Chapter 6
Shati/Nat8l and *N*-acetylaspartate (NAA) Have Important Roles in Regulating Nicotinic Acetylcholine Receptors in Neuronal and Psychiatric Diseases in Animal Models and Humans

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Abstract Shati/Nat8l was originally isolated as a methamphetamine-related-molecule from the nucleus accumbens of mice. Since then, Shati/Nat8l has been characterized as an *N*-acetyltransferase-8-like protein (Nat8l) that catalyzes *N*-acetylaspartate (NAA) synthesis from aspartate and acetyl-coenzyme A. It has been shown that elevated NAA levels detected by proton magnetic resonance spectroscopy (^{1}H-MRS) brain imaging indicates increased neuronal activity. Our group produced Shati/Nat8l knock out mice (Shati/Nat8l KO mice), which exhibit hyper locomotion, anxiety behaviors, and social dysfunction. These mice have a high sensitivity to methamphetamine, as evidenced by their results in assessments of locomotor activity and conditioned place preference, as well as their elevated dopamine levels. We used an adeno-associated virus (AAV) vector containing *Shati/Nat8l* (AAV-*Shati/Nat8l*) to overexpress the protein in different brain regions such as the striatum and the nucleus accumbens, in order to investigate their involvement in methamphetamine-induced behavioral and pharmacological changes. We showed that overexpression of accumbal Shati/Nat8l attenuates methamphetamine-induced behaviors.

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Recent clinical studies have revealed further novel roles of Shati/Nat8l in psychiatric and neuronal diseases. We are just beginning to appreciate the various actions of this intriguing, recently discovered molecule in the central nervous system.

Keywords Shati/Nat8l · Methamphetamine · Addiction · Depression · Alzheimer's disease · ATP

6.1 Introduction

Shati is a molecule originally isolated from the nucleus accumbens of mice that had received repeated administration of methamphetamine (Niwa et al. 2007, 2008). Shati was later identified as an *N*-acetyltransferase-8-like protein (Nat8l) and was found to catalyze *N*-acetylaspartate (NAA) synthesis from aspartate and acetyl-coenzyme A (Ariyannur et al. 2010) (Fig. 6.1). We renamed the novel molecule from Shati to Shati/Nat8l following this finding. NAA is present at high concentrations in the central nervous system and is combined 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 (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; Fig. 6.1). A postmortem study showed that the levels of NAA and NAAG are significantly lower in the brains of subjects with major depressive disorder, schizophrenia and bipolar disorder (Reynolds and Reynolds 2011). In patients with pre-Alzheimer's disease, NAA levels are significantly reduced in the cingulate gyrus. A clinical study using magnetic resonance spectroscopy (MRS) showed that NAA was significantly increased in adult patients with autism spectrum disorder when compared with a control group (Aoki et al. 2012). Together, these observations suggest that the NAA synthetase, Shati/Nat8l, may play important roles in psychiatric, neurodegenerative, and neurodevelopmental disorders.

Furthermore, treatment of a neuroblastoma-derived cell line with a physiological level of NAA resulted in apoptosis of cancerous cells and enhanced neuronal differentiation (Mazzoccoli et al. 2016). NAA is of unique clinical significance and hence is exploited in MRS. Treatment of an SH-SY5Y neuroblastoma-derived cell line with sub-cytotoxic physiological concentrations of NAA has been shown to inhibits cell growth (Mazzoccoli et al. 2016). This effect is partly due to enhanced apoptosis, which is indicated by a decrease in the anti-apoptotic factors survivin and Bcl-xL, and partly due to the arrest of the cell-cycle progression, linked to enhanced expression of the cyclin-inhibitors p53, p21Cip1/Waf1, and p27Kip1 (Mazzoccoli et al. 2016)**.** NAA-pre-treated SH-SY5Y cells were more sensitive to the cytotoxic effect of the chemotherapeutic drugs cisplatin and 5-fluorouracil (Mazzoccoli et al. 2016).

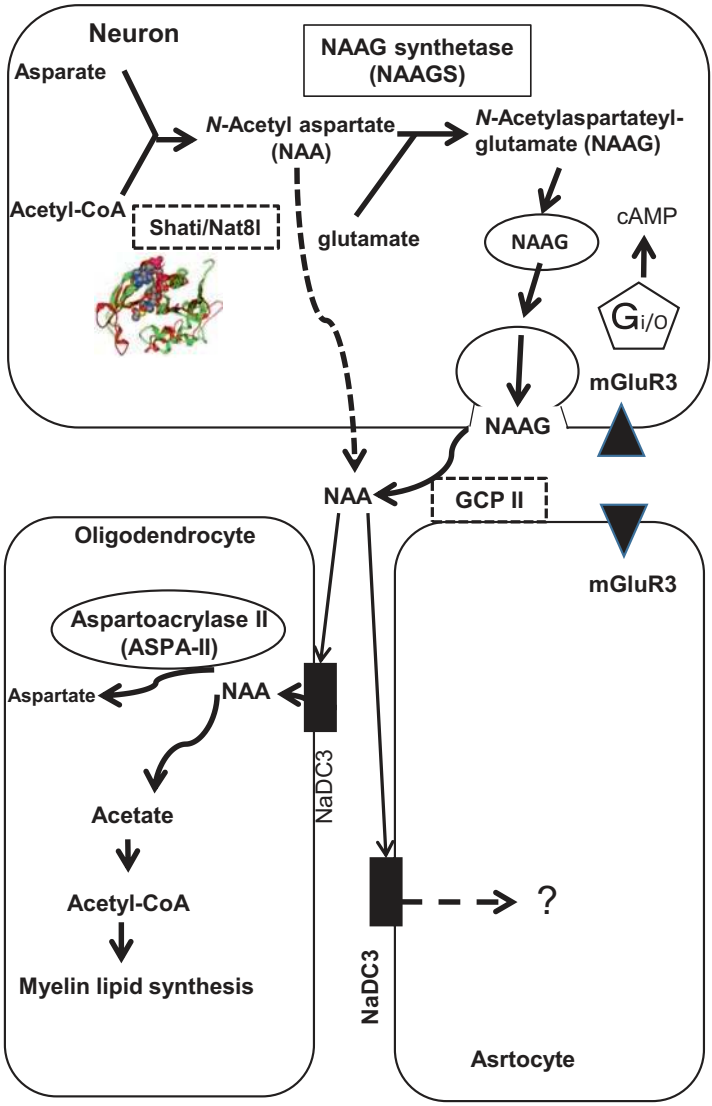

Fig. 6.1 Schematic overview of Shati/Nat8l function. *Shati/Nat8l* catalyzes the *N*-acetylation of aspartate, forming *N*-acetylaspartate (NAA). The condensation of NAA and glutamate is catalyzed by *N*-acetylaspartylglutamate (NAAG) synthetase (NAAGS). NAAG is released from nerve terminals most likely via synaptic vesicles. The transporter responsible for the translocation of NAAG into synaptic vesicles is unknown. Released NAAG can be degraded by glutamate carboxypeptidase II (GCP-II), membrane-bound enzymes mainly expressed by astrocytes, liberating NAA and glutamate (Moffett et al. 2007). NAAG may also bind to metabotropic glutamate receptor 3 (mGluR3) on presynaptic membranes and astrocytes. mGluR3 is coupled to a G_i protein and negatively coupled to adenylyl cyclase (Conn and Pin 1997). The NAA transporter, sodium-dependent dicarboxylate (NaDC3), is expressed by astrocytes and oligodendrocytes (Huang et al. 2000). In oligodendrocytes, NAA can be degraded by aspartoacylase II (ASPA-II, liberating aspartate and acetate (Moffett et al. 2007). The released acetate may be used for lipid synthesis by myelinating oligodendrocytes (Burri et al. 1991; Namboodiri et al. 2006). To what extent NAA is taken up by astrocytes *in vivo* or its metabolic fate in these cells is unclear

In this review, we will introduce the various functions of Shati/Nat8l and NAA in psychiatric behaviors, especially addictive behaviors, and summarize their roles in neuronal and psychiatric diseases.

6.2 Shati/Nat8l and Drug Reward

6.2.1 Function of Accumbal Shati/Nat8l in Nicotinic Effects

We previously reported that overexpression of Shati/Nat8l in the nucleus accumbens (NAc) of mice depressed the pharmacological effects of methamphetamine, especially addiction-related behaviors, hyperactivity, and place preference. In an in vivo microdialysis experiment, the extracellular dopamine (DA) level was increased by 200–300% of the base line by peripheral methamphetamine injection. In the NAc of these mice, NAA and NAAG levels were significantly reduced (Miyamoto et al. 2014). The mGluR3 antagonist, LY341495, cancelled the Shati-Nat8l-indeuced reduction of hyperlocomotion and conditioned place preference after methamphetamine treatment. Furthermore, LY341495 also cancelled the Shati/Nat8l-associated increase in extracellular DA levels in the NAc of methamphetamine-treated mice. These results indicate that the overexpression of Shati/Nat8l in the NAc suppresses the increase in dopamine release caused by methamphetamine *via* mGluR3 (Miyamoto et al. 2014).

We also investigated the effects of Shati/Nat8l on nicotine preference using a three-bottle paradigm. Experiments were performed as follows: I. Both NAc-Mock and NAc-Shati/Nat8l overexpressing mice were habituated to an experimental chamber which contained three water bottles on days 1–3. II. The tap water in all three bottles was changed to 2% saccharin to habituate animals to saccharin on days 4–6. III. The contents of all three bottles were replaced with a mixture of 75 μg/mL and 2% saccharin on days 8–14 (Fig. 6.2). During the subsequent test phase, three bottles containing either 0 μg/mL nicotine +2% saccharin, 75 μg/mL nicotine +2% saccharin or 150 μg/mL nicotine +2% saccharin, were presented to each mouse, and the total intake of nicotine was measured in each mouse on days 15–21 (Fig. 6.2). The daily amounts of nicotine intake are shown in Fig. 6.3a. On the first day of the test phase, an average of 300 μg of nicotine was consumed by both groups. In the NAc-Mock mice, the amount of nicotine consumed increased each day during days 15–17 (Fig. 6.3a). In contrast, the Shati/Nat8l mice showed a lower intake of nicotine enriched solution during this period (Fig. 6.3a). These results suggest that overexpression of Shati/Nat8l in NAc lowers nicotine intake and preference. The 2% of saccharin were essential for the experimental protocol, since nicotine also has aversive effects due to its bitter taste. While overexpression of Shati/Nat8l in NAc lowered nicotine preference during days 16–17 (Fig. 6.3a), total intake (water and nicotine solution) was not changed (Fig. 6.3c, d).

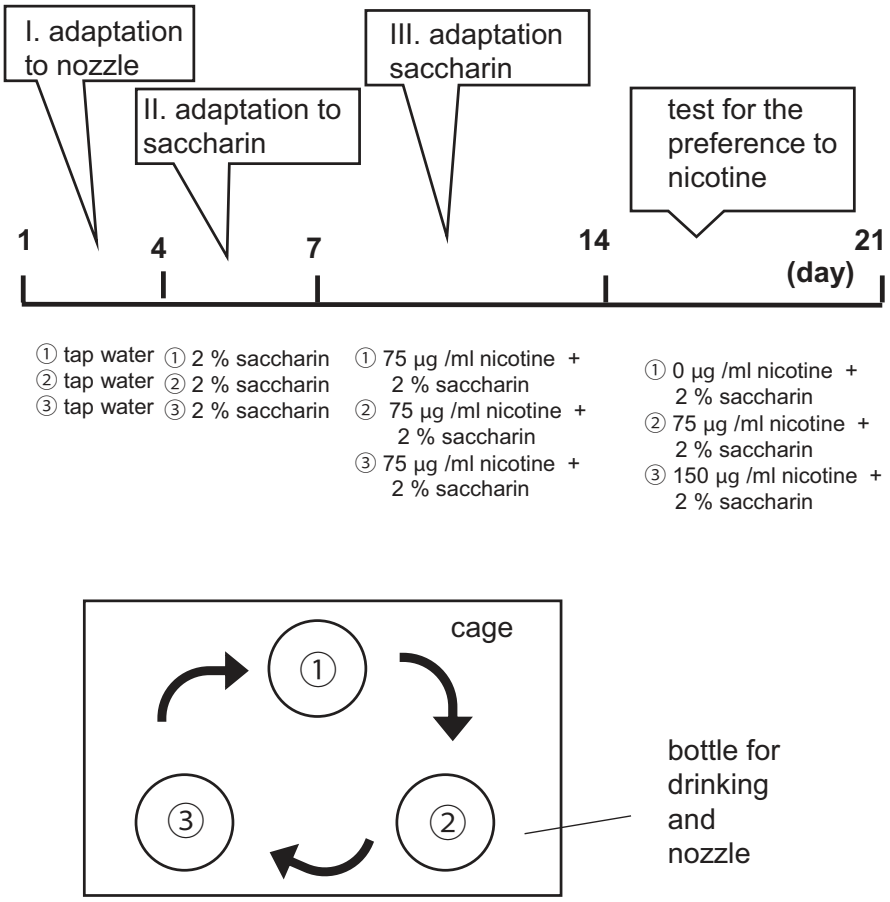

Fig. 6.2 Procedure for the three bottle test to evaluate nicotine preference. I. Both NAc-Mock and NAc-Shati/Nat8l mice were habituated to an experimental chamber which contained three water bottles on days 1–3. II. The tap water in all three bottles was changed to 2% saccharin to habituate animals to saccharin on days 4–6. III. The contents of all three bottles were replaced with a mixture of 75 μg/mL nicotine and 2% saccharin on days 8–14. During the test phase, three bottles containing either 0 μg/ mL nicotine +2% saccharin, 75 μg/mL + 2% saccharin or 150 μg/mL + 2% saccharin were presented to each mouse, and the total intake of nicotine was measured in each mouse on days 15–21

Next, we performed in vivo microdialysis experiments to measure the amount of extracellular DA induced by nicotine in the system (Fig. 6.4a). Basal levels of extracellular DA in the NAc of Shati/Nat8l-overexpressing mice were significantly lower than those of NAc-Mock mice (Fig. 6.4b). In the NAc-Mock mice group, the extracellular DA level was significantly increased 60–120 min after nicotine injection, up to a level of approximately 170% of that of the saline injection. Nicotine-injected NAc-Shati/Nat8l mice showed low levels of extracellular DA at the 60–120 min time point similar to saline-injected groups (Fig. 6.5). Furthermore, the suppressive

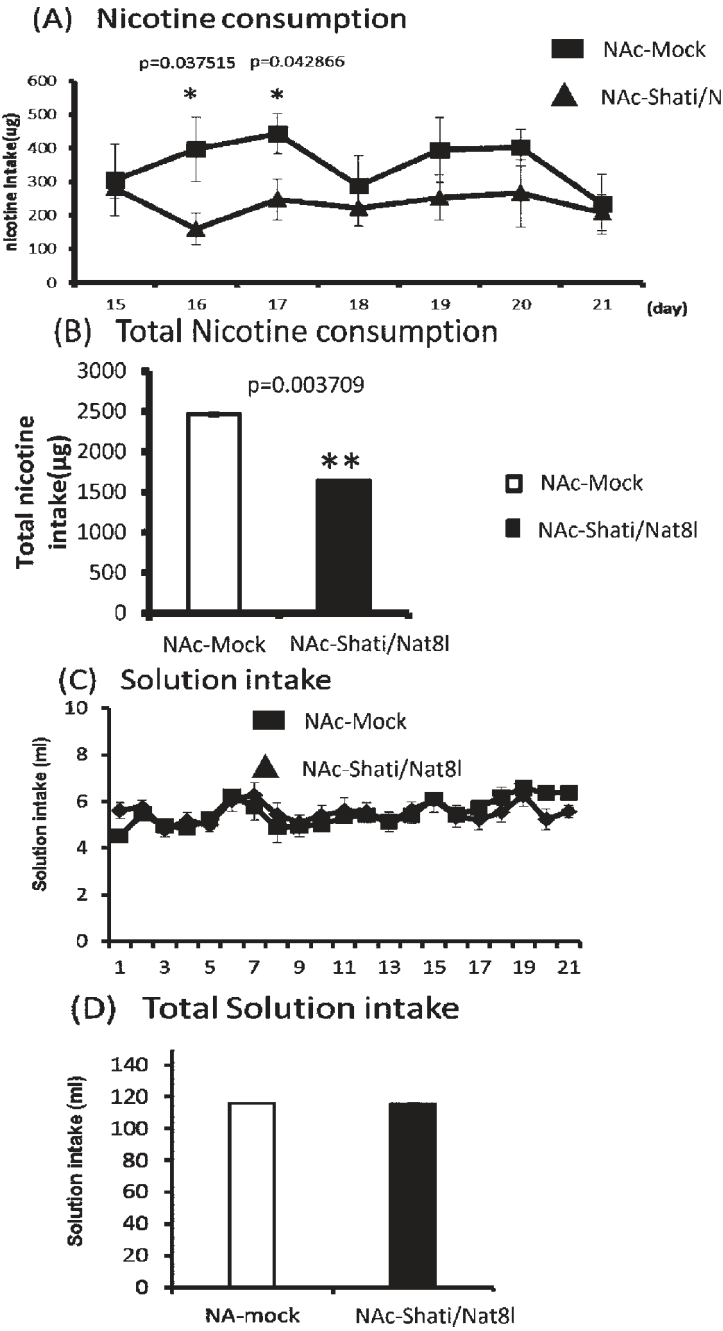

Fig. 6.3 Effects on nicotine preference of overexpression of Shati/Nat8l in NAc of mice. (**a**) Daily nicotine consumption in the test phase from days 15–21. (**b**) Total nicotine consumption during the test phase. (**c**) Daily solution intake in the test phase. (**d**) Total solution intake during the test phase

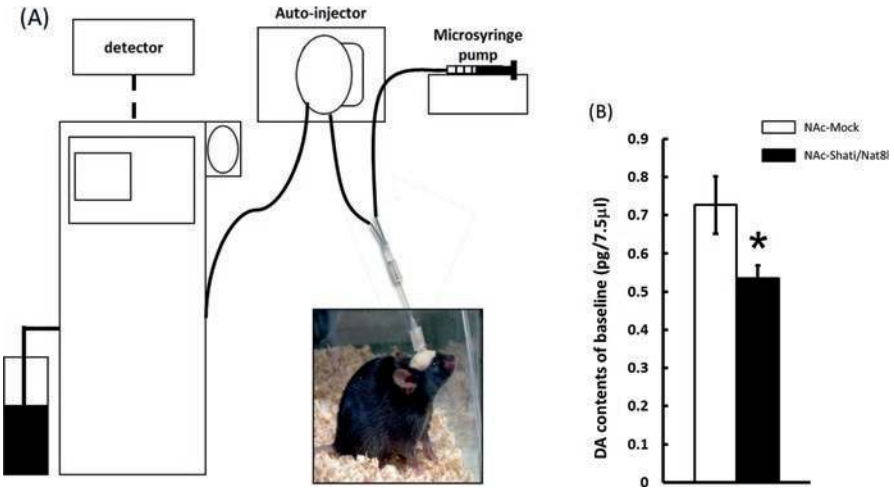

Fig. 6.4 Apparatus for in vivo microdialysis and quantification of basal DA levels. (**a**) Apparatus for in vivo microdialysis to measure dopamine (DA). (**b**) Effects of Shati/Nat8l overexpression in the basal levels of extracellular DA. Each column represents the mean ± S.E.M. n = 10, *P < 0.05 vs. NAc-Mock, Student's t-test)

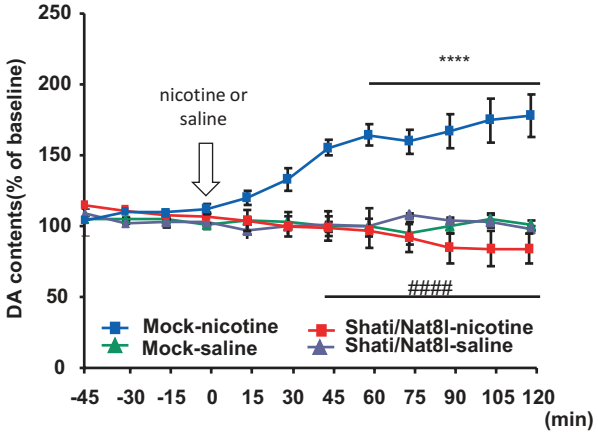

Fig. 6.5 Effect of Shati/Nat8l overexpression on nicotine (NIC)-induced dopamine (DA) release in the NAc. At 0 min, NAc-Shati/Nat8l and NAc-Mock mice were injected with either nicotine (0.4 mg/kg as free base) or saline (sal) subcutaneously. Each value represents the mean ± S.E.M. (n = 5, ****P < 0.001 vs. Mock-sal group, #P < 0.05 vs. Mock-NIC group, two- way repeated measure ANOVA with Bonferroni's post hoc test)

effects of Shati/Nat8l on nicotine-induced DA elevation were partially reversed by LY341495, an mGluR2/3 antagonist (Fig. 6.6). These results indicate that the function of Shati/Nat8l to suppress the effect of nicotine-induced extracellular DA in the NAc is partially dependent on mGluR3. These results are similar to those showing

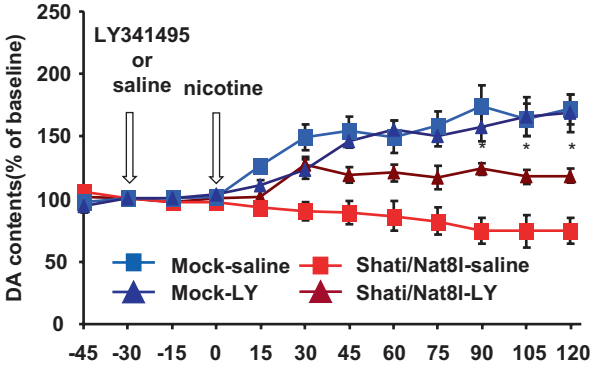

Fig. 6.6 The suppressant effect of Shati/Nat8l overexpression on nicotine-induced dopamine (DA) elevation was partially blocked by the mGluR2/3 antagonist, LY341495. At −30 min, NAc-Shati/Nat8l and NAc-Mock mice were injected with the mGluR2/3 antagonist, LY341495 (LY, 0.1 mg/kg, *i.p.*). Mice were injected with nicotine (NIC) (0.4 mg/kg, s.c.) 30 min after LY341495 injection. Each value represents the mean ± SEM. (n = 5, *P < 0.05 vs. Shati/Nat8l-Saline group, two-way repeated measures ANOVA with Bonferroni post hoc test)

the action of Shati/Nat8l against the pharmacological effects of methamphetamine. One difference is the complete or partial contribution of mGluR3 in methamphetamine and nicotine use, respectively. Both nicotine and methamphetamine regulate the DA reward system in the brain. Methamphetamine directly alters dopamine uptake and release, while nicotine first binds to the nicotinic acetylcholine receptor (nAChR) and, following signal transduction contributes to the potentiation of DA release. The suppressive effects of Shati/Nat8l on the DA release might happen downstream of the mGluR3 pathway. Further studies are required to discern whether there is a mechanistic crossover between the pharmacological actions of nicotine and methamphetamine. We attempted to investigate *Shati/Nat8l* mRNA changes in the NAc, the hippocampus and the frontal cortex, related to reward pathways following single or repeated treatments with nicotine. Unfortunately, we could not reproduce the results of the mRNA measurements, potentially because the levels of Shati/Nat8l or NAAG do not change based on the activation of nAChR. In relation to Shati/Nat8l production, methamphetamine and nicotine most likely act through different pathways.

Taken together, our results show that Shati/Nat8l in the NAc has a protective effect against the deleterious physiological changes associated with nicotine or methamphetamine administration.

6.2.2 Striatal Shati/Nat8l and the Reward System

We produced Shati/Nat8l transgenic mice (*Shati/Nat8l*-Tg) to investigate global overexpression in the brain. A targeting vector was used to produce the *Shati/Nat8l*-Tg mice, which ubiquitously expressed his-tagged *Shati/Nat8l* gene. A transgene

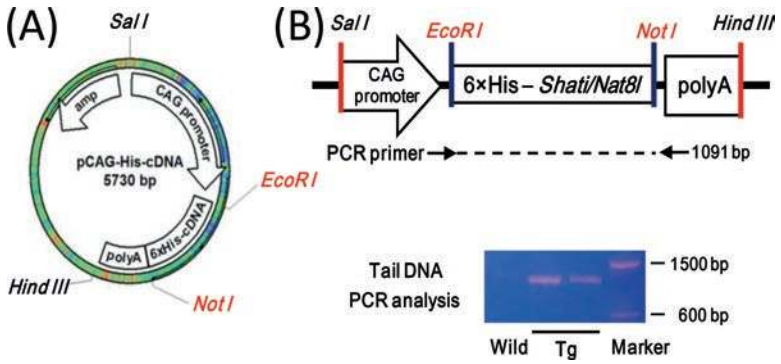

Fig. 6.7 Genetic scheme of *Shati/Nat8l*-Tg mice. (**a**) Targeting vector used to produce *Shati/Nat8l*-Tg mice. Transgenic mice ubiquitously expressing the His-tagged *Shati/Nat8l* gene were produced by Unitech (Chiba, Japan). Briefly, the transgene cassette including the CAG promoter followed by the his-*Shati/Nat8l* sequence was obtained from the CAG promoter his-*Shati/Nat8l* expression plasmid. The transgene cassette was microinjected into fertilized eggs from C57BL/6J females mated with males. (**b**) Genotyping results of *Shati/Nat8l*-Tg mice using a wild type mouse

cassette including the CAG promoter followed by the his-*Shati/Nat8l* sequence was obtained from a CAG promoter the his-*Shati/Nat8l* expression plasmid (Fig. 6.7a). The transgene cassette was microinjected into fertilized eggs from C57BL/6 J females mated with males. Genotyping confirmation of *Shati/Nat8l*-Tg mice using a wild type mouse as a control is shown in Fig. 6.7b. *Shati/Nat8l* mRNA levels were measured by quantitative real-time reverse transcriptase (RT)-PCR in the brain and are presented relative to the expression of the housekeeping gene, 36B4. *Shati/Nat8l* mRNA was highly expressed in the whole brain of 8-week-old transgenic mice (Fig. 6.8). However, *Shati/Nat8l* mRNA levels were increased in relation to wild type expression in the striatum only, not in other brain regions, such as the olfactory bulb, the prefrontal cortex or the NAc (Fig. 6.9). These *Shati/Nat8l*-Tg mice were therefore used for striatal Shati/Nat8l-overexpression mice. The mice showed no differences in basal locomotor activity compared to wild-type mice when observed in a novel environment (Fig. 6.10). These mice also performed a Y maze task as well as novel object recognition tests, to assess their learning abilities (Fig. 6.11). In the Y maze task, neither the number of total entries nor spontaneous alternation behaviors were different between *Shati/Nat8l*-Tg and wild type mice (Fig. 6.12). In the novel recognition test, both time and trial exploratory preference (in %) were similar between wild type and *Shati/Nat8l*-Tg mice (Fig. 6.12). Next, anxiety-like emotional behaviors in *Shati/Nat8l*-Tg mice were investigated using the light/dark box test, and no difference in the time spent on the light side was observed between *Shati/Nat8l*-Tg mice and wild type mice (Fig. 6.13). Results from both the light/dark box and elevated plus maze tests indicated no effect of increased levels of striatal Shati/Nat8l on anxiety-like behaviors. We also investigated the social abilities of the *Shati/Nat8l*-Tg mice. The experimental schedule of the three-chamber social interaction test is shown in Fig. 6.13a. In trial 1, the test mouse was placed in the

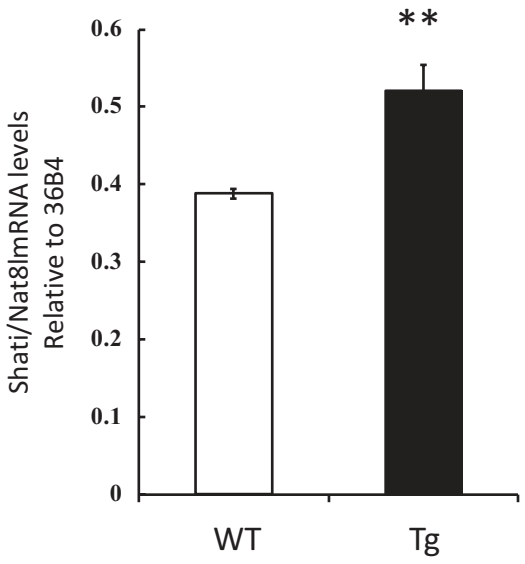

Fig. 6.8 Expression of *Shati/Nat8l* mRNA in the whole brain of *Shati/Nat8l*-Tg mice. *Shati/Nat8l* mRNA levels were measured by quantitative real-time RT-PCR in the brain and are presented as relative to the expression of the housekeeping gene, 36B4. Values are mean ± SEM (n = 5 mice/group, $^{**}P < 0.01$ vs. wild type group, Student's t-test)

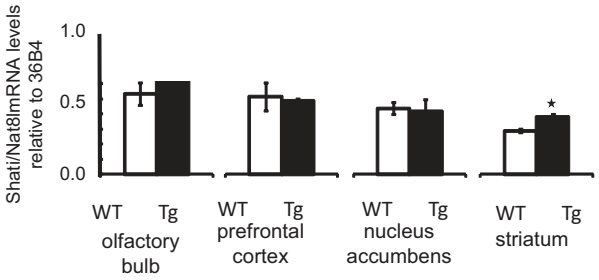

Fig. 6.9 Expression of *Shati/Nat8l* mRNA in various brain regions of *Shati/Nat8l*-Tg mice. *Shati/Nat8l* mRNA levels were measured by quantitative real-time RT-PCR in the brain and are presented as relative to 36B4. Values are mean ± SEM (n = 3 mice/group, $^{*}P < 0.05$ vs. wild type group, Student's t-test)

center of the chamber, while the other side of the chamber remained empty and no wire cage was placed. Both *Shati/Nat8l*-Tg and wild type mice were more interested in the novel object (Fig. 6.13b, left). In trial 2, another novel object was placed in the wire cage on one side, and an unfamiliar mouse (C57BL/6J) was placed in a wire cage on the opposite side. The *Shati/Nat8l*-Tg mice and wild type mice showed a similar level of interest in the unfamiliar mouse (Fig. 6.13b, right). In a prepulse inhibition (PPI) test, auditory startle response (Fig. 6.14a) and sensory motor control function (Fig. 6.14b) were not changed in *Shati/Nat8l*-Tg mice. Furthermore, in forced swimming and tail suspension tests, the immobility times of *Shati/Nat8l*-Tg mice were the same as those found for wild type mice (Figs. 6.15 and 6.16). These

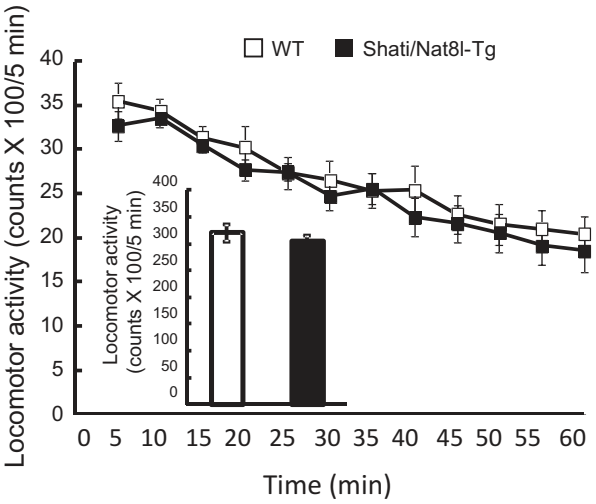

Fig. 6.10 Locomotor activity in *Shati/Nat8l*-Tg mice. No difference in basal locomotor activity was observed in a novel environment between *Shati/Nat8l*-Tg mice and wild type mice. Values are mean ± SEM (n = 10 or 14 mice/group)

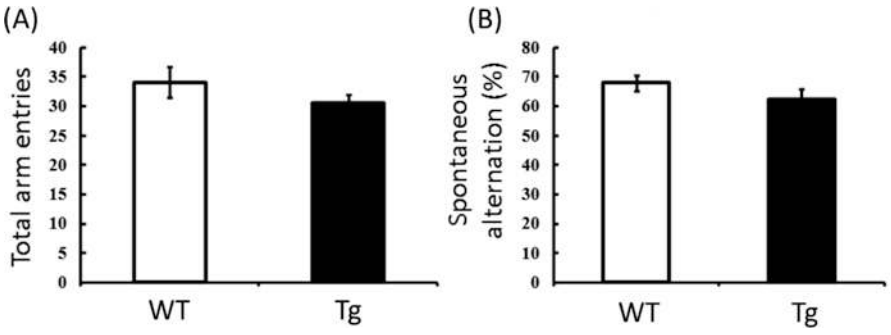

Fig. 6.11 Spontaneous alternation behavior in *Shati/Nat8l*-Tg mice. There was no difference in (**a**) total arm entries or (**b**) spontaneous alternation behavior in the Y-maze test observed between *Shati/Nat8l*-Tg mice and wild type mice. Values are mean ± SEM (n = 10 or 14 mice/group)

results indicate *Shati/Nat8l*-Tg mice do not demonstrate schizophrenia or depression-like behaviors.

Shati/Nat8l-Tg mice were not stable for the overexpression of Shati/Nat8l in the striatum, depending on their generation. In general, mouse lines generated with transgenes are often not stable, since transecting genes is a process that does not happen naturally. Therefore, to measure the effect of increased striatal Shati/Nat8l on the reward system, we injected an EGFP-tagged AAV containing *Shati/Nat8l* into the striatum of mice (Str-Shati/Nat8l) and confirmed localized overexpression by *in situ* hybridization and EGFP immunohistochemistry in the dorsal striatum

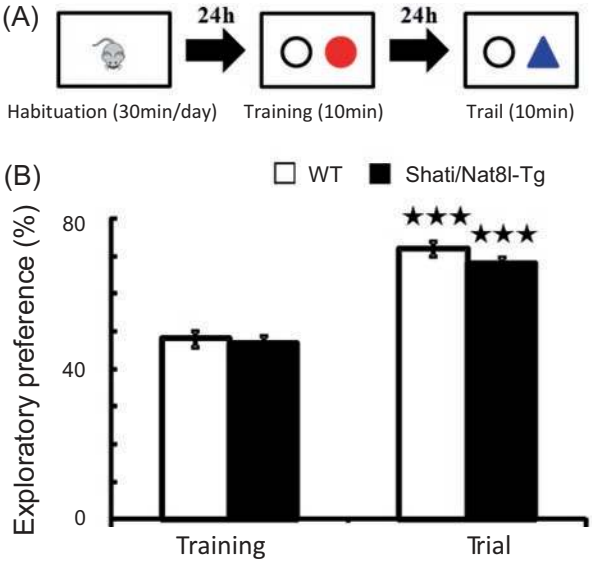

Fig. 6.12 Novel object recognition in *Shati/Nat8l*-Tg mice. (**a**) Experimental schedule of the novel object recognition test. (**b**) No difference in exploratory preference in the novel object recognition test was observed between *Shati/Nat8l*-Tg mice and wild type mice. Values are mean ± SEM (n = 10 or 14 mice/group, $^{**}P < 0.001$ vs. training phase for each group, Student's t-test)

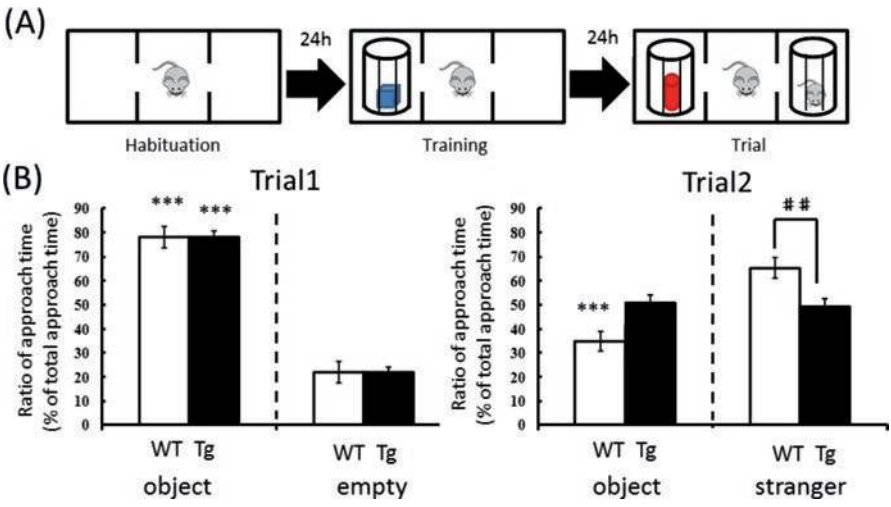

Fig. 6.13 Social interaction in *Shati/Nat8l*-Tg mice. (**a**) Experimental schedule of three chamber social interaction test. (**b**), left A novel object was placed in a wire cage on one side of the chamber, and no wire cage was placed on the other side of the chamber. Both *Shati/Nat8l*-Tg mice and wild type mice were more interested in the novel object. Values are mean ± SEM (n = 10 or 14 mice/group, $^{***}P < 0.001$ vs. empty side for each group, Student's t-test). WT: wild type, Tg: transgenic. (**b**), right Another novel object was placed in a wire cage on one side of the chamber, and an unfamiliar mouse (C57BL/6 J) was placed in a wire cage on the other side of the chamber. *Shati/Nat8l*-Tg mice were not more interested in the unfamiliar mouse than the wild type group. Values are mean ± SEM (n = 10 or 14 mice/group, $^{***}P < 0.001$ vs. unfamiliar mouse, $^{\#\#}P < 0.01$ vs. wild type group, Student's t-test)

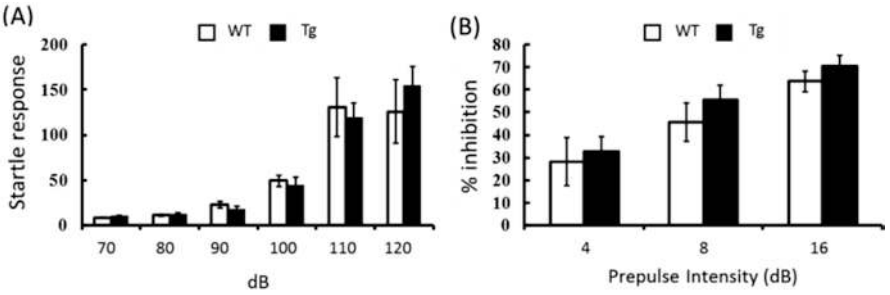

Fig. 6.14 Startle response and PPI in *Shati/Nat8l*-Tg mice. **a** Startle response was measured for 70, 80, 90, 100, 110 and 120 dB (background noise: 70 dB). Values are mean ± SEM. **b** PPI was measured with 4, 8 and 16 dB of prepulse intensity (background noise: 70 dB). Values are mean ± SEM (n = 8 or 13 mice/group)

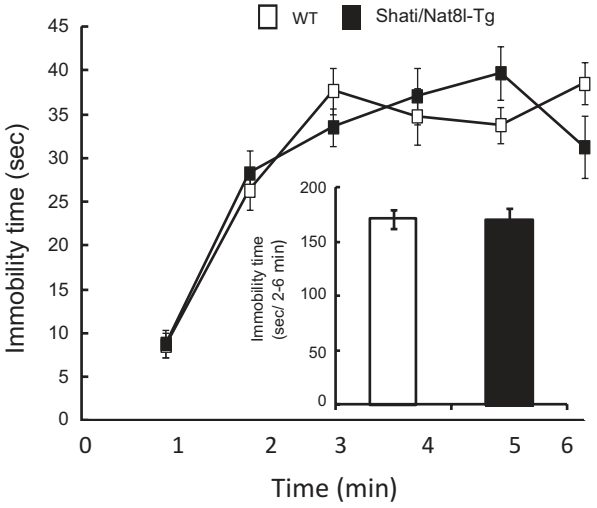

Fig. 6.15 Depressive behavior in *Shati/Nat8l*-Tg mice in the tail suspension test. No difference in immobility time was observed during the tail suspension test between *Shati/Nat8l*-Tg mice and wild type mice. Values are mean ± SEM (n = 10 or 14 mice/group)

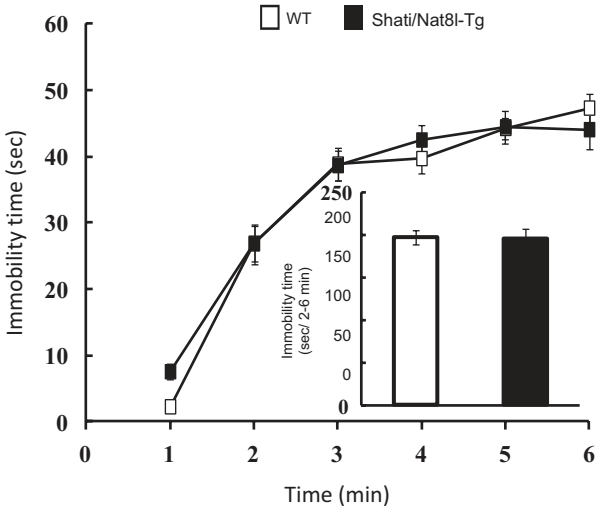

Fig. 6.16 Depressive behavior in *Shati/Nat8l*-Tg mice in the forced swimming test. No difference in immobility time was observed in the forced swimming test between *Shati/Nat8l*-Tg mice and wild type mice. Values are mean ± SEM (n = 10 or 14 mice/group)

area. Str-Shati/Nat8l mice showed no difference in methamphetamine-induced hyperactivity compared to mock-injected mice (Fig. 6.17a). Methamphetamine-induced conditioned place preference was also not changed in Str-Shati/Nat8l mice (Fig. 6.17b). Therefore, we conclude that Shati/Nat8l in the striatum does not contribute to reward effects in mice.

6.3 Shati/Nat8l in Learning and Memory

6.3.1 Hippocampal Shati/Nat8l in Learning and Memory

Shati/Nat8l produces NAA from acetyl-coA and aspartic acid, and represents one of the amino acids with the highest-concentration amino acids in the brain. Recently, a change in NAA concentration measured by MRS was proposed as a biomarker for early-stage Alzheimer's (Murray et al. 2014). This suggests that Shati/Nat8l and/or NAA are related in some way to the onset of Alzheimer's disease. However, learning ability was not changed in Y maze and novel object tests conducted in Shati/Nat8l KO mice (Furukawa-Hibi et al. 2012). We overexpressed Shati/Nat8l in the hippocampus *via* AAV injection (Hip-Shati/Nat8l), and conducted learning and memory tests. In the novel object trial session, Hip-Shati/Nat8l mice preferred to explore the novel object for a significantly prolonged time compared to mock injection mice (Fig. 6.18). This result suggests that cognitive ability might be potentiated by the overexpression of Shati/Nat8l. Murray et al. (2014) and Guo et al. (2017)

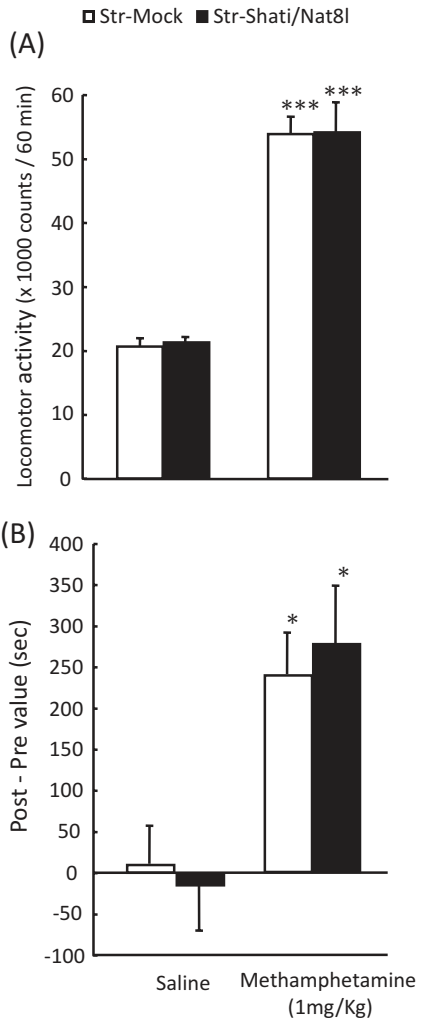

Fig. 6.17 Pharmacological changes to locomotor activity and place conditioned preference induced by methamphetamine. No difference was seen in (**a**) the locomotor activity after treatment with methamphetamine (1 mg/kg) or (**b**) conditioned place preference with methamphetamine (1 mg/kg) between Str-Mock and Str-Shati/Nat8l

demonstrated that a change in NAA level is a clinical marker for Alzheimer's disease, although these authors looked at only the posterior cingulate gyri, the inferior precunei and the posterior cingulate cortex. Proton magnetic resonance spectroscopy (^{1}H-MRS) showed low NAA/creatine ratios in the posterior cingulate cortices of patients with Alzheimer's disease, which is a typical region for the accumulation of amyloid beta. Vulnerability to shifts in NAA is not the same in all brain regions in patients with Alzheimer's disease. Our results using Hip-Shati/Nat8l mice are partly in agreement with these clinical observations. There are also other clinical studies that measure NAA levels in relation to Alzheimer's disease using the MRS technique (Zhong et al. 2014; Zhang et al. 2015). Further studies are required to

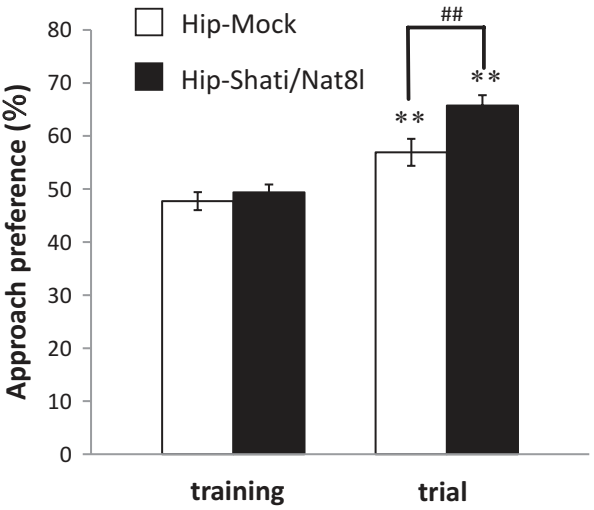

Fig. 6.18 Enhanced approach preference to novel object in Hip-Shati/Nat8l mice in the novel object recognition test. Hip-Shati/Nat8l mice significantly preferred to approach the novel object compared to Hip-mock mice. (**P < 0.01 vs. trial for each group, ##P < 0.01 vs Hip-Mock group, Student's t-test)

investigate changes in Shati/Nat8l itself in the brain of patients with Alzheimer's disease, since Shati/Nat8l has also functions, apart from being synthetase for NAA.

6.3.2 Function of Accumbal Shati/Nat8l on Learning Memory and Emotional Behaviors

We have shown that nicotine reversed scopolamine-induced impairment in the passive avoidance task in rats through its action on the dopaminergic neuronal system (Nitta et al. 1994). The study did not mention which brain areas are important for the nicotine-potentiated learning ability. If altered DA levels triggered by Shati/Nat8l overexpression had any effect on cognitive function, *Shati/Nat8l*-Tg mice would show potentiated cognitive function over normal mice. However, their cognitive functions were not changed in Y maze or novel object tests (Figs. 6.8 and 6.9).

6.3.3 Function of Shati/Nat8l in Axon Outgrowth

In vitro studies were performed to evaluate the function of Shati/Nat8l in neuronal cells, since *Shati/Nat8l* mRNA signals were observed in all brain regions of mice (Sumi et al. 2015). Especially strong signals were found in cortical pyramidal cells,

dentate granule cells, hippocampal pyramidal cells, and cerebellar granule cells. Neuronal cells of all brain regions showed positive *Shati/Nat8l* mRNA signals. Hippocampal neurons were selected for their easy evaluation and high cell density. *Shati/Nat8l* mRNA positive cells colocalized with NeuN (a marker for neurons)-positive cells but not with GFAP (a marker for astrocytes)- or Iba1 (a marker for microglia)-positive cells. These results show that *Shati/Nat8l* mRNA is only expressed in neuronal cells of the mouse hippocampus. An AAV vector containing *Shati/Nat8l* was transfected into primary cultured mouse neurons. Overexpression of Shati/Nat8l in primary cultured neurons induced axonal growth but not dendrite elongation. Treatment with a selective group II mGluR antagonist did not eliminate Shati/Nat8l-induced axon outgrowth, and NAAG itself did not induce axon outgrowth. Overexpression of Shati/Nat8l also increased the ATP content in the cultured hippocampal neurons. These results suggest that neuronal Shati/Nat8l induces axon outgrowth via ATP synthesis independently of the mGluR3 signaling pathway. Shati/Nat8l is associated with microtubule structure when overexpressed in COS7 cells and primary mouse cultured neurons (Toriumi et al. 2013). On the other hand, it was also reported that Shati/Nat8l co-localizes with a mitochondrial marker in SH-SH5Y cells (Ariyannur et al. 2010), and that Shati/Nat8l is localized in the endoplasmic reticulum (Wiame et al. 2009). Shati/Nat8l may have novel functions in neuronal cells. NAA is produced in the mitochondria because it is associated with the tricarboxylic acid cycle (TCA) related to cell metabolism (Madhavarao et al. 2003). The levels of NAA and ATP were increased in primary cultured neurons overexpressed with Shati/Nat8l. The TCA cycle produces ATP molecules at the highest rate in terms of cell metabolism, and ATP in the growth cone is known to promote neurite elongation in cultured neurons (Höpker et al. 1996). Neuronal dendrite length in Shati/Nat8l KO mice was significantly shorter than in wildtype mice (Berent-Spillson et al. 2004). Shati/Nat8l appears to play a major role in ATP-induced neurite elongation. Shati/Nat8l is an indicator of the stimulation of mGluR3. However, neither NAAG nor LY341495, the endogenous agonist of mGluR3 and an antagonist of mGluR3, respectively, affected axon outgrowth. Shati/Nat8l is thus associated with neurite elongation and the ATP synthesis pathway during NAA synthesis.

6.4 Shati/Nat8l and Psychiatric Disease

6.4.1 Patients with Depression and NAA

NAA is used as a biomarker of depression in specific regions of the human brain as it is an indicator of neuronal activity in ^{1}H–MRS. NAA is significantly decreased in the anterior cingulate gyrus of patients with depression. Brain-derived neurotrophic factor (BDNF) has been reported to be one of the biomarkers for patients with depressive (Rogóż et al. 2017; Zhao et al. 2016). Anti-depressant drugs rescue the reduction in NAA, in addition to BDNF. Interestingly, *BDNF* mRNA is increased in

the prefrontal cortex, the NAc and the hippocampus in the brains of Shati/Nat8l KO mice (Furukawa-Hibi et al. 2012). Therefore, the changes in Shati/Nat8l and BDNF are not parallel, but independent. Although detailed mechanisms for the roles of Shati/Nat8l and/or BDNF in depression have not been clarified, they may nevertheless serve as reliable clinical markers.

6.4.2 *Shati/Nat8l and Depressive Behaviors in Mice*

Miyamoto et al. (2017) also demonstrated that striatal Shati/Nat8l induces depressive behaviors. We produced mice overexpressing Shati/Nat8l in the striatum (Str-Shati/Nat8l mice) using an AAV vector, as described earlier. The Str-Shati/Nat8l mice showed depression-like behaviors in forced swimming and tail suspension tests (Miyamoto et al. 2017) as measured by prolonged immobility, indicating that Shati/Nat8l is an inducer of depression. These dysfunctions are cancelled by the anti-depressant drug, fluvoxamine, and the mGluR3 antagonist, LY341495. Shati/Nat8l levels may be a factor in the vulnerability to stress. In the psychiatric field, mental diseases are often caused by two factors, genetic background and circumstance, and striatal Shati/Nat8l might thus represent one of the genetic factors.

6.4.3 *Shati/Nat8l and Postpartum Depression*

Postpartum depression is observed in about 13% of postpartum women, and is defined as a depressive disorder leading to a substantial impairment of daily life. It also has large impacts on the patients' families, including the promotion of depressive tendencies in the husband, the abuse or neglect of the child and/or delayed cognitive development or increased psychopathological issues in the child (Stumbo et al. 2015).

The occurrence of postpartum depression has been related to fluctuations in the levels of steroid hormones and glucocorticoids (cortisol in humans, corticosterone in animals) occurring during pregnancy and after delivery. Mice that received chronic ultra-mild stress (CUMS, procedure described in Fig. 6.19) demonstrated similar symptoms to the depression symptoms related to pregnancy in humans. Pregnant female mice subjected to CUMS could be a model for human postpartum depression, since the mice show prolonged immobility in forced swimming and tail suspension tests and anhedonia against sucrose (Shang et al. 2016). The dams exposed to CUMS during gestation showed depression-like behavior during their postpartum period. *Shati/Nat8l* mRNA expression was increased in the striatum of the dams, but not in the NAc or the frontal cortex (Fig. 6.20). The relationship between *Shati/Nat8l* in the striatum and postpartum depression-like behavior induced by CUMS was investigated. The dams that overexpressed *Shati/Nat8l* spe-

	Morning (11:30 to 12:30)	Afternoon (14:30 to 16:30)	Night (18:30 to 8:30)
Monday	Confinement	Cage tilt (30°)	Soiled cage
Tuesday	Cage tilt	Paired housing	Overnight illumination
Wednesday	Cage tilt	Confinement	Soiled cage
Thursday	Confinement	Paired housing	Cage tilt
Friday	Confinement	Cage tilt	Reversed light/dark cycle
Weekend	Reversed light / dark cycle	Reversed light/dark cycle	Reversed light/dark cycle

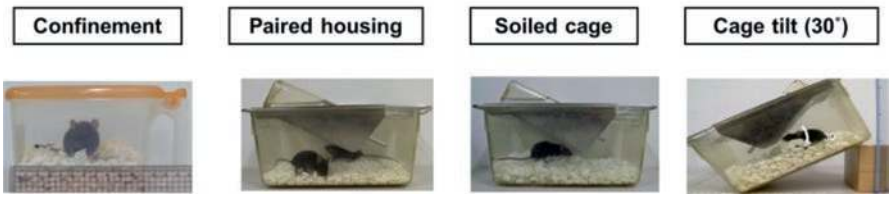

Fig. 6.19 Chronic ultramild stress protocol. The chronic ultramild stress (CUMS) regimen consisted of five ultramild stressors: period of cage tilt (30°), confinement in a small box (11 × 8 × 8 cm), paired housing, one overnight period with soiled cage (50 ml water on 1 L paper pellet) and permanent light. The animals were also placed on a reversed light/dark cycle from Friday evening through the next Monday. This procedure was scheduled over a 1-week period and repeated throughout the period from the time of separation from the male until parturition

cifically in the bilateral striatum showed increased sensitivity to stress, and exacerbated depression-like symptoms such as despair behaviors.

We also carried out a clinical study among pregnant women. Serum concentrations of Shati/Nat8l were measured in pregnant womean during late pregnancy and at 5 days and 1 month after delivery, respectively. The women were divided into two groups, a non-depressive and a depressive group, based on their score on the Edinburgh Postnatal Depression Scale (EPDS). Serum concentrations of Shati/Nat8l were higher in the depressive group than in the non-depressive group at the time point before delivery (Nitta et al. unpublished data). Serum concentrations of Shati/Nat8l at the late pregnancy stage could be one predictive biomarker for postnatal depression, and the appropriate preventive and early interventions might thus be undertaken for pregnant and postpartum women. We also measured serum concentrations of Shati/Nat8l in these women after delivery, but observed no significant differences.

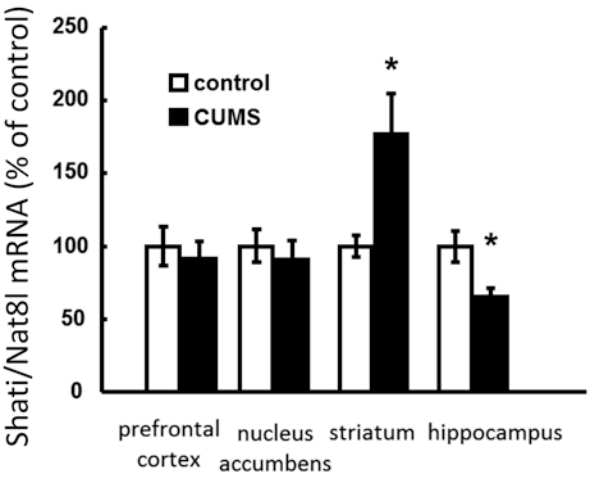

Fig. 6.20 Expression of *Shati/Nat8l* mRNA in CUMS mice. The changes in *Shati/Nat8l* mRNA expression in various brain regions of stressed postpartum mice and control mice. (*P < 0.05 vs control group in each brain region)

6.5 Conclusions

Shati/Nat8l and NAA have various functions in the central nervous system. In addition to the neuronal system, Shati/Nat8l is also highly expressed in the adipose tissue, and NAA pathway could similarly serve as an acetyl-CoA metabolizing mechanism in adipocytes (Pessentheiner et al. 2013). An increase in lipolysis followed by an activation of β-oxidation can restore acetyl-CoA back to the mitochondria. Lipid turnover can raise the oxidative potential of the brown fat cells and thereby boost the brown adipogenic phenotype. However, the physiological stimuli contributing to the regulation of the NAA pathway are unknown (Pessentheiner et al. 2013). These results suggest the possibility that Shati/Nat8l also plays a role in lifestyle diseases such as diabetes.

Because of their widespread occurrence in the human body, Shati/Nat8l and NAA have the potential to be used as treatment tools for a variety of diseases in the near future.

Conflicts of Interest The authors have declared that no competing interests exist.

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