Available online at www.sciencedirect.com
SCIENCE DIRECT®
Neuroscience Research 49 (2004) 205–217
Neuroscience Research
www.elsevier.com/locate/neures

# Antisense knockdown of drebrin A, a dendritic spine protein, causes stronger preference, impaired pre-pulse inhibition, and an increased sensitivity to psychostimulant

Rika Kobayashi [a], Yuko Sekino [b], Tomoaki Shirao [b], Satoshi Tanaka [b], Taichi Ogura [a], Ken Inada [c], Makoto Saji [a,d,*]

[a] *Division of Brain Science, Kitasato University Graduate School of Medical Sciences, Sagamihara 228-8555, Japan*
[b] *Department of Neurobiology and Behavior, Gunma University School of Medicine, Maebashi 371-8511, Japan*
[c] *Department of Psychiatry, Kitasato University School of Medicine, Sagamihara 288-8555, Japan*
[d] *Department of Physiology, Kitasato University School of Allied Health Sciences, Sagamihara 288-8555, Japan*

Received 7 January 2004; accepted 25 February 2004

Available online 24 April 2004

**Abstract**

Drebrin located in dendritic spines regulates their morphological changes and plays a role in the synaptic plasticity via spine function. Reduced drebrin has been found in the brain of patients with Alzheimer's disease or Down's syndrome. To examine whether the down-regulation of drebrin protein levels causes deficits in higher brain function, such as memory or cognition, we performed antisense-induced knockdown of drebrin A expression in rat brain using an hemagglutinating virus of Japan (HVJ)-liposome gene transfer technique. We investigated the effects of drebrin in vivo knockdown on spatial memory in a water-maze task, sensorimotor gating in a pre-pulse-inhibition test, adaptive behaviors in an open-field test, and sensitivity to psychostimulant in an amphetamine-induced locomotor response. Rats with drebrin A in vivo knockdown displayed a stronger preference for a previous event due to perseverative behavior, impaired pre-pulse inhibition (PPI), increased locomotor activity, anxiety-like behavior, and an increased sensitivity to psychostimulant, suggesting behaviors related to schizophrenia. These findings indicated that decreased drebrin produces deficits in cognitive function but not in spatial memory, probably via hypofunction of dendritic spines.


*Keywords:* Actin-binding protein; Antisense oligodeoxynucleotides; HVJ-liposome-mediated gene transfer; Water-maze task; Locomotor activity; Cognitive function

## 1. Introduction

Many types of neurons have dendrites with small protrusions termed spines. In the cerebral cortex, about 75% of neurons have dendrites with a high density of spines, and most of these spiny neurons are excitatory (Keller, 2002). Furthermore, most of the excitatory synapses in the cortex make contact with the dendritic spines of other excitatory neurons (Marrs et al., 2001; Okabe et al., 2001). Since dendritic spines are specialized compartments that serve as the structural base for synaptic signaling, integration, and plasticity in mature brain, the ability of spines to alter their shape or length and the stability of synaptic contacts at spines provide a powerful mechanism for synaptic plasticity in the excitatory circuitry in the cerebral cortex and the limbic regions, such as the hippocampus and the amygdala (Engert and Bonhoeffer, 1999; Lendvai et al., 2000; Shirao and Sekino, 2001).

Drebrin A, an actin-binding protein, accumulates specifically in the dendritic spines (Hayashi et al., 1996; Shirao et al., 1987; Shirao, 1995). In recent in vitro studies, it has been demonstrated that drebrin A regulates changes in the shape and density of dendritic spines, probably via the rearrangement of cytoskeletal actin filaments (Hayashi and Shirao, 1999; Shirao et al., 1994). In particular, high expression of drebrin A is specifically observed in the cerebral cortex, hippocampus, amygdala, thalamus, and striatum (Hayashi et al., 1996; Shirao and Obata, 1986), which are responsible

* Corresponding author. Tel.: +81-427-78-8311; fax: +81-427-78-8153.
*E-mail address:* sajim@ahs.kitasato-u.ac.jp (M. Saji).

0168-0102/$ – see front matter 
doi:10.1016/j.neures.2004.02.014

for higher brain functions. Clinically, a marked decrease of drebrin A was found in brains with Alzheimer's disease (Harigaya et al., 1996; Hatanpaa et al., 1999) or Down's syndrome (Shim and Lubec, 2002). The synaptic plasticity of the excitatory circuitry underlying higher brain functions, such as learning, memory, or cognition is considered to involve a stimulus-dependent change of the shape and density of dendritic spines (Keller, 2002; Marrs et al., 2001; Okabe et al., 2001; Shirao and Sekino, 2001). Therefore, it is hypothesized that experimentally induced knockdown of drebrin A, dendritic spine protein, in the whole brain could cause some defects in memory formation, sensorimotor gating function, or cognitive function.

To examine whether the dysfunction or hypofunction of dendritic spine-mediated synaptic function in the excitatory circuitry in the forebrain causes defects in spatial reference memory, adaptive response to a novel environment, and sensorimotor gating, we performed antisense-induced down-regulation of drebrin A expression in the forebrain regions. In rats with long-lasting knockdown of drebrin A expression in the forebrain regions, such as the neocortex or hippocampus, we tested spatial learning using the Morris water-maze task, an adaptive response to a novel environment in an open-field test, psychostimulant sensitivity using an amphetamine-induced locomotor response, and sensorimotor gating function in the pre-pulse inhibition (PPI) test of the acoustic startle response, all of which are sensitive to anti-glutaminergic drugs and lesions of the hippocampus (Bakshi and Geyer, 1998; Carlsson and Carlsson, 1990; Ellison, 1995; Morris et al., 1982). To achieve a long-lasting in vivo knockdown of drebrin A expression by a single intra-ventricular injection of antisense oligodeoxynucleotides (ODNs), we chose to use a combination of the antisense technique and the hemagglutinating virus of Japan (HVJ)-liposome-mediated gene transfer method. HVJ-liposome-mediated gene transfer is a method that enables delivery of the contents (ODNs) of the HVJ-liposome vector directly into cells by means of the Sendai virus (HVJ)-cell fusion machinery (Inada et al., 2003; Iwakuma et al., 2003; Kobayashi et al., 2002; Saeki et al., 1997; Yamada et al., 1996). Down-regulation of drebrin A by the antisense ODNs we used in the present study has induced the attenuation of synaptic clustering of PSD-95 as well as clustering of drebrin and F-actin in cultured hippocampal neurons (Takahashi et al., 2003).

## 2. Materials and methods

### 2.1. Animal care

Ninety six naïve, 10-week-old, male Wistar rats weighing 230–280 g (Japan SLC, Kanagawa, Japan) were used in the present study. The animals were housed in clear plastic cages in groups of two or three and were allowed access to food and water throughout the experiment. The animals were maintained in a temperature-, humidity-, and light-controlled environment with a 12 h light/dark cycle. On arrival in the colony, the experimenter holds the animal gently by the body rubbing its head and back for about 5 min. Not to be in fear of the experimenter, this type of handling was performed once a day for several days or a week until the surgical operation for the intra-ventricular injection of HVJ-liposomes containing ODNs was conducted.

All experiments conformed to Japanese and international guidelines on the ethical use of animals, and every effort was made to minimize the number of animals and their suffering.

### 2.2. Oligodeoxynucleotides (ODNs)

Phosphotioated ODNs (24 mers) corresponding to a specific segment in the 5′-coding region of drebrin cDNA were designed to selectively deacrese byosynthesis of the drebrin A as antisense ODNs to drebrin A (Dre-AS: 5′-AGGA-AGGCCCACTGTCCGATGCCT-3′)(Takahashi et al., 2003; Tanaka et al., 2001). Reversed antisense ODNs (Dre-RE: 5′-TCCGTAGCCTGTCACCCGGAAGGA-3′), in which the bases of the 24 mers corresponding to Dre-AS were reversed, were synthesized for use as a control. A search of the Genebank/EMBL database determined that none of the ODN sequence overlapped with other mammalian sequences.

### 2.3. Preparation of HVJ-liposome containing ODNs

The detailed preparation of anionic HVJ-liposome containing ODNs has been described elsewhere (Iwakuma et al., 2003; Saeki et al., 1997; Yamada et al., 1996). Briefly, three kinds of lipids (phosphatidylserine, phosphatidylcholine, and cholesterol) dissolved in chloroform (1 mg/ml) were mixed in a weight ratio of 1:4.8:2. One milligram of the lipid mixture was transferred into a glass tube and dried as a thin lipid film using a rotary evaporator filled with nitrogen gas at 40 °C. The lipid thin film layering on the bottom of a glass tube was hydrated in 200 μl of a balanced salt solution (BSS: 137 mM NaCl, 5.4 mM KCl, 10 mM Tris–HCl, pH 7.5) containing 100 μg of ODNs which were dispersed in the aqueous phase at room temperature. The mixture of the hydrated lipid thin film and ODNs was agitated by vortexing for 30 s and then incubated at 37 °C for 30 s. This procedure was repeated eight times, making fragments of lipid thin film into liposomes in which ODNs were packaged up. The liposome suspension was sonicated for 20 s and vortexed for 30 s. Then 300 μl of BSS was added to the liposomes suspension and incubated at 37 °C for 30 min shaking. The liposome suspension prepared above (500 μl) was mixed with 1 ml HVJ suspension (more than 10,000 hemagglutinating units), whose RNA genome had been inactivated by ultraviolet irradiation (198 mJ/cm$^2$) just before use. The mixture was incubated at 4 °C for 10 min and at 37 °C for 1 h shaking to facilitate the fusion between the inactivated HVJ and the liposomes. The ODNs–HVJ–liposome complex was loaded onto a discontinuous sucrose gradient and

centrifuged at 25,000 rpm at 4 °C for 1.5 h to separate the ODNs–HVJ–liposome complex from free HVJ. The purified ODNs–HVJ–liposomes suspension was adjusted to OD 1.0 (540 nm) with 200–250 μl of BSS. Since 10–30% of ODNs (10–30 μg) are available for packing into the liposomes (Kaneda, 1999), it is estimated that 1 μl of the purified ODNs–HVJ–liposomes (OD 1.0) contains about 40–120 ng of ODNs.

### 2.4. Intra-ventricular injection of an HVJ-liposome vector containing ODNs

Rats were anesthetized with an intra-peritoneal injection of pentobarbital sodium (40 mg/kg). A total of 30 μl (1.2–3.6 μg ODNs) of HVJ-liposome vectors containing ODNs was stereotaxically injected into both sides of the lateral ventricle in the rats. The coordinates for intra-ventricular injection in mm in respect to ear bar were; (1) AP: 8.2; L: +1.4; D: 3.8 and (2) AP: 8.2; L: −1.4; D: 3.8. A glass micropipette (tip size: 30–40 μm; volume: 2.5 μl/mm) made from a disposable micropipette (20 μl, Drummond), which was connected to an air pressure system with polyethylene tubing, was used for a single intra-ventricular injection of the HVJ-liposome vectors containing ODNs (Iwakuma et al., 2003).

### 2.5. Western blot analysis

For quantification of the protein expression levels, Western blot analysis was used. Rats were anesthetized with diethyl ether and decapitated at various days after the intra-ventricular injection of HVJ-liposome vectors containing antisense ODNs or reversed antisense ODNs. The hippocampus, neocortex, cerebellum and thalamus were quickly dissected out of the removed brain and stored at −80 °C before use. The tissue (100 μg per 1 μl buffer) was sonicated in cold sample buffer containing 40% glycerol, 20% Tris-glycine, 2% sodium dodecylsulfate (SDS), 5% 2-mercaptoethanol until the homogenate was uniform. The homogenate was centrifuged at 15,000 rpm for 10 min at 4 °C and denatured for 3 min. The supernatant of 4 μl was loaded on 8% sodium dodecylsulfate–polyacrylamide gel (40 μg protein per lane) and separated by electrophoresis. Separated proteins were transferred electrophoretically from the gel to a polyvinylidine difluoride (PVDF) membrane (ATTO, Tokyo, Japan). After being blocked with blocking buffer (Block Ace, Dainihon Seiyaku, Japan) at 4 °C for 1 h for drebrin A or overnight for β-actin, the membrane was processed with the primary antibodies overnight at room temperature for more than 1 h. Two primary antibodies were used, a rabbit polyclonal antibody against drebrin A (1:1000, produced by Dr. T Shirao) (Shirao et al., 1994) and a monoclonal antibody against β-actin (1:3000, Abcom, UK). After being washed in PBS containing 0.1% Tween for 1 h, the membrane was incubated with the second antibody, peroxidase-conjugated goat anti-rabbit IgG (1:2000 for drebrin A, 1:10000 for β-actin, Leinco Technologies) at room temperature for 1 h. Then the membrane was processed with enhanced chemiluminescence reagents to visualize the antibody reaction using an ECL detection kit (Amersham International, UK) and finally exposed to X-ray film (Kodak). Western blots were analyzed with computer densitometory by using NIH image software for quantification of drebrin A protein levels. For the measurement of β-actin as control protein, the membrane was reprobed with monoclonal antibody against β-actin to confirm equal protein loading in each lane. The mean optical densities of bands for two samples per animal were determined, and the film background was subtracted. The band densities for drebrin A or β-actin protein in treated rats were represented as relative percentages of those in non-treated control rats. The mean band densities thus obtained were statistically analyzed with one-way ANOVA and post-hoc Fisher's test to determine the significance.

### 2.6. Open field test for spontaneous locomotor activity, adaptive behaviors and amphetamine-induced locomotor response

A rat was placed in the center of a white square box (width, 1.0 m; height, 0.4 m). The locomotor behavior of the rat was recorded by a video camera during the initial 30 min period following the start of spontaneous locomotion in the open field. In experiment for psychostimulant-sensitivity, the locomotor behavior of the rat was recorded during the total 90 min period that consists of the initial 30 min period (pre-injection) after the start of spontaneous locomotion and the following 60 min period (post-injection) of amphetamine-induced locomotor response by the intra-peritoneal administration of amphetamine (1.0 mg/kg). From the 30 min or 90 min recording of locomotor behavior, rat position was tracked by computer and the locomotor distance during every 5 min period was measured as a locomotor activity using computer-guided image software (Kurihara Medical Instrument, Tokyo).

As for typical behaviors in adaptive response to a novel environment, a total duration of being stationary, being exploratory or grooming during the 30 min test period was measured from the 30 min recording of behaviors following the start of spontaneous locomotion in an open field, respectively. Being exploratory means both horizontal locomotion and vertical locomotion including rearing, while being stationary is defined as a stationary state without grooming or rearing.

### 2.7. Prepulse inhibition test of acoustic startle response

A startle chamber with a floor equipped with an electric weighing machine and a speaker mounted 24 cm above the floor for presenting acoustic noise burst was used. Rats were placed in the startle chamber and allowed to acclimate for 5 min before the test session. Background noise

was set at 65 dB of white noise throughout the acclimation period and test session. In a test session, 10 trials of three types of acoustic stimulation (total 30 trials) were given in pseudo-random order after initial startle-stimuli (20 ms burst of 120 dB). One of the three types was a startle pulse alone (P alone) trail, which involved a 20 ms burst of 120 dB, and the other two types were a combined trial of prepulse and startle pulse (PP70 &P and PP80 &P), which involved a 20 ms burst of 70 dB or 80 dB as a prepulse, respectively, followed by the same pulse as in the P alone trial 100 ms later. The inter-trial intervals averaged 40 s (20–60 s) were given in pseudo-random order. In addition to these three types of trials, no-stimulus (non-stim) trials were inserted between trials to check the baseline amplitude without stimulation. The startle response was measured for 100 ms from the startle pulse presentation, and the average value was defined as the startle amplitude. The startle amplitudes in response to repetitions of each trial type were averaged over the session. The test schedule was controlled by a microcomputer.

The percent prepulse inhibition (% PPI) of a startle response was calculated by the following formula:

$$\%\text{PPI at PP80} = \left\{1 - \frac{\text{PP80 \&P}}{\text{P alone}}\right\} \times 100$$

$$\%\text{PPI at PP70} = \left\{1 - \frac{\text{PP70 \&P}}{\text{P alone}}\right\} \times 100$$

### 2.8. Water-maze task training for spatial reference memory

We used the Morris water-maze task to test for an acquisition of spatial reference memory. The apparatus used for the hidden platform task in water-maze consisted of a white circular pool (diameter, 1.5 m; height, 0.5 m) filled to a depth of 20 cm with clear water ($24 \pm 2\,^{\circ}$C) and a transparent plastic platform (diameter, 15 cm; height, 0.3 m) submerged 2 cm below the water surface. Rats were given 6 consecutive training trials (a session) per day with an inter-trial interval of 60 s, from random starting location, with a hidden platform in a fixed position. Training sessions were performed over 5 consecutive days (total 30 trials). For each trial, rats had a maximum of 60 s swim time to mount the platform and they were then allowed to rest on the platform for additional 15 s. Rats were placed on the platform for 15 s manually if they elapsed. Rat position was tracked by computer, and the swim time was measured as the escape latency some rats that stayed longer making circle along the periphery of the pool in the session 1 were eliminated from the further training sessions.

On the last day of training session, a spatial probe test was conducted 60 s after the end of the last training session. Rats were given a 60 s-probe trial in which the hidden platform had been removed from the pool. For each probe test rat position was tracked by computer and the time spent in each quadrant zone was measured as the place preference. Each quadrant zone was defined as follows: target, the quadrant in which the hidden platform had been positioned; opposite, the quadrant opposite to the target; right, the quadrant on the right side of the target; left, the quadrant on the left side of the target.

### 2.9. Statistics

All statistical analysis was carried out by one-way ANOVA. Post–hoc individual comparison of paired groups was carried out by using Fisher's PLSD or the Student's *t*–test. A value of $P < 0.05$ was considered to represent a significant difference.

## 3. Results

### 3.1. Antisense-induced down-regulation of drebrin A protein levels and its time course

We first used Western blot analysis with antibodies against drebrin A and β-actin to examine the regional differences in the levels of drebrin A protein expression in the forebrain and cerebellum. Fig. 1 shows representative blots and quantitation of relative band densities for drebrin A and β-actin obtained from the neocortex, cerebellum, hippocampus, and thalamus of non-treated control rats. As shown in Fig. 1, drebrin A protein expression was clearly rich in the neocortex, hippocampus, and thalamus but poor in the cerebellum, while β-actin protein levels were almost uniform in all four brain regions.

We then examined the antisense-induced change in the protein levels in the two drebrin-rich regions of the forebrain, the neocortex, and hippocampus, at 4, 8, and 18 days after the intra-ventricular injection of HVJ-liposome vectors containing Dre-AS or Dre-RE. The levels of drebrin A protein in the neocortex of the rats treated with Dre-AS decreased markedly at 4 days after injection, remained reduced for several days, and returned to the original level by 18 days post-injection, while the levels of drebrin A protein in the Dre-RE-treated rats remained unchanged at 4 days post-injection. On the other hand, in the neocortex of the Dre-AS-treated rats and Dre-RE-treated rats, the β-actin protein levels did not change at any time post-injection (Fig. 2A). As shown in the quantitative analysis for drebrin A protein levels (Fig. 2B), a significant reduction of $53.7 \pm 13.2\%$ (mean ± S.E.M., $n = 7$, 4 days post-injection) or $44.7 \pm 12.1\%$ (mean ± S.E.M., $n = 7$, 8 days post-injection) in drebrin A protein level was specifically found in the neocortex of the Dre-AS-treated rats and was sustained for more than a week, while the Dre-RE treatment did not affect the drebrin A protein levels. As shown in the quantitative analysis for β-actin protein levels (Fig. 2C), a significant change of the β-actin protein levels was not found in the neocortex of the rats treated with Dre-AS or Dre-RE at any time post-injection.

The amount of drebrin A in the hippocampus of the rats treated with Dre-AS decreased markedly at 4 days after

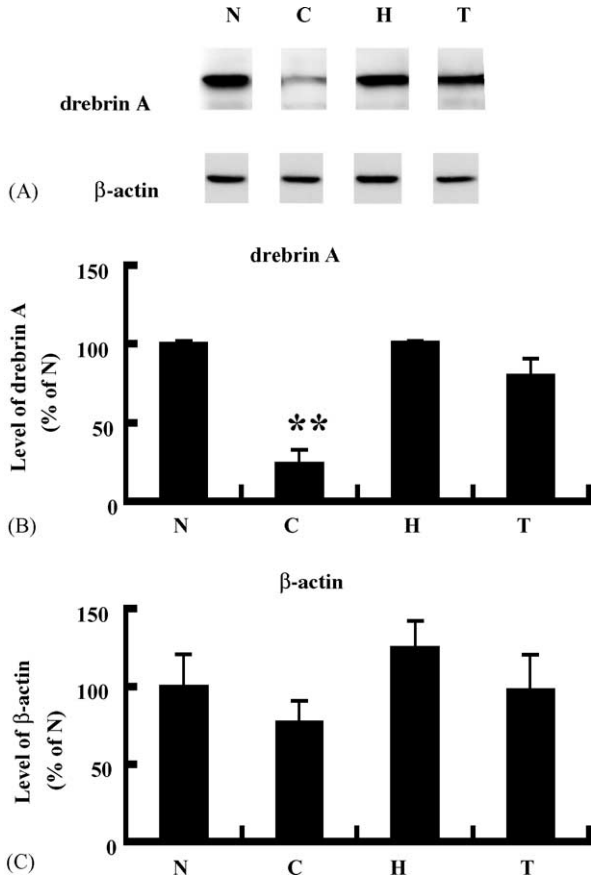


Fig. 1. Regional difference in the level of drebrin A protein expression in the brain of non-treated control rat. (A), Representative blots for drebrin A and β-actin obtained from the neocortex (N), cerebellum (C), hippocampus (H) and thalamus (T). Four μl of the homogenate (40 μg protein per lane) was loaded on 8% SDS gel for separation by electrophoresis. For measurement of β-actin as control protein, the membrane used for drebrin A was reprobed with monoclonal antibody against β-actin to confirm equal protein loading in each lane. (B–C) Quantitative analysis of relative band densities for drebrin A protein (B) and that for β-actin protein (C) obtained from the four brain structures. Level of drebrin A and β-actin protein in the four brain structures was represented as a percentage of that in the neocortex (N). Each value is an average of relative abundance of drebrin A protein or β-actin protein obtained from four non-treated rats ($n = 4$, mean ± S.E.M.). **Significantly different from the other three forebrain structures ($P < 0.01$).

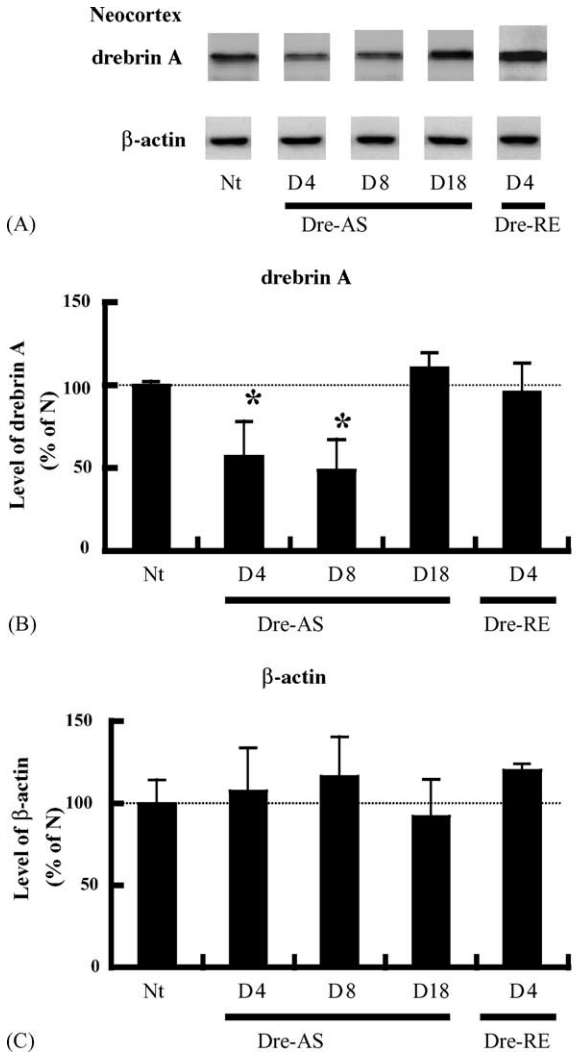


Fig. 2. Antisense-induced down-regulation of drebrin A protein expression in the neocortex and its time course following a single intra-ventricular injection of antisense ODNs to drebrin A by using HVJ-liposome mediated gene transfer method. Each treatment group: Nt, non-treated control rats; Dre-AS, rats that received an intra-ventricular injection of HVJ-liposome vectors containing antisense ODNs to drebrin A; Dre-RE, rats that received an intra-ventricular injection of HVJ-liposome vectors containing reversed antisense ODNs to drebrin A. (A) Representative blots for drebrin A and β-actin obtained from the neocortex of rats in each treatment group at 4 days (D4), 8 days (D8) or 18 days (D18) after an intra-ventricular injection of HVJ-liposome vectors containing ODNs. (B–C) Quantitative analysis of relative band densities for drebrin A protein (B) and β-actin protein (C) from the neocortex of rats in each treatment group. The relative density of each band was expressed as a percentage of the averaged density of bands in the Nt. Each value is an average of relative abundance of drebrin A or β-actin protein obtained from seven rats in each treatment group ($n = 7$, mean ± S.E.M.). *Significantly different from the Dre-RE (D4) ($P < 0.05$), and from the Nt and Dre-AS (D18) ($P < 0.01$).

injection, remained reduced for several days, and returned to the original level by 18 days post-injection, while the drebrin A protein levels in the Dre-RE-treated rats remained unchanged at 4 days post-injection. On the other hand, in the hippocampus of the Dre-AS-treated rats and Dre-RE-treated rats, the β-actin protein levels did not change at any time post-injection (Fig. 3A). As shown in the quantitative analysis for drebrin A protein levels (Fig. 3B), a significant reduction of 40.5 ± 8.9% (mean ± S.E.M., $n = 7$, 4 days post-injection) or 28.7 ± 11.8% (mean ± S.E.M., $n = 7$, 8 days post-injection) in drebrin A protein was specifically found in the hippocampus of the Dre-AS-treated rats and was sustained for more than a week, while the Dre-RE treatment did not affect the drebrin A protein levels. As shown in the quantitative analysis for β-actin protein levels (Fig. 3C), no significant change of the β-actin protein levels was found in the hippocampus of rats treated with Dre-AS or Dre-RE at any time post-injection.

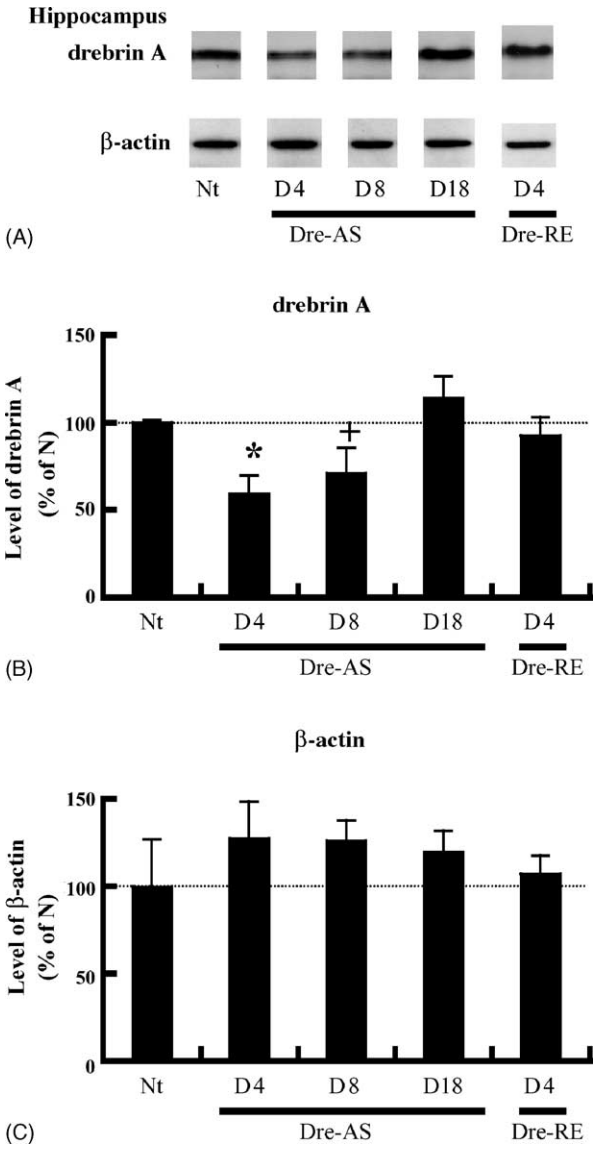


Fig. 3. Antisense-induced down-regulation of drebrin A protein expression in the hippocampus and its time course following a single intra-ventricular injection of antisense ODNs to drebrin A by using HVJ-liposome mediated gene transfer method. Each treatment group: Nt, non-treated control rats; Dre-AS, rats that received an intra-ventricular injection of HVJ-liposome vectors containing antisense ODNs to drebrin A; Dre-RE, rats that received an intra-ventricular injection of HVJ-liposome vectors containing reversed antisense ODNs to drebrin A. (A) Representative blots for drebrin A and β-actin obtained from the hippocampus of rats in each treatment group at 4 days (D4), 8 days (D8) or 18 days (D18) after an intra-ventricular injection of HVJ-liposome vectors containing ODNs. (B–C) Quantitative analysis of relative band densities for drebrin A protein (B) and β-actin protein (C) from the hippocampus of rats in each treatment group. The relative density of each band was expressed as a percentage of the averaged density of bands in the Nt. Each value is an average of relative abundance of drebrin A or β-actin protein obtained from seven rats in each treatment group ($n = 7$, mean ± S.E.M.). (+) Significantly different from the Nt ($P < 0.05$), and from the Dre-AS (D18) ($P < 0.01$). *Significantly different from the Dre-RE(D4) ($P < 0.05$), and from the Nt and Dre-AS (D18) ($P < 0.01$).

### *3.2. Increased spontaneous locomotion and abnormal adaptive behaviors in response to a novel environment in rats with drebrin A in vivo knockdown*

To investigate the effects of antisense in vivo knockdown of drebrin A expression on adaptive behaviors in response to a novel environment, we recorded spontaneous locomotion and typical adaptive behaviors during a 30 min period in an open field test in rats that had received an intra-ventricular injection of HVJ-liposome vectors containing Dre-AS or Dre-RE 4 days prior to the open field test. The spontaneous locomotor activity was estimated by measuring the distance of locomotion during every 5 min period in the open field test of 30 min. On the other hand, the degree of typical adaptive behaviors, such as being exploratory, being stationary, or grooming in an open field, was estimated by measuring the total duration of being exploratory, being stationary, or grooming during the 30 min test period, respectively. The inserted figure in Fig. 4A shows the locomotor activity estimated by the total distance of spontaneous locomotion during the 30 min test period. As shown in the inserted figure, the locomotor activity in the Dre-AS-treated rats ($n = 9$) was significantly higher than that in the control Dre-RE-treated rats ($n = 6$). The time course of locomotor activity during the 30 min test period indicates that the significant increase of locomotor activity in the Dre-AS-treated rats is seen only in the earliest 5 min period during the 30 min open field test (Fig. 4A). As shown in Fig. 4B, the Dre-AS-treated rats ($n = 6$) were significantly less stationary and exhibited more grooming than the control Dre-RE-treated rats ($n = 5$).

### *3.3. Alterations in amphetamine-induced locomotor response in rats with drebrin A in vivo knockdown*

To test whether abnormality of dopamine-related cortical functioning, which is believed to underlie some mental illnesses, is involved in behavioral alterations by the drebrin in vivo knockdown, we assessed the sensitivity to psychostimulant of the Dre-AS-treated rats using amphetamine-induced locomotor response. We measured locomotor activity in an open field during the 30 min period before amphetamine-injection (pre-injection phase) and the 60 min period after amphetamine-injection (post-injection phase) in rats that had received an intra-ventricular administration of HVJ-liposome vectors containing Dre-AS or Dre-RE 4 days prior to the amphetamine-induced locomotion test. The locomotor activity was estimated by measuring the locomotion during every 5 min period obtained from the total 90 min recording of locomotion in the open field. As shown in the post-injection phase in Fig. 5, a marked increase of the amphetamine-induced locomotor response was observed in the Dre-AS-treated rats. This increase by the Dre-AS treatment suggests that the Dre-AS-treated rats exhibit enhancement in the sensitivity to psychostimulant, compared with the sensitivity of the control Dre-RE-treated rats. It is notable that the degree of this increase of the

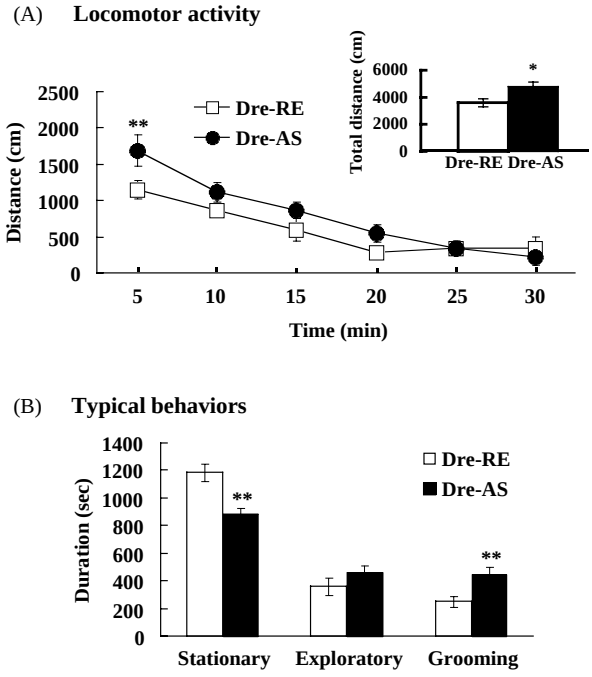


Fig. 4. Behavioral abnormalities in an open field test observed in rats with antisense in vivo knockdown of drebrin A expression. Treatment groups were: Dre-AS, rats that received a single intra-ventricular injection of HVJ-liposome vectors containing antisense ODNs to drebrin A 4 days prior to the open field test; Dre-RE, rats that received a single intra-ventricular injection of HVJ-liposome vectors containing reversed antisense ODNs to drebrinA 4 days prior to the open field test. The Dre-RE group was used as a control for the Dre-AS. (A) Increased locomotor activity in the Dre-AS rats during the open field test. Time-dependent change of spontaneous locomotor activity was estimated by locomotor distance during every 5 min following the start of locomotion in the open field. The inserted figure indicates the total locomotor distance during the 30 min test period. (B) Abnormal behavioral manifestations observed in the Dre-AS rats. Three typical behaviors that are characteristic of adaptive response to a novel environment: being stationary, being exploratory and grooming. Total duration of each behavioral manifestation was measured from the 30 min recording of behaviors in the open field by a chronometer, respectively. Each value represents mean ± S.E.M. ($n = 5$). *Significantly different from the Dre-RE ($P < 0.05$). **Significantly different from the Dre-RE ($P < 0.01$).

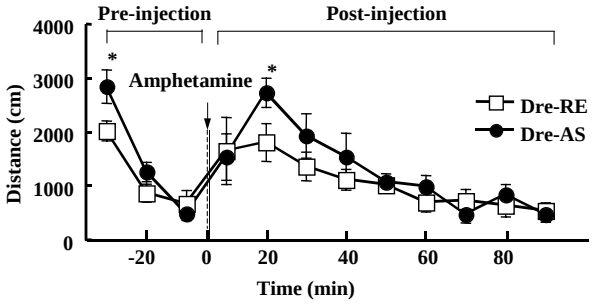


Fig. 5. Increased sensitivity to psychostimulant in an amphetamine-induced locomotor response observed in rats with antisense in vivo knockdown of drebrin A expression. Time-dependent change of locomotor activity before and after the intra-peritoneal injection of amphetamine was estimated by locomotor distance for every 5 min during the 30 min pre-injection and 60 min post-injection period. Treatment groups were: Dre-AS, rats that received a single intra-ventricular injection of HVJ-liposome vectors containing antisense ODNs to drebrin A 4 days prior to the amphetamine-induced locomotion test; Dre-RE, rats that received a single intra-ventricular injection of HVJ-liposome vectors containing reversed antisense ODNs to drebrinA 4 days prior to the amphetamine-induced locomotion test. The Dre-RE group was used as a control for the Dre-AS. Each value represents mean ± S.E.M. ($n = 6$). *Significantly different from the Dre-RE ($P < 0.05$).

amphetamine-induced locomotor response by the Dre-AS treatment and its recovery process are quite similar to that of the antisense-induced increase of the spontaneous locomotion in response to a novel environment, which is shown in the pre-injection phase in Fig. 5.

### 3.4. Reduction of pre-pulse inhibition and its recovery in rats with drebrin A in vivo knockdown

To examine whether the drebrin A in vivo knockdown causes a defect in the cognitive function, we used a PPI test of acoustic startle response. The PPI test was performed 4 and 18 days after the Dre-AS or Dre-RE treatment. The averaged startle amplitudes are shown in Table 1. The results from the PPI test performed 4 days after the treatment showed a significant disruption of PPI upon stimulation at PP70 but no significant disturbance of PPI at PP80 even

Table 1
Acoustic startle amplitudes in three types of stimulus trial and baseline amplitude in non-stimulus trial

| Experiment | Treatment | Amplitudes in stimulus trial | | | |
|---|---|---|---|---|---|
| | | B alone | P alone | PP70 | PP80 |
| Day 4 | Dre-RE | 5.2 ± 0.2 | 101.1 ± 15.1 | 40.5 ± 7.2 | 16.4 ± 1.7 |
| | Dre-AS | 4.7 ± 0.2 | 117.1 ± 20.9 | 71.0 ± 15.8 | 25.2 ± 4.9 |
| Day 18 | Dre-RE | 5.0 ± 0.1 | 114.9 ± 23.9 | 46.2 ± 5.2 | 29.7 ± 7.2 |
| | Dre-AS | 6.3 ± 0.4 | 96.6 ± 4.2 | 45.0 ± 5.2 | 21.0 ± 1.5 |

Type of stimulus trial: B alone, non-stimulus trial to check the baseline amplitude without stimulation; P alone, stimulus trial with startle pulse alone; PP70, stimulus trial with the combination of startle pulse and 70 dB prepulse; PP80, stimulus trial with the combination of startle pulse and 80 dB prepulse. Experiment: Day 4, PPI test performed 4 days after the treatment with Dre-RE or Dre-AS; Day 18, PPI test performed 18 days after the treatment with Dre-RE or Dre-AS. Treatment group: Dre-RE, rats with intra-ventricular injection of reversed antisense ODNs to drebrin (Dre-RE); Dre-AS, rats with intra-ventricular injection of antisense ODNs to drebrin (Dre-AS). Each value represents mean ± S.E.M. ($n = 9$ for Day 4, $n = 6$ for Day 18).

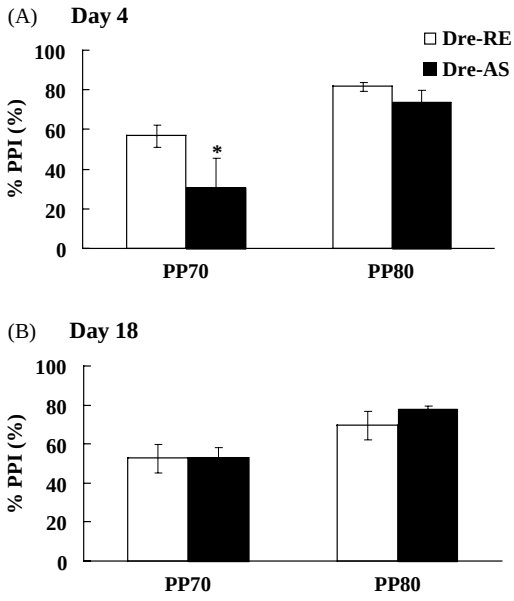


Fig. 6. Deficit of PPI of acoustic startle response and its recovery observed in rats with antisense in vivo knockdown of drebrin A expression. Treatment groups were: Dre-AS, rats that received a single intra-ventricular injection of HVJ-liposome vectors containing antisense ODNs to drebrin A 4 days or 18 days prior to the PPI test; Dre-RE, rats that received a single intra-ventricular injection of HVJ-liposome vectors containing reversed antisense ODNs to drebrinA 4 days or 18 days prior to the PPI test. The Dre-RE group was used as a control for the Dre-AS. The PPI was performed under two types of stimulation referred as PP70 and PP80. PP70 is an acoustic stimulation consisted of prepulse (20 ms burst of 70 dB) and startle-pulse (20 ms burst of 120 dB), while PP80 is that consisted of prepulse (20 ms burst of 80 dB) and startle-pulse (20 ms burst of 120 dB). (A) Percent prepulse inhibition in the Dre-AS or the Dre-RE 4 days after the ODNs-treatment. The rats treated with Dre-AS exhibited a significant disruption of PPI at the stimulation of PP70, but no disturbance of PPI at PP80 even though a slight tendency toward a reduced PPI. Each value represents mean ± S.E.M. ($n = 9$). *Significantly different from the Dre-RE ($P < 0.05$). (B) %PPI in the Dre-AS or the Dre-RE 18 days after the ODNs-treatment, when the down-regulated drebrin A expression has completely recovered to the original levels. The rats with Dre-AS treatment did not exhibit disruption of PPI at either PP70 or PP80, suggesting a recovery from the antisense-induced PPI deficit. Each value represents mean ± S.E.M. ($n = 6$).

though a slight tendency toward a reduced PPI was apparent compared with the control Dre-RE-treated rats (Fig. 6A). On the other hand, 18 days after the Dre-AS treatment, when the down-regulated drebrin A protein expression had completely recovered to the original levels, the Dre-AS-treated rats did not exhibit disruption of PPI at either PP70 or PP80 (Fig. 6B), suggesting a recovery from the antisense-induced PPI deficit.

### *3.5. Abnormality in the water-maze test in rats with drebrin A in vivo knockdown*

To examine whether the antisense-induced down-regulation of drebrin A protein expression in the forebrain regions, such as the neocortex or hippocampus, causes an alteration in the acquisition of spatial reference memory, we used the Morris water-maze test. The rats received an intra-ventricular injection of HVJ-liposome vectors containing Dre-AS or Dre-RE 4 days prior to undergoing training in a water-maze. All of the rats in the treatment groups learned to swim directly to a hidden platform in five training sessions well before training was completed, as indicated in Fig. 7A. Compared with the rats treated with Dre-RE as a control group, a significant reduction in the escape latency was observed only in the earliest training session in the rats treated with Dre-AS.

On the last day of training, a spatial probe test was conducted just after the final training session. As shown in Fig. 7B, both Dre-AS-treated rats and control Dre-RE-treated rats spent more time in the target quadrant than in any other quadrant. However, surprisingly, the preference for the target quadrant observed in the Dre-AS-treated rats was significantly stronger than that in the control Dre-RE-treated rats, implicating that better spatial memory was acquired by the Dre-AS treatment.

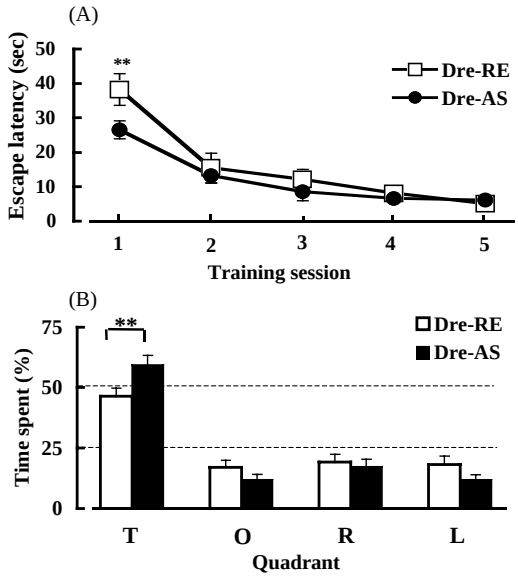


Fig. 7. Better memory in the hidden platform water-maze observed in rats with antisense in vivo knockdown of drebrin A. Treatment groups were: Dre-AS ($n = 9$), rats that received a single intra-ventricular injection of HVJ-liposome vectors containing antisense ODNs to drebrin A 4 days prior to the water-maze training; Dre-RE ($n = 6$), rats that received an intra-ventricular injection of HVJ-liposome vectors containing reversed antisense ODNs to drebrin A 4 days prior to the water-maze training. The Dre-RE group was used as a control for the Dre-AS. (A) Escape latency in water-maze training. Each value represents the averaged escape latency for 6 trials in each session (mean ± S.E.M.). The Dre-AS-treated rats displayed the greater learning ability during the first session in water-maze training than that of the Dre-RE. (B) Place preference in the spatial probe test conducted just after the end of training sessions. The Dre-AS-treated rats exhibited stronger preference for the target quadrant than that of the Dre-RE, suggesting that the Dre-AS-treated rats acquired better spatial memory. Quadrant was: T, target quadrant where the hidden platform was previously located; R, right quadrant; L, left quadrant; O, quadrant opposite to the target. **Significantly different from the Dre-RE ($P < 0.01$).

To clarify why the stronger preference for the target quadrant in Dre-AS-treated rats seems abnormal compared with that of non-treated control rats or Dre-RE-treated rats, we examined the time course of the place preference during 60 s probe trial. The upper panel of Fig. 8A shows a representative swimming track of a control Dre-RE-treated rat during the whole 60 s period of the probe test and its three divided tracks. The lower panel of Fig. 8A shows the place preference during every 20 s period following the start of the 60 s probe trial observed in control Dre-RE-treated

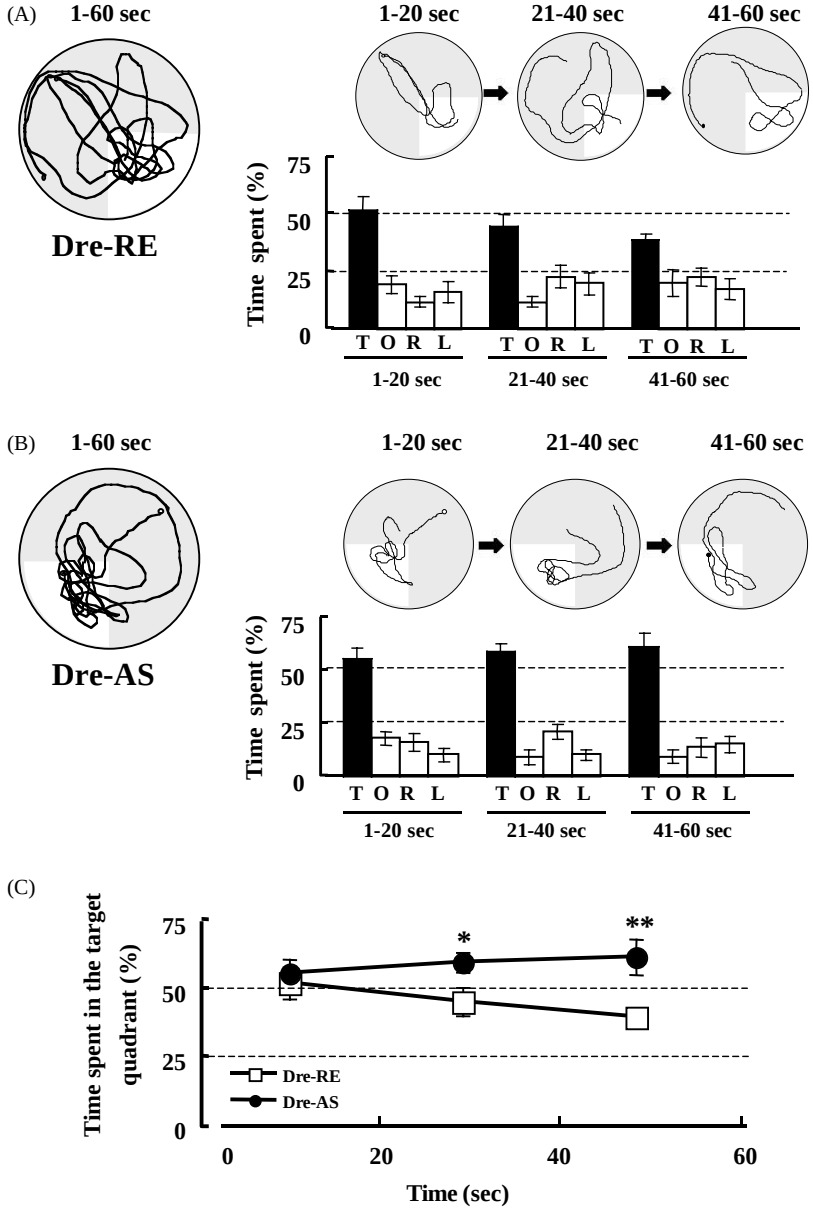


Fig. 8. Stronger preference for the target quadrant during the 60 s probe trial observed in rats with antisense in vivo knockdown of drebrin A. Treatment groups were: Dre-AS ($n = 9$), rats that received a single intra-ventricular injection of HVJ-liposome vectors containing antisense ODNs to drebrin A 4 days prior to the water-maze training; Dre-RE ($n = 6$), rats that received an intra-ventricular injection of HVJ-liposome vectors containing reversed antisense ODNs to drebrin A 4 days prior to the water-maze training. The Dre-RE group was used as a control for the Dre-AS. (A) Upper panel, representative swimming track of the control Dre-RE-treated rat during the 60 s period of the probe test and its three divided tracks; Lower panel, place preference during every 20 s period following the start of the 60 s probe trial observed in the Dre-RE-treated rats. The strong preference for the target quadrant in the Dre-RE rats declined within 60 s period of the probe trial. (B) Upper panel, representative swimming track of the Dre-AS-treated rat during the 60 s period of the probe test and those during every 20 s period following the start of a 60 s probe trial; Lower panel, place preference during every 20 s period following the start of the 60 s probe trial observed in the Dre-AS-treated rats. The strong preference for the target quadrant slightly increased within 60 s period of the probe trial. Unshadowed zone in the circle indicated the target quadrant, where the dense swimming track was depicted. (C) Time dependency of the strong preference for the target quadrant during 60 s period of the probe trial. In contrast to a decline in the strong preference for the target quadrant found in the Dre-RE-treated rats, the Dre-AS rats exhibited an abnormal persistence in the strong preference for the target quadrant. **Significantly different from the Dre-RE ($P < 0.01$). *Significantly different from the Dre-RE ($P < 0.05$).

rats ($n = 9$). As shown in the lower panel of Fig. 8A, the Dre-RE-treated rats as a control group exhibited a strong preference for the target quadrant zone in every 20 s period of the 60 s probe trial, but the level of preference for the target quadrant declined. This 60 s spatial probe test is originally designed to confirm an accomplishment of spatial learning by selection of a place preference in the water-maze without a hidden platform; that is, the same 60 s probe trial is not hypothesized to involve an additional behavioral response to the disappearance of the hidden platform task, which constitutes a problem that involves matching to sample. However, the decline in the preference for the target quadrant within the 60 s test period observed in the control rats suggests that behaviors in the 60 s spatial-probe test involve an adaptive response to a change of situation (namely, the disappearance of the hidden platform in the water-maze task), which is displayed by starting the searching behavior in quadrants other than the target quadrant in the water-maze. On the other hand, the upper panel of Fig. 8B shows a representative swimming track of the Dre-AS-treated rat during 60 s period of the probe test and its divided three tracks. The lower panel of Fig. 8B shows place preference during every 20 s period following the start of a 60 s probe observed in the Dre-AS-treated rats ($n = 9$). As shown in lower panel of Fig. 8B, the Dre-AS-treated rats exhibited a strong preference for the target quadrant in every 20 s period of the 60 s probe trial, and their preference for the target quadrant was sustained at the same level or slightly increased. As indicated in Fig. 8C, in contrast to the rapid decline that the control Dre-RE-treated rats exhibited in the preference for the target quadrant, the Dre-AS-treated rats displayed a rather slight increase in the level of their preference for the target quadrant within the 60 s probe trial. Thus, this abnormal persistence in the strong preference for the target quadrant zone in 60 s probe trial (Fig. 8C) suggests that the better spatial memory of the Dre-AS rats observed in Fig. 7B results from a cognitive deficit or worse judgment, which is revealed in their inability to inhibit inappropriate response to novel environment (namely, the disappearance of the hidden platform in the water-maze task).

## 4. Discussion

### 4.1. Antisense in vivo knockdown of drebrin A expression

We describe here the development of a rat with antisense-induced down-regulation of drebrin A protein levels in the drebrin-rich forebrain regions, such as the neocortex or hippocampus. The down-regulation of drebrin A protein levels that expressed 45–55% in the neocortex and 30–40% in the hippocampus was sustained for more than a week. The blocking effect of this intra-ventricular injection of antisense ODNs by using the HVJ-liposome method on the biosynthesis of drebrin A protein and the long-lasting action of antisense ODNs are equal to those of a repeated application or chronic infusion of antisense ODNs to NMDA-NR1 (Zapata et al., 1997). In a previous study, it was demonstrated that the Dre-AS we used here prevented the biosynthesis of drebrin A protein by about 48% in cultured cortical neurons (Takahashi et al., 2003; Tanaka et al., 2001). The present rate (45–55% in the cortex and 30–40% in the hippocampus) of an in vivo knockdown of drebrin A induced by the intra-ventricular administration of HVJ-liposome vectors containing Dre-AS was consistent with the rate of the in vitro knockdown of drebrin A induced by De-AS. In addition, since the transfection efficiency in vivo of HVJ-liposomes containing a plasmid DNA of constitutive nitric oxide synthase (c-NOS) has been reported to be 40–50% (Kaneda, 1999), the rate of an antisense in vivo knockdown of drebrin A by using HVJ-liposome vectors suggests that the biosynthesis of drebrin A in the transfected neurons is almost completely prevented by the long-lasting action of antisense ODNs, which are introduced into neurons bypassing the lysosomal attack. Thus, the degree of in vivo knockdown of drebrin A by a single intra-ventricular infusion of HVJ-liposome containing Dre-AS and its long-lasting action are sufficient to allow behavioral analysis for investigating the role of some specific proteins in higher brain functions without the stressful implantation of a cannula for repetitive or chronic administration.

### 4.2. Behavioral alterations induced by a drebrin A in vivo knockdown

Drebrin A in vivo knockdown rats display increased locomotor activity, a decreased stationary state, and enhanced grooming as adaptive behaviors in an open field and marked increase in an amphetamine-induced locomotor response (see Figs. 4 and 5). It could be considered that the decreased stationary state simply reflects the increase of locomotor activity due to unchanged locomotor velocity. Both increased locomotor activity in the earliest session and enhanced grooming in the open field test are a typical anxiety-like behavior for rodents as an adaptive response to a novel environment. It is commonly believed that changes in dopaminergic tone are highly related to alterations in locomotor activity (Amara and Kuhar, 1993; Giros et al., 1996), which explains the amphetamine-induced locomotor response. Therefore, the increase in amphetamine-induced locomotor response in the Dre-AS knockown rats, indicating their hypersensitivity, is thought to be an outcome of the increased sensitivity to psychostimulant in the drebrin knockdown rats. In addition to alteration in an emotional state, such as anxiety, alterations in locomotor behavior due to an increased sensitivity to psychostimulant have also been correlated with a positive symptom of schizophrenia (Corbett et al., 1995; Malhotra et al., 1997). Thus, an antisense-induced in vivo knockdown of drebrin A expression results in a number of altered behaviors that have been

traditionally characterized as modeling behaviors associated with schizophrenia. Indeed, mice treated with NMDA receptor antagonist PCP or MK-801, which have been regarded as animal models of schizophrenia, display an increase in both motor activity and sensitivity to psychostimulant (Abi-Saab et al., 1998; Duncan et al., 1999; Ellison, 1995; Javitt and Zukin, 1991; Malhotra et al., 1997).

In this study, we demonstrated that drebrin A knockdown rats display impaired PPI of the acoustic startle response at the stimulation type of PP70. Among a wide available range (65–85 dB) of pre-pulse intensity in acoustic stimulation, the pre-pulse intensity optimal to reveal the dysfunction of sensorimotor gating has been dependent on the types of treatment or disorder (Braff and Geyer, 1990; Braff et al., 2001; Inada et al., 2003). Thus, the present result indicates that the stimulation type of PP70 was more optimal than that of 80 dB for revealing PPI deficit in drebrin A knockdown rats. As shown in Fig. 6, the recovery from the deficit of PPI at PP70 in antisense-treated rats was observed at 18 days after the antisense treatment, when the down-regulated expression of drebrin A protein recovered to the original level. This recovery from the PPI deficit suggests that the disruption of PPI is dependent on the antisense-induced down-regulation of drebrin A in the forebrain such as the neocortex or hippocampus. It has been considered that the PPI reflects the function of the sensorimotor gating system, which partly overlaps with the cognitive function (Bakshi and Geyer, 1998; Braff and Geyer, 1990; Braff et al., 1992; Braff et al., 2001; Geyer, 1998), and that the disruption of PPI provides one of the modeling behaviors observed in schizophrenia, as schizophrenic patients display a significant impairment of PPI (Braff and Geyer, 1990; Braff et al., 1992; Braff et al., 2001; Geyer, 1998; Inada et al., 2003). Recently, it has been reported that an injection of non-competitive NMDA receptor antagonist into the dorsal hippocampus or amygdala produces the disruption of PPI in normal rats, suggesting that hypofunction of excitatory synapses in the multiple limbic regions mediates the PPI deficit (Bakshi and Geyer, 1998). Therefore, it is likely that the PPI deficit found in the drebrin in vivo knockdown rats results from dysfunction of excitatory synapses via antisense-induced modulation of dendritic spine function in the multiple limbic regions, such as the dorsal hippocampus or amygdala.

In the present study, global processes associated with spatial memory were impaired in drebrin A knockdown rats. In the spatial probe test, the preference for the target quadrant was stronger in the Dre-AS rats, suggesting that better memory was acquired by the Dre-AS treatment (see Fig. 7B). However, analysis of the perseverative behavior in the spatial probe test suggested that the stronger preference for the previous target in a novel environment observed in the Dre-AS rats (see Fig. 8) might rather result from a cognitive deficit or worse judgment than better memory, by which these rats are unable to inhibit inappropriate response or inattentive behavior to the sudden disappearance of the target. Another interpretation on the better spatial memory is that the abnormally strong preference for the previous target in the probe test may be due to a deficit in the naturally occurring extinction of memory. However, this interpretation is nearly the same as the cognitive deficit or worse judgment because the extinction of memory will occur when the animal is able to recognize being in any situation different from that memory event. Thus, the “better memory” observed in drebrin knockdown rats should be replaced by the term of “stronger preference” for previous experience due to cognitive deficit leading to perseverative tendencies. This behavioral abnormality in the water-maze resembles the cognitive dysmetria of the schizophrenic symptoms in the inability to receive and process information rapidly that has been associated with dysfunction of prefrontal–thalamic–cerebellar circuitry (Andreasen et al., 1996; Hecker et al., 1998; Wiser et al., 1998).

Because the swimming velocity of the Dre-AS rats in the first session was equal to that of the Dre-RE controls (data not shown), the reduced escape latency observed in the first session in the analysis of spatial memory formation seems to indicate that the drebrin A knockdown rats might have enhanced learning ability (see Fig. 7A). However, since the escape latency in the earliest training session depends largely on an animal’s adaptive behaviors in a different situation from the previous event, this behavioral alteration may result from changes in the emotional or cognitive state rather than from increased learning ability. One possible interpretation of the shorter escape latency is also the stronger preference for the previous experience; that is, for the early trials when a maximum of 60 s swim time elapsed, rats were placed on the platform manually for 15 s, in an effort to force them to learn the hidden-platform task. Since most of the rats tested experienced this forced learning at least once, the stronger preference for the previous experience in the Dre-AS rats could make the escape latency shorter in the earlier session.

Due to the coincidence between several abnormal behaviors in drebrin in vivo knockdown rats and schizophrenia-related behaviors in rodents, there has been considerable interest in identifying the relationship between schizophrenic symptoms and dysfunction of excitatory synapses via antisense modulation of the drebrin–mediated dendritic spine function. The brain structures that have been correlated with the positive symptoms of schizophrenia, such as the prefrontal cortex, striatum, thalamus, dorsal hippocampus, or amygdala, largely overlap with the brain regions that have been characterized by high expression of dendritic spine protein drebrin A (see Fig. 1) (Hayashi et al., 1996) and the abundance of spiny neurons. Therefore, the combination of impaired adaptive behaviors, increased sensitivity to psychostimulant, and impaired PPI with stronger preference due to perseverative behavior observed in drebrin in vivo knockdown rats may provide a good animal model of schizophrenia. Further study is needed to demonstrate other schizophrenia-related behaviors, such as behavioral abnormalities in a forced-swim test or the negative symptoms of defective social interaction.

## Acknowledgements

This study was supported by the Health Science Research Grants (no. H12-brain-018) from the Ministry of Health, Labor and Welfare, Japan. This investigation was also supported in part by the Grant for Scientific Research from Kitasato University Graduate School of Medical Science, Japan. We would like to thank Mr. Sakaguchi of Kitasato University for his kind help in the data analysis of water-maze test.

## References

Abi-Saab, W.M., D'Souza, D.C., Moghaddam, B., Krystal, J.H., 1998. The NMDA antagonist model or schizophrenia: promise and pitfalls. Pharmacopsychiatry 31 (Suppl 2), 104–109.

Amara, S.G., Kuhar, M.J., 1993. Neurotransmitter transporters: recent progress. Annu. Rev. Neurosci. 16, 73–93.

Andreasen, N.C., O'Learly, D.S., Cizadlo, T., Arndt, S., Rezai, K., Ponto, L.L., Watkins, G.L., Hichwa, R.D., 1996. Schizophrenia and cognitive dysmetria: a positron-emission tomography study of dysfunctional prefrontal–thalamic–cerebellar circuitry. Proc. Natl. Acad. Sci. U.S.A. 93, 9985–9990.

Bakshi, V.P., Geyer, M.A., 1998. Multiple limbic regions mediate the disruption of prepulse inhibition produced in rats by the noncompetitive NMDA antagonist dizocilpine. J. Neurosci. 18, 8394–8401.

Braff, D.L., Geyer, M.A., 1990. Sensorimotor gating and schizophrenia: human and animal model studies. Arch. Gen. Psychiatry 47, 181–188.

Braff, D.L., Grillon, C., Geyer, M.A., 1992. Gating and habituation of the startle reflex in schizophrenic patients. Arch. Gen. Psychiatry 49, 206–215.

Braff, D.L., Geyer, M.A., Light, G.A., Sprock, J., Perry, W., Cadenhead, K.S., Swerdlow, N.R., 2001. Impact of prepulse characteristics on the detection of sensorimotor gating deficits in schizophrenia. Schizophr. Res. 49, 171–178.

Carlsson, M., Carlsson, A., 1990. Interaction between glutamatergic and monoaminergic systems within the basal ganglia: implications for schizophrenia and Parkinson's disease. Trends Neurosci. 13, 272–276.

Corbett, R., Camacho, F., Woods, A.T., Kerman, L.L., Fishkin, R.J., Brooks, K., Dunn, R.W., 1995. Antisychotic agents antagonize non-competitive *N*-methyl-D-aspartate antagonist-induced behaviors. Psychopharmacology (Berlin) 120, 67–74.

Duncan, G.E., Zorn, S., Lieberman, J.A., 1999. Mechanisms of typical and atypical antipsychotic drug action in relation to dopamine and NMDA receptor hypofunction hypotheses of schizophrenia. Mol. Psychiatry 4, 418–428.

Ellison, G., 1995. The *N*-methyl-D-aspartate antagonists phencyclidine, ketamine, dizoclipine as both behavioral and anatomical models of the dementias. Brain Res. Brain Res. Rev. 20, 250–267.

Engert, F., Bonhoeffer, T., 1999. Dendritic spine changes associated with hippocampal long-term synaptic plasticity. Nature 399, 66–70.

Geyer, M.A., 1998. Behavioral studies of hallucinohenic drugs in animals: implications for schizophrenia research, Pharmacopsychiatry Suppl. 2, 73–79.

Giros, B., Jaber, M., Jones, S.R., Wightman, R.M., Caron, M.G., 1996. Hyperlocomotion and indifference to cocaine and amphetamine in mice lacking the dopamine transporter. Nature 379, 606–612.

Harigaya, Y., Shoji, M., Shirao, T., Hirai, S., 1996. Disappearance of actin-binding protein drebrin, from hippocampal synapses in Alzheimer's disease. J. Neurosci. Res. 43, 87–92.

Hatanpaa, K., Isaacs, K.R., Shirao, T., Brady, D.R., Rapoport, S.I., 1999. Loss of proteins regulating synaptic plasticity in normal aging of the human brain and in Alzheimer disease. J. Neuropathol. Exp. Neurol. 58, 637–643.

Hayashi, K., Ishikawa, R., Ye, L.H., Takata, K., Kohama, K., Shirao, T., 1996. Modulatory role of drebrin in the cytoskeleton within dendritic spines in the rat cerebral cortex. J. Neurosci. 16, 7161–7170.

Hayashi, K., Shirao, T., 1999. Change in the shape of dendritic spines caused by overexpression of drebrin in cultured cortical neurons. J. Neurosci. 19, 3918–3925.

Hecker, S., Rauch, S.L., Goff, D., Savage, C.R., Schacter, D.L., Fischman, A.J., Alpert, N.M., 1998. Impaired recruitment of the hippocampus during conscious recollection in schizophrenia. Nat. Neurosci. 1, 318–322.

Inada, K., Ishigooka, J., Anzai, T., Suzuki, E., Miyaoka, H., Saji, M., 2003. Antisense hippocampal knockdown of NMDA-NR1 by HVJ-liposome vector induces deficit of prepulse inhibition but not of spatial memory. Neurosci. Res. 45, 473–481.

Iwakuma, M., Anzai, T., Kobayashi, S., Ogata, M., Kaneda, Y., Ohno, K., Saji, M., 2003. Antisense in vivo knockdown of synaptotagmin I and synapsin I by HVJ-liposome mediated gene transfer modulates ischemic injury of hippocampus in opposing ways. Neurosci. Res. 45, 285–296.

Javitt, D.C., Zukin, S.R., 1991. Recent advances in the phencyclidine model of Schizophrenia. Am. J. Psychiatry 148, 1301–1308.

Kaneda, Y., 1999. Development of a novel fusogenic viral liposome system (HVJ-liposomes) and its applications to the treament of acquired diseases. Mol. Memb. Biol. 16, 119–122.

Keller, A., 2002. Use-dependent inhibition of dendritic spines. Trends Neurosci. 25, 541–544.

Kobayashi, S., Ohno, K., Iwakuma, M., Kaneda, Y., Saji, M., 2002. Synaptotagmin I hypothalamic knockdown prevents amygdaloid seizure-induced damage of hippocampal neurons but not of entorhinal neurons. Neurosci. Res. 44, 455–465.

Lendvai, B., Stern, E.A., Chen, B., Svoboda, K., 2000. Experience-dependent plasticity of dendritic spines in the developing rat barrel cortex in vivo. Nature 404, 876–881.

Malhotra, A.K., Pinals, D.A., Adler, C.M., Elman, I., Clifton, A., Pickar, D., Breier, A., 1997. Ketamine-induced exacerbation of psychotic symptoms and cognitive impairment in neuoleptic-free schizophrenics. Neuropsychopharmacology 17, 141–150.

Marrs, G.S., Steven, H.G., Dailey, M.E., 2001. Rapid formation and remodeling of postsynaptic dendrites in developing dendrites. Nat. Neurosci. 4, 1006–1013.

Morris, R.G., Garrud, P., Rawlins, J.N.P., O'Keef, J., 1982. Place navigation impaired in rats with hippocampal lesions. Nature 297, 681–683.

Okabe, S., Miwa, A., Okado, H., 2001. Spine formation and correlated assembly of presynaptic and postsynaptic molecules. J. Neurosci. 21, 6105–6114.

Saeki, Y., Matsumoto, N., Nakano, Y., Mori, M., Awai, K., Kaneda, Y., 1997. Development and characterization of cationic liposomes conjugated with HVJ (Sendai virus): reciprocal effect of cationic lipid for in vitro and in vivo gene transfer. Hum. Gene Ther. 8, 2133–2144.

Shim, K.S., Lubec, G., 2002. Drebrin, a dendritic spine protein, is manifold decreased in brains of patients with Alzheimer's disease and Down syndrome. Neurosci. Lett. 324, 209–212.

Shirao, T., Obata, K., 1986. Immunochemical homology of 3 developmentally regulated brain proteins and their developmental change in neuronal distribution. Brain Res. 394, 233–244.

Shirao, T., Inoue, H.K., Kano, Y., Obata, K., 1987. Localization of a developmentally regulated neuron-specific protein S54 in dendrites as revealed by immunoelectron microscopy. Brain Res. 413, 374–378.

Shirao, T., Hayashi, K., Ishikawa, R., Isa, K., Asada, H., Ikeda, K., Uyemura, K., 1994. Formation of thick curving bundles of actin by drebrin A expressed in fibroblasts. Exp. Cell Res. 215, 145–153.

Shirao, T., 1995. The roles of microfilament-associated proteins, drebrins, in brain morphogenesis: a review. J. Biochem. (Tokyo) 117, 231–236.

Shirao, T., Sekino, Y., 2001. Clustering and anchoring mechanisms of molecular constituents of postsynaptic scaffolds in dendritic spines. Neurosci. Res. 40, 1–7.

Takahashi, H., Sekino, Y., Tanaka, S., Mizui, T., Kishi, S., Shirao, T., 2003. Drebrin-dependent actin clustering in dendritic filopodia governs synaptic targeting of postsynaptic density-95 and dendritic spine morphogenesis. J. Neurosci. 23, 6586–6595.

Tanaka S., Sekino Y., Shirao, T., 2001. Change of dendritic spine length induced by drebrin A knockdown using antisense oligonucleotides in vitro. Soc. Neurosci. Abstr. 29.

Wiser, A.K., Andreasen, N.C., O'Learly, D.S., Watkins, G.L., Boles Ponto, L.L., Hichwa, R.D., 1998. Dysfunctional cortico-cerebellur circuits cause 'cognitive dysmetria' in schizophrenia. Neuroreport 9, 1895–1899.

Yamada, K., Moriguchi, A., Morishita, R., Aoki, M., Mikami, H., Oshima, T., Ninomiya, M., Kaneda, Y., Higaki, J., Ogihara, T., 1996. Efficient oligonucleotide delivery using the HVJ-liposome method in the central nervous system. Am. J. Physiol. 271, R1212–R1220.

Zapata, A., Capdevilla, J.L., Tarrason, G., Martinez, J.M., Piulats, J., Trulla, R., 1997. Effects of NMDA-R1 antisense oligonucleotides administration: behavioral and radioligand binding studies. Brain Res. 745, 114–120.