Diacylglycerol kinase β knockout mice with impairment of spine conformation show an abnormal response on psychostimulant-induced behavioral change

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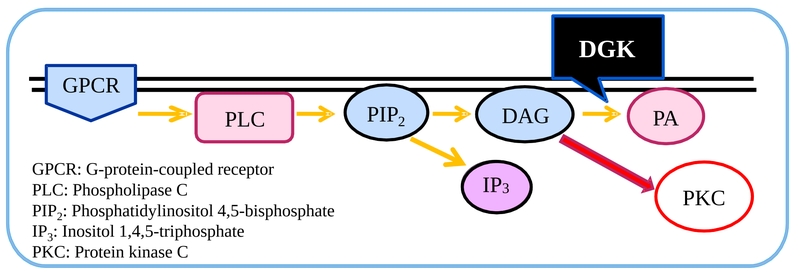
Objective

DGKβ

Diacylglycerol kinase (DGK) phosphorylates diacylglycerol (DAG) to produce phosphatidic acid (PA). DGKβ is widely distributed in the central nervous system, such regions as the olfactory bulb, cerebral cortex, striatum and hippocampus.



1. **DGKβ expression rapidly increases after 14 days of age, which is coincident with the synapse formation in the brain** (1).
2. **In bipolar disorder patients, DGKβ protein displays a COOH-terminal truncation downstream of the catalytic domain** (2).

Previously, we generated DGKβ KO mouse (3) and investigated they exhibited lithium-sensitive behavioral changes.

Attention-deficit hyperactivity disorder (ADHD)

ADHD is a disease characterized by hyperactive motor movements, impulsivity and inability to pay attention to what is important. As a drug treatment, methylphenidate (MPD) is an commonly used drug. MPD has a paradoxically effect on activity. That is, for normal person MPD shows locomotor-promorting (psychostimulant) effect. In contrast, MPD antagonize hyperactivity for ADHD patient. However, the detailed mechanism of such a paradoxically effect is still unknown.

Methods

1. Cognitive function and spine conformation

1-1. Y maze test

Each mouse was placed at the end of one arm and allowed to move freely during an 8 min session. The sequence of arm entries was recorded manually. The alternations ratio was calculated as
(actual alternations/maximum alternations) × 100.

1-2. Morris water maze tesr

Mice were placed in the water facing the wall and trained with 4 trials per day for 5 days. Twenty-four hours after the last training trial, the mice were given a prove test without the platform. In this test, each mouse was placed in the pool once and allowed to search for 60 s.

1-3. Electrophysiology

Electrophysiology analysis was performed as described previously (4).

1-4. Golgi staining

Each sample was further immersed in 30% sucrose for 2–3 days. The tissue block was placed in 2% potassium dichromate for 2 days at 4°C and then in 2% silver nitrate solution for 2 days at 4 °C in the dark. The block was cut into 60 µm thick sliced into distilled water. Finally, the sections were mounted onto slides, dried for 10 min, and dehydrated through 95% alcohol, 100% alcohol, clear in xylene.

1-5. Primary culture of mouse hippocampal neurons

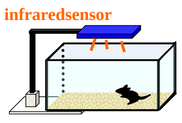
Fetuses were removed on embryonic 17–18 days. Hippocampi were dissected and placed in Ca^{2+}- and Mg^{2+}-free HEPES-buffered Hanks salt solution at pH 7.45. Primary culturing of hippocampal neurons was carried out using Nerve Cell Culture System. After culturing for 3, 10, or 15 days, adenoviruses NSE-tTA, TetOp-GFP, or TetOP-GFP-DGKβ were applied to a dish culturing hippocampal neurons. After 1 hr incubation, the medium was washed well and cultured for a further 48 hr. After fixation with 4% PFA and 0.2% picric acid at 4 °C and washing with PBS-T, the fluorescence of GFP was monitored under confocal microscopy.

1-6. Cell culture and transfection to SH-SY5Y cells

SH-SY5Y cells were cultured in DMEM/F-12 medium supplemented with 10% fetal bovine serum, penicillin (100 units/ml), and streptomycin (100 µg/ml). All cells were cultured at 37 °C in a humidified atmosphere containing 5% CO_2. The fetal bovine serum used was not heat inactivated. SH-SY5Y cells (1 × 105) were plated onto a glass-bottom culture dish and then transfected with 2 µg of plasmid encoding GFP or GFP-DGKβ on the following day by lipofection using FuGene 6 according to the manufacturer's protocol. After culturing for 48 hr, the cells were fixed with 4% paraformaldehyde (PFA) and 0.2% picric acid for 1 hr at 4 °C, and observed using confocal microscopy.

2. ADHD like behaviors

2-1. Locomotor activity test

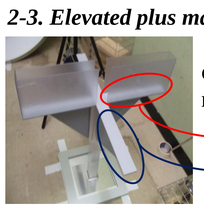


A mouse was placed in a transparent plastic cage (175 × 245 × 125 mm) with a sawdust bedding on floor. Locomotion was measured every hour for 1 day using digital counter with infrared sensor (NS-ASS01; Neuroscience, Inc, Tokyo).

2-2. Open field test

Each mouse was placed in the periphery of the open field apparatus for 2 hr. The total distance mice walked was recorded using EthoVision XT system (Noldus, Wageningen, The Netherlands). The number of scratching behavior was manually counted for the first 10 min of test session in a blind manner by a single observer.

2-3. Elevated plus maze test



Each mouse was placed in the central platform, facing one of the open arms. During a 10 min test session, mouse behavior was recorded using EthoVision XT.

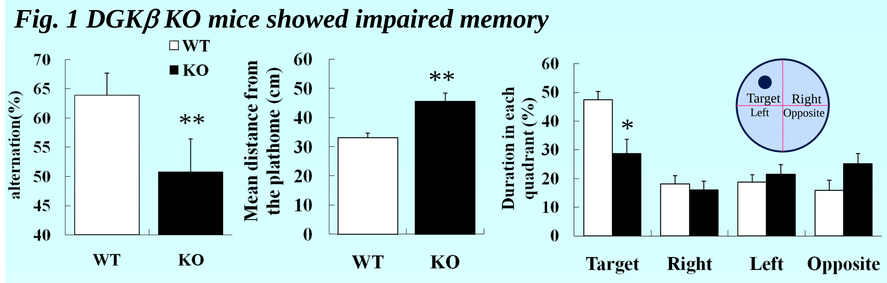
The number of entries into the each arm, the time spent on the open arms, and number of falling were scored.

2-4. Psychostimulant-induced hyperactivity

Each mouse was placed in the periphery of the open field apparatus and left for 2 hours. After 30 min habituation, each mouse was administered methylphenidate (30 mg/kg, dissolved in saline, i.p.), MK-801 (0.3 mg/kg, dissolved in saline, i.p.) or vehicle and continuously monitored their locomotor for 90 min. Five minutes after drug administration, we then removed mouse brain and separated it into striatum subsection for a sample of Western blot analysis.

Cognitive function and spine conformation

Fig. 1 DGKβ KO mice showed impaired memory



Each column and bar represent mean ± S.E.M.(n =8-9), *; p < 0.05, **;p < 0.01, vs.WT

Fig. 2 DGKβ KO mice showed impairment of LTP

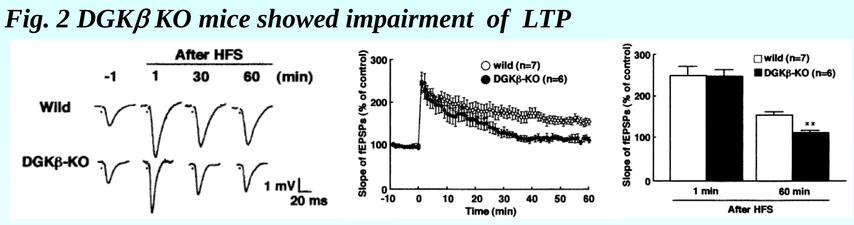
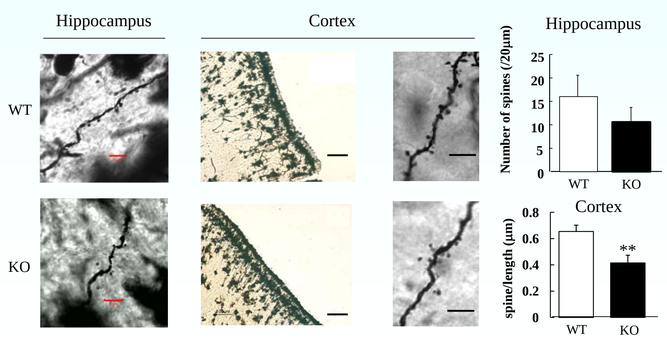


Fig. 3 Synapse formation of DGKβ KO and WT mice



Each column and bar represent mean ± S.E.M.(n =10 or 11), *; p < 0.05, **;p < 0.01, vs.WT

Fig.4 Overexpression of DGKβ rescued the neuronal deficit of DGKβ KO mice

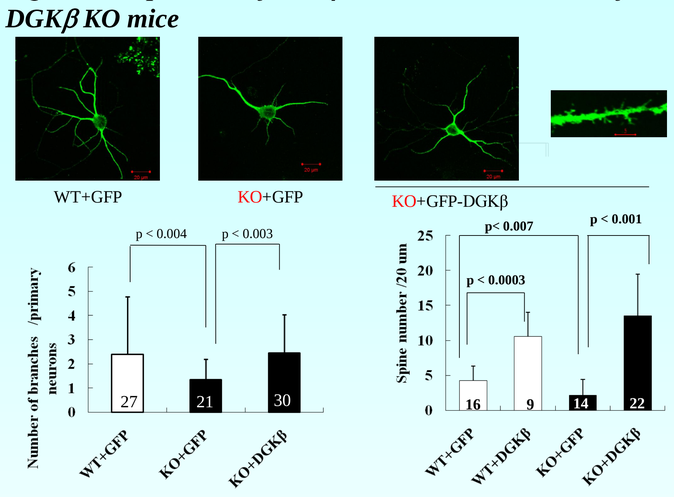
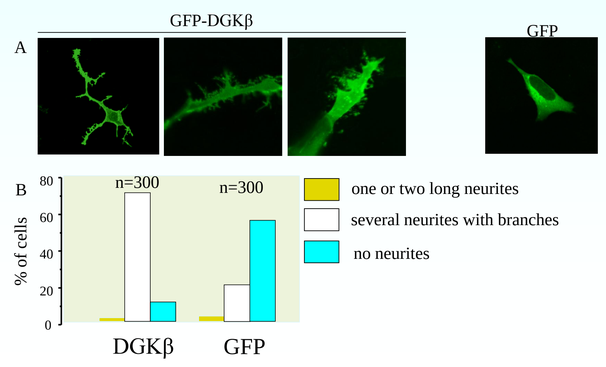
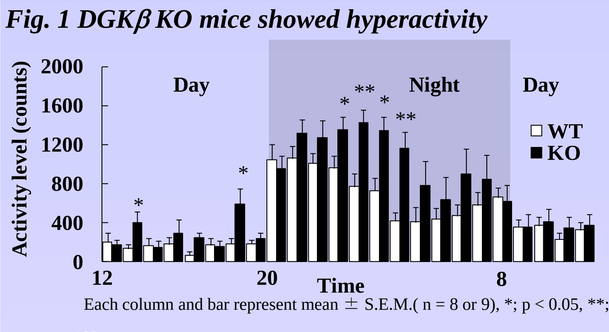


Fig. 5 Overexpression of DGKβ in SH-SY5Y

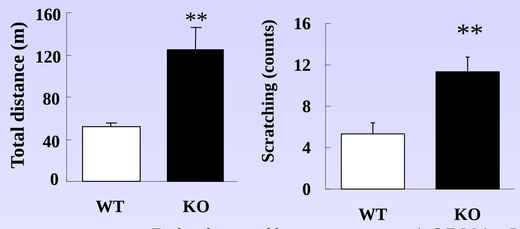


ADHD like behaviors

Fig. 1 DGKβ KO mice showed hyperactivity

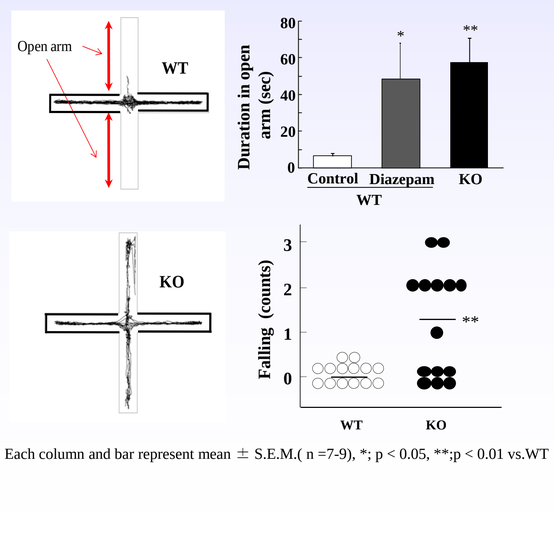


Each column and bar represent mean ± S.E.M.(n = 8 or 9), *; p < 0.05, **;p < 0.01 vs.WT



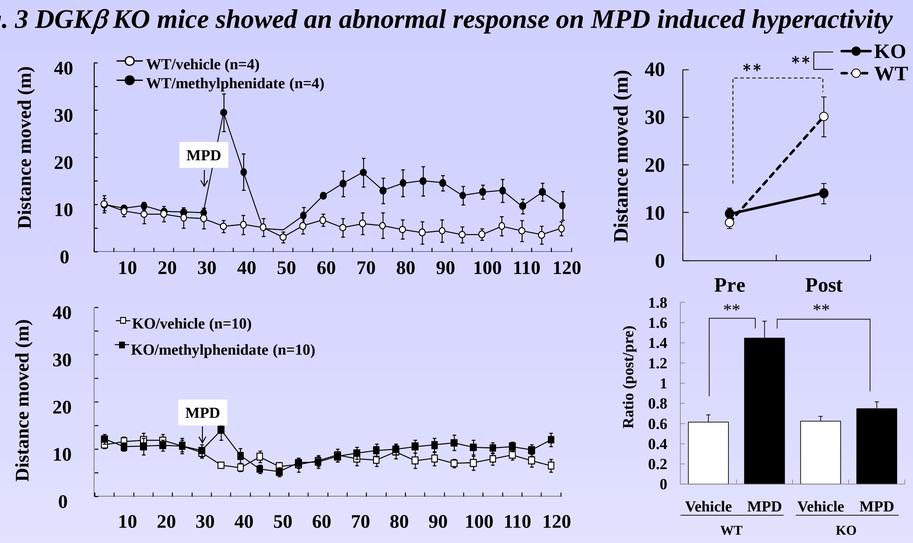
Each column and bar represent mean ± S.E.M.(n=5-7), **; p < 0.01 vs.WT

Fig. 2 DGKβ KO mice showed less anxiety



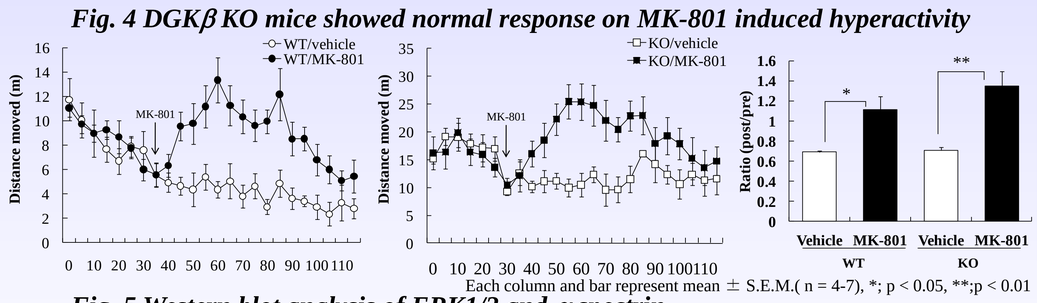
Each column and bar represent mean ± S.E.M.(n =7-9), *; p < 0.05, **;p < 0.01 vs.WT

Fig. 3 DGKβ KO mice showed an abnormal response on MPD induced hyperactivity



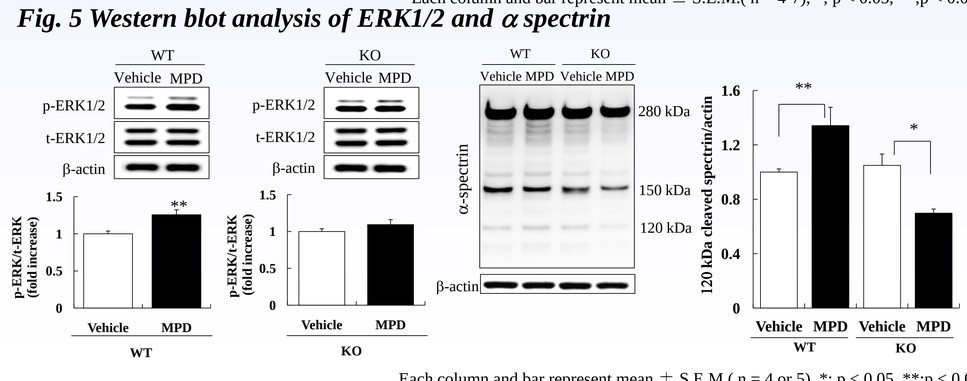
Each column and bar represent mean ± S.E.M.(n = 4 or 10), *; p < 0.05, **;p < 0.01

Fig. 4 DGKβ KO mice showed normal response on MK-801 induced hyperactivity



Each column and bar represent mean ± S.E.M.(n = 4-7), *; p < 0.05, **;p < 0.01

Fig. 5 Western blot analysis of ERK1/2 and α spectrin



Each column and bar represent mean ± S.E.M.(n = 4 or 5), *; p < 0.05, **;p < 0.01

Results

Impairment of spine conformation

1. DGKβ KO mice exhibited impaired cognitive function in the Y-maze test and Morris water maze test (Fig. 5)
2. The LTP in the hippocampal CA1 region of DGKβ KO mice was reduced in comparison with that of WT mice.
3. The hippocampal and cortical spine conformation of DGKβ KO mice was deficit.
4. Overexpression of DGKβ enhanced the dendrite maturation in hippocampal primary neuron derived from DGKβ KO mice and SH-SY5Y cells.

Abnormal response on psychostimulant-induced behavioral change

1. DGKβ KO mice showed hyperactivity and less-anxiety.
2. The psychostimulant effect of MPD was weaker in DGKβ KO mice than WT mice.
3. Using aother psychostimulant MK-801 (noncompetitive inhibitor of NMDA receptor), DGKβ KO mice shwoed normal response.
4. After MPD treatment, activation of ERK1/2 was not occurred in the striatum of DGKβ KO mice.
5. After MPD treatment, spectrin proteolysis was induced in the striatum of WT nice. On the other hand, the product of proteolysis was decreased in KO mice.

Conclusion

DGKβ KO mice may show LTP reduction and consequently cognitive impairment by incompleteness of spine conformation. Furthermore, DGKβ KO mice showed the abnormal response on psychostimulant-induced behavioral change, suggesting that hyperactivity and careless behavior of DGKβ KO mice may relate the pathogenesis of ADHD.

References

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Disclosure

No potential conflict of interest.