

**Studies on mechanisms of depression and blunted circadian
rhythm in Spontaneously Diabetic Torii (SDT) fatty rats**

KATSUYA SAKIMURA

2019

Contents

Chapter 1 -----	1
General Introduction	
 Chapter 2 -----	7
Depression-related behavioural and neuroendocrine changes in the SDT fatty rat, an animal model of Type 2 Diabetes Mellitus	
 Chapter 3 -----	26
SDT fatty rat, a novel animal model of type 2 diabetes mellitus, shows blunted circadian rhythms and melatonin secretion	
 Chapter 4 -----	43
A novel Na ⁺ -independent alanine-serine-cysteine transporter 1 inhibitor inhibits both influx and efflux of D-serine	
 Chapter 5 -----	63
General Discussion	
 Acknowledgements -----	68
 References -----	69

List of Abbreviations

Abbreviation	Term
Aanat	Arylalkylamine-N-acetyltransferase
ACPP	(1)-amino(1-(3,5-dichlorophenyl)-3,5-dimethyl-1H-pyrazol-4-yl)acetic acid
ANOVA	Analysis of variance
Asc-1	Alanine-serine-cysteine transporter 1
ASCT1	Alanine-serine-cysteine transporter 1
ASCT2	Alanine-serine-cysteine transporter 2
Asmt	Acetylserotonin O-methyltransferase
BBB	Blood-brain barrier
cDNA	Complementary deoxyribonucleic acid
ChCl	Choline chloride
CHO	Chinese Hamster Ovary
CNS	Central nervous system
CRSWD	Circadian rhythm sleep-wake deficit
CSF	Cerebrospinal fluid
DCS	D-cycloserine
DEX	Dexamethasone
DM	Diabetes Mellitus
DSM-5	Diagnostic and Statistical Manual of Mental Disorders-5

Abbreviation	Term
DST	Dexamethasone suppression test
ELISA	Enzyme-linked immunosolvent assay
FST	Forced swimming test
GABA	Gamma-aminobutyric acid
GK	Goto-Kakizaki
hAsc-1	Human asc-1
HIP	Human islet amyloid polypeptide
HPA	Hypothalamic-pituitary-adrenal
LTP	Long-term potentiation
NaCl	Sodium chloride
MDD	Major depressive disorder
NA	Noradrenaline
NMDA	N-methyl-D-asparate
PFC	Prefrontal cortex
QOL	Quality of life
qRT-PCR	Quantitative reverse transcription polymerase chain reaction
RNA	Ribonucleic acid
SCN	Suprachiasmatic nucleus
SD	Sprague-Dawley
SDT	Spontaneously Diabetic Torii
SEM	Standard error of the mean

Abbreviation	Term
SLA	Spontaneously locomotor activity
SMLC	S-methyl-L-cysteine
STAR*D	Sequenced Treatment Alternatives to Relieve Depression
STZ	Streptozotocin
TC	Total cholesterol
TG	Triglyceride
T1DM	Type 1 Diabetes Mellitus
T2DM	Type 2 Diabetes Mellitus
WHO	World Health Organization
5-HT	5-hydroxytryptophane

Chapter 1

General Introduction

Overview of the mental diseases in worldwide

As population numbers and average age increase worldwide, so do the number of people with mental disorders. Mental health is defined by the World Health Organization (WHO) as “a state of well-being in which every individual realizes his or her own potential, can cope with the normal stresses of life, can work productively and fruitfully, and is able to make a contribution to her or his community” (WHO, a). Unfortunately, one in three individuals experience a disrupted state of well-being during their lifetime (Ginn and Horder, 2012; Steel et al., 2014). According to Marquez and Saxena (2016), the social costs of mental- and substance-use disorders are striking, with reported estimates of at least 10 percent of the world’s population affected. In addition to their health impact, mental disorders cause a significant economic burden. In Japan, the number of patients with mental disorders including neurodevelopmental disorder, major depressive disorder (MDD) and dementia is also increasing, and according to the estimation using a disability-adjusted life years estimate, MDD will be placed as second highest and dementia on third highest among all diseases by 2020 (Ministry of Health, Labor and Welfare of Japan, 2013). It is therefore clear that mental/ psychiatric disorders have medical needs that should be addressed as soon as possible. Recently, accumulating evidence suggests that there are the metabolic deficits underlying the pathophysiology of the mental disorders including MDD and the deficits of circadian rhythms. Here I overview the relationships between MDD, circadian rhythm deficit and the metabolic disorders as a representative of mental health disorders.

MDD and metabolic deficits

MDD is one of the most common and severe mental illnesses, with WHO estimates at 300 million individuals affected globally (WHO, b). In addition to mood symptoms, individuals with MDD experience impaired physical, occupational, and social functioning (Kessler et al., 2003). According to the Diagnostic and Statistical Manual of Mental Disorders-5 (DSM-5), individuals are diagnosed with MDD when five (or more) of the nine symptoms, including continuous depressed mood and recurrent suicidal ideation, have been present during the same two-week period (American Psychiatric Association, 2013). The most severe consequence of MDD is suicide, and up to 15% of individuals with recurrent MDD commits suicide (Kessler and Bromet, 2013; Whiteford et al., 2013). Suicidality (ideation, plans, and attempts) is a significant cause of injury and mortality worldwide, ranking as the 14th most common cause of death by the WHO (Nock et al., 2008). In Japan, more than 30,000 individuals commit suicide every year.

There are many approaches for the treatment of MDD, such as cognitive behavioral therapy, transcranial magnetic stimulation, and bright light therapy. However, the main treatment is drug therapy using antidepressants. Many antidepressants such as selective serotonin (5-hydroxytryptamine: 5-HT) reuptake inhibitor and serotonin noradrenaline (NA) reuptake inhibitor have been developed based on “monoamine hypothesis” – the impaired 5-HT or NA neurotransmission in the brain. However, these drugs take several weeks to exert their antidepressant effects in clinic. Furthermore, 30% patients with MDD do not respond to any treatment as revealed by the Sequenced Treatment Alternatives to Relieve Depression (STAR*D) Study funded by the National Institute of Mental Health (Rush et al., 2006; Sinyor et al., 2010). Thus, we are only beginning to understand the full complexity of the pathophysiology of MDD, with even

the detailed mechanisms underlying existing antidepressants yet to be revealed.

A large body of evidence gathered over the past two decades suggests close links between mental disorders including MDD and circadian rhythms defects and metabolic disorders such as Diabetes Mellitus (DM) and obesity. For example, Anderson et al. (2011) estimated that one-quarter of patients with DM experience depressive episodes during their lifetime. In addition, Timonen et al. (2005) found that insulin resistance and severity of depressive symptoms were positively correlated. Thus, approaches based on metabolism might reveal new strategies for treating MDD.

Circadian rhythm and metabolic deficits

Circadian rhythm is the term used to describe the physiological and behavioral 24-hour cycle experienced by most organisms, including the sleep/wake cycle. It is known to be affected by many other factors such as hormonal levels, eating, and drinking. The main role of circadian rhythms is hypothesized as adjusting to the environmental changes associated with light/dark cycles, e.g., increasing body temperature and blood pressure at the time when an organism is most active. The circadian rhythms have been known to be controlled by the suprachiasmatic nucleus (SCN), and the secretion of melatonin (Mohawk et al., 2012; Sollars and Pickard, 2015; Tordjman et al., 2017). The production and secretion of melatonin in the pineal gland is strictly regulated by light: lower plasma melatonin levels maintained during the day and peaking late at night.

Misalignment between the circadian system and the external environment can have a pronounced impact on physiologic functioning. The circadian rhythm sleep-wake deficit (CRSWD) such as jet lag disorder is one of the example of this misalignment (Williams et al., 2016). Millions of people in the world suffer from this disorder because

of its substantial negative impact on the quality of life (QOL) (Drake et al., 2004; Sack et al., 2007; Williams et al., 2016). Several mental disorders such as bipolar disorder and schizophrenia have also been linked to dysfunctional circadian rhythms (Brissos et al., 2013; Soreca, 2014). The treatment of sleep disorders including insomnia and CRSWD varies from cognitive behavioral therapy to drug therapy: primary treatments for insomnia such as cognitive behavioral therapy, benzodiazepines. However, about 40% of patients with chronic insomnia do not reach sustained remission with these treatments (Morin et al., 2009). For CRSWDs, melatonin agonists and/or supplementation and light exposure are applied, although there is only scant data on the effectiveness of these treatments (Morgenthaler et al., 2007).

Evidence has been accumulating regarding the relationship between the circadian deficits and peripheral metabolic dysregulation. For example, an epidemiological study revealed that shift workers such as nurses are at high risk of developing obesity and metabolic syndrome (Brum et al., 2015; Laermans and Depoortere, 2016). Specifically, there have been reported the relationship between DM and sleep disorder (Watanabe et al., 2010; Holliday et al., 2013; McNeil et al., 2013). In addition, melatonin regulation is impaired both in patients with DM (Peschke et al., 2006) and in streptozotocin (STZ)-treated diabetic rats (Velasco et al., 1988). In contrast, a study demonstrated that human islet amyloid polypeptide (HIP) rats showed normal circadian rhythms and melatonin secretion (Qian et al., 2017). Thus, the relationship between metabolic disorders, including hyperglycemia, and aberrant circadian rhythms of melatonin is controversial.

D-serine and mental disorders

There is abundant evidence implicating dysfunction of neurotransmitters in the brain with the pathophysiology of a wide variety of the central nervous system (CNS) diseases. For example, the serotonergic and noradrenergic neurotransmissions are known markedly dysregulated in MDD (Agren, 1980; Syvalahti, 1987). In the brain, these neurotransmitters are mainly synthesized mainly from amino acids via enzymatic reaction. Thus, amino acids are important molecules for the function of the brain.

D-Serine is a unique endogenous substance enriched in the brains of mammals at high concentrations (Hashimoto et al., 1992; Nishikawa, 2011). D-serine have been found to function as a co-agonist of NMDA receptor (Mothet et al., 2000; Stevens et al., 2003), and be involved in a variety of higher brain functions including learning and memory. In addition, accumulating evidence suggests that impaired of D-serine signaling is involved in a variety of mental disorders including schizophrenia and MDD (Bendikov et al., 2007; Malkesman et al., 2012). Specifically, the involvement of reduced D-serine signaling in schizophrenic patients has been investigated. For example, the D-serine levels and D/L-serine ratio were significantly decreased in the cerebrospinal fluid (CSF) (Bendikov et al., 2007) and in plasma (Yamada et al., 2005) of schizophrenia patients.

As described above, D-serine has received recent attention because of its relevance to a variety of mental disorders. However, generally when testing the effects of D-serine on specific behavioral changes in studies, the effective dose of D-serine was very high both in humans (30 mg/kg/day) and in animals (0.8–1.0 g/kg) due its low blood-brain barrier (BBB) penetration potency. This will lead to induce off-target related peripheral side effects. Thus, selective increases in the extracellular D-serine levels in the target brain region are desirable. One approach to achieve this is to inhibit the uptake of D-serine into the cells in the brain (Helboe et al., 2003; Brown et al., 2014).

Purpose

In order to reveal the pathophysiology and the relationship between metabolic disorder and the mental diseases, I studied behavioral deficits and the underlying pathophysiology in Spontaneously Diabetic Torii (SDT) fatty rats, which mimic the T2DM comorbid with obesity. In Chapter 2, I investigated whether SDT fatty rats exhibit a depression-like phenotype, impaired hypothalamic-pituitary-adrenal (HPA) axis and altered neurotransmitter levels in several brain regions such as cortex and hippocampus. In Chapter 3, I examined the circadian rhythms of behavior as well as plasma metabolic parameters (glucose, triglyceride (TG), total cholesterol (TC)) and melatonin concentrations in SDT fatty rats. Moreover, I also investigated the mRNA expressions of melatonin-synthesizing enzymes in the pineal gland.

In chapter 4, in order to get a better understanding of regulation mechanism of the synaptic D-serine, I focused on Na⁺-independent alanine-serine-cysteine transporter1 (asc-1), a transporter involved in the regulation of extracellular D-serine levels (Fukasawa et al., 2000) using a novel compound, (1)-amino(1-(3,5-dichlorophenyl)-3,5-dimethyl-1H-pyrazol-4-yl) acetic acid (ACPP).

Chapter 2

Depression-related behavioural and neuroendocrine changes in the SDT fatty rat, an animal model of Type 2 Diabetes Mellitus

INTRODUCTION

Depression is known to be frequently associated with DM. One report estimated that one-quarter of patients with DM experience depressive episodes during their lifetime (Anderson et al., 2001). Moreover, depression and DM are known to interact reciprocally. For example, DM affects the brain negatively, leading to impaired cognitive function not only in the clinical setting (Strachan et al., 1997; Akisaki et al., 2006), but also in animal models of DM (Liu et al., 2016; Cai et al., 2017). Similarly, depression can lead to decreased quality of life and worse long-term prognosis in patients with DM by increasing the incidence of diabetic microangiopathies (Schmitz et al., 2014). Thus, it is important that comorbid depression in patients with DM is diagnosed and treated as early as possible. However, the underlying brain pathophysiology of DM patients with depression remains incompletely understood.

Several studies have investigated depression-related changes in animal models of DM. For example, STZ-induced diabetic mice were reported to show longer periods of immobility in FST, suggesting depression-like behaviour (Amorim et al., 2017; Aswar et al., 2017). In addition, STZ-diabetic rats exhibit an abnormal stress response, characterised by the excessive secretion of corticosterone when exposed to acute stress; this abnormal stress response is frequently observed in depressed patients, suggesting hyperactivation of the HPA axis (Chan et al., 2002).

Moreover, recent studies have revealed biochemical changes in the brains of animal models of DM. For example, Badawy et al. reported that markers of oxidative stress and neurotransmitter concentrations were elevated in the brains of STZ-diabetic rats (Badawy et al., 2015). Changes in neurotransmitter concentrations in STZ-diabetic rats have also been reported by Ezzeldin et al., who demonstrated altered levels of dopamine, NA, and 5-HT in several brain regions, including the cerebral cortex, hypothalamus and brain stem (Ezzeldin et al., 2014). These findings suggest that changes in various neurotransmitters may play a role in the pathophysiology of comorbid depression in DM. However, such findings are based on artificially induced animal models of DM. A growing body of evidence suggests that DM is a complicated disease that involves both genetic and environmental factors. Moreover, metabolic deficits, including insulin resistance, are frequently observed in the condition. Thus, it is preferable to use animal models that comprise the same features.

The SDT fatty rat is a novel animal model of T2DM that develops not only hyperglycaemia, but also hyperlipidaemia and insulin resistance from a young age (Katsuda et al., 2015). In the present study, we investigate whether SDT fatty rats show depression-like behaviour using a forced swimming test (FST). The FST has frequently been used for the screening of antidepressants and assessment of depressive features in animal models of depression and transgenic or knockout rodents (Cryan and Mombereau, 2004; Reus et al., 2011; Zhao et al., 2017). In addition, we examined several neuroendocrine features, including HPA axis activity and associated neurotransmitters levels in the brains of SDT fatty rats.

MATERIALS AND METHODS

Animals

All experimental procedures were conducted in compliance with the Guidelines for Animal Experimentation of Japan Tobacco Biological/Pharmacological Research Laboratories. The animal protocol was designed to minimize pain or discomfort to the animals.

Experiments were conducted in 8- to 12-week old male SDT fatty rats and age-matched SD rats as the control group (CLEA Japan, Tokyo, Japan). Two to three rats were group housed in bracket cages in a climate-controlled room (mean $\pm$ standard deviation) temperature $23 \pm 3^{\circ}\text{C}$, humidity $55 \pm 15\%$) under a 12-hour light–dark cycle (lights on at 0800 hours). Rats had free access to a commercial diet (CRF-1; Charles River Japan, Yokohama, Japan) and water.

Forced swimming test

The FST procedure used in the present study was based on that described by Porsolt et al. (Porsolt et al., 1977; Porsolt et al., 1978), although the pretest was not conducted in the present study. Briefly, a cylinder (20 cm $\times$ 50 cm) was filled with water to 40 cm above the bottom and the temperature was maintained at $22 \pm 1^{\circ}\text{C}$. Rats were habituated in the testing environment in the experimental room for more than 1 hour, after which they were gently placed into the cylinder and assessment began.

The duration of immobility was measured automatically for 5 min using an automated video tracking and analysing system (EthoVision XT). The water was replaced between each test. After the test, rats were dried with paper towels and returned to their home cage. Behaviour was classified as immobile, mobile or high mobile based on the system's algorithm.

Locomotor activity

The spontaneous locomotor activity (SLA) of rats was assessed using a Supermex apparatus (Muromachi Kikai, Tokyo, Japan). An infrared beam sensor was placed on top of an acrylic cage. Rats were placed individually into the cage and the number of movements over a 3-hour period (1400–1700 hours) was counted in 10-min bins. Rats had free access to food and water during measurements.

Stress exposure, Dexamethasone (DEX) challenge and measurement of corticosterone

Swimming stress-induced changes in plasma corticosterone concentrations were assessed. The experiment was performed in the morning (0900–1200 hours) and rats were acclimated to the experimental room for 1 hour before exposure to stress. The apparatus to induce swimming stress was the same as that used in the FST described above. Rats were subjected to 2-min swimming stress. Immediately after stress exposure, rats were roughly dried with paper towels and returned to their home cages. Blood samples were collected from the tail vein by cutting the edge with razors at 0 min (baseline; before exposure to stress), and subsequently 30, 60 and 120 min thereafter. Plasma was separated by centrifugation (15,000 g, 5 min, 4°C) and stored at –80°C until analysis.

The DST was performed as follows. First, SDT fatty and control rats were injected with DEX (50 µg/kg, s.c., in 0.1% dimethylsulphoxide saline) or vehicle at 1200 hours and returned to their home cages. At 1700 hours, blood samples were collected from the tail vein as described above. Plasma corticosterone concentrations were

determined by Enzyme-linked immunosolvent assay (ELISA) (YK240; Yanaihara Institute, Shizuoka, Japan) according to the manufacturer's instructions.

Measurement of brain neurotransmitters

To measure neurotransmitters in the brain, after behavioural testing rats were decapitated and the PFC, hippocampus and brain stem were dissected out and stored at -80°C until use. Rat brain tissues were homogenized using a multi-bead shaker (MB701C; Yasui Kikai, Osaka, Japan) in ice-cold methanol by adding 4 parts methanol per gram brain tissue. 5-HT, NA, glutamate and GABA were extracted from the brain homogenate (20 μL) with deproteinization and concentrations were simultaneously measured by liquid chromatography (UFLC; Shimadzu, Kyoto, Japan) coupled with tandem mass spectrometry (API5000, AB Sciex, Framingham, MA, USA).

Statistical analysis

Results are expressed as the mean $\pm$ standard of error of the mean (SEM). Comparisons between two groups were made using unpaired t-tests or Welch tests. The time-courses of locomotor activity and stress-induced increases in corticosterone was analysed using two-way repeated analysis of variance (ANOVA). All data were analysed using Excel (Microsoft, Bellevue, WA, USA) or EXSUS software (CAC Croit, Tokyo, Japan). Two-sided $P < 0.05$ was considered significant.

RESULTS

Forced swimming test

First, we performed the FST to investigate whether SDT fatty rats exhibit depression-like behaviour. The swimming pattern was automatically classified as immobile, mobile or highly mobile using an EthoVision system (Noldus, Wageningen, The Netherlands), as described in the Materials and Methods. The SDT fatty rats were observed to spend significantly longer periods immobile ($P < 0.001$) and to exhibit significantly shorter mobile ($P < 0.001$) and highly mobile ($P < 0.001$) periods than control SD rats (Figure 1A–C).

To rule out the possibility that these observations were due to decreased motor activity, SLA was measured for 3 hours (from 1400 to 1700 hours) when the FST was performed. The SDT fatty rats showed a tendency for greater locomotor activity than control SD rats (Figure 2A; $P = 0.061$), with mean $\pm$ SEM total activity counts of 4884.3 ± 651.3 and 3285.6 ± 461.9 , respectively (Figure 2B).

Changes in basal and stress-induced plasma corticosterone concentrations

Because it is known that patients with depression exhibit hyperactivation of basal HPA activity, as well as aberrant hormonal responses to stress, we measured changes in basal and stress-induced plasma corticosterone concentrations in SDT fatty rats. As shown in Figure 3A, basal corticosterone levels were significantly higher in SDT fatty rats than in control SD rats ($P < 0.05$).

Conversely, the extent of increased corticosterone induced by 2-min swimming stress was almost the same in SDT fatty and control SD rats (Figure 3B). However, plasma corticosterone concentrations 120 min after exposure to swimming stress exhibited a tendency to remain higher than baseline in SDT fatty rats, whereas they

returned to baseline levels in control SD rats. However, the difference between the two groups was not significant because of wide inter-individual variability ($P = 0.38$).

DST

The results reported above suggest that the negative feedback function of the HPA axis may be impaired in SDT fatty rats. Therefore, a DST was used to evaluate the effects of DEX on natural increases in plasma corticosterone levels in the evening. Significantly higher basal corticosterone concentrations were observed in SDT fatty rats than control SD rats in the early evening (1730 hours; Figure 4; $P < 0.05$). However, plasma corticosterone levels were significantly suppressed by DEX to a similar extent in SDT fatty and control SD rats (Figure 4; $P < 0.001$).

Neurotransmitter levels

To provide an insight into the mechanisms that result in depression-like behaviour in SDT fatty rats, we investigated 5-HT, NA, glutamate and GABA concentrations (Figure 5). Changes to these neurotransmitters are known to be involved in depression in several brain regions, including the prefrontal cortex (PFC), hippocampus and brain stem.

In SDT fatty rats, 5-HT concentrations in the PFC were significantly lower (by ~50%) than in control SD rats (Figure 5A; $P < 0.01$). In addition, glutamate levels were significantly lower in the PFC of SDT fatty than in control SD rats ($P < 0.05$). In the hippocampus, glutamate and GABA levels were significantly higher in SDT fatty than control SD rats ($P < 0.01$ and $P < 0.05$, respectively; Figure 5B). However, there was no change in NA concentrations in either brain region in SDT fatty compared with control

SD rats. We did not observe any changes in neurotransmitter levels in the brain stem (Figure 5C).

DISCUSSION

In the present study, we found that SDT fatty rats exhibited a depression-like behavioural and biochemical phenotype, as shown by increased immobility in the FST, aberrant plasma corticosterone concentrations and altered neurotransmitter levels in the brain. These results suggest that SDT fatty rats have at least some depression-like features.

In the FST, SDT fatty rats exhibited depression-like behaviour characterised by increased time spent immobile, as well as decreased time spent mobile or highly mobile. These behavioural changes may result from the ‘fatty’ features of SDT fatty rats. However, body weight did not differ between the control SD and SDT fatty rats. Moreover, there were no differences in SLA between the control SD and SDT fatty rats, suggesting that increased immobility of SDT fatty rats in the FST was not due to decreased locomotor activity. Taking these results into consideration, we suggest that SDT fatty rats exhibit depression-like features.

Several behavioural tests to assess depression-like phenotypes have been developed other than the FST, including the sucrose preference test and the novelty-induced suppression of feeding test (Cryan and Mombereau, 2004). Therefore, whether SDT fatty rats also exhibit depression-like features in these tests should be investigated in future studies. There are also well-known sex differences in stress vulnerability, as well as in the incidence of stress-related disorders in men and women (Seney and Sibille, 2014). Female SDT fatty rats exhibit DM from a young age, as reported previously (Ishii et al.,

2010), and further investigation is needed to elucidate sex differences in depression-like behaviours in SDT fatty rats.

Several studies have suggested a relationship between DM and psychiatric deficits, demonstrated by increased immobility time in the FST in STZ-diabetic mice (Amorim et al., 2017; Aswar et al., 2017), and decreased time spent in an open arm in the elevated plus maze test (anxiety-like behaviour) (Thorre et al., 1997). More recently, another study demonstrated that rats with diabetes induced by a combination of high fat diet and STZ exhibit various depression-like behavioural deficits, such as decreased sucrose preference and increased latency to feeding in the novelty suppression of feeding test (Qiu et al., 2016). Details of the depression-like phenotypes exhibited by DM models have been described elsewhere (Reagan, 2012). Our finding that SDT fatty rats show depression-like behavioural changes provides further support for previous studies that have used classical animal models of DM.

We also observed that SDT fatty rats show higher basal plasma corticosterone concentrations in both the morning and early evening. Enhanced basal secretion of corticosterone throughout the light phase may reflect disrupted circadian rhythms associated with corticosterone secretion, which are frequently observed in diabetic patients (Cameron et al., 1984; Roy et al., 1993), although nocturnal corticosterone levels in SDT fatty rats remain to be determined.

Aberrant basal HPA activity has also been observed in other models of DM. For example, STZ-diabetic rats, as well as obese Zucker rats, have increased basal corticosterone levels (De Nicola et al., 1976; Meehan et al., 1986; Plotsky et al., 1992; Chan et al., 2002). In addition, changes in the circadian corticosterone cycle have been observed in the urine of STZ-diabetic rats (Scribner et al., 1991). The findings of the

present study confirm these previous observations. However, when exposed to 2 min of swimming stress, the extent by which plasma corticosterone concentrations increased in SDT fatty rats was comparable between the SDT fatty and control SD rats, although there was a tendency for a delayed return to baseline levels in the SDT fatty compared with control SD rats. Unfortunately, we did not observe a significant delay in corticosterone compared with control rats due to wide inter-individual variation. This variability in SDT fatty rats may result from differences in the development of hyperglycaemia. Indeed, although previous studies suggested that SDT fatty rats develop hyperglycaemia from a young age (Katsuda et al., 2015), the animals used in the present study showed a wide range of plasma glucose concentrations.

Finally, we found that 5-HT and glutamate levels were significantly lower in the PFC of SDT fatty compared with control SD rats, while hippocampal glutamate and GABA levels were significantly higher in SDT fatty rats. A large body of evidence suggests deficits in the serotonergic system as one underlying pathophysiology of depression. For example, Korf and van Praag reported decreased levels of 5-hydroxyindole acetic acid, a 5-HT metabolite, in the CSF of depressed patients (Korf and van Praag, 1971). In addition, 5-HT_{2A} receptor binding is increased in the PFC of postmortem brains of depressed patients and patients who have committed suicide, although this has not been observed in all studies (Hrdina et al., 1993; Ono et al., 2001; Oquendo et al., 2006). The finding of decreased 5-HT levels in the PFC of SDT fatty rats in the present study probably partly reflects these clinical findings. In contrast, STZ rats reportedly have normal baseline 5-HT levels in the PFC, in contrast with a blunted increase in 5-HT observed in response to acute stress (Miyata et al., 2007). The mechanisms underlying these differences in basal 5-HT levels in the PFC between SDT

fatty and STZ rats remain unclear. However, one possibility is that the differences may be due to deficits in metabolic parameters in SDT fatty rats, such as insulin resistance or hyperleptinaemia (Masuyama et al., 2005). Consistent with this hypothesis, leptin is known to attenuate 5-HT synthesis and the firing of serotonergic neurons in the brain (Yadav et al., 2009). Further investigations are required to determine whether serotonergic activity is altered in the SDT fatty rat.

Contrary to the observed decreases in 5-HT and glutamate in the PFC, we observed significant increases in glutamate and GABA in the hippocampus of SDT fatty rats compared with controls. Considerable evidence suggests that impaired glutamatergic/GABAergic signalling can lead to a variety of psychiatric diseases, including depression, anxiety disorders and schizophrenia (Olkola and Ahonen, 2008; Murrough et al., 2017). This is because both these neurotransmitters are critical for the modulation of excitation and inhibition of neuronal activity in the brain (Fee et al., 2017; Foss-Feig et al., 2017). The SDT fatty rat appears to provide a novel animal model for psychiatric diseases associated with an excitatory–inhibitory imbalance.

A few studies have reported deficits in glutamate signalling in the hippocampus of rat models of DM. For example, Yang et al. demonstrated that glutamate and D-serine levels were significantly higher in the hippocampus of STZ rats than in the control group (Yang et al., 2015). The authors also observed functional changes in glutamatergic signalling with marked impairment of the induction of long-term potentiation (LTP) by high-frequency stimulation in the hippocampal CA1 area of STZ rats, which may reflect the neural basis of cognitive deficits in STZ rats. Taking these findings into consideration, SDT fatty rats may also exhibit deficits in LTP and cognitive function. Future studies are needed to investigate cognitive functions, as well as to elucidate the details of glutamate

signalling. In addition, studies are needed to investigate the cause of these neurotransmitter impairments; that is, whether the effects of hyperglycaemia on neurotransmitters are direct or indirect, via factors such as inflammation and/or oxidative stress.

In conclusion, SDT fatty rats exhibited depression-like behaviours, as well as altered baseline HPA axis activity. Furthermore, neurotransmitter levels were impaired in brain regions associated with the pathophysiology of depression. The findings of the present study suggest that the SDT fatty rat is a useful model for studies trying to elucidate the pathophysiology of DM with comorbid depression.

Table 1. Body weight and plasma parameters in control SD and SDT fatty rats.

parameter	SD	SDT fatty
Body weight (g)	423.5 ± 6.1	443.6 ± 7.6
Glucose (mg/dL)	157.2 ± 7.4	516.0 ± 92.8 **
Total cholesterol (mg/dL)	86.9 ± 4.3	120.4 ± 12.4
Triglyceride (mg/dL)	220.4 ± 38.2	592.6 ± 91.9 †

Data are represented as the mean ± SEM. ** $P < 0.01$ compared with control SD rats (Welch test); † $P < 0.01$ compared with control SD rats (Student's t-test).

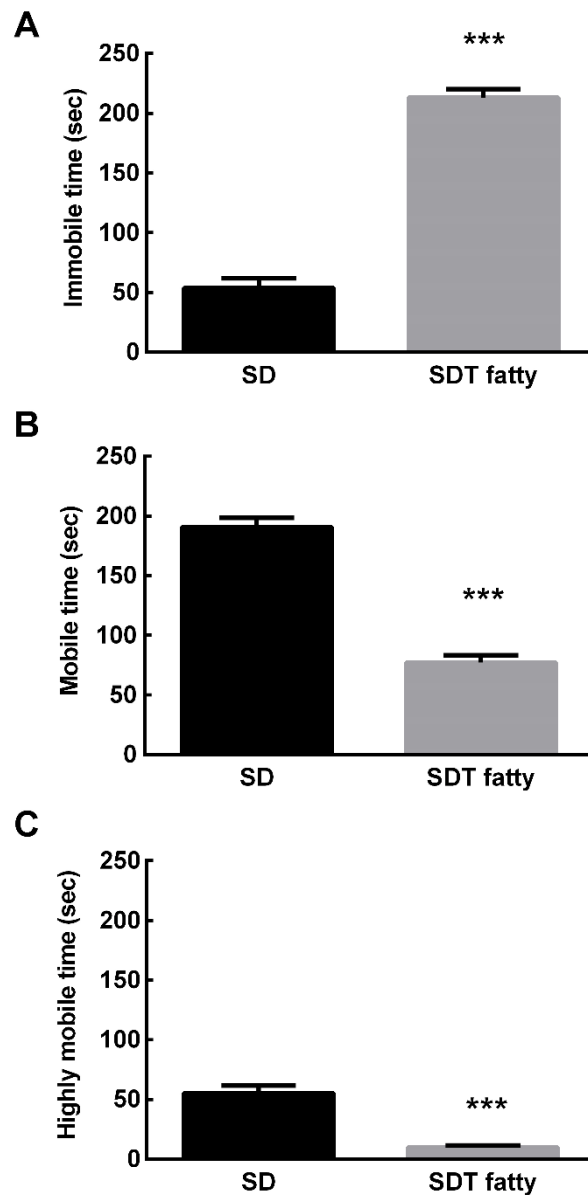


Figure 1. Depression-like behaviour of SDT fatty compared with control SD rats in the forced swimming test (A–C). Behaviour was automatically classified as immobile (A), mobile (B), or highly mobile (C). Data are represented as the mean $\pm$ SEM ($n = 10$ per group). *** $P < 0.001$ compared with control SD rats (Student's t -test).

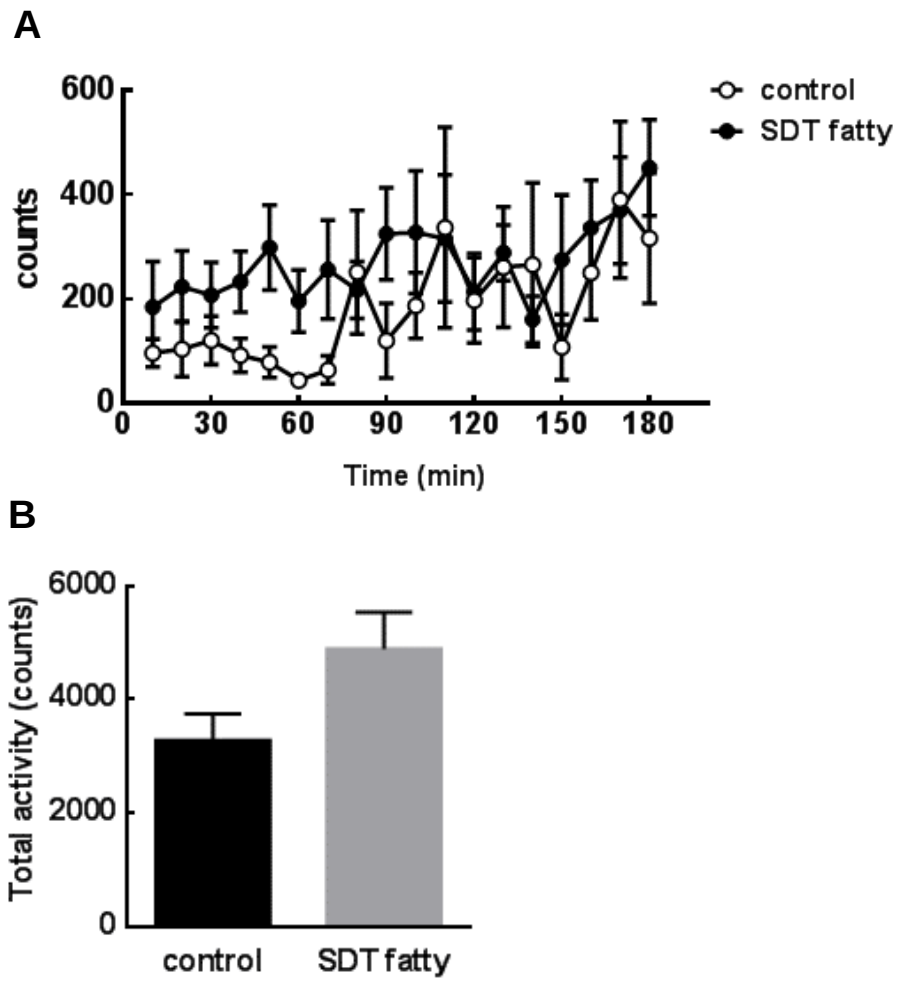


Figure 2. Time course of spontaneous locomotor activity (A) and total activity (B) over the 3-hours observation period. Data are represented as the mean $\pm$ SEM (n = 10 per group).

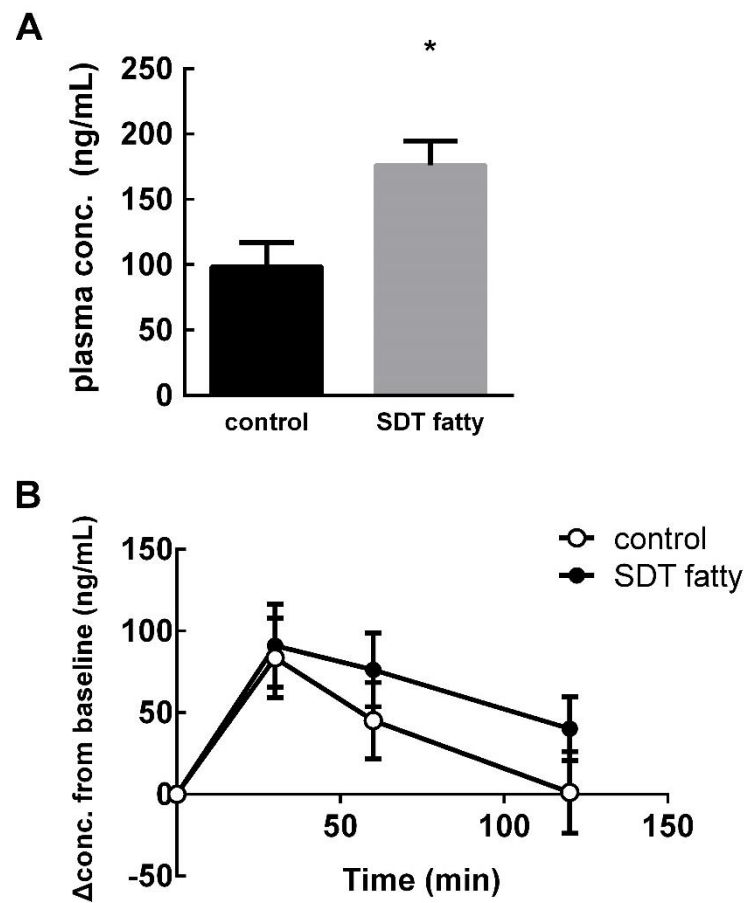


Figure 3. Plasma corticosterone concentrations (A) at baseline and (B) after 2-minutes swimming stress in control SD and SDT fatty rats. The time course of plasma corticosterone concentrations after swimming stress is represented as changes from baseline. Data are represented as the mean $\pm$ SEM (n = 10 per group). *P < 0.05 compared with control SD rats with (Student's t-test).

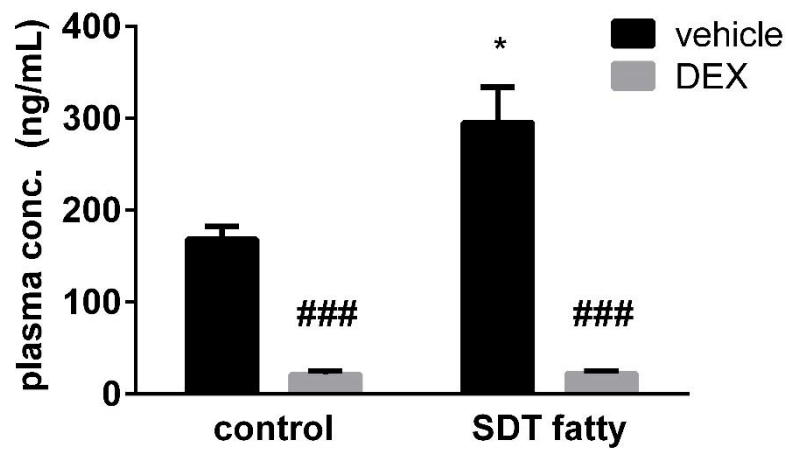


Figure 4. Plasma corticosterone concentrations after vehicle or DEX administration in control SD and SDT fatty rats. Data are represented as the mean $\pm$ SEM (n = 8 per group). *P < 0.05 compared with vehicle-treated control SD rats (Welch test). ###P < 0.001 compared with vehicle-treated rats of the same strain (Welch test).

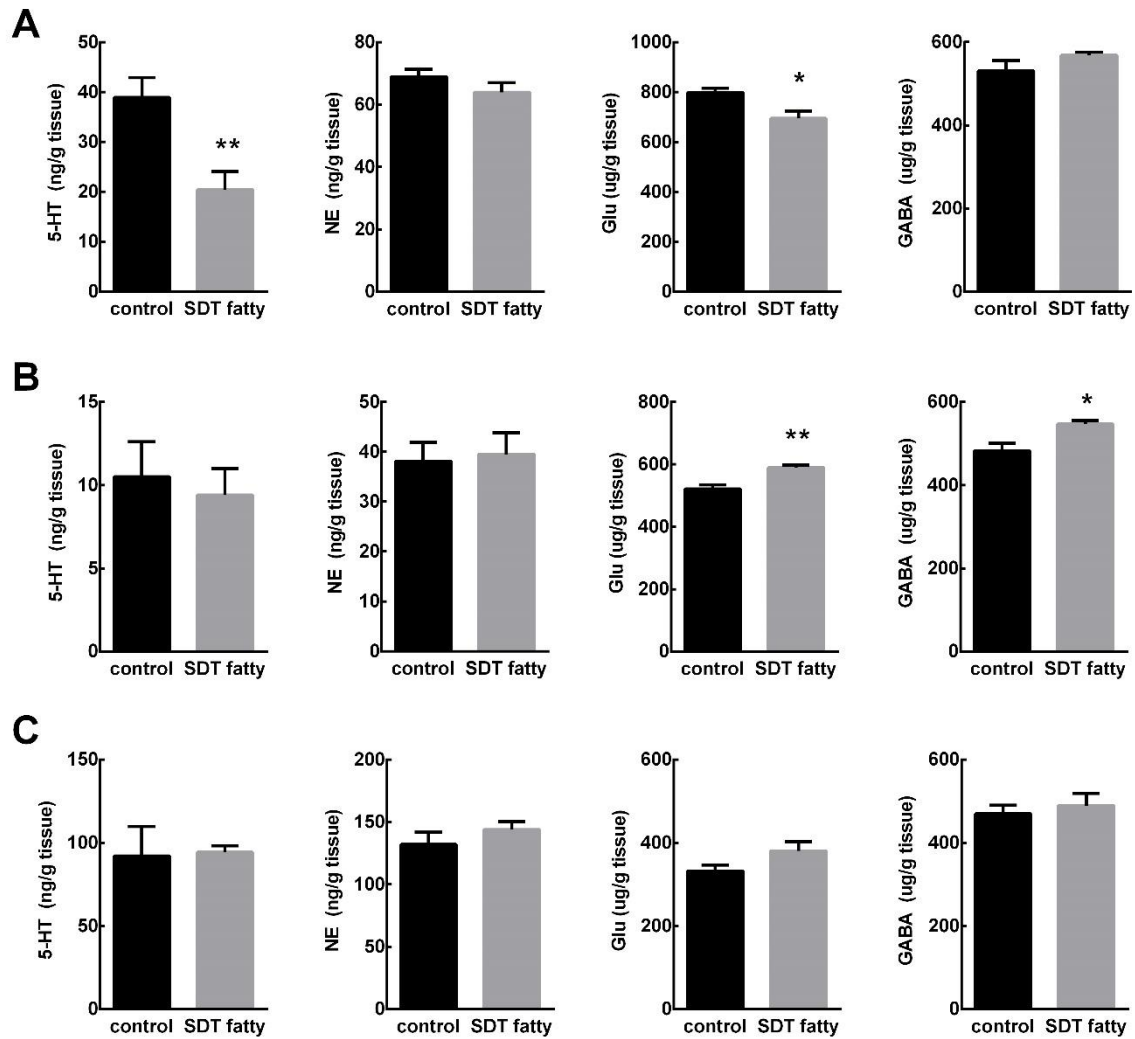


Figure 5. 5-HT, NA, glutamate (Glu), and GABA levels in the (A) frontal cortex, (B) hippocampus and (C) brain stem of control SD and SDT fatty rats. Data are represented as the mean $\pm$ SEM ($n = 4-5$ per group). * $P < 0.05$, ** $P < 0.01$ compared with control SD rats (Student's t-test).

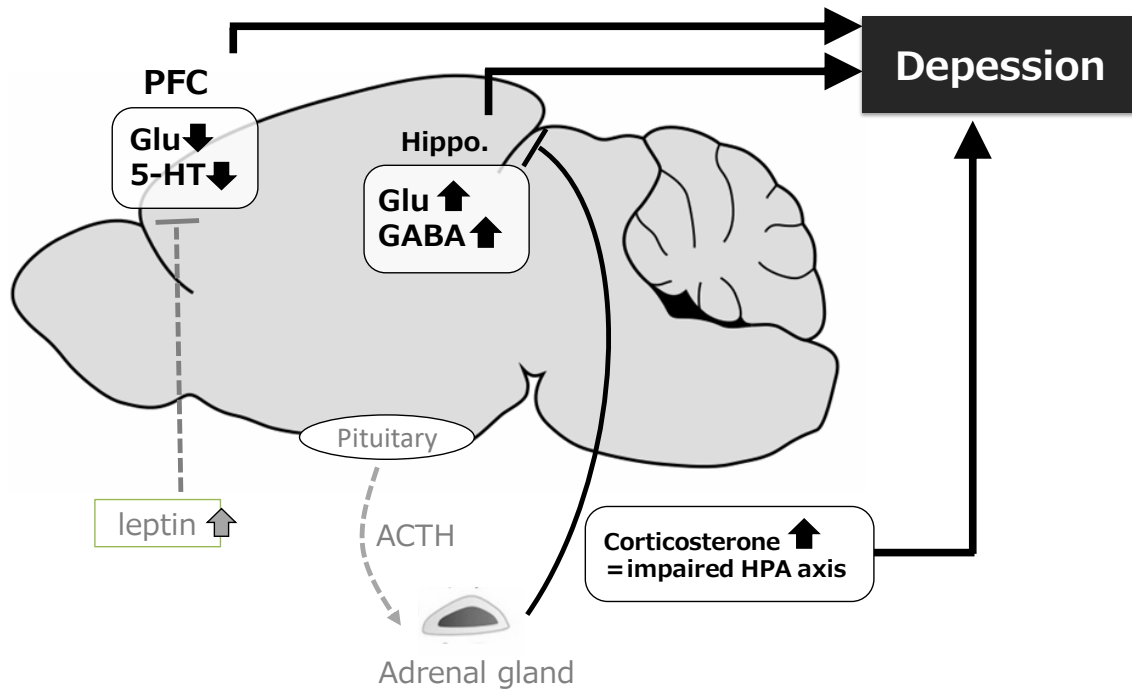


Figure 6. Schematic illustration of the putative pathophysiology underlying diabetes-related depression. Hyperactivation of HPA axis as well as the impaired neurotransmission of 5-HT, glutamate (Glu) and GABA in the PFC and hippocampus (Hippo) observed in the SDT fatty rats may play roles in diabetes-related depression-like behavior.

Chapter 3

SDT fatty rat, a novel animal model of type 2 diabetes mellitus, shows blunted circadian rhythms and melatonin secretion

INTRODUCTION

It is well-known that mammals have circadian rhythms not only for many kinds of behaviors, such as the sleep–wake cycle and feeding behavior, but also for several fundamental physiological functions, including hormone secretion. The circadian rhythm is organized by the so-called clock genes (e.g. period, circadian locomotor output cycles kaput, and brain and muscle Arnt-like protein, among others) in the SCN of the brain as “master regulators”, with the amplitude and/or phase of the rhythm modulated in response to extracellular stimuli such as light (Brainard et al., 1988), feeding (Asher et al., 2010), and melatonin (Akerstedt et al., 1979).

Circadian rhythms and metabolic function are known to be reciprocally linked (Bass and Takahashi, 2010). For example, it has been reported that impairment of circadian rhythms leads to the development of diabetes via the induction of abnormal insulin secretion, decreased sensitivity to insulin, and exacerbated inflammation (Van Cauter et al., 1991; Boden et al., 1996). In contrast, patients with diabetes reportedly exhibit circadian rhythms of blood pressure and plasma cortisol concentrations (Cuspidi et al., 2006; Pistrosch et al., 2007), suggesting that metabolic functions substantially affect circadian rhythms. However, the mechanisms underlying the disrupted circadian rhythms in diabetes remain to be fully elucidated, because the metabolic disorders are very complex, with many factors, including hyperglycemia, obesity, and hypertension, involved. Several animal models of DM have been reported to exhibit deficits in circadian

rhythms. For example, STZ-diabetic rats exhibit a blunted circadian rhythm of locomotor activity with a normal phase entrainment (Velasco et al., 1988), whereas Zucker obese rats, an animal model of T2DM, exhibit a phase advance and blunted amplitude of locomotor activity (Mistlberger et al., 1998).

Melatonin, a hormone secreted from the pineal gland in the brain, is known as a regulator of many physiological circadian rhythms including the sleep–wake cycle (Cajochen et al., 2003). Growing evidence suggests an important role for melatonin in DM. For example, melatonin is known to decrease plasma insulin concentrations in humans (Boden et al., 1996) and rodents (Rasmussen et al., 1999; Nishida et al., 2003; Tresguerres et al., 2013). Moreover, the physiological increase in nocturnal plasma melatonin concentrations is not observed in diabetic patients, especially those with neuropathy (O'Brien et al., 1986). Similarly, Peschke et al. reported reduced diurnal circulating melatonin levels in patients with T2DM, suggesting that melatonin secretion is impaired in DM patients (Peschke et al., 2006). Similarly, several animal models of DM have been reported to exhibit aberrant plasma melatonin concentrations. For example, plasma melatonin concentrations at midnight are attenuated in Goto-kakizaki (GK) rats, and melatonin synthesis efficiency is decreased (Frese et al., 2009). Conversely, Peschke et al. reported that melatonin synthesis was increased in the pineal glands of STZ-diabetic rats (Peschke et al., 2008). Moreover, another study demonstrated that diabetic rats transgenic for HIP rats, an established non-obese model of T2DM, showed normal circadian rhythms and melatonin secretion (Qian et al., 2017). Thus, the relationship between metabolic disorders, including hyperglycemia, and aberrant circadian rhythms of melatonin is still controversial.

SDT fatty rat is a novel animal model of T2DM, that not only hyperglycemia,

but also hyperlipidemia and insulin resistance from a young age (Katsuda et al., 2015). In the present study we investigated whether SDT fatty rats show impaired circadian rhythms of SLA, plasma metabolic parameters, and plasma melatonin concentrations, as well as changes in the expression of Arylalkylamine-N-acetyltransferase (Aanat) and acetylserotonin O-methyltransferase (Asmt) mRNA, which encode the rate-limiting enzymes for melatonin synthesis (Ebihara et al., 1986; Roseboom et al., 1998; Rath et al., 2016) in the pineal glands of SDT fatty rats.

MATERIALS AND METHODS

Animals

The present study was performed in compliance with the Guidelines for Animal Experimentation of Japan Tobacco Biological/Pharmacological Research Laboratories. The animal protocol was designed to minimize pain or discomfort to the animals. SDT fatty rats and age-matched SD rats (as controls) were used in the study. The age of animals used in each experiment were as follows: 8 weeks of age for SLA, measurement of plasma glucose, TG, TC and melatonin concentrations; 12 weeks of age for measurement of mRNA expressions. All rats were obtained from CLEA Japan (Tokyo, Japan). Rats were housed in groups of two to three per bracket cage in a climate-controlled room (temperature $23\text{ }^{\circ}\text{C} \pm 3\text{ }^{\circ}\text{C}$, humidity $55\% \pm 15\%$) under a 12-hour light–dark cycle (lights on at 0800 hours), with free access to a commercial diet (CRF-1; Charles River Japan, Yokohama, Japan) and water.

Locomotor activity

The SLA of rats was assessed using a Supermex apparatus (Muromachi Kikai, Tokyo, Japan). An infrared beam sensor was set on top of a Plexiglas cage and the number of movements was counted. Activity was integrated every 1 hour. SLA was measured during the 12-hour light–dark cycle for 5 days. Rats had free access to food and water during the measurements.

Blood sampling and measurement of metabolic parameters and melatonin

Blood samples were collected from the tail vein at 1000, 1600, 2100 and 0400 hours by cutting the edge of the tail with a razor. Plasma was separated by centrifugation (15,000 ×g for 5 min at 4°C) and stored at -80°C until analysis. Metabolic parameters, namely plasma glucose, TG, and TC concentrations, were measured using an automatic analyzer (HITACHI Clinical analyzer 7180; Hitachi, Tokyo, Japan). Plasma melatonin concentrations were determined using a commercially available ELISA kit (RE54021; IBL International, Hamburg, Germany) according to the manufacturer's instructions.

Sample preparation for mRNA measurement

Rats were killed by decapitation, and the pineal gland was dissected at 0400 hours under dim red light. Ribonucleic acid (RNA) from the pineal gland was extracted using the RNeasy mini kit (Qiagen, Valencia, CA, USA). RNA was quantified using the Nanodrop D8000 (Thermo-Scientific, Wilmington, DE, USA). The purity of RNA samples was assessed using the ratio of absorbance at 260/280 nm and samples with an absorbance ratio of 1.8–2.0 were used to prepare complementary deoxyribonucleic acid (cDNA). The RNA was transcribed into cDNA using high-capacity cDNA reverse-transcription kits with RNA inhibitors (Applied Biosystems). The reaction mixture was

incubated for 10 min at 25°C, for 2 h at 37°C, and then for 5 s at 85°C. The cDNA was stored at -20°C until use.

Quantitative reverse transcription polymerase chain reaction

Quantitative reverse transcription polymerase chain reaction (qRT-PCR) was performed in a 20-μL reaction mixture with an automated sequence detector combined with StepOne plus (Applied Biosystems). The reaction mixture was created using TaqMan Gene Expression Master Mix (Applied Biosystems) and contained approximately 50 ng synthesized cDNA, 0.9 μmol/L primers, 0.25 μmol/L probes or TaqMan gene expression assays on demand and Universal Maser Mix (primer/probe set). The reaction mixture was incubated for 2 min at 50°C and then 10 min at 95°C, followed by 40 cycles of 15 s at 95°C and 60 s at 60°C. The expression of beta-actin (Actb; purchased from Applied Biosystems), Aanat (Rn00664873_g1) and Asmt (Rn00595341_m1) was investigated using TaqMan gene expression assays and the Universal Maser Mix (primer/probe set), with the expression of Aanat and Asmt in each sample normalized against that of Actb.

Statistical analysis

Results are expressed as the mean ± SEM. Comparisons between control SD and SDT fatty rats were performed using two-way ANOVA with post hoc tests where appropriate for SLA and plasma parameters over time, whereas qRT-PCR data were compared using unpaired t-tests. All data were analyzed using Excel (Microsoft Corp., Bellevue, WA, USA) or EXSUS software (CAC Croit, Tokyo, Japan). Two-sided P value

of < 0.05 was considered significant. A P value of < 0.05 was considered statistically significant.

RESULTS

Circadian rhythms of SLA in SDT fatty rats

In order to investigate whether there were any disruptions to circadian rhythms of SDT fatty rats, the SLA of control SD and SDT fatty rats was monitored over a period of 5 consecutive days. As shown in Figure 7, SLA during the dark phase was significantly lower in SDT fatty than control SD rats ($P < 0.001$). In addition, SLA during the first 1 hour during the light phase (0800–0900 hours) was markedly decreased in SDT fatty compared with control SD rats. However, the SLA in the light phase of SDT fatty rats was slightly high compared with control SD rats, though total activity did not differ significantly between the two groups.

Circadian rhythms of metabolic parameters in SDT fatty rats

It is well known that several metabolic parameters, including glucose and TG, show circadian rhythms regulated by diet and/or hormones. This led us to investigate whether glucose, TG, and TC exhibit aberrant circadian rhythms in SDT fatty rats. Comparisons of plasma glucose, TG, and TC concentrations in control SD and SDT fatty rats are shown in Figure 8A–C. Two-way ANOVA of the circadian pattern of plasma glucose revealed significant effects of group ($F_{1,8} = 16.0$, $P < 0.01$), time ($F_{3,24} = 29.3$, $P < 0.001$) and their interaction ($F_{3,24} = 14.4$, $P < 0.001$). Post hoc analysis using the Aspin-Welch test revealed that plasma glucose concentrations were significantly higher in SDT fatty than control SD rats at 1600 ($P < 0.05$), 2100 ($P < 0.01$) and 0400 hours ($P < 0.05$).

Two-way ANOVA of the circadian pattern of plasma TG showed significant effects of group ($F_{1,8} = 17.0$, $P < 0.05$) and time ($F_{3,24} = 4.6$, $P < 0.05$). Post hoc analysis using the Aspin-Welch test revealed that plasma TG concentrations were significantly higher in SDT fatty than control SD rats at all time points measured (i.e. 1000, 1600, and 0400 hours [$P < 0.05$ for all], as well as at 2100 hours [$P < 0.01$]). Unlike glucose and TG concentrations, there were no obvious changes in TC in either strain, with TC concentrations in the range 80–100 mg/dL in control SD rats and 100–120 mg/dL in SDT fatty rats. Plasma TC levels peaked at 0400 hours in control SD rats, compared with 1000 hours in SDT fatty rats. Two-way ANOVA of the circadian pattern of plasma TC showed significant effects of group ($F_{1,8} = 8.9$, $P < 0.05$), but not time ($F_{3,24} = 2.7$, $P > 0.05$). Post hoc analysis using the Aspin-Welch test revealed that plasma TC concentrations were significantly higher in SDT fatty than control SD rats at 1000 ($P < 0.01$) and 2100 ($P < 0.05$).

Circadian rhythms of plasma melatonin

In order to obtain an insight into the mechanism(s) underlying the aberrant circadian rhythms in SDT fatty rats, we measured plasma melatonin concentrations. As shown in Figure 9, there was an obvious circadian rhythm for melatonin in control SD rats, with low concentrations during the light phase (1000 and 1600 hours) and early dark phase (2100 hours), with the greatest increase in contrast during the night (0400 hours). Two-way ANOVA and subsequent post hoc analysis revealed significant effects of time ($F_{3,42} = 16.0$, $P < 0.001$) but not group ($F_{1,14} = 1.9$, $P = 0.19$). Their interaction was significant ($F_{3,42} = 7.4$, $P < 0.001$) on changes over time in plasma melatonin concentrations between the control SD and SDT fatty rats. Plasma melatonin

concentrations were significantly higher in SDT fatty than control SD rats at 1600 ($P < 0.05$) and 2100 hours ($P < 0.01$), but were significantly lower at 0400 hours ($P < 0.01$). In addition, the total melatonin production was also analyzed and found that there was a tendency for higher in SDT fatty rats than control SD rats, with the area under curve value of 1949.1 ± 163.0 pg·day/mL, 2422.7 ± 206.2 pg·day/mL (SD rats v.s. SDT fatty rats, $P = 0.093$).

Expression of melatonin-synthesizing enzymes

The observation that SDT fatty rats showed blunted circadian rhythms of plasma melatonin led us to investigate whether the expression of Aanat and Asmt mRNA, enzymes important for melatonin synthesis, in the pineal gland of SDT fatty rats was impaired compared with control SD rats at night, when melatonin expression was highest. Although there were no significant differences in Aanat and Asmt mRNA expression in the pineal glands of the two groups, there was a tendency for higher Aanat and Asmt mRNA expression in SDT fatty compared with control SD rats (Figure 10).

DISCUSSION

In the present study, we found that 1) SDT fatty rats showed aberrant circadian rhythms of SLA, 2) the circadian fluctuations of plasma glucose, TG, and TC concentrations were found more apparent in SDT fatty rats and 3) these rats exhibited blunted circadian rhythms of plasma melatonin secretion, even though the mRNA expression of melatonin-synthesizing enzymes did not differ significantly from that in the control SD rats.

Because rats are generally nocturnal, their activity increases in the dark phase and decrease markedly during the light phase. However, SLA in SDT fatty rats was decreased during the dark phase, with a tendency for higher SLA compared with control SD rats during the light phase. This observation suggests that SDT fatty rats have blunted circadian rhythms of SLA. In addition, the entrainment by light stimuli seemed normal in the SDT fatty rats, as revealed by the observation that the SDT fatty rats exhibited the same SLA pattern was observed in SDT fatty rats over a period of 5 consecutive days of monitoring, although actograms were not measured in the present study. The findings of the present study confirm those of previous reports demonstrating impaired circadian rhythms in animal models of T2DM. For example, Zucker obese rats, an animal model for T2DM with mutations in the leptin receptor, exhibit a phase advance and a decreased amplitude of activity and body temperature (Mistlberger et al., 1998). In addition, Otsuka Long-Evans Tokushima Fatty rats have been reported to show decreased nocturnal locomotor activity before developing hyperglycemia (Sei et al., 1999). Furthermore, STZ-diabetic rats, an animal model of type 1 DM, also show a blunted circadian rhythm for locomotor activity, but no phase shift (Velasco et al., 1988). Together, these findings suggest that hyperglycemia and/or obesity may induce blunted circadian rhythms while phase entrainment is preserved.

Circadian rhythms are known to be affected by food-taking behavior (Abe et al., 1989; Stephan, 2002). As our previous studies have revealed that SDT fatty rats show hyperphagia (Ohta et al., 2014; Toriniwa et al., 2018), this feature may underlie the deficits of circadian rhythm in the SDT fatty rats. Although there is no data on the food intake pattern of SDT fatty rats, the relatively higher SLA during light phase may reflect

the food-taking. Consistent with this, plasma glucose, TG and TC concentrations are significantly higher than control SD rats even in the light phase as well as the dark phase.

As mentioned in the Introduction, diabetes and impaired circadian rhythms are known to be closely linked (Bass and Takahashi, 2010). In addition, Yoda et al. recently reported that the increased HbA1c associated with poor glycemic control in the T2DM induces sleep disorder with exacerbated sleep quality (Yoda et al., 2015). Furthermore, those authors demonstrated that sleep disorders in T2DM can increase the risk of cardiovascular events via aberrant hypertension in the morning. Future study investigating the relationship between hyperglycemia and the quality of sleep in SDT fatty rats are needed in order to elucidate the underlying pathophysiology.

In addition to the impaired circadian rhythm of SLA in SDT fatty rats, the rhythm of melatonin secretion was blunted in SDT fatty compared with control SD rats. Melatonin signaling, including its synthesis and secretion is known to be suppressed by light inputs from eyes, and usually reaches peak values at night both in humans and rodents (Lewy et al., 1980; Bartness et al., 1993; Aoki et al., 1998). Consistent with this, in the present study, melatonin concentrations in control SD rats were low during the light and early dark phase and were highest in samples collected at 0400 hours. In contrast, melatonin concentrations during the light phase (1600 hours) and at 2100 hours were higher in SDT fatty than control SD rats, whereas they were lower at 0400 hours in the SDT fatty rats, suggesting that SDT fatty rats have blunted circadian rhythms of melatonin secretion. To get an insight into the mechanism underlying this aberrant melatonin secretion, we investigated the mRNA expression of melatonin-synthesizing enzymes in the pineal glands of SDT fatty rats by focusing on the peak time of melatonin secretion (0400 hours). Unexpectedly, there was no significant difference in the expression of Aanat

and Asmt between the SDT fatty and control SD rats, while there was a tendency for higher expression in the SDT fatty rats.

In the present study, we did not investigate the levels of Aanat and Asmt proteins nor the precursors of melatonin including tryptophan and 5-HT in the pineal gland of SDT fatty rats. Thus, further study is required in order to expand the understanding about the alteration of melatonin synthesis of SDT fatty rats. One possibility is that no obvious changes in the mRNA expression in the SDT fatty rats was due to a time lag between the mRNA expression and the protein production. Thus, in addition to the time-course measurement of melatonin protein, the time-course of mRNA expression is needed in order to unveil the exact melatonin synthesis regulation in the SDT fatty rats. Similar findings have been reported by Frese et al., who demonstrated that melatonin synthesis was impaired in the pineal glands of GK rats (Frese et al., 2009). They also found that the mRNA expression of three of four melatonin-synthesizing enzymes (i.e. tryptophan hydroxylase, aromatic amino-acid decarboxylase, and Asmt) was significantly increased in the pineal gland of GK rats. Based on these findings, the authors suggested that the upregulated mRNA expression may be a compensatory response to a decrease in the efficiency of melatonin synthesis. Although melatonin content and its synthesis in the pineal gland of SDT fatty rats were not investigated in the present study, it may be that a similar compensatory response occurs in SDT fatty rats. Meanwhile, the mRNA expression of Aanat was decreased by approximately 50% in GK rats in contrast to SDT fatty rats. The findings from the present study confirm the findings of Frese et al in part, however, the opposite direction of changes in Aanat mRNA expression between the two studies suggests that the mechanism regulating melatonin synthesis in the pineal gland may differ between these two rat strains.

Altered melatonin secretion in diabetic patients has also been reported. For example, the physiological increase in nocturnal plasma melatonin concentrations was not observed in diabetic patients, especially those with neuropathy (O'Brien et al., 1986). Similarly, Peschke et al. reported reduced diurnal circulating melatonin levels in T2DM patients (Peschke et al., 2006). The findings of the present study support these clinical findings. Moreover, a relatively large clinical case control study suggested that the decreased nocturnal melatonin secretion may be the cause of T2DM (McMullan et al., 2013). It is important to investigate any intervention such as anti-diabetics will improve the circadian rhythm deficits as well as the symptoms of T2DM. Such an investigation should be performed in the future in order to judge the validity of this model as a T2DM comorbid with circadian rhythm deficit.

The results of the present study provide important evidence of the deficits in the circadian rhythms, as well as dysregulation of melatonin secretion, in animal models of T2DM. Unlike other animal models of DM, SDT fatty rats develop not only hyperglycaemia, but also hyperlipidaemia and insulin resistance from a young age as described in the Introduction. Thus, given the findings in this study, SDT fatty rats may be a useful animal model for investigating the relationship between deficits in the circadian rhythm and metabolic dysfunction.

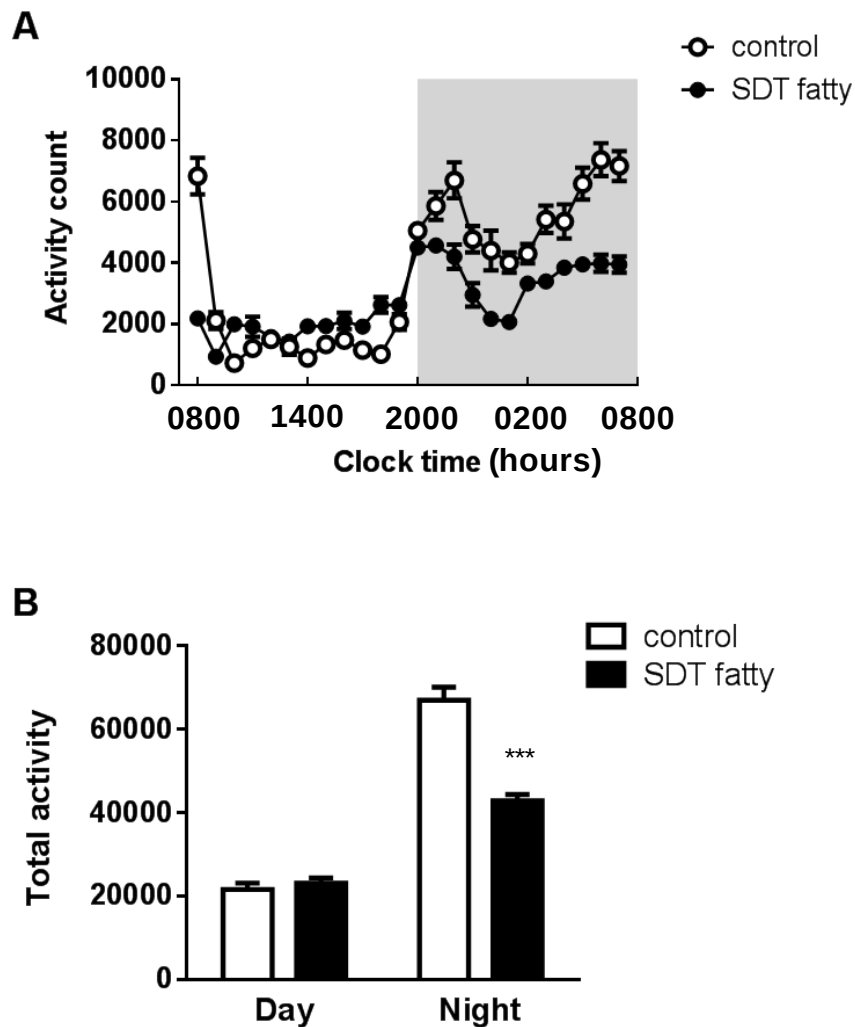


Figure 7. Circadian rhythm of SLA in control SD and SDT fatty rats. (A) Hourly averages of SLA over a 24 hour-period for 5 consecutive days under a standard light–dark cycle (the shaded area indicates the dark phase). (B) Total activity during the light and dark phases in control SD and SDT fatty rats. Data are the mean $\pm$ SEM (n=8 in each group). ***P < 0.001 compared with control SD rats (Aspin-Welch test).

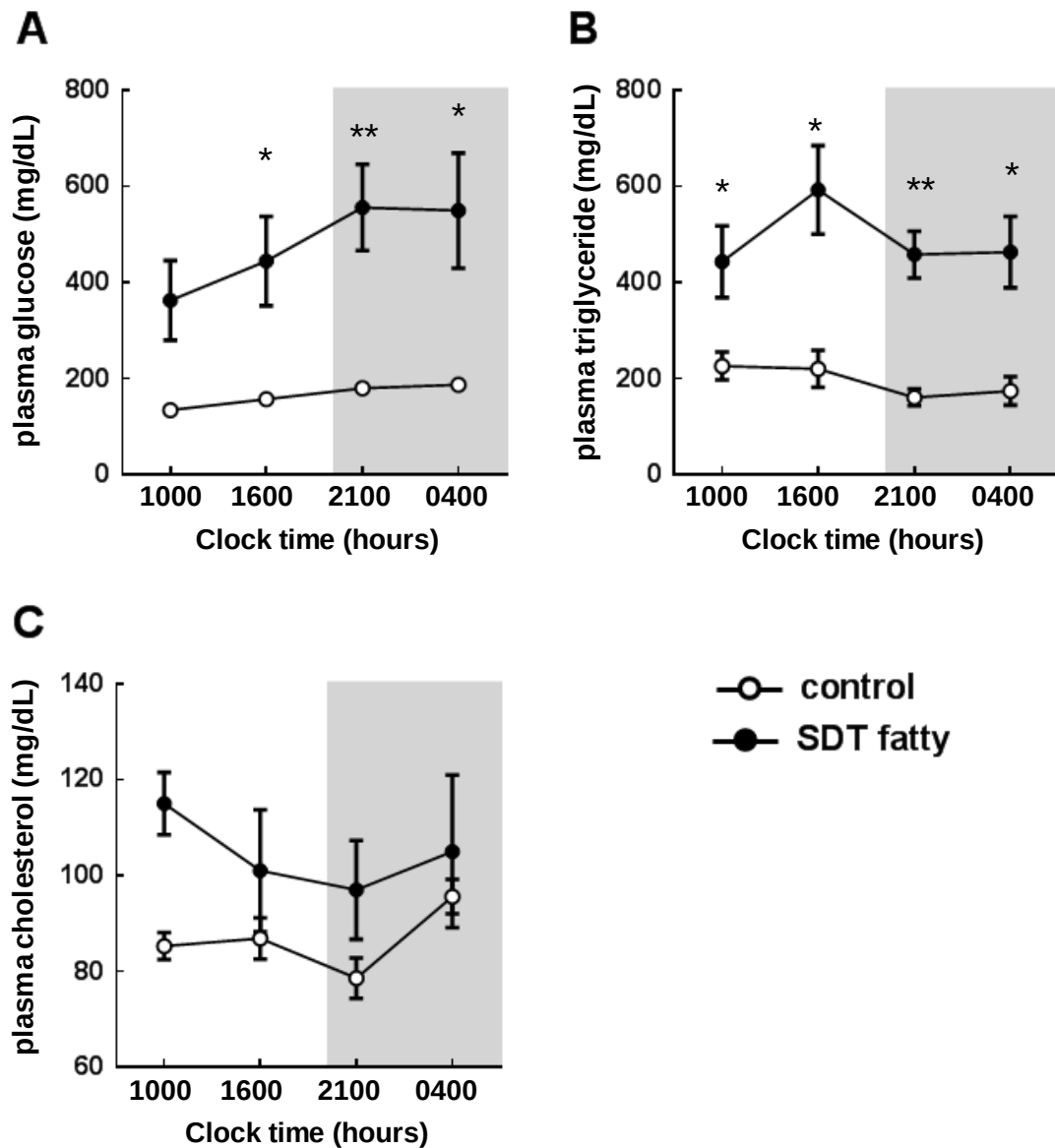


Figure 8. Circadian rhythms of plasma metabolic parameters in control SD and SDT fatty rats: (A) glucose, (B) triglyceride, and (C) total cholesterol. Data are the mean $\pm$ SEM (n=5 in each group). * $P < 0.05$, ** $P < 0.01$ compared with the control group at the same time point (two-way ANOVA with post hoc Aspin-Welch tests).

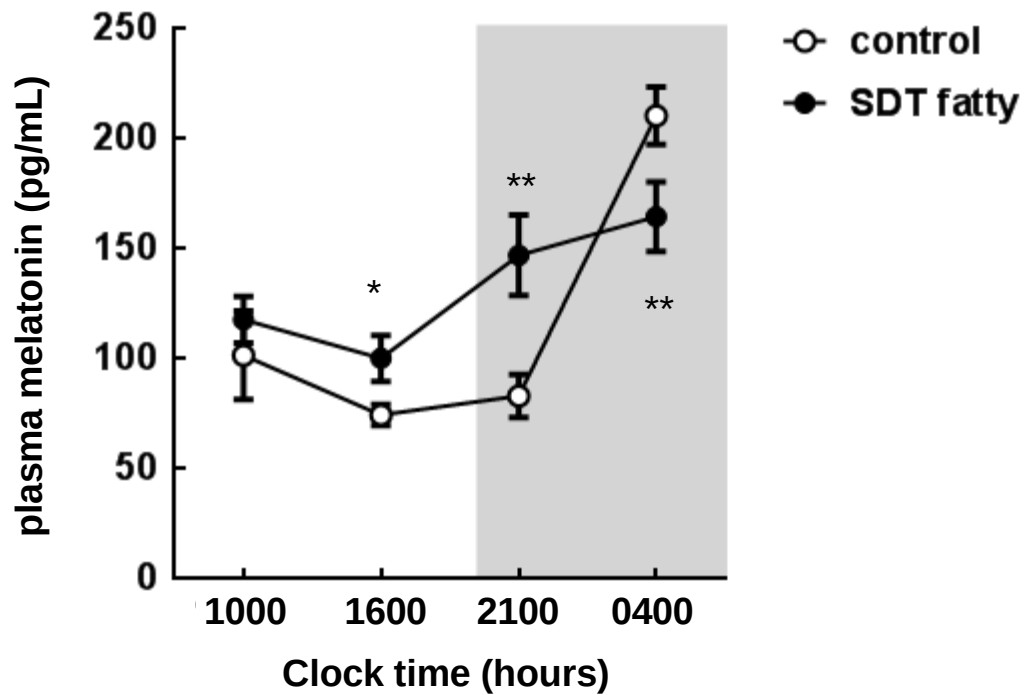


Figure 9. Circadian rhythm of plasma melatonin concentrations in control SD and SDT fatty rats. Data are the mean $\pm$ SEM (n=5 in each group). *P < 0.05, **P < 0.01 compared with the control group at the same time point (two-way ANOVA with post hoc Aspin-Welch tests).

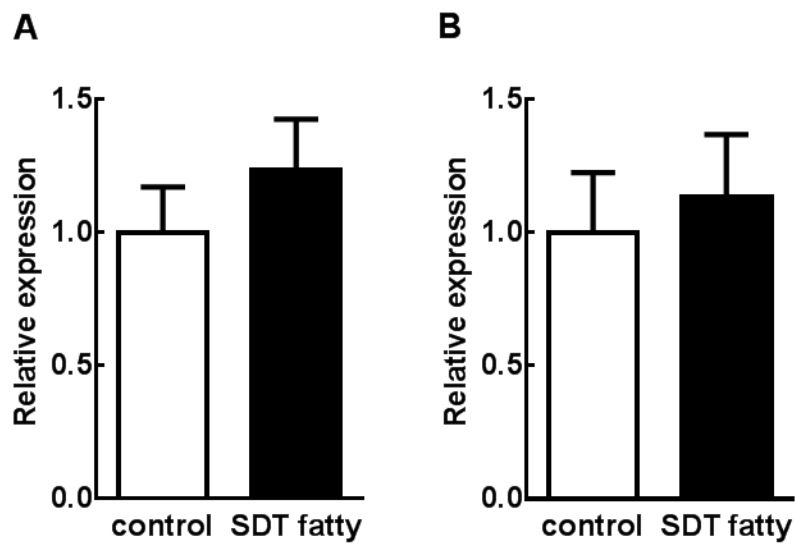


Figure 10. Relative expression of (A) *Aanat* and (B) *Asmt* mRNA in the pineal gland of control SD and SDT fatty rats at night (0400 hours). Data are the mean + SEM (n=6 in each group). There were no significant differences in *Aanat* and *Asmt* expression between the two strains ($P > 0.05$, Student's t-test).

Chapter 4

A novel Na⁺-independent alanine-serine-cysteine transporter 1 inhibitor inhibits both influx and efflux of D-serine

INTRODUCTION

Schizophrenia is a devastating neuropsychiatric disorder characterized by positive symptoms, negative symptoms, and cognitive deficits. The cause of this disease remains unclear, but several hypotheses have been proposed from clinical and preclinical studies (Ross et al., 2006, WHO, c). Dopaminergic hyperactivation is considered to be involved mainly in the positive symptom of schizophrenia because this symptom is improved by treatment with dopamine D2 antagonists (Kuepper et al., 2012). Hypofunction of NMDA receptors may underlie all three symptoms because NMDA receptor antagonists, such as phencyclidine or ketamine, induce both psychotic symptoms and cognitive deficits in rodents and primates as well as in humans (Krystal et al., 1994; Taffe et al., 2002). In addition, glutamate levels in the CSF of schizophrenic patients are reportedly lower than those in healthy controls (Hashimoto et al., 2005). These clinical and preclinical findings suggest that the activation of NMDA receptors is a promising strategy for schizophrenia treatment. D-serine and glycine are NMDA receptor co-agonists known to activate the NMDA receptor allosterically.

It has been reported that the number of glycine binding sites increased in the postmortem brains of schizophrenic patients, whereas the D-/L-serine ratio decreased (Ishimaru et al., 1994; Bendikov et al., 2007), suggesting that decreases in D-serine or glycine signaling may play a role in the pathophysiology of schizophrenia (Labrie et al., 2012; Nunes et al., 2012). In fact, adjunctive treatments with D-Serine or glycine in

addition to the stable antipsychotic regimen of patients ameliorated several symptoms, including cognitive deficits, in small clinical trials (Tsai et al., 1998). Moreover, D-serine improved the cognitive dysfunction induced by an NMDA receptor antagonist, MK-801, in rats (Karasawa et al., 2008; Shimazaki et al., 2010). The effective dose of D-serine was very high in both humans (30 mg/kg/day) and animals (0.8–1.0 g/kg), and the possibility of off-target and D-serine related peripheral side effects are concerns. Thus, selective increases in the extracellular D-serine levels in the target brain region are desirable, in which one approach is to inhibit the uptake of D-serine into the cells in the brain (Brown et al., 2014).

Transporters involved in the regulation of extracellular D-serine levels have been identified, i.e., asc-1, ASCT1 and ASCT2. These transporters are known to transport small neutral amino acids without stereoselectivity (Fukasawa et al., 2000; Ribeiro et al., 2002). In particular, asc-1 exhibits a CNS-enriched expression pattern including spinal cord and selective expression in neurons (Fukasawa et al., 2000). Furthermore, the uptake of D-serine was greatly reduced in the synaptosomes obtained from the hippocampus of asc-1 knockout mice (Rutter et al., 2007). These observations suggest that asc-1 is a major contributor to extracellular D-Serine uptake in the brain. It has been reported that SMLC, an asc-1 inhibitor patented by Lundbeck (Deerfield, IL; patent No. WO2003077998A1), inhibits D-serine uptake in human asc-1-expressing HEK293 cells as well as increasing the extracellular D-serine levels in the rat ventral hippocampus and medial prefrontal cortex (Ishiwata et al., 2013), which suggests that asc-1 inhibition can increase the extracellular D-Serine levels in the brain. Recently, Sason et al. (2017) reported that a novel asc-1 inhibitor, Lu AE00527, decreased the release of D-serine from rat brain slices and inhibited long-term potentiation, suggesting that asc-1 mediates tonic D-serine

release (Sason et al., 2017).

For the current study, we conducted high throughput screening to obtain a potent asc-1 inhibitor. After optimization of the hit compound, we identified ACPP. Modification of amine and carboxylic acid moieties in ACPP decreased asc-1 inhibitory activity, and introduction of substituents on the benzene ring in ACPP increased asc-1 inhibitory activity. We then examined the role of asc-1 in rat brains using ACPP as a chemical probe.

MATERIALS AND METHODS

Animals

Wistar rats (CLEA-Japan, Shizuoka, Japan) were used for primary neuronal culture. SD rats (Charles River, Tokyo, Japan) were used for primary astrocyte culture and in the microdialysis experiments. The rats were given food and water ad libitum and were maintained in groups under a 12-hour light–dark cycle (lights on at 0700). The rats were allowed to adapt to the environment for at least 1 week before the experiments. All animals were maintained and sacrificed according to the guidelines of the Takeda experimental animal care and use committee.

Chemical Treatment

ACPP was synthesized in our laboratory (>99.9% ee, tR1, CHIROBIO T). The specific optical rotation, $[\alpha]_{25}^{D166.08}$ (c50.407, MeOH), was measured by Sumika Chemical Analysis Service (Osaka, Japan). D-Serine and choline chloride (ChCl) were purchased from Wako Pure Chemical Industries (Osaka, Japan), and SMLC was purchased from Sigma-Aldrich (St. Louis MO). D-[^3H]Serine was obtained from

PerkinElmer Life and Analytical Sciences (Waltham, MA). For the in vitro experiments, the compounds were dissolved in assay buffer containing sodium chloride (NaCl) or ChCl before application to the cells after dilution with the assay buffer at the indicated concentrations. For the in vivo microdialysis experiments, the compounds were dissolved in Ringer's solution at the indicated concentrations.

Construction of Chinese Hamster Ovary (CHO) Cells That Stably Expressed human asc-1

A CHO cell line that stably expressed human asc-1 (hAsc-1; NCBI reference sequence NM_019849) was constructed by transfecting cells with hAsc-1-pcDNA3.1 expression plasmid, followed by selection with 500 µg/ml Geneticin. Cells were cultured routinely in Ham's F-12 medium supplemented with 10% heat-inactivated fetal bovine serum (FBS) and 1% G418 in a 5% CO₂/95% air atmosphere.

Rat Primary Cultures

Primary cortical neurons were prepared from embryonic day 18 Wistar rat embryos (CLEA-Japan), as described previously (Onishi et al., 2011). Briefly, cortical fragments of eight litters were dissociated into single cells with a papain-containing nerve cell dispersion kit (Sumitomo Bakelite, Akita, Japan) and resuspended in nerve cell culture medium (Sumitomo Bakelite). The neurons were seeded onto poly-D-lysine/laminin-coated 96-well plates (BD Biosciences, Franklin Lakes, NJ) at a density of 30,000 cells per well and cultured in Neurobasal medium supplemented with 2% B27 for days in vitro 13–19 to perform the uptake assay.

Primary astrocyte cultures were prepared from the cortex of eight pups of SD

rats at postnatal day 3. Briefly, the cells were isolated with a papain-containing nerve cell dispersion kit, as described above, and then cultured in DMEM/F12 with 10% heat-inactivated FBS. After the cells had grown to confluence, they were shaken for 1 hour at 200 rpm to remove the microglial cells and oligodendrocytes and then cultured for an additional 2 weeks. After the cells had grown to confluence at passage 1, they were cryopreserved at -80°C as a stock. Before the assays, cryopreserved astrocytes were thawed in plating medium and transferred to noncoated 96-well plates at a density of 30,000 viable cells per well and then used at DIV 3.

D-Serine Uptake and Efflux Assay with Cell Cultures

Primary cultures or CHO cells that stably expressed hAsc-1 were cultured in 96-well plates and loaded with 100–400 nM D- ^3H Serine in NaCl-containing buffer (NaCl-buf; 135 mM NaCl, 1.5 mM KCl, 1.0 mM KH_2PO_4 , 1.2 mM MgSO_4 , 1.2 mM CaCl_2 , 5.0 mM D-glucose, and 5 mM HEPES; pH 7.4) or ChCl-containing buffer (ChCl-buf) in which the NaCl in the NaCl-buf was replaced with ChCl. The uptake assay was performed with 100 nM D- ^3H Serine for 15 min with hAsc-1-expressing CHO cells with 100 nM D- ^3H Ser for 30 min in the primary neurons or with 200 nM D- ^3H Serine for 30 min in the primary astrocytes. In all of the experiments, the uptake of D- ^3H Serine was terminated by washing the cells twice with ice cold buffer. The radioactivity was monitored by adding Microscint PS (PerkinElmer) to the cells. Kinetic constants were calculated by a nonlinear regression curve fitting in Prism 5 (GraphPad Software; LaJolla, CA). Competitive inhibition was analyzed with the equation $K_m(\text{app}) = K_m \times (1 + [\text{I}]/K_i)$, where $K_m(\text{app})$ is the K_m in the presence of the inhibitor. In the D- ^3H Serine release experiments, the cells were preloaded with 400 nM D- ^3H Serine for 30 min.

Subsequently, the wells were washed twice with ice-cold buffer and exposed to ChCl-buf containing ACPP, SMLC, and D-Serine for 30 min at room temperature. The intracellular D-[³H]Serine level (intra-D-Serine) immediately after preloading was regarded as the baseline (no efflux), and the remaining intra-D-Serine after incubation with drugs was determined after lysing with Microscint PS. The degree of D-serine release was calculated with the formula (no efflux – intra-D-serine x 100)/no efflux.

Quantitative TaqMan PCR Analysis

The primer and probe sequences were as follows: (rat asc-1) probe, Fam-5'-CTCCATGGCCTGCCTGATGCTCC-3'; F-primer, 5'-CAGCCACAGCCTCTCGAGTAC-3'; R-primer, 5'-TGGAGCTGTTCACCCATGTC-3', and (rat ASCT2) probe, Fam-5'-CTGCCCATCGGCGCCACC-3'; F-primer, 5'-CAAACACATCAGCCGGTTCAT-3'; R-primer, 5'-GCTGCCCCGTCCATATTG-3'. These sequences were designed in Primer Express (Applied Biosystems, Foster City, CA). Standard curves were generated with serial dilutions of known quantities of standard template DNA (Sigma Genosys, Spring, TX). TaqMan PCR assays for each gene target were performed in duplicate with cDNA samples or standard DNA in 384-well optical plates with an ABI Prism 7700 sequence detection system (Applied Biosystems). For each TaqMan reaction, the cDNA or standard DNA was mixed with 2 x TaqMan Universal PCR master mix (Applied Biosystems), 100 μM of sense primer, 100 μM antisense primer, and 10 μM TaqMan probe. The PCR parameters were 50°C for 2 min, 95°C for 10 min, 40 cycles at 95°C for 15 sec, and 60°C for 1min. The data were analyzed in SDS 2.2.2 (Applied Biosystems). The increase in the fluorescence intensity of the reporter dye (DRn) was plotted against the cycle number. The threshold cycle (Ct) was calculated as the cycle number where

DRn crossed the threshold. The cDNA copy number in each sample was calculated from the Ct by linear interpolation with the standard curve; the unit used in Table I is the copy number.

Microdialysis Technique

Male SD rats were anesthetized with 50 mg/kg (intraperitoneal) somnopenyl (Dainippon Sumitomo Pharma, Osaka, Japan), and guide cannulas (AG-8; Eicom, Kyoto, Japan) were stereotactically implanted into the ventral hippocampus (coordinates 5.6 mm posterior to bregma, 25.0 mm lateral, 7.0 mm ventral to dura). The rats were allowed to recover from surgery for at least 2 days. On the day of the experiment, rats were randomly assigned to eight groups: vehicle-treated group (n = 5), ACPP 10 μ M-treated group (n = 6), ACPP 100 μ M-treated group (n = 4), and ACPP 1,000 μ M-treated group (n = 5) for the test with ACPP and vehicle-treated group (n = 6), 0.1 mM SMLC-treated group (n = 6), 1 mM SMLC-treated group (n = 6), and 10 mM SMLC-treated group (n = 6) for the test with SMLC. A microdialysis probe (A-I-8-02; Eicom) was inserted through the guide cannula. The probes were connected via a dual-channel swivel to a microinjection pump. Filtered Ringer's solution (145 mM NaCl, 3 mM KCl, 1 mM MgCl₂, 1.2 mM CaCl₂) was perfused for the duration of the experiment at a constant flow rate of 1 μ L/min. After perfusion for more than 2 hr to allow baseline stabilization, a sampling of the dialysate was initiated. A 40-min sampling regime was used throughout the experimental period. SMLC and ACPP were dissolved in filtered Ringer's solution and diluted at the indicated concentration. Ringer's solution was infused locally into the ventral hippocampus via the implanted dialysis probe for 2 hr before switching to Ringer's solution containing SMLC or ACPP, with additional infusion for 2 hr to allow sample collection. The collected

samples were stored at -80°C until analysis by HPLC. After the experiments, the dye was injected into the site of the dialysis probe to determine whether the dialysis probe was inserted correctly into the ventral hippocampus (data not shown). No rats were excluded because of misinsertion in this study. The average of the D-serine concentrations during the period preceding vehicle or drug treatment (three measurements were performed every 40 min) was used as the baseline (100%). Individual data were expressed as percentages relative to this baseline period. Mean and SEM were obtained for the results with four to six animals and were calculated for the corresponding periods. Area under the curve (AUC) was calculated for the concentration vs. the time in plots for the dialysate D-Serine levels at 0–120 min post injection.

Measurement of D-Serine in Rat Brain Dialysate by HPLC

To quantify the D-serine level by HPLC with fluorometric detection, an aliquot of each sample was derivatized for 2.5 min with N-acetyl-L-cysteine (Wako Pure Chemical Industries) and o-phthaldialdehyde (Wako Pure Chemical Industries) at room temperature. The derivatized sample was applied immediately to the HPLC system (HTEC-500; Eicom). The column (EX-3; Eicom) was operated at a constant flow rate of 0.5 mL/min at room temperature. Mobile phase A was 0.1M acetate buffer (pH 6.0) containing 18% methanol, and mobile phase B was acetate buffer containing 40% methanol. The amino acid derivatives were separated with a linear gradient from mobile phase A to B over 28 min.

Statistical Analysis

Data are expressed as mean $\pm$ SEM and were analyzed by one-tailed Williams'

test; $P < 0.025$ was considered significant. All analyses were performed in SAS System 8 (SAS Institute, Cary, NC).

RESULTS

Inhibitory Activity of ACP

We performed in vitro high-throughput screening using a D-[^3H]Serine uptake assay in cells that stably expressed hAsc-1, and we identified ACP from the Takeda Chemical Library (Fig. 12). As shown in Figure 13A, the hAsc-1-expressing cells used for high-throughput screening exhibited robust uptake of D-[^3H]Serine, which was attenuated almost completely (98.3%) by 10 mM cold D-Serine ($n = 3$). ACP inhibited the uptake of D-[^3H]Serine in a concentration-dependent manner, with an IC_{50} of $0.72 \pm 0.13 \mu\text{M}$ ($n = 3$), and SMLC had an inhibitory effect, with an IC_{50} of $78.9 \pm 18.0 \mu\text{M}$ ($n = 3$; Figure 13B). As shown in Figure 13C, D, ACP had a competitive inhibition pattern according to the Lineweaver-Burk plot analysis. The K_m value for D-serine was $8.68 \mu\text{M}$, and treatment with ACP increased the K_m to $71.3 \mu\text{M}$. To evaluate ACP in native cells, we examined the effects of ACP on the D-[^3H]Serine uptake activity using primary neurons and primary astrocytes derived from rat cortex. As shown in Figure 14A, both ACP and SMLC showed a concentration-dependent inhibition of D-[^3H]Serine uptake in the primary neurons under Na^+ -free conditions, with IC_{50} values of $0.89 \pm 0.30 \mu\text{M}$ ($n = 3$) and $50.7 \pm 14.4 \mu\text{M}$ ($n = 3$), respectively. In contrast, in the uptake assay with primary astrocytes under NaCl-existing condition, ACP had no inhibitory effect, whereas SMLC inhibited the uptake of D-[^3H]Serine in a concentration-dependent manner, with an IC_{50} of $27.4 \pm 3.6 \mu\text{M}$ ($n = 3$; Figure 14B). Some previous studies have also indicated that ASCT2 plays a role in the control of D-serine in astrocytes (Shao et al., 2009). Gene

expression analysis by TaqMan PCR demonstrated that asc-1 mRNA was selectively detected in rat primary neurons, whereas ASCT2 mRNA was more robustly expressed than asc-1 mRNA in rat primary astrocytes (Table 2). This observation suggests that ACPD selectively affects the activity of asc-1 in neurons.

Effects of asc-1 Inhibitors on the Extracellular D-Serine in Rat Hippocampus

To investigate the effects of ACPD on the extracellular D-serine levels in the brain, we monitored the extracellular D-serine levels by microdialysis in rats. SMLC and ACPD were infused locally into the ventral hippocampus via a dialysis probe, and the D-serine levels in the collected dialysate were determined by HPLC. As reported previously (Ishiwata et al., 2013), SMLC significantly increased the extracellular D-serine levels up to approximately 150% of the baseline levels in a concentration-dependent manner ($n = 6$) relative to vehicle treatment ($n = 6$, $P < 0.025$; Figure 15A, C), as revealed by Williams' test. In contrast, ACPD significantly decreased the extracellular D-serine levels ($n = 4-6$) relative to vehicle treatment ($n = 5$, $P < 0.025$; Figure 15B, D), as revealed by Williams' test.

Effects of asc-1 Inhibitors on the Efflux of D-Serine

asc-1 has been reported to function as an exchanger in which the release of ^{14}C -labeled amino acids preloaded into oocytes expressing asc-1 was enhanced by adding its substrate extracellularly, i.e., alanine (Fukasawa et al., 2000). In addition, Rosenberg et al. (2010) showed that D-alanine enhanced the release of endogenous D-serine via asc-1 in both asc-1-expressing cells and rat hippocampal slices (Rosenberg et al., 2010). Thus, we investigated the effects of ACPD and SMLC on the efflux of D- ^3H serine using D-

[³H]Serine-preloaded hAsc-1-expressing CHO cells. The asc-1-expressing CHO cells exhibited the spontaneous efflux of preloaded D-[³H]Serine, with approximately 30–40% efflux during incubation for 30 min. As shown in Figure 16A, the extracellular addition of cold D-Serine significantly enhanced the efflux of D-[³H]Serine in a concentration-dependent manner (n = 6, P < 0.025). SMLC also significantly enhanced the efflux of D-[³H]Serine in a concentration-dependent manner (n = 6, P < 0.025; Figure 16B). In contrast, ACPP did not affect D-[³H]Serine efflux under the same experimental conditions (n = 6; Figure 16C).

Figure 14 illustrates the difference of mode of action between SMLC and ACPP. From the finding from in vitro and in vivo experiments, we found a clear difference specifically on the D-serine efflux from the neuron, suggesting asc-1 plays a critical role in tonic regulation of the synaptic D-serine levels in the brain.

DISCUSSION

To obtain a novel asc-1 inhibitor, we performed in vitro D-[³H]Serine uptake assays with cells that stably expressed hAsc-1, and we identified ACPP. SMLC had a moderate inhibitory activity, with an IC₅₀ of 78.9 ± 18.0 μM (Figure 14B), which was similar to the potency reported previously, whereas ACPP had an inhibitory activity, with an IC₅₀ of 0.72 ± 0.13 μM in the same assay. This result suggests that ACPP has an inhibitory activity that is approximately 100 times more potent than that of SMLC.

ACPP had inhibitory effects on hAsc-1-expressing cells and rat primary neurons in a competitive manner with similar IC₅₀ values (Figure 13B, 14A) but no inhibitory effect on rat primary astrocytes (Figure 14B). In this study, primary neurons isolated from Wistar rats and astrocytes isolated from SD rats were used. These data are in line with

our previous observation of asc-1 expression in primary neurons but not in the primary astrocytes in SD rats (Matsuo et al., 2004). Gene expression analysis by TaqMan PCR demonstrated that asc-1 was intensely expressed in primary neurons but was rarely expressed in primary astrocytes (Table 2). In addition, ASCT1 and volume-regulated anion channels, which are known to play roles in D-Serine regulation, are also expressed in astrocytes (Yamamoto et al., 2001). Given these observations, it is possible that ACPP selectively acts on neuronal asc-1 against other transporters involved in D-serine transport. Our results are also in line with previous studies demonstrating that asc-1 is restricted to neurons and plays an important role in the dynamics of its substrates in neurons (Fukasawa et al., 2000; Rosenberg et al., 2010). On the other hand, SMLC inhibited the uptake of D-serine in both neurons and astrocytes (Figure 14A, B). In vitro, ACPP inhibited both uptake and efflux of D-Serine, suggesting that asc-1 has both of these activities. Therefore, whether asc-1 inhibitor results in upregulation or downregulation of extracellular D-serine levels may be affected by equilibration determined by intracellular and extracellular levels.

Inhibition of asc-1 by SMLC in the brain is considered to increase the extracellular D-serine levels according to microdialysis analyses (Ishiwata et al., 2013). We replicated SMLC increase of the extracellular D-serine levels in the rat ventral hippocampus in a concentration-dependent manner (Figure 15A, C). We were surprised to discover that ACPP, unlike SMLC, significantly decreased the extracellular D-serine levels in a concentration-dependent manner (Figure 15B, D). asc-1 is reported to work as an exchanger that mediates both the uptake and efflux of asc-1 substrates (Fukasawa et al., 2000), so we hypothesized that the opposite effects of ACPP and SMLC on the hippocampal extracellular D-serine levels were the result of differences in their impacts

on the substrate efflux function of asc-1 rather than influx. ACPD prevented the efflux of D-[³H]Serine in hAsc-1-expressing CHO cells, whereas SMLC enhanced the substrate efflux in a concentration-dependent manner (Figure 16A,B). These results suggest that ACPD might decrease the extracellular D-serine levels by inhibiting the efflux of endogenous D-Serine from the neurons, whereas SMLC increases the extracellular D-serine levels by enhancing the efflux of D-serine, and that SMLC is not an inhibitor but works as the substrate of asc-1. We cannot explain the difference in these mechanisms of action, but a previous analytical study with a choline transporter showed that the velocity of a substrate analog affected the transport rate of the labeled substrate when it was presented on the trans side of the membrane (Deves and Krupka, 1979). Assuming that asc-1 follows this proposed model, it is possible that ACPD is a substrate with a different transport velocity compared with D-serine and SMLC. Additional kinetic studies with radiolabeled ACPD and SMLC are required to test this hypothesis.

A large body of evidence supports the idea that NMDA hypofunction is associated with the pathophysiology of schizophrenia, and NMDA activation via increasing D-serine levels is an attractive approach. We originally hypothesized that the main function of asc-1 in the brain is D-Serine uptake and that inhibition of asc-1 can increase the synaptic D-serine levels (Helboe et al., 2003; Xie et al., 2005; Rutter et al., 2007). However, ACPD was found to decrease the extracellular D-serine levels. Our data are in line with a very recent study showing that a novel asc-1 inhibitor, Lu AE00527, decreased the release of D-serine from rat brain slices and inhibited LTP (Sason et al., 2017). These data indicate that asc-1 modulates synaptic D-serine levels via efflux mechanisms and that inhibition of asc-1 results in the reduction of D-serine levels in the brain. This is an unexpected finding; however, NMDA inhibition is also useful for some

disorders other than schizophrenia, such as stroke and MDD. For example, chronic corticosterone exposure and isolation stress significantly reduced NMDA receptor subunit 1 (NR1) expression in rodents (Cohen et al., 2011; Hermes et al., 2011). Malkesman et al. (2012) showed the antidepressant-like effects of D-serine in multiple behavioral tests including the FST, female urine sniffing test, which is a non-operant test for measuring reward-seeking behavior in rodents based on interest in sniffing pheromone odors from the opposite sex and learning helplessness model in 5-HT-depleted rodents. In addition, they also tested the behavior of NR1 knockout mice and found that NR1 knockout mice and found depression-like behaviors in the above tests that did not respond to D-serine (Malkesman et al., 2012). Additional preclinical characterization of ACPP for these disorders should be considered in the future. The upregulation of the D-Serine levels by SMLC observed in the current study and by others may result from the inhibition of another transporter. In fact, SMLC inhibited D-serine uptake in astrocytes, in which asc-1 expression level is low (Figure 14B) (Sason et al., 2017).

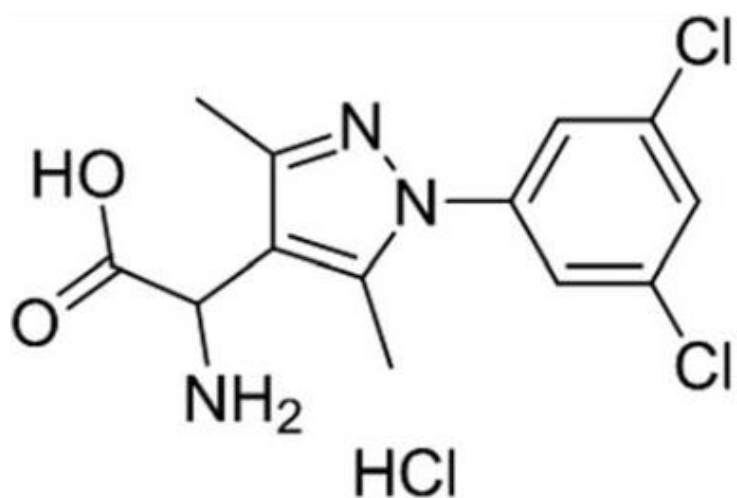


Figure 12. Chemical structure of ACPP.

Table 2 Different Expression Patterns of asc-1 and ASCT2.

parameter	asc-1	ASCT2
Neuron	3.2×10^4	UDL [†]
Astrocyte	5.8×10^1	5.7×10^2

*Data are expressed as the copy number.

[†]Under detection limit.

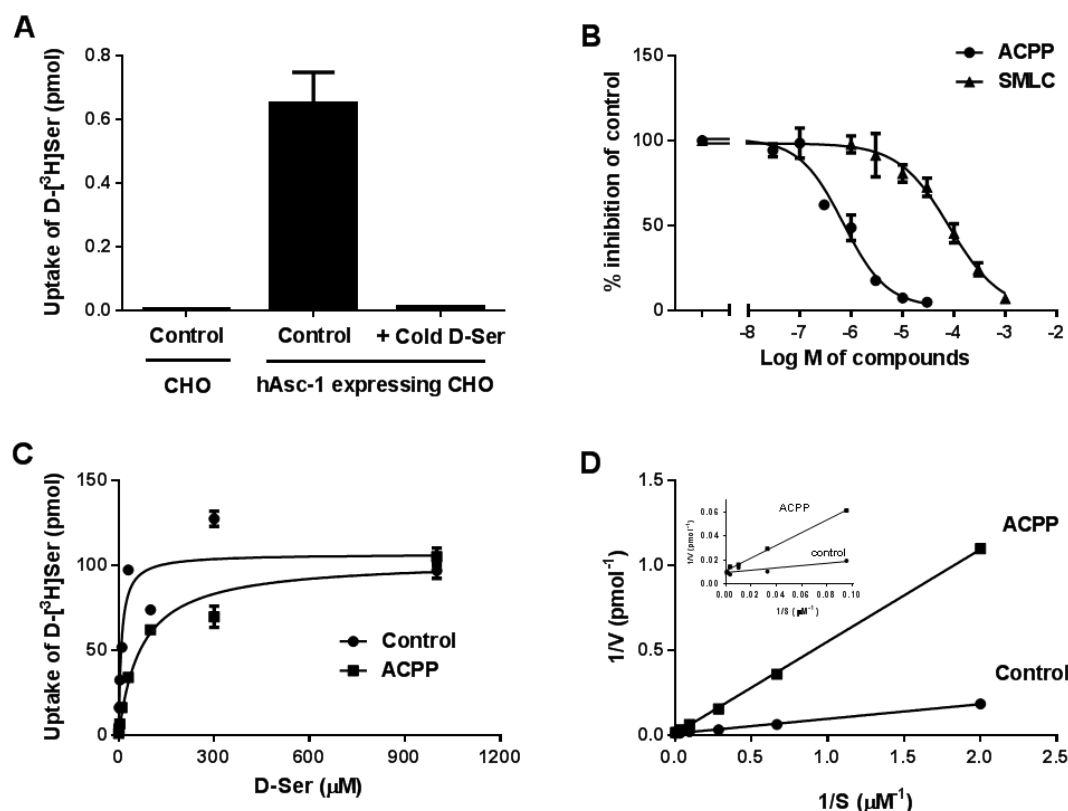


Figure 13. Inhibitory effect of ACPP on D-[³H]Serine in the uptake assay with hAsc-1-expressing CHO cells. A: Uptake of D-[³H]Serine by hAsc-1-expressing CHO cells and inhibition by 10 mM cold D-Ser. Data are mean $\pm$ SEM from three independent experiments expressed as percentages relative to the control. B: Concentration-dependent inhibition of uptake by ACPP and SMLC. C: Uptake of D-[³H]Serine in the absence (control) and presence (ACPP) of 3 μ M ACPP. Each point represents mean $\pm$ SEM from of four to six wells. D: Lineweaver-Burk plot of the data from C. Inset: Plot of the data without three data points of high 1/S for clarity.

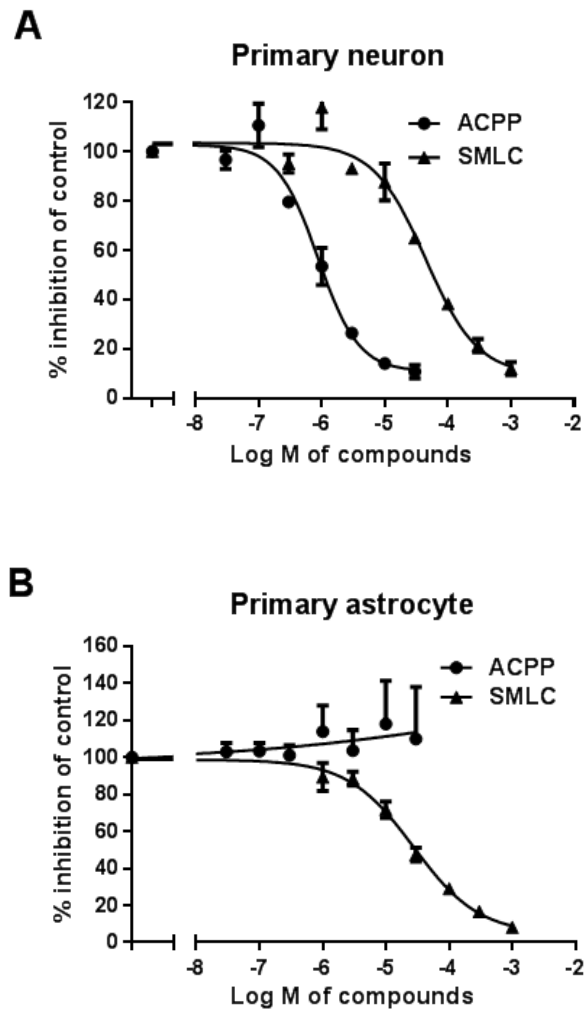


Figure 14. Effects of ACP and SMLC on the uptake of D-[³H]Serine in rat primary neurons (A) and primary astrocytes (B). Data are mean $\pm$ SEM from three independent experiments expressed as percentages relative to the control.

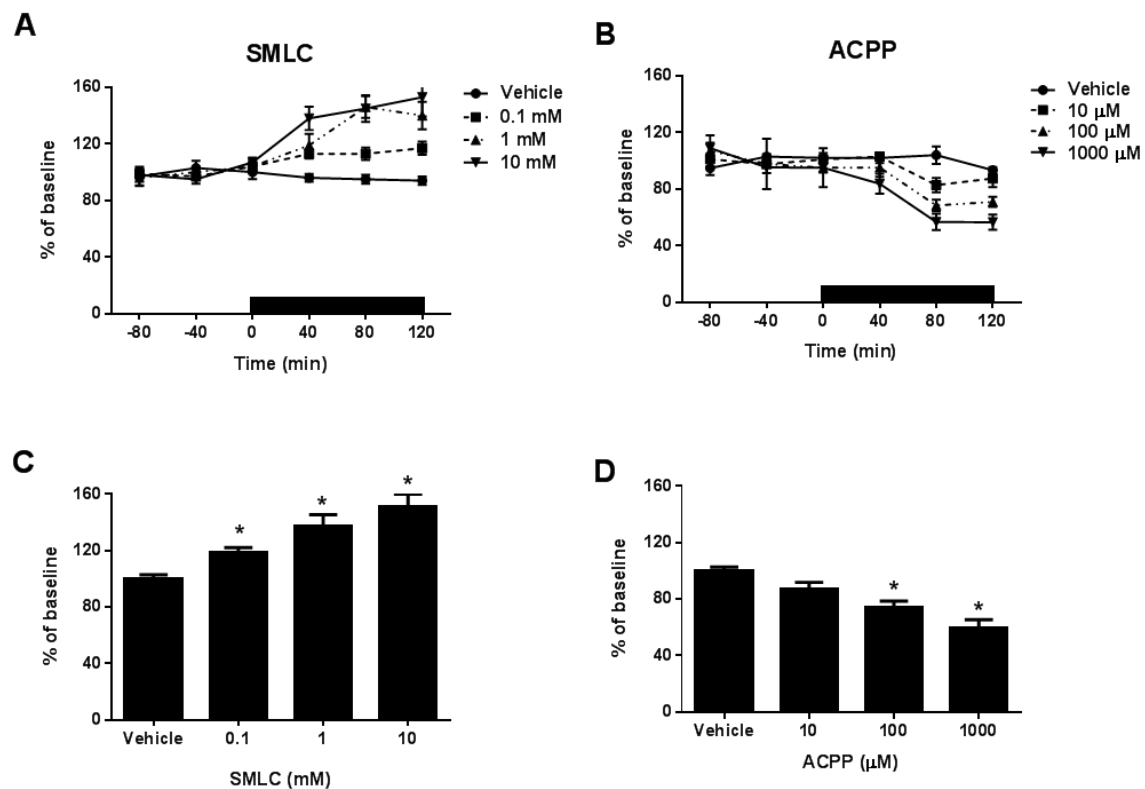


Figure 15. Effects of intrahippocampal perfusion of SMLC and ACPP on the extracellular D-serine levels in freely moving rats. The time course is shown for the D-serine levels (A, B) and AUCs over 0–120 minutes (C, D). Bars in A, B represent the duration of local drug infusion. Data are mean $\pm$ SEM, $n = 4$ –6 per group. * $P < 0.025$ vs. vehicle-treated group, parametric Williams' test.

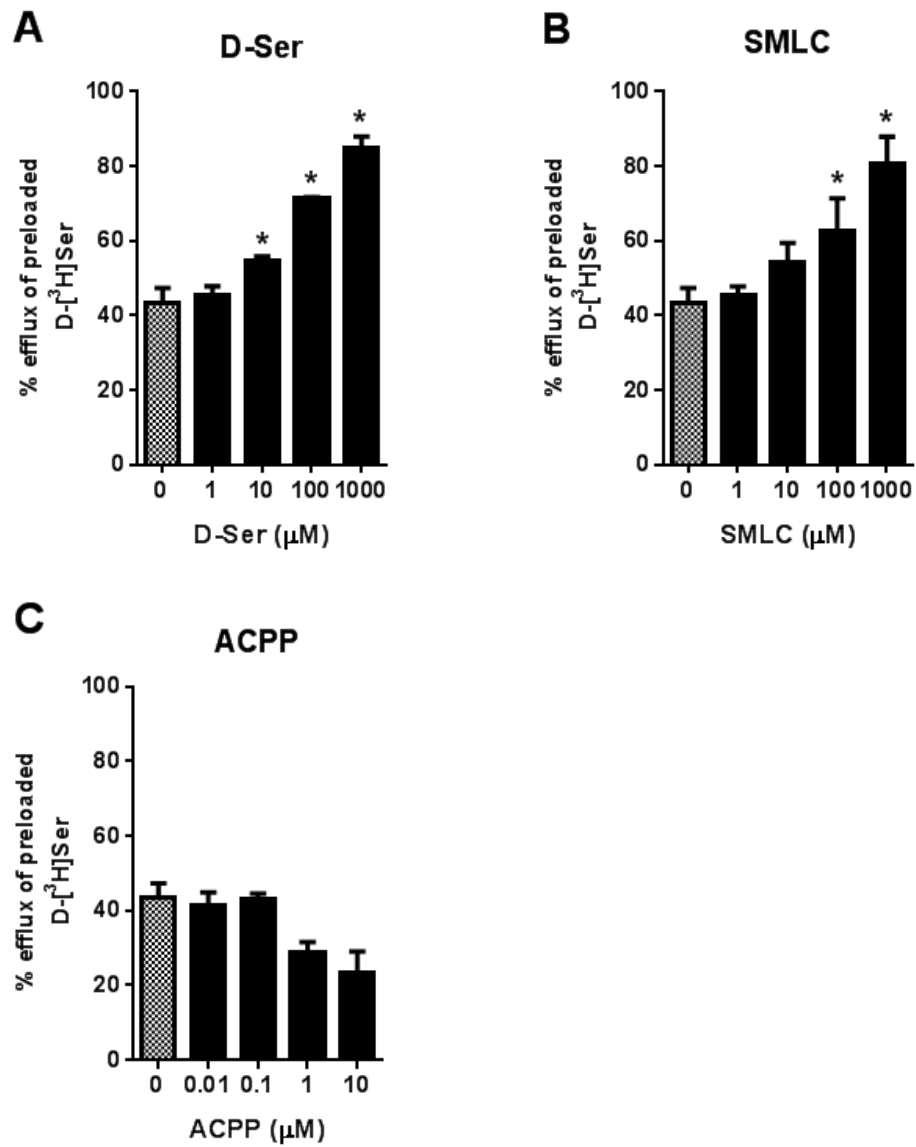


Figure 16. Effects of D-Ser (A), SMLC (B), and ACPP (C) on the efflux of D-[3 H]Serine preloaded into hAsc-1-expressing CHO cells. Data are the percentage efflux of preloaded D-[3 H]Serine. *P < 0.025 vs. vehicle-treated group, parametric Williams' test.

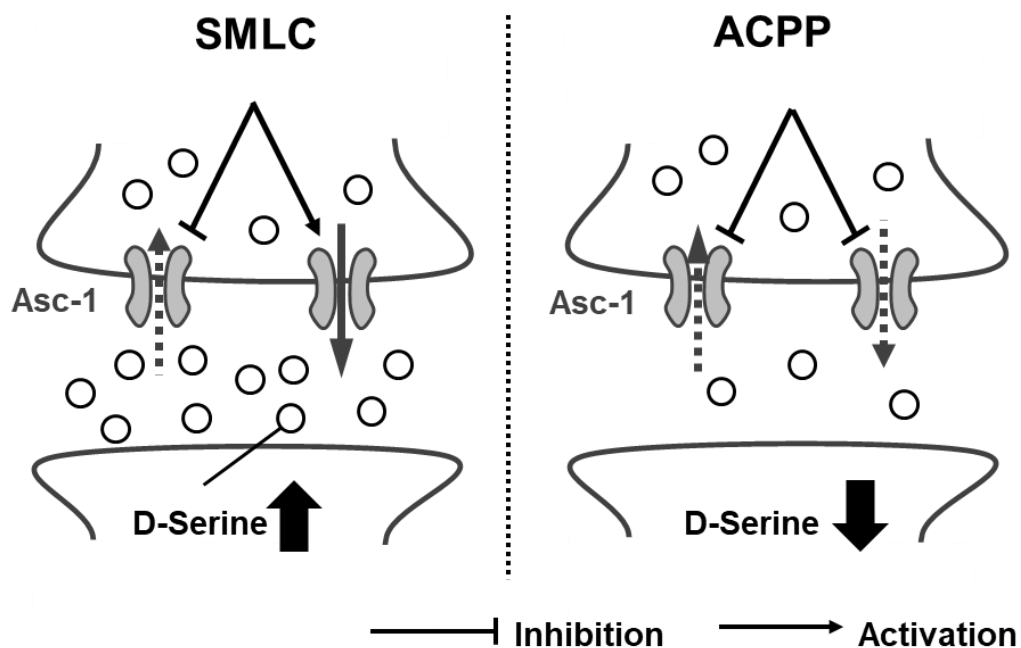


Figure 17. Illustration of the difference of mode of action between SMLC and ACPD. ACPD has a unique mechanism of action in vitro as revealed by the observation that ACPD inhibited the efflux as well as uptake of D-serine in contrast to a well-known asc-1 inhibitor, SMLC, which inhibited only uptake. Under these profiles, ACPD reduced the extracellular D-serine levels in the rat brain whereas SMLC increased it.

Chapter 5

General Discussion

Mechanism of depression

Mental disorders severely affect patient QOL, and the number of people with mental disorders is expected to grow as the world population increases and society becomes more stressful. Although a variety of antidepressants have been developed, populations of patients remain whose symptoms are not improved even using drug combinations. For example, approximately 30% of patients with depression are defined as treatment-resistant and show no response to any of the antidepressant drug classes (Mitani et al., 2006). Some of the most common symptoms of MDD are characterized by decreases in motivation and blunted ability to feel reward (anhedonia) (Nestler et al., 2002). Specifically, excessive anhedonia leads to recurrent suicidal ideation without a specific plan, which is one of the features of MDD (American Psychiatric Association, 2013). Thus, the finding in this study should be a clue to investigate the pathophysiology of depressive state observed in the T2DM using SDT fatty rats. In addition, a large amount of evidence suggests that the HPA axis is hyperactivated in patients with MDD (Knorr et al., 2010; Carroll et al., 1976; Choi et al., 2018). Moreover, corticosterone has been known to increase plasma glucose. According to this, the increased corticosterone secretion increases the plasma glucose, which probably accelerates hyperglycemia shown in SDT fatty rats. In addition, the increase in the plasma corticosterone is reported to reduce the hippocampal volume (Zhang et al., 2015). Although it remains unclear what causes the hypersecretion of corticosterone, this vicious cycle between high plasma corticosterone and hyperglycemia may underlie the pathophysiology of the depression-like behavior in

SDT fatty rats.

Moreover, there is also an evidence suggesting that patients with MDD exhibit cognitive impairments in its acute phase (Hammar and Ardal, 2009; Rock et al., 2014). It is reported that the high plasma corticosterone secretion probably plays a role in such impairment (Egeland et al., 2005). In addition, the impaired neurotransmissions such as 5-HT and glutamate found in this study are known to play roles in the cognitive functions (Logue and Gould, 2014; Volk et al., 2015). Although cognitive functions were not tested in this study, it is worth testing whether SDT fatty rats exhibit cognitive impairments in the future because it may reveal the mechanism of the cognitive deficits in MDD.

Mechanism of blunted circadian rhythm

There have been reported that STZ-treated rats/mice and GK rats, which is a non-obese insulin-independent DM model, exhibit the deficits in the circadian rhythms (Velasco et al., 1988; Frese et al., 2009). Unlike these models, SDT fatty rats has many features of the insulin resistance as well as obesity, which is the majority of T2DM in clinic (Katsuda et al., 2015). Thus, the finding of deficits in the circadian rhythms in SDT fatty rats seems worthwhile in terms that SDT fatty rat may be a suitable animal model to help more deep understanding of the pathophysiology in human. Consistent with the previous report which demonstrated the deficits in the melatonin secretion in GK rats (Frese et al., 2009), the circadian rhythms of plasma melatonin were blunted in SDT fatty rats. In contrast, this is the first report that the circadian rhythms of plasma metabolic parameter were impaired in the animal models of DM. Consistent with these observations, studies in shift workers provide evidence for the link between disrupted sleep cycles and elevated triglyceride levels, even after adjusting for other factors such as dietary intake

(Karlsson et al., 2001; Mohebbi et al., 2012). Interestingly, Tsujino et al. (2006) demonstrated that 5-HT levels in the PFC were significantly higher in the animal model of autism, which also shows an aberrant sleep–wake cycle (Bendikov et al., 2007). In this study, the SDT fatty rats showed reduced 5-HT contents in the PFC. However, although the direction of change is opposite to the previous findings, the deficits in 5-HT transmission might disturb the sleep–wake cycle.

As mentioned above, the deficits in the circadian rhythms is suggested to closely linked with a variety of mental disorders as well as metabolic disorders. The findings that SDT fatty rats exhibited not only depression-like behavior but also the deficits in the circadian rhythms suggest that SDT fatty rats can be a good tool for investigating the interrelationship between depression, circadian rhythm and metabolic disorder.

D-serine signaling in CNS diseases

The importance of D-serine in normal brain function and its fluctuation in many CNS diseases has been gaining attention year by year (Guercio and Panizzutti, 2018). Thus, a more comprehensive understanding of synaptic D-serine in the brain is highly needed. Most of the approaches thus far involved activating D-serine signaling to investigate the role of D-serine have been dosing D-serine itself both in clinical and preclinical testing. However, the dosage of D-serine was high due to its low BBB penetration (Karasawa et al., 2008; Shimazaki et al., 2010). As expected, dosing high amount of D-serine is reported to induce off-target related peripheral side effects. For example, a single administration of D-serine at a dose of 750 mg/kg caused a selective necrosis to the pars recta region of the renal proximal tubules in the rat (Ganote et al., 1974).

To investigate the regulation mechanism at synapse, I focused on asc-1, which is specifically located at synapse. Asc-1 has long been an attractive therapeutic drug target for schizophrenia according to one report showing the increase of synaptic D-serine levels by SMLC (Helboe et al., 2003). However, the findings in this study has revealed that the role of asc-1 was contrary to what it used to believed. Moreover, these findings potentially provide the usefulness of ACPP as a good tool compound for investigating the role of synaptic D-serine in a variety of CNS diseases. Indeed, accumulating evidence suggest that activation of D-serine signaling plays a role in the pathophysiology of MDD or circadian rhythm deficits. For example, significantly higher D-serine levels in serum (Griffin, 1989) and in CSF (Madeira et al., 2015) have been reported in the patients with MDD. In a very recent study performed by Ishiwata et al. (2018), the D-serine levels in the CSF of patients with MDD correlated with the severity. In contrast, ketamine is also known to exert an antidepressive effect both in humans and rodents (Berman et al., 2000; Lai et al., 2014; Murrough et al., 2015). Thus, the precise role of NMDA receptor signaling in modulation of mood is yet to be understood.

In addition to the role of D-serine as a critical regulator in the brain function, D-serine is known to have a potential role in the metabolic function. Long-term (8-weeks) treatment with D-serine is reported to effectively suppress high fat diet intake resulting in reduced weight gain (Sasaki et al., 2015; Suwandhi et al., 2018). However, D-serine also induces hyperglycemia, and strongly impairs glucose tolerance due to impaired insulin secretion from pancreatic beta cells (Suwandhi et al., 2018). Thus, further investigation is required to elucidate the role of D-serine in the pathophysiology of depression-like behavior and the deficits of circadian rhythms observed in SDT fatty rats.

Conclusion

In conclusion, I found the CNS-related behavioral deficits and the pathophysiology of SDT fatty rat, a novel animal model of T2DM. Furthermore, I also found the ideal tool compound for testing physiological role of D-serine and found a novel role of asc-1 in the brain. As described above, the metabolic deficits including DM and obesity substantially affect the brain function and can lead to CNS diseases such as MDD and the deficits in circadian rhythm and vice versa. It can be hypothesized that an aberrant D-serine signaling may be a central key factor that links CNS diseases including MDD, deficits in circadian rhythms and schizophrenia with metabolic dysfunction. It is very important to modulate the levels of D-serine to a physiologically achievable level in the appropriate site - close to the NMDA receptor. In the future, this hypothesis should be investigated using ACPP, which is the only compound available for above testing at this time, in order to unveil the pathophysiology underlying the above hypothesis.

Acknowledgements

I would like to thank Dr. Shigenori Ohkawa, Dr. Mutsuyoshi Matsushita and Dr. Jun Nishiu from Japan Tobacco Inc. Central Pharmaceutical Research Institute and Dr. Haruhide Kimura, Dr. Keisuke Hirai from Takeda Pharmaceutical Company Ltd. Pharmaceutical Research Division for providing the opportunity to conduct this study. I would also like to express my gratitude to Dr. Takeshi Ohta, who provided constructive comments and suggestions, and would also like to thank Mr. Tatsuya Maekawa, Mr. Kazuo Sasagawa, Dr. Yukihiro Ishii, Dr. Kenji Nakao, Dr. Masato Yoshikawa and Dr. Motohisa Suzuki for their advice and involvement with parts of the experiments. I also wish to express my sincere gratitude to Professor Shinichi Kume from Kyoto University for providing helpful advice for the completion of this study.

References

- Abe, H., Kida, M., Tsuji, K. and Mano, T. 1989. Feeding cycles entrain circadian rhythms of locomotor activity in CS mice but not in C57BL/6J mice. *Physiol Behav* 45, 397-401.
- Agren, H. 1980. Symptom patterns in unipolar and bipolar depression correlating with monoamine metabolites in the cerebrospinal fluid: I. General patterns. *Psychiatry Res* 3, 211-223.
- Akerstedt, T., Froberg, J. E., Friberg, Y. and Wetterberg, L. 1979. Melatonin excretion, body temperature and subjective arousal during 64 hours of sleep deprivation. *Psychoneuroendocrinology* 4, 219-225.
- Akisaki, T., Sakurai, T., Takata, T., Umegaki, H., Araki, A., Mizuno, S., Tanaka, S., Ohashi, Y., Iguchi, A., Yokono, K. and Ito, H. 2006. Cognitive dysfunction associates with white matter hyperintensities and subcortical atrophy on magnetic resonance imaging of the elderly diabetes mellitus Japanese elderly diabetes intervention trial (J-EDIT). *Diabetes Metab Res Rev* 22, 376-384.
- American Psychiatric Association (2013) Major depressive disorder. In: *Diagnostic and statistical manual of mental disorders (DSM-5)*, 5th ed, pp160–168. Washington, DC: American Psychiatric Association.
- Amorim, D., Puga, S., Braganca, R., Braga, A., Pertovaara, A., Almeida, A. and Pinto-Ribeiro, F. 2017. Minocycline reduces mechanical allodynia and depressive-like behaviour in type-1 diabetes mellitus in the rat. *Behav Brain Res* 327, 1-10.
- Anderson, R. J., Freedland, K. E., Clouse, R. E. and Lustman, P. J. 2001. The prevalence of comorbid depression in adults with diabetes: a meta-analysis. *Diabetes Care* 24,

1069-1078.

- Aoki, H., Yamada, N., Ozeki, Y., Yamane, H. and Kato, N. 1998. Minimum light intensity required to suppress nocturnal melatonin concentration in human saliva. *Neurosci Lett* 252, 91-94.
- Asher, G., Reinke, H., Altmeyer, M., Gutierrez-Arcelus, M., Hottiger, M. O. and Schibler, U. 2010. Poly(ADP-ribose) polymerase 1 participates in the phase entrainment of circadian clocks to feeding. *Cell* 142, 943-953.
- Aswar, U., Chepurwar, S., Shintre, S. and Aswar, M. 2017. Telmisartan attenuates diabetes induced depression in rats. *Pharmacol Rep* 69, 358-364.
- Badawy, E. A., Rasheed, W. I., Elias, T. R., Hussein, J., Harvi, M., Morsy, S. and Mahmoud Yel, L. 2015. Flaxseed oil reduces oxidative stress and enhances brain monoamines release in streptozotocin-induced diabetic rats. *Hum Exp Toxicol* 34, 1133-1138.
- Bartness, T. J., Powers, J. B., Hastings, M. H., Bittman, E. L. and Goldman, B. D. 1993. The timed infusion paradigm for melatonin delivery: what has it taught us about the melatonin signal, its reception, and the photoperiodic control of seasonal responses? *J Pineal Res* 15, 161-190.
- Bass, J. and Takahashi, J. S. 2010. Circadian integration of metabolism and energetics. *Science* 330, 1349-1354.
- Bendikov, I., Nadri, C., Amar, S., Panizzutti, R., De Miranda, J., Wolosker, H. and Agam, G. 2007. A CSF and postmortem brain study of D-serine metabolic parameters in schizophrenia. *Schizophr Res* 90, 41-51.
- Berman, R. M., Cappiello, A., Anand, A., Oren, D. A., Heninger, G. R., Charney, D. S. and Krystal, J. H. 2000. Antidepressant effects of ketamine in depressed patients.

- Biol Psychiatry 47, 351-354.
- Boden, G., Ruiz, J., Urbain, J. L. and Chen, X. 1996. Evidence for a circadian rhythm of insulin secretion. *Am J Physiol* 271, E246-252.
- Brainard, G. C., Lewy, A. J., Menaker, M., Fredrickson, R. H., Miller, L. S., Weleber, R. G., Cassone, V. and Hudson, D. 1988. Dose-response relationship between light irradiance and the suppression of plasma melatonin in human volunteers. *Brain Res* 454, 212-218.
- Brissos, S., Afonso, P., Canas, F., Bobes, J., Bernardo Fernandez, I. and Guzman, C. 2013. Satisfaction with Life of Schizophrenia Outpatients and Their Caregivers: Differences between Patients with and without Self-Reported Sleep Complaints. *Schizophr Res Treatment* 2013, 502172.
- Brown, J. M., Hunihan, L., Prack, M. M., Harden, D. G., Bronson, J., Dzierba, C. D., Gentles, R. G., Hendricson, A., Krause, R., Macor, J. E. and Westphal, R. S. 2014. In vitro Characterization of a small molecule inhibitor of the alanine serine cysteine transporter -1 (SLC7A10). *J Neurochem* 129, 275-283.
- Brum, M. C., Filho, F. F., Schnorr, C. C., Bottega, G. B. and Rodrigues, T. C. 2015. Shift work and its association with metabolic disorders. *Diabetol Metab Syndr* 7, 45.
- Cai, X. S., Tan, Z. G., Li, J. J., Gao, W. H., Li, S. J., Li, J. L., Tang, Y. M., Li, H. W. and Hui, H. X. 2017. Glucagon-Like Peptide-1 (GLP-1) Treatment Ameliorates Cognitive Impairment by Attenuating Arc Expression in Type 2 Diabetic Rats. *Med Sci Monit* 23, 4334-4342.
- Cajochen, C., Krauchi, K. and Wirz-Justice, A. 2003. Role of melatonin in the regulation of human circadian rhythms and sleep. *J Neuroendocrinol* 15, 432-437.
- Cameron, O. G., Kronfol, Z., Greden, J. F. and Carroll, B. J. 1984. Hypothalamic-

- pituitary-adrenocortical activity in patients with diabetes mellitus. *Arch Gen Psychiatry* 41, 1090-1095.
- Carroll, B. J., Curtis, G. C. and Mendels, J. 1976. Cerebrospinal fluid and plasma free cortisol concentrations in depression. *Psychol Med* 6, 235-244.
- Chan, O., Inouye, K., Vranic, M. and Matthews, S. G. 2002. Hyperactivation of the hypothalamo-pituitary-adrenocortical axis in streptozotocin-diabetes is associated with reduced stress responsiveness and decreased pituitary and adrenal sensitivity. *Endocrinology* 143, 1761-1768.
- Choi, K. W., Na, E. J., Fava, M., Mischoulon, D., Cho, H. and Jeon, H. J. 2018. Increased adrenocorticotrophic hormone (ACTH) levels predict severity of depression after six months of follow-up in outpatients with major depressive disorder. *Psychiatry Res* 270, 246-252.
- Cohen, J. W., Louneva, N., Han, L. Y., Hodes, G. E., Wilson, R. S., Bennett, D. A., Lucki, I. and Arnold, S. E. 2011. Chronic corticosterone exposure alters postsynaptic protein levels of PSD-95, NR1, and synaptopodin in the mouse brain. *Synapse* 65, 763-770.
- Cryan, J. F. and Mombereau, C. 2004. In search of a depressed mouse: utility of models for studying depression-related behavior in genetically modified mice. *Mol Psychiatry* 9, 326-357.
- Cuspidi, C., Meani, S., Lonati, L., Fusi, V., Valerio, C., Sala, C., Magnaghi, G., Maisaidi, M. and Zanchetti, A. 2006. Short-term reproducibility of a non-dipping pattern in type 2 diabetic hypertensive patients. *J Hypertens* 24, 647-653.
- De Nicola, A. F., Fridman, O., Del Castillo, E. J. and Foglia, V. G. 1976. The influence of streptozotocin diabetes on adrenal function in male rats. *Horm Metab Res* 8, 388-392.
- Deves, R. and Krupka, R. M. 1979. A general kinetic analysis of transport. Tests of the

- carrier model based on predicted relations among experimental parameters. *Biochim Biophys Acta* 556, 533-547.
- Drake, C. L., Roehrs, T., Richardson, G., Walsh, J. K. and Roth, T. 2004. Shift work sleep disorder: prevalence and consequences beyond that of symptomatic day workers. *Sleep* 27, 1453-1462.
- Ebihara, S., Marks, T., Hudson, D. J. and Menaker, M. 1986. Genetic control of melatonin synthesis in the pineal gland of the mouse. *Science* 231, 491-493.
- Egeland, J., Lund, A., Landro, N. I., Rund, B. R., Sundet, K., Asbjornsen, A., Mjelle, N., Roness, A. and Stordal, K. I. 2005. Cortisol level predicts executive and memory function in depression, symptom level predicts psychomotor speed. *Acta Psychiatrica Scand* 112, 434-441.
- Ezzeldin, E., Souror, W. A., El-Nahas, T., Soudi, A. N. and Shahat, A. A. 2014. Biochemical and neurotransmitters changes associated with tramadol in streptozotocin-induced diabetes in rats. *Biomed Res Int* 2014, 238780.
- Fee, C., Banasr, M. and Sibille, E. 2017. Somatostatin-Positive Gamma-Aminobutyric Acid Interneuron Deficits in Depression: Cortical Microcircuit and Therapeutic Perspectives. *Biol Psychiatry* 82, 549-559.
- Foss-Feig, J. H., Adkinson, B. D., Ji, J. L., Yang, G., Srihari, V. H., McPartland, J. C., Krystal, J. H., Murray, J. D. and Anticevic, A. 2017. Searching for Cross-Diagnostic Convergence: Neural Mechanisms Governing Excitation and Inhibition Balance in Schizophrenia and Autism Spectrum Disorders. *Biol Psychiatry* 81, 848-861.
- Frese, T., Bach, A. G., Muhlbauer, E., Ponicke, K., Bromme, H. J., Welp, A. and Peschke, E. 2009. Pineal melatonin synthesis is decreased in type 2 diabetic Goto-Kakizaki rats. *Life Sci* 85, 526-533.

- Fukasawa, Y., Segawa, H., Kim, J. Y., Chairoungdua, A., Kim, D. K., Matsuo, H., Cha, S. H., Endou, H. and Kanai, Y. 2000. Identification and characterization of a Na(+)-independent neutral amino acid transporter that associates with the 4F2 heavy chain and exhibits substrate selectivity for small neutral D- and L-amino acids. *J Biol Chem* 275, 9690-9698.
- Ganote, C. E., Peterson, D. R. and Carone, F. A. 1974. The nature of D-serine--induced nephrotoxicity. *Am J Pathol* 77, 269-282.
- Ginn, S. and Horder, J. 2012. "One in four" with a mental health problem: the anatomy of a statistic. *BMJ* 344, e1302.
- Griffin, T. W. 1989. Status of clinical trials with neutron irradiation. *Important Adv Oncol* 221-234.
- Guercio, G. D. and Panizzutti, R. 2018. Potential and Challenges for the Clinical Use of d-Serine As a Cognitive Enhancer. *Front Psychiatry* 9, 14.
- Hammar, A. and Ardal, G. 2009. Cognitive functioning in major depression--a summary. *Front Hum Neurosci* 3, 26.
- Hashimoto, A., Nishikawa, T., Hayashi, T., Fujii, N., Harada, K., Oka, T. and Takahashi, K. 1992. The presence of free D-serine in rat brain. *FEBS Lett* 296, 33-36.
- Hashimoto, K., Engberg, G., Shimizu, E., Nordin, C., Lindstrom, L. H. and Iyo, M. 2005. Elevated glutamine/glutamate ratio in cerebrospinal fluid of first episode and drug naive schizophrenic patients. *BMC Psychiatry* 5, 6.
- Helboe, L., Egebjerg, J., Moller, M. and Thomsen, C. 2003. Distribution and pharmacology of alanine-serine-cysteine transporter 1 (asc-1) in rodent brain. *Eur J Neurosci* 18, 2227-2238.
- Hermes, G., Li, N., Duman, C. and Duman, R. 2011. Post-weaning chronic social

- isolation produces profound behavioral dysregulation with decreases in prefrontal cortex synaptic-associated protein expression in female rats. *Physiol Behav* 104, 354-359.
- Holliday, E. G., Magee, C. A., Kritharides, L., Banks, E. and Attia, J. 2013. Short sleep duration is associated with risk of future diabetes but not cardiovascular disease: a prospective study and meta-analysis. *PLoS One* 8, e82305.
- Hrdina, P. D., Demeter, E., Vu, T. B., Sotonyi, P. and Palkovits, M. 1993. 5-HT uptake sites and 5-HT₂ receptors in brain of antidepressant-free suicide victims/depressives: increase in 5-HT₂ sites in cortex and amygdala. *Brain Res* 614, 37-44.
- Ishii, Y., Ohta, T., Sasase, T., Morinaga, H., Ueda, N., Hata, T., Kakutani, M., Miyajima, K., Katsuda, Y., Masuyama, T., Shinohara, M. and Matsushita, M. 2010. Pathophysiological analysis of female Spontaneously Diabetic Torii fatty rats. *Exp Anim* 59, 73-84.
- Ishimaru, M., Kurumaji, A. and Toru, M. 1994. Increases in strychnine-insensitive glycine binding sites in cerebral cortex of chronic schizophrenics: evidence for glutamate hypothesis. *Biol Psychiatry* 35, 84-95.
- Ishiwata, S., Hattori, K., Sasayama, D., Teraishi, T., Miyakawa, T., Yokota, Y., Matsumura, R., Nishikawa, T. and Kunugi, H. 2018. Cerebrospinal fluid D-serine concentrations in major depressive disorder negatively correlate with depression severity. *J Affect Disord* 226, 155-162.
- Ishiwata, S., Ogata, S., Umino, A., Shiraku, H., Ohashi, Y., Kajii, Y. and Nishikawa, T. 2013. Increasing effects of S-methyl-L-cysteine on the extracellular D-serine concentrations in the rat medial frontal cortex. *Amino Acids* 44, 1391-1395.
- Karasawa, J., Hashimoto, K. and Chaki, S. 2008. D-Serine and a glycine transporter

- inhibitor improve MK-801-induced cognitive deficits in a novel object recognition test in rats. *Behav Brain Res* 186, 78-83.
- Karlsson, B., Knutsson, A. and Lindahl, B. 2001. Is there an association between shift work and having a metabolic syndrome? Results from a population based study of 27,485 people. *Occup Environ Med* 58, 747-752.
- Katsuda, Y., Sasase, T., Tadaki, H., Mera, Y., Motohashi, Y., Kemmochi, Y., Toyoda, K., Kakimoto, K., Kume, S. and Ohta, T. 2015. Contribution of hyperglycemia on diabetic complications in obese type 2 diabetic SDT fatty rats: effects of SGLT inhibitor phlorizin. *Exp Anim* 64, 161-169.
- Kessler, R. C., Berglund, P., Demler, O., Jin, R., Koretz, D., Merikangas, K. R., Rush, A. J., Walters, E. E. and Wang, P. S. 2003. The epidemiology of major depressive disorder: results from the National Comorbidity Survey Replication (NCS-R). *JAMA* 289, 3095-3105.
- Kessler, R. C. and Bromet, E. J. 2013. The epidemiology of depression across cultures. *Annu Rev Public Health* 34, 119-138.
- Knorr, U., Vinberg, M., Kessing, L. V. and Wetterslev, J. 2010. Salivary cortisol in depressed patients versus control persons: a systematic review and meta-analysis. *Psychoneuroendocrinology* 35, 1275-1286.
- Korf, J. and van Praag, H. M. 1971. Endogenous depressions with and without disturbances in the 5-hydroxytryptamine metabolism: A biochemical classification? *Psychopharmacologia* 19, 148-152.
- Krystal, J. H., Karper, L. P., Seibyl, J. P., Freeman, G. K., Delaney, R., Bremner, J. D., Heninger, G. R., Bowers, M. B., Jr. and Charney, D. S. 1994. Subanesthetic effects of the noncompetitive NMDA antagonist, ketamine, in humans. Psychotomimetic,

- perceptual, cognitive, and neuroendocrine responses. *Arch Gen Psychiatry* 51, 199-214.
- Kuepper, R., Skinbjerg, M. and Abi-Dargham, A. 2012. The dopamine dysfunction in schizophrenia revisited: new insights into topography and course. *Handb Exp Pharmacol* 1-26.
- Labrie, V., Wong, A. H. and Roder, J. C. 2012. Contributions of the D-serine pathway to schizophrenia. *Neuropharmacology* 62, 1484-1503.
- Laermans, J. and Depoortere, I. 2016. Chronobesity: role of the circadian system in the obesity epidemic. *Obes Rev* 17, 108-125.
- Lai, R., Katalinic, N., Glue, P., Somogyi, A. A., Mitchell, P. B., Leyden, J., Harper, S. and Loo, C. K. 2014. Pilot dose-response trial of i.v. ketamine in treatment-resistant depression. *World J Biol Psychiatry* 15, 579-584.
- Lewy, A. J., Wehr, T. A., Goodwin, F. K., Newsome, D. A. and Markey, S. P. 1980. Light suppresses melatonin secretion in humans. *Science* 210, 1267-1269.
- Liu, X., Mo, Y., Gong, J., Li, Z., Peng, H., Chen, J., Wang, Q., Ke, Z. and Xie, J. 2016. Puerarin ameliorates cognitive deficits in streptozotocin-induced diabetic rats. *Metab Brain Dis* 31, 417-423.
- Logue, S. F. and Gould, T. J. 2014. The neural and genetic basis of executive function: attention, cognitive flexibility, and response inhibition. *Pharmacol Biochem Behav* 123, 45-54.
- Madeira, C., Lourenco, M. V., Vargas-Lopes, C., Suemoto, C. K., Brandao, C. O., Reis, T., Leite, R. E., Laks, J., Jacob-Filho, W., Pasqualucci, C. A., Grinberg, L. T., Ferreira, S. T. and Panizzutti, R. 2015. d-serine levels in Alzheimer's disease: implications for novel biomarker development. *Transl Psychiatry* 5, e561.

- Malkesman, O., Austin, D. R., Tragon, T., Wang, G., Rompala, G., Hamidi, A. B., Cui, Z., Young, W. S., Nakazawa, K., Zarate, C. A., Manji, H. K. and Chen, G. 2012. Acute D-serine treatment produces antidepressant-like effects in rodents. *Int J Neuropsychopharmacol* 15, 1135-1148.
- Marquez P.V. and Saxena S. 2016. Making Mental Health a Global Priority. *Cerebrum*. pii: cer-10-16.
- Masuyama, T., Katsuda, Y. and Shinohara, M. 2005. A novel model of obesity-related diabetes: introgression of the *Lepr(fa)* allele of the Zucker fatty rat into nonobese Spontaneously Diabetic Torii (SDT) rats. *Exp Anim* 54, 13-20.
- Matsuo, H., Kanai, Y., Tokunaga, M., Nakata, T., Chairoungdua, A., Ishimine, H., Tsukada, S., Ooigawa, H., Nawashiro, H., Kobayashi, Y., Fukuda, J. and Endou, H. 2004. High affinity D- and L-serine transporter Asc-1: cloning and dendritic localization in the rat cerebral and cerebellar cortices. *Neurosci Lett* 358, 123-126.
- McMullan, C. J., Schernhammer, E. S., Rimm, E. B., Hu, F. B. and Forman, J. P. 2013. Melatonin secretion and the incidence of type 2 diabetes. *JAMA* 309, 1388-1396.
- McNeil, J., Doucet, E. and Chaput, J. P. 2013. Inadequate sleep as a contributor to obesity and type 2 diabetes. *Can J Diabetes* 37, 103-108.
- Meehan, W. P., Leedom, L. J., Nagayama, T. and Zeidler, A. 1986. Agonistic behavior patterns in mice with streptozotocin-induced diabetes mellitus. *Physiol Behav* 38, 301-306.
- Ministry of Health, Labor and welfare. Report "Health Japan 21." http://www1.mhlw.go.jp/topics/kenko21_11/s1.html (accessed at Oct 30, 2018)
- Mistlberger, R. E., Lukman, H. and Nadeau, B. G. 1998. Circadian rhythms in the Zucker obese rat: assessment and intervention. *Appetite* 30, 255-267.

- Mitani, H., Shirayama, Y., Yamada, T., Maeda, K., Ashby, C. R., Jr. and Kawahara, R. 2006. Correlation between plasma levels of glutamate, alanine and serine with severity of depression. *Prog Neuropsychopharmacol Biol Psychiatry* 30, 1155-1158.
- Miyata, S., Yamada, N., Hirano, S., Tanaka, S. and Kamei, J. 2007. Diabetes attenuates psychological stress-elicited 5-HT secretion in the prefrontal cortex but not in the amygdala of mice. *Brain Res* 1147, 233-239.
- Mohawk, J. A., Green, C. B. and Takahashi, J. S. 2012. Central and peripheral circadian clocks in mammals. *Annu Rev Neurosci* 35, 445-462.
- Mohebbi, I., Shateri, K. and Seyedmohammadzad, M. 2012. The relationship between working schedule patterns and the markers of the metabolic syndrome: comparison of shift workers with day workers. *Int J Occup Med Environ Health* 25, 383-391.
- Moller, H. J. 2007. Clinical evaluation of negative symptoms in schizophrenia. *Eur Psychiatry* 22, 380-386.
- Morgenthaler, T. I., Kapur, V. K., Brown, T., Swick, T. J., Alessi, C., Aurora, R. N., Boehlecke, B., Chesson, A. L., Jr., Friedman, L., Maganti, R., Owens, J., Pancer, J. and Zak, R. 2007. Practice parameters for the treatment of narcolepsy and other hypersomnias of central origin. *Sleep* 30, 1705-1711.
- Morin, C. M., Vallieres, A., Guay, B., Ivers, H., Savard, J., Merette, C., Bastien, C. and Baillargeon, L. 2009. Cognitive behavioral therapy, singly and combined with medication, for persistent insomnia: a randomized controlled trial. *JAMA* 301, 2005-2015.
- Mothet, J. P., Parent, A. T., Wolosker, H., Brady, R. O., Jr., Linden, D. J., Ferris, C. D., Rogawski, M. A. and Snyder, S. H. 2000. D-serine is an endogenous ligand for the glycine site of the N-methyl-D-aspartate receptor. *Proc Natl Acad Sci U S A* 97, 4926-

4931.

- Murrough, J. W., Abdallah, C. G. and Mathew, S. J. 2017. Targeting glutamate signalling in depression: progress and prospects. *Nat Rev Drug Discov* 16, 472-486.
- Murrough, J. W., Soleimani, L., DeWilde, K. E., Collins, K. A., Lapidus, K. A., Iacoviello, B. M., Lener, M., Kautz, M., Kim, J., Stern, J. B., Price, R. B., Perez, A. M., Brallier, J. W., Rodriguez, G. J., Goodman, W. K., Iosifescu, D. V. and Charney, D. S. 2015. Ketamine for rapid reduction of suicidal ideation: a randomized controlled trial. *Psychol Med* 45, 3571-3580.
- Nestler, E. J., Barrot, M., DiLeone, R. J., Eisch, A. J., Gold, S. J. and Monteggia, L. M. 2002. Neurobiology of depression. *Neuron* 34, 13-25.
- Nishida, S., Sato, R., Murai, I. and Nakagawa, S. 2003. Effect of pinealectomy on plasma levels of insulin and leptin and on hepatic lipids in type 2 diabetic rats. *J Pineal Res* 35, 251-256.
- Nishikawa, T. 2011. Analysis of free D-serine in mammals and its biological relevance. *J Chromatogr B Analyt Technol Biomed Life Sci* 879, 3169-3183.
- Nock, M. K., Borges, G., Bromet, E. J., Cha, C. B., Kessler, R. C. and Lee, S. 2008. Suicide and suicidal behavior. *Epidemiol Rev* 30, 133-154.
- Nunes, E. A., MacKenzie, E. M., Rossolatos, D., Perez-Parada, J., Baker, G. B. and Dursun, S. M. 2012. D-serine and schizophrenia: an update. *Expert Rev Neurother* 12, 801-812.
- O'Brien, I. A., Lewin, I. G., O'Hare, J. P., Arendt, J. and Corrall, R. J. 1986. Abnormal circadian rhythm of melatonin in diabetic autonomic neuropathy. *Clin Endocrinol (Oxf)* 24, 359-364.
- Ohta, T., Katsuda, Y., Miyajima, K., Sasase, T., Kimura, S., Tong, B. and Yamada, T. 2014.

- Gender differences in metabolic disorders and related diseases in Spontaneously Diabetic Torii-Lepr(fa) rats. *J Diabetes Res* 2014, 841957.
- Olkola, K. T. and Ahonen, J. 2008. Midazolam and other benzodiazepines. *Handb Exp Pharmacol* 335-360.
- Onishi, T., Iwashita, H., Uno, Y., Kunitomo, J., Saitoh, M., Kimura, E., Fujita, H., Uchiyama, N., Kori, M. and Takizawa, M. 2011. A novel glycogen synthase kinase-3 inhibitor 2-methyl-5-(3-{4-[(S)-methylsulfinyl]phenyl}-1-benzofuran-5-yl)-1,3,4-oxadiazole decreases tau phosphorylation and ameliorates cognitive deficits in a transgenic model of Alzheimer's disease. *J Neurochem* 119, 1330-1340.
- Ono, H., Shirakawa, O., Nishiguchi, N., Nishimura, A., Nushida, H., Ueno, Y. and Maeda, K. 2001. Serotonin 2A receptor gene polymorphism is not associated with completed suicide. *J Psychiatr Res* 35, 173-176.
- Oquendo, M. A., Russo, S. A., Underwood, M. D., Kassir, S. A., Ellis, S. P., Mann, J. J. and Arango, V. 2006. Higher postmortem prefrontal 5-HT_{2A} receptor binding correlates with lifetime aggression in suicide. *Biol Psychiatry* 59, 235-243.
- Peschke, E., Frese, T., Chankiewitz, E., Peschke, D., Preiss, U., Schneyer, U., Spessert, R. and Muhlbauer, E. 2006. Diabetic Goto Kakizaki rats as well as type 2 diabetic patients show a decreased diurnal serum melatonin level and an increased pancreatic melatonin-receptor status. *J Pineal Res* 40, 135-143.
- Peschke, E., Wolgast, S., Bazwinsky, I., Ponicke, K. and Muhlbauer, E. 2008. Increased melatonin synthesis in pineal glands of rats in streptozotocin induced type 1 diabetes. *J Pineal Res* 45, 439-448.
- Pistrosch, F., Reissmann, E., Wildbrett, J., Koehler, C. and Hanefeld, M. 2007. Relationship between diurnal blood pressure variation and diurnal blood glucose

- levels in type 2 diabetic patients. *Am J Hypertens* 20, 541-545.
- Plotsky, P. M., Thrivikraman, K. V., Watts, A. G. and Hauger, R. L. 1992. Hypothalamic-pituitary-adrenal axis function in the Zucker obese rat. *Endocrinology* 130, 1931-1941.
- Porsolt, R. D., Anton, G., Blavet, N. and Jalfre, M. 1978. Behavioural despair in rats: a new model sensitive to antidepressant treatments. *Eur J Pharmacol* 47, 379-391.
- Porsolt, R. D., Le Pichon, M. and Jalfre, M. 1977. Depression: a new animal model sensitive to antidepressant treatments. *Nature* 266, 730-732.
- Qian, J., Thomas, A. P., Schroeder, A. M., Rakshit, K., Colwell, C. S. and Matveyenko, A. V. 2017. Development of diabetes does not alter behavioral and molecular circadian rhythms in a transgenic rat model of type 2 diabetes mellitus. *Am J Physiol Endocrinol Metab* 313, E213-E221.
- Qiu, Z. K., He, J. L., Liu, X., Zhang, G. H., Zeng, J., Nie, H., Shen, Y. G. and Chen, J. S. 2016. The antidepressant-like activity of AC-5216, a ligand for 18KDa translocator protein (TSPO), in an animal model of diabetes mellitus. *Sci Rep* 6, 37345.
- Rasmussen, D. D., Boldt, B. M., Wilkinson, C. W., Yellon, S. M. and Matsumoto, A. M. 1999. Daily melatonin administration at middle age suppresses male rat visceral fat, plasma leptin, and plasma insulin to youthful levels. *Endocrinology* 140, 1009-1012.
- Rath, M. F., Coon, S. L., Amaral, F. G., Weller, J. L., Moller, M. and Klein, D. C. 2016. Melatonin Synthesis: Acetylserotonin O-Methyltransferase (ASMT) Is Strongly Expressed in a Subpopulation of Pinealocytes in the Male Rat Pineal Gland. *Endocrinology* 157, 2028-2040.
- Reagan, L. P. 2012. Diabetes as a chronic metabolic stressor: causes, consequences and clinical complications. *Exp Neurol* 233, 68-78.

- Reus, G. Z., Stringari, R. B., Ribeiro, K. F., Cipriano, A. L., Panizzutti, B. S., Stertz, L., Lersch, C., Kapczinski, F. and Quevedo, J. 2011. Maternal deprivation induces depressive-like behaviour and alters neurotrophin levels in the rat brain. *Neurochem Res* 36, 460-466.
- Ribeiro, C. S., Reis, M., Panizzutti, R., de Miranda, J. and Wolosker, H. 2002. Glial transport of the neuromodulator D-serine. *Brain Res* 929, 202-209.
- Rock, P. L., Roiser, J. P., Riedel, W. J. and Blackwell, A. D. 2014. Cognitive impairment in depression: a systematic review and meta-analysis. *Psychol Med* 44, 2029-2040.
- Roseboom, P. H., Namboodiri, M. A., Zimonjic, D. B., Popescu, N. C., Rodriguez, I. R., Gastel, J. A. and Klein, D. C. 1998. Natural melatonin 'knockdown' in C57BL/6J mice: rare mechanism truncates serotonin N-acetyltransferase. *Brain Res Mol Brain Res* 63, 189-197.
- Rosenberg, D., Kartvelishvily, E., Shleper, M., Klinker, C. M., Bowser, M. T. and Wolosker, H. 2010. Neuronal release of D-serine: a physiological pathway controlling extracellular D-serine concentration. *FASEB J* 24, 2951-2961.
- Ross, C. A., Margolis, R. L., Reading, S. A., Pletnikov, M. and Coyle, J. T. 2006. Neurobiology of schizophrenia. *Neuron* 52, 139-153.
- Roy, M. S., Roy, A., Gallucci, W. T., Collier, B., Young, K., Kamilaris, T. C. and Chrousos, G. P. 1993. The ovine corticotropin-releasing hormone-stimulation test in type I diabetic patients and controls: suggestion of mild chronic hypercortisolism. *Metabolism* 42, 696-700.
- Rush, A. J., Trivedi, M. H., Wisniewski, S. R., Nierenberg, A. A., Stewart, J. W., Warden, D., Niederehe, G., Thase, M. E., Lavori, P. W., Lebowitz, B. D., McGrath, P. J., Rosenbaum, J. F., Sackeim, H. A., Kupfer, D. J., Luther, J. and Fava, M. 2006. Acute

- and longer-term outcomes in depressed outpatients requiring one or several treatment steps: a STAR*D report. *Am J Psychiatry* 163, 1905-1917.
- Rutter, A. R., Fradley, R. L., Garrett, E. M., Chapman, K. L., Lawrence, J. M., Rosahl, T. W. and Patel, S. 2007. Evidence from gene knockout studies implicates Asc-1 as the primary transporter mediating d-serine reuptake in the mouse CNS. *Eur J Neurosci* 25, 1757-1766.
- Sack, R. L., Auckley, D., Auger, R. R., Carskadon, M. A., Wright, K. P., Jr., Vitiello, M. V. and Zhdanova, I. V. 2007. Circadian rhythm sleep disorders: part II, advanced sleep phase disorder, delayed sleep phase disorder, free-running disorder, and irregular sleep-wake rhythm. An American Academy of Sleep Medicine review. *Sleep* 30, 1484-1501.
- Sasaki, T., Kinoshita, Y., Matsui, S., Kakuta, S., Yokota-Hashimoto, H., Kinoshita, K., Iwasaki, Y., Kinoshita, T., Yada, T., Amano, N. and Kitamura, T. 2015. N-methyl-d-aspartate receptor coagonist d-serine suppresses intake of high-preference food. *Am J Physiol Regul Integr Comp Physiol* 309, R561-575.
- Sason, H., Billard, J. M., Smith, G. P., Safory, H., Neame, S., Kaplan, E., Rosenberg, D., Zubedat, S., Foltyn, V. N., Christoffersen, C. T., Bundgaard, C., Thomsen, C., Avital, A., Christensen, K. V. and Wolosker, H. 2017. Asc-1 Transporter Regulation of Synaptic Activity via the Tonic Release of d-Serine in the Forebrain. *Cereb Cortex* 27, 1573-1587.
- Schmitz, N., Gariepy, G., Smith, K. J., Clyde, M., Malla, A., Boyer, R., Strychar, I., Lesage, A. and Wang, J. 2014. Recurrent subthreshold depression in type 2 diabetes: an important risk factor for poor health outcomes. *Diabetes Care* 37, 970-978.
- Scribner, K. A., Walker, C. D., Cascio, C. S. and Dallman, M. F. 1991. Chronic

- streptozotocin diabetes in rats facilitates the acute stress response without altering pituitary or adrenal responsiveness to secretagogues. *Endocrinology* 129, 99-108.
- Sei, M., Sei, H. and Shima, K. 1999. Spontaneous activity, sleep, and body temperature in rats lacking the CCK-A receptor. *Physiol Behav* 68, 25-29.
- Seney, M. L. and Sibille, E. 2014. Sex differences in mood disorders: perspectives from humans and rodent models. *Biol Sex Differ* 5, 17.
- Shao, Z., Kamboj, A. and Anderson, C. M. 2009. Functional and immunocytochemical characterization of D-serine transporters in cortical neuron and astrocyte cultures. *J Neurosci Res* 87, 2520-2530.
- Shimazaki, T., Kaku, A. and Chaki, S. 2010. D-Serine and a glycine transporter-1 inhibitor enhance social memory in rats. *Psychopharmacology (Berl)* 209, 263-270.
- Sinyor, M., Schaffer, A. and Levitt, A. 2010. The sequenced treatment alternatives to relieve depression (STAR*D) trial: a review. *Can J Psychiatry* 55, 126-135.
- Sollars, P. J. and Pickard, G. E. 2015. The Neurobiology of Circadian Rhythms. *Psychiatr Clin North Am* 38, 645-665.
- Soreca, I. 2014. Circadian rhythms and sleep in bipolar disorder: implications for pathophysiology and treatment. *Curr Opin Psychiatry* 27, 467-471.
- Steel, Z., Marnane, C., Iranpour, C., Chey, T., Jackson, J. W., Patel, V. and Silove, D. 2014. The global prevalence of common mental disorders: a systematic review and meta-analysis 1980-2013. *Int J Epidemiol* 43, 476-493.
- Stephan, F. K. 2002. The "other" circadian system: food as a Zeitgeber. *J Biol Rhythms* 17, 284-292.
- Stevens, E. R., Esguerra, M., Kim, P. M., Newman, E. A., Snyder, S. H., Zahs, K. R. and Miller, R. F. 2003. D-serine and serine racemase are present in the vertebrate retina

- and contribute to the physiological activation of NMDA receptors. *Proc Natl Acad Sci U S A* 100, 6789-6794.
- Strachan, M. W., Deary, I. J., Ewing, F. M. and Frier, B. M. 1997. Is type II diabetes associated with an increased risk of cognitive dysfunction? A critical review of published studies. *Diabetes Care* 20, 438-445.
- Suwandhi, L., Hausmann, S., Braun, A., Gruber, T., Heinzmann, S. S., Galvez, E. J. C., Buck, A., Legutko, B., Israel, A., Feuchtinger, A., Haythorne, E., Staiger, H., Heni, M., Haring, H. U., Schmitt-Kopplin, P., Walch, A., Caceres, C. G., Tschop, M. H., Rutter, G. A., Strowig, T., Elsner, M. and Ussar, S. 2018. Chronic d-serine supplementation impairs insulin secretion. *Mol Metab* 16, 191-202.
- Syvalahti, E. 1987. Monoaminergic mechanisms in affective disorders. *Med Biol* 65, 89-96.
- Taffe, M. A., Davis, S. A., Gutierrez, T. and Gold, L. H. 2002. Ketamine impairs multiple cognitive domains in rhesus monkeys. *Drug Alcohol Depend* 68, 175-187.
- Thorre, K., Chaouloff, F., Sarre, S., Meeusen, R., Ebinger, G. and Michotte, Y. 1997. Differential effects of restraint stress on hippocampal 5-HT metabolism and extracellular levels of 5-HT in streptozotocin-diabetic rats. *Brain Res* 772, 209-216.
- Timonen, M., Laakso, M., Jokelainen, J., Rajala, U., Meyer-Rochow, V. B. and Keinanen-Kiukaanniemi, S. 2005. Insulin resistance and depression: cross sectional study. *BMJ* 330, 17-18.
- Tordjman, S., Chokron, S., Delorme, R., Charrier, A., Bellissant, E., Jaafari, N. and Fougere, C. 2017. Melatonin: Pharmacology, Functions and Therapeutic Benefits. *Curr Neuropharmacol* 15, 434-443.
- Toriniwa, Y., Saito, T., Miyajima, K., Ishii, Y., Uno, K., Maekawa, T., Matsui, T., Kume,

- S., Yamada, T. and Ohta, T. 2018. Investigation of pharmacological responses to anti-diabetic drugs in female Spontaneously Diabetic Torii (SDT) fatty rats, a new nonalcoholic steatohepatitis (NASH) model. *J Vet Med Sci* 80, 878-885.
- Tresguerres, J. A., Cuesta, S., Kireev, R. A., Garcia, C., Acuna-Castroviejo, D. and Vara, E. 2013. Beneficial effect of melatonin treatment on age-related insulin resistance and on the development of type 2 diabetes. *Horm Mol Biol Clin Investig* 16, 47-54.
- Tsai, G., Yang, P., Chung, L. C., Lange, N. and Coyle, J. T. 1998. D-serine added to antipsychotics for the treatment of schizophrenia. *Biol Psychiatry* 44, 1081-1089.
- Tsujino N, Nakatani Y, Seki Y, Nakasato A, Nakamura M, Sugawara M, Arita H. 2007. Abnormality of circadian rhythm accompanied by an increase in frontal cortex serotonin in animal model of autism. *Neurosci Res.* 57(2), 289-95.
- Van Cauter, E., Blackman, J. D., Roland, D., Spire, J. P., Refetoff, S. and Polonsky, K. S. 1991. Modulation of glucose regulation and insulin secretion by circadian rhythmicity and sleep. *J Clin Invest* 88, 934-942.
- Velasco, A., Huerta, I. and Marin, B. 1988. Plasma corticosterone, motor activity and metabolic circadian patterns in streptozotocin-induced diabetic rats. *Chronobiol Int* 5, 127-135.
- Volk, L., Chiu, S. L., Sharma, K. and Huganir, R. L. 2015. Glutamate synapses in human cognitive disorders. *Annu Rev Neurosci* 38, 127-149.
- Watanabe, M., Kikuchi, H., Tanaka, K. and Takahashi, M. 2010. Association of short sleep duration with weight gain and obesity at 1-year follow-up: a large-scale prospective study. *Sleep* 33, 161-167.
- Whiteford, H. A., Degenhardt, L., Rehm, J., Baxter, A. J., Ferrari, A. J., Erskine, H. E., Charlson, F. J., Norman, R. E., Flaxman, A. D., Johns, N., Burstein, R., Murray, C. J.

- and Vos, T. 2013. Global burden of disease attributable to mental and substance use disorders: findings from the Global Burden of Disease Study 2010. *Lancet* 382, 1575-1586.
- WHO, a. Mental health: a state of well-being. http://www.who.int/features/factfiles/mental_health/en/ (accessed Oct 30, 2018)).
- WHO, b. Depression: Fact sheets. <http://www.who.int/news-room/fact-sheets/detail/depression> (accessed Oct 30, 2018))
- WHO, c. Mental health: Schizophrenia. http://www.who.int/mental_health/management/schizophrenia/en/ (accessed Oct 30, 2018)).
- Williams, W. P., 3rd, McLin, D. E., 3rd, Dressman, M. A. and Neubauer, D. N. 2016. Comparative Review of Approved Melatonin Agonists for the Treatment of Circadian Rhythm Sleep-Wake Disorders. *Pharmacotherapy* 36, 1028-1041.
- Xie, X., Dumas, T., Tang, L., Brennan, T., Reeder, T., Thomas, W., Klein, R. D., Flores, J., O'Hara, B. F., Heller, H. C. and Franken, P. 2005. Lack of the alanine-serine-cysteine transporter 1 causes tremors, seizures, and early postnatal death in mice. *Brain Res* 1052, 212-221.
- Yadav, V. K., Oury, F., Suda, N., Liu, Z. W., Gao, X. B., Confavreux, C., Klemenhagen, K. C., Tanaka, K. F., Gingrich, J. A., Guo, X. E., Tecott, L. H., Mann, J. J., Hen, R., Horvath, T. L. and Karsenty, G. 2009. A serotonin-dependent mechanism explains the leptin regulation of bone mass, appetite, and energy expenditure. *Cell* 138, 976-989.
- Yamada, K., Ohnishi, T., Hashimoto, K., Ohba, H., Iwayama-Shigeno, Y., Toyoshima, M., Okuno, A., Takao, H., Toyota, T., Minabe, Y., Nakamura, K., Shimizu, E., Itokawa, M., Mori, N., Iyo, M. and Yoshikawa, T. 2005. Identification of multiple serine

- racemase (SRR) mRNA isoforms and genetic analyses of SRR and DAO in schizophrenia and D-serine levels. *Biol Psychiatry* 57, 1493-1503.
- Yamamoto, N., Tomita, U., Umino, A. and Nishikawa, T. 2001. Uptake of D-serine by synaptosomal P2 fraction isolated from rat brain. *Synapse* 42, 84-86.
- Yang, J., Song, Y., Wang, H., Liu, C., Li, Z., Liu, Y. and Kong, Y. 2015. Insulin treatment prevents the increase in D-serine in hippocampal CA1 area of diabetic rats. *Am J Alzheimers Dis Other Dement* 30, 201-208.
- Yoda, K., Inaba, M., Hamamoto, K., Yoda, M., Tsuda, A., Mori, K., Imanishi, Y., Emoto, M. and Yamada, S. 2015. Association between poor glycemic control, impaired sleep quality, and increased arterial thickening in type 2 diabetic patients. *PLoS One* 10, e0122521.
- Zhang, H., Zhao, Y. and Wang, Z. 2015. Chronic corticosterone exposure reduces hippocampal astrocyte structural plasticity and induces hippocampal atrophy in mice. *Neurosci Lett* 592, 76-81.
- Zhao, Z., Zhang, L., Guo, X. D., Cao, L. L., Xue, T. F., Zhao, X. J., Yang, D. D., Yang, J., Ji, J., Huang, J. Y. and Sun, X. L. 2017. Rosiglitazone Exerts an Anti-depressive Effect in Unpredictable Chronic Mild-Stress-Induced Depressive Mice by Maintaining Essential Neuron Autophagy and Inhibiting Excessive Astrocytic Apoptosis. *Front Mol Neurosci* 10, 293.