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Ganglioside GQ1b improves spatial learning and memory of rats as measured by the Y-maze and the Morris water maze tests

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ABSTRACT

Gangliosides are major components of cell membranes and are particularly enriched in the mammalian brain where they represent the major lipid constituents of the neuronal cell surface. In the central nervous system, gangliosides have a close connection to many neurophysiological functions related to neurogenesis, proliferation, synaptogenesis, and synaptic transmission. The previously reported effect of the tetra-sialoganglioside GQ1b in hippocampal CA1 neurons of brain slices showed that GQ1b enhanced ATP-induced long-term potentiation (LTP). However, there has been no clear evidence of the effects of GQ1b on learning and memory as measured using behavioral test. In the present study, we performed the Y-maze and the Morris water maze (MWM) tests to reveal the effects of GQ1b on spatial learning and memory following intracerebroventricular (ICV) injection of GQ1b. GQ1b-treated rats showed highly increased performance on the Y-maze and the MWM tests without any significant alteration of basal locomotor activity. Therefore, our behavioral data strongly suggest that GQ1b improves spatial learning and memory in rats. Also, these data support the previous finding that GQ1b treatment in hippocampal CA1 neurons of rodent brain slices increased ATP-induced LTP.

Gangliosides are components of most cell membranes, and they are particularly enriched in the brain where they are the major lipid constituents of the neuronal cell surface [16,18]. In the central nervous system, gangliosides have a close connection with many neurophysiological functions, such as neurogenesis, proliferation, synaptogenesis, and synaptic transmission [8,13,16].

Long-term potentiation (LTP) is a well-known phenomenon and is assumed to be responsible for the cellular mechanism of learning and memory processes [2]. In hippocampal CA1 neurons from rodent brains, treatment with the tetra-sialoganglioside GQ1b improved LTP, especially ATP-induced LTP in vitro [8,9]. The effects of GQ1b on ATP-induced LTP may involve the modulation of *N*-methyl-D-aspartate (NMDA) receptors by extracellular phosphorylation [9].

In cultured cortical neurons, the biosynthesis of gangliosides, including GQ1b, is inhibited by a ceramide analog, D-threo-1-phenyl-2-decanoylamino-3-morpholino-1-propranol (D-PDMP) and is stimulated by its L-enantiomer (L-PDMP) [10,12]. L-PDMP stimulates the formation of functional synapses in primary cultures of cortical neurons. The spatial memory performance of ischemic rats on the eight-arm radial maze is restored by L-PDMP. L-PDMP induces de novo synthesis of most b-series gangliosides such as GD3, GD2, GD1b, GT1b and GQ1b in neurons. Elevation of synaptic activity by L-PDMP is correlated with the stimulation of ganglioside biosynthesis through activating GM3, GD3 and especially GQ1b synthases [10]. When GM3, GM1, GD3, GD1b, GT1b, or GQ1b were added to cells depleted of glycosphingolipids by D-PDMP, only GQ1b was able to restore the decreased synaptic activity. Therefore, de novo synthesis of GQ1b is necessary for synapse formation and synaptic activity [12].

A two-trial Y-maze test has been developed to study recognition processes in rats [4,6]. Discrimination of novelty versus familiarity can then be studied by comparing exploring behavior in the three arms. This task does not involve the learning of a rule and thus enables specific testing of spatial working memory. Importantly, the influence of locomotor activity on memory performance is limited in this procedure since the dependent measure is principally based on the choice between a novel place and familiar places.

The Morris water maze (MWM) test is designed to measure spatial learning and memory performance. It is generally used today to investigate the role of the hippocampus in the formation of spatial memory [14].

There are several lines of evidence that indicate an effect of GQ1b on learning and memory [8,9,12]. Nevertheless, any behavioral effects of GQ1b on learning and memory performance have not yet been reported. Thus, in the present study, we investigated

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the effects of GQ1b on learning and memory using the Y-maze and MWM tests.

Twenty one male Sprague–Dawley rats (7-week old) were purchased from Orient Bio (Seoul, Korea) and acclimated for 1 week. Rats were housed four per cage and had free access to water and food until brain surgery was performed. Rats were maintained under standard conditions: 12 h light–dark cycle, 22 ± 2 °C. All procedures were performed following the Guide for the care and use of laboratory animals, published by the U.S. National Institutes of Health.

For the intracerebroventricular (ICV) injection of GQ1b, rats were surgically implanted with a CMA/12 guide cannula (CMA, Sweden). Anaesthetized (ketamine 56.04 mg/kg, and xylazine 7.05 mg/kg) rats were secured in a stereotaxic instrument (Stoelting, US) and implanted with the cannula secured 1.2 mm above the final target injection site as follows: AP: −0.4; ML: +1.5; V: −4.0 mm from the skull.

Following insertion of a guide cannula into the brain of each rat, all rats were housed one per cage and maintained under standard conditions to be stabilized for 1 week. GQ1b gangliosides (Alexis, Switzerland) were dissolved in artificial cerebrospinal fluid (aCSF; pH 7.4) before testing and kept on ice. GQ1b (1 μg in 2 μl of aCSF) or aCSF as a control was transferred into the rat brain by ICV injection. ICV injection was performed through a 28-gauge cannula. The cannula was attached via polyethylene tubing to a 1-ml Hamilton syringe with a CMA 7002 microdialysis pump (CMA, Sweden). One microgram of GQ1b was injected once prior to the Y-maze and MWM tests. The cannula was left in place for 30 s after infusion to allow diffusion and was then removed.

Rats were tested on the Y-maze after ICV injection of GQ1b. The Y-maze was constructed of black plexiglass with three arms. Visual cues were located outside the maze. The floor of the maze was covered with soiled animal bedding that was mixed between trials to reduce the utility of odor as a cue. The three arms of the maze were designated as the start arm, the other arm, and the novel arm (Fig. 1A).

The first trial was conducted 6 h following the ICV injection. For this trial rats were placed inside the start arm while the novel arm was blocked with a block. Therefore, rats were able to explore the start and other arms, but not the novel arm during a 15-min period. Six hours after trial 1, the block was removed, rats were placed in the start arm and they were allowed free access to all

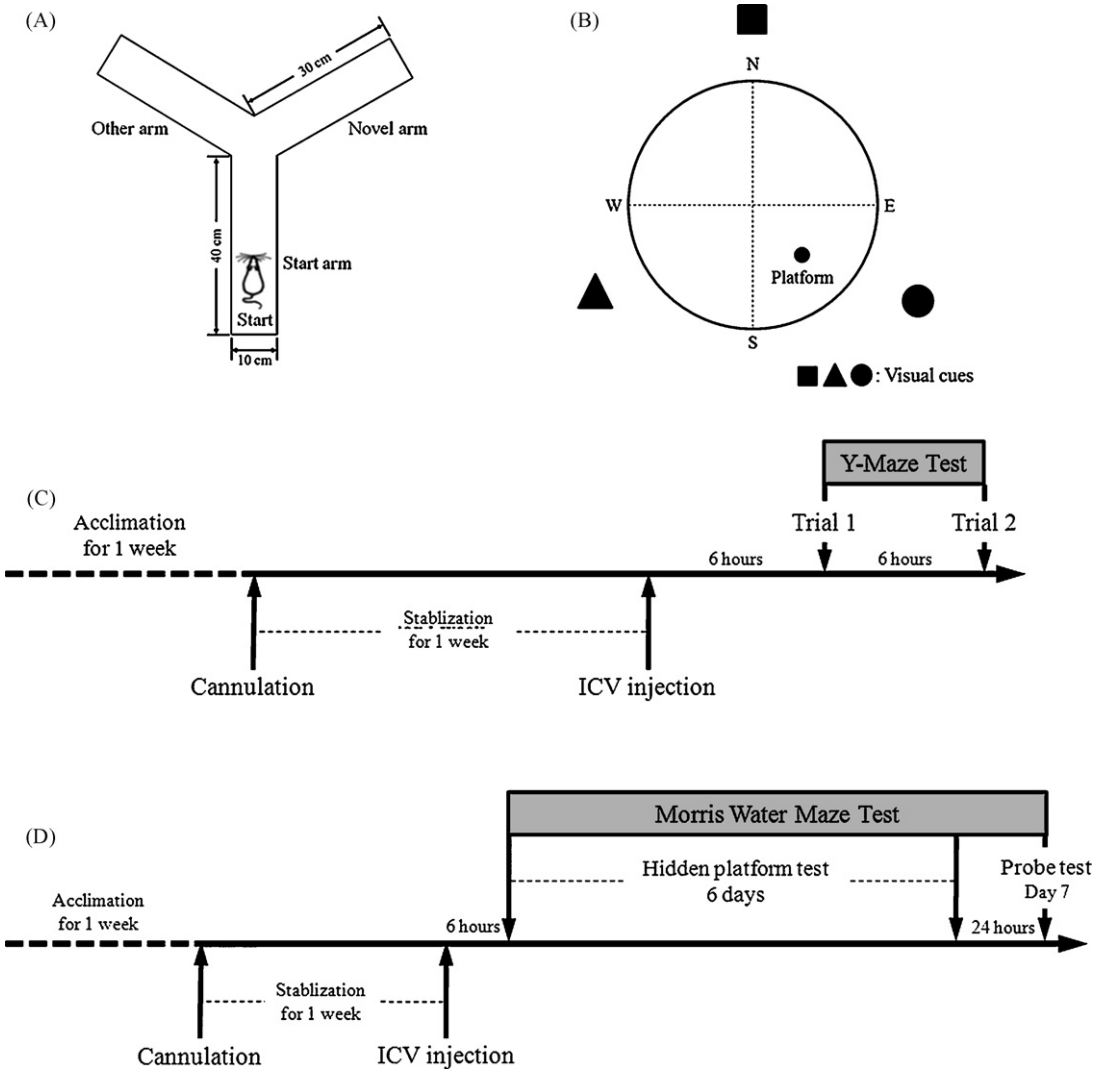

Fig. 1. A schematic diagram of the Y-maze test and the MWM test. The apparatus (A) and flowchart (C) of the Y-maze test were designed for assessing spatial memory. The novel arm of the Y-maze test was blocked in the first trial. During the second trial, rats were allowed free access to all three arms of Y-maze for 5 min. The apparatus of the MWM test was composed of a 180-cm diameter pool, a 10-cm diameter hidden platform, and extra-maze visual cues (B). Three training trials of the MWM test per day were conducted for six consecutive days (D). All behavioral tests were performed 6 h following a single ICV injection of GQ1b.