 deficiency renders the subjects more susceptible to CIH/OSA-induced behavior deficits, including cognitive impairment. Our ongoing studies indicate that the pathogenic impact of GRK5 deficiency can be effectively prevented with pharmaceutical means. Therefore, the same pharmaceutical interventions may be preventative for the OSA-induced neurological consequences in the subjects with GRK5 deficiency.

Acknowledgments

This work was supported by grants to W.Z.S. from the Medical Research and Development Service, Department of Veterans Affairs (Merit Review 1I01 BX001067-01A2), the Alzheimer's Association (NPSPAD-11-202149), and resources from the Midwest Biomedical Research Foundation, as well as support from KU ADC (NIH P30 AG035982).

References

1. Gale SD, Hopkins RO. Effects of hypoxia on the brain: neuroimaging and neuropsychological findings following carbon monoxide poisoning and obstructive sleep apnea. J Int Neuropsychol Soc. 2004; 10:60–71. [PubMed: 14751008]
2. Daulatzai MA. Quintessential risk factors: their role in promoting cognitive dysfunction and Alzheimer's disease. Neurochemical research. 2012; 37:2627–58. [PubMed: 22886562]
3. Gagnon K, Baril AA, Gagnon JF, Fortin M, Decary A, Lafond C, et al. Cognitive impairment in obstructive sleep apnea. Pathologie-biologie. 2014; 62:233–40. [PubMed: 25070768]
4. Osorio RS, Gumb T, Pirraglia E, Varga AW, Lu SE, Lim J, et al. Sleep-disordered breathing advances cognitive decline in the elderly. Neurology. 2015; 84:1964–71. [PubMed: 25878183]
5. Association As. 2015 Alzheimer's disease facts and figures. Alzheimers Dement. 2015; 11:332–84. [PubMed: 25984581]

6. Gozal D, Capdevila OS, Kheirandish-Gozal L, Crabtree VM. APOE epsilon 4 allele, cognitive dysfunction, and obstructive sleep apnea in children. Neurology. 2007; 69:243–9. [PubMed: 17636061]
7. Cosentino FI, Bosco P, Drago V, Prestianni G, Lanuzza B, Iero I, et al. The APOE epsilon4 allele increases the risk of impaired spatial working memory in obstructive sleep apnea. Sleep medicine. 2008; 9:831–9. [PubMed: 18083630]
8. Wilde MC, Castriotta RJ, Lai JM, Atanasov S, Masel BE, Kuna ST. Cognitive impairment in patients with traumatic brain injury and obstructive sleep apnea. Archives of physical medicine and rehabilitation. 2007; 88:1284–8. [PubMed: 17908570]
9. Kinugawa K, Nguyen-Michel VH, Mariani J. [Obstructive sleep apnea syndrome: a cause of cognitive disorders in the elderly?]. La Revue de medecine interne / fondee. 2014; 35:664–9.
10. Bombois S, Derambure P, Pasquier F, Monaca C. Sleep disorders in aging and dementia. The journal of nutrition, health & aging. 2010; 14:212–7.
11. Landry GJ, Liu-Ambrose T. Buying time: a rationale for examining the use of circadian rhythm and sleep interventions to delay progression of mild cognitive impairment to Alzheimer's disease. Frontiers in aging neuroscience. 2014; 6:325. [PubMed: 25538616]
12. Stormer VS, Passow S, Biesenack J, Li SC. Dopaminergic and cholinergic modulations of visual-spatial attention and working memory: insights from molecular genetic research and implications for adult cognitive development. Developmental psychology. 2012; 48:875–89. [PubMed: 22103306]
13. Graef S, Schonknecht P, Sabri O, Hegerl U. Cholinergic receptor subtypes and their role in cognition, emotion, and vigilance control: an overview of preclinical and clinical findings. Psychopharmacology. 2011; 215:205–29. [PubMed: 21212938]
14. Bartus RT, Dean RL 3rd, Beer B, Lippa AS. The cholinergic hypothesis of geriatric memory dysfunction. Science (New York, NY. 1982; 217:408–17.
15. Jellinger KA. The cholinergic basal forebrain in Lewy body dementia and Alzheimer's disease. Journal of neurology. 2015; 262:479–80. [PubMed: 25504308]
16. He M, Singh P, Cheng S, Zhang Q, Peng W, Ding XF, et al. GRK5 Deficiency Leads to Selective Basal Forebrain Cholinergic Neuronal Vulnerability. Nature communications. 2016; 6 in press.
17. Gainetdinov RR, Bohn LM, Walker JK, Laporte SA, Macrae AD, Caron MG, et al. Muscarinic supersensitivity and impaired receptor desensitization in G protein-coupled receptor kinase 5-deficient mice. Neuron. 1999; 24:1029–36. [PubMed: 10624964]
18. Suo Z, Cox AA, Bartelli N, Rasul I, Festoff BW, Premont RT, et al. GRK5 deficiency leads to early Alzheimer-like pathology and working memory impairment. Neurobiology of aging. 2007; 28:1873–88. [PubMed: 17011668]
19. Ding XF, Zhao YQ, Liu SH, Zhao T, Zhu LL, Suo WZ, et al. An improved elevated platform for simultaneously assessing rodent locomotor activity and anxiety. CNS neuroscience & therapeutics. 2015; 21:536–8. [PubMed: 25879537]
20. Alamed J, Wilcock DM, Diamond DM, Gordon MN, Morgan D. Two-day radial-arm water maze learning and memory task; robust resolution of amyloid-related memory deficits in transgenic mice. Nature protocols. 2006; 1:1671–9. [PubMed: 17487150]
21. Suo WZ, Ding XF, Peng W, Zhang Q, Wang F, Fan M. Heightened anxiety level of APPsw mice unveiled by a novel elevated platform task. Alzheimer's & Dementia. 2014; 10:p632.
22. Suo WZ, Li L. Dysfunction of G protein-coupled receptor kinases in Alzheimer's disease. The Scientific World Journal. 2010; 10:1667–78. [PubMed: 20730384]
23. Suo WZ. Accelerating Alzheimer's pathogenesis by GRK5 deficiency via cholinergic dysfunction. Adv Alzheimer's Dis. 2013; 2:148–60.
24. Chiang AA. Obstructive sleep apnea and chronic intermittent hypoxia: a review. The Chinese journal of physiology. 2006; 49:234–43. [PubMed: 17294831]
25. Nair D, Dayyat EA, Zhang SX, Wang Y, Gozal D. Intermittent hypoxia-induced cognitive deficits are mediated by NADPH oxidase activity in a murine model of sleep apnea. PloS one. 2011; 6:e19847. [PubMed: 21625437]

26. Nair D, Ramesh V, Li RC, Schally AV, Gozal D. Growth hormone releasing hormone (GHRH) signaling modulates intermittent hypoxia-induced oxidative stress and cognitive deficits in mouse. Journal of neurochemistry. 2013; 127:531–40. [PubMed: 23815362]

27. Xu W, Chi L, Row BW, Xu R, Ke Y, Xu B, et al. Increased oxidative stress is associated with chronic intermittent hypoxia-mediated brain cortical neuronal cell apoptosis in a mouse model of sleep apnea. Neuroscience. 2004; 126:313–23. [PubMed: 15207349]

28. Kheirandish L, Row BW, Li RC, Brittian KR, Gozal D. Apolipoprotein E-deficient mice exhibit increased vulnerability to intermittent hypoxia-induced spatial learning deficits. Sleep. 2005; 28:1412–7. [PubMed: 16335482]

Highlights

- GRK5 deficiency leads to increased anxiogenic tendency induced by CIH
- GRK5 deficiency interacts with CIH to impair fine motor coordination of balance
- GRK5 deficiency renders susceptibility to CIH-induced cognitive impairments

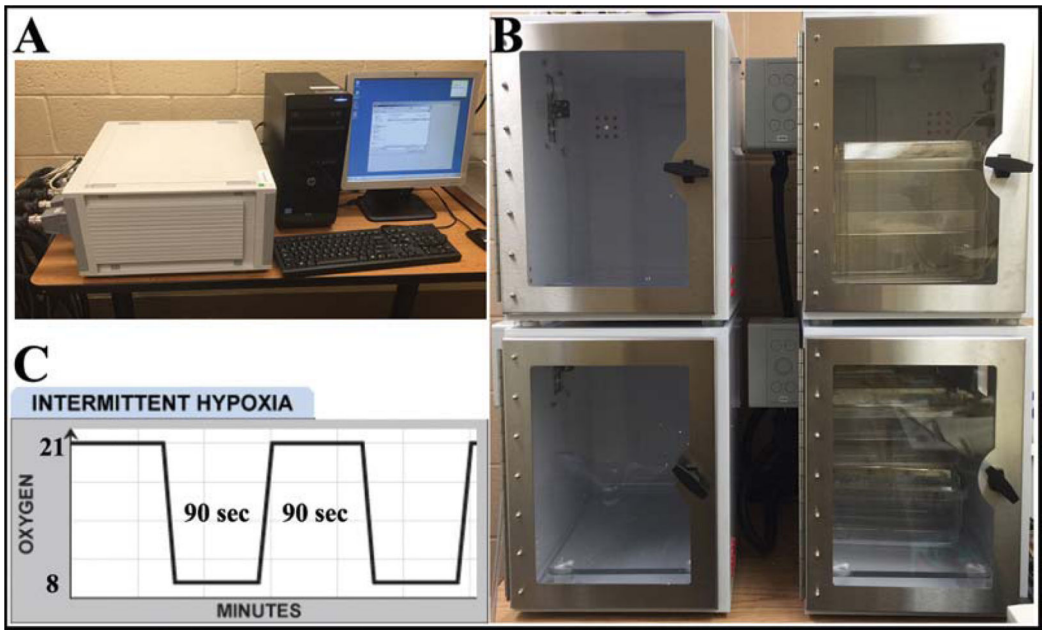

Fig. 1. Illustration of the OxyCyler and the IH cycles

Panel A shows the controller; Panel B shows the hypoxia chambers; and panel C shows an example of the intermittent hypoxia cycles that we used in this experiment.

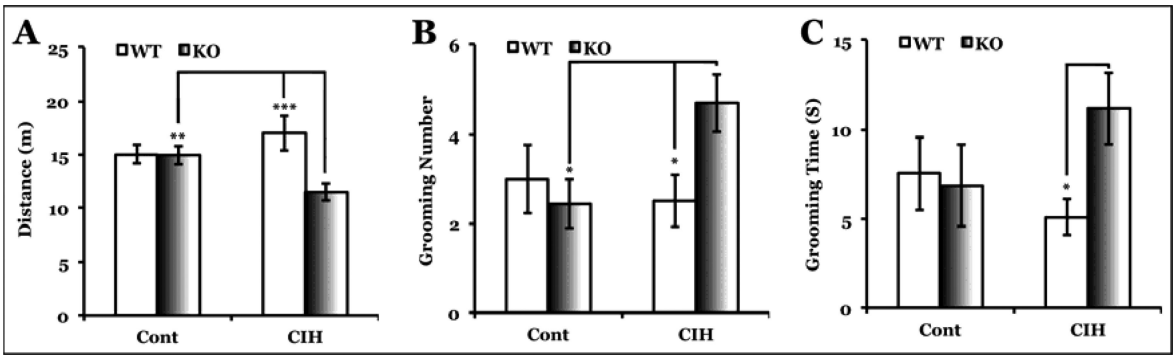

Fig. 2. Decreased travel distance and increased grooming behavior induced by CIH in GRK5KO mice

The open field was used for measuring anxiety and exploration as well as locomotion, in which panels A, B, and C show the total travel distance, grooming number and time, respectively. Cont= normoxic control; CIH=chronic intermittent hypoxia; WT=wild type; KO=GRK5KO. *=p<0.05, **=p<0.01, ***=p<0.001 for the comparisons as indicated.

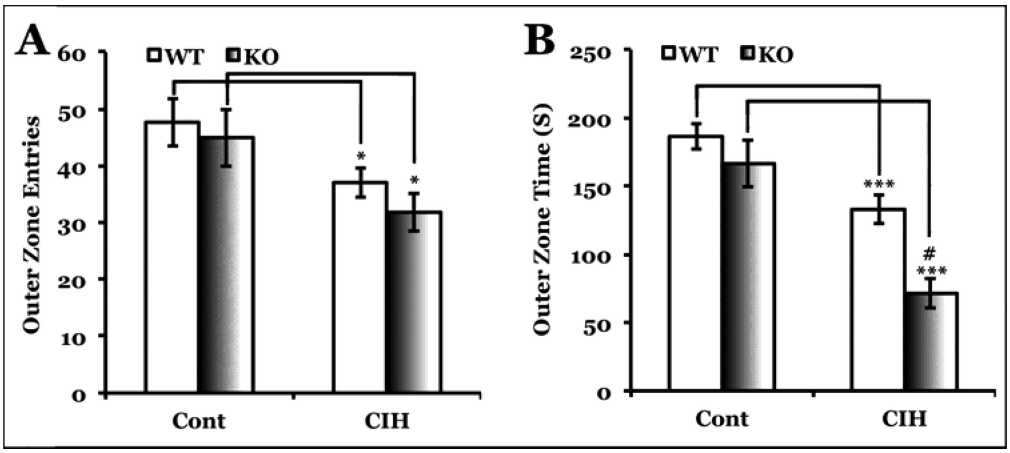

Fig. 3. Elevated anxiogenic tendency induced by CIH and GRK5 deficiency

The elevated platform task was used to assess the animal's anxiogenic tendency. Panels A and B show the entry numbers and time, respectively, in the outer zone of the elevated platform. *=p<0.05, ***=p<0.001 for the comparisons as indicated. #=p<0.001 for comparison between the CIH-treated WT and KO mice.

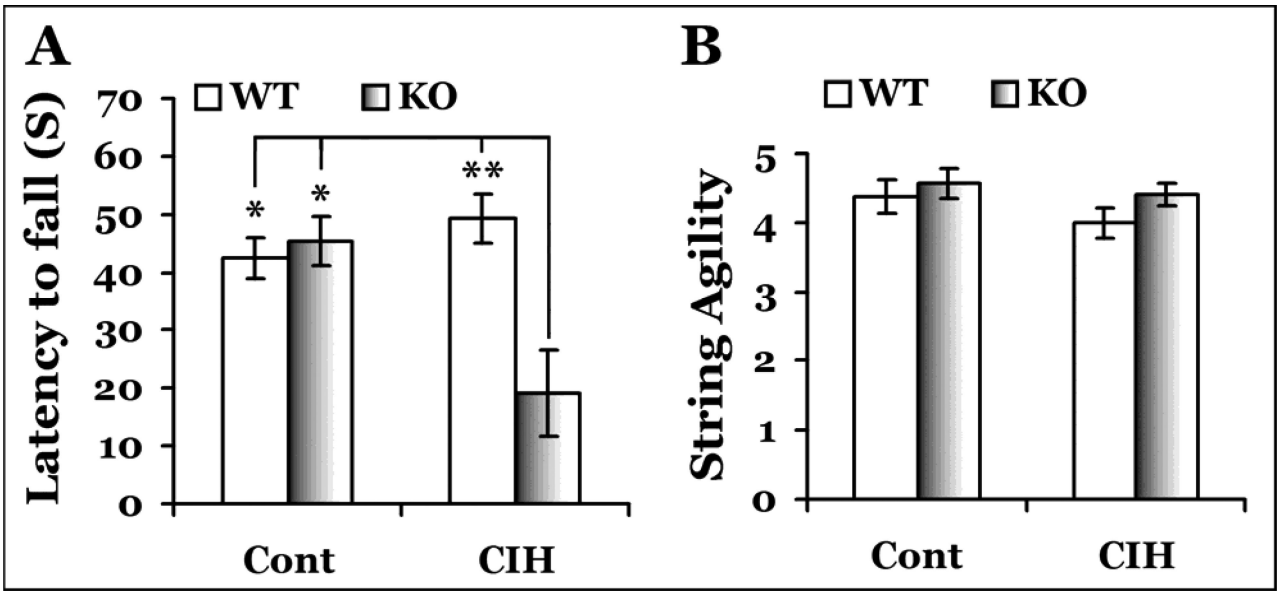

Fig. 4. Impaired motor coordination of balance by CIH and GRK5 deficiency

The balance beam (A) and string agility (B) tasks were used to assess animal's sensorimotor functions. The results revealed no change in the string agility but there was a significant decrease of the latency to fall off the beam for the CIH-treated KO mice. Two-way ANOVA also revealed a significant interaction between GRK5 and CIH ($p=0.006$) in addition to the differences between their means. *=$p<0.05$, **=$p<0.01$ for the comparisons as indicated.

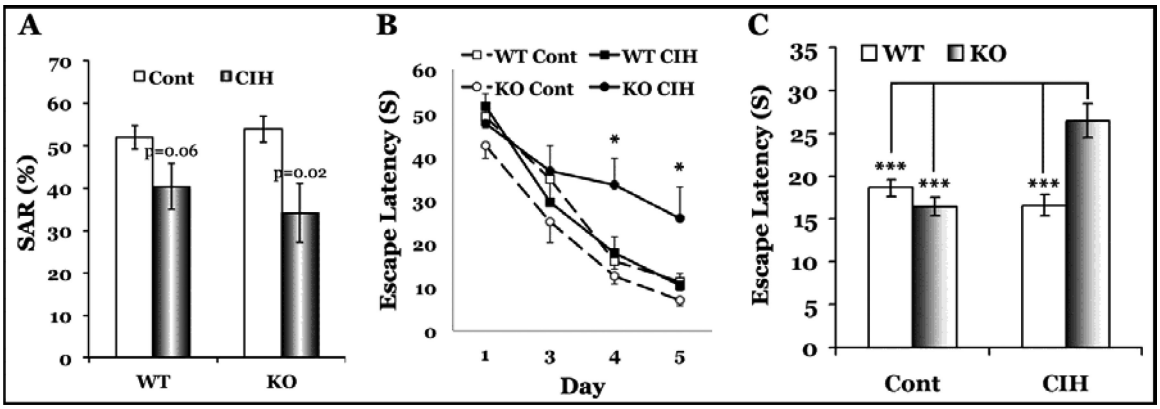

Fig. 5. Short-term spatial memory deficit induced by CIH in GRK5KO mice

The Y-maze spontaneous alternation (A), Morris water maze (B), and radial arm water maze (C) tasks were used to assess the animal's cognitive status. SAR=spontaneous alternation rate. The p values indicate the comparisons between the CIH versus control for the same genotype in the Y-maze task. For the MWM task, *=p<0.05 as compared with all other three groups at the same day. For the RAWM task, all the 15 trials for each mouse with the hidden platform in all the three blocks of the second day were averaged to represent the animal's performance on that day. The panel C shows the escape latency on the day 2. ***=p<0.001 for the comparisons as indicated.