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REGULAR RESEARCH ARTICLE

Striatal N-Acetylaspartate Synthetase Shati/Nat8l Regulates Depression-Like Behaviors via mGluR3-Mediated Serotonergic Suppression in Mice

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Abstract

Background: Several clinical studies have suggested that *N*-acetylaspartate and *N*-acetylaspartylglutamate levels in the human brain are associated with various psychiatric disorders, including major depressive disorder. We have previously identified Shati/Nat8l, an *N*-acetyltransferase, in the brain using an animal model of psychosis. Shati/Nat8l synthesizes *N*-acetylaspartate from L-aspartate and acetyl-coenzyme A. Further, *N*-acetylaspartate is converted into *N*-acetylaspartylglutamate, a neurotransmitter for metabotropic glutamate receptor 3.

Methods: Because *Shati/Nat8l* mRNA levels were increased in the dorsal striatum of mice following the exposure to forced swimming stress, Shati/Nat8l was overexpressed in mice by the microinjection of adeno-associated virus vectors containing *Shati/Nat8l* gene into the dorsal striatum (dS-Shati/Nat8l mice). The dS-Shati/Nat8l mice were further assessed using behavioral and neurochemical tests.

Results: The dS-Shati/Nat8l mice exhibited behavioral despair in the forced swimming and tail suspension tests and social withdrawal in the 3-chamber social interaction test. These depression-like behaviors were attenuated by the administration of a metabotropic glutamate receptor 2/3 antagonist and a selective serotonin reuptake inhibitor. Furthermore, the metabolism of *N*-acetylaspartate to *N*-acetylaspartylglutamate was decreased in the dorsal striatum of the dS-Shati/Nat8l mice. This finding corresponded with the increased expression of glutamate carboxypeptidase II, an enzyme that metabolizes *N*-acetylaspartylglutamate present in the extracellular space. Extracellular serotonin levels were lower in the dorsal striatum of the dS-Shati/Nat8l and normal mice that were repeatedly administered a selective glutamate carboxypeptidase II inhibitor.

Conclusions: Our findings indicate that the striatal expression of *N*-acetylaspartate synthetase Shati/Nat8l plays a role in major depressive disorder via the metabotropic glutamate receptor 3-mediated functional control of the serotonergic neuronal system.

Keywords: Shati/Nat8l, behavioral despair, social withdrawal, mGluR3, serotonin

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Significance Statement

N-acetylaspartate (NAA) and *N*-acetylaspartylglutamate (NAAG) are present at high levels in mammalian brain. The alteration of those levels has been demonstrated in patients with major depressive disorder. NAA is synthesized by an *N*-acetyltransferase Shati/Nat8l, and NAAG is subsequently generated by NAAG synthetase from NAA. In the mice exposed to physical stress, *Shati/Nat8l* mRNA levels elevated in the dorsal striatum. In mice with striatal Shati/Nat8l-overexpression, NAA but not NAAG level was elevated, and therefore NAAG peptidase expression was enhanced. The Shati/Nat8l-overexpression mice revealed diminished motivation and sociability, and these depressive phenotypes were ameliorated by treatment with LY341495, a potent antagonist of the group II metabotropic glutamate receptors including mGluR3 as the binding target of NAAG, and antidepressant drug fluvoxamine. The extracellular serotonin levels were lower in the Shati/Nat8l-overexpression mice. Thus, the NAA-NAAG-mGluR3 pathway regulated by Shati/Nat8l in the striatum may be a target for the pharmacotherapies of major depressive disorder.

Introduction

The worldwide increase in the number of patients with psychiatric disorders, such as major depressive disorder, autism spectrum disorder, schizophrenia, and bipolar disorder, has garnered significant attention. However, current medical treatments for these disorders have notable limitations. In particular, the antidepressants that are widely prescribed for major depressive disorder, which affects approximately 16% of the population (Kupfer et al., 2012) and has an age of onset that encompasses the entire lifespan (Kessler et al., 2007), require 2 to 3 weeks to produce therapeutic benefits and are only moderately effective. Furthermore, a portion of these patients is resistant to these therapeutic drugs (Trivedi et al., 2006). A functional insertion deletion promoter variant in the serotonin (5-hydroxytryptamine [5-HT]) transporter gene SLC6A4 (Huezo-Diaz et al., 2009; Wilkie et al., 2009; Kato and Serretti, 2010) may explain the inefficient outcome of these medical treatments. Therefore, additional studies are required to understand the pathophysiology of major depressive disorder and develop new therapeutic strategies.

Shati was isolated from the brain of animals with methamphetamine-induced psychosis (Niwa et al., 2007). Shati was later identified as an *N*-acetyltransferase-8 like protein (Nat8l) that catalyzes *N*-acetylaspartate (NAA) synthesis from aspartate and acetyl-coenzyme A (Ariyannur et al., 2010). Thus, we refer to this enzyme as Shati/Nat8l in the present study. NAA exists at high levels in the human brain (Moffett et al., 1991) and is condensed with glutamate and converted into *N*-acetylaspartylglutamate (NAAG) by NAAG synthetase (NAAGS) (Becker et al., 2010). NAAG is widely distributed in the brains of mammals (Moffett and Namboodiri 1995; Passani et al., 1997; Neale et al., 2000) and acts as a highly selective neurotransmitter for group II metabotropic glutamate receptor 3 (mGluR3) (Neale et al., 2011). Following the release of NAAG into the synaptic cleft, NAAG binds mGluR3 and is metabolized to NAA and glutamate by glutamate carboxypeptidase II (GCPII) (Bzdega et al., 1997). A postmortem study has reported that NAA and NAAG levels are significantly lower in the brains of patients with major depressive disorder, schizophrenia, and bipolar disorder (Reynolds and Reynolds, 2011). Alternatively, a clinical study using magnetic resonance spectroscopy has reported that the NAA level was significantly increased in adult patients with autism spectrum disorder compared with that in the control group (Aoki et al., 2012). Therefore, these observations indicate that NAA synthetase Shati/Nat8l plays an important role in psychiatric disorders.

In the present study, we observed increased *Shati/Nat8l* mRNA expression in the dorsal striatum of mice that were exposed to forced swimming stress inducing behavioral despair. We produced genetically manipulated mice that specifically overexpressed Shati/Nat8l in the dorsal striatum (dS-Shati/Nat8l mice), using an adeno-associated virus (AAV) vector. We demonstrated that the dS-Shati/Nat8l mice exhibited depression-like behaviors and striatal mGluR3-related neuronal alterations. Our study suggests that Shati/Nat8l-regulated mGluR3 neurotransmission and the serotonergic neuronal system are associated with major depressive disorder.

Methods

Animals

Male C57Bl/6J strain mice (Nihon SLC) at 8 weeks of age (22–27 g) were used. The mice were housed in a regulated environment (25±1°C; 50±5% humidity) with a 12-hour-light/-dark cycle (lights on at 8:00 AM). Food and water were available ad libitum. All experiments followed the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals and were approved by the Committee for Animal Experiments of the University of Toyama (A2015PHA-20).

Drugs

LY341495 and 2-(phosphonomethyl) pentanedioic acid (2-PMPA) were purchased from Tocris Bioscience (Ellisville) and dissolved in 0.125 M phosphate buffer (pH 8.0) and saline (0.9% NaCl), respectively. Fluvoxamine was obtained from Sigma-Aldrich and dissolved in saline. Other drugs were obtained from standard commercial sources. For behavioral experiments, the doses of LY341495 (0.3 mg/kg, i.p.) and fluvoxamine (10 mg/kg, i.p.) were determined on the basis of the maximum dose that did not affect the immobility time of wild-type mice in the forced swimming test. For neurochemical experiments, the dose of 2-PMPA (30 mg/kg, i.p.) was determined as the amount that did not affect the locomotor activity in wild-type mice.

Production and Microinjection of the AAV Vector

The study design of the AAV vector was approved by the Board of Safety Committee for Recombination DNA Experiments of the University of Toyama (G2015PHA-12). The method used for the production and microinjection of AAV vector was previously reported (Krzyzosiak et al., 2010; Miyazaki et al., 2012). The AAV vector plasmids contained an expression cassette that included cDNA encoding either Shati/Nat8l (Shati: GenBank accession no. NM_001001985) or EGFP. The recombinant AAV-Shati/Nat8l or AAV-Mock vectors were produced by co-transfection of the AAV2 rep and AAV1 vp expression plasmid and pHelper (Agilent Technologies) in HEK293 cells. The suspension of AAV-Shati/Nat8l or AAV-Mock vectors (10^{10}–10^{12} unit) was injected bilaterally into the dorsal striatum of anesthetized mice (0.5 mm anterior and 2.0 mm lateral from bregma, 3.5 mm below skull surface) using the coordinates of a mouse brain atlas

(Franklin and Paxinos, 2008). Three weeks after the microinjection, the mice were used for experiments.

Quantitative RT-PCR

Whole brains were removed and divided into 1-mm-thick sections using a mouse brain matrix (Neuroscience Idea). The tissues of the dorsal striatum were collected with a 1-mm punch from the relevant section referencing to the plates of the mouse brain atlas (Franklin and Paxinos, 2008). The tissue samples were placed on dry ice and stored at -80°C until use. The quantitative RT-PCR protocol and primers for *Shati/Nat8l* and *36B4* mRNA were previously reported (Miyamoto et al., 2014).

In Situ Hybridization

In situ hybridization was performed as previously reported (Sumi et al., 2015). Coronal sections of the paraformaldehyde-fixed whole brain (20-μm thickness) were cut using a cryostat (Leica Biosystems). The coronal sections were hybridized to digoxigenin-labeled antisense and sense cRNA probes for Shati/Nat8l (1133–1557 bp) using standard protocols. The staining signal of the developed image was observed using an AxioObserver Z1 (Carl Zeiss).

Locomotor Activity Test

We measured the locomotor activity of mice using previously published methods (Miyamoto et al., 2002, 2014). Briefly, a mouse was placed in a transparent acrylic cage with a black frosted Plexiglas floor (45 × 45 × 40 cm), and locomotion was measured for 60 minutes using digital counters with infrared sensors (SCANET MV-40; MELQUEST).

Forced Swimming Test

The forced swimming test was performed as previously described (Miyamoto et al., 2002). Briefly, individual mice were placed in a transparent polycarbonate cylinder (21 cm in diameter × 22.5 cm high) containing water at 22°C to a depth of 18 cm. Mice were then forced to swim for 6 minutes. The duration of immobility was measured in 1-minute bins using a SCANET MV-40AQ (MELQUEST).

Tail Suspension Test

The tail suspension test was performed as previously described (Furukawa-Hibi et al., 2010). Briefly, individual mice were suspended by their tail for 6 minutes. The duration of immobility was recorded manually in 1-minute bins using a stopwatch.

Three-Chamber Social Interaction Test

The social testing apparatus comprised a rectangular, 3-chambered box (Nadler et al., 2004). Each chamber measured 20 × 40 × 22 cm, and the dividing walls were made of clear Plexiglas with small square openings (5 × 3 × 3 cm) that allowed access to each chamber. Each individual mouse was habituated to the chamber without the wire cage for a single 20-minute session. During trial 1, a novel object was placed in a wire cage in one side chamber and an empty wire cage was placed in the chamber on the other side. The subject mouse was placed in the middle chamber for 10 minutes and then allowed to explore the entire social test box for 10 minutes. The amount of time spent in the quadrant around each wire cage was recorded. During trial 2, another novel object was placed in the wire cage in one side chamber, and an intruder mouse (male C57Bl/6J strain) was placed in the wire cage in the chamber on the other side. The subject mouse was placed in the middle chamber for 10 minutes and then allowed to explore the entire social test box for 10 minutes. The amount of time spent in the quadrant around each wire cage was recorded. A preference index was used to assess social interaction, that is, the ratio of the approach time either to the wire cage with the object (trial 1) or the intruder mouse (trial 2) relative to the total approach time.

Measurement of NAA and NAAG

The measurement of NAA and NAAG by HPLC was performed as previously described (Reynold et al., 2005; Takanashi et al., 2012; Miyamoto et al., 2014). Deproteinized samples for HPLC were applied to pretreated Bond Elut SAX anion exchange columns (Agilent Technologies) followed by extraction with phosphoric acid (85%).

Western Blotting

Western blotting was performed using standard protocols. Brain homogenates (20 μg total proteins) were separated using sodium dodecyl sulfate-polyacrylamide gel electrophoresis and blotted onto a polyvinylidene difluoride membrane. The membranes were incubated with primary antibodies, and the proteins were detected by horseradish peroxidase-conjugated secondary antibodies and the ECL Prime kit (GE Healthcare). The anti-mGluR2/3 antibody (#06-676, 1/1000) was obtained from Millipore. The anti-NAAGS (#ab74284, 1/1000) and GCPII (#ab80554, 1/1000) antibodies were obtained from Abcam. The antibody against GAPDH (#M171-3, 1/5000) was purchased from MBL.

In Vivo Microdialysis for 5-HT

We performed in vivo microdialysis as previously described (Miyamoto et al., 2014). The guide cannula was placed into the dorsal striatum of anesthetized mice (0.5 mm anterior and 2.0 mm lateral from bregma, 3.5 mm below skull surface) according to the mouse brain atlas (Franklin and Paxinos, 2008). The day following the operation, a dialysis probe (1-mm membrane length, EICOM) was inserted through the guide cannula and perfused with Ringer's solution (147 mM NaCl, 4 mM KCl, and 2.3 mM $CaCl_2$) at a flow rate of 0.5 μL/min. The dialysates were collected in 12-minute fractions from the dorsal striatum and injected into an HPLC system equipped with a reversed-phase ODS column (PP-ODS; EICOM) connected to an electrochemical detector (EICOM). Three samples were used to establish the baseline levels of extracellular 5-HT.

Statistical Analysis

The data were expressed as the mean ± SEM. Statistical differences between 2 groups were determined using the Student's *t* test. Statistical differences among values for individual groups were determined using ANOVA, followed by the Student-Newman-Keuls posthoc test when the *F* value was significant ($P < .05$).

Results

Overexpression of *Shati/Nat8l* mRNA in the Dorsal Striatum

We initially investigated the association of Shati/Nat8l with depression-like behavior using normal wild-type mice. Two

hours after behavioral despair induced by forced swimming stress, which reflects a motivational decline, the expression levels of *Shati/Nat8l* mRNA were increased in the dorsal striatum ($P = .0115$) but not in the prefrontal cortex ($P = .0931$), nucleus accumbens ($P = .7421$), or hippocampus ($P = .2922$) of the normal wild-type mice (Figure 1A). Following these results, we produced dS-Shati/Nat8l mice via the microinjection of AAV-Shati/Nat8l vectors into the dorsal striatum. *Shati/Nat8l* mRNA expression was greatly increased 9.9±0.2-fold in the dorsal striatum ($P = .0003$) but not in the nucleus accumbens ($P = .7228$) of the dS-Shati/Nat8l mice compared with that in the control dS-Mock mice (Figure 1B). Furthermore, in situ hybridization detected the enhanced expression of *Shat/Nat8l* mRNA in the dorsal striatum of the dS-Shati/Nat8l mice (Figure 1C).

Behavioral Despair in the dS-Shati/Nat8l Mice

We performed various behavioral tests to assess the dS-Shati/Nat8l mice. There were no differences in the total locomotor activity counts between the dS-Mock and dS-Shati/Nat8l mice during a 60-minute observation period (dS-Mock mice, 22821.8±1045.7 counts; dS-Shati/Nat8l mice, 21570.9±976.3 counts; Student's *t* test, $P = .5154$), suggesting that the motor function in the dS-Shati/Nat8l mice was unaffected. Further, we evaluated behavioral despair. The dS-Shati/Nat8l mice exhibited a significant increase in immobility time during the forced swimming and tail suspension tests compared with the dS-Mock mice (Figure 2A, D) (forced swimming test, $P = .0228$; tail suspension test, $P = .0028$).

Shati/Nat8l synthesizes NAA, which is then converted to NAAG, and NAAG acts as a neurotransmitter for mGluR3. Therefore, we examined the effect of a potent group II mGluR2/3 antagonist LY341495 on the behavioral despair of the dS-Shati/Nat8l mice. In both the forced swimming and tail suspension tests, the increased immobility time of the dS-Shati/Nat8l mice was attenuated with the same dose of LY341495 administration (0.3 mg/kg, i.p. 30 minutes prior to each behavioral test) (Figure 2B, E) (forced swimming test, dS-Shati/Veh vs dS-Shati/LY; $F_{3,42} = 3.318$; $P < .05$; tail suspension test, dS-Shati/Veh vs dS-Shati/LY; $F_{3,34} = 9.412$; $P < .01$). Furthermore, we tested whether the behavioral despair of the dS-Shati/Nat8l mice responded to a common antidepressant, a selective serotonin reuptake inhibitor (SSRI), fluvoxamine. The administration of fluvoxamine (10 mg/kg, i.p. 30 minutes prior to each behavioral test) also attenuated the increased immobility time of the dS-Shati/Nat8l mice in the forced swimming and tail suspension tests (Figure 2C, F) (forced swimming test, dS-Shati/Veh vs dS-Shati/Flu; $F_{3,33} = 5.527$; $P < .05$; tail suspension test, dS-Shati/Veh vs dS-Shati/Flu; $F_{3,31} = 18.97$; $P < .01$).

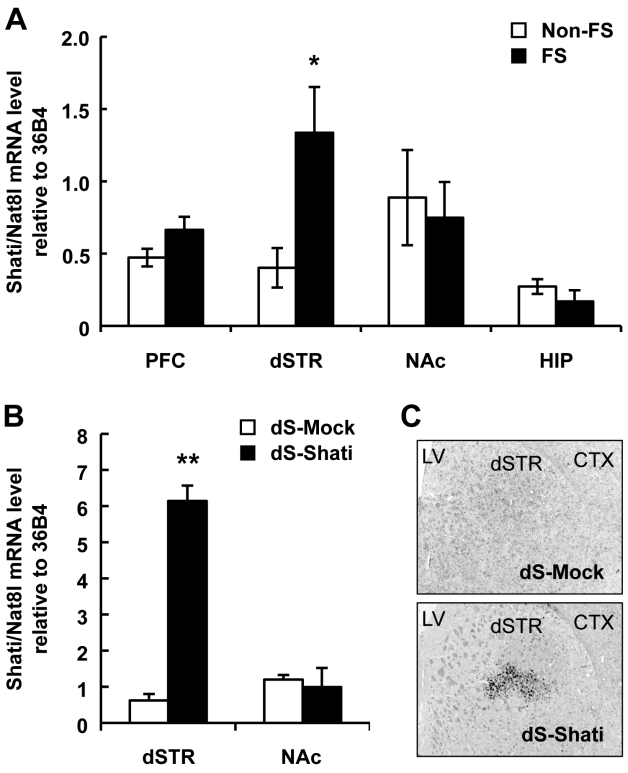

Figure 1. *Shati/Nat8l* mRNA expression in the dorsal striatum of mice that were exposed to forced swimming stress and of the dS-Shati/Nat8l mice. (A) *Shati/Nat8l* mRNA in the dorsal striatum (dSTR) but not in the prefrontal cortex (PFC), nucleus accumbens (NAc), or hippocampus (HIP) was increased 2 hours after the forced swimming (FS) test (n=6–8). *$P < .05$ vs Non-FS (Student's *t* test). (B) *Shati/Nat8l* mRNA was higher in the dSTR but not in the NAc of the dS-Shati/Nat8l mice compared with that in the dS-Mock mice (n=3). **$P < .01$ vs dS-Mock (Student's *t* test). (C) In situ hybridization revealed that *Shat/Nat8l* mRNA was overexpressed only in the dSTR of the dS-Shati/Nat8l mice. CTX, cortex; LV, lateral ventricle. *Shati/Nat8l* mRNA expression was measured by quantitative real-time RT-PCR and expressed relative to the expression of the housekeeping gene 36B4. The data are shown as the mean ± SEM.

Social Withdrawal in the dS-Shati/Nat8l Mice

We performed a 3-chamber social interaction test to examine sociability in mice (Nadler et al., 2004; Nakatani et al., 2009). In trial 1, the time spent exploring a novel object was significantly longer than that spent in the empty wire cage for both the dS-Mock and dS-Shati/Nat8l mice (dS-Mock mice: novel object, 57.60±3.38%; empty, 42.40±3.38%; Student's *t* test, $P = .0038$; dS-Shati/Nat8l mice: novel object, 55.66±2.07%; empty, 44.34±2.07%; Student's *t* test, $P = .0006$). However, there was no difference in the time spent exploring a novel object between the dS-Mock and dS-Shati/Nat8l mice. In trial 2, the dS-Mock mice spent significantly longer time with an intruder mouse than with the novel object (Figure 3A). In contrast, the dS-Shati/Nat8l mice exhibited no difference in the time spent exploring the intruder mouse or the novel object (Figure 3A). Moreover, the time taken to approach the intruder mouse was significantly lower in the dS-Shati/Nat8l mice than that in the dS-Mock mice (Figure 3A).

We also assessed whether the social withdrawal of the dS-Shati/Nat8l mice was attenuated by the administration of group II mGluR2/3 antagonist LY341495 or the antidepressant SSRI fluvoxamine. The administration of LY341495 (0.3 mg/kg, i.p. 30 minutes prior to trial 2) or fluvoxamine (10 mg/kg, i.p. 30 minutes prior to trial 2) attenuated the decreased sociability of the dS-Shati/Nat8l mice (Figure 3B, C) (dS-Shati/Veh vs dS-Shati/LY, $F_{3,34} = 4.236$, $P < .05$; dS-Shati/Veh vs dS-Shati/Flu, $F_{3,32} = 5.358$, $P < .01$).

Thus, these pharmacological results suggest that depression-like behaviors, including behavioral despair and social withdrawal, in the dS-Shati/Nat8l mice are induced by the activation of group II mGluR-mediated neurotransmission and the dysfunction of the serotonergic neuronal system.

NAAG-mGluR3 Neurotransmission in the Dorsal Striatum of the dS-Shati/Nat8l Mice

To investigate mGluR3-mediated neurotransmission in the dorsal striatum of the dS-Shati/Nat8l mice, we measured the tissue content of NAA and NAAG using HPLC. NAA exhibited a

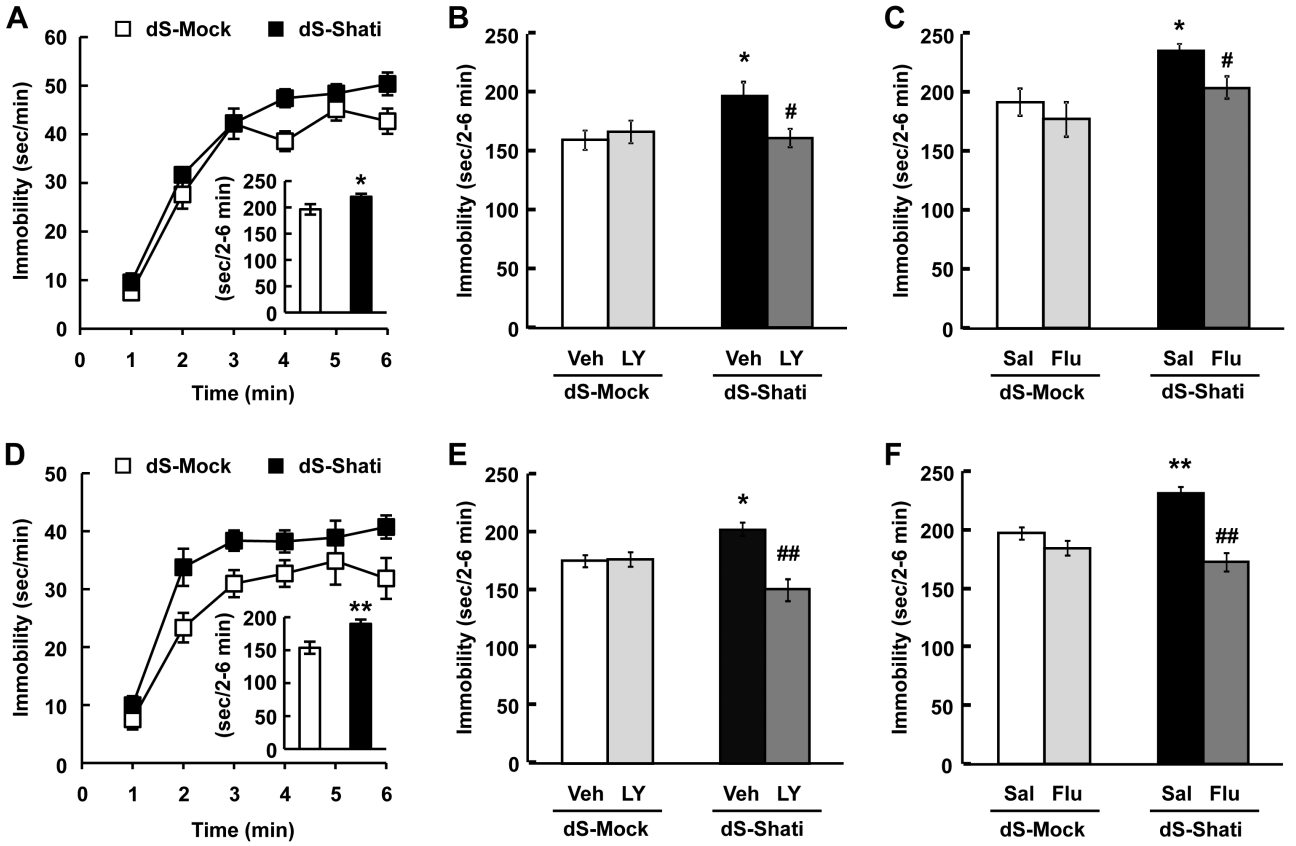

Figure 2. Behavioral despair in the dS-Shati/Nat8l mice. (A) The immobility time in the forced swimming test was increased in the dS-Shati/Nat8l mice compared with the dS-Mock mice (n = 8–10). *P < .05 vs dS-Mock (Student's *t* test). (B) The behavioral despair observed in the dS-Shati/Nat8l mice in the forced swimming test was attenuated by LY341495. Thirty minutes after treatment with vehicle (Veh) or LY341495 (LY, 0.3 mg/kg, i.p.), the immobility time was measured in the dS-Mock and dS-Shati/Nat8l mice (n = 11–12). *P < .05 vs dS-Mock (Veh), #P < .05 vs dS-Shati (Veh) (ANOVA followed by Student-Newman-Keuls posthoc test). (C) The behavioral despair observed in the dS-Shati/Nat8l mice in the forced swimming test was attenuated by fluvoxamine. Thirty minutes after treatment with saline (Sal) or fluvoxamine (Flu, 10 mg/kg, i.p.), the immobility time was measured in the dS-Mock and dS-Shati/Nat8l mice (n = 8–10). *P < .05 vs dS-Mock (Sal), #P < .05 vs dS-Shati (Sal) (ANOVA followed by Student-Newman-Keuls posthoc test). (D) The immobility time in the tail suspension test was increased in the dS-Shati/Nat8l mice compared with the dS-Mock mice (n = 12–15). **P < .01 vs dS-Mock (Student's *t* test). (E) The behavioral despair observed in the dS-Shati/Nat8l mice in the tail suspension test was attenuated by LY341495. Thirty minutes after treatment with vehicle (Veh) or LY341495 (LY, 0.3 mg/kg, i.p.), the immobility time was measured in the dS-Mock and dS-Shati/Nat8l mice (n = 9–10). *P < .05 vs dS-Mock (Veh), ##P < .01 vs dS-Shati (Veh) (ANOVA followed by Student-Newman-Keuls posthoc test). (F) The behavioral despair observed in the dS-Shati/Nat8l mice in the tail suspension test was attenuated by fluvoxamine. Thirty minutes after treatment with saline (Sal) or fluvoxamine (Flu, 10 mg/kg, i.p.), the immobility time was measured in the dS-Mock and dS-Shati/Nat8l mice (n = 8–9). **P < .01 vs dS-Mock (Sal), ##P < .01 vs dS-Shati (Sal) (ANOVA followed by Student-Newman-Keuls posthoc test). The data are shown as the mean ± SEM.

tendency to increase in the dS-Shati/Nat8l mice compared with the dS-Mock mice (dS-Mock mice, 2.973 ± 0.295 μmol/g tissue; dS-Shati/Nat8l mice, 3.738 ± 0.262 μmol/g tissue; Student's *t* test, *P* = .0885). There was no change in NAAG between the dS-Mock and dS-Shati/Nat8l mice (dS-Mock mice, 0.151 ± 0.012 μmol/g tissue; dS-Shati/Nat8l mice, 0.136 ± 0.010 μmol/g tissue; Student's *t* test, *P* = .3852). However, the ratio of NAA to NAAG was significantly lower in the dorsal striatum of the dS-Shati/Nat8l mice compared with that in the dS-Mock mice (Figure 4A) (*P* = .0070). In addition, we examined the expression levels of NAAG-related proteins, including NAAGS, mGluR2/3, and GCPII, in the dorsal striatum. The expression of NAAGS and mGluR2/3 was similar for the dS-Mock and dS-Shati/Nat8l mice (supplementary Figure 1A, B) (NAAGS, *P* = .6849; mGluR2/3, *P* = .6844). The expression of the GCPII, which is a glial ectoenzyme that hydrolyzes NAAG to glutamate and NAA, was significantly increased in the dS-Shati/Nat8l mice compared with the dS-Mock mice (Figure 4B) (*P* = .0047).

These results indicate that the release efficacy of NAAG into the synaptic cleft, which influences extracellular NAAG metabolism, is upregulated in the dS-Shati/Nat8l mice.

mGluR3-Mediated Serotonergic Regulation in the Dorsal Striatum of the dS-Shati/Nat8l Mice

To confirm serotonergic neuronal dysfunction in the dS-Shati/Nat8l mice, we measured 5-HT levels using in vivo microdialysis. The basal levels of extracellular 5-HT in the dorsal striatum were significantly lower in the dS-Shati/Nat8l mice compared with the dS-Mock mice (Figure 4C) (*P* = .0064). This neurochemical result supports the observation that the behavioral abnormalities of the dS-Shati/Nat8l mice improved with fluvoxamine treatment.

To verify our findings on mGluR3-mediated serotonergic regulation, we estimated the influence of mGluR3 activation on the serotonergic neuronal system in wild-type mice. A single administration of a selective GCPII inhibitor 2-PMPA (30 mg/kg, i.p.) after pretreatment with vehicle for 6 days did not significantly affect extracellular 5-HT levels in the dorsal striatum of wild-type mice (Figure 4D: Repeat Veh) (at 0 minutes vs 120 minutes, *P* = .4118). Alternatively, wild-type mice that were repeatedly pretreated with 2-PMPA for 6 days (30 mg/kg/day, i.p.), showed a significant reduction in extracellular 5-HT levels

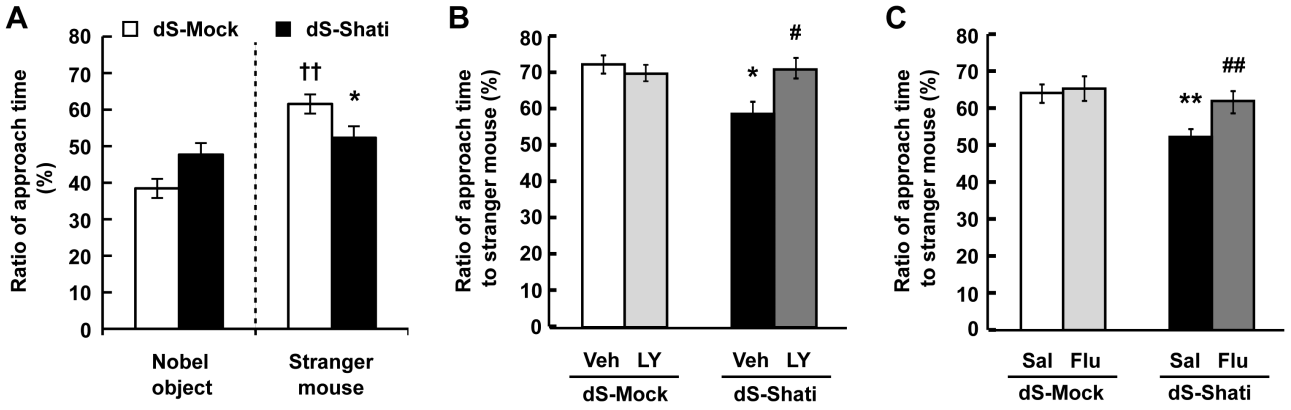

Figure 3. Social withdrawal in the dS-Shati/Nat8l mice. (A) In trial 2 of the 3-chamber social interaction test, the dS-Shati/Nat8l mice were not more interested in the stranger mouse than the dS-Mock mice (n = 14–16). ††$P < .01$ vs dS-Mock (Novel object), *$P < .05$ vs dS-Shati (Stranger mouse) (Student's *t* test). (B) Social withdrawal observed in the dS-Shati/Nat8l mice in the 3-chamber social interaction test was attenuated by LY341495. Thirty minutes after the treatment with vehicle (Veh) or LY341495 (LY, 0.3 mg/kg, i.p.), the approach time was measured in the dS-Mock and dS-Shati/Nat8l mice (n = 9–10). *$P < .05$ vs dS-Mock (Veh), #$P < .05$ vs dS-Shati (Veh) (ANOVA followed by Student-Newman-keuls posthoc test). (C) Social withdrawal observed in the dS-Shati/Nat8l mice in the 3-chamber social interaction test was attenuated by fluvoxamine. Thirty minutes after treatment with saline (Sal) or fluvoxamine (Flu, 10 mg/kg, i.p.), the approach time was measured in the dS-Mock and dS-Shati/Nat8l mice (n = 8–9). **$P < 0.01$ vs dS-Mock (Sal), ##$P < .01$ vs dS-Shati (Sal) (ANOVA followed by Student-Newman-Keuls posthoc test). The data are shown as the mean ± SEM.

in the dorsal striatum 120 minutes after the seventh administration compared with that before administration (Figure 4D: Repeat 2-PMPA) (at 0 minutes vs 120 minutes, $P = .0010$) without an effect on basal 5-HT levels (6-day vehicle repeated pretreatment, 0.275 ± 0.019 pg/μL; 6-day 2-PMPA repeated pretreatment, 0.296 ± 0.023 pg/μL).

These findings suggest that the continuous enhancement of mGluR3 neurotransmission induced by extracellular NAAG elevation suppresses serotonergic neuronal function in the dorsal striatum.

Discussion

In the present study, the dS-Shati/Nat8l mice exhibited both behavioral despair and social withdrawal, and these depression-like behaviors were successfully attenuated by the administration of SSRI fluvoxamine. The dS-Shati/Nat8l mice also exhibited decreased extracellular 5-HT levels in the dorsal striatum, and the administration of group II mGluR2/3 antagonist LY341495 ameliorated depression-like behaviors in the dS-Shati/Nat8l mice. In our previous study, NAA and NAAG levels were significantly increased by the infusion of an AAV-Shati/Nat8l vector into the nucleus accumbens (Miyamoto et al., 2014), whereas in the present study, there was no significant change in NAA or NAAG levels in the dorsal striatum of the dS-Shati/Nat8l mice. Notably, the conversion efficiency of NAA to NAAG was altered and the extracellular NAAG peptidase GCPII, but not NAAGS, was overexpressed in the dorsal striatum of the dS-Shati/Nat8l mice. Thus, NAAG-mGluR3 neurotransmission could be activated by the increased extracellular NAAG levels in the dorsal striatum of the dS-Shati/Nat8l mice. Alternatively, considering the possibility that NAAG is not a direct endogenous agonist for mGluR3 (Chopra et al., 2009), the extracellular glutamate levels may be increased because of NAAG metabolism by GCPII, and therefore glutamate neurotransmission may be activated. However, the continuous activation of NAAG-mGluR3 neurotransmission by GCPII inhibitor in the wild-type mice induced serotonergic neuronal dysfunction in the dorsal striatum. Our observations suggest that the Shati/Nat8l-associated system in the dorsal striatum regulates depression-like behaviors via mGluR3-mediated serotonergic suppression.

Postmortem NAA levels are significantly lower in the striatum of the brains of patients with major depressive disorder (Reynolds and Reynolds, 2011). In the present study, depression-like behaviors induced by Shati/Nat8l-overexpression in the dorsal striatum were observed; however, there were no considerable changes in NAA levels in the brain region. This discrepancy may be because of the chronic treatment of human patients with antidepressants leading to decreased NAA levels. Regardless, NAA metabolism in the striatum appears to be involved in major depressive disorder. In addition, in clinical studies, major depressive disorder has been reported to be associated with polymorphisms in the mGluR3 gene GRM3 (Tsunoka et al., 2009). Thus, functional alterations of the NAA synthetase Shati/Nat8l and its downstream mGluR3 neurotransmission might be the pathological aspects of major depressive disorder. The activation of mGluR3 neurotransmission proposed by us is supported by much evidence, which have demonstrated that group II mGluR2/3 antagonists ameliorate depression-like behaviors in animal models (Bespalov et al., 2008; Engers et al., 2015; Podkowa et al., 2016; Chaki 2017).

In major depressive disorder, the cerebrospinal fluid levels of 5-hydroxyindoleacetic acid, a metabolite of 5-HT, are low (Asberg et al., 1976; Banki et al., 1981), and such patients have higher 5-HT transporter potentials than healthy individuals (Meyer et al., 2004). SSRIs are generally prescribed as antidepressants in clinics. Therefore, the decrease of 5-HT levels in the brain is considered a pathological aspect of major depressive disorder. In the present study, both behavioral despair and social withdrawal, which reflect the symptoms of major depressive disorder, were induced by Shati/Nat8l-overexpression in the dorsal striatum. These phenomena were ameliorated by the administration of antidepressant SSRI or group II mGluR2/3 antagonist. Moreover, we observed decreased extracellular 5-HT levels in the dorsal striatum of the dS-Shati/Nat8l mice and decreased 5-HT levels in the dorsal striatum induced by continuous mGluR3 activation in the wild-type mice. Taken together, our observations suggest that Shati/Nat8l in the dorsal striatum regulates depression-like behaviors by controlling serotonergic neuronal function via mGluR3 neurotransmission.

The beneficial effects of group II mGluR2/3 antagonist on depression-like behaviors are associated with the serotonergic

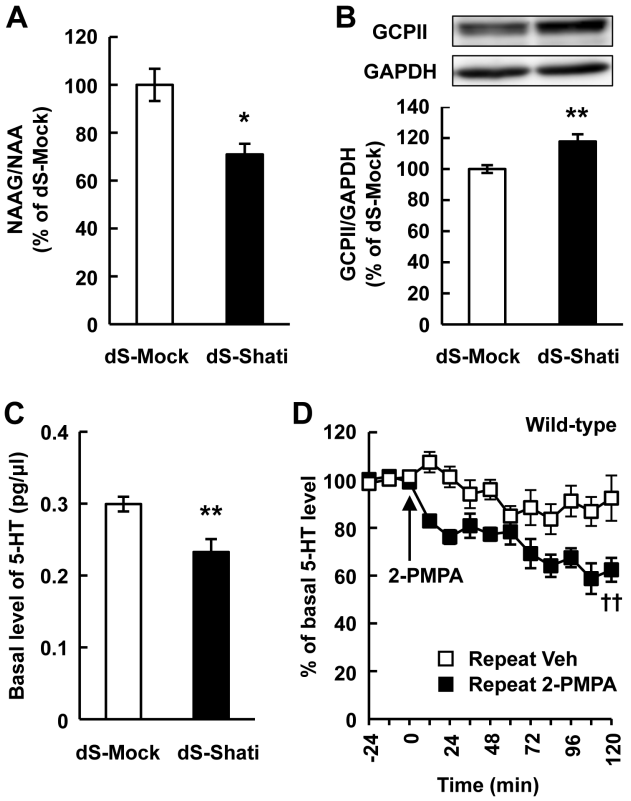

Figure 4. mGluR3-mediated serotonergic neuronal signaling in the dorsal striatum of the dS-Shati/Nat8l mice. (A) The ratio of NAAG/NAA was decreased in the dorsal striatum of the dS-Shati/Nat8l mice compared with that in the dS-Mock mice. The tissue content of NAA and NAAG was evaluated using HPLC (n = 5). *P < .05 vs dS-Mock (Student's *t* test). (B) GCPII expression was increased in the dorsal striatum of the dS-Shati/Nat8l mice compared with that in the dS-Mock mice. GCPII expression relative to GAPDH was assessed by western blotting (n = 6–8). **P < .01 vs dS-Mock (Student's *t* test). (C) The basal levels of extracellular serotonin (5-HT) were decreased in the dorsal striatum of the dS-Shati/Nat8l mice compared with that in the dS-Mock mice. The extracellular levels of 5-HT were measured by in vivo microdialysis (n = 6–7). **P < .01 vs dS-Mock (Student's *t* test). (D) The repeated administration of 2-PMPA for 6 days (30 mg/kg/day, i.p.) decreased extracellular 5-HT levels in the dorsal striatum of wild-type mice (n = 5). ††P < .01 vs Repeat 2-PMPA (at 0 min) (Student's *t* test). The data are shown as the mean ± SEM.

neuronal system in the medial prefrontal cortex (Fukumoto et al., 2014; Pehrson and Sanchez 2014; Chaki 2017). However, functional roles of the dorsal striatum in depression remain unclear. The dorsal striatum receives projections from the serotonergic neurons in the dorsal raphe nucleus and supplies GABAergic projections to the serotonergic neurons in the dorsal raphe nucleus; this neuronal circuit is associated with reward-seeking and motivational behaviors (Michelsen et al., 2007; Nakamura 2013; Pollak Dorocic et al., 2014). Furthermore, there are distinct types of GABAergic interneurons in the dorsal striatum (Tepper et al., 2010). Therefore, group II mGluR2/3 antagonists may directly or indirectly activate the serotonergic neuronal system in the dorsal striatum via stimulating the GABAergic interneurons or projections to the dorsal raphe nucleus. The synaptic mechanism of mGluR2/3-mediated serotonergic neuronal modulation in the dorsal striatum should be investigated to elucidate the neuronal mechanism of the dorsal striatum in major depressive disorder.

Incidentally, impaired social interaction is one of the core symptoms of autism spectrum disorder. Individuals with autism spectrum disorder exhibit impaired 5-HT-mediated neuronal function (Huang and Santangelo, 2008). Furthermore, proton magnetic resonance spectroscopy has revealed that NAA levels were higher in the brains of patients with autism spectrum disorder than in the control group (Aoki et al., 2012). Both social withdrawal and 5-HT depletion were induced by Shati/Nat8l-overexpression in the dorsal striatum. Although the detailed pathology of autism spectrum disorder is unclear, the serotonergic neuronal dysfunction indirectly affected by Shati/Nat8l may be associated with autism spectrum disorder.

In conclusion, the present study is the first to demonstrate that the overexpression of NAA synthetase Shati/Nat8l in the dorsal striatum induces behavioral despair, social withdrawal, and extracellular 5-HT depletion. Our results indicate that the regulation of motivation and social interaction via the striatal mGluR3-mediated control of the serotonergic neuronal system plays an important role in the pathogenesis of major depressive disorder. Therefore, Shati/Nat8l and its downstream influence on mGluR3 neurotransmission may be potential new targets for the development of pharmacotherapies for major depressive disorder.

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Statement of Interest

None.

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