The present results suggest that sequence variations in 5′UTR, exon 1, and part of intron 1 of OPRM are not genetic markers for MAP dependence/psychosis. Further studies could usefully look for novel polymorphisms in the downstream sequence of the OPRM gene and for any association between the polymorphisms and MAP dependence/psychosis.

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REFERENCES

1. UHL, G.R., F.S. HALL & I. SORA. 2002. Cocaine, reward, movement and monoamine transporters. Mol. Psychiatry **7:** 21–26.
2. WOOD, P.L. 1983. Opioid regulation of CNS dopaminergic pathways: a review of methodology, receptor types, regional variations and species differences. Peptides **4:** 595–601.
3. HAYASHI, T. *et al.* 1999. [D-Ala2, D-Leu5]enkephalin blocks the methamphetamine-induced c-fos mRNA increase in mouse striatum. Eur. J. Pharmacol. **366:** R7–8.
4. EL DALY, E. *et al.* 2000. Modulation of the neurotoxic effects of methamphetamine by the selective kappa-opioid receptor agonist U69593. J. Neurochem. **74:** 1553–1562.
5. MAYER, P. & V. HOLLT. 2001. Allelic and somatic variations in the endogenous opioid system of humans. Pharmacol. Ther. **91:** 167–177.
6. UHL, G.R., I. SORA & Z. WANG. 1999. The mu opiate receptor as a candidate gene for pain: polymorphisms, variations in expression, nociception, and opiate responses. Proc. Natl. Acad. Sci. USA **96:** 7752–7755.
7. HOEHE, M.R. *et al.* 2000. Sequence variability and candidate gene analysis in complex disease: association of mu opioid receptor gene variation with substance dependence. Hum. Mol. Genet. **9:** 2895–2908.
8. BOND, C. *et al.* 1998. Single-nucleotide polymorphism in the human mu opioid receptor gene alters beta-endorphin binding and activity: possible implications for opiate addiction. Proc. Natl. Acad. Sci. USA **95:** 9608–9613.
9. BERGEN, A.W. *et al.* 1997. Mu opioid receptor gene variants: lack of association with alcohol dependence. Mol. Psychiatry **2:** 490–494.
10. SANDER, T. *et al.* 1998. Human mu-opioid receptor variation and alcohol dependence. Alcohol Clin. Exp. Res. **22:** 2108–2110.
11. GELERNTER, J. *et al.* 1999. Genetics of two mu opioid receptor gene (OPRM1) exon I polymorphisms: population studies, and allele frequencies in alcohol- and drug-dependent subjects. Mol. Psychiatry **4:** 476–483.
12. FRANKE, P. *et al.* 2001. Nonreplication of association between mu-opioid-receptor gene (OPRM1) A118G polymorphism and substance dependence. Am. J. Med. Genet. **105:** 114–119.
13. SHI, J. *et al.* 2002. Sequence variations in the mu-opioid receptor gene (OPRM1) associated with human addiction to heroin. Hum. Mutat. **19:** 459–460.
14. SCHINKA, J.A. *et al.* 2002. A functional polymorphism within the mu-opioid receptor gene and risk for abuse of alcohol and other substances. Mol. Psychiatry **7:** 224–228.
15. SCHNEIDER, S., D. ROESSLI & L. EXCOFFIER. 2000. Arlequin: a software for population genetics data analysis. Ver 2.000. Genetics and Biometry Lab., Dept. of Anthropology, Univ. of Geneva. Switzerland.
16. SHAM, P.C. & D. CURTIS. 1995. Monte Carlo tests for associations between disease and alleles at highly polymorphic loci. Ann. Hum. Genet. **59:** 97–105.

17. BECKER, A. *et al.* 2002. Rewarding effects of ethanol and cocaine in mu opioid receptor-deficient mice. Naunyn Schmiedeberg's Arch. Pharmacol. **365:** 296–302.
18. CONTARINO, A. *et al.* 2002. Lack of reward and locomotor stimulation induced by heroin in mu-opioid receptor-deficient mice. Eur. J. Pharmacol. **446:** 103–109.
19. BERRENDERO, F. *et al.* 2002. Attenuation of nicotine-induced antinociception, rewarding effects, and dependence in mu-opioid receptor knock-out mice. J. Neurosci. **22:** 10935–10940.

Food-Reinforced Operant Behavior in Dopamine Transporter Knockout Mice

Enhanced Resistance to Extinction

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ABSTRACT: **Dopamine (DA) plays roles in circuits that are important for brain reward and in striatal brain regions that are important for certain types of habit learning. These processes in wildtype, heterozygous, and homozygous dopamine transporter knockout (DAT-KO) mice, which were mildly food deprived and allowed to make nose-poke responses for food reinforcement, were studied. The mice were given 20-min sessions of daily (a) baseline exposure to the operant chambers, (b) acquisition of nose-poke responses in which responses were reinforced under a fixed ratio (FR5) schedule, (c) a progressive ratio schedule in which the number of responses required to obtain food was gradually increased, and (d) extinction of responses in which nose pokes were not followed by food. Neither heterozygous nor homozygous DAT-KO mice differed from their wildtype litter mates in the number of nose pokes displayed during baseline exposures to the chambers, the number of sessions required for acquisition, the number of responses under the FR5 schedule, or the number of responses under the progressive ratio schedule. Interestingly, however, in the five extinction sessions in which food was no longer delivered by nose poking, homozygous DAT-KO mice exerted significantly more responses than mice of either of the other two genotypes. These lines of evidence suggest a greater resistance of DAT-KO mice to the elimination of the response and support roles of dopaminergic systems in habit memory.**

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KEYWORDS: **habit learning; extinction; food reinforcement; dopamine transporter**

INTRODUCTION

The mesolimbic dopamine (DA) system is known to play a critical role in mediating the reinforcing effects of abused drugs.[1] Recently, the growing evidence indicates that DA in the striatum also plays an important role in habit learning, which is crucial in drug-taking behavior.[2,3] Dopamine transporter knockout mice (DAT-KO) are a valuable model to study the roles of DA in these systems. DAT-KO mice, in which the clearance of DA from the synaptic cleft is about 100 times longer than the normal mice, are known to be hyperactive in a novel environment and insensitive to the motor-stimulating effect of cocaine.[4] Nevertheless, the reinforcing effect of cocaine is manifested in these mice.[5] So far, the behavioral characteristics of DAT-KO mice pertaining to natural reward have not been well documented. In the present study, we have examined the behavior of DAT-KO mice regarding learning and motivation for food reward using the operant conditioning paradigm.

MATERIALS AND METHODS

Animals

A total of 24 female DAT-KO mice (8 homozygous, 8 heterozygous, and 8 wild-types that were 134 to 218 (average 192.7) days old were used. The details of the generation of DAT-KO mice have been described previously.[5] These three genotypes were obtained by crossing adult heterozygotes. Throughout the experimental period, they were housed individually and their food supply was restricted to maintain approximately 90% of their free-feeding body weight. Tap water was freely available in their home cages.

Apparatus

A standard operant chamber for mice was used (O'Hara & Co. Ltd.). One wall of the chamber had a hole equipped with a dim light and a photo beam. Poking the hole interrupted the beam and resulted in the delivery of a 20-mg food pellet into the hole. Four identical chambers were used in sound-attenuating boxes. The experiment was controlled by the MED-PC system (MED-Associates, Inc.) using in-house software.

Procedure

The mice were given 20-min sessions of the following five stages daily:

(1) *Baseline exposure to the chambers*: The number of spontaneous nose-poke responses was recorded.

(2) *Training of the food-reinforced response*: A food pellet was delivered contingent on the nose poke. Initially, the response was reinforced under a fixed ratio (FR) 1 schedule, in which each response was followed by the delivery of food. The training continued until the animal got at least 10 reinforcements in a session (the

response criterion). When the criterion was met, the ratio was set to two and finally to five. The number of sessions required to meet the criterion under an FR5 schedule for three consecutive sessions was recorded.

(3) *Progressive ratio (PR) schedule*: The number of responses required to obtain food was gradually increased. Initially, the number was set to five. Every time the animal got the food within 300 s, the ratio was increased to 7, 10, 14, 20, 28, 40, 57, 80, and 113. If the animal failed to get food within the limited time, the test was terminated and the ratio immediately before termination was defined as the breaking point.

(4) *Retraining of the response under an FR5*: Before going to the next stage, it was confirmed that the responses had not deteriorated.

(5) *Extinction of response*: Nose pokes were not followed by food. Five extinction sessions were given.

RESULTS AND DISCUSSION

Throughout the experiment the body weight of the homozygotes was significantly lower than the other two genotypes (mean ± SD: homo: 19.2 ± 1.2 g; hetero: 22.3 ± 1.7 g; wild: 22.5 ± 1.1 g) (ANOVA $F(2, 21) = 14.16$, $P = .0002$, with the *post hoc* Fisher's PLSD test).

Baseline Level

Mean numbers of responses in a 20-min session are shown in the leftmost column of TABLE 1. Although there was little difference among genotypes in the number of responses, the difference was not statistically significant. Thus, the homozygous DAT-KO mice were not spontaneously hyperactive in this particular experimental situation.

Training of the Food Reinforced Response

Two out of eight wildtype mice failed to obtain 10 reinforcements under the FR1 schedule despite the extensive training by more than 15 sessions. The data of these mice were not included in the further analysis. This might be attributable to one of the background strains of DAT-KO (129/sv). The median number of sessions required to meet the criterion under the FR5 schedule was 7.5 (range 5–16) for homozygous, 5 (range 3–12) for heterozygous, and 7.5 (range 3–21) for wildtype mice. The mean numbers of responses under the FR5 schedule are shown in TABLE 1. No statistical difference among genotypes was found in both of these measures.

Progressive Ratio (PR) Schedule

The median value of the breaking point was 34 (range 5–113) for homozygous, 70.5 (range 20–113) for heterozygous, and 40 (range 14–80) for the wildtype mice. There was no significant difference among genotypes. We also calculated the response rate per minute under each response requirement, and again there was no significant difference among genotypes (data not shown). The PR schedule test is known to be a standard method for testing the reward value and/or motivation to get

TABLE 1. Mean numbers of nose-poke responses in a 20-min session, with standard deviation in parentheses

	Baseline response	FR5 (average of 3 days)	Extinction day 1	2	3	4	5
Homozygous ($n = 8$)	43.3 (12.2)	110.3 (30.0)	235.1 (91.9)	221.8 (80.3)	93.6 (52.7)	120.4 (70.0)	110.3 (72.2)
Heterozygous ($n = 8$)	51.3 (12.7)	92 (49.2)	360.8 (154.9)	191 (111.4)	126.3 (49.7)	91.5 (32.9)	83.5 (34.2)
Wildtype ($n = 6$)	35.8* (9.1)	80.7 (18.5)	251.2 (98.5)	112 (50.9)	65.3 (60.2)	45.5 (23.8)	50.7 (28.5)

*$n = 8$.

the reward.[6] Thus, the present data suggest that the DAT-KO mice were the same as the wildtype mice with respect to motivation to get the food reward.

Retraining Under an FR5 Schedule

No response deterioration was found in any of the three genotypes.

Extinction of Response

During the course of extinction, the number of responses decreased within a session and also across sessions in all of the animals. However, the homozygous mice exerted more responses relative to other genotypes. TABLE 1 shows the mean daily

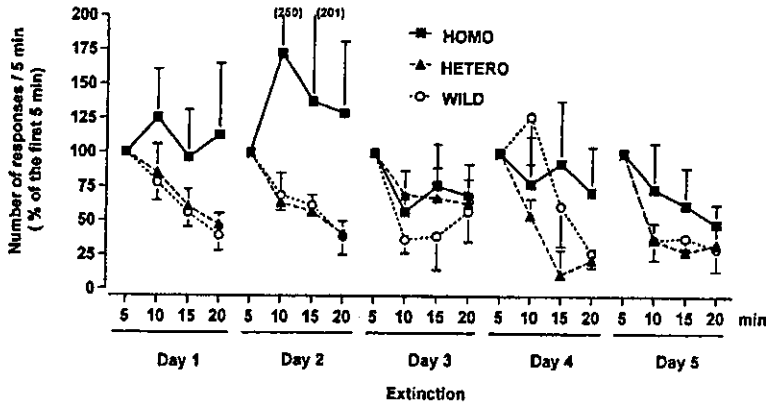

FIGURE 1. Nose-poke response of homozygous ($n = 8$), heterozygous ($n = 8$), and wildtype ($n = 6$) DAT-KO mice during five extinction sessions. Number of responses in a 5-min period was recorded and converted into the percentage of the number of responses in the first 5-min period in each day. Homozygous mice showed greater resistance to resonse elimination of response than other genotypes, especially on days one and two.

number of responses for each genotype. There was a statistically significant interaction between genotypes and the number of extinction sessions (ANOVA with repeated measures, $df = 8$, $F = 3.161$, $P = .0038$), though the main effect of genotype was not significant ($df = 2$, $F = 2.300$, $P = .1275$). The *post hoc* Fisher's PLSD test revealed that the homozygotes exerted significantly more responses than the wildtype mice during extinction days two, four, and five. There was no significant difference between homozygotes and heterozygotes on these days, and between heterozygotes and wildtype mice due to the large individual difference among heterozygotes. When we looked at the response decrement within the day, the decrement was not apparent in homozygotes except for day three (FIG. 1). These data indicate that the homozygotes were resistant to extinction of response. Thus, these mice showed a stronger habit. Another measure confirmed this point. If we employ the arbitrary criterion of extinction of response as no response for five consecutive minutes, only one homozygous mouse out of eight met the criterion. In contrast, six out of eight heterozygous and six out of six in wildtype mice met the criterion.

SUMMARY

Homozygous DAT-KO mice showed no clear evidence of hyperactivity in this operant conditioning situation. Acquisition and maintenance of responses for food reinforcement as well as the motivation to get food tested by the PR schedule were not markedly altered in these mice. However, greater resistance to extinction was found in these mice. Although this study was preliminary in nature, the results indicate that the DA system is involved in the habit memory system when we used food as a reward. Since resistance to extinction of response induced by environmental cues is important for drug-seeking behavior,[7] further behavioral phenotyping of DAT-KO mice related to learning and extinction might provide useful information concerning drug dependence.

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REFERENCES

1. ALTMAN, J., B.J. EVERITT, S. GLAUTIER, *et al.* 1996. The biological, social and clinical bases of drug addiction: commentary and debate. Psychopharmacology **125:** 285–345.
2. ITO, R., J.W. DALLY, T.W. ROBBINS, *et al.* 2002. Dopamine release in the dorsal striatum during cocaine-seeking behavior under the control of a drug-associated cue. J. Neurosci. **22:** 6247–6253.
3. GERDEMAN, G.L., J.G. PARTRIDGE, C.R. LUPICA, *et al.* 2003. It could be habit forming: drugs of abuse and striatal synaptic plasticity. Trends Neurosci. **26:** 184–192.
4. GIROS, B., M. JABER, S.R. JONES, *et al.* 1996. Hyperlocomotion and indifference to cocaine and amphetamine in mice lacking the dopamine transporter. Nature **379:** 606–612.

5. SORA, I., C. WICHEMS, N. TAKAHASHI, *et al.* 1998. Cocaine reward models: conditioned place preference can be established in dopamine- and in serotonin-transporter knockout mice. Proc. Natl. Acad. Sci. USA **96:** 7699–7704.
6. STAFFORD, D., M.G. LESAGE & J.R. GLOWA. 1998. Progressive-ratio schedules of drug delivery in the analysis of drug self-administration: a review. Psychopharmacology **139:** 169–184.
7. WEISS, F., R. MARTIN-FARDON, R. CICCOCIOPPO, *et al.* 2001. Enduring resistance to extinction of cocaine-seeking behavior induced by drug-related cues. Neuropsychopharmacology **25:** 361–372.

Study of Association between α-Synuclein Gene Polymorphism and Methamphetamine Psychosis/Dependence

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ABSTRACT: **Methamphetamine (MAP) dissipates proton gradients across the membranes of synaptic vesicles, enhances cytoplasmic dopamine (DA) concentrations, and causes calcium-independent, nonvesicular DA release into synapses. MAP is taken into the cytosol by the dopamine transporter (DAT) on the synaptic terminals of DA neurons, and endogenous DA is concurrently released through the transporter by carrier exchange mechanisms, resulting in a robust increase in DA concentration in the synaptic clefts. The enhanced DA release through DAT by MAP is the main mechanism for the reinforcing ef-**

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fects of MAP. The complexes of α-synuclein and DAT facilitate membrane clustering of the DAT, thereby accelerating DA uptake *in vitro*. α-Synuclein has been shown to be overexpressed in the midbrain DA neurons of chronic cocaine abusers. The present study was performed to study the association between the α-synuclein gene polymorphisms and MAP psychosis/dependence in Japanese population. Since the T10A7 polymorphic site at the 5′ end of the noncoding exon 1′ in the α-synuclein gene is highly polymorphic, we analyzed the noncoding exon 1′ and intron 1, including this polymorphic site by sequencing. We confirmed four single nucleotide polymorphisms (SNPs) within 1.38 kbp of the T10A7 polymorphic site. No significant difference was found in genotype or allele frequencies in the T10A7 polymorphic site between MAP psychotic/dependent and control subjects. We found significant association between three SNPs in the vicinity of this polymorphic site in intron 1 and MAP psychosis/dependence in female subjects, but not in males. These results suggest an association of the α-synuclein gene polymorphisms with MAP psychosis/dependence in our female subjects. Further analyses are necessary to clarify the gender difference, by using a larger sample size and/or different ethnic groups, as well as functional variations in the α-synuclein gene.

KEYWORDS: **methamphetamine; dopamine transporter; α-synuclein; mesolimbic dopaminergic pathway**

INTRODUCTION

α-Synuclein is a major component of nigral Lewy bodies in Parkinson's disease.[1,2] α-Synuclein is a soluble presynaptic protein and is abundant in neurons,[3] but its function is yet to be elucidated. Lee and colleagues found that complexes of α-synuclein and dopamine transporter (DAT) facilitate membrane clustering of the DAT, thereby accelerating dopamine (DA) uptake *in vitro*.[4] Excess α-synuclein potentiates production of reactive oxygen species by DA, which may cause cell death.[5–8] Modulation of DA transmission by α-synuclein is probably involved with neurodegenerative and neuropsychiatric disorders such as drug dependence.

The mesolimbic dopaminergic pathway has an important role in addiction to psychostimulants and reinforcement. [^{3}H]-WIN 35428 binding sites, which reflect DAT protein amount and/or function, were increased in postmortem brains of cocaine abusers.[9] Mash and colleagues found overexpression of α-synuclein protein in DA neurons in cocaine abusers.[10] These findings provide further support for the involvement of α-synuclein in regulating dopaminergic neurons.[9,10] Methamphetamine (MAP) dissipates proton gradients across the membranes of synaptic vesicles, enhances cytoplasmic DA concentrations, and causes calcium-independent, nonvesicular DA release into synapses. MAP is taken into cytosol by DAT on the synaptic terminals of DA neurons, and endogenous DA is concurrently released through the transporter by carrier exchange mechanisms, resulting in a robust increase of DA concentration in the synaptic clefts. The enhanced DA release through DAT by MAP is the main mechanism for the reinforcing effects of MAP.[11,12] It has been reported that long-term MAP abuse induced development of psychosis. These findings suggested the importance of α-synuclein on MAP abusers and prompted us to study the association between the α-synuclein gene and MAP psychosis/dependence in Japa-

nese population. A highly polymorphic sequence variation (T10A7) has been reported at the 5′ end of the noncoding exon 1′ of α-synuclein gene.[13] In the present study, we have investigated whether the polymorphic sites in the noncoding exon 1′ and intron 1, including T10A7, are associated with MAP psychosis/dependence in Japan.

MATERIALS AND METHODS

Subjects

This study was performed following approval from the ethics committees of each institute of the Japanese Genetics Initiative for Drug Abuse (JGIDA); all subjects provided written informed consent for the use of their DNA samples for this research. The subjects were 170 unrelated patients with MAP-dependence disorder meeting ICD-10-DCR criteria (F15.2 and F15.5), who were outpatients or inpatients of psychiatric hospitals of JGIDA, and also 161 geographical origin-matched healthy controls, mostly medical staff who had no past or family history of drug dependence or psychotic disorders. Patients were excluded if they had a clinical diagnosis of schizophrenia, another psychotic disorder, or an organic mental syndrome. All subjects were Japanese, born and living in certain areas of Japan, including northern Kyusyu, Setouchi, Chukyou, Toukai, and Kantou. Blood samples were drawn for DNA extraction from 170 patients (male 138, female 32) and 161 controls (male 83, female 78). The mean age of the patients was 37.6 ± 12.0 years (male: 39.5 ± 12.0 years; female: 29.4 ± 7.4 years). The mean age of the controls was 38.6 ± 12.0 years (male: 38.2 ± 11.1 years; female: 39.1 ± 12.9 years). Genomic DNA was extracted from peripheral blood by the phenol/chloroform method.

Defining Variation with the α-Synuclein Gene

The 5′ end of the noncoding exon 1′ in the α-synuclein gene (accession no. AF163864) was amplified by PCR, and the products were sequenced in both directions using BigDye terminators (Applied Biosystems). Amplification primer pairs were 11F: CAT CTC CCA TCC ATC TTG GC and 12F: AGA AGC TCT GAC AAA TCA GCG GTG. The PCR product was 1.38 kbp and was sequenced using four primers (11F, 11R: AAA TCT GTC TGC CCG CTC TC, 12F, 12R: ACC CGG TGT TCT CCA GGA TTT CCA). Genotyping and sequencing were performed on an ABI3100 Genetic Analyzer (Applied Biosystems). The position numbers of polymorphic variants are quoted with respect to the National Center for Biotechnology Information (NCBI) single nucleotide polymorphism (SNP) consortium database.

Statistical Analysis

Data for each locus were used to estimate allele and genotype frequencies and to test for Hardy-Weinberg equilibrium (HWE), using the chi-squared method or the Arlequin program available from http://anthropologie.unige.ch/arlequin.[14] The allele and genotype frequencies of patients and control groups were compared using the chi-squared method and the Monte Carlo type CLUMP analysis program.[15]

RESULTS

Our subjects were 170 MAP psychotic/dependent patients and 161 controls. DNA samples from 16 of the patients were sequenced in 1.38 kbp around the T10A7 polymorphic site at the 5′ end of the noncoding exon 1′ of the α-synuclein gene. We confirmed four SNPs (rs#1372520, 3756063, 2870027, 3756059) in these patients in addition to the T10A7 polymorphic site. All these four SNPs were in intron 1. The genotype and allele frequencies of these four SNPs were all in Hardy-Weinberg equilibrium (HWE), indicating no sample bias in our case and control samples. These four SNPs showed no association in genotypic or allelic analysis according to the chi-squared test (TABLE 1a). We found four allelic variations in the T10A7 polymorphic site (TABLE 2a). The genotype frequencies of the MAP psychotic/dependent group and control group were in HWE (control $P = .73$, MAP $P = .77$). Genotype frequencies were compared using the CLUMP analysis program and showed no association ($P = .677$, T1 = 4.00). Allele frequencies also showed no association based on the chi-squared test ($P = .622$, chi-squared = 1.77).

Since there were many more MAP psychotic/dependent males than females, we analyzed the associations in each gender. In males, there was no difference in the four SNPs and the T10A7 polymorphic site between patients and control samples (TABLES 1b and 2b). In females, genotype frequencies were significant in rs#1372520 ($P = .03$), rs#3756063 ($P = .03$), and rs#3756059 ($P = .03$) (TABLES 1c and 2c).

DISCUSSION

We have analyzed the sequence variation (T10A7) at the 5′ end of the noncoding exon 1′ in the α-synuclein gene and found no significant difference in genotype or allele frequencies between MAP psychotic/dependent subjects and controls. We confirmed four SNPs in intron 1 and found a significant difference of genotype in three SNPs in MAP psychotic/dependent females, but not in males. Association in the T10A7 polymorphism was first studied by Autere and colleagues. They found no statistically significant differences in Parkinson's disease patients in Finland.[13] Since the T10A7 polymorphic site has many variations, this site is thought to be a good marker for an association study of the α-synuclein gene. Our results at this site do not suggest any role for the α-synuclein gene in MAP psychosis/dependence. We nevertheless found significant association between three SNPs in the vicinity of this polymorphic site and MAP psychosis/dependence in female subjects, though not males. The reason for this gender difference is not clear, although recent evidence suggests women and men differ in their progression to dependence and abuse. In preclinical and clinical studies, it has been suggested that ovarian hormones, particularly estrogen, are involved in gender differences in drug abuse.[16] Koizumi and colleagues also found a correlation between glutathione S-transferase M1 gene deletion and MAP abuse by females (Koizumi and Iyo, unpublished data). The data in our study should be carefully treated, as the samples were divided into two groups by gender. The significance was corrected to $P = .025$ by Bonferroni corrections, and the P value of these sites was .03, suggesting weak association.

The functional alterations caused by these SNPs are not clear in the present study, but there are several possibilities. First, the SNPs or relating linkage disequilibrium

TABLE 1. Genotype and allele distribution of α-synuclein gene SNPs in patient and control groups

SNP	Group	*N*	Genotype (Percent)			*P*	Allele (Percent)		*P*
a. Male and Female									
rs#1372520			GG	GA	AA		G	A	
	Control	161	141 (88)	18 (11)	2 (1)		300 (93)	22 (7)	
	MAP	170	142 (84)	27 (16)	1 (1)	.389	311 (92)	29 (5)	.502
rs#3756063			CC	CG	GG		C	G	
	Control	161	2 (1)	18 (11)	141 (88)		22 (7)	300 (93)	
	MAP	170	1 (1)	29 (17)	140 (82)	.263	31 (9)	309 (91)	.348
rs#2870027			GG	GA	AA		G	A	
	Control	161	55 (34)	75 (47)	31 (19)		185 (58)	137 (43)	
	MAP	170	60 (35)	88 (52)	22 (13)	.281	208 (61)	132 (39)	.371
rs#3756059			CC	CT	TT		C	T	
	Control	161	2 (1)	18 (11)	141 (88)		22 (7)	300 (93)	
	MAP	170	1 (1)	28 (17)	141 (83)	.323	30 (9)	310 (91)	.420
b. Male									
rs#1372520			GG	GA	AA		G	A	
	Control	83	71 (86)	11 (13)	1 (1)		153 (92)	13 (8)	
	MAP	138	119 (86)	18 (13)	1 (1)	.932	256 (93)	20 (7)	1.000
rs#3756063			CC	CG	GG		C	G	
	Control	83	1 (1)	11 (13)	71 (86)		13 (8)	153 (92)	
	MAP	138	1 (1)	20 (14)	117 (84)	.909	22 (8)	254 (92)	.888
rs#2870027			GG	GA	AA		G	A	
	Control	83	27 (32)	40 (48)	16 (19)		94 (57)	72 (43)	
	MAP	138	46 (33)	76 (55)	16 (12)	.274	168 (61)	108 (39)	.435
rs#3756059			CC	CT	TT		C	T	
	Control	83	1 (1)	11 (13)	71 (86)		13 (8)	153 (92)	
	MAP	138	1 (1)	19 (14)	118 (86)	.932	21 (8)	255 (92)	.920

TABLE 1. (*continued*) **Genotype and allele distribution of α-synuclein gene SNPs in patient and control groups**

SNP	Group	*N*	Genotype (Percent)			*P*	Allele (Percent)		*P*
c. Female									
rs#1372520			GG	GA	AA		G	A	
	Control	78	70 (90)	7 (9)	1 (1)		147 (94)	9 (6)	
	MAP	32	23 (72)	9 (28)	0 (0)	.030	55 (86)	9 (14)	.077
rs#3756063			CC	CG	GG		C	G	
	Control	78	1 (1)	7 (9)	70 (90)		9 (6)	147 (94)	
	MAP	32	0 (0)	9 (28)	23 (72)	.030	9 (14)	55 (86)	.077
rs#2870027			GG	GA	AA		G	A	
	Control	78	28 (36)	35 (45)	15 (19)		91 (58)	65 (42)	
	MAP	32	14 (44)	12 (38)	6 (19)	.719	40 (63)	24 (38)	.671
rs#3756059			CC	CT	TT		C	T	
	Control	78	1 (1)	7 (9)	70 (90)		9 (6)	147 (94)	
	MAP	32	0 (0)	9 (28)	23 (72)	.030	9 (14)	55 (86)	.077

TABLE 2a. Genotype and allele frequencies of the T10A7 polymorphism of the α-synuclein gene in MAP psychosis/dependence: male and female

Subjects	*N*	Genotype (Percent)						
		T10A7/ T10A7	T10A8/ T10A8	T11A6/ T11A6	T10A7/ T10A8	T10A7/ T11A6	T10A8/ T11A6	T11A6/ T12A5
Control	161	18 (11)	7 (4)	31 (19)	30 (19)	46 (29)	29 (18)	0 (0)
MAP	170	20 (12)	10 (6)	22 (13)	29 (17)	53 (31)	35 (21)	1 (1)
		Allele (Percent)						
		T10A7	T10A8	T11A6	T12A5			
Control	322	112 (35)	73 (23)	137 (43)	0 (0)			
MAP	340	122 (36)	84 (25)	133 (39)	1 (0)			

NOTE: *N*, number of genotypes and alleles in MAP psychotic/dependent subjects and controls. MAP and control genotypes of both sexes were in Hardy-Weinberg equilibrium (control $P = .73$, MAP $P = .77$). CLUMP analysis for genotype frequencies: $P = .677$ (T1 = 4.00). Chi-squared test for allele frequencies: $P = .622$ (chi-squared = 1.77).

TABLE 2b. Genotype and allele frequencies of the T10A7 polymorphism of the α-synuclein gene in MAP psychosis/dependence: male

Subjects	*N*	Genotype (Percent)						
		T10A7/ T10A7	T10A8/ T10A8	T11A6/ T11A6	T10A7/ T10A8	T10A7/ T11A6	T10A8/ T11A6	T11A6/ T12A5
Control	83	7 (8)	4 (5)	16 (19)	16 (19)	23 (28)	17 (20)	0 (0)
MAP	138	14 (10)	9 (7)	16 (12)	22 (16)	45 (33)	31 (22)	1 (1)
		Allele (Percent)						
		T10A7	T10A8	T11A6	T12A5			
Control	166	53 (32)	41 (25)	72 (43)	0 (0)			
MAP	276	95 (34)	71 (26)	109 (39)	1 (0)			

NOTE: *N*, number of genotypes and alleles in MAP psychotic/dependent subjects and controls. MAP and control genotypes of males were in Hardy-Weinberg equilibrium (control $P = .82$, MAP $P = .34$). CLUMP analysis for genotype frequencies: $P = .682$ (T1 = 3.96). Chi-squared test for allele frequencies: $P = .748$ (chi-squared = 1.22).

TABLE 2c. Genotype and allele frequencies of the T10A7 polymorphism of the α-synuclein gene in MAP psychosis/dependence: female

Subjects	*N*	Genotype (Percent)						
		T10A7/ T10A7	T10A8/ T10A8	T11A6/ T11A6	T10A7/ T10A8	T10A7/ T11A6	T10A8/ T11A6	T11A6/ T12A5
Control	78	11 (14)	3 (4)	15 (19)	14 (18)	23 (29)	12 (15)	0 (0)
MAP	32	6 (19)	1 (3)	6 (19)	7 (22)	8 (25)	4 (13)	0 (0)

		Allele (Percent)			
		T10A7	T10A8	T11A6	T12A5
Control	156	59 (38)	32 (21)	65 (42)	0 (0)
MAP	64	27 (42)	13 (20)	24 (38)	0 (0)

NOTE: *N*, number of genotypes and alleles in MAP psychotic/dependent subjects and controls. MAP and control genotypes of females were in Hardy-Weinberg equilibrium (control $P = .87$, MAP $P = .33$). CLUMP analysis for genotype frequencies: $P = .991$ (T1 = 0.82). Chi-squared test for allele frequencies: $P = .936$ (chi-squared = 0.42).

positions may change DAT and α-synuclein complex formation. MAP is taken into cytosol by DAT on the synaptic terminals of DA neurons, and endogenous DA is concurrently released through the transporter by carrier exchange mechanisms.

α-Synuclein forms functional protein–protein complexes, thereby modifying dopaminergic neurotransmission.[4] Overexpression of α-synuclein in mice increased the density of the DAT.[17] Mutation of the α-synuclein gene may affect complex formation with DAT, modulating dopaminergic neurotransmission. Modulated expression from the mutated α-synuclein gene may then alter the development of MAP psychosis/dependence.

As a second possibility, the SNPs or relating linkage disequilibrium positions may change the transcriptional expression level. Several positron emission tomography studies found that DAT in the caudate/putamen of MAP abusers was significantly reduced.[18,19] Some patients showed a lasting reduction of DAT for several months after detoxication. Sekine and colleagues also showed reduction of DAT in the caudate/putamen, and also in the nucleus accumbens and prefrontal cortex of MAP dependents.[19] Elevated DA concentration in the synaptic clefts is removed rapidly by reuptake through DAT. Reduced DAT density in MAP dependence may delay DA clearance and contribute to the persistence of a hyperdopaminergic state. Cocaine potentiates dopaminergic neurotransmission in a different way from MAP, binding to the DAT, blocking neurotransmitter uptake, and giving rise to marked elevations in synaptic DA. It has been reported that chronic cocaine abuse increases α-synuclein levels in midbrain DA neurons.[10] α-Synuclein levels in the DA cell groups of the substantia nigra/ventral tegmental complex were elevated threefold in chronic cocaine users compared with normal age-matched subjects. These results suggest that overexpression of α-synuclein may occur as a protective response to changes in DA turnover. Since the three SNPs were in intron 1, it is possible that these variants contribute to changes in expression of the α-synuclein gene.

In conclusion, our findings suggest a weak association of the α-synuclein gene with MAP psychosis/dependence in our female samples. Further work is necessary to clarify the gender difference, using a larger sample size and/or different ethnic groups of MAP psychotic/dependent subjects as well as functional variations in the α-synuclein gene.

REFERENCES

1. SPILLANTINNI, M.G., M.L. SCHMIDT, V.M.Y. LEE, *et al.* 1997. α-Synuclein in Lewy bodies. Nature **388:** 839–840.
2. SPILLANTINI, M.G., R.A. CROWTHER, R. JAKES, *et al.* 1998. α-Synuclein in filamentous inclusions of Lewy bodies from Parkinson's disease and dementia with Lewy bodies. Proc. Natl. Acad. Sci. USA **95:** 6469–6473.
3. CLAYTON, D.F. & J.M. GEORGE. 1999. Synucleins in synaptic plasticity and neurodegenerative disorders. J. Neurosci. Res. **58:** 120–129.
4. LEE, F.J., F. LIU, Z.B. PRISTUPA, *et al.* 2001. Direct binding and functional coupling of α-synuclein to the dopamine transporters accelerate dopamine-induced apoptosis. FASEB J. **15:** 916–926.
5. ZHOU, W., M.S. HURLBERT, J. SCHAACK, *et al.* 2000. Overexpression of human α-synuclein causes dopamine neuron death in rat primary culture and immortalized mesencephalon-derived cells. Brain Res. **866:** 33–43.
6. ZHOU, W., J. SCHAACK, W.M. ZAWADA, *et al.* 2002. Overexpression of human α-synuclein causes dopamine neuron death in primary human mesencephalic culture. Brain Res. **926:** 42–50.

7. XU, J., S.Y. KAO, F.J. LEE, *et al.* 2002. Dopamine-dependent neurotoxicity of α-synuclein: a mechanism for selective neurodegeneration in Parkinson disease. Nat. Med. **8:** 600–606.
8. LOTHARIUS, J. & P. BRUNDIN. 2002. Pathogenesis of Parkinson's disease: dopamine, vesicles and α-synuclein. Nat. Rev. Neurosci. **3:** 932–942.
9. LITTLE, K.Y., D.P. MCLAUGHLIN, L. ZHANG, *et al.* 1998. Brain dopamine transporter messenger RNA and binding sites in cocaine users: a postmortem study. Arch. Gen. Psychiatry **55:** 793–799.
10. MASH, D.C., Q. OUYANG, J. PABLO, *et al.* 2003. Cocaine abusers have an overexpression of α-synuclein in dopamine neurons. J. Neurosci. **23:** 2564–2571.
11. UJIKE, H. 2002. Stimulant-induced psychosis and schizophrenia: the role of sensitization. Curr. Psychiatry Rep. **4:** 177–184.
12. UJIKE, H., M. HARANO, T. INADA, *et al.* 2003. Nine- or fewer repeat alleles in VNTR polymorphism of the dopamine transporter gene is a strong risk factor for prolonged methamphetamine psychosis. Pharmacogenomics J. **3:** 242–247.
13. AUTERE, J.M., M.J. HILTUNEN, A.J. MANNERMAA, *et al.* 2002. Molecular genetic analysis of the α-synuclein and the parkin gene in Parkinson's disease in Finland. Eur. J. Neurol. **9:** 479–483.
14. SCHNEIDER, S., D. ROESSLI & L. EXCOFFIER. 2000. Arlequin: a software for population genetics data analysis. Ver 2.000. Genetics and Biometry Lab, Department of Anthropology. University of Geneva. Switzerland.
15. SHAM, P.C. & D. CURTIS. 1995. Monte Carlo tests for associations between disease and alleles at highly polymorphic loci. Ann. Hum. Genet. **59:** 97–105.
16. LYNCH, W.J., M.E. ROTH & M.E. CARROLL. 2002. Biological basis of sex differences in drug abuse: preclinical and clinical studies. Psychopharmacology (Berl.) **164:** 121–137.
17. RICHFIELD, E.K., M.J. THIRUCHELVAM, D.A. CORY-SLECHTA, *et al.* 2002. Behavioral and neurochemical effects of wild-type and mutated human α-synuclein in transgenic mice. Exp. Neurol. **175:** 35–48.
18. VOLKOW, N.D., L. CHANG, G.J. WANG, *et al.* 2001. Association of dopamine transporter reduction with psychomotor impairment in methamphetamine abusers. Am. J. Psychiatry **158:** 377–382.
19. SEKINE, Y., M. IYO, Y. OUCHI, *et al.* 2001. Methamphetamine-related psychiatric symptoms and reduced brain dopamine transporters studied with PET. Am. J. Psychiatry **158:** 1206–1214.

アルコールと脳機能

アルコールが脳に与える影響は，酔いをはじめとして様々あり興味ぶかくかつ重要である。ここではアルコールの鎮痛作用とイオンチャネルの一つであるGIRKチャネルとの関係を中心に紹介していただいた。また，アルコールは，人の情動などを脳機能として調べる上でも重要な糸口を与えてくれる物質であるようだ。

池田和隆・小林　徹・曽良一郎

1．アルコールと脳

酒は古くより世界各地で親しまれてきたが，これはアルコールが特に脳に大きな影響を与えるからだと考えられる。アルコールは味覚や嗅覚を刺激して人を楽しませ，食文化において欠かせないものであるだけでなく，ストレス解消，コミュニケーションの促進，発想の転換，食欲増進，睡眠誘導，鎮痛など多くのメリットを持つ。これらのメリットの多くはアルコールが脳機能に影響することによる。もちろんアルコールには，判断力の低下，意識混濁，運動失調，嘔気，中毒や依存，脳萎縮など様々なデメリットもあるが，これらデメリットの多くも脳機能に影響した結果である。アルコールの脳における作用機序を解明することで，アルコールの持つマイナス面を減らしてプラス面をより増やすことが可能になると考えられる。

2．アルコールの脳への作用

脳の進化は第1図に示す模式図で下側から進んできたが，アルコールの影響は主に上側から進むと考えられている。まず理性を司る大脳皮質の機能が抑制されて，通常大脳皮質によって抑制されている大脳辺縁系や視床下部の活動性が上り感情が表れやすくなる。そしてアルコール過量摂取により呼吸や覚醒などを制御している脳幹部分までも麻痺して生命の危険に到る。なぜこの順でアルコールが脳に影響するのかは詳しくはまだ分かっていない。脳は主に神経細胞（ニューロン）と神経膠細胞（グリア）によって構成されており，特に神経細胞が主要な脳機能を担っている。神経細胞は多種多様であるが，第2図に示すような特徴的形態がある。神経細胞は離れた神経細胞や筋肉に神経繊維を伸ばしているので，細胞膜の占める割合が非常に高い。細胞膜は主に脂肪酸で出来ているので，脳は脂肪の割合が高い臓器である。アルコールは水酸基を持つので親水性であると同時に，炭素鎖を持つので脂溶性の特徴も合わせ持っていることなどから，10年ほど前まではアルコールは脳の中の細胞膜部分に非特異的に作用していろいろな効果を発揮するものと考えられてきた。しかし最近，細胞膜の流動性に影響する濃度よりもはるかに低い濃度において，アルコールが脳機能に影響することが明らかになってきた[1]。アルコールの新たな脳内標的分子としては，イオンの通り道を作る蛋白質であるイオンチャネルの中の一群や蛋白質の機能を修飾する働きがある燐酸化酵素の中の一群などが発見されている。

3．アルコールによるGIRKチャネルの開口

このようなアルコールの作用点の一つにGIRKチャネルというイオンチャネルが有ることを最近著者らが発見した[2]。GIRKチャネルとは，G-protein activated inwardly rectifying potassium channelの略で，G蛋白質という蛋白質によって活性が制御される内向き整流性の特徴を持ったカリウムチャネルである[3]。カリウムチャネルとはカリウムイオンが選択的

Alcohol Effects on Brain Functions
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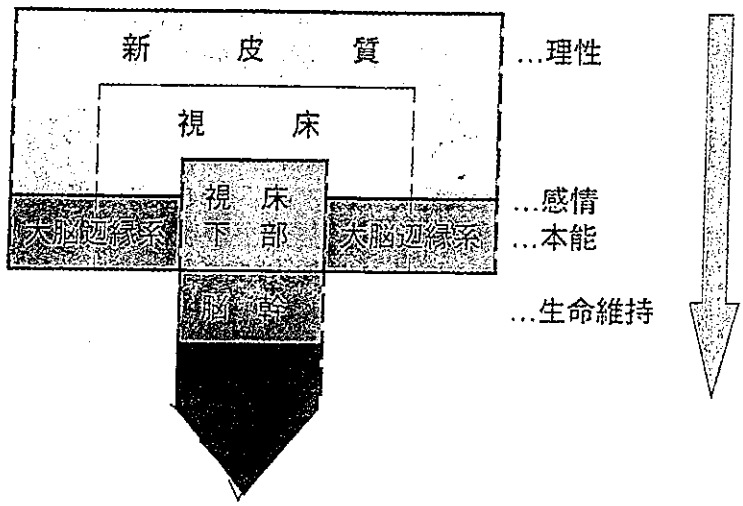
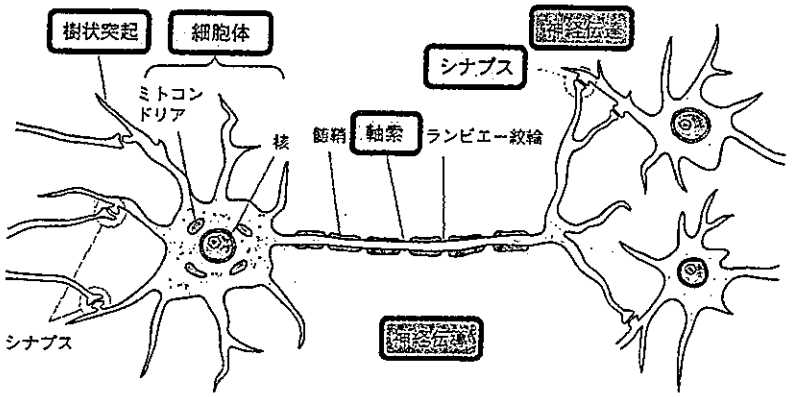


第1図　アルコールが脳に作用する順序



<脳神経科学イラストレイテッドより改変>

第2図　神経細胞の模式図

脳には150億以上の神経細胞が存在し，各神経細胞はそれぞれ千から1万のシナプス入力を受ける。

に通過することを意味する。また，内向き整流性とは細胞膜の外側から内側に電流が流れやすい特性のことであるが，実際の神経細胞では細胞内のカリウム濃度が高いのでカリウムは外向きに流れ，その神経細胞の活動が抑制される。このチャネルの開口は通常，モルヒネの生体内標的であるオピオイド受容体やマリファナの作用点であるカナビノイド受容体などGi/o型のG蛋白質と共役する受容体が活性化したときに，その共役するG蛋白質を介して起こる[4]。GIRKチャネルの構造は第3図に示すように，4つのサブユニットが1つのチャネルを形成し，それぞれのサブユニットは細胞膜を2回貫通して一部分が膜に食い込む構造を持つ。GIRKチャネルのサブユニットには4種類があり，脳ではGIRK 1，2，3サブユニットが発現している[5]。なお，GIRK4は主に心臓で発現するサブユニットである。著者らはGIRKチャネルサブユニットをコードするDNAをクローニングし，人工的にアフリカツメガエル卵母細胞膜上にこのチャネルを発現させることで，チャネル機能の解析を行った。チャネルを発現した卵母細胞は低濃度のエタノールに触れた時にカリウムイオンが流れることが明らかになった。GIRKチャネルの開口はエタノール濃度が10mM以上で認められることから，ほろ酔い期の血中アルコール濃度でGIRKチャネルの機能が影響を受けていると考えられる。メタノール，プロパノール，ブタノールなどもGIRKチャネルの開閉に影響することや，G蛋白質の機能を阻害した条件下でもGIRKチャネルの開口が認められることなどがわかり，アルコールは他の分子を介さずに直接GIRKチャネルに作用することが示唆された。そこで著者らは，一つのチャネル分子の挙動を電気生理学的に解析するパッチクランプ法を用いることで，確かにエタノールがGIRKチャネルを直