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Forebrain-specific deletion of Cdk5 in pyramidal neurons results in mania-like behavior and cognitive impairment

Susan C. Su [1], Andrii Rudenko [1], Sukhee Cho, Li-Huei Tsai *

The Picower Institute for Learning and Memory, Department of Brain and Cognitive Sciences, Massachusetts Institute of Technology, Cambridge, MA, United States
Howard Hughes Medical Institute, Massachusetts Institute of Technology, Cambridge, MA, United States

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ABSTRACT

Cyclin-dependent kinase 5 (Cdk5) is associated with synaptic plasticity and cognitive function. Previous reports have demonstrated that Cdk5 is necessary for memory formation, although others have reported Cdk5 conditional knockout mouse models exhibiting enhanced learning and memory. Furthermore, how Cdk5 acts in specific cell populations to affect behavior and cognitive outcomes remains unclear. Here we conduct a behavioral characterization of a forebrain-specific Cdk5 conditional knockout mouse model under the αCaMKII promoter, in which Cdk5 is ablated in excitatory pyramidal neurons of the forebrain. The Cdk5 conditional knockouts exhibit hyperactivity in the open field, reduced anxiety, and reduced behavioral despair. Moreover, the Cdk5 conditional knockouts also display impaired spatial learning in the Morris water maze and are severely impaired in contextual fear memory, which correspond to deficits in synaptic transmission. Remarkably, the hyperactivity of the Cdk5 conditional knockouts can be ameliorated by the administration of lithium chloride, an inhibitor of GSK3β signaling. Collectively, our data reveal that Cdk5 ablation from forebrain excitatory neurons results in deleterious effects on emotional and cognitive behavior and highlight a key role for Cdk5 in regulating the GSK3β signaling pathway.

1. Introduction

The neuronal cyclin-dependent kinase 5 (Cdk5) is a serine/threonine kinase with a well-established role during embryonic neuronal migration and in cytoskeletal dynamics (Dhavan & Tsai, 2001). More recently, Cdk5 has been implicated in a variety of synaptic functions including the synaptic vesicle cycle and synapse formation (Samuels et al., 2007; Su & Tsai, 2011). In addition to targeting NMDA receptors and postsynaptic scaffolding proteins such as PSD-95 (Li et al., 2001; Morabito, Sheng, & Tsai, 2004), Cdk5 activity is critical for the formation of synaptic plasticity as measured by long-term potentiation (LTP) at hippocampal synapses (Fischer, Sananbenesi, Pang, Lu, & Tsai, 2005; Li et al., 2001). Furthermore, several intriguing reports have uncovered a prominent role for Cdk5 in regulating synaptic transmission to fine-tune homeostatic plasticity (Kim & Ryan, 2010; Mitra, Mitra, & Tsien, 2011; Seeburg, Feliu-Mojer, Gaiottino, Pak, & Sheng, 2008). Despite the growing evidence highlighting Cdk5 and its influence on synaptic plasticity, recent studies on how Cdk5 loss-of-function affects behavioral outcome have reported diverging findings.

One study examining how loss of Cdk5 affects behavior reports an increase in learning and memory in a Cdk5 conditional knockout mouse line (Hawasli et al., 2007). The Cdk5 mutant animals were generated under the prion protein promoter, and they displayed enhanced spatial memory, increased memory formation during fear conditioning, and significantly enhanced LTP in synaptic plasticity experiments. However, other studies have demonstrated that Cdk5 is necessary for associative learning within the septohippocampal system, as inhibition of Cdk5 activity prevents contextual-fear memory formation (Fischer, Sananbenesi, Schrick, Spiess, & Radulovic, 2002). Subsequent studies indicated that loss of Cdk5 in pyramidal neurons of specific hippocampal regions under the αCaMKII promoter, or pharmacological inhibition of Cdk5 activity, is detrimental to acquisition of spatial and contextual memory and results in deficits in LTP (Guan et al., 2011; Li et al., 2001). It is interesting to note that mouse models in which p35, the regulatory subunit of Cdk5, has been deleted show cortical lamination deficits, seizures, and adult lethality (Chae et al., 1997). The p35 knockout animals also exhibit hyperactivity and alterations in the dopaminergic and cholinergic systems (Drerup et al., 2010; Krapacher et al., 2010). In the p35 knockout animals, the contribution of p39, another co-activator of Cdk5, cannot be

* Corresponding author. Address: Massachusetts Institute of Technology, Building 46, Room 4235, 77 Massachusetts Avenue, Cambridge, MA 02139, USA. Fax: +1 617 324 1657.

E-mail address: lhtsai@mit.edu (L.-H. Tsai).

[1] These authors contributed equally to this work.

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excluded from participating in a compensatory role to regulate Cdk5 function.

Given the uncertainty of how loss of Cdk5 affects the behavioral and cognitive outcomes, we have now examined a conditional knockout mouse model in which Cdk5 is selectively ablated in forebrain excitatory neurons. Through a series of behavioral tasks, we examined parameters such as motor activity, cognitive function, and synaptic plasticity after temporal, widespread Cdk5 ablation in a specific neuronal subtype. We show here that Cdk5 loss-of function in αCaMKII-positive pyramidal neurons results in strong hyperactivity, severely impaired cognitive function and marked deficits in neurotransmitter release. Remarkably, treatment of the Cdk5 conditional knockouts with lithium chloride can alleviate their hyperactivity. Further evaluation of the molecular mechanisms underlying the behavioral phenotype in these Cdk5 conditional knockouts will be useful to better understand how Cdk5 regulates behavior and cognition.

2. Materials and methods

2.1. Animals

Cdk5 conditional knockout animals were generated by crossing the αCaMKII-Cre (CW2) line with the Cdk5 floxed/floxed (Cdk5f/f) line to generate αCaMKII-Cre;Cdk5fl/fl animals, referred to as Cdk5 conditional knockout (Cdk5f/f/CW2) animals. Mice were generated and maintained in a C57Bl/6J background and group-housed in a 12 h light–dark cycle, with food and water *ad libitum* and handled according to the National Institutes of Health Care and Use of Laboratory Animals. Experiments were conducted using adult, 3–5 month old male mice.

2.2. Immunohistochemistry

Mice were transcardially perfused with phosphate-buffered saline (PBS) followed by 4% paraformaldehyde (PFA) in PBS, and brains were postfixed in 4% PFA at 4 °C overnight. Free floating coronal sections (40 μm) were prepared using a vibratome. Sections were incubated in a blocking solution of 10% normal donkey serum, 3% bovine serum albumin, 0.2% Triton-X 100, 0.02% sodium azide in PBS for 1–2 h at room temperature (RT), followed by incubation with primary antibody (Cdk5, clone C-8, 1:100, Santa Cruz Biotechnology) in blocking solution overnight at 4 °C with the appropriate Cy3-congugated (1:1000, Jackson Labs) secondary antibody for 2 h at RT. Hoechst dye was added to label cell nuclei. Images were acquired using high-resolution multi-channel scanning confocal microscopy (Zeiss LSM 510).

2.3. Behavioral training

2.3.1. Open field test

Animals were placed into 40 × 40 × 30 cm arenas with orthogonal lasers to track position and locomotor activity for 60 min (VersaMax, AccuScan Instruments). Activity was measured by movement across a grid of infrared light beams and automatically recorded. The margin was defined as the distance within 1 cm of the walls of the open field arena.

2.3.2. Light/dark exploration

Animals were placed into a light–dark chamber apparatus and allowed to explore the arenas for 10 min. Latency to enter the light for the first time, duration spent in the light chamber and the number of crossings from the dark to the light chamber were recorded.

2.3.3. Forced swim

Animals were placed in a 4000 ml Pyrex cylindrical container (10.2 × 45.7 cm) filled half-way full with water at a temperature of 24 °C, and various parameters such as swim velocity, mobile duration and time during which the animal is swimming around the tank, immobile duration and time during which the animal remains almost completely still with its head above water, were automatically recorded using video tracking software for 6 min (EthovisionXT, Noldus). Immobility duration and time, parameters used to analyze learned helplessness or despair, was measured during the last 4 min of the test.

2.3.4. Pre-pulse inhibition

To assess sensorimotor gating, animals were habituated to the equipment for two days (Startle Monitor System, Hamilton Kinder). On the third day, the mouse was placed into the chamber, and after 5 min exposure to the white noise (65 dB), a series of trials were performed at random intervals: no acoustic stimulus, acoustic stimulus alone, or prepulse stimuli (70, 75, 80, 85 dB) followed by an acoustic startle stimulus. Percent PPI was calculated as [100 – (response amplitude for prepulse stimulus with startle stimulus/amplitude for the startle stimulus) × 100].

2.3.5. Morris water maze

Spatial memory was performed using a circular tank (1.2 m in diameter) filled with opaque water at 22 °C. The walls contained spatial reference cues, and inside the tank was a fixed platform (10 cm in diameter) in a target quadrant. During pre-training (Day 1), animals were placed in the water, guided to the platform, and allowed to stay in the platform for 15 s. In the following days, animals were placed into the maze randomly and allowed to search for the platform for 60 s. Two trials a day were conducted with a 2 min interval. Mouse behavior was recorded and analyzed, and escape latency was scored (TSE Systems). On day 9, the platform was removed and a probe trial was conducted to assess spatial learning.

2.3.6. Fear conditioning

Both contextual and cued fear conditioning was conducted over the course of 3 days. On day 1, animals were placed in a fear conditioning apparatus chamber for 3 min and then presented with a 2 s foot shock delivery at 0.8 mA preceded by a 30 s, 75 dB tone (TSE Systems). After the animals were placed in their homecage for 24 h, they were assessed for contextual learning by recording the freezing behavior for 3 min in the training context. On day 3, the animals were presented with a different context but levels of freezing were assessed after exposure to the same tone. Prior to each trial, each context was cleaned with 70% ethanol except for on day 3, where it was cleaned with acetic acid.

2.4. Electrophysiology

To record field excitatory postsynaptic potentials, transverse hippocampal slices were prepared from 8 to 12 week old adult mice. Briefly, the brain was rapidly removed and transferred to ice-cold, oxygenated (95% O_2 and 5% CO_2) cutting solution containing (mM) 211 sucrose, 3.3 KCl, 1.3 NaH_2PO_4, 0.5 $CaCl_2$, 10 $MgCl_2$, 26 $NaHCO_3$ and 11 glucose. Hippocampal slices were cut with a Leica VT1000S vibratome (Leica) and transferred to a holding chamber containing oxygenated artificial cerebrospinal fluid (ACSF) consisted of (mM) 124 NaCl, 3.3 KCl, 1.3 NaH_2PO_4, 2.5 $CaCl_2$, 1.5 $MgCl_2$, 26 $NaHCO_3$ and 11 glucose at 28–30 °C for at least 1 h before recording. CA1 field potentials evoked by Schaffer collateral stimulation were measured. Recordings were performed using a MultiClamp 700B amplifier and a Digidata 1440A A–D converter and analyzed by pClamp 10 software (Axon Instruments).