Altered hippocampal place cell activity in conditional Cdk5 knockout mice

E. S. Lovitz, J. R. Jones, G. E. Farmer, A. M. Savage[1], J. A. Bibb[1], and L. T. Thompson

Aging & Memory Research Lab., School of Behavioral & Brain Sciences, Univ. of Texas at Dallas, Richardson, TX
[1]Psychiatry, Univ. of Texas Southwestern Medical Center, Dallas, TX

Fig. 1. Examples of activity of two place-cells from Cdk5(-) mice.

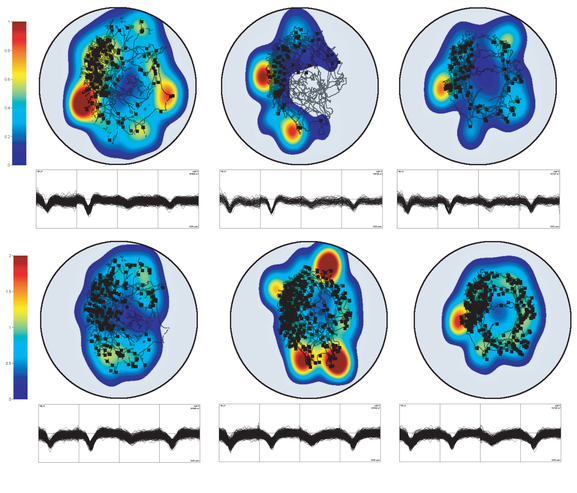


Fig. 2. Examples of activity of two place-cells from control mice.

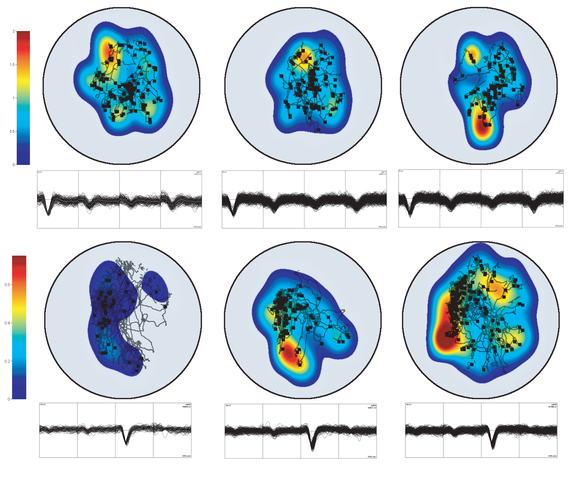


Fig. 3. The grand mean, peak in-field, and mean in-field firing rates were significantly higher in Cdk5(-) than in control mice ($p < 0.01$, $p < 0.05$, $p < 0.05$ respectively). Mean out-of-field firing rates were comparable between groups ($p > 0.25$).

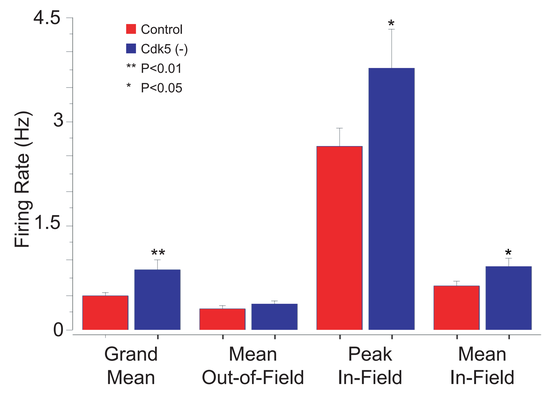
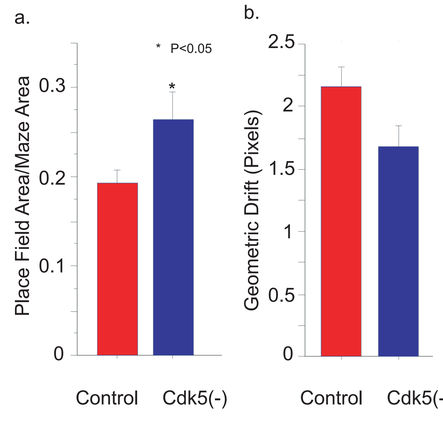


Fig. 4. Stability measures of place-fields. (a) Place-fields encompassed significantly larger maze areas in Cdk5(-) than control animals ($p < 0.05$). (b) Geometric center of place-fields drifted less from session to session in knockout than control animals ($p = 0.0568$).



Background

Many pyramidal neurons in the hippocampus (termed "place cells") selectively fire when a rat visits specific environmental locales (location-specific "place-field" firing). In young rats, these fields remain remarkably stable when the animal revisits that same location across multiple sessions and multiple days (Thompson & Best, 1989, 1990). Rapid place-field plasticity is widely reported in response to environmental manipulations and task-specific cognitive processing. Place-field activity has also been studied in mice, an attractive knockout model, with place-field size typically larger than that observed in rats (cf. Cacucci *et al*., 2007). Conditional knockout of cyclin-dependent kinase 5 (Cdk5) in mice has been shown to improve spatial learning on the Morris water maze, a hippocampal-dependent task (Hawasli *et al*., 2007). Cdk5 knockout reduces the threshold for induction of LTP by attenuating calpain-dependent degradation of the NR2B subunit of NMDA receptors, which can also alter intrinsic excitability. Here we test the hypothesis that the Cdk5 knockout improves spatial learning by mediating the stability, specificity, or frequency of hippocampal place-cells monitored in mice with chronic tetrode implants.

Methods

Subjects: Experiments were performed using a total of 4 male mice, 2 Cdk5 knockouts (Cdk5(-)) and 2 sham control animals. Conditional knockouts were performed in Dr. James Bibb's laboratory at UTSW, and maintained in our laboratory at UTD under conditions approved by the ACUC on a 12 hr/12 hr light/dark schedule. After stereotaxic implantation, mice were housed individually with *ad libitum* access to food and water, and daily health was monitored.

Chronic Implants: Animals were implanted with drivable Scribe microelectrodes (adapted from Bilkey, 1999), four bundles of 38 µm Formvar-insulated Nichrome microwire tetrodes inserted into a 27 ga stainless steel cannula in a non-rotating driveable assembly. The electrodes were implanted stereotaxically above dorsal CA1, then later driven into *s. pyramidale* of CA1.

Spatial Behavior: Mice were trained to navigate a open field barrel or drum maze baited with 22 mg dehydrated milk pellets. The maze (0.91 m in diameter, 0.6 m high) was located in a dimly lit 6.4 m^2 room. Each session used included navigation of all accessible parts of the maze for the mouse connected to the multichannel recording tether. Sessions were 5–10 min in length.

Place-Cell Recording and Data Analysis: Mice performing the task were followed for multiple daily recording sessions across multiple days during which hippocampal activity was monitored. Implanted electrodes were attached to a recording headstage. LEDs were affixed to a source-follower preamp headstage each session to monitor the mouse's location.

Amplification and filtering of unit signals used a Multichannel Acquisition Processor (MAP) System (Plexon Inc., Dallas, TX). Spike wave forms were recorded and template sorted using Plexon's RASPUTIN and OfflineSorter software. Spatial location was recorded using Plexon's Video Tracker System. Place fields were analyzed with NeuroExplorer and NexScript (Nex Technologies, Littleton, MA). After sorting, place fields were defined as a set of at least 5 contiguous pixels with a firing rate more than 2 standard deviations above the grand mean (i.e., the firing for the entire session) of that single unit. Pixel to pixel comparison analyses were performed in MATLAB (Mathworks, Natick, MA) and used to compare place-field stability between sessions.

ANOVA statistical comparisons were performed with StatView (SAS Institute Inc., Cary, NC) to assess differences between knockout (i.e. experimental) and control animals. ANOVA was performed on in-field and out-of-field firing rates, grand-mean firing rates, and place-field stability measures. A post-hoc Scheffe's analysis was performed on all analyses.

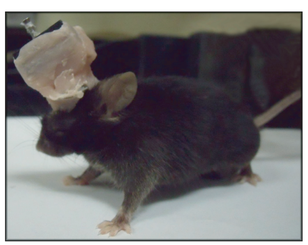


Fig. 5: Mouse implanted with scribe electrode.

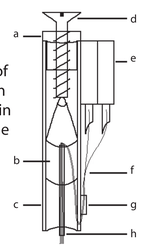


Fig. 6: Schematic of electrode. (a) Bottom half of ball-point pen nib, (b) Top half of ball-point pen nib, (c) Aluminum tube, (d) Screw, (e) 2 ea. 10-pin omnetics connectors, (f) 4 ea. formvar-nichrome microwire tetrodes, (g) Plastic guide cannula, (h) Stainless steel cannula.

Results

All data sets in which an animal successfully navigated the barrel maze were used, a total of 18 neurons from knockout mice and 40 neurons from controls. It was observed that Cdk5(-) mice learned to navigate the barrel maze faster than control mice. Cdk5(-) mice had increased firing rates, in terms of grand mean, peak in-field, and mean in-field firing ($F(1,167)=7.4$, $p=0.0072$; $F(1,167)=4.57$, $p=0.034$; $F(1,167)=4.95$, $p=0.028$ respectively). Mean out-of-field firing rates, however, were not different between groups ($F(1,167)=1.11$, $p=0.294$) (Figure 3). Increased firing rates of Cdk5(-) neurons was accompanied by an increase in place-field size (control=0.193, Cdk5(-)=0.259; $F(1,167)=4.64$, $p=0.033$) (Figure 4a). The drift or relative instability of the geometric center of place-fields in the two groups was not statistically different (control=2.164 pixels, Cdk5(-)=1.683 pixels; $F(1,167)=3.68$, $p=0.057$), although place-fields in Cdk5(-) mice consistently showed less geometric drift than in control mice (Figure 4b). Control and Cdk5(-) mice exhibited no differences in the stability of grand mean firing rates across multiple sessions ($F(1,164)=0.24$, $p=0.626$).

Summary

- Cdk5(-) place-cells are more excitable in specific places, but not out-of-field.
- Cdk5(-) mice have place-fields that are larger than controls.
- Place-field location for Cdk5(-) animals was more stable than in controls.

Acknowledgements

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