Vulnerability to depressive behavior induced by overexpression of striatal Shati/Nat8l via the serotonergic neuronal pathway in mice

Kyosuke Uno[1,2], Hajime Miyanishi[1], Kengo Sodeyama[1], Toshiyuki Fujiwara[1], Toh Miyazaki[1], Shi-ichi Muramatsu[3,4], Atsumi Nitta[1]

Affiliations

[1]Department of Pharmaceutical Therapy and Neuropharmacology, Faculty of Pharmaceutical Sciences, Graduate School of Medicine and Pharmaceutical Sciences, University of Toyama, Toyama 930-0194, Japan.

[2]Laboratory of Molecular Pharmacology, Faculty of Pharmaceutical Sciences, Setsunan University, Hirakata, Japan.

[3]Division of Neurology, Department of Medicine, Jichi Medical University, Shimotsuke, Tochigi, Japan.

[4]Center for Gene & Cell Therapy, Institute of Medical Science, The University of Tokyo, Tokyo, Japan.

Correspondence

Atsumi Nitta, Ph.D.
Department of Pharmaceutical Therapy and Neuropharmacology, Faculty of Pharmaceutical Sciences, University of Toyama, Toyama, Japan
Telephone: +81-7-6415-8823
Fax Number: +81-7-6415-8826
E mail; nitta@pha.u-toyama.ac.jp

Abstract

The number of patients with depressive disorders is increasing. However, the mechanism of depression onsets has not been completely revealed. We previously identified Shati/Nat8l, an *N*-acetyltransferase, in the brain using an animal model of psychosis. In this study, we revealed the involvement of Shati/Nat8l in the vulnerability to major depression. Shati/Nat8l mRNA was increased only in the striatum of mice, which were exposed to chronic social defeat stress. Shati/Nat8l-overexpressed mice showed impairment in social interaction and sucrose preference after the subthreshold social defeat (microdefeat) stress. These depression-like behaviors were restored by fluvoxamine and LY341495 injection prior to these tests. Furthermore, the intracerebral administration of only fluvoxamine, but not of LY341495, to the dorsal striatum and direct infusion of LY341495 to the dorsal raphe also rescued. Taken together, Shati/Nat8l in the striatum has an important role in the vulnerability to depression onsets by regulating the origin of serotonergic neuronal system via GABAergic projection neuron in the dorsal raphe from the dorsal striatum.

Key Words: Shati/Nat8l, depression, stress, striatum

Abbreviations

WMH: World Mental Health, SSRI: selective serotonin reuptake inhibitor, SNRI: serotonin and/or noradrenaline reuptake inhibitor, NAA: N-acetylaspartate, NAAG: N-acetylaspartylglutamate, NAAGS: NAAG synthetase, mGluR3: metabotropic glutamate type 3 receptor, MRS: magnetic resonance spectroscopy, NAc: nucleus accumbens, AAV: adeno-associated virus, CSDS: Chronic social defeat stress, MDS: microdefeat stress, HPLC: high performance liquid chromatography, FLX: fluvoxamine, HPA axis: hypothalamic-pituitary-adrenal axis

1. Introduction

The number of patients with psychiatric disorders increases year by year. These disorders include schizophrenia and major depressive, autism spectrum, and bipolar disorders. Among them, major depressive disorders are considered a disease with many patients. According to a World Mental Health (WMH) investigation, the prevalence of this disorder was 6.6 % of the population. Major depressive disorder is one of the most common and debilitating mental disorders with traumatic symptoms of sadness, hopelessness, pessimism, loss of interest in life, and reduced emotional well-being. The disorder also comes with disturbances of sleep and appetite, decreased energy, and often, cognitive disturbances, such as difficulty concentrating and remembering. Furthermore, depressed individuals are at a significantly elevated risk of suicide.

In major depressive disorder, the patients have higher 5-HT transporter potentials than that in healthy individuals [1]. Moreover, cerebrospinal fluid levels of 5-hydroxyindoleacetic acid, a metabolite of 5-HT, are lower than those in healthy individuals [2,3]. Thus, the reduction of 5-HT in the brain is considered a pathological aspect of major depressive disorder. A selective serotonin reuptake inhibitor (SSRI) and a serotonin and/or noradrenaline reuptake inhibitor (SNRI) have become the first-line therapeutic drugs for the depression. Furthermore, environmental factors such as the physical or psychological stressful environment and emotional trauma are thought to be the etiology of the depression and it is therefore, named a stress-related disease [4]. However, the detailed onset mechanism remains unidentified.

In this study, we used social defeat stress to cause depression in mice, as the methods reflect the stress in the clinical and human situation [5]. Many studies focus on the prefrontal cortex related to stress-induced social

avoidance [6-10] in depressive model mice.

We have previously identified Shati/Nat8l in the brain of an animal model of psychosis [11]. Shati/Nat8l was reported as the synthetic enzyme that produces *N*-acetylaspartate (NAA) from L-aspartate and acetyl-coenzyme A [12]. Further, synthesized NAA is converted into *N*-acetylaspartylglutamate (NAAG) by NAAG synthetase (NAAGS) [13]. Finally, NAAG acts as a highly selective endogenous agonist for the metabotropic glutamate type 3 receptor (mGluR3) [14]. A postmortem study has reported that both NAA and NAAG levels are significantly lower in the brains of schizophrenia, major depressive disorder, and bipolar disorder patients [15]. In contrast, a clinical study using magnetic resonance spectroscopy (MRS) has reported that the NAA level significantly increased in adult autism patients compared with that in control subjects [16]. These reports indicate that "Shati/Nat8l" or its downstreaming plays a pivotal role in psychiatric disorders. We recently found that overexpression of Shati/Nat8L in nucleus accumbens (NAc) regulates the dopaminergic neuronal system via the activation of group II mGluRs by elevated N-acetylaspartylglutamate following increased N-acetylaspartate [17].

There are some reports that in the prefrontal cortex, the group II mGluR2/3 antagonist is associated with the serotonergic neuronal system in depression-like behaviors [18-20]. However, functional effect of the dorsal striatum on depression-like behaviors is still unknown. In this study, we revealed the involvement of Shati/Nat8l via the serotonergic system in the vulnerability to social defeat stress by using depression model mice.

2. Material and Methods

2.1 Animals

Male C57Bl/6J mice (Nihon SLC) at 8 weeks of age 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 7:00 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-23, 2018PHA-5).

2.2 Drugs

LY341495 was purchased from Tocris Bioscience (Bristol, UK) and dissolved in 0.125 M phosphate buffer (pH 8.0). Fluvoxamine was purchased from Sigma-Aldrich (St.Louis, Missouri) and dissolved in saline. Other drugs were obtained from standard commercial sources. For behavioral experiments, the doses of LY341495 (0.03 mg/kg, i.p. or 2.12 ng/kg/site (direct brain infusion)) and fluvoxamine (10 mg/kg, i.p. or 0.32 μg/kg/site (direct brain infusion)) were determined based on the maximum dose that did not affect the locomotor activity in wild-type mice. These drugs were administrated 30 minutes prior to each behavioral experiment [17, 21].

2.3 Production and Microinjection of the AAV Vector and Microinjection of Retrograde tracing Beads

The method used for the production and microinjection of AAV vector has been previously reported [22, 23]. Briefly, the AAV vector plasmids contained an expression cassette that included the CMV promoter and cDNA encoding, either 3'-6xHis-tagged Shati/Nat8l (NM_001001985.3) or EGFP sequence. The recombinant AAV-

Shati/Nat8l or AAV-Mock vectors were produced by co-transfection of the AAV plasmid, AAV3 rep/AAV9 vp expression plasmid, and pHelper (Agilent Technologies; Santa Clara, CA) in HEK293 cells. The study was approved by the Board of Safety Committee for Recombination DNA Experiments of the University of Toyama (G2015PHA-12).The suspension of AAV-Shati/Nat8l or AAV-Mock vectors (1×10^{10}–10^{12} units) were injected bilaterally into the dorsal striatum of anesthetized mice (0.5 mm anterior and 2.0 mm lateral from bregma, 3.5 mm below the skull surface) by referring to a reference image [24]. Four weeks after the microinjection, the mice were used for experiments. Retrograde tracing RetroBeadsTM (Lumafluor; Durham, NC) were injected in the same area of the dorsal striatum.

2.4 Quantitative RT-PCR

Whole brains were removed and divided into 1-mm-thick sections using a mouse brain matrix (Brainscience Idea; Osaka, Japan). The tissues were collected with a 1-mm punch from the relevant section referencing to the plates of the mouse brain atlas [24]. 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 have been previously reported [25].

2.5 Immunostaining of mice brains

Immunostaining was performed as previously described [26]. The frozen brains were cut into 30 μm sections. After fixation, permeabilization, washing, and blocking steps, sections were incubated with primary antibodies (anti-NeuN, 500 times dilution MBL, Nagoya, Japan; anti-His, 500 times dilution MBL, Nagoya, Japan) with

10 % goat serum in TBS-T at 4 °C overnight, washed with TBS-T, and then incubated with CFTM 594 goat anti-mouse IgG (H + L) (1000 times dilution Biotium, Hayward, CA) and CFTM 488 goat anti-rabbit IgG (H + L) (1000 times dilution; Biotium, Hayward, CA) at 25 °C for 2 h. After being washed, the sections were mounted using Fluoromount (Diagnostic BioSystems, Pleasanton, CA).

2.6 Chronic social defeat stress

Chronic social defeat stress (CSDS) was performed as previously described [5]. Briefly, single-housed, ICR-retired mice (4-5 months of age) were screened for aggressive behavior. Experimental mice (C57BL/6J mice; subjects) were exposed to a novel ICR aggressor for 10 min each day for 10 consecutive days. After each 10 min physical interaction, subjects and aggressors were separated by a perforated translucent divider, allowing sensory but not physical contact during the next 24 h. Control mice, housed two mice per cage, were separated by a perforated translucent divider and rotated daily. Experimental mice and undefeated control mice were single-housed immediately after the last defeat session and tested for depression-like behaviors.

2.7 Microdefeat stress

The microdefeat stress (MDS) protocol was performed as previously described [5]. Briefly, a C57BL/6J mouse is subjected to subthreshold levels of social defeat that consist of three 5-min defeat sessions given consecutively on a single day with 15 min of rest between each session. Social interaction is then tested 24 h later and does not produce any significant avoidance in wild-type C57BL/6J mice.

2.8 Social interaction test

Social interaction test was performed as previously described [5,27]. All social interaction testing was performed under light conditions. Mice were placed in a new interaction open-field area custom crafted from opaque plexiglass (40 cm × 40 cm × 30 cm) with a small animal cage placed at one end. Their movements were then automatically monitored and recorded for 2.5 min in the absence (target absent phase) of a new ICR mouse, and measured the time spent in the interaction zone with the target absent. This phase was used to determine baseline exploratory behavior. Mice were returned to their home cage for 30 s. We then immediately recorded and monitored 2.5 min of exploratory behavior in the presence of a caged ICR mouse (target present phase), and measured the total distance traveled and duration of time spent in the interaction and corner zones. Social interaction behavior was then calculated as total time spent in each zone or as a ratio of the time spent in the interaction with the target present divided by the time spent in the interaction zone with the target absent. All mice were classified as resilient (social interaction ratio > 1.0), and susceptible (social interaction ratio < 1.0).

2.9 Sucrose preference test

To determine whether mice acquired anhedonic responses to any experimental manipulations, we performed a standard sucrose preference assay. Immediately after the final CSDS or MDS session or the final microdefeat bout, mice had their standard water bottle removed and replaced with two 15-ml conical tubes with sipper tops filled with water. After a 24-h habituation period, water from one 15-ml conical tube was replaced with 1 % sucrose. All tubes were weighed and mice were allowed 12 h to drink. Tubes were then re-weighed and their

locations in the wire tops were switched before a second 12-h period of drinking. At the end of the sucrose testing, sucrose preference was calculated as the total amount of sucrose consumption divided by the total amount of fluid consumed.

2.10 Direct infusion of drugs into striatum and dorsal raphe nucleus

Under anesthetic conditions, the guide cannula (AG-4, Eicom, Kyoto, Japan) was implanted into the dorsal striatum of mice (0.5 mm anterior and 2.0 mm lateral from bregma, 3.5 mm below brain surface) or dorsal raphe nucleus (-4.3 mm anterior from bregma, 2.0 mm below brain surface). After 6 days, the mice were exposed to microdefeat stress. Then, 24 h later, the drugs or ringer solution (147 mM NaCl, 4 mM KCl, and 2.3 mM CaCl2, 0.5 μl/site) were infused into the striatum by inserting the broken dialysis probe (A-I-4-01, Eicom, Kyoto, Japan) into the guide cannula. We also used the injector EPS-64 micro syringe pump (EICOM, Kyoto, Japan) for infusion.

2.11 Statistical analysis

All data are expressed as the mean ± S.E.M. Statistical differences between the two groups were determined with a Student's *t*-test or Chi-squared test. Correlation was calculated using Pearson's *r*. Statistical differences among values for individual groups were determined by two-way analysis of variance (ANOVA), followed by the Bonferroni's post-hoc test (Prism version 5).

3. Results

3.1 Shati/Nat8l expression in the dorsal striatum induced by chronic social defeat stress.

In the first experiment, to investigate whether Shati/Nat8l expression is regulated by chronic social defeat stress (CSDS), we quantified Shati/Nat8l mRNA levels in male mice immediately after the social interaction tests. As shown in Fig. 1a and b, compared to unstressed control mice, social interaction time was significantly reduced in CSDS-treated mice ($p = 0.008$), and time in corner zone was also significantly increased in CSDS-treated mice ($p = 0.022$). In CSDS-treated mice, Shati/Nat8l mRNA levels were significantly increased in the dorsal striatum (Fig. 1c) ($p = 0.00015$) and correlated with social behavior (Fig. 1d) ($r = -0.410$, $p = 0.0417$). This upregulation was not observed in other brain regions, such as the prefrontal cortex, nucleus accumbent, and hippocampus (Fig. 1e).

3.2 Vulnerability and increased susceptibility against chronic social defeat stress induced by overexpression of Shati/Nat8l in the dorsal striatum.

Following the above results, we produced Shati/Nat8l overexpressed in the dorsal striatum mice (AAV-Shati mice) using the microinjection technique. As shown in Fig. 2a, His-positive cells (green) were detected in the dorsal striatum of AAV-Shati mice (red signals indicated NeuN-positive cells). We also measured NAA content in the dorsal striatum tissue using High performance liquid chromatography (HPLC) methods. NAA exhibited an increase in the AAV-Shati mice compared with the AAV-Mock injected mice (AAV-Mock mice) (Fig. 2b) ($p = 0.015$). While there is no difference between AAV-Mock and AAV-Shati in both social interaction test and sucrose preference test without social defeat stress, AAV-Shati mice with social defeat showed significantly lower interaction compared with AAV-Mock mice (Fig. 2c) ($p = 0.035$). Furthermore, the ratio

of susceptible mice significantly increased in Shati mice compared with Mock mice (Fig. 2d) ($p = 0.002$).

3.3 Susceptibility to subthreshold social defeat (microdefeat) stress (MDS) increased by overexpression of Shati/Nat8l on dorsal striatum.

As Shati mRNA was induced in the dorsal striatum of susceptible mice, and overexpression of Shati protein on the dorsal striatum induced vulnerability to CSDS, we next investigated whether genetic induction of Shati on the dorsal striatum confers stress susceptibility. Four weeks after virus injection, the mice underwent the MDS, which consisted of three consecutive 5 min physical interaction sessions followed by 15 min sensory contact across a perforated divider. The interaction time to a novel social target of AAV-Shati mice, which underwent MDS, was reduced significantly compared with AAV-Mock mice and non-stressed control mice (Fig. 3a). A two-way ANOVA demonstrated a significant main effect of virus ($F_{(1,60)} = 5.371$, $p = 0.0239$) and significant main effect of stress ($F_{(1,60)} = 5.943$, $p = 0.0178$). A significant interaction effect ($F_{(1,60)} = 4.676$, $p = 0.0346$) was observed. A pairwise comparison using a Bonferroni post hoc test resulted in a significant difference, $p < 0.05$ when comparing the AAV-Shati MDS group to all other groups ($p = 0.0081$, non-stress Mock; $p = 0.0145$, microdefeat Mock; $p = 0.0113$, non-stress Shati). These mice also showed lower sucrose preference (Fig. 3b). A two-way ANOVA demonstrated a significant main effect of stress ($F_{(1,32)} = 11.07$, $p = 0.002$) and significant main effect of virus ($F_{(1,32)} = 6.609$, $p = 0.0150$). A significant interaction effect ($F_{(1,32)} = 4.591$, $p = 0.0399$) was observed. A pairwise comparison using a Bonferroni post hoc test resulted in a significant difference, $p < 0.01$, when comparing the AAV-Shati MDS group to all other groups ($p = 0.0010$, non-stress Mock; $p = 0.0078$, microdefeat Mock; $p = 0.0024$, non-stress Shati).

3.4 Depression-like behavior of AAV-Shati MDS mice rescued by intraperitoneal injection of anti-depressant and mGluR3 antagonist.

We also assessed whether the vulnerability to depression in AAV-Shati MDS mice was attenuated by the administration of the antidepressants, fluvoxamine or the group II mGluR2/3 antagonist, LY341495 (LY). The administration of fluvoxamine (FLX) (10 mg/kg i.p.) and LY341495 (0.03 mg/kg, i.p.) 30 min prior to the social interaction test attenuated the decreased interaction time (Fig. 3c) ($p = 0.0035$, vehicle Mock; $p = 0.0245$, LY Mock; $p = 0.0264$, FLX Mock; $p = 0.0233$, LY Shati; $p = 0.0143$, FLX Shati). A two-way ANOVA demonstrated a significant main effect of virus ($F_{(1,48)} = 4.401$, $p = 0.0412$), a significant interaction effect ($F_{(2, 48)} = 4.676$, $p = 0.0093$), and sucrose preference (Fig. 3d) of the AAV-Shati MDS mice ($p < 0.0001$ to other groups). A two-way ANOVA demonstrated a significant effect of the drugs ($F_{(2,62)} = 10.47$, $p = 0.0001$), a significant main effect of virus ($F_{(1,62)} = 8.535$, $p = 0.0049$), and a significant interaction effect ($F_{(2, 62)} = 10.84$, $p < 0.0001$).

Thus, these pharmacological results suggest that depression-like behaviors in the AAV-Shati mice are induced by the dysfunction of the serotonergic neuronal system and the activation of group II mGluR-mediated neurotransmission.

3.5 Depression-like behavior of AAV-Shati MDS mice rescued by direct infusion of anti-depressant, but not mGluR3 antagonist, into the striatum.

To clarify which brain region is effective against depression-like behavior, we also assessed whether the

vulnerability to depression in AAV-Shati MDS mice attenuated by the direct infusion of fluvoxamine or LY341495. The direct infusion of FLX (0.008μg/0.5 μl/side), but not of 0.3 μM LY341495, attenuated the decreased interaction time (Fig. 4a);a two-way ANOVA demonstrated a significant main effect of virus ($F_{(2,61)}$ =13.51, p = 0.0005) and a significant interaction effect ($F_{(2, 61)}$ = 3.446, p = 0.0382) of the AAV-Shati MDS mice (p = 0.0077, Ringer Mock; p = 0.0307, FLX Shati). To clarify which part of the brain LY341495 affected, we focused on the dorsal raphe nucleus. The dorsal striatum receives projections from the serotonergic neurons and supplies GABAergic projections to the serotonergic neurons in the dorsal raphe nucleus [28-30]. Firstly, we confirmed their existence. As shown in Fig. 4b and c, retrograde positive cells (Red color) and GFP positive cells were detected in the dorsal striatum (injection site) (Fig. 4b) and the dorsal raphe nucleus (projection site) (Fig. 4c). Then, we directly infused LY341495 into the dorsal raphe nucleus. The direct infusion of LY341495 into the dorsal raphe (0.5 μl) attenuated the decreased interaction time (Fig. 4d) (p = 0.0245, Ringer Mock; p = 0.0104, LY Shati) of the AAV-Shati MDS mice. A two-way ANOVA demonstrated a significant main effect of stress ($F_{(1,28)}$ = 6.667, p = 0.0153) and significant main effect of virus ($F_{(1,28)}$ = 4.966, p = 0.0341). A significant interaction effect ($F_{(1,28)}$ = 5.445, p = 0.020) was observed.

4. Discussion

In this study, we generated a depression model of mice by exposing social defeat stress. The social defeat stressed mice showed not only depression-like behavior in the forced swim and tail suspension test [31], but also various physiologic changes such as weight loss, loss of sucrose preference, and loss of sociability [32]. Furthermore, it was reported that such dysregulations as social behaviors were restored by the tricyclic antidepressant, imipramine, and the selective serotonin reuptake inhibitor, FLX [33]. The social defeat stressed mice are model animals indicating the condition of a patient of the depression, and it may be useful for the elucidation of the condition.

In the present study, expression of Shati/Nat8l mRNA increases only in the dorsal striatum that exposed CSDS. It was reported that expression of Shati/Nat8l in the nucleus accumbens is dependent on the restraint action [17], but it is thought that this was because the input of the dopamine nerves from the ventral tegmental area to the nucleus accumbens affects the dependence formation [34]. Alternatively, it was thought that the part of the brain reactive to the stress of Shati/Nat8l expression is the striatum as it is a part of the basal nuclei and is important to the adjustment of emotional behaviors.

A recent study proposed the 2-Hit hypothesis, the combination of a hereditary factor and an environmental factor [35] in the development of a mental disease and that vulnerability to the onset of a mental disease is because of a genetic factor (mental disease-related genes; 1st Hit) and an environmental factor (stress in the prenatal or juvenile period; 2nd Hit) and it is thought that the mental disease developed is revealed later by a new stress. This is the microdefeat method, which we used as one of the methods to examine the genetic factor using social defeat stress. This method examines the weakness in social interaction by revealing the degree of

subthreshold social defeat stress at which social interaction does not decrease in normal mice compared to the gene expression mice operated on using virus vectors [5]. In this study, locally overexpressed Shati/Nat8l mice showed vulnerability to subthreshold stress in the social interaction and sucrose preference tests. These results indicate the possibility that overexpression of Shati/Nat8l in the striatum became one of the genetic factors of the onset of depression.

It has been demonstrated that mice lacking serotonin in the brain hereditarily showed weakness for subthreshold social defeat stress [36]. In this study, the decrease in social interaction and sucrose preference caused by subthreshold social defeat stress recovered with intraperitoneal administration of the SSRI, fluvoxamine in figure 3c and d. These results indicate that overexpression of Shati/Nat8l in the dorsal striatum controls serotonin release and therefore, influences the vulnerability to onset of depression-like behavior. Direct infusion of fluvoxamine also rescued the decrease in social interaction in figure 4a. It was suggested that serotonin release in the dorsal striatum is important to influences the vulnerability to onset of depression-like behavior. It is reported that there were no changes in levels or functionality of serotonin-receptors at the level of dorsal raphe nucleus neurons in socially stressed rats that show behavioral symptoms of depression [37]. It was also correspondence with our result which show the importance of serotonin in the striatum, not dorsal raphe nucleus. Furthermore, the decrease in social interaction and sucrose preference caused by subthreshold social defeat stress recovered with intraperitoneal administration of the mGluR3 antagonist, LY341495. From these results, it was suggested the decreased in social interaction and sucrose preference caused by Shati/Nat8l overexpression via the mGluR3 pathway. As shown in Fig. 3c and 4a, however, intraperitoneal administration of LY341495, but not direct infusion of LY341495, attenuated the decreased

interaction time of the AAV-Shati MDS mice. It means that LY341495 did not affect in the striatum but other brain area, and control the serotonin release in the dorsal striatum. Therefore, to clarify which part of the brain contribute to LY341495 function, we focused on the dorsal raphe nucleus because the dorsal striatum receives projections from the serotonergic neurons and supplies GABAergic projections to the serotonergic neurons in the dorsal raphe nucleus [28-30]. We did direct infusion of LY341495 into the dorsal raphe nucleus. Direct infusion of LY341495 into the dorsal raphe nucleus completely attenuated the decreased interaction time of the AAV-Shati MDS mice. These results mean the overexpressed Shati/Nat8l in the dorsal striatum is regulating serotonin release via projection neurons in the dorsal raphe nucleus. Serotonergic neurons in the dorsal raphe nucleus regulated the GABAergic interneuron monosynaptically [38]. Considering our results, the synaptic mechanism of mGluR3-mediated serotonergic neuronal modulation in the striatum should be investigated to elucidate the neuronal mechanism of the raphe nucleus in major depressive disorder.

Although we showed mainly the effects of the serotonergic systems on the depressive-like behavior, in our study, other neuronal systems also effect on the depressive-like behavior. It is reported that fluvoxamine and LY341495 affected on dopaminergic signaling [17,39], and it is showed that dopaminergic signal is important for depression-like behaviors [9]. We might need to study the other neuronal systems on the depression-like behavior in the future study.

In conclusion, this study is the first to demonstrate that the overexpression of Shati/Nat8l in the dorsal striatum induces vulnerability to depression-like behavior. Our results indicate that the regulation of mGluR3 signaling in dorsal raphe-mediated control of the serotonergic neuronal system in the dorsal raphe plays a pivotal role in the pathogenesis of depression disorder. Therefore, Shati/Nat8l and its downstreaming of

mGluR3 neurotransmission may be potential new targets for the development of pharmacotherapies for major depressive disorder.

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Competing interests

The authors declare no competing financial interests.

Author contributions

KU and AN conceived the projects. KU, HM, and TM designed and conducted the experiments. HM, KU, KS, and TF conducted the experiments. SM provided the AAV-Shati vectors. KU wrote the draft of the manuscript and AN confirmed the final version.

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Figure legends

Fig. 1 Region specific regulation of Shati/Nat8l by chronic social defeat stress (CSDS)**.**

(a) Social interaction test was performed after the chronic social defeat stress (CSDS). There was a significant difference in time in interaction zone between the two groups under the condition that ICR mice were present. **p < 0.001 vs control mice (Student's *t*-test). CSDS included resilient and susceptible groups. Social interaction test and tissue collection were performed 24 h and 48 h after the last social defeat session, respectively. (b) There was a significant difference in time in corner zone between the two groups under the condition that ICR mice were present. *p < 0.05 vs control mice (Student's *t*-test). (c) Shati/Nat8l mRNA in the dorsal striatum was increased 24 h after the social interaction tests. ***$p < 0.005$ vs control mice (Student's *t*-test). (d) Shati/Nat8l mRNA expression in the dorsal striatum of CSDS-treated was strongly correlated with social avoidance behavior (Pearson's *r*). (e) Shati/Nat8l mRNA in the prefrontal cortex (PFC), nucleus accumbens (NAc), or hippocampus (Hip) were not changed 24 h after CSDS compared with control. The number of the mice used in the experiments are included in each figure.

Fig. 2 Vulnerability to depression-like behavior in chronic social defeat stress induced by overexpression of Shati/Nat8l on the dorsal striatum.

(a) Immunohistochemistry revealed that Shat/Nat8l (His-positive cells, green) was overexpressed in the dorsal striatum of the AAV-Shati mice (NeuN-positive cells, red). (b) The content of NAA was increased in the dorsal

striatum of the AAV-Shati mice compared with that in the AAV-Mock mice. The tissue content of NAA was evaluated using HPLC. *$p < 0.05$ vs AAV-Mock (Student's *t*-test). (c) Social interaction test was performed after the chronic social defeat stress. This score was measured under the condition that ICR mice were present. There was a significant difference between the two groups. *p < 0.05 vs AAV-Mock. (d) Proportion of susceptible and resilient mice (AAV-Mock and AAV-Shati) by evaluation of the social interaction test. There was a significant difference between the two groups. p < 0.005 vs AAV-Mock. (Chi-squared test). The number of the mice used in the experiments are included in the figure.

Fig. 3 Vulnerability to depression-like behavior in microdefeat stress induced by overexpression of Shati/Nat8l on the dorsal striatum.

(a) Social interaction test was performed after the microdefeat stress. Time in the interaction zone was significantly decreased in AAV-Shati mice that were exposed to microdefeat stress. There was no significant decrease of time in the interaction zone in AAV-Mock-none-stress, AAV-Mock-microdefeat, and AAV-Shati-none-stress mice. *$p < 0.05$ vs. all other groups. (b) Sucrose preference test was performed 24 h after the social interaction test. The ratio of sucrose solution intake to total intake was significantly decreased in AAV-Shati mice that were exposed to microdefeat stress. There was no significant decrease of time in the interaction zone in AAV-Mock-none-stress, AAV-Mock-microdefeat, and AAV-Shati-none-stress mice. **$p < 0.01$ vs. all other groups. (c) Mice were injected fluvoxamine (10 mg/kg i.p.), LY341495 (0.03 mg/kg, i.p.), or vehicle 30 min before the social interaction test. Treatment of fluvoxamine and LY341495 recovered decreased time in the

interaction zone in AAV-Shati mice which were exposed to microdefeat stress. *$p < 0.05$ vs. AAV-Mock-vehicle mice, #$p < 0.05$ vs. AAV-Shati-vehicle mice. (d) Treatment of fluvoxamine and LY341495 recovered decreased sucrose preference in AAV-Shati mice which were exposed to microdefeat stress. *$p < 0.05$ vs. AAV-Mock-vehicle mice, ###$p < 0.005$ vs. AAV-Shati-vehicle mice. The number of the mice used in the experiments are included in the figure.

Fig. 4 Regulation of serotonergic neuron via mGluR3 in the dorsal raphe by Shati/Nat8l overexpressed striatal projection neurons.

(a) Mice were directly infused fluvoxamine (50 μM, 0.5 μl/side (0.32 μg/kg/site)), LY341495 (0.3 μM, 0.5 μl/side (2.12 ng/kg/site)) or ringer solution to dorsal striatum 30 min before the social interaction test. Treatment of fluvoxamine, but not LY341495, recovered decreased time in the interaction zone in AAV-Shati mice which were exposed to MDS. **$p < 0.01$ vs. AAV-Mock-Ringer mice, #$p < 0.05$ vs. AAV-Shati -Ringer mice. Dorsal striatum microinjection of AAV-Mock vector and retrograde beads showed EGFP florescent and red fluorescent in the (b) dorsal striatum and projection site of (c) dorsal raphe. (d) Mice were directly infused LY341495 (0.3 μM, 0.5 μl (2.12 ng/kg)) or ringer solution to the dorsal raphe 30 min before the social interaction test. Treatment of LY341495 recovered decreased time in the interaction zone in AAV-Shati mice which were exposed to MDS. **$p < 0.01$ vs. AAV-Mock-Ringer mice, #$p < 0.05$ vs. AAV-Shati-Ringer mice. The number of the mice used in the experiments are included in the figure.