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References

Alonso, M., Bekinschtein, P., Cammarota, M., Vianna, M.R., Izquierdo, I., Medina, J.H., 2005. Endogenous BDNF is required for long-term memory formation in the rat parietal cortex. Learn. Memory 12, 504–510.

Amano, M., Suemaru, K., Cui, R., Umeda, Y., Li, B., Gomita, Y., Kawasaki, H., Araki, H., 2007. Effects of physical and psychological stress on 5-HT2A receptor-mediated wet-dog shake responses in streptozotocin-induced diabetic rats. Acta Med. Okayama 61, 205–212.

Bekinschtein, P., Cammarota, M., Katche, C., Slipczuk, L., Rossato, J.I., Goldin, A., Izquierdo, I., Medina, J.H., 2008. BDNF is essential to promote persistence of long-term memory storage. Proc. Natl. Acad. Sci. U. S. A. 105, 2711–2716.

Berton, O., McClung, C.A., Dileone, R.J., Krishnan, V., Renthal, W., Russo, S.J., Graham, D., Tsankova, N.M., Bolanos, C.A., Rios, M., Monteggia, L.M., Self, D.W., Nestler, E.J., 2006. Essential role of BDNF in the mesolimbic dopamine pathway in social defeat stress. Science 311, 864–868.

Bimonte-Nelson, H.A., Hunter, C.L., Nelson, M.E., Granholm, A.C., 2003. Frontal cortex BDNF levels correlate with working memory in an animal model of Down syndrome. Behav. Brain Res. 139, 47–57.

Chourbaji, S., Hellweg, R., Brandis, D., Zörner, B., Zacher, C., Lang, U.E., Henn, F.A., Hörtnagl, H., Gass, P., 2004. Mice with reduced brain-derived neurotrophic factor expression show decreased choline acetyltransferase activity, but regular brain monoamine levels and unaltered emotional behavior. Brain Res. Mol. Brain Res. 121, 28–36.

Egan, M., Goldman, D., Weinberger, D., 2002. The human genome: mutations. Am. J. Psychiatry 159, 12.

Egan, M.F., Weinberger, D.R., 1997. Neurobiology of schizophrenia. Curr. Opin. Neurobiol. 7, 701–707.

Evenden, J.L., Robbins, T.W., 1983. Increased response switching, perseveration and perseverative switching following D-amphetamine in the rat. Psychopharmacology (Berl) 80, 67–73.

Einat, H., Szechtman, H., 1995. Perseveration without hyperlocomotion in a spontaneous alternation task in rats sensitized to the dopamine agonist quinpirole. Physiol. Behav. 57, 55–59.

Fumagalli, F., Racagni, G., Colombo, E., Riva, M.A., 2003. BDNF gene expression is reduced in the frontal cortex of dopamine transporter knockout mice. Mol. Psychiatry 8, 898–899.

Gainetdinov, R.R., Wetsel, W.C., Jones, S.R., Levin, E.D., Jaber, M., Caron, M.G., 1999. Role of serotonin in the paradoxical calming effect of psychostimulants on hyperactivity. Science 283, 397–401.

Gaspar, P., Bloch, B., Le Moine, C., 1995. D1 and D2 receptor gene expression in the rat frontal cortex: cellular localization in different classes of efferent neurons. Eur. J. NeuroSci. 7, 1050–1063.

Goldman-Rakic, P.S., Lidow, M.S., Smiley, J.F., Williams, M.S., 1992. The anatomy of dopamine in monkey and human prefrontal cortex. J. Neural Transm., Suppl. 36, 163–177.

Goldman-Rakic, P.S., 1996. Regional and cellular fractionation of working memory. Proc. Natl. Acad. Sci. U. S. A. 93, 13473–13480.

Hellweg, R., Lohmann, P., Huber, R., Kühl, A., Riepe, M.W., 2006. Spatial navigation in complex and radial mazes in APP23 animals and neurotrophin signaling as a biological marker of early impairment. Learn. Memory 13, 63–71.

Hironaka, N., Ikeda, K., Sora, I., Uhl, G.R., Niki, H., 2004. Food-reinforced operant behavior in dopamine transporter knockout mice: enhanced resistance to extinction. Ann. N. Y. Acad. Sci. 1025, 140–145.

Hughes, R.N., 2004. The value of spontaneous alternation behavior (SAB) as a test of retention in pharmacological investigations of memory. Neurosci. Biobehav. Rev. 28, 497–505.

Kesner, R.P., Rogers, J., 2004. An analysis of independence and interactions of brain substrates that subserve multiple attributes, memory systems, and underlying processes. Neurobiol. Learn. Mem. 82, 199–215.

Korte, M., Kang, H., Bonhoeffer, T., Schuman, E., 1998. A role for BDNF in the late-phase of hippocampal long-term potentiation. Neuropharmacology 37, 553–559.

Kozlov, A.P., Druzin, M.Y., Kurzina, N.P., Malinina, E.P., 2001. The role of D1-dependent dopaminergic mechanisms of the frontal cortex in delayed responding in rats. Neurosci. Behav. Physiol. 31, 405–411.

Krejcova, G., Patocka, J., Slaninova, J., 2004. Effect of humanin analogues on experimentally induced impairment of spatial memory in rats. J. Pept. Sci. 10, 636–639.

Küppers, E., Beyer, C., 2001. Dopamine regulates brain-derived neurotrophic factor (BDNF) expression in cultured embryonic mouse striatal cells. NeuroReport 12, 1175–1179.

Lalonde, R., 2002. The neurobiological basis of spontaneous alternation. Neurosci. Biobehav. Rev. 26, 91–104.

Lessmann, V., 1998. Neurotrophin-dependent modulation of glutamatergic synaptic transmission in the mammalian CNS. Gen. Pharmacol. 31, 667–674.

Lewis, D.A., Sesack, S.R., Levey, A.I., Rosenberg, D.R., 1998. Dopamine axons in primate prefrontal cortex: specificity of distribution, synaptic targets, and development. Adv. Pharmacol. 42, 703–706.

Li, B., Suemaru, K., Cui, R., Kitamura, Y., Gomita, Y., Araki, H., 2006. Repeated electroconvulsive stimuli increase brain-derived neurotrophic factor in ACTH-treated rats. Eur. J. Pharmacol. 529, 114–121.

Li, B., Suemaru, K., Cui, R., Araki, H., 2007a. Repeated electroconvulsive stimuli have long-lasting effects on hippocampal BDNF and decrease immobility time in the rat forced swim test. Life Sci. 80, 1539–1543.

Li, B., Suemaru, K., Kitamura, Y., Cui, R., Gomita, Y., Araki, H., 2007b. Strategy to develop a new drug for treatment-resistant depression–role of electroconvulsive stimuli and BDNF. Yakugaku Zasshi 127, 735–742.

Lidow, M.S., Goldman-Rakic, P.S., Gallager, D.W., Rakic, P., 1991. Distribution of dopaminergic receptors in the primate cerebral cortex: quantitative autoradiographic analysis using [3H]raclopride, [3H]spiperone and [3H]SCH23390. Neuroscience 40, 657–671.

Luine, V., Villegas, M., Martinez, C., McEwen, B.S., 1994. Repeated stress causes reversible impairments of spatial memory performance. Brain Res. 639, 167–170.

Ma, M.X., Chen, Y.M., He, J., Zeng, T., Wang, J.H., 2007. Effects of morphine and its withdrawal on Y-maze spatial recognition memory in mice. Neuroscience 147, 1059–1065.

Mamiya, T., Ukai, M., 2001. [Gly(14)]-Humanin improved the learning and memory impairment induced by scopolamine in vivo. Br. J. Pharmacol. 134, 1597–1599.

Mehta, M.A., Swainson, R., Ogilvie, A.D., Sahakian, J., Robbins, T.W., 2001. Improved short-term spatial memory but impaired reversal learning following the dopamine D(2) agonist bromocriptine in human volunteers. Psychopharmacology (Berl) 159, 10–20.

Mizuno, M., Yamada, K., Olariu, A., Nawa, H., Nabeshima, T., 2000. Involvement of brain-derived neurotrophic factor in spatial memory formation and maintenance in a radial arm maze test in rats. J. Neurosci. 20, 7116–7121.

Mogensen, J., Divac, I., 1993. Behavioural changes after ablation of subdivisions of the rat prefrontal cortex. Acta Neurobiol. Exp. (Wars) 53, 439–449.

Murer, M.G., Yan, Q., Raisman-Vozari, R., 2001. Brain-derived neurotrophic factor in the control human brain, and in Alzheimer's disease and Parkinson's disease. Prog. Neurobiol. 63, 71–124.

Pioli, E.Y., Meissner, W., Sohr, R., Gross, C.E., Bezard, E., Bioulac, B.H., 2008. Differential behavioral effects of partial bilateral lesions of ventral tegmental area or substantia nigra pars compacta in rats. Neuroscience 153, 1213–1224.

Perona, M.T., Waters, S., Hall, F.S., Sora, I., Lesch, K.P., Murphy, D.L., Caron, M., Uhl, G.R., 2008. Animal models of depression in dopamine, serotonin, and norepinephrine transporter knockout mice: prominent effects of dopamine transporter deletions. Behav. Pharmacol. 19, 566–574.

Poo, M.M., 2001. Neurotrophins as synaptic modulators. Nat. Rev., Neurosci. 2, 24–32.

Sawaguchi, T., Goldman-Rakic, P.S., 1991. D1 dopamine receptors in prefrontal cortex: involvement in working memory. Science 251, 947–950.

Sawaguchi, T., Goldman-Rakic, P.S., 1994. The role of D1-dopamine receptor in working memory: local injections of dopamine antagonists into the prefrontal cortex of rhesus monkeys performing an oculomotor delayed-response task. J. Neurophysiol. 71, 515–528.

Schaaf, M.J., de Jong, J., de Kloet, E.R., Vreugdenhil, E., 1998. Downregulation of BDNF mRNA and protein in the rat hippocampus by corticosterone. Brain Res. 813, 112–120.

Shen, H.W., Hagino, Y., Kobayashi, H., Shinohara-Tanaka, K., Ikeda, K., Yamamoto, H., Yamamoto, T., Lesch, K.P., Murphy, D.L., Hall, F.S., Uhl, G.R., Sora, I., 2004. Regional differences in extracellular dopamine and serotonin assessed by in vivo microdialysis in mice lacking dopamine and/or serotonin transporters. Neuropsychopharmacology 29, 1790–1799.

Sora, I., Wichems, C., Takahashi, N., Li, X.F., Zeng, Z., Revay, R., Lesch, K.P., Murphy, D.L., Uhl, G.R., 1998. Cocaine reward models: conditioned place preference can be established in dopamine- and in serotonin-transporter knockout mice. Proc. Natl. Acad. Sci. U.S.A. 95, 7699–7704.

Sora, I., Hall, F.S., Andrews, A.M., Itokawa, M., Li, X.F., Wei, H.B., Wichems, C., Lesch, K.P., Murphy, D.L., Uhl, G.R., 2001. Molecular mechanisms of cocaine reward: combined dopamine and serotonin transporter knockouts eliminate cocaine place preference. Proc. Natl. Acad. Sci. U. S. A. 98, 5300–5305.

Sora, I., Li, B., Fumushima, S., Fukui, A., Arime, Y., Kasahara, Y., Tomita, H., Ikeda, K., 2009. Monoamine transporter as a target molecule for psychostimulants. Int. Rev. Neurobiol. 85, 29–33.

Tyler, W.J., Alonso, M., Bramham, C.R., Pozzo-Miller, L.D., 2002. From acquisition to consolidation: on the role of brain-derived neurotrophic factor signaling in hippocampal-dependent learning. Learn. Memory 9, 224–237.

Yamashita, M., Fukushima, S., Shen, H.W., Hall, F.S., Uhl, G.R., Numachi, Y., Kobayashi, H., Sora, I., 2006. Norepinephrine transporter blockade can normalize the prepulse inhibition deficits found in dopamine transporter knockout mice. Neuropsychopharmacology 31, 2132–2139.

Yang, C.R., Seamans, J.K., Gorelova, N., 1999. Developing a neuronal model for the pathophysiology of schizophrenia based on the nature of electrophysiological actions of dopamine in the prefrontal cortex. Neuropsychopharmacology 21, 161–194.

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Research report

Progesterone reduces hyperactivity of female and male dopamine transporter knockout mice

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ABSTRACT

There are gender differences in prevalence, course, and/or prognosis of schizophrenia. Yet, neurobiological factors that may account for the more favorable outcomes of women with schizophrenia are not well understood. Evidence that the steroid hormone, progesterone (P_4), may influence mood and/or arousal among some people with schizophrenia led us to examine the effects of P_4 on dopamine transporter knockout (DATKO) mice, an animal model of schizophrenia. Our hypothesis was that P_4 would have greater effects than vehicle to improve the behavioral phenotype of DATKO, more so than wildtype, mice. Young adult, male and female DATKO mice and their wildtype counterparts were subcutaneously administered P_4 (10 mg/kg) or vehicle 1 h prior to testing in pre-pulse inhibition (PPI), activity monitor, or open field. DATKO mice had impaired PPI compared to their wildtype counterparts, but there was no effect of P_4. In the activity monitor, DATKO mice showed significantly greater distance traveled during the 60 min test compared to wildtype controls. In the open field, DATKO mice made a significantly greater number of total, but fewer central, entries than did wildtype mice. Administration of P_4 decreased the hyperactivity of DATKO mice in the activity monitor and open field, but did not alter motor behavior of wildtype mice. P_4 increased the number of central entries made by DATKO and wildtype mice. Thus, P_4 administration to DATKO female or male mice partially attenuated their hyperactive phenotype.

1. Introduction

Schizophrenia is characterized by deficits in social, affective and cognitive functioning. Gender differences in schizophrenia include that some women have a lower incidence, later age of onset, fewer hospitalizations, and better response to antipsychotics than do some men [24,27,31,58]. As with other gender/sex differences that are influenced by hormonal status, there is evidence for a role of hormones to influence expression of symptoms in schizophrenia. In support, some women have a greater occurrence of negative symptoms and/or may require higher dosages of antipsychotics, when endogenous progesterone (P_4) levels are low, during the follicular phase or post-menopause [21,24,58]. Thus, there is clinical evidence for P_4 to influence the pathophysiology of schizophrenia.

In animal models, there is evidence that P_4 can modulate the expression of some behaviors that are altered in schizophrenia. First, P_4 facilities sexual and/or social behaviors of female rodents, in part through its actions in the Ventral Tegmental Area (VTA), which is notable for the many dopamine (DA) cell bodies located there. Some of P_4's actions in this region may be through DA signaling. Activation and/or attenuation of P_4's actions via DA type 1 receptors in the VTA, respectively, facilitates and inhibits, sexual responses of rodents [61]. Second, P_4 also has effects on arousal and affective measures. In support, when administered to aged mice, P_4 (10 mg/kg, subcutaneously, SC), compared to vehicle, increased: the number of central entries in the open field, open quadrant time in the elevated zero maze, time spent in the mirror-chamber, time spent in light chamber in the dark/light transition task, and increased punished drinking in the Vogel conflict task [14]. Interestingly, similar effects of P_4 to enhance sexual receptivity and arousal were observed in older C57BL/6 mice and also P_4 receptor knockout mice (PRKOs) [15]. Third, P_4 can enhance cognitive function in tasks mediated by the nucleus accumbens (conditioned place preference), cortex (object recognition; T maze), and/or hippocampus (object recognition, water maze, conditioned fear), areas which are DA sensitive and that the VTA projects to [8,10,12,65]. Moreover, P_4 can have these effects in wildtype and PRKO mice. Together, these data suggest that P_4 has effects on some normative functional process that are atypical in schizophrenia and that some of these effects may be at least/in part, independent of action at PRs.

Dopaminergic inputs from the VTA play a key role in arousal and affective function in animals. The DA and serotonin (5-HT)

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systems have been implicated in striatal dysfunction associated with schizophrenia and may also play a role in reward and/or susceptibility to drug abuse [4,26]. As such, a greater understanding of hormones' effects through these systems is important.

The DA transporter (DAT) is a plasma membrane transport protein that controls extracellular DA concentrations and is an important target for a variety of therapeutic agents [51,53,59,61,62]. DAT knockout mice (DATKOs) exhibit elevated interstitial levels of dopamine and a range of behavioral alterations, including poor cognitive function [40], hyperactivity, and some stereotyped and/or perseverative behavior [61,62]. DATKO mice also have impaired pre-pulse inhibition (PPI), a model of sensorimotor gating in schizophrenia [67]. To date, the effects of P_4 on behavior of DATKO mice and their wildtype counterparts have not been reported. Evidence from our laboratory suggests that P_4 may influence sexual, social, cognitive and/or affective behaviors in part through its actions on the DA systems. As such, we wished to test the hypothesis that P_4 may reduce hyperactivity of DATKO, but not wildtype, mice.

2. Methods

These methods were pre-approved by The Institutional Animal Care and Use Committees at the University at Albany, State University of New York and The Tohoku University Graduate School of Medicine (Sendai, Japan).

2.1. Subjects

DATKO mice are used as a model for schizophrenia [50,59]. Individual DATKO strains used in this report have been described previously [61,62]. Intact male and female wildtype and DATKO mice ($N = 120$; $n = 15$/group) were bred and obtained from the colony at Tohoku University Graduate School of Medicine. DATKO and wildtype littermates were obtained by heterozygote crosses that had been previously generated on 129Sv-C57BL/6J mixed genetic background [60].

2.2. Housing

Offspring were weaned at postnatal day 28 and group housed, segregated by sex, in a temperature- and light-controlled colony (lights on at 0800 h, lights off at 2000 h), with food and water available *ad libitum*.

2.3. Genotype

Mice were genotyped using multiplex polymerase chain reaction methods on DNA extracted from tails, as previously described [14–16].

2.4. Subcutaneous P_4-priming

All mice were randomly assigned to either P_4 (10 mg/kg) or vehicle (sesame oil) condition. P_4 was obtained from Sigma Chemical Co. (St. Louis, MO) and dissolved in sesame oil to a concentration of 10 mg/ml. Mice received SC P_4 or vehicle injections 1 h prior to behavioral testing. This P_4 regimen was utilized because it increases plasma and central progestogen levels akin to that seen during behavioral estrus of rodents, without producing gross alterations in motor behavior and/or coordination [14,16]. After mice were injected with P_4 or vehicle, they were returned to their home cages for 1 h until behavioral testing.

2.5. Behavioral testing

Limited numbers of DATKO mice were available. Given this, a repeated-measures design was utilized. Therefore, all mice were once tested in either the P_4 or vehicle condition, then they were re-tested in the opposite condition five days later. Whether P_4 or vehicle was received initially was counterbalanced across subjects [63,65]. All animals were tested for PPI and for behavior in the activity monitor and open field following vehicle and P_4 conditions.

2.5.1. Handling and habituation

To minimize the effects of handling associated with the repeated testing, on day 1 mice were picked up by the tail and placed back in the home cage. On day 2, mice were transferred to another clean home cage. On day 3, mice were picked up, weighed, and transferred on a cart to another room and then the mouse was moved to another clean home cage. On day 4, the experimenter put the mouse in their home cage on a cart, and mice were transported to another room and were placed in novel environment for 2 min. On day 5, mice cages were put in their home cages on a cart, the cart and cage were moved to another room, mice were SC injected with sesame oil, then mice were placed in a novel environment, and then returned to their home cage.

2.5.2. Pre-pulse inhibition

Following the handling period, arousal behavior was assessed in the PPI task. Experiments were conducted based upon previously reported methods [7,17,67]. Startle chambers (SR-LAB, San Diego Instruments, San Diego, CA) were used to measure the startle response. Each chamber consisted of a non-restrictive Plexiglas cylinder mounted on a frame inside a lighted, ventilated box (35 cm × 35 cm × 47.5 cm). Movement within the cylinder was detected by piezoelectric accelerometers attached to the cylinder's bottom. Force detected by the accelerometer was converted into analog signals that were digitized and stored electronically. In all experiments, 65 readings were recorded at 1 ms intervals beginning at stimulus onset; the average amplitude was used to describe the acoustic startle response. A high-frequency loudspeaker inside the chamber, mounted above the cylinder, generated broadband background noise and acoustic stimuli, which were controlled by the SR-LAB software system and interface. Sound levels (dB (A) scale) and accelerometer sensitivity were calibrated routinely, as described previously [7,17]. Mice were tested initially for baseline PPI and pseudo-randomly assigned to hormone treatment groups based on these measurements. Mice were treated with vehicle or P_4 60 min before testing. Experimental sessions consisted of a 5 min acclimatization period with 65 dB broadband background noise followed by PPI sessions. Sessions consisted of five different trial types: no stimulus trials (nostim); startle pulse alone, 40 ms duration at 120 dB (p120); and three pre-pulse + pulse trials, 20 ms duration pre-pulse at 68 dB (pp3), 71 dB (pp6), or 77 dB (pp12), followed by a 40 ms duration startle stimulus at 120 dB after a 100 ms delay. The nostim trial consisted of only background broadband noise. All test sessions started and concluded with six presentations of the p120 trial, while the remainder of the session consisted of 12 presentations of the p120 trial type, 10 presentations of the nostim, the pp3, pp6, and pp12 trial types, in a pseudorandom order, with varying inter-trial intervals (mean 15 s, range 8–23 s). Each animal was tested on a PPI session for 21 min with a total of 64 trials.

2.5.3. Activity monitor

Locomotion was assessed in an activity monitor. Mice were first habituated to the apparatus (40 cm × 30 cm × 26 cm clear plastic chamber) for 180 min and then subcutaneously injected with P_4 or vehicle. After 1 h, mice were placed back in the apparatus and locomotor activity was measured in 5-min increments using digital counters with passive infrared sensors (Supermex System, Tokyo, Japan).

2.5.4. Open field

Behavior was assessed in the open field, which can be used to determine total motor activity as well as anxiety behavior. Rodents typically avoid open, bright areas so propensity to move in the center of the open field indicates a reduced anxiety-like response. Mice were placed in the open field arena (39 cm × 39 cm × 30 cm) that had a 16-square grid floor and an overhead light illuminating the central squares [14,16]. Total numbers of entries and central entries were recorded for 5 min. The total number of entries made in the open field is used as an index of general motor activity, whereas the number of central entries made is an indicator of anti-anxiety-like behavior.

2.6. Statistical analyses

Analyses of variance (ANOVAs) were used to evaluate the effects of the two between-subjects variables (genotype-wildtype or DATKO; sex-male or female), and one-within subject (P_4 or vehicle) variables. Where appropriate, one-way analyses and Fisher's *post hoc* tests were utilized to evaluate groups that were different. The α level for statistical significance was $p < 0.05$.

3. Results

3.1. PPI

There was a main effect of genotype, but neither an effect of sex, nor P_4, on the magnitude of the startle responses. As has previously been demonstrated, DATKO mice had a diminished startle response following 20 ms duration pre-pulse at 68 dB (pp3) ($F(1,112) = 4.55$, $p \leq 0.01$), 71 dB (pp6) ($F(1,112) = 14.07$, $p \leq 0.01$), or 77 dB (pp12) ($F(1,112) = 24.29$, $p \leq 0.01$), followed by a 40 ms duration startle stimulus at 120 dB after a 100 ms delay. See Fig. 1.

3.2. Activity monitor

There were main effects of genotype ($F(1,112) = 72.15$, $p \leq 0.01$) and P_4 ($F(1,112) = 3.64$, $p \leq 0.05$), but not sex, on the total distance moved in the activity monitor. The interaction between genotype

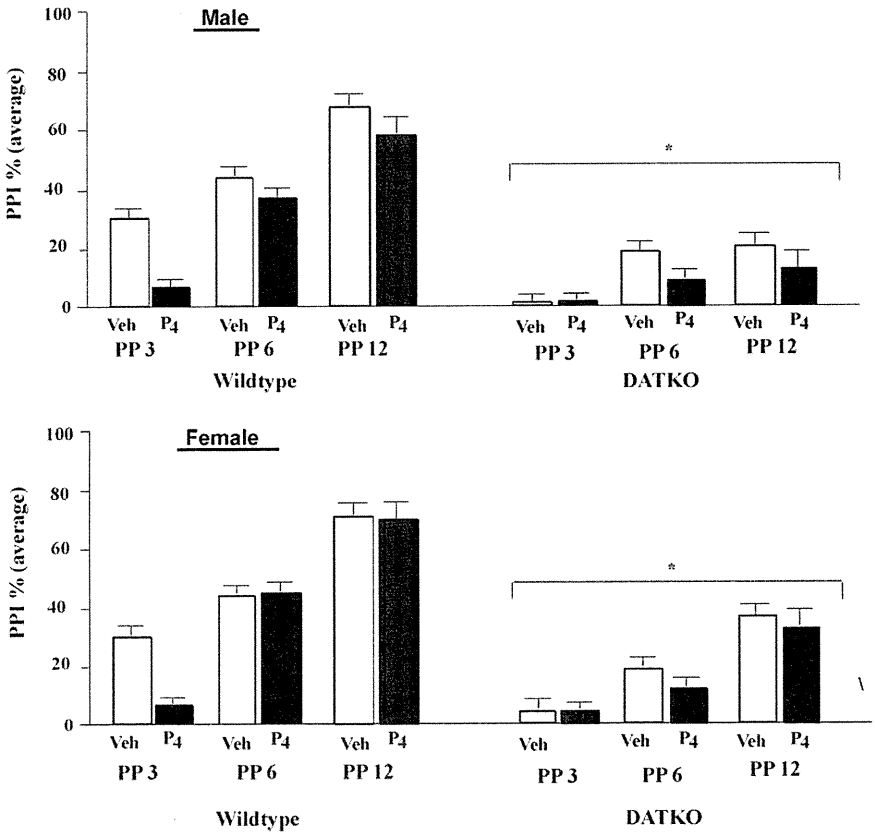

Fig. 1. The mean (+sem) startle magnitude of male (top panel) and female (bottom panel) mice administered vehicle (open bars) or P_4 (closed bars) before testing at pre-pulse at 68 dB (pp3), 71 dB (pp6), or 77 dB (pp12). * Above bar compared to wildtype (left) and DATKO (right) mice ($p < 0.05$; $n = 15$/group).

and hormonal status ($F(1,112) = 9.71$, $p \leq 0.01$) was attributed to P_4 decreasing the distances DATKO mice traveled, but also tending to increase the distances traveled by the wildtype mice. See Fig. 2.

3.3. Open field

There were main effects of genotype ($F(1,112) = 43.56$, $p \leq 0.01$) and P_4 ($F(1,112) = 30.12$, $p \leq 0.01$), but not sex, on the total number of entries in the open field. Total entries were lower in wildtype compared to DATKO mice. The interaction between genotype and hormonal status ($F(1,112) = 22.95$, $p \leq 0.01$) was due to P_4 decreasing the number of entries of DATKO, but not wildtype, mice. See Fig. 3.

Genotype ($F(1,112) = 56.58$, $p \leq 0.01$) and P_4 ($F(1,112) = 36.26$, $p \leq 0.01$), but not sex, influenced the number of central entries in the open field. The interaction between genotype and hormonal status ($F(1,112) = 13.57$, $p \leq 0.01$) was attributed to P_4 having a much greater effect to increase the number of central entries made by wildtype compared to DATKO mice. See Fig. 4.

4. Discussion

Findings from this study partially supported our hypothesis that progesterone would normalize hyperactivity of DATKO mice. Locomotion in the activity monitor and open field was significantly greater among DATKO, compared to wildtype, mice and P_4 dampened the hyperactivity of DATKO mice. Central entries in the open field were greater among wildtype, compared to DATKO, mice and P_4 significantly increased central entries of wildtype mice. DATKO, compared to wildtype, mice showed a dampened PPI response, but P_4 did not alter this effect. There were no sex differences in these effects. Thus, P_4 had circumspect effects to reduce hyperactivity in the activity monitor and open field of female and male DATKO mice.

The present findings are relevant for previous research that has shown that a prohormone for P_4, pregnenolone, is lower among schizophrenics, compared to healthy controls and is associated with trait-anxiety scores independent of acute anxiety symptoms [48,56]. Pregnenolone is synthesized during early developmental [20,28,41,49] and, when administered neonatally, influences DA turnover in the striatum [41]. Neonatal pregnenolone-treated rats are considered an animal model of cortical/subcortical dysfunction. Indeed, DA metabolites in the fronto-parietal cortex were similarly increased in pregnenolone-treated female and male rats and resulted in hyperactivity in the open field [42]. These findings and others, in conjunction with the present results that acute P_4 can normalize hyperactivity in DATKO mice, suggest that progestogens may play a role in the pathophysiology of schizophrenia.

One explanation for the effects of progestogens may be due to their effects on stress responses. Schizophrenia is characterized by dysregulation in stress responses. Although diagnosis of schizophrenia is based upon both positive (hallucinations, delusions) and negative symptoms (avolition, alogia) [44,45,57,58,66], there has been a recent emphasis on negative symptoms, which correlate with loss of social function [32], and plasma levels of the stress hormone, cortisol, albeit not P_4 [41–45,58,64,66]. How dysfunction of the hypothalamic-pituitary adrenal (HPA) axis contributes to the pathophysiology of schizophrenia needs to be better understood. Of interest is whether there are differences in HPA responses of DATKO mice compared to their wildtype counterparts in the present study.

One possible mechanism that may underlie some of the effects of progestogens to normalize behavior of DATKO mice are their effects on steroid biosynthesis. This may be particularly important

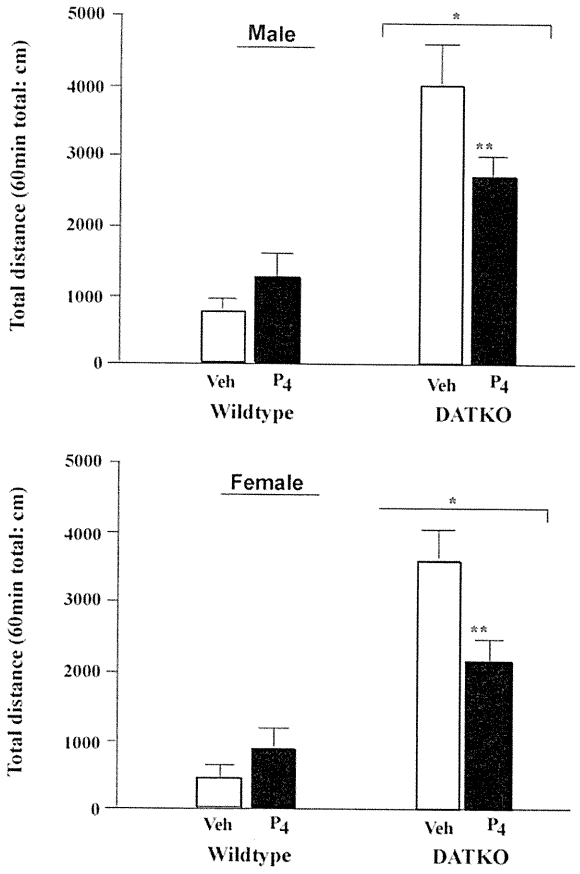

Fig. 2. The mean (+sem) total distance moved in the Super Mex activity chamber of male (top) and female (bottom) mice administered vehicle (open) or P_4 (closed bars). * Above bar compared to wildtype (left) versus DATKO (right) mice ($p < 0.05$). ** Above bar difference compared to vehicle ($p < 0.05$; $n = 15$/grp).

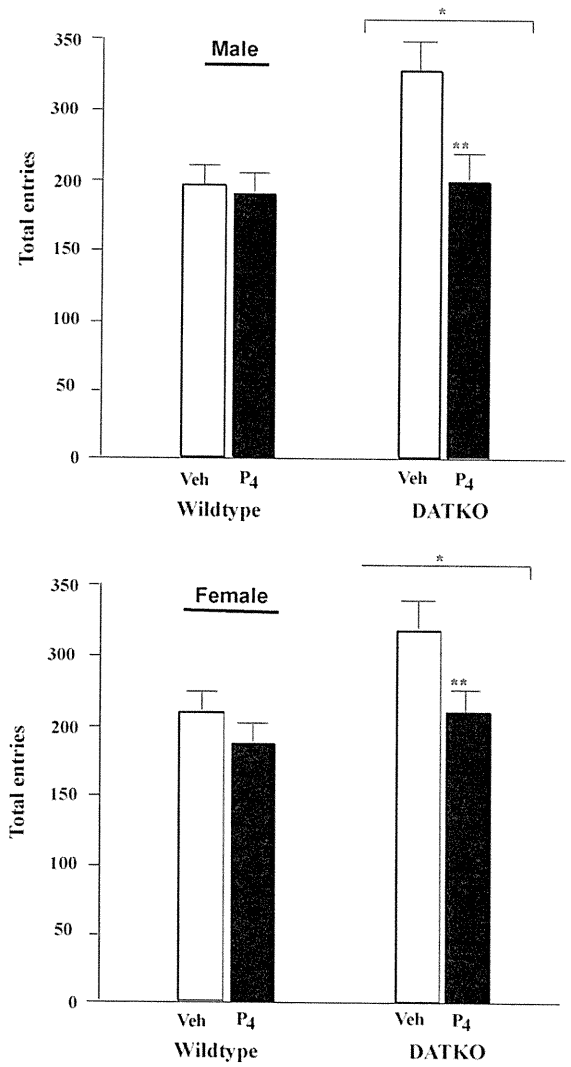

Fig. 3. The mean (+sem) total squares entered in the open field of male (top) and female (bottom) mice administered vehicle (open) or P_4 (filled bars). * Above bar compared to wildtype (left) and DATKO (right) mice ($p < 0.05$). ** Above bar difference compared to vehicle ($p < 0.05$; $n = 15$/grp).

because no sex differences were observed in the present study, suggesting that there may be a greater role of brain-derived versus ovary/gonad-derived steroids. Neurosteroids, steroid hormones produced in the brain, such as 3α-hydroxy-5α-pregnan-20-one (3α,5α-THP), are important endogenous modulators of the HPA that may serve to buffer stress responses. In response to stress, plasma and brain concentrations of 3α,5α-THP are elevated [46,47]. Stress-induced elevations in 3α,5α-THP dampen hyperactivity of the HPA axis [34,52]. 3α,5α-THP has potent agonist-like actions at $GABA_A$ receptors in the brain [52], which underlie its effects to reverse sympathetic activity. 3α,5α-THP from central metabolism of ovarian and/or adrenal P_4 [9] also mitigates stress responses. Although basal levels of 3α,5α-THP are similar for females in the follicular phase and males, during the luteal phase and pregnancy, plasma and hippocampal 3α,5α-THP levels are higher for females than males [25]. Further evidence that 3α,5α-THP mediates HPA responses include that 3α,5α-THP administration to female or male rats attenuates the elevation of plasma adrenocorticotropin (ACTH) and serum corticosterone secretion produced by emotional stress [46]. These data suggest that 3α,5α-THP, produced by glia in response to stress, and/or metabolized in neurons from peripheral sources, may serve as an important mediator of stress responses. It would be of interest to investigate how 3α,5α-THP may be mediating the reduction in hyperactivity that was observed in the present study among DATKO mice administered P_4.

Evidence suggests that 3α,5α-THP may be important in the pathophysiology of schizophrenia. First, neonatal 3α,5α-THP administration to rats disrupts the normal development of the prefrontal cortex and medial dorsal thalamus [18,20], implying that there may be a critical window of vulnerability to neurosteroid insult across development [41,42]. Second, stress-reactivity may underlie the etiology and/or manifestation of schizophrenia. Among people with schizophrenia, dysregulation of the HPA axis is common [33,35,43–45] and stress can precipitate psychiatric episodes related to schizophrenia [45]. Third, stress-induced 3α,5α-THP production can be disrupted in schizophrenia. A novel polymorphism and genetic mutation in the sequence encoding the gene for the mitochondrial benzodiazepine receptor (MBR), which is necessary for 3α,5α-THP biosynthesis in glial cells, has been demonstrated among some schizophrenics, and may create a predisposition to over-sensitivity to stress [34,44]. As well, social isolation (an animal model of schizophrenia) decreases 3α,5α-THP biosynthesis, in the frontal cortex of male Swiss-Webster mice, compared to group-housed controls [6]. Third, there is evidence that 3α,5α-THP metabolized in the brain from peripheral prohormones may reduce the incidence and/or expression of schizophrenia. Women, compared to men, typically have higher levels of 3α,5α-THP, are more likely to have schizophrenia with later onset, better prognosis, and therapeutic response to lower dosages of antipsychotics [23]. When 3α,5α-THP levels are low perimenstrually, first onset, or recurrence of psychotic episodes are

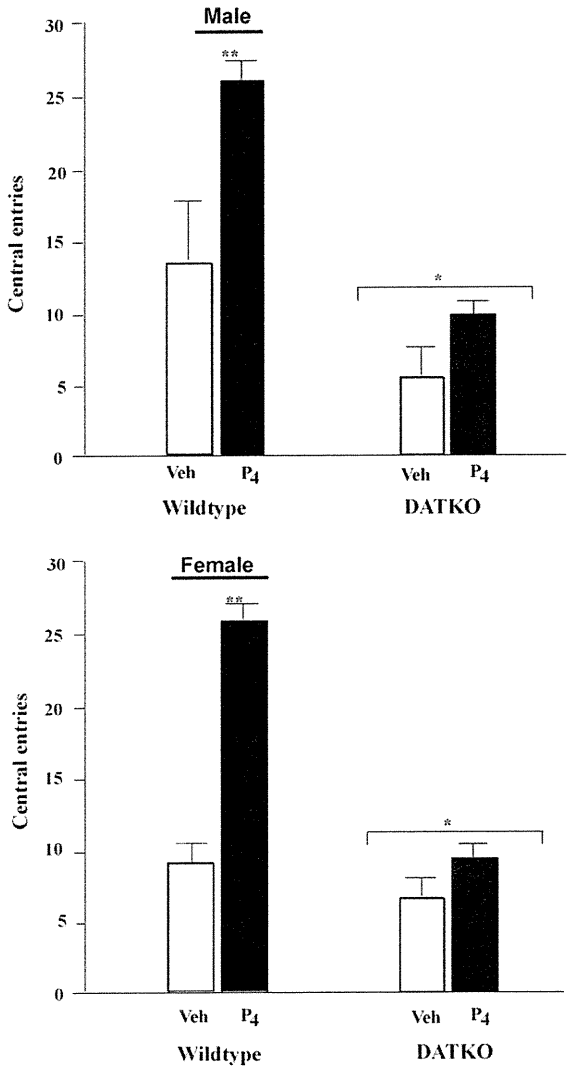

Fig. 4. The mean (+sem) central squares entered in the open field of male (top) and female (bottom) mice administered vehicle (open) or P_4 (filled). * Above bar compared to wildtype (left) and DATKO mice ($p < 0.05$). ** Above bar compared to vehicle ($p < 0.05$; $n = 15$/grp).

more likely and more negative symptoms are reported [21,24,27]. After menopause, when 3α,5α-THP levels are lower, there is a greater recurrence of psychiatric episodes than pre-menopause [8]. Fourth, effective pharmacotherapies for schizophrenia can alter 3α,5α-THP levels. The atypical antipsychotic drug, olanzapine, enhances social functioning and increases 3α,5α-THP levels [13,36]. In an animal model, clozapine can have similar neurosteroidogenic effects; however, it has not been demonstrated to alter circulating neurosteroids levels concomitant with therapeutic effects [41,42]. Together, these data suggest that schizophrenia may involve a reduced capacity to synthesize 3α,5α-THP in the brain, which may increase sensitivity to stress. Although 3α,5α-THP may underlie etiology and/or expression of schizophrenia, the present findings suggest that progestogen-based therapeutics may have a role to normalize the schizophrenic-like behavioral phenotypes [3].

3α,5α-THP may influence the function of the prefrontal cortex (PFC) to mitigate negative symptoms of schizophrenia [30]. Schizophrenia involves PFC hypofunction, poor social function, and disrupted working memory [32]. The PFC is integral for decisions related to social interactions [12] and working memory, a key component of human reasoning and judgment [32]. Notably, the PFC is sensitive to progestogens. Systemic administration of precursors of 3α,5α-THP enhance working memory [5,12] and 3α,5α-THP enhances dopamine release in the PFC in response to stress [5]. A question is why P_4 did not improve PPI among DATKO mice. It is not that DATKO mice are unresponsive to pharmacotherapies as psychostimulants; NET and SERT inhibitors improve PPI of DATKO mice, and impair PPI of wildtype mice [67]. Given the effects of progestogens for dopamine release in the PFC, it may be that P_4 administration elevated DA in the PFC without the DAT, contributing to a lack of effect of P_4 on PPI among DATKO mice. This may also underlie the apparent (but not significant) effect of P_4 to reduce PPI particularly in the pp3 trial. Further investigation of the potential of hyperdopaminergic action underlying these effects is necessary. Indeed, whether these effects are due to direct actions of progestogens on the PFC or indirect actions of progestogens on the hippocampus and/or VTA, which projects to the PFC, has not been established.

Schizophrenia is characterized by deficits in social functioning and progestogens mediate social behavior in part through actions in the VTA and its projection areas. For example, administration of 3α,5α-THP to the VTA increases time spent in interaction with a conspecific and blocking 3α,5α-THP's formation in the VTA attenuates social behavior. We have also shown that the neurosteroidogenic effects of mating can increase 3α,5α-THP and DA in the midbrain, hippocampus, striatum, and prefrontal cortex. Thus, 3α,5α-THP-enhanced social interactions may involve the VTA and its projections to the mesolimbic dopamine system. A question for future studies is the role of 3α,5α-THP in DATKO and wildtype mice for their social responding.

This is a particularly important area of research. There are differences in plasma levels of pregnenolone, a precursor for 3α,5α-THP, between those with schizophrenia and healthy controls [56]. In a recent study of 21 patients with schizophrenia or schizoaffective disorder, those with the lowest natural levels of pregnenolone, reported the best memory and concentration. In this study, patients took placebo for two weeks and were then randomly assigned to take pregnenolone, as a health supplement, or placebo for eight weeks in conjunction with an antipsychotic. Those taking pregnenolone had about a ~20% reduction in their negative symptoms than did the placebo group [37]. Other studies have also shown that plasma levels of neurosteroids correlate with the severity of negative symptoms among some men with schizophrenia [60]. Thus, it is important to understand further the role of progestogens, such as pregnenolone, P_4, or 3α,5α-THP, given the emerging evidence of their role in the etiology, expression, or treatment of schizophrenia and/or schizoaffective disorders.

Affective, and cognitive, processes are also disrupted in schizophrenia and progestins can influence these behaviors through actions in the hippocampus. In the present study, there was a clear anti-anxiety effect of P_4 among wildtype mice, as demonstrated by an increase in central entries in the open field, independent of an increase in total entries. This same effect was not observed in the DATKO mice, suggesting that there may be some involvement of the DAT for progestogens to increase anti-anxiety responding. However, it may also be that no effects of P_4 were found for central entries of DATKO mice because of the robust effect of P_4 to reduce their motor behavior. A question for follow-up studies would be the effects of P_4 to DATKO and wildtype mice in other typical measures of anxiety behavior of mice (e.g. elevated plus maze, light–dark transition, etc.). As well, whether this effect was due to 3α,5α-THP is of interest. 3α,5α-THP is increased in the hippocampus concomitant with reduced anxiety behavior and enhanced cognitive performance [54,55,65]. Blocking the formation of 3α,5α-THP in the hippocampus increases anxiety behaviors and impairs cognitive performance [5,11,53]. Given that the hippocampus projects to the PFC, an important question is whether 3α,5α-THP has direct actions in the PFC to mitigate stress and/or

behavioral responses. Another possibility is that these effects occur indirectly through connections of the PFC with the VTA and/or striatum. These questions are the topics of ongoing investigation in our laboratory.

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References

[3] Castner SA, Goldman-Rakic PS, Williams GV. Animal models of working memory: insights for targeting cognitive dysfunction in schizophrenia. Psychopharmacology (Berl) 2004;174:111–25.
[4] Cooper DC. The significance of action potential bursting in the brain reward circuit. Neurochem Int 2002;41:333–40.
[5] Dazzi L, Serra M, Seu E, Cherchi G, Pisu MG, Purdy RH, et al. Progesterone enhances ethanol-induced modulation of mesocortical dopamine neurons: antagonism by finasteride. J Neurochem 2002;83:1103–9.
[6] Dong E, Matsumoto K, Uzunova V, Sugaya I, Takahata H, Nomura H, et al. Brain 5α-dihydroprogesterone and allopregnanolone synthesis in a mouse model of protracted social isolation. Proc Natl Acad Sci 2001;98:2849–54.
[7] Dulawa SC, Hen R, Scearce-Levie K, Geyer MA. Serotonin1B receptor modulation of startle reactivity, habituation, and prepulse inhibition in wild-type and serotonin1B knockout mice. Psychopharmacology 1997;132:125–34.
[8] Frye CA. Progestins influence motivation, reward, conditioning, stress, and/or response to drugs of abuse. Pharmacol Biochem Behav 2007;86:209–19.
[9] Frye CA, Bayon LE. Mating stimuli influence endogenous variations in the neurosteroids 3α,5α-THP and 3α-Diol. J Neuroendocrinol 1999;11:839–47.
[10] Frye CA, Duffy CK, Walf AA. Estrogens and progestins enhance spatial learning of intact and ovariectomized rats in the object placement task. Neurobiol Learn Mem 2007;88:208–16.
[11] Frye CA, Edinger KL. Testosterone's metabolism in the hippocampus may mediate its anti-anxiety effects in male rats. Pharmacol Biochem Behav 2004;78:473–81.
[12] Frye CA, Lacey EH. Progestins influence performance on cognitive tasks independent of changes in affective behavior. Psychobiology 2000;28:550–63.
[13] Frye CA, Seliga AM. Olanzapine's effects to reduce fear and anxiety and enhance social interactions coincide with increased progestin concentrations of ovariectomized rats. Psychoneuroendocrinology 2003;28:657–73.
[14] Frye CA, Sumida K, Dudek BC, Harney JP, Lydon JP, O'Malley BW, et al. Progesterone's effects to reduce anxiety behavior of aged mice do not require actions via intracellular progestin receptors. Psychopharmacology 2006;186:312–22.
[15] Frye CA, Sumida K, Lydon JP, O'Malley BW, Pfaff DW. Mid-aged and aged wild-type and progestin receptor knockout (PRKO) mice demonstrate rapid progesterone and 3α,5α-THP-facilitated lordosis. Psychopharmacology 2006;185:423–32.
[16] Frye CA, Walf AA, Rhodes ME, Harney JP. Progesterone enhances motor, anxiolytic, analgesic, and antidepressive behavior of wild-type mice, but not those deficient in type 1 5α-reductase. Brain Res 2004;1004:116–24.
[17] Geyer MA, Dulawa SC. Assessment of murine startle reactivity, prepulse inhibition, and habituation. In: Crawley J, Gerfen C, editors. Current protocols in neuroscience. New Jersey: John Wiley & Sons Inc.; 2003. p. 8171–215.
[18] Gizerian SS, Morrow AL, Lieberman JA, Grobin AC. Neonatal neurosteroid administration alters parvalbumin expression and neuron number in medial dorsal thalamus of adult rats. Brain Res 2004;1012:66–74.
[20] Grobin AC, Gizerian S, Lieberman JA, Morrow AL. Perinatal allopregnanolone influences prefrontal cortex structure, connectivity and behavior in adult rats. Neuroscience 2006;138:809–19.
[21] Häfner H, Riecher-Rössler A, Maurer K, Fätkenheuer B, Löffler W. First onset and early symptomatology of schizophrenia. A chapter of epidemiological and neurobiological research into age and sex differences. Eur Arch Psychiatry Clin Neurosci 1992;242:109–18.
[23] Hallonquist JD, Seeman MV, Lang M, Rector NA. Variation in symptom severity over the menstrual cycle of schizophrenics. Biol Psychiatry 1993;33:207–9.
[24] Hendrick V, Altshuler LL, Burt VK. Course of psychiatric disorders across the menstrual cycle. Harv Rev Psychiatry 1996;4:200–7.
[25] Holzbauer M. Physiological variations in the ovarian production of 5α-pregnane derivatives with sedative properties in the rat. J Steroid Biochem 1975;6:1307–10.
[26] Hornykiewicz O. Neurohumoral interactions and basal ganglia function and dysfunction. Res Publ Assoc Res Nerv Ment Dis 1976;55:269–80.
[27] Huber TJ, Rollnik J, Wilhelms J, von zur Mühlen A, Emrich HM, Schneider U. Estradiol levels in psychotic disorders. Psychoneuroendocrinology 2001;26:27–35.
[28] Jung-Testas I, Renoir M, Bugnard H, Greene GL, Baulieu EE. Demonstration of steroid hormone receptors and steroid action in primary cultures of rat glial cells. J Steroid Biochem Mol Biol 1992;41:621–31.
[30] Laruelle M, Kegeles LS, Abi-Dargham A. Glutamate, dopamine, and schizophrenia: from pathophysiology to treatment. Ann N Y Acad Sci 2003;1003:138–58.
[31] Leung A, Chue P. Sex differences in schizophrenia, a review of the literature. Acta Psychiatr Scand Suppl 2000;401:3–38.
[32] Liddle PF. Cognitive impairment in schizophrenia: its impact on social functioning. Acta Psychiatr Scand Suppl 2000;400:11–6.
[33] Lukoff D, Snyder K, Ventura J, Nuechterlein KH. Life events, familial stress, and coping in the developmental course of schizophrenia. Schizophr Bull 1984;10:258–92.
[34] Majewska MD, Harrison NL, Schwartz RD, Barker JL, Paul SM. Steroid hormone metabolites are barbiturate-like modulators of the GABA receptor. Science 1986;232:1004–7.
[35] Malla AK, Cortese L, Shaw TS, Ginsberg B. Life events and relapse in schizophrenia. A one year prospective study. Soc Psychiatr Epidemiol 1990;25:221–4.
[36] Marx CE, Duncan GE, Gilmore JH, Lieberman JA, Morrow AL. Olanzapine increases allopregnanolone in the rat cerebral cortex. Biol Psychiatry 2000;47:1000–4.
[37] Marx CE, Keefe RS, Buchanan RW, Hamer RM, Kilts JD, Bradford DW, et al. Proof-of-concept trial with the neurosteroid pregnenolone targeting cognitive and negative symptoms in schizophrenia. Neuropsychopharmacology 2009;34:1885–903.
[40] Morice E, Billard JM, Denis C, Mathieu F, Betancur C, Epelbaum J, et al. Parallel loss of hippocampal LTD and cognitive flexibility in a genetic model of hyperdopaminergia. Neuropsychopharmacology 2007;32:2108–16.
[41] Muneoka KT, Shirayama Y, Minabe Y, Takigawa M. Effects of a neurosteroid, pregnenolone, during the neonatal period on adenosine A1 receptor, dopamine metabolites in the fronto-parietal cortex and behavioral response in the open field. Brain Res 2002;956:332–8.
[42] Muneoka KT, Takigawa MA. Neuroactive steroid, pregnenolone, alters the striatal dopaminergic tone before and after puberty. Neuroendocrinology 2002;75:288–95.
[43] Myin-Germeys I, van Os J, Schwartz JE, Stone AA, Delespaul PA. Emotional reactivity to daily life stress in psychosis. Arch Gen Psychiatry 2001;58:1137–44.
[44] Newcomer JW, Faustman WO, Whiteford HA, Moses Jr JA, Csernansky JG. Symptomatology and cognitive impairment associate independently with post-dexamethasone cortisol concentrations in unmedicated schizophrenic patients. Biol Psychiatry 1991;29:855–64.
[45] Norman RM, Malla AK. Stressful life events and schizophrenia. I: a review of the research. Br J Psychiatry 1993;162:161–6.
[46] Patchev VK, Hassan AH, Holsboer DF, Almeida OF. The neurosteroid tetrahydroprogesterone attenuates the endocrine response to stress and exerts glucocorticoid-like effects on vasopressin gene transcription in the rat hypothalamus. Neuropsychopharmacology 1996;15:533–40.
[47] Paul SM, Purdy RH. Neuroactive steroids. FASEB J 1992;6:2311–22.
[48] Pisu MG, Serra M. Neurosteroids and neuroactive drugs in mental disorders. Life Sci 2004;74:3181–97.
[49] Pomata PE, Colman-Lerner AA, Barañao JL, Fiszman ML. *In vivo* evidences of early neurosteroid synthesis in the developing rat central nervous system and placenta. Dev Brain Res 2000;120:83–6.
[50] Powell SB, Young JW, Ong JC, Caron MG, Geyer MA. Atypical antipsychotics clozapine and quetiapine attenuate prepulse inhibition deficits in dopamine transporter knockout mice. Behav Pharmacol 2008;19:562–5.
[51] Prata DP, Mechelli A, Picchioni MM, Fu CH, Toulopoulou T, Bramon E, et al. Altered effect of dopamine transporter 3′UTR VNTR genotype on prefrontal and striatal function in schizophrenia. Arch Gen Psychiatry 2009;66:1162–72.
[52] Purdy RH, Morrow AL, Moore Jr PH, Paul SM. Stress-induced elevations of gamma-aminobutyric acid type A receptor-active steroids in the rat brain. Proc Natl Acad Sci 1991;88:4553–7.
[53] Read J, Perry BD, Moskowitz A, Connolly J. The contribution of early traumatic events to schizophrenia in some patients: a traumagenic neurodevelopmental model. Psychiatry 2001;64:319–45.
[54] Rhodes ME, Frye CA. Estrogen has mnemonic-enhancing effects in the inhibitory avoidance task. Pharmacol Biochem Behav 2004;78:551–8.
[55] Rhodes ME, Frye CA. Inhibiting progesterone metabolism in the hippocampus of rats in behavioral estrus decreases anxiolytic behaviors and enhances exploratory and antinociceptive behaviors. Cogn Affect Behav Neurosci 2001;1:287–96.
[56] Ritsner M, Maayan R, Gibel A, Weizman A. Differences in blood pregnenolone and dehydroepiandrosterone levels between schizophrenia patients and healthy subjects. Eur Neuropsychopharmacol 2007;17:358–65.
[57] Schultz SK, Andreasen NC. Schizophrenia. Lancet 1999;353:1425–30.
[58] Seeman MV. Psychopathology in women and men: focus on female hormones. Am J Psychiatry 1997;154:1641–7.
[59] Shen HW, Hagino Y, Kobayashi H, Shinohara-Tanaka K, Ikeda K, Yamamoto H. Regional differences in extracellular dopamine and serotonin assessed by in vivo microdialysis in mice lacking dopamine and/or serotonin transporters. Neuropsychopharmacology 2004;29:1790–9.

[60] Shirayama Y, Hashimoto K, Suzuki Y, Higuchi T. Correlation of plasma neurosteroid levels to the severity of negative symptoms in male patients with schizophrenia. Schizophr Res 2002;58:69–74.
[61] Sora I, Kobayashi H, Numachi Y. Genetically modified animals as models for psychiatric diseases. Seishin Shinkeigaku Zasshi 2005;107:285–9.
[62] Sora I, Wichems C, Takahashi N, Li XF, Zeng Z, Revay R, et al. Cocaine reward models: conditioned place preference can be established in dopamine- and in serotonin-transporter knockout mice. Proc Natl Acad Sci 1998;95: 7699–704.
[63] Sumida K, Walf AA, Frye CA. Progestin-facilitated lordosis of hamsters may involve dopamine-like type 1 receptors in the ventral tegmental area. Behav Brain Res 2005;161:1–7.
[64] Tandon R, Ribeiro SC, DeQuardo JR, Goldman RS, Goodson J, Greden JF. Covariance of positive and negative symptoms during neuroleptic treatment in schizophrenia: a replication. Biol Psychiatry 1993;34:495–7.
[65] Walf AA, Rhodes ME, Frye CA. Ovarian steroids enhance object recognition in naturally cycling and ovariectomized, hormone-primed rats. Neurobiol Learn Mem 2006;86:35–46.
[66] Walker EF, Diforio D. Schizophrenia: a neural diathesis-stress model. Psychol Rev 1997;104:667–85.
[67] Yamashita M, Fukushima S, Shen HW, Hall FS, Uhl GR, Numachi Y, et al. Norepinephrine transporter blockade can normalize the prepulse inhibition deficits found in dopamine transporter knockout mice. Neuropsychopharmacology 2006;31:2132–9.

Association Analysis of the Adenosine A1 Receptor Gene Polymorphisms in Patients with Methamphetamine Dependence/Psychosis

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Abstract: Several lines of evidence suggest that the dopaminergic nervous system contributes to methamphetamine (METH) dependence, and there is increasing evidence of antagonistic interactions between dopamine and adenosine receptors in METH abusers. We therefore hypothesized that variations in the A1 adenosine receptor (*ADORA1*) gene modify genetic susceptibility to METH dependence/psychosis. In this study, we identified 7 single nucleotide polymorphisms (SNPs) in exons and exon-intron boundaries of the *ADORA1* gene in a Japanese population. A total of 171 patients and 229 controls were used for an association analysis between these SNPs and METH dependence/psychosis. No significant differences were observed in either the genotypic or allelic frequencies between METH dependent/psychotic patients and controls. A global test of differentiation among samples based on haplotype frequencies showed no significant association. In the clinical feature analyses, no significant associations were observed among latency of psychosis, prognosis of psychosis, and spontaneous relapse. These results suggest that the *ADORA1* gene variants may make little or no contribution to vulnerability to METH dependence/psychosis.

Keywords: Single nucleotide polymorphism, SNP, variation, human, Japanese, MAP, abuse, dopamine.

INTRODUCTION

Methamphetamine (METH) is a psychomotor stimulant with high liability for abuse, and METH abuse has become a very serious social problem in Japan [1]. Chronic METH abusers have been shown to have persistent dopaminergic deficits [2, 3]. Amphetamines are thought to produce their stimulant effects mainly *via* the dopaminergic system [4, 5], although other systems may also be involved. Dopamine D1 and D2 receptors form heterodimeric complexes with adenosine A1 and A2a receptors respectly, which modulate their responsiveness [6-9], suggesting that responses to amphetamines may also depend on adenosinergic function.

Several lines of evidence suggest that adenosine A1 receptors play a role in inhibiting the effects of METH. Adenosine receptor antagonists potentiate the effects of lower METH doses and substitute for the discriminative stimulus effects of METH [10, 11]. Adenosine receptor agonists protect against METH-induced neurotoxicity, and amphetamine-induced stereotypy and locomotor activity, and reduce the acquisition of conditioned place preference induced by amphetamine [12-15]. These results suggest that adenosine A1 receptors play important roles in the expression of METH-induced neurotoxicities and behaviors.

To date, however, there has been no association analysis between A1 adenosine receptor (*ADORA1*) gene variants and drug addiction. The purpose of this study was (1) to identify novel sequence variants in all coding exons as well as exon-intron boundaries of the *ADORA1* gene in Japanese, and (2) to investigate whether these polymorphisms and/or haplotypes were associated with METH dependence/psychosis.

MATERIALS AND METHODS

Subjects

One-hundred seventy-one unrelated patients with METH dependence/psychosis (138 males and 33 females; mean age 37.5±12.0 years) meeting ICD-10-DCR criteria (F15.2 and F15.5) were used as case subjects; they were outpatients or inpatients of psychiatric hospitals. The 229 control subjects (119 males and 110 females; mean age 41.2±12.3 years) were mostly medical staff members who had neither per-

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sonal nor familial history of drug dependence or psychotic disorders, as verified by a clinical interview. All subjects were Japanese, born and living in the northern Kyushu, Setouchi, Chukyo, Tokai, and Kanto regions. This study was approved by the ethical committees of each institute of the Japanese Genetics Initiative for Drug Abuse (JGIDA), and all subjects provided written informed consent for the use of their DNA samples for this research [16]. After informed consent was obtained, blood samples were drawn and genomic DNA was extracted by the phenol/chloroform method.

Defining Variants of the *ADORA1* Gene

Initially, DNA samples from 16 METH dependent/psychotic patients were used to identify nucleotide variants within the *ADORA1* gene (GenBank accession no. AC105940). Exon numbers were based on the report by Ren and colleagues [17]. Exons 1A, 1B, 2, 3 and exon-intron boundaries were amplified by polymerase chain reaction (PCR) using a thermal cycler (Astec, Fukuoka, Japan), and the products were sequenced in both directions using BigDye terminators (Applied Biosystems, Foster City, CA) by an ABI Genetic analyzer 3100 (Applied Biosystems). The primer sequences used in this study are shown in Table **1**.

Genotyping of IVS1A+182 (rs56298433) was performed by PCR amplification using 2F-2R primers followed by restriction enzyme *Nla* III digestion. Genotyping of Exon2+363 (rs10920568) was performed by PCR amplification using 4F-4R primers followed by sequencing with the same primers. IVS2+35826 (rs5780149) was performed by PCR amplification using 5F-9R primers followed by sequencing with 5F and 5R primers. Genotyping of Exon3+937 (rs6427994), Exon3+987 (rs41264025), and Exon3+1064 (rs16851030) was performed by PCR amplification using 5F-9R primers followed by sequencing with 7F and 7R primers.

Patient Subgroups

For the clinical category analysis, the patients were divided into two subgroups by three different clinical features. (A) Latency of psychosis from first METH intake: less than 3 years or more than 3 years. The course of METH psychosis varied among patients, with some patients showing psychosis sooner after the first METH intake, as previously reported [16, 18]. Because the median latency was 3 years, this time point was used as the cutoff in defining the two groups. (B) Duration of psychosis after the last METH intake: transient (<1 month) or prolonged (≧1 month). Some patients showed continuous psychotic symptoms even after METH discontinuation, as previously reported [16, 18]. Patients with the transient type showed a reduction of psychotic symptoms within one month after the discontinuation of METH consumption and the beginning of treatment with neuroleptics. Patients with the prolonged type showed a psychotic symptoms continued for more than one month even after the discontinuation of METH consumption and the beginning of neuroleptic treatment. (C) Spontaneous relapse: present or not. It has been well documented that once METH psychosis has developed, patients in the remission phase are liable to spontaneous relapse without reconsumption of METH [16, 18].

Statistical Analysis

The Hardy-Weinberg equilibrium of genotypic frequencies in each SNP was tested by the chi-square test. The level of statistical significance was set at $\alpha = 0.05$. The allelic and genotypic frequencies of the patient and control groups were compared using the chi-square test. Haplotype frequencies were calculated by the Arlequin program available from http://anthropologie.unige.ch/arlequin [19]. Locus by locus linkage disequilibrium (LD) was evaluated by D' and r^2, which were calculated by the haplotype frequencies using the appropriate formula in the Excel program. A global test of differentiation among samples based on haplotype frequencies was also performed by the Arlequin program.

RESULTS

Analysis of the *ADORA1* Gene Variants

To identify polymorphisms in the *ADORA1* gene, exons 1A, 1B, 2, and 3, and exon-intron boundaries were analyzed using genomic DNA from Japanese METH dependent/psychotic subjects. Seven SNPs were identified (Table **2**). Five out of seven of these SNPs were previously reported by Deckert [20]. In the two SNPs, the frequencies of the minor

Table 1. Primers Used in this Study

Exon	Forward		Reverse	
Exon1A	1F:	TGG ACT GGA TGC CTT ATG GCT TAG	1R:	GGC GCA GGA GCT GAG TGA CAA TCG
	2F:	TCT CAC CCA GTA TCA CTT CCT TTG	2R:	ATC ACA TGG TAC GGC AGA GAC TCA
Exon1B	3F:	AAT AGG GAG AAA CGC CCC AGC CTT	3R:	AAG CAC CTG TGT GGT CAG GGA AGC
Exon2	4F:	GGT AGG AGC TGC ATG TGA CAA GTG	4R:	GCA GAG TGA GGA CTG GAG CAC GAT
Exon3	5F:	GGC TGT CAT GAA GCA ATG ATG AGA	5R:	CCA GCG ACT TGG CGA TCT TCA GCT
	6F:	TCT ACC TGG AGG TCT TCT ACC TAA	6R:	CCC TGA AGC TCT GGA CTG CTC ATG
	7F:	GTG GTC CCT CCA CTA GGA GTT AAC	7R:	ACA GGT AAT TAC ACT CCA AGG CTC
	8F:	CTG ATA TTT GCT GGA GTG CTG GCT	8R:	ACA CCT GCA ACA GAG CTT CCA AAG
	9F:	CCT TGC TGT CAT GTG AAT CCC TCA	9R:	CAA GAG GAA GAT GCC AAT GGG AGA

alleles differed between our patients and those of Deckert. In the Exon2+363 (rs10920568) SNP, the G allele was present in 15.5% of our Japanese controls (Table **3**) and 36.9% of the German controls [20]. In the Exon3+1064 (rs16851030) SNP, the T allele was present in 35.8% of our Japanese controls and 1.2% of the German controls [20]. These differences were suggested to be related to the difference in ethnicity between the two cohorts. One SNP, Exon2+363 (rs10920568), was a synonymous mutation (Ala to Ala) (Table **2**). All the other SNPs were located either in the introns or an untranslated region in the exon 3. Two SNPs (Exon3+937 (rs6427994) and Exon3+1454 (rs11315020)) were in linkage disequilibrium (LD) in the sense that the genotypic patterns of the 16 samples examined were the same, representing Exon3+937 (rs6427994) for these two SNPs. IVS1A+182 (rs56298433), Exon2+363 (rs10920568), IVS2+35826 (rs5780149), Exon3+937 (rs6427994), Exon3+987 (rs41264025), and Exon3+1064 (rs16851030) were chosen for further analysis.

Table 2. ***ADORA1*** **Gene Variants Found in the Japanese Population**

Location	Variants	rs#	SNP Name	Function
IVS1A+182	G/T	rs56298433		intron
Exon2+363	T/G	rs10920568	805T/G	synonymous (Ala->Ala)
IVS2+35826	T4/T5	rs5780149		intron
Exon3+937	A/C	rs6427994	1777C/A	untranslated
Exon3+987	C/T	rs41264025	1827C/T	untranslated
Exon3+1064	C/T	rs16851030	1904C/T	untranslated
Exon3+1454	T/del	rs11315020	2294insT	untranslated

The nucleotide sequence of the *ADORA1* gene was refereneed to the NCBI nucleotide database under accession number AC105940. Exon numbers were based on the report by Ren and colleagues [17]. The column labelled rs# shows SNP numbers from the NCBI SNP database. The data in the column labelled SNP name are from the report by Deckert [20].

Table 3. Genotypic and Allelic Distribution of the *ADORA1* Gene SNPs in the METH Subjects and the Controls

SNP	Group	N	Genotype (%)			P	Allele (%)		P
IVS1A+182 (rs56298433)			G	G/T	T		G	T	
	Control	224	222 (99.1%)	2 (0.9%)	0 (0.0%)	0.961	446 (99.6%)	2 (0.4%)	0.823
	METH	168	166 (98.8%)	2 (1.2%)	0 (0.0%)		334 (99.4%)	2 (0.6%)	
Exon2+363 (rs10920568)			T	T/G	G		T	G	
	Control	229	162 (70.7%)	63 (27.5%)	4 (1.7%)	0.333	387 (84.5%)	71 (15.5%)	0.233
	METH	171	132 (77.2%)	36 (21.1%)	3 (1.8%)		300 (87.7%)	42 (12.3%)	
IVS2+35826 (rs5780149)			T4	T4/T5	T5		T4	T5	
	Control	229	150 (65.5%)	69 (30.1%)	10 (4.4%)	0.887	369 (80.6%)	89 (19.4%)	0.708
	METH	171	108 (63.2%)	55 (32.2%)	8 (4.7%)		271 (79.2%)	71 (20.8%)	
Exon3+937 (rs6427994)			A	A/C	C		A	C	
	Control	229	2 (0.9%)	46 (20.1%)	181 (79.0%)	0.248	50 (10.9%)	408 (89.1%)	0.222
	METH	171	5 (2.9%)	38 (22.2%)	128 (74.9%)		48 (14.0%)	294 (86.0%)	
Exon3+987 (rs41264025)			C	C/T	T		C	T	
	Control	229	215 (93.9%)	14 (6.1%)	0 (0.0%)	0.937	444 (96.9%)	14 (3.1%)	0.888
	METH	171	162 (94.7%)	9 (5.3%)	0 (0.0%)		333 (97.4%)	9 (2.6%)	
Exon3+1064 (rs16851030)			C	C/T	T		C	T	
	Control	229	89 (38.9%)	116 (50.7%)	24 (10.5%)	0.071	294 (64.2%)	164 (35.8%)	0.572
	METH	171	80 (46.8%)	67 (39.2%)	24 (14.0%)		227 (66.4%)	115 (33.6%)	

N: number of samples.
P: Significance values between the METH subjects and the controls.

Relationship Between the *ADORA1* Gene SNPs and METH Dependence/Psychosis

Association analyses between these SNPs in the *ADORA1* gene and METH dependence/psychosis were performed using DNA samples from 171 METH dependent/psychotic subjects and 214 control subjects (Table **3**). Among them, the genotypes of five control samples and three METH samples could not be determined at IVS1A+182 (rs56298433). The genotypic frequencies in these SNPs were within the Hardy-Weinberg expectations. No significant differences of the genotypic and allelic distributions of these SNPs in these samples were observed. As the minor allele frequencies of two SNPs, IVS1A+182 (rs56298433) and Exon3+987 (rs41264025), were less than 5%, another four SNPs, Exon 2+363 (rs10920568), IVS2+35826 (rs5780149), Exon3+937 (rs6427994), and Exon3+1064 (rs16851030), were used for further analyses.

A global test of differentiation among samples based on haplotype frequencies was performed using the Arlequin program, but no significant association with METH dependence/psychosis was observed (P=0.590). Haplotype frequencies were estimated by the Arlequin program, and locus by locus LD was calculated by using the appropriate formula in the Excel program. Most of the SNPs in exon 2 and exon 3 were in LD, suggesting that the locus from exon 2 to exon 3 was in a LD block (Table **4**).

Subcategory analyses were conducted on the clinical parameters (latency of psychosis, prognosis of psychosis, and spontaneous relapse) (Table **5**). Significant differences were observed in the shorter latency of psychosis (P=0.025) at Exon3+937 (rs6427994). However, this significance disappeared after Bonferroni correction by the sub-group numbers, two ($P < 0.025$).

DISCUSSION

We analyzed the *ADORA1* gene variations in a Japanese population and found seven SNPs in exons and exon-intron boundaries. However, no significant associations were

Table 4. Linkage Disequilibrium Mapping of the *ADORA1* Gene

	Exon2+363 (rs10920568)	IVS2+35826 (rs5780149)	Exon3+937 (rs6427994)	Exon3+1064 (rs16851030)	
Exon2+363		0.807	0.729	0.374	
IVS2+35826	0.029		1.000	0.676	D'
Exon3+937	0.012	0.030		1.000	
Exon3+1064	0.014	0.061	0.068		
		r^2			

D' and r^2 values for Controls are shown in the upper right and lower left, respectively.

Table 5. Genotypic Distribution of the *ADORA1* Gene SNPs in Subcategorized METH Subjects

		SNP	Exon2+363 (rs10920568)				IVS2+35826 (rs5780149)				Exon3+937 (rs6427994)				Exon3+1064 (rs16851030)			
		Genotype	T	T/G	G		T4	T4/T5	T5		A	A/C	C		C	C/T	T	
Group		N				P				P				P				P
Control		229	162	63	4		150	69	10		2	46	181		89	116	24	
METH	Latency of Psychosis																	
	<3 years	67	48	16	3	0.387	46	17	4	0.684	4	10	53	0.025	30	26	11	0.173
	≧3 years	71	56	15	0	0.275	40	29	2	0.229	0	22	49	0.124	35	28	8	0.237
	Prognosis of Psychosis																	
	Transient (<1 month)	91	70	19	2	0.465	59	29	3	0.883	3	22	66	0.190	42	37	12	0.269
	Prolonged (≧1 month)	56	41	14	1	0.932	33	20	3	0.654	1	11	44	0.835	27	21	8	0.205
	Spontaneous Relapse																	
	Not present	104	81	22	1	0.381	64	34	6	0.733	4	25	75	0.107	52	39	13	0.081
	Present	60	45	13	2	0.519	39	19	2	0.923	1	11	48	0.831	25	24	11	0.163

N: number of samples.
P: Significance values between the METH subjects and the controls.

observed between these SNPs and METH dependence/psychosis in the genotypic, allelic, haplotypic or clinically subcategorized analyses.

This is the first association analysis between *ADORA1* gene variants and drug addiction. We failed to find associations between the *ADORA1* gene SNPs and METH dependence/psychosis. While the significant difference (P=0.025) in the shorter latency of psychosis at Exon3+937 (rs6427994) disappeared after Bonferroni correction, this may have been due to the sample size, and thus further analysis with a larger sample is warranted.

The variants we found were one synonymous SNP, two intron SNPs and four exon SNPs in the untranslated region. These SNPs are unlikely to affect receptor function because they are not non-synonymous SNPs or promoter SNPs. Because several animal studies have suggested a modulatory role of adenosine receptors for dopamine systems, it remains possible that another region in the *ADORA1* gene, such as a promoter region or intron regions, contributes to the alteration of *ADORA1* gene function.

Although a few association analyses of the *ADORA1* gene and psychiatric diseases have been performed, no significant association has been reported between *ADORA1* variants and bipolar affective disorder or panic disorder [20, 21]. As caffeine is a nonselective adenosine receptor antagonist, the association between the psychoactive effects of caffeine and gene variants of adenosine receptors have also been studied. However, the anxiogenic response to an acute dose of caffeine in healthy, infrequent caffeine users was not associated with *ADORA1* gene polymorphism [22]. Interindividual variation in the anxiety response to amphetamine has also been studied in healthy volunteers, but no association was observed with *ADORA1* gene variants [23]. These results suggest that the *ADORA1* gene variations have little effect on psychiatric symptoms and/or personality traits.

In conclusion, our data suggest that the *ADORA1* gene variants may not play a major role in the development of METH dependence/psychosis.

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REFERENCES

[1] Matsumoto, T.; Kamijo, A.; Miyakawa, T.; Endo, K.; Yabana, T.; Kishimoto, H.; Okudaira, K.; Iseki, E.; Sakai, T.; Kosaka, K. Methamphetamine in Japan: the consequences of methamphetamine abuse as a function of route of administration. *Addiction,* **2002**, *97*(7), 809-817.

[2] Volkow, N.D.; Chang, L.; Wang, G.J.; Fowler, J.S.; Leonido-Yee, M.; Franceschi, D.; Sedler, M.J.; Gatley, S.J.; Hitzemann, R.; Ding, Y.S.; Logan, J.; Wong, C.; Miller, E.N. Association of dopamine transporter reduction with psychomotor impairment in methamphetamine abusers. *Am. J. Psychiatry*, **2001**, *158*(3), 377-382.

[3] Wilson, J.M.; Kalasinsky, K.S.; Levey, A.I.; Bergeron, C.; Reiber, G.; Anthony, R.M.; Schmunk, G.A.; Shannak, K.; Haycock, J.W.; Kish, S.J. Striatal dopamine nerve terminal markers in human, chronic methamphetamine users. *Nat. Med.*, **1996**, *2*(6), 699-703.

[4] Di Chiara, G.; Imperato, A. Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. *Proc. Natl. Acad. Sci. USA,* **1988**, *85*(14), 5274-5278.

[5] Giros, B.; Jaber, M.; Jones, S.R.; Wightman, R.M.; Caron, M.G. Hyperlocomotion and indifference to cocaine and amphetamine in mice lacking the dopamine transporter. *Nature,* **1996**, *379*(6566), 606-612.

[6] Ferre, S.; Fredholm, B.B.; Morelli, M.; Popoli, P.; Fuxe, K. Adenosine-dopamine receptor-receptor interactions as an integrative mechanism in the basal ganglia. *Trends Neurosci.,* **1997**, *20*(10), 482-487.

[7] Ferre, S.; Fuxe, K.; von Euler, G.; Johansson, B.; Fredholm, B.B. Adenosine-dopamine interactions in the brain. *Neuroscience*, **1992**, *51*(3), 501-512.

[8] Gines, S.; Hillion, J.; Torvinen, M.; Le Crom, S.; Casado, V.; Canela, E.I.; Rondin, S.; Lew, J.Y.; Watson, S.; Zoli, M.; Agnati, L.F.; Verniera, P.; Lluis, C.; Ferre, S.; Fuxe, K.; Franco, R. Dopamine D1 and adenosine A1 receptors form functionally interacting heteromeric complexes. *Proc. Natl. Acad. Sci. USA*, **2000**, *97*(15), 8606-8611.

[9] O'Neill, C.; Nolan, B.J.; Macari, A.; O'Boyle, K.M.; O'Connor, J.J. Adenosine A1 receptor-mediated inhibition of dopamine release from rat striatal slices is modulated by D1 dopamine receptors. *Eur. J. Neurosci.*, **2007**, *26*(12), 3421-3428.

[10] Munzar, P.; Justinova, Z.; Kutkat, S.W.; Ferre, S.; Goldberg, S.R. Adenosinergic modulation of the discriminative-stimulus effects of methamphetamine in rats. *Psychopharmacology* (Berl), **2002**, *161*(4), 348-355.

[11] Justinova, Z.; Ferre, S.; Segal, P.N.; Antoniou, K.; Solinas, M.; Pappas, L.A.; Highkin, J.L.; Hockemeyer, J.; Munzar, P.; Goldberg, S.R. Involvement of adenosine A1 and A2A receptors in the adenosinergic modulation of the discriminative-stimulus effects of cocaine and methamphetamine in rats. *J. Pharmacol. Exp. Ther.*, **2003**, *307*(3), 977-986.

[12] Delle Donne, K.T.; Sonsalla, P.K. Protection against methamphetamine-induced neurotoxicity to neostriatal dopaminergic neurons by adenosine receptor activation. *J. Pharmacol. Exp. Ther.*, **1994**, *271*(3), 1320-1326.

[13] Poleszak, E.; Malec, D. Influence of adenosine receptor agonists and antagonists on amphetamine-induced stereotypy in rats. *Pol. J. Pharmacol.*, **2000**, *52*(6), 423-429.

[14] Turgeon, S.M.; Pollack, A.E.; Schusheim, L.; Fink, J.S. Effects of selective adenosine A1 and A2a agonists on amphetamine-induced locomotion and c-Fos in striatum and nucleus accumbens. *Brain Res.*, **1996**, *707*(1), 75-80.

[15] Poleszak, E.; Malec, D. Effects of adenosine receptor agonists and antagonists in amphetamine-induced conditioned place preference test in rats. *Pol. J. Pharmacol.*, **2003**, *55*(3), 319-326.

[16] Ujike, H.; Harano, M.; Inada, T.; Yamada, M.; Komiyama, T.; Sekine, Y.; Sora, I.; Iyo, M.; Katsu, T.; Nomura, A.; Nakata, K.; Ozaki, N. Nine- or fewer repeat alleles in VNTR polymorphism of the dopamine transporter gene is a strong risk factor for prolonged methamphetamine psychosis. *Pharmacogenomics. J,* **2003**, *3*(4), 242-247.

[17] Ren, H.; Stiles, G.L. Separate promoters in the human A1 adenosine receptor gene direct the synthesis of distinct messenger RNAs that regulate receptor abundance. *Mol. Pharmacol.*, **1995**, *48*(6), 975-980.

[18] Ujike, H. Stimulant-induced psychosis and schizophrenia: the role of sensitization. *Curr. Psychiatry Rep.*, **2002**, *4*(3), 177-184.

[19] Schneider, S.; Roessli, D.; Excoffier, L. Arlequin: a software for population genetics data analysis. Version 2.000. Genetics and Biometry Lab, Department of Anthropology, University of Geneva, **2000**.

[20] Deckert, J.; Nothen, M.M.; Albus, M.; Franzek, E.; Rietschel, M.; Ren, H.; Stiles, G.L.; Knapp, M.; Weigelt, B.; Maier, W.; Beckmann, H.; Propping, P. Adenosine A1 receptor and bipolar

affective disorder: systematic screening of the gene and association studies. *Am. J. Med. Genet.*, **1998**, *81*(1), 18-23.

[21] Deckert, J.; Nothen, M.M.; Franke, P.; Delmo, C.; Fritze, J.; Knapp, M.; Maier, W.; Beckmann, H.; Propping, P. Systematic mutation screening and association study of the A1 and A2a adenosine receptor genes in panic disorder suggest a contribution of the A2a gene to the development of disease. *Mol. Psychiatry*, **1998**, *3*(1), 81-85.

[22] Alsene, K.; Deckert, J.; Sand, P.; de Wit, H. Association between A2a receptor gene polymorphisms and caffeine-induced anxiety. *Neuropsychopharmacology*, **2003**, *28*(9), 1694-1702.

[23] Hohoff, C.; McDonald, J.M.; Baune, B.T.; Cook, E.H.; Deckert, J.; de Wit, H. Interindividual variation in anxiety response to amphetamine: possible role for adenosine A2A receptor gene variants. *Am. J. Med. Genet. B Neuropsychiatry. Genet.*, **2005**, *139*B(1), 42-44.

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Association Analysis of the Tryptophan Hydroxylase 2 Gene Polymorphisms in Patients with Methamphetamine Dependence/Psychosis

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Abstract: There is a growing evidence that serotoninergic systems modulate dopaminergic neurotransmission. We analyzed the association between the variations in the brain tryptophan hydroxylase 2 (*TPH2*) gene, a rate limiting enzyme for serotonin biosynthesis, and methamphetamine (METH) dependence/psychosis in a Japanese population. We found ten single nucleotide polymorphisms (SNPs) and two polynucleotide polymorphisms in *TPH2* gene exons and exon-intron boundaries. A total of 162 patients and 243 controls were used for the association analysis between these polymorphisms and METH dependence/psychosis. No significant differences were observed in either genotypic or allelic frequencies between METH dependent/psychotic patients and controls. A global test of differentiation among samples based on haplotype frequencies showed no significant association. With respect to latency of psychosis, prognosis of psychosis, and spontaneous relapse, we found no significant association with these SNPs. These results suggest that the *TPH2* gene variants may not be a factor in vulnerability to METH dependence/psychosis.

Keywords: Single nucleotide polymorphism, SNP, variation, serotonin, human, Japanese, MAP, abuse.

INTRODUCTION

Methamphetamine (METH) is a psychomotor stimulant with high liability for abuse, and METH abuse has become a very serious social problem in Japan [1]. Chronic METH abusers have been shown to have persistent dopaminergic deficits [2, 3]. In animals, amphetamine elevates extracellular dopamine levels in the mesolimbic circuits [4, 5]. There is growing evidence that serotonergic systems modulate dopaminergic neurotransmission. For example, the mesocorticolimbic dopamine system is under inhibitory control by the serotonin system, which exerts its actions *via* serotonin receptor subtypes [6, 7].

Acute and chronic administration of METH markedly decreases the activity of tryptophan hydroxylase (TPH) [8, 9], the rate-limiting enzyme in the biosynthesis of serotonin [10]. TPH2 (or neuronal TPH) was identified as a second isoform of TPH in 2003 [11, 12]. In contrast to TPH1, which is expressed predominantly in the pineal gland and the periphery, TPH2 mRNA is expressed in the raphe nuclei [11]. Since the identification of TPH2, there have been numerous association analyses between *TPH2* gene variants and psychiatric diseases. For example, associations have been observed between *TPH2* variants and bipolar disorder [13-18], suicidal behavior in major depression [19-21], the response to selective serotonin reuptake inhibitors (fluoxetine and/or citalopram) [22, 23] and emotional regulation in healthy subjects [24-28]. These reports indicate that polymorphic variants in the *TPH2* gene may have a role in the pathophysiology of a wide range of psychiatric disorders and emotional regulation. A recent study of heroin addiction also showed an association with *TPH2* variants in Hispanics and African-Americans [29].

The purpose of this study was (1) to identify novel sequence variations in all coding exons as well as exon-intron boundaries of the *TPH2* gene in Japanese, and (2) to investigate whether these polymorphisms and/or haplotypes were associated with METH dependence/psychosis.

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MATERIALS AND METHODS

Subjects

One-hundred sixty-two unrelated patients with METH dependence/psychosis (130 males and 32 females; mean age 37.4±12.0 years) meeting ICD-10-DCR criteria (F15.2 and F15.5) were used as case subjects; they were outpatients or inpatients of psychiatric hospitals. The 243 control subjects (168 males and 75 females; mean age 35.4±11.5 years) were mostly medical staff members who had neither personal nor familial history of drug dependence or psychotic disorders, as verified by a clinical interview. All subjects were Japanese, born and living in the northern Kyushu, Setouchi, Chukyo, Tokai, and Kanto regions. This study was approved by the ethical committees of each institute of the Japanese Genetics Initiative for Drug Abuse (JGIDA), and all subjects provided written informed consent for the use of their DNA samples for this research [30]. After informed consent was obtained, blood samples were drawn and genomic DNA was extracted by the phenol/chloroform method.

Defining Variants of the *TPH2* Gene

Initially, 16 METH dependent/psychotic patient samples were used to identify nucleotide variants within the *TPH2* gene (GenBank accession no. AC090109). Exons 1 to 11 and exon-intron boundaries were amplified by polymerase chain reaction (PCR) using a thermal cycler (Astec, Fukuoka, Japan), and the products were sequenced in both directions using BigDye terminators (Applied Biosystems, Foster City, CA) by an ABI Genetic analyzer 3100 (Applied Biosystems).

Genotyping of each polymorphism except in exon 11 was performed by PCR amplification using the relevant primers listed in Table **1** followed by sequencing using the same primers in both directions. Genotyping of polymorphisms in exon 11 was performed by PCR amplification using 9F and 11R primers followed by sequencing using 10F, 11F, and 11R primers.

Patient Subgroups

For the clinical category analysis, the patients were divided into two subgroups by three different clinical features. (A) Latency of psychosis from first METH intake: less than 3 years or more than 3 years. The course of METH psychosis varied among patients, with some patients showing psychosis sooner after the first METH intake, as previously reported [30, 31]. Because the median latency was three years, this time point was used as the cutoff in defining the two groups. (B) Duration of psychosis after the last METH intake: transient (<1 month) or prolonged (≧1 month). Some patients showed continuous psychotic symptoms even after METH discontinuation, as previously reported [30, 31]. Patients with the transient type showed a reduction of psychotic symptoms within one month after the discontinuation of METH consumption and the beginning of treatment with neuroleptics. Patients with the prolonged type showed a psychotic symptoms continued for more than one month even after the discontinuation of METH consumption and the beginning of neuroleptic treatment. (C) Spontaneous relapse: present or not. It has been well documented that once METH psychosis has developed, patients in the remission phase are liable to spontaneous relapse without reconsumption [30, 31].

Statistical Analysis

The Hardy-Weinberg equilibrium of genotypic frequencies in each SNP was tested by the chi-square test. The level of statistical significance was set at $\alpha = 0.05$. The allelic and genotypic frequencies of patients and control groups were compared using the chi-square test. Locus by locus linkage disequilibrium (LD) was evaluated by D' and r^2, which were calculated by the haplotype frequencies using the appropriate formula in the Excel program. A global test of differentiation among samples based on haplotype frequencies was performed using the Arlequin program available from http://anthropologie.unige.ch/arlequin [32].

Table 1. Primers Used in this Study

Exon	Forward		Reverse	
Exon 1	1F	CCT TAT GTA TTG TTC TCC ACC ACC	1R	GTT GAG CAC GCA GTG ATT GGC ACA
Exon 2	2F	CCA CTA GAT GAT GTC TTA GAC CAT	2R	CTG ACC TCC TAA CCT GGC AAT AGT
Exon 3,4	3F	GTA CTT GGC ACC TTG CTT AAG ATG	3R	TGG AAG TCT GCT CTC AGT TAT GGG
Exon 5	4F	GCT CAA CTA AGC CAT TCT GCT TAC	4R	GTA GCA CTT GGC ATG TGG CTC ACA
Exon 6	5F	GAT CCT TTC AGA CGC TCA TGT GCT	5R	CAT ACT CAT GTA GCC CAG CAC AGC
Exon 7	6F	GTG CGG TAA GCA TCA CTT TCG ATT	6R	CAG ATG AGG AGT CTG ATC CTT CAG
Exon 8	7F	GAA GTC CCA GCA TTG ATG AAC TGT	7R	GGC TAA GCT GAG TAA TTC TGA CAG
Exon 9	8F	CAG GAA GCG TAA GAC TCT TAG TAG	8R	GTC AGT AGG ATC ACT GCT AGC TCA
Exon 10, 11	9F	CCT GCA CAC AGG AGA GTT CCA TAT	9R	CAT GCT GGC AAC AAC ATA GTT CCA
	10F	CAA TCC CTA CAC ACA GAG TAT TGA	10R	CAT TCC AAC TGC TGT GTT ACC TCA
	11F	GAT CTA AGC CTT TCC TCT GTG TTC	11R	GAC ACA GAA ACA CAT GCA AGC ACT

RESULTS

To identify polymorphisms in the *TPH2* gene, all coding exons (1 to 11) and exon-intron boundaries were analyzed using genomic DNA from 16 Japanese METH-dependent/psychotic subjects. Ten single nucleotide polymorphisms (SNPs) and two insertion / deletion polymorphisms were identified. One polymorphism, Exon11+(C3) 500(C2), was novel (Table **2**). Two SNPs, rs7305115 (Exon7+A131G) and rs4290270 (Exon9+A57T), were synonymous mutations and Eon2+C18A was a non-synonymous mutation. Three linkage disequilibrium (LD) regions were found, rs11178998 (Exon1-A42G) to rs41265611 (IVS1+60 (I/D)), rs11179003 (IVS4+C4821T) to rs10879348 (IVS6+G144A), and rs4760816 (IVS6+C6106T) to rs7305115 (Exon7+A131G), in the sense that all genotypic patterns in all 16 samples analyzed were the same. Each one of the SNPs was chosen and a total of nine SNPs were genotyped for further analysis. LD mapping was analyzed by using SNPs having minor allele frequencies of over 10% in both samples (Table **4**). LD was observed from rs17110566 (IVS6+G152A) to rs17110747 (Exon11+G654A) and from rs4290270 (Exon9+A57T) to rs41317114 (IVS11+G128C) (Fig. **1** and Table **3**).

Association analyses were performed on these nine polymorphic positions using 162 METH dependent/psychotic patients and 243 controls. Genotypic frequencies in these

Table 2. ***TPH2*** **Gene Variants Found in the Japanese Population**

Position[1)]	Location	rs Number[2)]	SNP Name	Variation	Function
30029	5' side	rs11178998	Exon1-A42G	A/G	
30241	Intron 1	rs41265611	IVS1+60(I/D)	TCT/del	
32694	Exon 2		Exon2+C18A[3)]	C/A	nonsynonymous (Ser41Tyr)
40601	Intron 4	rs11179003	IVS4+C4821T	C/T	
63953	Intron 6	rs10879348	IVS6+G144A	G/A	
63961	Intron 6	rs17110566	IVS6+G152A	G/A	
69915	Intron 6	rs4760816	IVS6+C6106T	C/T	
70176	Exon 7	rs7305115	Exon7+A131G	A/G	synonymous (Pro312Pro)
113549	Exon 9	rs4290270	Exon9+A57T	A/T	synonymous (Ala375Ala)
123114	Exon 11		Exon11+(C3)500(C2)	C3/C2	
123268	Exon 11	rs17110747	Exon11+G654A	G/A	
123663	3' side	rs41317114	IVS11+G128C	G/C	

[1)] Position: nucleotide position number in the NCBI nucleotide database under accession number AC090109. [2)] rs number: NCBI SNP database. [3)] This SNP was reported as C2755A [14].

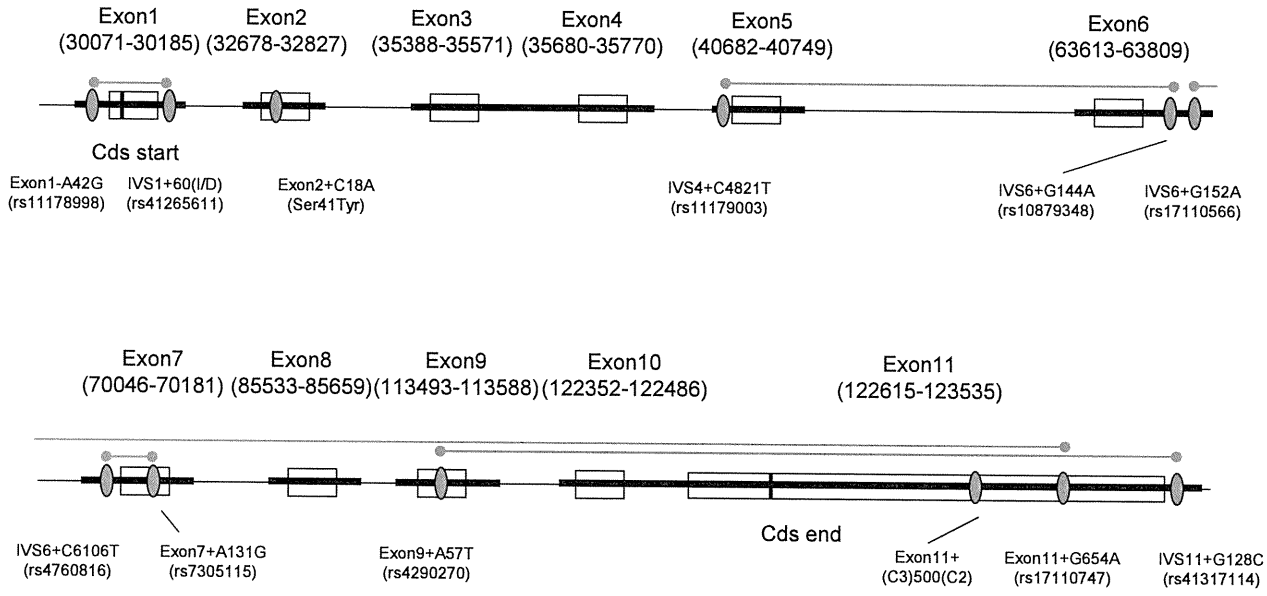

Fig. (1). Location and linkage disequilibrium mapping of the *TPH2* gene polymorphisms. All the coding exons and their regions were taken from the NCBI database under accession number AC090109. Red ovals indicate the polymorphic positions, solid black lines the analyzed regions, and solid red lines the LD block.

Table 3. Linkage Disequilibrium Mapping of the *TPH2* Gene

	rs17110566 (IVS6+G152A)	rs4760816 (IVS6+C6106T)	rs4290270 (Exon9+A57T)	rs17110747 (Exon11+G654A)	rs41317114 (IVS11+G128C)	
rs17110566		0.9392	0.6138	0.8581	0.0348	
rs4760816	0.9724		0.7301	0.9253	0.0092	
rs4290270	0.5262	0.5881		0.9284	0.6051	D'
rs17110747	0.8437	0.7885	0.9774		0.9399	
rs41317114	0.0111	0.2179	0.6284	0.9123		
			r^2			

D' and r^2 values for Control samples are shown in the upper right and lower left, respectively.

Table 4. Genotypic and Allelic Distribution of the *TPH2* gene SNPs in the METH Dependent/Psychotic Patients and the Control Groups

SNP	Group	Genotype (%)			P	Allele (%)		P
rs11178998 (Exon1-A42G)		A/A	A/G	G/G		A	G	
	METH	130 (80%)	29 (18%)	3 (2%)	0.102	289 (89%)	35 (11%)	0.617
	Control	197 (81%)	46 (19%)	0 (0%)		440 (91%)	46 (9%)	
Exon2+C18A		C/C	C/A	A/A		C	A	
	METH	146 (90%)	16 (10%)	0 (0%)	0.914	308 (95%)	16 (5%)	0.807
	Control	222 (91%)	21 (9%)	0 (0%)		465 (96%)	21 (4%)	
rs10879348 (IVS6+G144A)		G/G	G/A	A/A		G	A	
	METH	136 (84%)	26 (16%)	0 (0%)	0.975	298 (92%)	26 (8%)	0.920
	Control	206 (85%)	37 (15%)	0 (0%)		449 (92%)	37 (8%)	
rs17110566 (IVS6+G152A)		G/G	G/A	A/A		G	A	
	METH	123 (76%)	35 (22%)	4 (2%)	0.552	281 (87%)	43 (13%)	0.406
	Control	173 (71%)	64 (26%)	6 (2%)		410 (84%)	76 (16%)	
rs4760816 (IVS6+C6106T)		C/C	C/T	T/T		C	T	
	METH	28 (17%)	85 (52%)	49 (30%)	0.314	141 (44%)	183 (56%)	0.200
	Control	57 (23%)	121 (50%)	65 (27%)		235 (48%)	251 (52%)	
rs4290270 (Exon9+A57T)		A/A	A/T	T/T		A	T	
	METH	29 (18%)	80 (49%)	53 (33%)	0.840	138 (43%)	186 (57%)	0.777
	Control	49 (20%)	115 (47%)	79 (33%)		213 (44%)	273 (56%)	
Exon11+(C3)500(C2)		C3/C3	C3/C2	C2/C2		C3	C2	
	METH	159 (98%)	3 (2%)	0 (0%)	0.357	321 (99%)	3 (1%)	0.357
	Control	242 (100%)	1 (0%)	0 (0%)		485 (100%)	1 (0%)	
rs17110747 (Exon11+G654A)		G/G	G/A	A/A		G	A	
	METH	92 (57%)	63 (39%)	7 (4%)	0.956	247 (76%)	77 (24%)	0.888
	Control	136 (56%)	95 (39%)	12 (5%)		367 (76%)	119 (24%)	
rs41317114 (IVS11+G128C)		G/G	G/C	C/C		G	C	
	METH	119 (73%)	38 (23%)	5 (3%)	0.719	276 (85%)	48 (15%)	0.462
	Control	187 (77%)	50 (21%)	6 (2%)		424 (87%)	62 (13%)	

Table 5. Genotypic Distribution of the *TPH2* Gene SNPs in Clinically Subcategorized METH Subjects

SNP	Groups	Subgroup		N	Genotype			P
rs17110566 (IVS6+G152A)					G	G/A	A	
	Control			243	173	64	6	
	METH	Latency of Psychosis	<3 years	64	53	10	1	0.172
			≧3 years	67	47	18	2	0.966
		Prognosis of Psychosis	Transient (<1 month)	87	67	17	3	0.421
			Prolonged (≧1 month)	52	38	13	1	0.951
		Spontaneous Relapse	Not present	101	78	21	2	0.517
			Present	56	42	12	2	0.694
rs4760816 (IVS6+C6106T)					C	C/T	T	
	Control			243	57	121	65	
	METH	Latency of Psychosis	<3 years	64	13	35	16	0.771
			≧3 years	67	9	35	23	0.165
		Prognosis of Psychosis	Transient (<1 month)	87	15	39	33	0.125
			Prolonged (≧1 month)	52	7	34	11	0.107
		Spontaneous Relapse	Not present	101	19	51	31	0.577
			Present	56	8	30	18	0.306
rs4290270 (Exon9+A57T)					A	A/T	T	
	Control			243	49	115	79	
	METH	Latency of Psychosis	<3 years	64	8	35	21	0.338
			≧3 years	67	13	32	22	0.990
		Prognosis of Psychosis	Transient (<1 month)	87	16	37	34	0.541
			Prolonged (≧1 month)	52	6	34	12	0.058
		Spontaneous Relapse	Not present	101	17	52	32	0.712
			Present	56	10	27	19	0.923
rs17110747 (Exon11+G654A)					G	G/A	A	
	Control			243	136	95	12	
	METH	Latency of Psychosis	<3 years	64	35	28	1	0.438
			≧3 years	67	37	26	4	0.947
		Prognosis of Psychosis	Transient (<1 month)	87	52	31	4	0.827
			Prolonged (≧1 month)	52	26	25	1	0.366
		Spontaneous Relapse	Not present	101	57	41	3	0.712
			Present	56	32	21	3	0.970
rs41317114 (IVS11+G128C)					G	G/C	C	
	Control			243	187	50	6	
	METH	Latency of Psychosis	<3 years	64	49	15	0	0.411
			≧3 years	67	48	16	3	0.552
		Prognosis of Psychosis	Transient (<1 month)	87	65	19	3	0.852
			Prolonged (≧1 month)	52	38	13	1	0.767
		Spontaneous Relapse	Not present	101	77	21	3	0.966
			Present	56	38	17	1	0.282

N: Number of samples.
P: Significance values between the METH subjects and the controls.

SNPs were within the Hardy-Weinberg expectations. No significant differences were found in the allelic or genotypic frequencies of these SNPs between the METH dependent/psychotic patients and the controls (Table **4**). Since the minor allele frequency of the Exon11+(C3)500(C2) SNP was less than 1% in controls, this SNP was excluded from the haplotype analysis. No significant difference (P=0.448) was observed in a differentiation test between all pairs of samples based on haplotype frequencies by the Arlequin program.

Subcategory analyses were conducted on the clinical parameters (latency of psychosis, prognosis of psychosis, and spontaneous relapse). SNPs having minor allele frequencies of over 10% in both samples were used for this analysis: rs17110566 (IVS6+G152A), rs4760816 (IVS6+C6106T), rs4290270 (Exon9+A57T), rs17110747 (Exon11+G654A), and IVS11+G129C. No significant associations with clinical parameters were observed (Table **5**).

DISCUSSION

We analyzed the *TPH2* gene polymorphisms in a Japanese population and found ten SNPs and two insertion/deletion variants, among which one variant was novel. However, we failed to identify any variants or haplotypes in the *TPH2* gene examined in this study which were associated with METH dependence/psychosis.

Exon2+C18A is a nonsynonymous SNP and the corresponding amino acid is changed from Ser to Tyr at peptide position 41 (S41Y). This SNP was reported as C2755A by Lin and colleagues in a Han Chinese population [14]. They transfected plasmids containing full-lengthTPH2 protein–encoding sequences with two alternative alleles into SH-SY5Y cells and found that the amount of serotonin in SH-SY5Y cells expressing the 41Y allele was about 36% lower than in cells expressing the 41S allele. Despite the strong scientific rationale for studying polymorphisms in the *TPH2* gene in METH dependence/psychosis, we could not identify any variants or haplotypes associated with the phenotype. These results were comparable to those for cocaine use. Both cocaine and METH increase extracellular dopamine in the brain, and increased dopamine in the nucleus accumbens is thought to underlie the reinforcing effects of drugs of abuse [5, 33]. The association of cocaine dependence in subjects of African descent with TPH2 SNPs was analyzed by Dahl and colleagues, but they failed to identify any SNPs that were associated with the cocaine-dependent phenotype [34]. The disparity between these results and the previously reported results for heroin addiction [29] suggest that the *TPH2* gene has little effect in psychostimulants with the characteristics of indirect dopaminergic agonists.

Our results indicate that the *TPH2* gene variations may not be vulnerability factors in METH dependence/psychosis, and indeed that they are likely to make a small or no contribution to the development of METH dependence/psychosis.

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REFERENCES

[1] Matsumoto, T.; Kamijo, A.; Miyakawa, T.; Endo, K.; Yabana, T.; Kishimoto, H.; Okudaira, K.; Iseki, E.; Sakai, T.; Kosaka, K. Methamphetamine in Japan: the consequences of methamphetamine abuse as a function of route of administration. *Addiction,* **2002**, *97*(7), 809-817.

[2] Volkow, N.D.; Chang, L.; Wang, G.J.; Fowler, J.S.; Leonido-Yee, M.; Franceschi, D.; Sedler, M.J.; Gatley, S.J.; Hitzemann, R.; Ding, Y.S.; Logan, J.; Wong, C.; Miller, E.N. Association of dopamine transporter reduction with psychomotor impairment in methamphetamine abusers. *Am. J. Psychiatry,* **2001**, *158*(3), 377-382.

[3] Wilson, J.M.; Kalasinsky, K.S.; Levey, A.I.; Bergeron, C.; Reiber, G.; Anthony, R.M.; Schmunk, G.A.; Shannak, K.; Haycock, J.W.; Kish, S.J. Striatal dopamine nerve terminal markers in human, chronic methamphetamine users. *Nat. Med.,* **1996**, *2*(6), 699-703.

[4] Di Chiara, G.; Bassareo, V.; Fenu, S.; De Luca, M.A.; Spina, L.; Cadoni, C.; Acquas, E.; Carboni, E.; Valentini, V.; Lecca, D. Dopamine and drug addiction: the nucleus accumbens shell connection. *Neuropharmacology,* **2004**, *47*(Suppl 1), 227-241.

[5] Di Chiara, G.; Imperato, A. Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. *Proc. Natl. Acad. Sci. USA,* **1988**, *85*(14), 5274-5278.

[6] Di Matteo, V.; De Blasi, A.; Di Giulio, C.; Esposito, E. Role of 5-HT(2C) receptors in the control of central dopamine function. *Trends Pharmacol. Sci.,* **2001**, *22*(5), 229-232.

[7] Higgins, G.A.; Fletcher, P.J. Serotonin and drug reward: focus on 5-HT2C receptors. *Eur. J. Pharmacol.,* **2003**, *480*(1-3), 151-162.

[8] Hotchkiss, A.J.; Gibb, J.W. Long-term effects of multiple doses of methamphetamine on tryptophan hydroxylase and tyrosine hydroxylase activity in rat brain. *J. Pharmacol. Exp. Ther.,* **1980**, *214*(2), 257-262.

[9] Knapp, S.; Mandell, A.J.; Geyer, M.A. Effects of amphetamines on regional tryptophan hydroxylase activity and synaptosomal conversion of tryptophan to 5-hydroxytryptamine in rat brain. *J. Pharmacol. Exp. Ther.,* **1974**, *189*(3), 676-689.

[10] Cooper, J.R.; Melcer, I. The enzymic oxidation of tryptophan to 5-hydroxytryptophan in the biosynthesis of serotonin. *J. Pharmacol. Exp. Ther.,* **1961**, *132*, 265-268.

[11] Patel, P.D.; Pontrello, C.; Burke, S. Robust and tissue-specific expression of TPH2 versus TPH1 in rat raphe and pineal gland. *Biol. Psychiatry,* **2004**, *55*(4), 428-433.

[12] Walther, D.J.; Peter, J.U.; Bashammakh, S.; Hortnagl, H.; Voits, M.; Fink, H.; Bader, M. Synthesis of serotonin by a second tryptophan hydroxylase isoform. *Science,* **2003**, *299*(5603), 76.

[13] Harvey, M.; Shink, E.; Tremblay, M.; Gagne, B.; Raymond, C.; Labbe, M.; Walther, D.J.; Bader, M.; Barden, N. Support for the involvement of TPH2 gene in affective disorders. *Mol. Psychiatry,* **2004**, *9*(11), 980-981.

[14] Lin, Y.M.; Chao, S.C.; Chen, T.M.; Lai, T.J.; Chen, J.S.; Sun, H.S. Association of functional polymorphisms of the human tryptophan hydroxylase 2 gene with risk for bipolar disorder in Han Chinese. *Arch Gen Psychiatry,* **2007**, *64*(9), 1015-1024.

[15] Harvey, M.; Gagne, B.; Labbe, M.; Barden, N. Polymorphisms in the neuronal isoform of tryptophan hydroxylase 2 are associated with bipolar disorder in French Canadian pedigrees. *Psychiatr. Genet.,* **2007**, *17*(1), 17-22.

[16] Grigoroiu-Serbanescu, M.; Diaconu, C.C.; Herms, S.; Bleotu, C.; Vollmer, J.; Muhleisen, T.W.; Prelipceanu, D.; Priebe, L.; Mihailescu, R.; Georgescu, M.J.; Sima, D.; Grimberg, M.; Nothen, M.M.; Cichon, S. Investigation of the tryptophan hydroxylase 2 gene in bipolar I disorder in the Romanian population. *Psychiatr. Genet.,* **2008**, *18*(5), 240-247.

[17] Van Den Bogaert, A.; Sleegers, K.; De Zutter, S.; Heyrman, L.; Norrback, K.F.; Adolfsson, R.; Van Broeckhoven, C.; Del-Favero, J. Association of brain-specific tryptophan hydroxylase, TPH2, with unipolar and bipolar disorder in a Northern Swedish, isolated population. *Arch. Gen. Psychiatry,* **2006**, *63*(10), 1103-1110.