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cGMP-dependent protein kinase type II knockout mice exhibit working memory impairments, decreased repetitive behavior, and increased anxiety-like traits

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ABSTRACT

Neuronal activity regulates AMPA receptor trafficking, a process that mediates changes in synaptic strength, a key component of learning and memory. This form of plasticity may be induced by stimulation of the NMDA receptor which, among its activities, increases cyclic guanosine monophosphate (cGMP) through the nitric oxide synthase pathway. cGMP-dependent protein kinase type II (cGKII) is ultimately activated via this mechanism and AMPA receptor subunit GluA1 is phosphorylated at serine 845. This phosphorylation contributes to the delivery of GluA1 to the synapse, a step that increases synaptic strength. Previous studies have shown that cGKII-deficient mice display striking spatial learning deficits in the Morris Water Maze compared to wild-type littermates as well as lowered GluA1 phosphorylation in the postsynaptic density of the prefrontal cortex (Serulle et al., 2007; Wincott et al., 2013). In the current study, we show that cGKII knockout mice exhibit impaired working memory as determined using the prefrontal cortex-dependent Radial Arm Maze (RAM). Additionally, we report reduced repetitive behavior in the Marble Burying task (MB), and heightened anxiety-like traits in the Novelty Suppressed Feeding Test (NSFT). These data suggest that cGKII may play a role in the integration of information that conveys both anxiety-provoking stimuli as well as the spatial and environmental cues that facilitate functional memory processes and appropriate behavioral response.

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1. Introduction

It is generally accepted that experience-dependent behavioral formation relies upon the modification of synaptic strength in the brain regions whose activities underlie the expression of behavior. Precise communication between the prefrontal cortex (PFC), striatum, amygdala, and hippocampus among other regions is critical in memory formation and retrieval as well as decision-making and appropriate action selection in processes such as executive function (Floresco, Seamans, & Phillips, 1997). The role of the PFC has been well studied in working memory, a component of executive function, and this controlled processing of information is necessary to achieve temporary goals (Baddeley, 1992; Ragozzino, Adams, & Kesner, 1998; Shimamura, 1995). The working memory system is thought to integrate perceptual information and long-term memory in order to generate motor commands and drive performance on complex tasks (Baddeley, 1992, 1996, 1998; Faw, 2003; Jones, 2002; Prabhakaran, Narayanan, Zhao, & Gabrieli, 2000). The PFC has a prominent role in the assimilation and communication of spatial information during tasks such as the Radial Arm Maze (RAM), a test designed to assess working memory (Floresco et al., 1997; Goldman-Rakic, 1990; Olton & Samuelson, 1976).

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At the cellular level, memory is thought to result from long-lasting changes in synaptic function, a process also known as synaptic 'plasticity' (Eccles, 1964; Hebb, 1949; Malinow & Malenka, 2002). Neural activity regulates the molecular changes that underlie these synaptic alterations and involve the insertion and removal of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs) (Kessels & Malinow, 2009). These ionotropic glutamate receptors are tetrameric and consist of GluA1–4 subunits (Hollmann & Heinemann, 1994; Malinow & Malenka, 2002). Receptors that lack the GluA2 subunit are Ca^{2+}-permeable and addition of these receptors postsynaptically may be triggered by N-Methyl-D-aspartic acid (NMDA) receptor activation. In one such pathway, a signaling cascade induced by the influx of Ca^{2+} promotes nitric oxide-dependent formation of cyclic guanosine monophosphate (cGMP) (Garthwaite & Boulton, 1995). cGMP-dependent protein kinase II (cGKII), a target of cGMP, phosphorylates serine 845 (S845) on the C-terminus of GluA1 which leads to increased levels of GluA1 in the plasma membrane (Incontro et al., 2013; Serulle et al., 2007). Phosphorylation of S845, as well as other critical residues on the C-terminus of GluA1, lead to increases in excitatory neuronal transmission and are believed to play a role in memory processes (Boehm et al., 2006; Lee et al., 2003).

Appropriate behavioral responses are reliant upon functional memory processes as well as top-down control over emotional reactions to stimuli (Bishop, 2007). Previous behavioral studies of cGKII knockout (KO) animals suggest that this kinase is important for emotional responses (Werner et al., 2004). Animals deficient in cGKII display heightened anxiety-like behaviors in the light–dark (L/D) box test as well as increased ethanol consumption in a two-bottle free choice test (Werner et al., 2004). cGKII may also be involved in learning and memory. Recent studies have shown striking learning and memory deficits in the Morris Water Maze in cGKII KO animals when compared to wild type (WT) controls (Wincott et al., 2013). Additionally, previous tests revealed a heightened acoustic startle response in cGKII KO animals as well as significant decreases in phosphorylation of GluA1 in the postsynaptic density (PSD) of PFC fractions (Wincott et al., 2013).

In the current study, we further characterize the role of cGKII in PFC-dependent behavior. We have investigated cGKII KO mice in a PFC-dependent, working memory task: the RAM (D'Ardenne et al., 2012; Fuster, 1973; Olton & Samuelson, 1976). This version of the task used in this study also assesses reference memory, a hippocampal-dependent process (Schmitt et al., 2004). Using spatial cues, animals learn which of four of the eight arms are baited over the course of shaping and acquisition phases (Borroni, Fichtenholtz, Woodside, & Teyler, 2000). Re-entry into arms from which baits have been consumed is recorded as working memory errors (WMEs) and entry into arms where baits have never been discovered are recorded as reference memory errors (RMEs).

Based on our recent findings showing spatial learning deficits and lowered phosphorylation of GluA1 in the PSD of PFC fractions, we hypothesized that disrupted functioning of cGKII-mediated phosphorylation of S845 of GluA1 and the effects on AMPA receptor trafficking in the PFC of cGKII KO animals would result in increased working memory errors in the RAM in cGKII KO animals (Wincott et al., 2013). Additionally, we performed other behavioral tasks dependent in part on the PFC as well as other structures where cGKII is highly expressed, such as the amygdala (Werner et al., 2004). The Novelty Suppressed Feeding Test (NSFT) is a conflict test used to assess anxiety-like behaviors in animals (Shephard & Broadhurst, 1982) but has also been shown to be dependent on PFC function (Banasr & Duman, 2008; Sun, Liu, Yuan, Li, & Chen, 2012). We performed the NSFT to further characterize the reported anxiety-like phenotype in cGKII KO mice (Werner et al., 2004). Food-deprived animals are presented with a choice whereby they can either consume a pellet of food that has been positioned in the center of a brightly lit novel arena or they can remain around the perimeter of the arena and refrain from eating. Longer latency to consume food in this assay is considered to display an anxious phenotype (Fukumoto, Iijima, & Chaki, 2014; Iijima, Fukumoto, & Chaki, 2012). We also performed experiments using the Marble Burying (MB) assay. While this test has been used to investigate anxiety-like traits, more recently it has been shown to more accurately assess perseverative and repetitive behaviors, in which the prefrontal cortex plays a role (Burguiere, Monteiro, Feng, & Graybiel, 2013; Ichimaru, Egawa, & Sawa, 1995; Okada, Ota, Iida, Kishimoto, & Kishimoto, 2013; Thomas et al., 2009). MB is a frequently used assay in animal models of obsessive–compulsive disorder, a disorder thought to arise from inadequate function of the PFC (Okada et al., 2013; Rauch et al., 1997; Shinomiya et al., 2005).

The proceeding set of experiments was conducted to gain further insight into the consequences of cGKII gene deletion on PFC-dependent behaviors. Building on our previous work with cGKII KO mice, we find that cGKII KO animals exhibit increased anxiety-like traits, lowered perseverative behavior, and spatial learning and memory deficits, suggesting that cGKII plays a role in behaviors that involve the PFC.

2. Materials and methods

2.1. Statistics

Analysis of variance (ANOVA) and *t* tests were applied to behavior tests as necessary and each analysis is identified in the representative Section 3. For the RAM, statistical analyses are two-tailed at a significance level of 0.05 and data on the graphs display mean ± SEM. Statistical analyses were performed using Graphpad Prism software, Version 6.0c (La Jolla, CA, USA).

2.2. Mice

cGKII KO generation has been previously described (Pfeifer et al., 1996): animals were backcrossed to C57BL/6 and bred at Taconic Farms in Germantown, NY. When the mice reached eight weeks of age, they were transferred to Memorial Sloan Kettering Cancer Center (MSKCC), Zuckerman Building, where they were housed under standard conditions of ~2–3 animals per cage (20–24 °C, 30–70% relative humidity, 12-h dark: 12-h light cycle, 0700–1900 h). All behavioral assays were conducted on adult male mice from the ages of 10–12 weeks at the beginning of testing during the latter part of the light cycle. Animal experiments were conducted in compliance with the Institutional Animal Care and Use Committee at the New York University School of Medicine. Assays took place in the Zuckerman Building Barrier Facility at MSKCC and were conducted in this order: MB, NSFT, RAM, Grip Strength, Wire-Hang and Nest-Building in two cohorts. For both cohorts, the time from the first test to the last was approximately five weeks.

2.3. Marble Burying

In MB (Broekkamp, Rijk, Joly-Gelouin, & Lloyd, 1986), each mouse was acclimated in a cage alone for half an h in the testing room before being placed into a 22 × 16 in. rectangular Tupperware tub filled with sterile off-white mouse bedding tamped down to make a flat, even surface. Atop the bedding, twelve black marbles (Land of Marbles) had been carefully aligned in a uniform manner (3 × 4 in the center). The distance between marbles was approximately two inches. The distance of each outer marble was approximately two inches from the perimeter of the container wall. Each mouse was allowed to explore the environment freely for 30 min. At the conclusion of the 30 min, each mouse was