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Serotonin 5-HT$_7$ Receptor Blockade Reverses Behavioral Abnormalities in PACAP-Deficient Mice and Receptor Activation Promotes Neurite Extension in Primary Embryonic Hippocampal Neurons

Therapeutic Implications for Psychiatric Disorders

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Abstract The serotonin 5-HT$_7$ receptor has been linked to various psychiatric disorders, including schizophrenia, anxiety and depression, and is antagonized by antipsychotics such as risperidone, clozapine and lurasidone. In this study, we examined whether inhibiting the 5-HT$_7$ receptor could reverse behavioral abnormalities in mice lacking pituitary adenylate cyclase-activating polypeptide (PACAP), an experimental mouse model for psychiatric disorders such as schizophrenia. The selective 5-HT$_7$ antagonist SB-269970 effectively suppressed abnormal jumping behavior in PACAP-deficient mice. SB-269970 tended to alleviate the higher immobility in the forced swim test in PACAP-deficient mice, although SB-269970 reduced the immobility also in wild-type mice. In addition, we found that mutant mice had impaired performance in the Y-maze test, which was reversed by SB-269970. In the mutant mouse brain, 5-HT$_7$ protein expression did not differ from wild-type mice. In primary embryonic hippocampal neurons, the 5-HT$_7$ agonist AS19 increased neurite length and number. Furthermore, SB-269970 significantly inhibited the increase in neurite extension mediated by the 5-HT$_{1A/7}$ agonist 8-OH-DPAT. These results indicate that 5-HT$_7$ receptor blockade ameliorates psychomotor and cognitive deficits in PACAP-deficient mice, providing additional evidence that the 5-HT$_7$ receptor is a rational target for the treatment of psychiatric disorders.

Keywords Serotonin 7 receptor · PACAP · Antipsychotics · Forced swimming test · Y-maze task · Neurite outgrowth

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Introduction

Serotonin (5-HT) has diverse physiological and pharmacological effects mediated by different receptor subtypes (Meltzer 1995; Roth et al. 2004). Among these is the 5-HT$_7$ receptor, which is a Gs-coupled receptor that activates adenylate cyclase. It is localized to brain regions that modulate mood, sleep, circadian rhythms, learning and memory, such as the cortex, hippocampus, thalamus and brainstem raphe nuclei (Ruat et al. 1993; Neumaier et al. 2001; Hedlund 2009). The 5-HT$_7$ receptor is associated with a number of psychiatric disorders, including schizophrenia and depression. 5-HT$_7$

receptor mRNA expression is decreased in the dorsolateral prefrontal cortex of schizophrenic patients (East et al. 2002), and in the Japanese population, 5-HT_7 receptor gene single nucleotide polymorphisms (SNPs) are associated with schizophrenia (Ikeda et al. 2006).

Antipsychotic drugs primarily target the dopamine D_2 and 5-HT_{2A} receptors. However, several antipsychotic drugs and antidepressants block the 5-HT_7 receptor, and particularly high affinities are observed with atypical antipsychotics such as clozapine and risperidone (Roth 1994). The selective 5-HT_7 receptor antagonist SB-269970 has an anxiolytic effect in the Vogel conflict drinking test (Wesolowska et al. 2006b, 2007). SB-269970 also exerts an antidepressive effect in the forced swimming test (FST) (Wesolowska et al. 2006a, b, 2007) which is arguably the most reliable model available for assessing the effects of antidepressants with strong predictive ability for a broad spectrum of antidepressant effects (Porsolt et al. 1977; Petit-Demouliere et al. 2005). 5-HT_7 receptor knockout or blockade enhances learning and memory (Gasbarri et al. 2008). In animal models of psychosis, SB-269970 decreases amphetamine and ketamine-induced hyperactivity and reverses amphetamine-induced prepulse inhibition disruption in mice, without changing startle amplitude (Galici et al. 2008). It also reverses phencyclidine (PCP)-induced deficits in the novel object recognition test in rats (Horiguchi et al. 2011). Together, these observations indicate that pharmacological blockade of the 5-HT_7 receptor has antipsychotic effects (Pouzet et al. 2002). Lurasidone, a recently approved atypical antipsychotic drug, has moderate to potent binding affinity for dopamine D_2, 5-HT_{1A}, 5-HT_{2A}, 5-HT_7 and adrenaline α_{2c} receptors (Ishiyama et al. 2007; Enomoto et al. 2008; Meltzer et al. 2011). Lurasidone reverses the cognitive impairment induced by subchronic administration of PCP (Horiguchi et al. 2011) and enhances *N*-methyl-D-aspartate (NMDA) receptor-mediated synaptic responses in rat frontal cortical pyramidal neurons (Yuen et al. 2012). Amisulpride was initially developed as a selective D_2/D_3 receptor antagonist for the treatment of schizophrenia (Perrault et al. 1997), but Abbas et al. (2009) reported that it is also a potent competitive antagonist of the 5-HT_{7a} receptor and that its antidepressive effect is due to blockade of this protein.

Pituitary adenylate cyclase-activating polypeptide (PACAP) is a multifunctional neuropeptide that functions as a neurotransmitter and a neuromodulator (Vaudry et al. 2009). Previously, we demonstrated that PACAP-deficient ($PACAP^{-/-}$) mice display notable psychomotor abnormalities, such as hyperlocomotion and jumping behavior in a novel open field, prolonged immobility time in the FST and prepulse inhibition deficits (Hashimoto et al. 2001; Tanaka et al. 2006; Hashimoto et al. 2009). These behavioral abnormalities were reversed by atypical antipsychotic drugs such as risperidone. In addition, PACAP gene SNPs are associated with schizophrenia and major depressive disorder (Hashimoto et al. 2007, 2009, 2010). These results suggest that PACAP might be a risk factor for psychiatric disorders such as schizophrenia and depression.

5-HT also regulates neuronal development and differentiation processes such as neurite outgrowth. In dissociated hippocampal neurons, the 5-HT_7 agonist 5-CT increases the length of neurites, which can be blocked by SB-269970, without changing the number of neurites (Kvachnina et al. 2005). In addition, in cultured striatal neurons, the mixed 5-$HT_{1A/7}$ agonist 8-OH-DPAT increases neurite length, which can be blocked by simultaneous treatment with SB-269970, without changing other morphological parameters, such as the number of neurites, branching, soma perimeter or total surface area (Leo et al. 2009). Furthermore, morphological changes induced by the stress hormone corticosterone in the hippocampal cell line HT-22 and the amygdaloid cell line AR-5 depend on 5-HT_7 signaling (Xu et al. 2011).

In this study, we examined whether 5-HT_7 antagonism could reverse behavioral impairment in $PACAP^{-/-}$ mice. In addition, we assessed whether the number and length of neurites was affected by 5-HT_7 receptor activation in primary cultured hippocampal neurons from mouse embryos.

Materials and Methods

Animals

Generation of $PACAP^{-/-}$ mice by gene targeting has been reported previously (Hashimoto et al. 2001). The null mutation was backcrossed into the genetic background of Crlj:CD1 mice (Charles River, Tokyo, Japan) at least ten times. All wild-type control and $PACAP^{-/-}$ mice used were obtained from the intercross of animals heterozygous for the mutant PACAP gene, and experiments were conducted with 10- to 17-week-old naïve male mice. Mice were housed in a temperature (22±1°C) and light-controlled room with a 12-h light/12-h dark cycle (lights on from 08:00 to 20:00), and allowed free access to water and food (DC-8; Crea Japan Inc., Tokyo, Japan) except during behavioral testing. All animal experiments were performed in accordance with protocols approved by the Animal Care and Use Committee of the Graduate School of Pharmaceutical Science, Osaka University.

Drugs

The following drugs were used: (2*R*)-1-[(3-hydroxyphenyl)-sulfonyl]-2-[2-(4-methyl-1-piperidinyl)ethyl] pyrrolidine hydrochloride (SB-269970; Tocris, Cookson, Bristol, UK), 8-hydroxy-2-(di-*n*-propylamino)-tetralin (8-OH-DPAT; Sigma-Aldrich, St. Louis, MO, USA) and (2*S*)-(+)-5-(1,3,5-trimethylpyrazol-4-yl)-2-(dimethylamino)-tetralin (AS-19; Tocris). All

other drugs and reagents were of the highest purity commercially available. For behavioral tests, SB-269970 was dissolved in saline and administered intraperitoneally (10 ml/kg body weight) 30 min before behavioral tests.

Open Field Locomotor Activity

Locomotor activity was quantified using an infrared photocell beam detection system (Acti-Track; Panlab, Barcelona, Spain). Following injection of drug or an equal amount of corresponding vehicle solution, the mice were placed in plastic activity monitoring boxes (45×45×30 cm) and tracked for 60 min, with data being stored permanently. Parameters indicative of locomotor activity, such as distance traveled and line crossing in the central area, were assessed. Each mouse was tested individually and had no contact with the other mice. The box was cleaned between tests.

FST

FST was performed as described previously (Porsolt et al. 1977; Matsuda et al. 1995), with minor modifications. Mice were forced to swim for 6 min individually in a vertical glass cylinder (height, 24 cm; diameter, 18.5 cm) filled with water maintained at 24–26 °C to a depth of 13 cm. After testing, mice were taken out of the cylinder and allowed to dry in a heated enclosure. Duration of immobility, swimming and climbing were measured from videotapes by a trained observer blind to the treatment and the genotype of the mice. The total duration of each of the three behavioral parameters was individually recorded. To examine the robustness of this method, we compared the immobility times measured separately and that calculated by subtracting the duration of swimming and climbing from total time.

Y-maze Test

Spontaneous alternation behavior in the Y-maze task reflects hippocampal spatial working memory, and is used to assess the ability of animals to remember arms that were previously entered (Sarter et al. 1988; Dudchenko 2004). Y-maze testing was performed as described previously (Hiramatsu and Inoue 2000), with minor modification. The Y-shaped maze was placed beneath a video camera. Each arm was 29.5 cm long, 14.5 cm high and 3 cm wide, and converged in an equilateral triangular central area. Each mouse, naïve to the maze, was placed at the end of one arm and allowed to move freely through the maze during an 8-min session. The series of arm entries was recorded visually. Events were counted as entry when the hind paws of the mouse had completely entered the arm. Alternation was defined as successive entries into the three different arms on overlapping triplet sets. The percentage of spontaneous alternation was calculated as the ratio of actual to possible alternations (defined as the total number of arm entries−2) multiplied by 100, as shown in the following equation: alternation (%) = number of alternations/(total arm entries − 2)×100.

Western Blot Analysis

Mouse brain sections were excised using a brain matrix (NeuroScience Idea, Osaka, Japan) and snap-frozen in liquid nitrogen. Tissues were homogenized in 10 volumes of buffer containing 20 mM Tris–HCl (pH 7.4), 1 mM EDTA, 0.15 M NaCl, 1 % Nonidet P-40, 5 % glycerol, 5 mM L-mercaptoethanol and 1 mM phenylmethylsulfonyl fluoride. The homogenates were centrifuged at 8,000 rpm for 15 min at 4 °C. The protein concentration in the supernatant was determined using the bicinchoninic acid assay (BCA) assay (Pierce, Rockford, IL, USA) and an aliquot was mixed with sodium dodecyl sulfate (SDS) sample buffer. The samples were separated by 10 % SDS-PAGE, transferred electrophoretically to nitrocellulose membranes and then blocked with 3 % skim milk in Tris-buffered saline containing 0.05 % Tween-20. After incubation with rabbit polyclonal anti-5-HT_7 antibody (1:500 dilution; Abcam, Cambridge, UK) or mouse monoclonal anti-GAPDH antibody (1:80,000 dilution; Millipore, Billerica, MA, USA), the membranes were incubated with horseradish peroxidase-conjugated anti-rabbit IgG (1:5,000 dilution; Cappel laboratories, Cochranville, PA, USA) or horseradish peroxidase-conjugated anti-mouse IgG (1:2,000 dilution; Cappel Laboratories). A chemiluminescence detection system (Perkin Elmer, Waltham, MA, USA) was used for visualization.

Preparation of Primary Cultured Hippocampal Neurons and Immunocytochemistry

Embryonic day (E) 16 fetuses were removed using aseptic techniques from timed pregnant ICR mice (Japan SLC Inc., Shizuoka, Japan), and the brains were isolated and placed in Neurobasal medium (Life Technologies, Carlsbad, CA, USA) supplemented with penicillin, streptomycin and $NaHCO_3$ (7.5 %). Dissected hippocampal tissue was incubated with 0.02 % EDTA for 15 min at 37 °C and dissociated by repeated trituration. Cells were plated in neurobasal medium supplemented with B27 (Life Technologies) and L-glutamine (0.5 mM) at 2.5×10^4 cells/well in 24-well dishes containing glass slides coated with poly-L-lysine. We confirmed that the cultures consisted primarily (90–95 %) of neurons. The cells were fixed with 4 % paraformaldehyde in phosphate-buffered saline (PBS) and permeabilized with 0.3 % Triton-X100 in PBS. Following two washes in PBS, cells were incubated with anti-MAP2 mouse monoclonal primary antibody (1:1,000 dilution; Millipore) and with Alexa 488-conjugated anti-mouse IgG (1:1,000 dilution;

Life Technologies). The total neurite length, the mean neurite length and the number of neurites were determined for each individual hippocampal neuron using the Bio-Revo analysis platform (Keyence, Osaka, Japan).

Statistical Analysis

Statistically significant differences were assessed by two-way analysis of variance (ANOVA) (with genotype and drug treatment as factors of variation) using Statview software (Statview J-5.0; SAS Institute Japan Ltd., Tokyo, Japan) followed by post hoc Tukey–Kramer test. All values were expressed as mean±SEM. Statistical significance was defined as $P<0.05$.

Results

SB-269970 Attenuates Abnormal Jumping Behavior in PACAP$^{-/-}$ Mice

Our previous study revealed that PACAP$^{-/-}$ mice show increased locomotor activity and entry into the central area, as well as abnormal jumping behavior, in an open field (Hashimoto et al. 2001; Tanaka et al. 2006). In the present study, we examined the effects of SB-269970 on these behaviors. Although PACAP$^{-/-}$ mice injected with only saline displayed significant increased distance traveled and center entries (center crossings) compared with wild-type mice, SB-269970 did not significantly change these parameters either in wild-type or PACAP$^{-/-}$ mice (Fig. 1a, b). For distance traveled, two-way ANOVA revealed a significant main effect of genotype ($F_{1,53}>15.8$, $P=0.002$), but not of drug ($F_{1,53}=0.002$, $P=0.966$). There was no significant interaction between these variables ($F_{1,53}=0.707$, $P=0.404$). For center entries, two-way ANOVA revealed a significant main effect of genotype ($F_{1,51}>22.6$, $P<0.001$), but not of drug ($F_{2,51}=1.331$, $P=0.273$). There was no significant interaction between these variables ($F_{2,51}=1.622$, $P=0.208$). In contrast, jumping behavior, which is almost exclusively observed in PACAP$^{-/-}$ mice, was effectively suppressed by SB-269970 in a dose-dependent manner (Fig. 1c). Two-way ANOVA revealed significant main effects of drug ($F_{1,53}=12.621$, $P=0.008$) and genotype ($F_{1,53}=64.745$, $P<0.001$), as well as a significant interaction between these variables ($F_{1,53}=12.697$, $P=0.008$).

Effect of SB-269970 on Immobility in the FST in Wild-type and PACAP$^{-/-}$ Mice

The effects of SB-269970 on immobility in the FST were then examined in PACAP$^{-/-}$ mice. SB-269970 at 1 mg/kg body weight decreased the immobility time and increased the swimming time in wild-type mice (Fig. 2a, b). PACAP$^{-/-}$ mice displayed a significantly increased duration of immobility and a reduced swimming time compared with wild-type mice (Fig. 2a, b), as previously observed (Hashimoto et al. 2009). SB-269970 decreased the duration of immobility and increased swimming time in PACAP$^{-/-}$ mice, to an extent similar to that in wild-type mice. For immobility, two-way ANOVA revealed significant main effects of genotype ($F_{1,44}=5.036$, $P=0.029$) and drug ($F_{1,44}=5.437$, $P=0.024$), but there was no significant interaction between these variables ($F_{1,44}=0.079$, $P=0.779$). For swimming time, two-way ANOVA revealed significant main effects of genotype ($F_{1,44}=7.895$, $P=0.007$) and drug ($F_{1,44}=4.551$, $P=0.039$), but there was no significant interaction between these variables ($F_{1,44}=0.099$, $P=0.754$). In contrast, SB-269970 did not change climbing time in either PACAP$^{-/-}$ or wild-type mice (Fig. 2c). Two-way ANOVA revealed no significant main effects of genotype ($F_{1,44}=7.793$, $P=0.077$) or drug ($F_{1,44}=0.179$, $P=0.675$), but there was a significant interaction between these variables ($F_{1,44}=6.121$, $P=0.0173$).

Deficits in Working Memory in the Spontaneous Alternation in the Y-Maze Task in PACAP$^{-/-}$ Mice and Its Reversal by SB-269970

We examined whether PACAP$^{-/-}$ mice are impaired in working memory as assessed by the spontaneous alternation in the Y-maze task. Wild-type mice exhibited alternation rates of over 60 %, irrespective of whether they were injected with saline or SB-269970 (Fig. 3a). In contrast, PACAP$^{-/-}$ mice injected with only saline spent an approximately equal amount of time in each arm, resulting in significantly reduced alternation rates compared with wild-type mice. SB-269970 strikingly reversed the lowered spontaneous alternation behavior in PACAP$^{-/-}$ mice (Fig. 3a). Two-way ANOVA revealed significant main effects of genotype ($F_{1,51}=9.616$, $P=0.031$) and drug ($F_{1,51}=6.637$, $P=0.0129$), but there was no significant interaction between these variables ($F_{1,51}=3.122$, $P=0.0832$). The total number of arm entries did not differ significantly between the different treatments or genotypes (Fig. 3b). Two-way ANOVA revealed no significant main effects of genotype ($F_{1,51}=0.149$, $P=0.7007$) or drug ($F_{1,51}=0.194$, $P=0.6617$), and no significant interaction between these variables ($F_{1,51}=0.907$, $P=0.3454$).

Expression of 5-HT$_7$ Receptor Protein in the PACAP$^{-/-}$ Mouse Brain

To examine possible changes in 5-HT$_7$ receptor expression in PACAP$^{-/-}$ mice, we measured the protein levels of the receptor in brain regions associated with behavior, including the prefrontal cortex, hippocampus, hypothalamus and

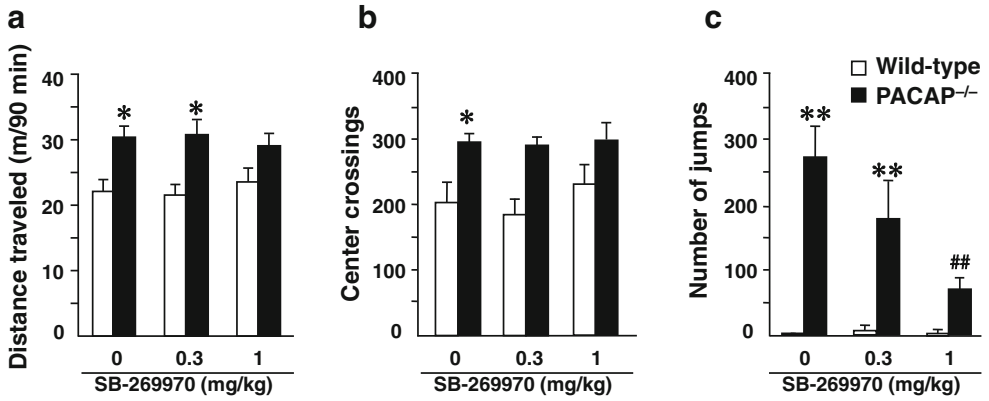

Fig. 1 SB-269970 effectively suppressed abnormal jumping behavior in PACAP$^{-/-}$ mice. Thirty minutes after mice were injected with SB-269970 (0.3, 1 mg/kg) or saline, they were placed in the open-field apparatus and their activity was measured. Total distance traveled (**a**), crossing events in the center field (**b**) and the number of jumps (**c**) during a 90-min session are shown. n=8–11 per genotype. *P<0.05, **P<0.01 versus wild-type mice; ##P<0.01 versus saline; two-way ANOVA, followed by post-hoc Tukey–Kramer test

amygdala. Quantitative Western blot analysis of samples from each brain region revealed that 5-HT$_7$ protein expression in PACAP$^{-/-}$ mice was not significantly different from wild-type mice (Fig. 4).

The 5-HT$_7$ Agonist AS19 Promotes Neurite Outgrowth and SB-269970 Inhibits 8-OH-DPAT-Induced Neurite Outgrowth in Primary Embryonic Hippocampal Neurons

We examined whether the number and length of neurites are increased by 5-HT$_7$ receptor activation in primary cultured hippocampal neurons from E16 mouse embryos. The neurons were treated with 5-HT$_7$ agonists and/or an antagonist for 72 h, and the total dendritic neurite length, mean dendritic neurite length and neurite number were determined (Fig. 5). The selective 5-HT$_7$ agonist AS19 significantly increased all three parameters in a dose-dependent manner. As expected, the 5-HT$_{1A/7}$ agonist 8-OH-DPAT also increased total and mean neurite length, and this effect was inhibited by the simultaneous addition of SB-269970 (Fig. 5).

Discussion

A growing body of evidence indicates that the 5-HT$_7$ receptor is involved in the pathophysiology of psychiatric diseases, and drugs acting on the receptor have attracted attention owing to their great therapeutic potential (Ruat et al. 1993; Roth 1994; Neumaier et al. 2001; East et al. 2002; Ikeda et al. 2006; Hedlund 2009; Horiguchi et al. 2011; Meltzer et al. 2011). However, the effects of these drugs have not been fully explored in animal models of psychiatric disorders. In the present study, we examined the effects of 5-HT$_7$ antagonism on behavioral abnormalities in PACAP$^{-/-}$ mice.

SB-269970 mitigated abnormal jumping behavior in PACAP$^{-/-}$ mice without influencing hyperlocomotion. These results appear to dovetail with a previous study that showed that inactivation of the 5-HT$_7$ receptor modulates prepulse inhibition deficits induced by PCP, but not those elicited by dopaminergic challenge (apomorphine and amphetamine) (Semenova et al. 2008). Although it is difficult to directly link the observed jumping behavior in mice to

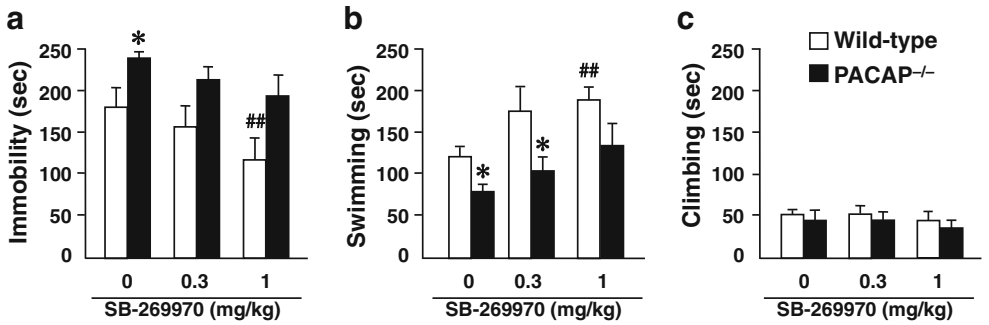

Fig. 2 Effects of SB-269970 on immobility in the FST in wild-type and PACAP$^{-/-}$ mice. Thirty minutes after mice were injected with the indicated doses of SB-269970 or saline, they were forced to swim for 6 min, and the durations of immobility (**a**), swimming (**b**) and climbing (**c**) were measured. n=8 per genotype. *P<0.05 versus wild-type mice; $^{\#}P$<0.05 versus saline

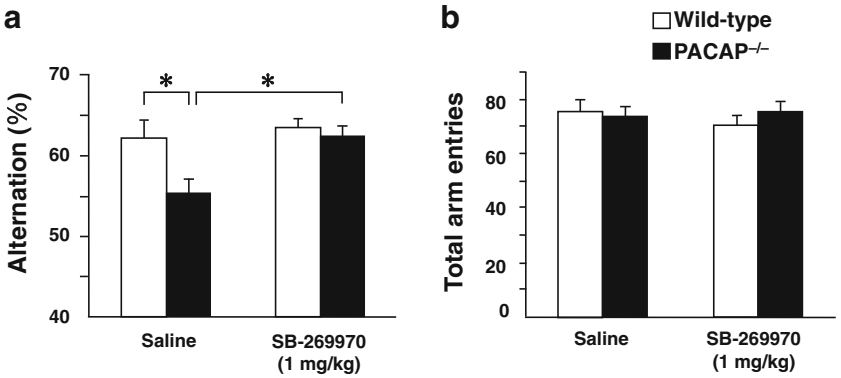

Fig. 3 Working memory deficits in PACAP$^{-/-}$ mice and its reversal by SB-269970. Thirty minutes after mice were injected with SB-269970 (1 mg/kg) or saline, they were placed at the end of one arm of a Y-maze and allowed to move freely through the maze during an 8-min session. Spontaneous alternation (**a**) and the number of total arm entries (**b**) are shown. n=12–15 per genotype. *P<0.05, two-way ANOVA, followed by post-hoc Tukey–Kramer test

human psychiatric symptoms, there are other mouse models that also display abnormal jumping. For example, in NIH Swiss mice, the NMDA-receptor antagonist MK-801 induces explosive jumping behavior that can be reversed by haloperidol (Rosse et al. 1995). In morphine-dependent mice, naloxone triggers withdrawal jumping, which is not attenuated by clozapine or haloperidol (Ballard and McAllister 1999). We previously demonstrated that fluoxetine and the 5-HT precursor 5-hydroxytryptophan diminishes the number of jumps in PACAP$^{-/-}$ mice (Shintani et al. 2006). Taken together, these results suggest that jumping behavior likely involves the serotonergic system and that SB-269970 may modulate behavioral deficits associated with NMDA receptor hypofunction.

We previously examined the effects of antidepressant drugs on FST behavior in PACAP$^{-/-}$ mice and observed that the tricyclic antidepressant desipramine increased climbing behavior, but not swimming behavior, to a similar extent in wild-type and PACAP$^{-/-}$ mice. In contrast, the atypical antipsychotic risperidone and the selective 5-HT$_2$ antagonist ritanserin decreased the duration of immobility and increased swimming time in PACAP$^{-/-}$ mice without having an effect on wild-type mice (Hashimoto et al. 2009). In the present study, although SB-269970 did not statistically significantly decrease immobility in PACAP$^{-/-}$ mice, it decreased immobility in wild-type mice. The result supports the previous conclusion that 5-HT$_7$ antagonists have a robust antidepressant effect (Wesolowska et al. 2006a, b, 2007).

To assess cognitive function in PACAP$^{-/-}$ mice and the effects of SB-269970, we examined spatial working memory using the Y-maze task in PACAP$^{-/-}$ mice. The mutant mice showed significantly decreased memory performance, but this impairment was strikingly reversed by SB-269970. It has been reported that amnesia induced by scopolamine or MK-801 can be reversed by 5-HT$_7$ antagonists (SB-269970 and DR 4004) in an autoshaping Pavlovian/instrumental learning test (Meneses 2004). Yuen et al. (2012) reported that SB-269970 and the newly developed atypical antipsychotic lurasidone enhance NMDA receptor-mediated synaptic responses in frontal cortical pyramidal neurons. Horiguchi et al. (2011) demonstrated that SB-269970 and lurasidone ameliorate deficits in novel object recognition memory produced by subchronic PCP treatment. They also showed that a subeffective dose of SB-269970 (0.1 mg/kg) in combination with subeffective doses of lurasidone (0.03 mg/kg) or sulpiride (20 mg/kg), a less potent 5-HT$_7$ antagonist, can reverse the PCP-induced memory deficits. Collectively, these results implicate 5-HT$_7$ receptors in cognitive deficits and

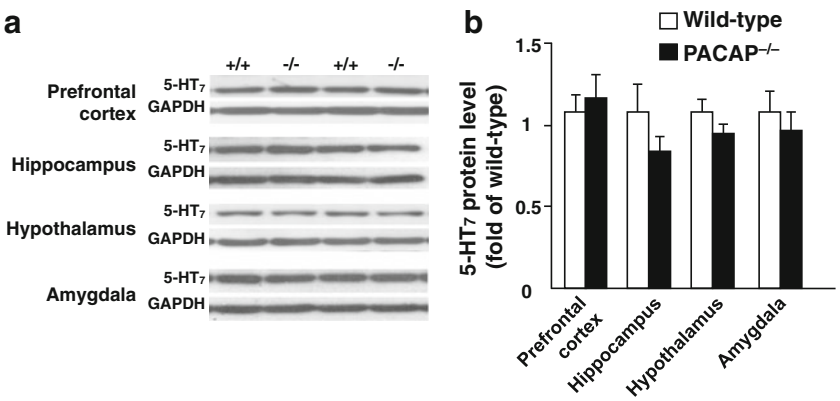

Fig. 4 5-HT$_7$ protein expression in the PACAP$^{-/-}$ mouse brain. 5-HT$_7$ receptor protein expression levels were determined by Western blot analysis in prefrontal cortex, hippocampus, hypothalamus and amygdala in wild-type (+/+) and PACAP$^{-/-}$ (−/−) mice. Representative images (**a**) and quantification (**b**) of Western blot analysis are shown. n=5–6 per genotype

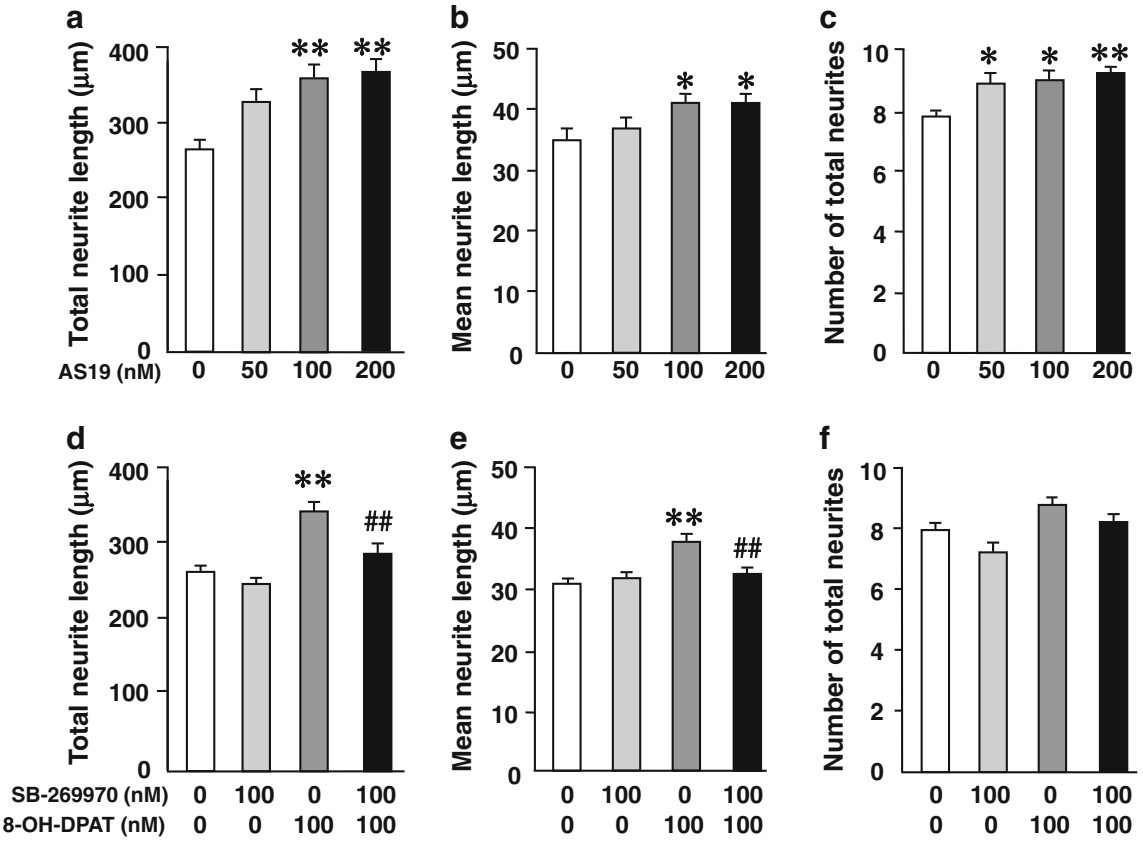

Fig. 5 The 5-HT$_7$ agonist AS19 increases the length and number of neurites, and SB-269970 inhibits neurite outgrowth induced by the mixed 5-HT$_{1A/7}$ agonist 8-OH-DPAT in primary embryonic hippocampal neurons. Primary cultured hippocampal neurons prepared from E16 mouse embryos were treated with the indicated concentrations of AS19 (**a**–**c**), 100 nM 8-OH-DPAT and/or 100 nM SB-269970 (**d**–**f**) for 72 h. The cells were then immunostained for MAP2, and total neurite length (**a**, **d**), mean neurite length (**b**, **e**) and the number of total neurites (**c**, **f**) were measured. n=95–100 neurons from four independent experiments. *P<0.05, **P<0.01 versus control; ##P<0.01 versus 8-OH-DPAT; two-way ANOVA, followed by post-hoc Tukey–Kramer test

suggest their involvement in the action of atypical antipsychotic drugs.

It has been reported that 5-HT$_7$ mRNA expression is decreased in the dorsolateral prefrontal cortex of schizophrenic patients (East et al. 2002). In the PACAP$^{-/-}$ mouse brain, the 5-HT metabolite 5-hydroxyindoleacetic acid is slightly decreased in the cortex and striatum (Hashimoto et al. 2001). In these mice, the hypothermic response to 5-HT$_{1A}$ agonists is considerably reduced (Tanaka et al. 2006) and 5-HT$_{2A}$ agonist-induced head twitch and ear scratch responses are increased (Hashimoto et al. 2009). In rats subjected to chronic unpredictable mild stress, 5-HT levels are reduced and 5-HT$_7$ mRNA levels are increased in the brain (Li et al. 2009). Therefore, we examined the expression of the 5-HT$_7$ receptor protein in the PACAP$^{-/-}$ mouse brain, but no obvious changes were seen, at least in the regions tested (prefrontal cortex, hippocampus, hypothalamus and amygdala). This result suggests that there may be alterations in signaling downstream of the 5-HT$_7$ receptor in PACAP$^{-/-}$ mice.

It has been suggested that several psychotropic drugs might have the ability to not only ameliorate abnormal behavior but also enhance neuronal development (Konradi and Heckers 2001; Lu and Dwyer 2005; Nandam et al. 2007). In dissociated hippocampal neurons, the 5-HT$_7$ agonist 5-CT increases the length of neurites (which can be blocked by SB-269970), without changing the number of neurites (Kvachnina et al. 2005). Furthermore, in cultured striatal neurons, 8-OH-DPAT increases neurite length (which can be blocked by SB-269970), without changing other morphological parameters, such as the number of neurites (Leo et al. 2009). In the present study, although treatment of primary cultured hippocampal neurons with SB-269970 did not change the length or number of dendritic neurites, the selective 5-HT$_7$ agonist AS19 significantly increased both the number and length of dendritic neurites. This result may indicate that the 5-HT$_7$ receptor-dependent increase in dendritic neurite outgrowth might be involved in pathological processes of psychiatric conditions. Xu et al. (2011) have demonstrated that the stress hormone corticosterone increases

5-HT_7 receptor mRNA levels and decreases neurite outgrowth in hippocampal HT-22 cells, while it has opposite effects in AR-5 amygdala cells. Although the exact role of the 5-HT_7 receptor-dependent neurite outgrowth is unclear, the present results, taken together with the previous studies (Kvachnina et al. 2005; Leo et al. 2009; Xu et al. 2011), suggest that the 5-HT_7 receptor signaling pathway is actively involved in neural plasticity, and therefore could be a target for therapeutic intervention.

In conclusion, our results indicate that 5-HT_7 receptor antagonists ameliorate behavioral abnormalities in PACAP-deficient mice, providing additional evidence for the 5-HT_7 receptor as a rational target for the treatment of psychiatric disorders.

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