



Spatial memory deficits and motor coordination facilitation in cGMP-dependent protein kinase type II-deficient mice

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ABSTRACT

Activity-dependent trafficking of AMPA receptors to synapses regulates synaptic strength. Activation of the NMDA receptor induces several second messenger pathways that contribute to receptor trafficking-dependent plasticity, including the NO pathway, which elevates cGMP. In turn, cGMP activates the cGMP-dependent protein kinase type II (cGKII), which phosphorylates the AMPA receptor subunit GluA1 at serine 845, a critical step facilitating synaptic delivery in the mechanism of activity-dependent synaptic potentiation. Since cGKII is expressed in the striatum, amygdala, cerebral cortex, and hippocampus, it has been proposed that mice lacking cGKII may present phenotypic differences compared to their wild-type littermates in emotion-dependent tasks, learning and memory, and drug reward salience. Previous studies have shown that cGKII KO mice ingest higher amounts of ethanol as well as exhibit elevated anxiety levels compared to wild-type (WT) littermates. Here, we show that cGKII KO mice are significantly deficient in spatial learning while exhibiting facilitated motor coordination, demonstrating a clear dependence of memory-based tasks on cGKII. We also show diminished GluA1 phosphorylation in the postsynaptic density (PSD) of cGKII KO prefrontal cortex while in hippocampal PSD fractions, phosphorylation was not significantly altered. These data suggest that the role of cGKII may be more robust in particular brain regions, thereby impacting complex behaviors dependent on these regions differently.

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1. Introduction

Environmental stimuli modify the functions of brain circuits that encode information surrounding the event. These modifications can be stabilized through the formation of memories. In rodents, the role of the hippocampus in memory has been studied extensively (Eichenbaum, 1999) but it is also thought that the prefrontal cortex, which connects to the hippocampus, plays a role in the maintenance of mental representations that guide goal-directed behaviors (Lynch, 2004). Activity within the prefrontal cortex is involved in the assignment of temporal position to spatial and nonspatial events alike (Lynch, 2004). The molecular mechanisms contributing to long-term potentiation (LTP), an activity-dependent form of synaptic plasticity that may underlie forms of nondeclarative memory, cognition and behavior, are only beginning to be understood (Mayford, Mansuy, Muller, & Kandel, 1997). Signaling pathways in the molecular component of memory have been

identified, and among those of particular interest are the pathways that convert short-term memories to long-term memories, including the cyclic nucleotide mediated protein kinase pathways that phosphorylate ionotropic glutamate receptors and control their subsequent trafficking to the synapse.

α -Amino-3-hydroxy-5-methylisoxazole-4-propionate receptors (AMPA receptors) play an important role in the regulation of synaptic strength, and are believed to be an essential component of the memory formation mechanism. AMPARs are tetramers that contain GluA1–4 subunits (Malinow & Malenka, 2002). Insertion of GluA2-lacking, Ca^{2+} -permeable AMPARs into the postsynaptic membrane may be induced by the activation of NMDA receptors (Derkach, Oh, Guire, & Soderling, 2007). The influx of Ca^{2+} induces signaling cascades that result in the phosphorylation of the intracellular C-terminal domain of GluA1 (Roche, O'Brien, Mammen, Bernhardt, & Huganir, 1996; Serulle et al., 2007). Increases in excitatory neuronal transmission that lead to spatial memory, learning, and retention are mediated by phosphorylation of GluA1, with phosphorylations of S831 and S845 contributing to the mechanism (Lee et al., 2003). Deletion of α CAMKII, the kinase that

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phosphorylates GluA1 at serine 831 (Fu, Zhang, Meng, & Zhang, 2004), impairs LTP in the hippocampus and disrupts spatial learning (Fu et al., 2004). It has also been shown that mice with knockin mutations substituting alanine for serines 831 and 845 of GluA1 exhibit deficient memory retention as well as disruptions in LTP and LTD (Lee et al., 2003).

Two closely-related cyclic nucleotide regulated protein kinases, cAMP-dependent PKA and cGMP-dependent protein kinase II (cGKII) phosphorylate serine 845, inducing delivery of GluA1 to the synapse (Serulle et al., 2007). PKA plays a key role in memory formation and the induction of LTP (Selcher, Weeber, Varga, Sweatt, & Swank, 2002). In transgenic mice expressing a dominant negative form of the regulatory subunit of PKA, deficits were observed in long-term contextual fear learning (Selcher et al., 2002). In addition to PKA's known role in GluA1 phosphorylation, it is also a regulator of cAMP response binding-element (CREB) and triggers signaling cascades that drive transcription of proteins necessary for strengthening synapses (Michel, Kemenes, Muller, & Kemenes, 2008). cGKII is inactive when cGMP is not present (Serulle et al., 2007). Upon NMDA receptor activation and the subsequent binding of cGMP to cGKII, a conformational change of the kinase occurs, whereby the autoinhibitory domain is auto-phosphorylated, released from the catalytic domain, and the kinase function is activated (Serulle et al., 2007). This series of molecular events enables the GluA1 C-terminus to bind to the kinase and GluA1 is phosphorylated at S845, leading to an increase in receptor surface levels (Serulle et al., 2007), a step that is required for receptor entry into the synapse.

The closely related roles of cGKII and PKA in GluA1 phosphorylation and trafficking raise the question of the relative contributions of these kinases to memory and behavior formation. Of the two, cGKII has been less well studied. Serulle et al. (2007) found that cGKII activation increased surface levels of GluA1 and that this effect was blocked by KT5823, a selective inhibitor of cGMP-dependent protein kinase. Also, LTP was blocked when KT5823 was applied to hippocampal slices. Interestingly, previous studies by Kleppisch et al. (1999) demonstrated that LTP was normal in the cGKII KO animals. Even though LTP was shown to be normal in cGKII KO animals, distinct phenotypes have been found in various behavioral assays. Werner et al. (2004) found that cGKII KO mice exhibited hyposensitivity and elevated preference for ethanol as well as heightened anxiety levels compared to wild-type littermates.

Modifications of kinase activity as well as phosphorylation sites on GluA1 have been shown to disrupt LTP and learning (Lee et al., 2003). S845 of GluA1 presents a novel situation in which two kinases under the control of the NMDA receptor and cyclic nucleotides, PKA and cGKII, both phosphorylate this critical residue. Furthermore, acute inhibition of cGKII blocks LTP in hippocampal slices, while KO of the gene leaves LTP apparently unchanged (Kleppisch et al., 1999; Serulle et al., 2007).

To begin to understand the role of cGKII in memory formation, we examined the behavior of cGKII KO mice in a battery of assays in order to identify phenotypic changes. The assays used to assess phenotypes were Prepulse Inhibition (PPI), Acoustic Startle (ASR), the Rotating Rod Test, Open Field Assay (OFA), and the Morris Water Maze (MWM). Although acute inhibition of cGKII has been shown to decrease levels of LTP significantly (Serulle et al., 2007) while knocking out the cGKII gene has revealed normal LTP (Kleppisch et al., 1999), we could not anticipate whether behavioral assays would indicate phenotypic differences in comparison to wild type. Here we have shown that cGKII KO mice exhibit deficits in spatial learning in the Morris Water Maze, heightened acoustic startle response, and improved motor coordination on the Rotating Rod Test as compared to wild-type (WT) littermates. Our immunoblot studies showed that GluA1 phosphorylation at serine 845 in

the hippocampus of cGKII KO animals was unchanged, but was significantly reduced in the prefrontal cortex. Thus, cGKII KO animals could show synaptic impairments in a brain region-specific manner.

2. Material and methods

2.1. Mice

The generation of cGKII knockout has been previously described (Pfeifer et al., 1996). cGKII KO mice were either kept on the Sv/129 background or back-crossed to C57BL/6N. Mice were bred in the Skirball Animal Facility at New York University School of Medicine. Mice were kept under standard housing conditions (20–24 °C, 30–70% relative humidity, 12-h dark:12-h light cycle) and were housed at an average of ~four animals per cage. At the conclusion of the behavioral assays, the animals were weighed and the following weight averages were recorded: cGKII KO: 24.7 ± 1.6 g and WT: 38.9 ± 0.6 g, $p < 0.0001$. All behavioral tests were performed on adult male mice from the ages of 27–41 weeks, with an average age of 33 weeks.

The animal experiments were conducted in accordance with the New York University School of Medicine's Institutional Animal Care and Use Committee, New York University School of Medicine. Experiments were conducted in the Smilow Barrier Facility. Assays were conducted in the following order: OFA, the Rotating Rod Test, ASR, PPI, and MWM using four cohorts in MWM and five cohorts for all other assays.

2.2. Prepulse inhibition and acoustic startle

In prepulse inhibition and acoustic startle (Graham, Putnam, & Leavitt, 1975; Lang, Bradley, & Cuthbert, 1990), the animal is removed from its home cage and placed into a soundproof chamber, where the startle response tone (120 decibels for 20 ms) is given and startle response is scored by an automated system (San Diego Instruments). Mild prepulse tones (74, 82, and 90 dB and 20 ms in duration) are then played shortly before response to the 120 dB tone is scored. Five trials of each prepulse and 120 dB tone pairing are performed in a randomized order with intervening segments and unpaired 120 dB test pulses.

Here, mice were acclimated in cages alone for half an hour before being placed into the soundproof chamber containing a Plexiglas tube sitting atop a plastic frame. An accelerometer detected the motion within the tube as animals were undergoing testing. The acoustic startle tone was controlled by startle software and delivered by a speaker mounted inside the chamber. Animals were placed inside the tube and the startle pulse was given. The animal's response was calibrated by the system. Five randomized trials were given from each pairing with interspersed silent periods and the animal's response was scored as described. In wild-type mice, the acoustic startle response decreases as the prepulse tone is increased.

2.3. The Rotating Rod Test

In the Rotating Rod Test (Bogo, Hill, & Young, 1981), the mouse is placed on a rotating horizontal rod (Columbus) which is approximately three cm in diameter. The rod accelerates from four to 40 rpm during the 5 min trial. A single test lasts from the time the animal is placed on the rod until it drops off (approximately 12 cm onto the rest platform) or until 5 min have elapsed. Promptly afterwards, the animal is returned to the home cage and the rod and the rest platform are cleaned with 70% ethanol and water. Each animal is tested for four trials per day for two

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