

Disruption of spatial cognition by intra-accumbens injection of Cav2.2 inhibitor

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Abstract

Cav2.1 and Cav2.2 channels have predominantly expressions at presynaptic neuronal terminals and mediate neurotransmitter release in the central nervous systems. Although it has been reported that spatial learning requires Cav2.1-regulated signaling in the nucleus accumbens (NAc), Cav2.2-regulated function remains unknown. In this study we examined whether Cav2.2-mediated signaling in NAc plays role in spontaneous alternation patterns of Y maze test. Mice received intra-accumbens injection of Cav2.2 blocker (ω -conotoxin GVIA, 5pg/side) showed impaired spontaneous alternation patterns. Our results indicate that Cav2.2-mediated signaling in the nucleus accumbens is also critical for spatial cognition.

Introduction

It has been reported that glutamatergic afferents from the hippocampus and prefrontal cortex provide the main source of information to the nucleus accumbens (NAc) during several types of cognitive activity [1-5]. Voltage gated calcium channels (VGCCs) controls neuronal circuits through a number of neuronal functions including neurotransmitter release, neuronal excitation, neurite outgrowth, synaptogenesis, neuronal survival, differentiation, plasticity, and regulation of gene expression [6-8]. Neuronal VGCCs include Cav2.1 (P/Q-type) and Cav2.2 (N-type) channels and have predominantly expressions at presynaptic neuronal terminals throughout the central nervous systems. It has been reported that glutamatergic system is one of the neurotransmitter systems regulated by Cav2.1 and Cav2.2 [9,10]. The role of different VGCCs in the neural circuits underlying spatial short-term memory has not been studied. An interesting question is whether there are differences in the effects observed after manipulation of different channel signaling. A study of different channel blockers and local infusions would be useful for inducing the effects of short-term spatial learning and for identifying functional signaling pathways in the specifically neuronal circuits.

We have shown that Cav2.1-regulated glutamatergic signaling in the hippocampus [11] and NAc [12] is important in short-term spatial learning. We have also demonstrated the importance of hippocampal Cav2.2-regulated signaling in spatial short-term memory [13]. Previous reports have showed that Cav2.1 and Cav2.2 are present at high concentrations in the hippocampus and NAc [14] involved in spatial short-term memory formation. Cav2.2 channels contribute at a variety of functional neuronal circuit systems [6,13]. These results indicate that Cav2.2 also plays an important role in the NAc. However, the physiological role of Cav2.2-regulated cognitive performance in the NAc has not been determined.

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, ω -conotoxin GVIA in the NAc. The studies presented here demonstrate the importance of Cav2.2-regulated cognitive signaling in the NAc.

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 were provided by Charles River Japan (Kanagawa, Japan). The mice were given free access to water and food pellets (CRF-1; Oriental Yeast Co. Ltd., Tokyo, Japan) and were housed under a 12/12-h light/dark cycle (lights on from 08:00 to 20:00) at $23 \pm 1^\circ\text{C}$ and $55 \pm 5\%$ humidity. Testing was performed during the light phase of the cycle. We used separate groups of male 2-month-old mice for each of the behavioral tests. All experiments were conducted blind to the treatment condition of the mouse.

Y maze test

Y maze test was conducted between 10:00 and 16:00 by a trained experimenter who was blind with regard to the mouse strains. The mice were moved into the behavioral testing room at least 2 h prior to testing. Y-maze apparatus consisted of three compartments (3 cm wide \times 40 cm long \times 25 cm high) radiating out from the center. Before

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infusion, the mice were placed in one of the compartments and allowed to move freely for 10 min. The light intensity in the experimental room was 35 lux. An arm entry was defined as three legs entering one of the arms, and the sequence of entries was manually recorded. Each mouse performed one trial. An alteration was defined as entry into all three arms in consecutive choices. The percentage of spontaneous alteration was calculated as (actual alteration/maximum alteration)×100. At the completion of the intrahippocampal injection experiments, frozen brain sections were examined under a light microscope. Mice with injection needle placements outside of the boundaries of targeted areas were excluded from behavioral analysis.

Infusion

For the infusion studies, Cav2.2 blocker, ω -conotoxin GVIA (10, 50, or 100 $\mu\text{g}/\mu\text{L}$, Peptide Institute, Osaka, Japan) were dissolved in saline (vehicle). Under anesthesia and using standard stereotaxic procedures, stainless-steel guide cannulae (22-gauge) were implanted into the NAc (anterior to bregma, +1.7 mm; lateral to midline, ± 1.0 mm; ventral from the dura, +2.3 mm). Mice were allowed to recover for at least 1 week following surgery. The drug doses were determined according to previous report [13,15]. Mice that were not treated with drugs received an equivalent volume of vehicle.

Histology

Histological verification of the cannula locations was performed at the end of behavioral testing. Mice were perfused transcardially with 0.9% saline, followed by 4% PFA. After extraction from the skull, the brains were postfixed in 4% PFA and then transferred to a 30% sucrose solution until sectioning. Coronal sections (40 μm thick, taken every 120 μm) were cut on a cryostat (-16°C) and mounted on glass microscope slides. After drying, the sections were stained with cresyl violet.

Data analysis

Data are presented as means \pm 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.

Results

To examine the effects of an intra-accumbens drug injection on spatial short-term memory in the Y-maze, we used four groups of male mice ($n=10$ each) given intra-accumbens injections of 0 (vehicle), 1, 5, or 10 $\mu\text{g}/\text{side}$ ω -conotoxin GVIA. There were no significant differences among groups in the number of arm entries [$F(3, 36)=1.2$, $P>0.05$] (Figure 1A). However, the groups significantly differed in the spontaneous alteration [$F(3, 36)=111.5$, $P<0.01$] (Figure 1B). The mice given 5 or 10 $\mu\text{g}/\text{side}$ ω -conotoxin GVIA showed fewer alterations than the mice given vehicle. Figure 2 shows the representative infusion cannula placement in the NAc. These results shows that blockade of Cav2.2-mediated NAc-dependent signaling impairs short-term memory.

Discussion

In previous study, we have shown that intra-cerebroventricular or intra-hippocampal injection of Cav2.1 blocker of levetiracetam blocked the spontaneous alteration behavior [11]. We have also shown that intra-cerebroventricular or intra-hippocampal injection of Cav2.2 blocker of ω -conotoxin GVIA blocked the spontaneous

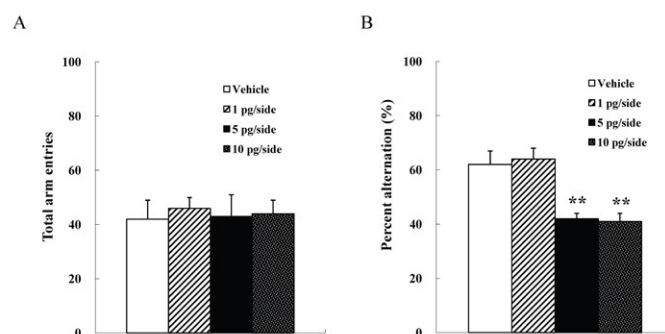


Figure 1. Effects of intra-accumbens injections of ω -conotoxin GVIA on the number of total arm entries (A) and spontaneous alteration (B) in the Y-maze test. The data are presented as the mean \pm standard error of the mean (SEM). ** $P<0.01$ compared with the appropriate control (Tukey's test).

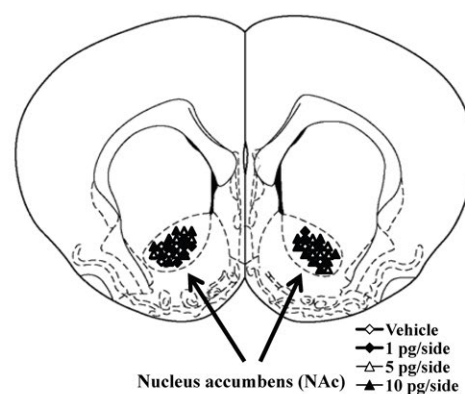


Figure 2. This coronal drawing shows the location of the ω -conotoxin GVIA injection aimed at the nucleus accumbens (NAc).

alteration behavior [13]. These results suggest that the hippocampal signal cascade activated by Cav2.1 and Cav2.2 plays a role in the spatial short-term memory. Neuronal VGCCs including Cav2.1 and Cav2.2 channels have predominantly expressions at high concentrations in the both hippocampus and NAc [14] involved in short-term memory formation [1-5]. Indeed, Cav2.1-regulated signaling in the NAc is also important in spatial memory formation [12]. However, the relationship between Cav2.2-mediated signaling in the NAc and short-term memory formation has not been studied. In this study, we examined whether intra-accumbens injection of ω -conotoxin GVIA disrupts the spontaneous alteration behavior.

The present study exhibited that mice received intra-accumbens injection of Cav2.2 blocker showed impaired spontaneous alteration patterns. Although we would need electrophysiological and biochemical studies to examine Ca^{2+} signaling through Cav2.2 in response to membrane depolarization and thus transduces electrical signals into chemical signals in the NAc, our results indicate that the hippocampus and NAc are important regions in spatial memory formation and that spatial information within the regions is processed by Cav2.2-regulated transmission.

NMDA receptor antagonists impair the spontaneous alteration of mice in the Y-maze test [16-19], while NMDA receptor agonists enhance performance in such memory tasks [11]. Additionally, local intra-accumbens administration of NMDA antagonists impairs the ability of mice to locate the hidden platform in a short-term version of the Morris water maze test [20]. These results suggest that NMDA

receptor signaling within the NAc is involved in short-term spatial memory. Electrophysiological studies have shown that Ca²⁺ currents through Cav2.1 are found in the isolated NAc [21]. Heterozygous rolling Nagoya (*roll*+) mice with a Cav2.1 α_1 mutation show normal Y-maze behavior [12]. In our previous study, although intra-accumbens injection of NMDA induced similar spontaneous alternations in wild-type and *roll*+/ mice, injections of NMDA receptor antagonist MK-801 (0.5 μ g/side) or Cav2.1 inhibitor levetiracetam (0.1 μ g/side) did not affect controls but decreased spatial cognition in *roll*+/ mice [12], suggesting that Cav2.1-mediated NMDA receptor signaling in the NAc is involved in short-term spatial learning and that combination of sub-threshold doses of pharmacological agents can be useful for inducing phenotypes in silent mutants and for identifying functional signaling pathways. It has been reported that glutamatergic system is one of the neurotransmitter systems regulated by Cav2.2 [9,10]. Thus, mice given microinfusions with Cav2.2 blocker and glutamatergic compounds into the NAc may be a useful model for delineating mechanisms in the spatial memory formation associated with Cav2.2-mediated NMDA receptor signaling.

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

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Authors' contributions

WL and ET designed and supervised the research, and wrote the manuscript. YZ and KN performed the surgeries and behavioral experiments. All authors read and approved the final version of the manuscript.

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