Effects of Cav2.2 inhibitor on hippocampal spatial short-term cognition

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Abstract

Neuronal voltage gated calcium channels (VGCCs) including Cav2.1 and Cav2.2 channels mediate the presynaptic machinery for vesicular release of neurotransmitters. However, the role of different VGCCs in the neural circuits underlying spatial short-term memory has not been studied. Although it has been reported that spatial cognition requires Cav2.1-regulated signaling in the hippocampus, Cav2.2-regulated function remains unknown. This study examined whether Cav2.2-mediated signaling plays role in spontaneous alternation patterns of Y maze test. Mice received intracerebroventricular injection of Cav2.2 blocker (ω-conotoxin GVIA, 5 pg/side) showed impaired spontaneous alternation patterns. Intrahippocampal injection of ω-conotoxin GVIA (5 pg/side) induced learning impairment. These results indicate that Cav2.2-mediated signaling is also critical in the hippocampus-dependent spatial cognition.

Introduction

Voltage gated calcium channels (VGCCs) mediate a number of neuronal functions including neurotransmitter release, neuronal excitation, neurite outgrowth, synaptogenesis, neuronal survival, differentiation, plasticity, and regulation of gene expression [1-3]. VGCCs have a molecular complex comprised of several subunits: α₁, α₂-δ, β, and γ [1]. The α₁ subunit is essential for channel functioning and determines fundamental channel properties [1]. The ~190 kDa pore-forming transmembrane α₁ subunit (~2000 amino acids), is organized in four homologous domains (I-IV), comprising six transmembrane α helices (S1–S6) and the pore-forming P loop between S5 and S6 [4]. Genes encoding 10 pore-forming α₁ as well as several splice variants have been identified and characterized [4]. Neuronal VGCCs include Cav2.1 and Cav2.2 channels and have predominantly expressions at presynaptic neuronal terminals throughout the central nervous systems [5]. Previous reports have showed that Cav2.1 and Cav2.2 are present at high concentrations in the hippocampus [5], which would be involved in memory formation.

To analyze memory mechanisms in rodents, animals usually learn rules through positive or negative reinforcement during the training session. On the other hand, human cognitive performance is not usually tested with reinforcers such as intense hunger. Y maze test is based on the spontaneous tendency of rodents to enter a novel arm more often than the other arms because of the innate tendency of mice to explore [6]. Thus, the Y maze test would be appropriate for memory formation.

Rolling Nagoya mice carrying a mutation in the α₁ subunit of the Cav2.1 channel (Cav2.1α¹) demonstrate severe ataxia after about 2 weeks of age [7,8]. In previous study, we showed cognitive alterations in heterozygous Rolling Nagoya (roll⁺) mice without apparent deficits in the Y maze test [9,10]. It has been thought that N-methyl-D-aspartate (NMDA)-dependent processes are involved in the mechanisms of working memory. The NMDA receptor (NMDAR) antagonists impaired spontaneous alternation of mice in the Y maze test [11-14]. Our previous reports have showed that systemic and intrahippocampal administration of NMDAR antagonist at a lower dose to roll⁺ mice would result in hippocampus-dependent cognitive dysfunction [10], suggesting that the importance of hippocampal Cav2.1/NMDAR signaling in spatial short-term memory.

It has been reported that glutamatergic system is one of the neurotransmitter systems regulated by Cav2.1 and Cav2.2 [15,16]. The role of different VGCCs in the neural circuits underlying spatial short-term memory has not been studied. In the present study, to examine the relationship between Cav2.2-mediated synaptic transmission and spatial short-term memory, we conducted the Y maze test using the mice treated with Cav2.2 inhibitor. The studies presented here demonstrate the importance of hippocampal Cav2.2-regulated signaling in spatial short-term memory.

Materials and methods

Mice

All animal procedures were approved by the Animal Experiments Committee of Shanghai Jiao Tong University and RIKEN. The C57BL/6J mice was provided by Charles River Japan (Kanagawa, Japan).
Results

Intracerebroventricular injection effects of ω-conotoxin GVIA on spatial short-term memory

We used four groups of male mice (n=10 each) given systemic injections of 0 (vehicle), 1, 5, or 10 pg/side ω-conotoxin GVIA. There were no significant differences among groups in the number of arm entries [F(3,36)=0.9, P>0.05] (Figure 1A). However, the groups significantly differed in the spontaneous alteration [F(3,36)=133.6, P<0.01] (Figure 1B). The mice given 5 or 10 pg/side ω-conotoxin GVIA showed fewer alterations than the mice given vehicle. These results shows that blockade of Cav2.2-mediated signaling impairs short-term memory.

Intrahippocampal injection effects of ω-conotoxin GVIA on spatial short-term memory

We used four groups of male mice (n=10 each) given intrahippocampal injections of 0 (vehicle), 1, 5, or 10 pg/side ω-conotoxin GVIA. There were no significant differences among groups in the number of arm entries [F(3,36)=0.9, P>0.05] (Figure 2A). However, the groups significantly differed in the spontaneous alteration [F(3,36)=129.5, P<0.01] (Figure 2B). The mice given 5 or 10 pg/side ω-conotoxin GVIA showed fewer alterations than the mice given vehicle. Figure 3 shows the representative infusion cannula placement in the dorsal hippocampus. These results shows that blockade of Cav2.2-mediated hippocampus dependent signaling impairs short-term memory.

Data analysis

Data are presented as means ± standard error on the mean (SEM). Statistical analyses for the behavioral tests were conducted using Excel Statistics 2006 (SSRI, Tokyo, Japan). Data were analyzed using repeated measures ANOVA with Tukey’s test.
Neuronal VGCCs including Cav2.1 and Cav2.2 channels mediates the presynaptic machinery for vesicular release of neurotransmitters. However, the role of different VGCCs in the neural circuits underlying spatial short-term memory has not been studied. In the present study, to examine the relationship between Cav2.2-mediated signaling in the hippocampus and short-term memory formation, we investigated the spontaneous alteration behavior using the wild-type mice received intracerebroventricular or intrahippocampal injection of Cav2.2 blocker (ω-conotoxin GVIA). It is generally accepted that ω-conotoxin GVIA binding site on the α1 subunit of the Cav2.2 channel (Cav2.2α1) has been mapped primarily to the external vestibule of the channel in the domain III pore-forming S5–S6 region [18,19].

We showed that intracerebroventricular injection of ω-conotoxin GVIA blocked the spontaneous alteration behavior, suggesting that the signal cascade activated by Cav2.2 plays a role in the spatial short-term memory. Because Cav2.2 channels are present at a variety of synapses [5], the systemic injection study is difficult to evaluate from the behavioral performance. Previous studies have demonstrated that the hippocampus is critical for spatial memory formation [20-22]. Intrahippocampal injection in the present study showed that ω-conotoxin GVIA impaired the spontaneous alternation behavior. These results indicate that Cav2.2-mediated signaling in the hippocampus is important in spatial memory formation.

Electrophysiological studies have showed that glutamate releases from presynaptic nerve terminals in hippocampal slices are elicited by Ca2+ influx through Cav2.1 and Cav2.2 [15]. Previous study has also indicated that the glutamatergic systems are both involved in cognitive processes [23]. It has been also reported that systemic and intrahippocampal administration of NMDAR antagonist showed cognitive dysfunction [10]. These results suggest the importance of hippocampal Cav2.2-mediated NMDA receptor signaling in spatial short-term memory. We have also showed that the combination study of different sub-threshold doses of agents and local infusions are useful for inducing the effects of short-term spatial learning and for identifying functional signaling pathways in the specifically neuronal circuits [10]. In the future study, the relationship between Cav2.2-mediated glutamatergic transmission in the hippocampus and spatial short-term memory would be investigated using the mice treated with both Cav2.2 inhibitor and NMDA receptor antagonist.

In conclusion, we found that Cav2.2 blocker disrupted the spontaneous alternation behavior in the Y maze test. Our results suggest that Cav2.2-mediated signaling in the hippocampus has an important role in spatial short-term memory.

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Authors’ contributions

The authors (W.L. and E.T.) designed and supervised the research, and wrote the manuscript. The authors (Y.Z. and K.N.) performed the surgeries and behavioral experiments. All authors read and approved the final version of the manuscript.

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