（ノルアドレナリン）、エピネフリン（アドレナリン）、セロトニンが知られている。モノアミンを神経伝達物質として使用するシナプスの割合は、他の神経伝達物質に比較して多くはないが、認知や情動などの高次神経機能の調節を担う重要な神経伝達系と考えられ、多くの向精神薬は脳内のモノアミン神経伝達を変化させる。ここではモノアミンを例に、シナプスにおける神経伝達の仕組みについて説明する（図1）。

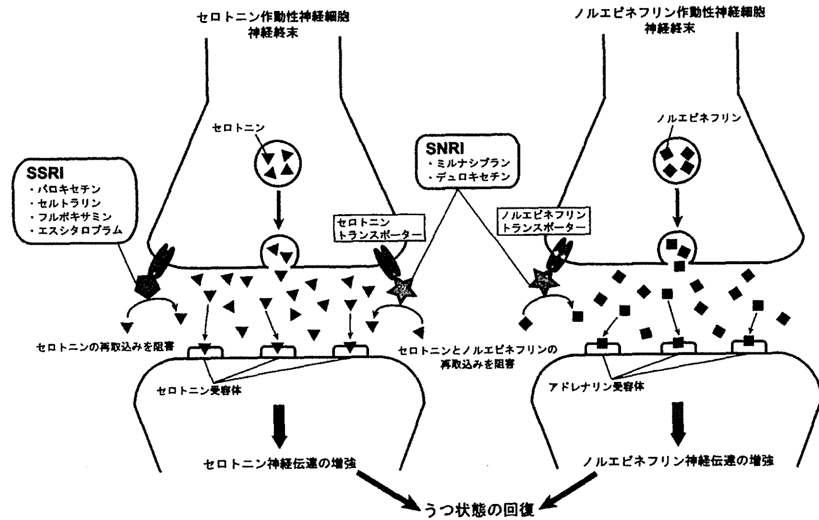
モノアミンは神経細胞内で合成された後、小胞モノアミントランスポーター（transporter：輸送体＝編集部）によってシナプス小胞に貯蔵され、電気信号が神経終末に到達すると小胞からシナプス間隙に放出される。放出されたモノアミンがシナプス後細胞の膜表面にあるモノアミン受容体に結合すると、これがきっかけとなりシナプス後細胞で電気信号が発生し、神経細胞間で情報が伝達される。受容体によって情報を伝えたモノアミンは、シナプス前細胞の膜表面にある細胞膜モノアミントランスポーターによって再び神経終末に取り込まれて、再利用される。あるいは、モノアミン酸化酵素により分解されたり、受動拡散によりシナプス間隙からモノアミンが減少することで神経伝達が終息する。

神経伝達が正常に行われるためには、シナプス間隙にモノアミンが過不足なく放出され、受容体がそれらを受け取り、トランスポーターによって再度取り込まれるという、一連の作業が円滑に行われる必要がある。

●●●モノアミン神経伝達への“くすり”の作用

既に述べたように、神経伝達が正常に行われるためには、モノアミンの放出、受容体へのモノアミンの結合、トランスポーターによるモノアミン再取込みが円滑に行われなければならない。向精神薬はこのいずれか、あるいは複数のステップに作用して、神経伝達を変化させると考えられる。例えば、細胞膜モノアミントランスポーターに作用してモノアミンの再取込みを阻害する、あるいはモノアミン酸化酵素に作用してその働きを阻害する薬物は、シナプス間隙のモノアミン量を増加させて神経伝達を増強させる。一方で、モノアミン受容体に作用する薬物は、受容体がモノアミンと

図2　セロトニンおよびノルエピネフリン神経伝達の調節：抗うつ薬の作用



SSRI：選択的セロトニン再取込み阻害薬
SNRI：セロトニン・ノルエピネフリン再取込み阻害薬
□で囲んだ分子は、各薬物の標的分子であることを示す。

SSRIやSNRIは、セロトニントランスポーターおよびノルエピネフリントランスポーターに結合して、セロトニンやノルエピネフリンの再取込みを阻害する。そのためシナプス間隙のモノアミン量が増加し、神経伝達が増強される。

結合できないようにして神経伝達を抑制する（図1）。このように、神経伝達の各ステップを調節することにより、モノアミン神経伝達を増強させたり、あるいは抑制することができる。特に、モノアミン神経伝達において、細胞膜モノアミントランスポーターとモノアミン受容体は、多くの向精神薬の標的となっている。

ここでは、三種類の細胞膜モノアミントランスポーター（ドーパミントランスポーター、セロトニントランスポーター、ノルエピネフリントランスポーター）に対して作用をもつ薬物を中心に取り上げ、モノアミン神経伝達に対して向精神薬が及ぼす影響について解説する。

(1)モノアミン神経伝達における抗うつ薬の作用

「うつ病」は、一生の間に六人中一人がかかるといわれるほど、私たちに身近な精神疾患である。うつ病は、一見心の問題のように思われがちであるが、実際には脳内の現象が原因で引き起こされる脳の病気である。うつ病にかかると、気分が落ち込んだり、何事にも意欲が無くなったり、自信を喪失したりといったうつ状態に陥る。

うつ病を回復させるために使用される抗うつ薬の一種が、選択的セロトニン再取込み阻害薬（SSRI）である。SSRIはその名の通り、セロトニン作動性神経細胞の神経終末にあるセロトニントランスポーターに選択的に結合してセロトニンの再取込みを阻害し、シナプス間隙におけるセロトニン量を増加させる。また、セロトニン・ノルエピネフリン再取り込み阻害薬（SNRI）はセロトニンの再取込みを阻害するだけでなく、ノルエピネフリントランスポーターに結合してノルエピネフリンの再取込みも阻害することにより、うつ病の症状を改善する。

このように、抗うつ薬は、細胞膜モノアミントランスポーターに作用してモノアミンの再取込みを阻害して、シナプス間隙におけるセロトニンやノルエピネフリンの量を増加させ、セロトニンおよびノルエピネフリン神経伝達を増強することで、結果としてうつ病を回復させる（図2）。この作用のため、脳内のモノアミン量が減少するとうつ病を引き起こすのではないか、という「モノアミン仮説」が提唱された。

しかし、うつ病患者において、脳内のセロトニンやノルエピネフリンの減少を示す報告は必ずしも一致しておらず、抗うつ薬の服用によってうつ病が回復するメカニズムについてもいまだ研究途上である。現在では、抗うつ薬により脳由来神経栄養因子が増加したり、神経新生が促進されたりすることが分かってきたため、抗うつ薬の長期服用により神経回路機能が修復されるからではないかという仮説が考えられている。

(2)モノアミン神経伝達における抗精神病薬の作用

実際には存在するはずのないものが見えたり、聞こえたり、あるいは感じられたりする体験を「幻覚」という。この幻覚や妄想などの特徴的な症状を示す代表

的な精神疾患が「統合失調症」であり、一〇〇人に一人の割合でかかるといわれている。

この統合失調症の治療に使用される抗精神病薬は、精神安定剤の一種であり、ドーパミン受容体のサブタイプの一つである、ドーパミンD2受容体に主に作用して効果を発揮する。定型抗精神病薬は、主にドーパミンD2受容体を遮断してドーパミン神経伝達を抑制する作用をもつ。一方、多くの非定型抗精神病薬は、このドーパミンD2受容体に対する遮断作用に加えて、セロトニン受容体のサブタイプの一つである、セロトニン2A受容体に対しても遮断作用を示すことが特徴である。

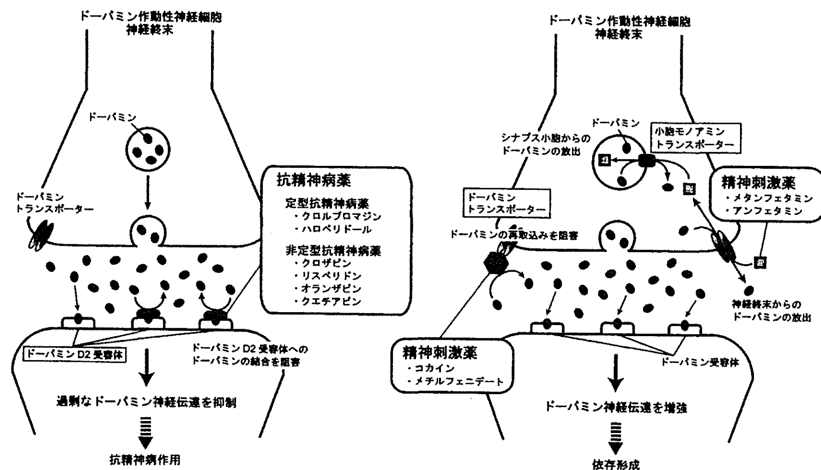
統合失調症の「ドーパミン仮説」では中脳辺縁系という領域でドーパミンの放出が多くなり、神経細胞が過剰に活性化されていると想定されており、定型・非定型抗精神病薬ともにドーパミンD2受容体に対する遮断作用を有していることから、この領域における過剰なドーパミン神経伝達を抑制することにより、幻覚や妄想などの統合失調症に特徴的な症状を改善すると考えられる[1]（図3左図）。

一方で、定型抗精神病薬は大脳基底核や脳下垂体などの他の領域におけるドーパミン神経伝達も抑制してしまうため、運動調節障害などの副作用を併発することが知られている。対して、非定型抗精神病薬は、ドーパミンD2受容体の遮断作用に加えてセロトニン2A受容体の遮断作用を併せ持つことから、抗精神病作用をもちながらも、こうした副作用は少ないとされる。そのため、現在では非定型抗精神病薬が、統合失調症治療の第一選択薬として使用される。

(3)モノアミン神経伝達における精神刺激薬の作用

私たちに快情動、いわゆる気持ち良さをもたらす仕組みの一つに、「ドーパミン報酬系」というメカニズムが知られている。これには、中脳の腹側被蓋野という領域から前脳の側坐核という領域に向かって伸びているドーパミン作動性神経細胞が関与している。快情動に伴い、腹側被蓋野のドーパミン作動性神経細胞から側坐核のシナプス間隙にドーパミンが放出される。放出されたドーパミンは、ドーパミントランスポーターを介して元の神経細胞に再び取り込まれるなどのメカ

図3　ドーパミン神経伝達の調節：抗精神病薬および精神刺激薬の作用



□で囲んだ分子は、各薬物の標的分子であることを示す。

抗精神病薬は、主にドーパミンD2受容体に結合して、過剰なドーパミン神経伝達を抑制することで、抗精神病作用を発揮する。

精神刺激薬のコカインやメチルフェニデートは、ドーパミントランスポーターに結合してドーパミン再取込みを阻害する。一方、メタンフェタミンやアンフェタミンは、小胞モノアミントランスポーターに作用してシナプス小胞からドーパミンを放出させるとともに、ドーパミントランスポーターを介してシナプス間隙にドーパミンを放出させる。

メチルフェニデートは、ADHDやナルコレプシーの治療薬として使用されている。

ニズムによりシナプス間隙から排除されるため、日常生活ではドーパミンが過剰になることはない。

　では、覚せい剤などの精神刺激薬は、どのようにして報酬効果をもたらすのだろうか。精神刺激薬の一種であるコカインやメチルフェニデートは、ドーパミントランスポーターに結合してドーパミンの再取込みを阻害する作用をもつ。一方で、同じ精神刺激薬でもメタンフェタミンやアンフェタミンは、ドーパミントランスポーターにより神経終末に取り込まれる際にドーパミンを神経終末より放出する。さらに、小胞モノアミントランスポーターに作用してシナプス小胞へのドーパミン貯蔵を阻害するとともに、貯蔵されているドーパミンを小胞外に放出させるという複雑な作用機序をもつ。

　このように、コカインやメタンフェタミンなどの精神刺激薬は、いずれもシナプス間隙のドーパミン量を増加させ側坐核の神経細胞を刺激し続けることにより、ドーパミン神経伝達を増強する結果、強い報酬効果をもたらすと考えられる[2]（図3右図）。

　現在、メチルフェニデートは、注意欠陥多動性障害（ADHD）やナルコレプシーの治療薬として用いられている。健常者へのメチルフェニデートの投与は、他の精神刺激薬と同様に興奮や過活動を引き起こすが、ADHD患者に対しては多動の抑制などの治療効果を示すためである。ADHD患者に対するメチルフェニデートの逆説的効果については詳しく解明されていないが、少なくともADHD患者の脳内における化学的異常がモノアミン神経伝達に関係しており、重要な治療標的と考えられる[3]。

＊

　向精神薬の作用機序は幅広く複雑であり、そのすべてが解明されているわけではない。ここで紹介した向精神薬は、主にモノアミントランスポーターやモノアミン受容体に作用して、モノアミン神経伝達を増強、あるいは抑制することで私たちの精神機能に影響を及ぼしている。

　昨今、自閉症スペクトラム障害やADHDなどの発達障害と診断される子どもが増加しており、症状を緩和させるために抗精神病薬や抗うつ薬を服用している場合も少なくない。また、覚せい剤などの精神刺激薬の乱用は若年層まで波及しつつあり、社会的な問題となっている。向精神薬がどのように私たちの脳に働くのか、その作用機序について今後の研究のさらなる進展が期待される。

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Association Between 5HT1b Receptor Gene and Methamphetamine Dependence

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Abstract: Several lines of evidence implicate serotonergic dysfunction in diverse psychiatric disorders including anxiety, depression, and drug abuse. Mice with a knock-out of the 5HT1b receptor gene (*HTR1B*) displayed increased locomotor response to cocaine and elevated motivation to self-administer cocaine and alcohol. Previous genetic studies showed significant associations of *HTR1B* with alcohol dependence and substance abuse, but were followed by inconsistent results. We examined a case-control genetic association study of *HTR1B* with methamphetamine-dependence patients in a Japanese population. The subjects were 231 patients with methamphetamine dependence, 214 of whom had a comorbidity of methamphetamine psychosis, and 248 age- and sex-matched healthy controls. The three single nucleotide polymorphisms (SNPs), rs130058 (A-165T), rs1228814 (A-700C) and rs1228814 (A+1180G) of *HTR1B* were genotyped. There was no significant difference in allelic and genotypic distributions of the SNPs between methamphetamine dependence and the control. Genetic associations of *HTR1B* were tested with several clinical phenotypes of methamphetamine dependence and/or psychosis, such as age at first abuse, duration of latency from the first abuse to onset of psychosis, prognosis of psychosis after therapy, and complication of spontaneous relapse of psychotic state. There was, however, no asscocation between any SNP and the clinical phenotypes. Haplotype analyses showed the three SNPs examined were within linkage disequilibrium, which implied that the three SNPs covered the whole *HTR1B*, and distribution of estimated haplotype frequency was not different between the groups. The present findings may indicate that *HTR1B* does not play a major role in individual susceptibility to methamphetamine dependence or development of methamphetamine-induced psychosis.

Keywords: Methamphetamine dependence, association study, *HTR1B*, haplotype.

INTRODUCTION

Family and twin studies have provided evidence that genetic factors can influence individual differences in vulnerability to substance abuse and dependence [1, 2]. We previously reported that patients with methamphetamine use disorders showed substantial individual differences in psychotomimetic and psychotogenic effects of methamphetamine consumption, e.g., intensity of subjective euphoric effects, latency to onset of methamphetamine-induced psychosis, and prognosis of psychosis after discontinuance of methamphetamine use [3], whose clinical variations should be affected by individual genetic background.

Pharmacological manipulation of serotonergic signaling can modulate the activity of brain reward pathways, and thus the effects of substance dependence to diverse classes of drugs. Fluoxetine, a selective serotonin uptake inhibitor, reduced self-administration of cocaine [4]. Ethanol intake decreased after the administration of 5-HT precursors, 5-HT uptake inhibitors, intracerebral 5-HT, and postsynaptic 5-HT agonists in animals and humans [5]. These serotonergic effects against drug abuse could be mediated by 5HT1b receptors at least. Administration of a 5HT1b agonist, CP-94,253, reduced ethanol self-administration and alcohol-induced aggressive behaviors *via* activation of postsynaptic 5HT1b receptors [6, 7]; in contrast, it facilitated cocaine reward by reducing 5HT release *via* 5HT1b autoreceptor stimulation at presynaptic sites [8]. Mice lacking 5HT1b receptors displayed increased locomotor response to and self-administration of cocaine [9], and elevated alcohol consumption [10].

Previous genetic studies indicated that the 5HT1b receptor gene (*HTR1B*, MIM 182131) was associated with drug dependence and related behaviors. Thus, *HTR1B* polymorphisms were reported to be associated significantly with al-

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coholism with antisocial behaviors [11, 12], whole alcoholism [13, 14], substance dependence [15] and heroin addiction [16], although there were also several inconsistent reports [17, 18]. Therefore, in order to investigate the roles of *HTR1B* in substance dependence, we examined a possible genetic association of *HTR1B* with methamphetamine dependence in a Japanese population.

METHODS

Subjects

The subjects consisted of 231 patients with methamphetamine dependence (184 male, 47 female; mean age±SD, 36.6±11.8) and 248 age-, sex-, and geographical origin-matched healthy controls (198 male, 50 female; mean age±SD, 36.6±10.6), who have no individual or family history of drug dependence or major psychotic disorders such as schizophrenia and bipolar disorders. Almost all patients (N=214) are or were co-morbid with methamphetamine-induced psychosis. All subjects were unrelated Japanese. Consensus diagnoses of methamphetamine dependence were made by two trained psychiatrists according to the ICD-10 criteria on the basis of interviews and medical records. The study protocol and purpose were explained to all subjects participating in the study, and written informed consent was obtained from all subjects. This study was approved by the Ethics Committee of each participating institute of Japanese Genetics Initiative for Drug Abuse (JGIDA) [19].

The patients with methamphetamine dependence and/or psychosis were divided into subgroups according to several clinical phenotypes that may indicate indirectly the severity of and liability to dependence and psychosis: 1) age at first abuse of methamphetamine: younger than 20 years old, which is underage in Japan, or older; 2) latency to onset of psychotic state after initial methamphetamine consumption: divided into two groups by median latency of 3 years; 3) Duration of psychotic state after discontinuance of abuse and therapy with antipsychotics: transient type and prolonged type, which were defined as psychosis that subsides within one month or lasts longer than one month, respectively; 4) complication of spontaneous psychosis after remission of methamphetamine-induced psychosis, and 5) multi-substance abuse status.

Genotyping

HTR1B consists of single exon and is a relatively small gene of about 11.7 Kb. HapMap data indicates that there is only one single nucleotide polymorphism (SNP) in the exon, rs 6297, which was proven polymorphic in a Japanese population. However, we did not examine this SNP because it is synonymous, Val3Val, indicating no or less physiological involvement. Instead, we genotyped three SNPs flanking the the gene, rs6297 (A-700C) and rs130058 (A-161T) in the 5' flanking region and rs1228814 (A+1180G) in the 3' flanking region, which have potential to be functional. Genotyping was performed by the PCR-RFLP method. The genomic DNA was extracted from peripheral leukocytes using a standard method. Each polymorphic site was amplified by PCR (the PCR primer sequence of each SNP is available on request) in a 15-ml volume containing 3% dimethyl sulfoxide and 0.75 units of Taq DNA polymerase (Promega Co., Japan) using a unique primer set. The PCR reaction was performed under the following conditions: 95°C for 5 min, then 35 cycles of 30 s of denaturing at 95°C, 1 min of annealing at the appropriate temperature, and 30 s of extension, and final elongation at 72°C for 10 min. The PCR products were digested with the corresponding restriction enzyme for each polymorphism, Nde*I* for rs6297, Nla*III* for rs130058 and Cfro13*I* for rs1228814, and then electrophoresed on 3.0% agarose gels and stained with GelStar (Takara Co., Japan). All genotyping was performed in a blinded fashion, with the control and cases samples mixed randomly. The genotyping of the SNPs were confirmed in part by direct sequencing.

Statistical Analysis

Statistical analysis of association was performed using SNPAlyze software (Dynacom Co., Japan). Deviation from Hardy-Weinberg equilibrium and the case-control study were tested using the χ^2 test for goodness of fit and χ^2 test for dependence, respectively. Linkage disequilibrium (LD) was tested using the χ^2 test, and D' and r^2 values were made the index in the authorization of LD. Case-control haplotype analysis was performed by the permutation method, and permutation *p*-values were calculated based on 100,000 replications.

RESULTS

The genotype distribution and allele frequencies of the each polymorphism are shown in Table **1**. The genotype distributions of patients and control subjects did not deviate from Hardy-Weinberg equilibrium at any SNP examined. We found no significant difference between the patients and controls in the frequencies of the genotype or allele at any SNP of *HTR1B* (rs6297: allele: p=0.37, genotype: p=0.38, rs130058: allele: p=0.30, genotype: p=0.33 rs1228814: allele: p=0.14, genotype: p=0.47).

We estimated the pairwise LD between the three SNPs of *HTR1B* using the D' and r^2 values as an index. A D' of more than 0.7 was found between all the SNPs (0.8455 between rs6297 and rs130058, 1.000 between rs6297 and rs1228814, 0.8216 between rs130058 and rs1228814) indicating that the three SNPs are in linkage disequilibrium (LD) and located within one LD block. Then, we performed case-control haplotype analysis (Table **2**). There were 5 kinds of haplotypes consisting of the three SNPs. There was no significant difference in distribution of haplotype between methamphetamine dependence and controls (overall permutation *p*=0.81). Neither haplotype consisting of the two SNPs in the promoter region (rs6297 and rs130058) showed a significant difference in distribution between the groups.

Additional analyses of subgroups of patients with methamphetamine dependence/psychosis stratified by five items of clinical phenotypes (Table **3**) revealed that there was no significant association of any SNP of *HTR1B* with any clinical phenotype of methamphetamine dependence and/or psychosis.

DISCUSSION

The 5HT1b receptors are expressed in the brain of rodents, and homologous 5HT1Dβ receptors are expressed in the human brain. The 5HT1b receptors are located at nerve

Table 1. Case-Control Association Analyses of *HTR1B*

Loci Groups	N	Genotype (%)			p	Allele (%)		p
SNP1 (rs6297)		A/A	A/G	G/G		A	G	
Control	248	73.4	23.8	2.8		85.3	14.7	
MAP-dependence	228	68.9	27.6	3.5	0.37	82.7	17.3	0.38
SNP2 (rs130058)		T/T	T/A	A/A		T	A	
Control	227	87.2	12.8	0		93.6	6.4	
MAP-dependence	229	89.5	10.5	0	0.3	94.8	5.2	0.33
SNP3 (rs1228814)		C/C	C/A	A/A		C	A	
Control	246	73.6	25.2	1.2		86.2	13.8	
MAP-dependence	225	70.7	27.5	1.8	0.14	84.4	15.6	0.47

Table 2. Haplotype Analysis of *HTR1B* in Methamphetamine Dependence

Haplotype	Controls	MAP-Dependence	p
A-T-C	0.6973	0.6721	0.41
G-T-C	0.1480	0.1673	0.43
A-T-A	0.0902	0.1095	0.33
A-A-A	0.0520	0.0461	0.68
A-A-C	0.0094	0.0034	0.26

Global permutation *p* value =0.81 (c^2=3.20).

terminals of various pathways and act as autoreceptors that are involved in the regulation of release of diverse neurotransmitters, including serotonin itself [20]. The 5HT1b receptors are also located at postsynaptic sites. A lot of studies suggest that 5HT1b receptors are implicated in several physiological functions, behaviors, and neuropsychiatric disorders including migraine, aggression, anxiety, depression, and substance dependence [20].

Genetic associations of HTR1B have been examined with various psychiatric conditions such as antisocial behaviors, suicide, depression, and schizophrenia. As to substance dependence, Lappapleinene *et al.* [11] found that rs6296, a synonymous SNP in exon 1 (G861C, Val3Val), was associated with antisocial alcoholism in two independent populations of alcoholic patients for the first time, but this was followed by consistent [12] and inconsistent results [15, 17, 18, 21]. Finally, Fehr *et al.* [13] reported that the risk allele of 861C reported by Lappapleinene *et al.* [11] was protective in their patients with alcoholism. These inconsistencies among alcoholism studies may indicate that status of co-morbidity with other substance abuse could influence the results because *HTR1B* was shown to be associated with substance abuse [15] and heroin addiction [16].

In the present study, we examined three SNPs in the 5' and 3' flanking regions of *HTR1B*, rs6297 (A-700C), rs130058 (A-161T), and rs1228814 (A+1180G) in patients with methamphetamine dependence and found no association at any loci. Neither was any association found with several clinical phenotypes, such as initial abuse of methamphetamine at a younger age, rapid onset of psychotic state induced by methamphetamine, longer duration of psychosis after discontinuance of methamphetamine abuse, complication of spontaneous relapse of psychosis after remission, and multi-substance abuse status. Haplotype analysis of the three SNPs also showed no significant difference in haplotype distribution between the patients and controls. As the LD block consisting of the three SNPs covers the whole of *HTR1B*, it is unlikely that any untested polymorphism including G861C in *HTR1B* could be associated with methamphetamine dependence or its clinical phenotypes. Our findings are consisted with a study of cocaine, another psychostimulant, which showed that T-261G, A-161T, and G861C of *HTR1B* was not associated with cocaine abuse [17].

Duan *et al.* [22] examined effects of common SNPs in the promoter region of *HTR1B* on its transcription activity by *in vitro* reporter assay and revealed that T-261G and A-161T (rs130058) potently affected gene expression. The haplotypes consisting of -261G and -161A enhanced transcriptional activity 2.3-fold compared with major haplotype consisting of -261T and -161T. The A-161T polymorphism altered characteristics of binding to AP-1 transcription factor.

Table 3. Association of *HTR1B* with Clinical Phenotypes of Methamphetamine Dependence and Psychosis

SNP1 (rs6297)	N	Genotype (%)			*p*	Allele (%)		*p*
		A/A	A/G	G/G		A	G	
Age at first use								
20y <=	111	0.68	0.26	0.05		0.82	0.18	
19y >=	113	0.68	0.30	0.02	0.37	0.83	0.17	0.38
Latency of psychosis								
3y >	103	0.64	0.31	0.05		0.80	0.20	
3y <=	83	0.70	0.28	0.02	0.57	0.84	0.16	0.31
Prognosis of psychosis								
Transient	114	0.68	0.30	0.03		0.82	0.18	
Prolonged	84	0.69	0.26	0.05	0.65	0.82	0.36	0.94
Spontaneous relapse of psychotic symptoms								
+	84	0.62	0.36	0.02		0.80	0.20	
−	129	0.71	0.24	0.05	0.15	0.83	0.17	0.35
Poly-substance abuse								
+	158	0.69	0.28	0.03		0.83	0.17	
−	63	0.68	0.27	0.05	0.85	0.82	0.18	0.77
SNP2 (rs130058)	**N**	**Genotype (%)**			***p***	**Allele (%)**		***p***
		T/T	**T/A**	**A/A**		**T**	**A**	
Age at first use								
20y <=	111	0.86	0.14	0.00		0.93	0.07	
19y >=	114	0.92	0.08	0.00	0.31	0.96	0.04	0.33
Latency of psychosis								
3y >	103	0.91	0.09	0.00		0.96	0.04	
3y <=	84	0.90	0.10	0.00	0.85	0.95	0.05	0.86
Prognosis of psychosis								
Transient	115	0.88	0.12	0.00		0.94	0.06	
Prolonged	84	0.93	0.07	0.00	0.24	0.96	0.04	0.26
Spontaneous relapse of psychotic symptoms								
+	85	0.92	0.08	0.00		0.96	0.04	
−	129	0.87	0.13	0.00	0.26	0.93	0.07	0.28
Poly-substance abuse								
+	159	0.90	0.10	0.00		0.95	0.05	
−	63	0.87	0.13	0.00	0.57	0.94	0.06	0.58

Table 3. contd....

SNP3 (rs1228814)	N	Genotype (%)			p	Allele (%)		p
		C/C	C/A	A/A		C	A	
Age at first use								
20y <=	110	0.72	0.25	0.04		0.84	0.16	
19y >=	111	0.68	0.32	0.00	0.14	0.84	0.16	0.47
Latency of psychosis								
3y >	100	0.76	0.22	0.02		0.87	0.13	
3y <=	83	0.66	0.33	0.01	0.27	0.83	0.17	0.23
Prognosis of psychosis								
Transient	113	0.71	0.28	0.01		0.85	0.15	
Prolonged	82	0.73	0.24	0.02	0.59	0.85	0.15	0.91
Spontaneous relapse of psychotic symptoms								
+	81	0.73	0.27	0.00		0.86	0.14	
−	129	0.69	0.28	0.03	0.27	0.83	0.17	0.34
Poly-substance abuse								
+	156	0.70	0.28	0.02		0.84	0.16	
−	62	0.69	0.29	0.02	0.98	0.84	0.16	0.98

Therefore, our negative findings may be significant and indicate that higher or lower density of the 5HT1b receptor due possession of -161A or -161T of *HTR1B* does not affect individual susceptibility to methamphetamine dependence and psychosis.

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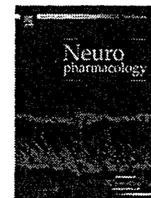

Serotonin 1A receptor gene is associated with Japanese methamphetamine-induced psychosis patients

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Background: Several investigations have reported associations the serotonin 1A (5-HT1A) receptor to schizophrenia and psychotic disorders, making 5-HT1A receptor gene (*HTR1A*) an adequate candidate gene for the pathophysiology of schizophrenia and methamphetamine (METH)-induced psychosis. Huang and colleagues reported that rs6295 in *HTR1A* was associated with schizophrenia. The symptoms of methamphetamine (METH)-induced psychosis are similar to those of paranoid type schizophrenia. It may indicate that METH-induced psychosis and schizophrenia have common susceptibility genes. In support of this hypothesis, we reported that the V-act murine thymoma viral oncogene homologue 1 (AKT1) gene was associated with METH-induced psychosis and schizophrenia in the Japanese population. Furthermore, we conducted an analysis of the association of *HTR1A* with METH-induced psychosis.
Method: Using one functional SNP (rs6295) and one tagging SNP (rs878567), we conducted a genetic association analysis of case-control samples (197 METH-induced psychosis patients and 337 controls) in the Japanese population. The age and sex of the control subjects did not differ from those of the methamphetamine dependence patients.
Results: Rs878567 was associated with METH-induced psychosis patients in the allele/genotype-wise analysis. Moreover, this significance remained after Bonferroni correction. In addition, we detected an association between rs6295 and rs878567 in *HTR1A* and METH-induced psychosis patients in the haplotype-wise analysis. Although we detected an association between rs6295 and METH-induced psychosis patients, this significance disappeared after Bonferroni correction.
Conclusion: *HTR1A* may play an important role in the pathophysiology of METH-induced psychosis in the Japanese population. However, because we did not perform a mutation scan of *HTR1A*, a replication study using a larger sample may be required for conclusive results.
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1. Introduction

Altered serotonergic neural transmission is hypothesized to be a susceptibility factor for schizophrenia (Geyer and Vollenweider, 2008; Meltzer et al., 2003). Several postmortem studies reported increased serotonin 1A (5-HT1A) receptor in the prefrontal cortex

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of schizophrenic patients (Burnet et al., 1996; Hashimoto et al., 1993, 1991; Simpson et al., 1996; Sumiyoshi et al., 1996). Huang and colleagues reported that rs6295 in an SNP (C-1019G: rs6295) in the promoter region of the 5-HT1A receptor gene (*HTR1A*), which regulate HTR1A transcription (Le Francois et al., 2008; Lemonde et al., 2003), was associated with schizophrenia (Huang et al., 2004). These facts suggest a crucial relationship between the 5-HT1A receptor and schizophrenia, and that *HTR1A* is an adequate candidate for the etiology of schizophrenia. *HTR1A* (OMIM*109 760, 1 exon in this genomic region spanning 2.069 kb) is located on 5q11.

The symptoms of methamphetamine (METH)-induced psychosis are similar to those of paranoid type schizophrenia (Sato et al., 1992). It may indicate that METH-induced psychosis and schizophrenia have common susceptibility genes (Bousman et al., 2009). In support of this hypothesis, we reported that the V-act murine thymoma viral oncogene homologue 1 (AKT1) gene was associated with METH-induced psychosis (Ikeda et al., 2006) and schizophrenia (Ikeda et al., 2004) in the Japanese population. Furthermore, we conducted an analysis of the association of these genes with METH-induced psychosis, using the recently recommended strategy of 'gene-based' association analysis (Neale and Sham, 2004).

2. Materials and methods

2.1. Subjects

The subjects in the association analysis were 197 METH-induced psychosis patients (164 males: 83.2% and 33 females; mean age ± standard deviation (SD) 37.6 ± 12.2 years) and 337 healthy controls (271 males: 80.4% and 66 females; 37.6 ± 14.3 years). The age and sex of the control subjects did not differ from those of the methamphetamine dependence patients. All subjects were unrelated to each other, ethnically Japanese, and lived in the central area of Japan. The patients were diagnosed according to DSM-IV criteria with consensus of at least two experienced psychiatrists on the basis of unstructured interviews and a review of medical records. METH-induced psychosis patients were divided into two categories of psychosis prognosis, the transient type and the prolonged type, which showed remission of psychotic symptoms within 1 month and after more than 1 month, respectively, after the discontinuance of methamphetamine consumption and beginning of treatment with neuroleptics; 112 patients (56.9%) were the transient type, and 85 patients (43.1%) were the prolonged type. One hundred thirty-seven subjects with METH-induced psychosis also had dependence on drugs other than METH. Cannabinoids were the most frequency abused drugs (31.4%), followed by cocaine (9.09%), LSD (9.09%), opioids (7.69%), and hypnotics (7.69%). Subjects with METH-induced psychosis were excluded if they had a clinical diagnosis of psychotic disorder, mood disorder, anxiety disorder or eating disorder. More detailed characterizations of these subjects have been published elsewhere (Kishi et al., 2008b). All healthy controls were also psychiatrically screened based on unstructured interviews. None had severe medical complications such as liver cirrhosis, renal failure, heart failure or other Axis-I disorders according to DSM-IV.

The study was described to subjects and written informed consent was obtained from each. This study was approved by the Ethics Committee at Fujita Health University, Nagoya University School of Medicine and each participating member of the Institute of the Japanese Genetics Initiative for Drug Abuse (JGIDA).

2.2. SNPs selection and linkage disequilibrium (LD) evaluation

We first consulted the HapMap database (release#23.a.phase2, Mar 2008, www.hapmap.org, population: Japanese Tokyo: minor allele frequencies (MAFs) of more than 0.05) and included 3 SNPs (rs6449693, rs878567 and rs1423691) covering *HTR1A* (5′-flanking regions including about 1 kb from the initial exon and about 2 kb downstream (3′) from the last exon: HapMap database contig number chr5: 63287418...63291774). Then one tagging SNP was selected with the criteria of an r^2 threshold greater than 0.8 in 'pair-wise tagging only' mode using the 'Tagger' program (Paul de Bakker, http://www/broad.mit.edu/mpg/tagger) of the HAPLOVIEW software (Barrett et al., 2005).

HTR1A has also been reported to have one biologically functional SNP (C-1019G: rs6295) (Albert et al., 1996; Albert and Lemonde, 2004; Lemonde et al., 2003). Rs6295 (C-1019G) in the promoter region regulate *HTR1A* transcription (Le Francois et al., 2008; Lemonde et al., 2003). The C allele is a part of a 26 palindrome that connect transcription factors (Deaf-1, Hes1 and Hes5) by NUDR (nuclear deformed epidermal autoregulatory factor), whereas the G allele abolishes repression by NUDR (Le Francois et al., 2008; Lemonde et al., 2003). This would lead to elevated levels of 5-HT1A receptor in the presynaptic raphe nucleus in GG genotypes, compared with CC genotype (Le Francois et al., 2008; Lemonde et al., 2003). Since no information about rs6295 was shown in the HapMap database, we included this SNP. These two SNPs were then used for the following association analysis.

2.3. SNPs genotyping

We used TaqMan assays (Applied Biosystems, Inc., Foster City, CA) for both SNPs. Detailed information, including primer sequences and reaction conditions, is available on request.

2.4. Statistical analysis

Genotype deviation from the Hardy–Weinberg equilibrium (HWE) was evaluated by chi-square test (SAS/Genetics, release 8.2, SAS Japan Inc., Tokyo, Japan).

Marker-trait association analysis was used to evaluate allele- and genotype-wise association with the chi-square test (SAS/Genetics, release 8.2, SAS Japan Inc., Tokyo, Japan), and haplotype-wise association analysis was conducted with a likelihood ratio test using the COCAPHASE2.403 program (Dudbridge, 2003). We used the permutation test option as provided in the haplotype-wise analysis to avoid spurious results and correct for multiple testing. Permutation test correction was performed using 1000 iterations (random permutations). In addition, Bonferroni's correction was used to control inflation of the type I error rate in the single marker association analysis and in the explorative analysis. For Bonferroni correction, we employed the following numbers for multiple testing: 2 for each sample set in allele- and genotype-wise analysis (2 examined SNPs). We had already performed a permutation test in the haplotype-wise analysis. Power calculation was performed using a genetic power calculator (Purcell et al., 2003).

The significance level for all statistical tests was 0.05.

3. Results

The LD from rs6449693, rs878567 and rs1423691 was tight in from the HapMap database samples ($r^2 = 1.00$). However, the LD structure of rs6295 (functional SNP) and rs878567 (tagging SNP) in our control samples was not tight ($r^2 = 0.160$). Genotype frequencies of all SNPs were in HWE (Table 1). Rs878567 was associated with METH-induced psychosis patients in the allele/genotype-wise analysis (P allele = 0.000122 and P genotype = 0.00103) (Table 1). Moreover, these significances remained after Bonferroni correction (P allele = 0.000244 and P genotype = 0.00203) (Table 1). In addition, we detected an association between rs6295 and rs878567 in *HTR1A* and METH-induced psychosis patients in the haplotype-wise analysis ($P = 0.0000643$) (Table 2). Although we detected an association between rs6295 and METH-induced psychosis patients (P allele = 0.0271), this significance disappeared after Bonferroni correction (P allele = 0.0542) (Table 1).

4. Discussion

We found associations between *HTR1A* and Japanese METH-induced psychosis patients. Therefore, we reasoned that *HTR1A* may play an important role in the pathophysiology of METH-induced psychosis in the Japanese population. However, our samples are small. Although Bonferroni's correction was used to control inflation of the type I error rate, we considered that there is a possibility of type I error in these results.

The 5-HT1A receptor is present in various regions of the brain, including the cortex, hippocampus, amygdala, hypothalamus and septum (Aznar et al., 2003; Barnes and Sharp, 1999; Le Francois et al., 2008; Varnas et al., 2004). Presynaptic 5-HT1A autoreceptors play an important role in the autoregulation of serotonergic neurons (Le Francois et al., 2008; Lemonde et al., 2003; Riad et al., 2000; Sotelo et al., 1990). The 5-HT1A receptor activation by serotonin induces the hyperpolarization of serotonergic neurons, decreasing their firing rate and consequently the release of serotonin in the brain (Le Francois et al., 2008; Lemonde et al., 2003; Riad et al., 2000; Sotelo et al., 1990). Also, the 5-HT1A receptor was associated hippocampal neurogenesis. The hippocampus is a part of the limbic system involved in cognitive function such as memory. Stimulation of 5-HT1A receptors has been known to reduce the

Table 1
Association analysis of *HTR1A* with methamphetamine-induced psychosis.

SNP[a]	Phenotype[b]	MAFs[c]	N	Genotype distribution[d]			P-value[f]			Corrected P-value[f,g]	
				M/M	M/m	m/m	HWE[e]	Genotype	Allele	Genotype	Allele
rs6295	Controls	0.254	336	192	117	27	0.132				
C > G	METH-induced psychosis	0.317	197	92	85	20	0.955	0.0657	**0.0271**		0.0542
rs878567	Controls	0.126	336	258	71	7	0.423				
C > T	METH-induced psychosis	0.216	197	124	61	12	0.233	**0.00103**	**0.000122**	**0.00203**	**0.000244**

[a] Major allele > minor allele.
[b] METH-induced psychosis: methamphetamine-induced psychosis.
[c] MAFs: minor allele frequencies.
[d] M: major allele, m: minor allele.
[e] Hardy–Weinberg equilibrium.
[f] Bold represents significant P-value.
[g] Calculated using Bonferroni's correction.

negative symptoms and cognitive dysfunction of schizophrenia (Meltzer et al., 2003; Meltzer and Sumiyoshi, 2008; Sumiyoshi et al., 2001, 2007). Mason and Reynolds (1992) reported that one of the major pharmacological therapeutic targets of clozapine is 5-HT1A receptors on cortical glutamatergic neurons. Several post-mortem studies reported increased 5-HT1A receptor in the prefrontal cortex of schizophrenic patients (Burnet et al., 1996; Hashimoto et al., 1993, 1991; Simpson et al., 1996; Sumiyoshi et al., 1996). NAN-190 (5-HT1A receptor antagonist) produced an inhibitory action on methamphetamine-induced hyperactivity (Ginawi et al., 2004; Millan and Colpaert, 1991). These facts suggest that altered serotonergic neural transmission caused by abnormalities in 5-HT1A receptor may be involved in the development of psychotic disorders such as schizophrenia and METH-induced psychosis (Geyer and Vollenweider, 2008; Meltzer et al., 2003).

Serretti et al. (2007) reported that rs878567 in *HTR1A* was associated with German and Italian suicidal attempters. Also, previous study have reported that rs878567 in *HTR1A* was found the interaction with childhood physical abuse in mood disorders (Brezo et al., 2009). These authors suggested rs878567 might influence hippocampus-mediated memory deficits in mood disorders (Brezo et al., 2009). The LD from rs6449693, rs878567 and rs1423691 was tight in from the HapMap database samples ($r^2 = 1.00$). As these results show, rs878567 covers a wide and important region including the exon and the promoter region in *HTR1A*. Because it is possible that rs878567 influences biological function in the brain, we suggest that functional analysis for rs878567 should be performed in future studies.

Rs6295 (C-1019G) in the promoter region regulate *HTR1A* transcription (Le Francois et al., 2008; Lemonde et al., 2003). The C allele is a part of a 26 palindrome that connect transcription factors (Deaf-1, Hes1 and Hes5) by NUDR (nuclear deformed epidermal autoregulatory factor), whereas the G allele abolishes repression by NUDR (Le Francois et al., 2008; Lemonde et al., 2003). This would lead to elevated levels of 5-HT1A receptor in the presynaptic raphe nucleus in GG genotyps, compared with CC genotype (Le Francois et al., 2008; Lemonde et al., 2003). This variant was associated with several studies, including major depressive disorder (Anttila et al., 2007; Kraus et al., 2007; Lemonde et al., 2003; Neff et al., 2009; Parsey et al., 2006) and panic disorder (Strobel et al., 2003) and antidepressant response in MDD (Arias et al., 2005; Hong et al., 2006; Lemonde et al., 2004; Parsey et al., 2006; Serretti et al., 2004; Yu et al., 2006). Huang et al. (2004) reported that rs6295 was associated with schizophrenia. Recent studies reported that rs6295 was associated with the improvement in negative symptoms from antipsychotics such as risperidone (Mossner et al., 2009; Reynolds et al., 2006; Wang et al., 2008) and that 5-HT1A receptor agonists such as tandospirone produced improvements in the cognitive impairment in schizophrenia (Meltzer and Sumiyoshi, 2008; Sumiyoshi et al., 2001, 2007).

A few points of caution should be mentioned with respect to our results. Firstly, the positive association may be due to small sample size. Ideal samples for this study are METH use disorder samples with and without psychosis. Because we had only a few METH use disorder samples without psychosis, and we wanted to avoid statistical error, we did not perform an association analysis with these samples. Secondly, we did not include a mutation scan to detect rare variants. We designed the study based on the common disease-common variants hypothesis (Chakravarti, 1999). However, Weickert et al. (2008) have shown associations between a common disease such as schizophrenia and rare variants. If the genetic background of METH-induced psychosis is described by the common disease-rare variants hypothesis, further investigation will be required, such as medical resequencing using larger samples. However, statistical power is needed to evaluate the association of rare variants. Lastly, our subjects did not undergo structured interviews. However, in this study patients were carefully diagnosed according to DSM-IV criteria with consensus of at least two experienced psychiatrists on the basis of a review of medical records (Kishi et al., 2008a,c, 2009). In addition, when we found misdiagnosis in a patient, we promptly excluded the misdiagnosed case to maintain the precision of our sample. To overcome these limitations, a replication study using larger samples or samples of other populations will be required for conclusive results.

In conclusion, our results suggest that *HTR1A* may play a major role in the pathophysiology of METH-induced psychosis in the Japanese population. However, because we did not perform a mutation scan of *HTR1A*, a replication study using a larger sample may be required for conclusive results.

Table 2
Haplotype-wise analysis of *HTR1A*.

Haplotype rs6295-rs878567	Phenotype[a]	Individual haplotype frequency	Individual P-value[b]	Phenotype[a]	Global P-value[b]
C–C	Control	0.811			
	METH-induced psychosis	0.694	**0.0000364**	METH-induced psychosis	**0.0000643**
G–C	Control	0.189			
	METH-induced psychosis	0.306	**0.0000364**		

[a] METH-induced psychosis: methamphetamine-induced psychosis.
[b] Bold numbers represent significant P-value.

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Chronic Treatment With Aripiprazole Prevents Development of Dopamine Supersensitivity and Potentially Supersensitivity Psychosis

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Background: **Long-term treatment of schizophrenia with antipsychotics is crucial for relapse prevention, but a prolonged blockade of D_2 dopamine receptors may lead to the development of supersensitivity psychosis. We investigated the chronic effects of aripiprazole (ARI) on dopamine sensitivity.** ***Methods:*** **We administered ARI (1.5 mg/kg/d), haloperidol (HAL; 0.75 mg/kg/d), or vehicle (VEH) via minipump for 14 days to drug-naive rats or to rats pretreated with HAL (0.75 mg/kg/d) or VEH via minipump for 14 days. On the seventh day following treatment cessation, we examined the effects of the treatment conditions on the locomotor response to methamphetamine and on striatal D_2 receptor density (N = 4-10/condition/experiment).** ***Results:*** **Chronic treatment with HAL led to significant increases in locomotor response and D_2 receptor density, compared with the effects of chronic treatment with either VEH or ARI; there were no significant differences in either locomotor response or D_2 density between the VEH- and ARI-treated groups. We also investigated the effects of chronic treatment with HAL, ARI, or VEH preceded by HAL or VEH treatment on locomotor response and D_2 density. ANOVA analysis indicated that the rank ordering of groups for both locomotor response and D_2 density was HAL-HAL > HAL-VEH > HAL-ARI > VEH-VEH.** ***Conclusions:*** **Chronic treatment with ARI prevents development of dopamine supersensitivity and potentially supersensitivity psychosis, suggesting that by reducing excessive sensitivity to dopamine and by stabilizing sensitivity for an extended period of time, ARI may be helpful for some patients with treatment-resistant schizophrenia.**

Key words: D_2 dopamine receptor/locomotor activity/partial agonist/radioligand binding assay/rat/striatum

Introduction

For decades, the standard schizophrenia treatment protocol has included the administration of D_2 dopamine receptor blockers as effective antipsychotics, especially for the amelioration of psychotic symptoms.[1] Long-term, continuous treatment with antipsychotic agents is emphasized as a treatment strategy to encourage remission in people with schizophrenia because the chance of relapse is decreased if pharmacotherapy continues uninterrupted.[2] However, even among stabilized patients maintained on optimal doses of antipsychotic depot therapy, significant rates of relapse have been reported.[3] It is widely recognized that a small reduction in antipsychotic dosage or a short-term interruption in antipsychotic drug therapy can induce an acute exacerbation of psychotic symptoms and that the dose of antipsychotics needed to reduce such symptoms tends to increase with each relapse.[4,5] There may be multiple causes for this phenomenon, including the development of the disease itself. One of the possible explanations, however, may be the development of supersensitivity psychosis associated with long-term treatment with D_2 receptor blockers.[6–10]

Aripiprazole (ARI), an atypical antipsychotic that is commercially known as a dopamine partial agonist, is clinically used to treat schizophrenia. Treatment with ARI has been associated with the lowest rate of rehospitalization (71% risk reduction) among antipsychotics in clinical use, including both first- and second-generation antipsychotics,[11] which suggests that, compared with other antipsychotics, ARI may more efficiently lower the risk of relapse or prevent a worsening of psychotic symptoms. We hypothesized that these clinical consequences might be related to certain unique effects of ARI on the development of supersensitivity psychosis.

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Dopamine supersensitivity in animals has been used as a model of supersensitivity psychosis in humans. Experimental rats chronically or subchronically treated with D_2 receptor antagonists develop dopamine supersensitivity in terms of behavior and movement, with increased striatal D_2 receptor density.[12–16] Based on the unique pharmacokinetic effects of ARI on D_2 receptors, eg, partial agonism, we hypothesized that chronic treatment with ARI not only does not induce dopamine supersensitivity but actually reduces the dopamine supersensitivity induced by D_2 receptor antagonists. In order to test these 2 hypotheses in the present study, we investigated the effects of chronic ARI treatment on (*a*) the behavioral sensitivity of experimental rats to methamphetamine (MAP) and (*b*) the density of striatal D_2 receptors.

Methods

Animals

Male Sprague Dawley rats (CLEA Japan Inc.) weighing 240-260 g were used. The animals were housed in groups of 2 per cage and were maintained under standard conditions (12 h-12 h light-dark cycle: lights on from 0700 to 1900 h; room temperature, 22 ± 2°C; humidity, 55 ± 5%) with free access to food and water. Experiments were performed in accordance with the *Guide for the Care and Use of Laboratory Animals* (1996).

Drugs

Aripiprazole (ARI; 1.5 mg/kg/d; a gift from Otsuka Pharmaceutical Co., Ltd.) and haloperidol (HAL; 0.75 mg/kg/d; Toronto Research Chemicals Inc.) were dissolved in a 2% glacial acetic acid/H_2O solution (pH adjusted to 3.0-3.8 with NaOH). These drugs were given via an Alzet osmotic minipump (model 2ML2; 14-day delivery; DURECT Corp.). Methamphetamine-HCl (MAP; 1.0 mg/kg; Dainippon Pharmaceutical, Ltd.) was dissolved in 0.85% saline and administered intraperitoneally (i.p.) in a volume of 1 ml/kg body weight.

The dose of HAL, 0.75 mg/kg/day, was determined based on data from a previous report,[16] and the dose of 1.5 mg/kg/day of ARI was equivalent to the dose of HAL, according to human clinical studies.[17] In a preliminary study, we examined the effects of these drugs on MAP-induced locomotion on the third day and the seventh day after the administration via minipump to the rats. As regards the total locomotor activity observed for 60 min after MAP injection, compared with the VEH-treated group, the ARI- and HAL-treated groups exhibited significantly smaller amounts of activity, ie, 74.2% (±SEM 0.5) and 94.9% (±SEM 0.2) less activity on the third day and 75.5% (±SEM 5.8) and 40.0% (±SEM 12.3) less activity on the seventh day, respectively. In other words, both the ARI and HAL treatments significantly suppressed MAP-induced hyperlocomotion ($P < .05$; one-way ANOVA).

[^{3}H]raclopride (80.1 Ci/mmol) was purchased from PerkinElmer Life Science. Other chemicals were purchased commercially.

Minipump Implantation

An Alzet osmotic minipump containing either vehicle (VEH; 2% glacial acetic acid/H_2O solution), HAL, or ARI was implanted under 5% pentobarbital sodium anesthesia. A 1.5-cm-wide incision was made in each animal's lower back, and hemostats were used to loosen connective tissue between the scapulae. Minipumps were inserted to lie on either side of the scapulae, with the flow moderator pointed away from incision. When a subsequent pump was implanted in exchange for a former one, the most recent pump was inserted on the other side of the scapulae across from the former pump. The incision was closed using 9-mm surgical staples and cleaned with 70% ethanol.

Groups and Procedures

Experiment 1 was designed to test whether or not chronic treatment with ARI induces dopamine supersensitivity (figure 1). Forty-five rats were divided into 3 groups (n = 15 each) that received the following treatments: (1) ARI at 1.5 mg/kg/d for 14 days (ARI group), (2) HAL at 0.75 mg/kg/d for 14 days (HAL group), and (3) VEH for 14 days (VEH group). Within each group, 10 rats were subjected to MAP-induced locomotion tests (Experiment 1a; n = 10 rats per treatment protocol), and the other 5 rats were used for radioligand binding assays (Experiment 1b; n = 5 rats per treatment protocol).

Experiment 2 was designed to determine whether chronic treatment with ARI reduces dopamine supersensitivity induced by chronic treatment with HAL (figure 1). Forty-eight rats were divided into 4 groups (n = 12 per group) that received the following treatments: (1) HAL at 0.75 mg/kg/d for 14 days, followed by ARI at 1.5 mg/kg/d for 14 days (HAL-ARI group); (2) HAL at 0.75 mg/kg/d for 14 days, followed by HAL at 0.75 mg/kg/d for 14 days (HAL-HAL group); (3) HAL at 0.75 mg/kg/d for 14 days, followed by VEH for 14 days (HAL-VEH group); and (4) VEH for 14 days, followed by VEH for 14 days (VEH-VEH group). All the drugs used for the first 14-day period were administered via minipump; the minipumps were then exchanged to administer the second set of drugs for the second 14-day period. Within each treatment group, 8 rats were subjected to MAP-induced locomotion tests (Experiment 2a; n = 8 per treatment protocol), and the other 4 rats were subjected to radioligand binding assays (Experiment 2b; n = 4 per treatment protocol).

Tests of MAP-Induced Locomotion

On the seventh day following treatment cessation (removal of the minipumps), MAP-induced locomotion

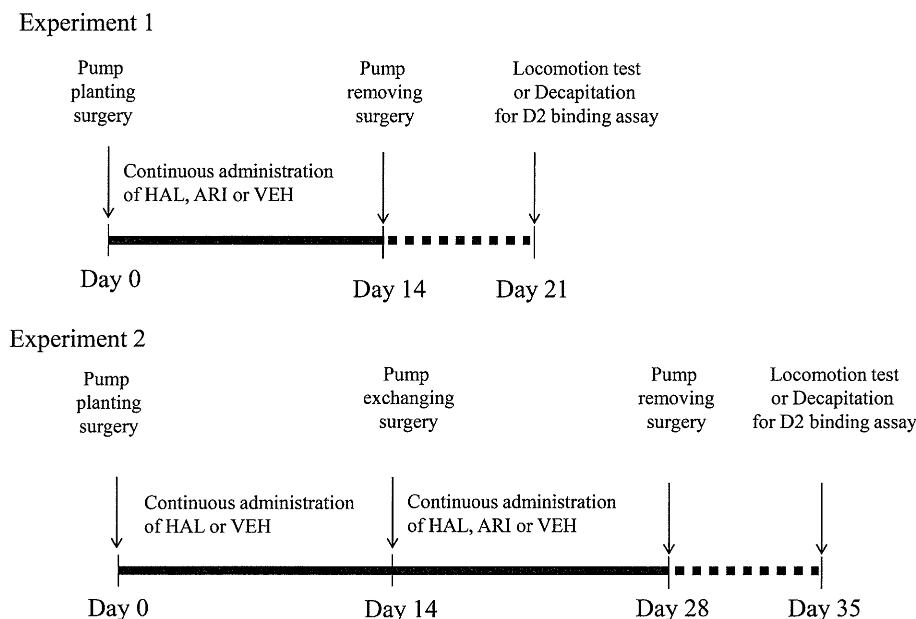

Fig. 1. Graphic depiction of the sequence of treatment and testing for experiments 1 and 2. In Experiment 1, an Alzet osmotic minipump was implanted into each rat, and drugs were administered starting on Day 0. The minipump was removed on Day 14, and the evaluation of either locomotor response to methamphetamine or the decapitation to evaluate D_2 receptor binding occurred on Day 21, ie, on the seventh day following treatment cessation. In Experiment 2, a minipump was implanted into each rat, and drugs were administered starting on Day 0; the first minipump was exchanged for a second minipump on Day 14. The second minipump was removed on Day 28, and the evaluation of either locomotor response to methamphetamine or the decapitation to evaluate D_2 receptor binding occurred on Day 35, ie, on the seventh day following the cessation of 2 consecutive treatment periods (total, 28 days). HAL indicates 0.75 mg/kg/d of haloperidol; ARI, 1.5 mg/kg/d of aripiprazole; and VEH, 2% glacial acetic acid/H_2O solution as vehicle.

was measured (Experiment 1a and 2a). Locomotor activity was assessed using an animal movement analysis system (Scanet SV-10; MATYS).[18] One hour before MAP injection, animals were placed in clear Plexiglas cages (30 × 48 × 60 cm), which were equipped with a row of 96 photocell beams placed 3 cm above the floor of the cage. Photocell beam breaks were detected and recorded by a computer. Data collected for 180 min were used for the analysis.

Radioligand Binding Assays

On the seventh day following treatment cessation (removal of the minipumps), the rats were sacrificed by decapitation, and the striata (rostal neo-striatum) were rapidly dissected. Tissues were stored at −70°C until use. Radioligand binding assays for striatal D_2 receptors were performed (Experiments 1b and 2b). Tissue samples (60-80 mg wet weight) of striata were homogenized for 15 s in 40 volumes (w/v) of ice-cold 50 mM Tris-HCl buffer (pH 7.4) containing 120 mM NaCl and 5 mM KCl, and the homogenates were then centrifuged (40 000*g*, 15 min, 4°C). The pellets were suspended and centrifuged twice in the same buffer.

The binding assays were carried out according to procedures described previously,[19] with slight modifications. Briefly, 100 μl of membrane homogenate was added to tubes containing 50 μl of [^{3}H]raclopride to yield a final assay volume of 500 μl. Binding to D_2 receptors was measured with 6 concentrations (0.25, 0.5, 1, 2,4, 8 nM) of [^{3}H]raclopride. The samples were incubated for 60 min at 25°C, and specific binding was determined in the presence of 300 μM (-)-sulpiride. The incubated samples were rapidly run through Whatman GF/B glass filters pretreated with 0.5% polyethyleneimine for at least 2 h, using a Brandell 24-channel cell harvester (Biochemical Research Laboratory). The filters were washed twice with 4 ml cold buffer. Radioactivity was determined using a liquid scintillation counter. In the final incubation tubes, the protein concentration was approximately 0.2 mg/ml, as determined by the Lowry method in 50 μl aliquots of membrane preparation. The Bmax and Kd values for D_2 receptors in each rat were calculated by a nonlinear regression curve fit using GraphPad Prism 5.01 software for Windows.

Statistical Analysis

In order to determine treatment effects, all data were analyzed using one-way ANOVA followed by 2-tailed Bonferroni's multiple comparison test or Fisher's least significant difference test (only for locomotion data from 3 groups). All statistical calculations were carried out with SPSS 12.0J software for Windows.

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Results

Experiment 1a: Effects of Chronic Treatment on Behavioral Sensitivity to MAP

In Experiment 1a, we examined the effects of chronic treatment with HAL (0.75 mg/kg/d), ARI (1.5 mg/kg/d), or VEH, delivered to rats via minipump for 14 days, on the locomotor response of rats to MAP (1.0 mg/kg/injection) on the seventh day following treatment cessation. The injection of MAP induced hyperlocomotion in each group. Hyperlocomotion peaked about 20 min after the administration of MAP and then gradually declined during the observation period (figure 2A). As regards the total locomotor activity for the 60-min period after the MAP injection, the HAL group exhibited a significantly greater amount of activity than either the VEH

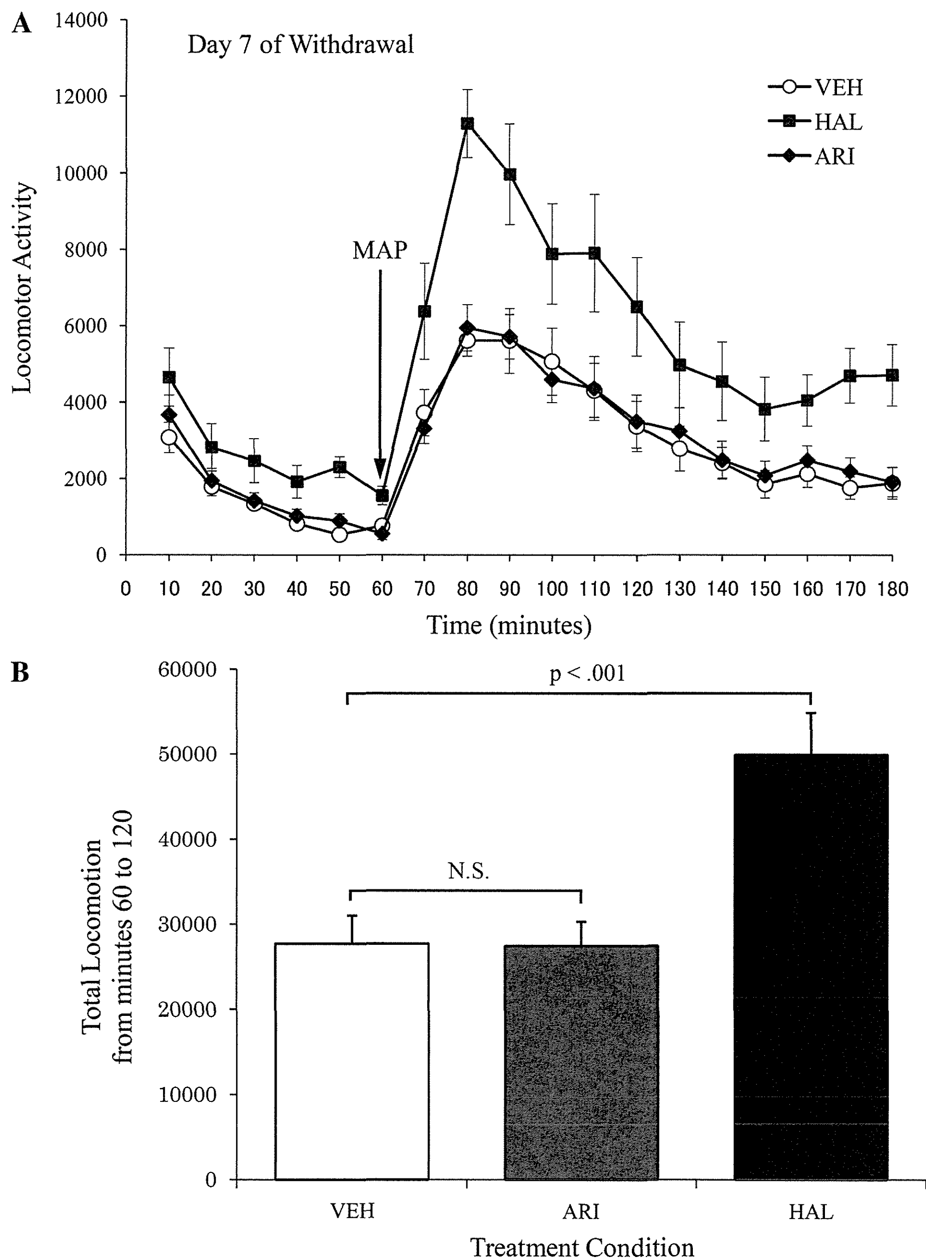

Fig. 2. The effects of chronic treatment with haloperidol (0.75 mg/kg/d, HAL), aripiprazole (1.5 mg/kg/d, ARI), or vehicle (VEH) on the locomotor response to methamphetamine (1.0 mg/kg i.p. injection, MAP). Drugs or vehicle were administered via an implanted minipump for 14 days, and locomotor tests were performed on the seventh day following treatment cessation. In (A), following MAP injection in each group, locomotor activity increased, peaked approximately 40 min after the injection of MAP, and then decreased with time. In (B), the total locomotor response was defined as the total locomotor activity measured for 60 min after MAP injection. The HAL group showed significantly higher activity levels than either the VEH or ARI group, whereas there was no significant difference in the activity levels between the VEH group and ARI group (one-way ANOVA with Bonferroni's tests; $F_2 = 11.44$; $P < .001$ [VEH vs HAL and ARI vs HAL]; $P = .999$ [VEH vs ARI]). $N = 10$ in each group. Error bars indicate the SEM.

group or the ARI group (ie, averaged about 80% more activity), whereas there was no significant difference in locomotor activity in response to MAP between the VEH group and ARI group ($P < .001$, figure 2B).

Experiment 1b: Effects of Chronic Treatment on Striatal D_2 Receptor Binding

In Experiment 1b, we examined the effects of chronic treatment with HAL (0.75 mg/kg/d), ARI (1.5 mg/kg/d), or VEH, delivered to rats via minipump for 14 days, on the density and affinity of striatal D_2 receptors, as determined on the seventh day following treatment cessation. As regards the Bmax value (ie, density) of the D_2 receptors, the HAL group showed a significantly higher Bmax value than either the VEH group or the ARI group (ie, averaged 153% and 126% higher, respectively), whereas there were no significant differences between the VEH group and the ARI group ($P < .001$; figure 3B). On the other hand, as regards the Kd value (ie, affinity) of the D_2 receptors, ANOVA revealed a slightly significant difference among the 3 groups, although post hoc analyses did not show any significant difference between any pairing of the 3 groups studied ($P < .05$; figure 3A).

Experiment 2a: Effects of Chronic Treatment Preceded by Chronic HAL Treatment on Behavioral Sensitivity to MAP

In Experiment 2a, we examined the effects of chronic treatment with HAL (0.75 mg/kg/d), ARI (1.5 mg/kg/d), or VEH, delivered to rats via minipump for 14 days, which had been preceded by a 14-day course of HAL (0.75 mg/kg/d) or VEH treatment, also delivered via minipump, on the locomotor response of rats to MAP (1.0 mg/kg/injection), as measured on the seventh day following treatment cessation. MAP injection induced hyperlocomotion in each group. Hyperlocomotion peaked approximately 20 min after the administration of MAP and then gradually declined during the observation period (figure 4A). As regards the total locomotor activity observed for 60 min after MAP injection, both the HAL-HAL group and the HAL-VEH group showed significantly greater amounts of activity (ie, averaged 120% and 99% more activity, respectively) than the VEH-VEH group, yet there was no significant difference between the VEH-VEH group and the HAL-ARI group in this regard ($P < .05$; figure 4B). Furthermore, the total locomotion value of the HAL-ARI group was significantly lower than that of the HAL-HAL group, ie, by an average of 38%, whereas there was no significant difference between the HAL-HAL group and the HAL-VEH group ($P < .05$; figure 4B). Consequently, the rank order of the total locomotor response to MAP was HAL-HAL > HAL-VEH > HAL-ARI > VEH-VEH.

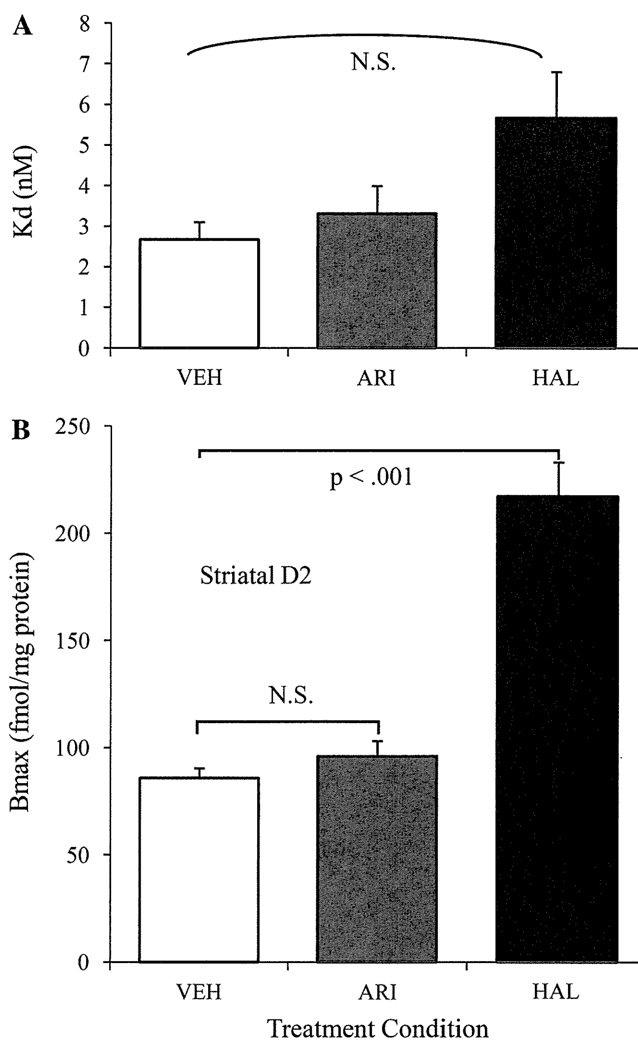

Fig. 3. The effects of chronic treatment with haloperidol (0.75 mg/kg/d, HAL), aripiprazole (1.5 mg/kg/d, ARI), or vehicle (VEH) on Kd (A) and Bmax (B) of striatal D_2 receptors. Drugs were administered via an implanted minipump for 14 days, and the animals were decapitated on the seventh day following treatment cessation. In (A), the Kd value showed significant treatment effects by one-way ANOVA ($P = .048$), although no significant difference was seen in the post hoc analysis by Bonferroni's multiple comparison tests. In (B), the Bmax value of the HAL group was significantly higher than that of either the VEH group or the ARI group, whereas there was no significant Bmax value difference between the VEH group and ARI group (one-way ANOVA with Bonferroni's tests; $F_2 = 50.55$; $P < .001$ [VEH vs HAL and ARI vs HAL]; $P = 1.00$ [VEH vs ARI]). $N = 5$ in each group. The error bars indicate the SEM.

Experiment 2b: Effects of Chronic Treatment Preceded by Chronic HAL Treatment on Striatal D_2 Receptor Binding

In Experiment 2b, we examined the effects of chronic treatment with HAL (0.75 mg/kg/d), ARI (1.5 mg/kg/d), or VEH, delivered to rats via minipump for 14 days, which had been preceded by a 14-day course of HAL (0.75 mg/kg/d) or VEH treatment, also delivered via minipump, on the density and affinity of striatal

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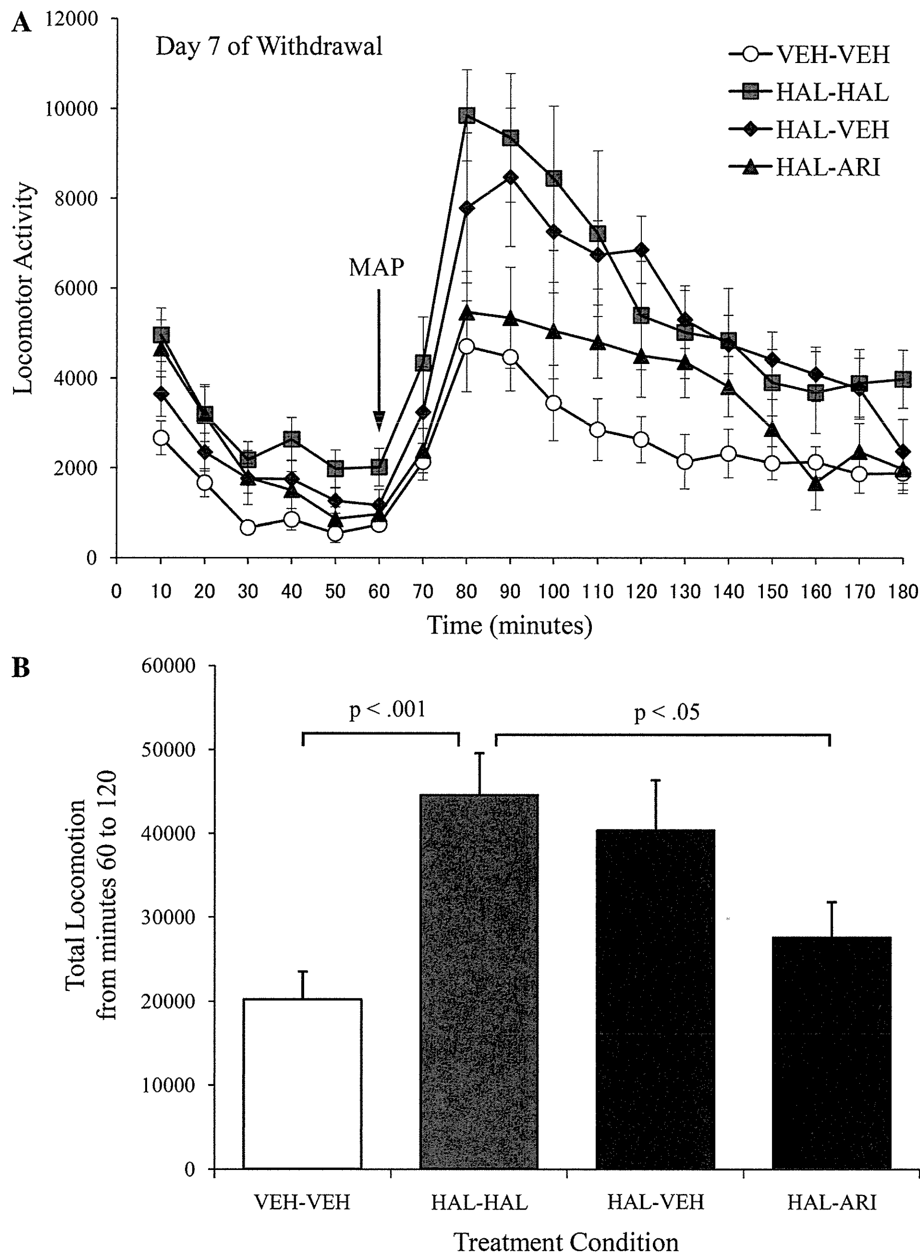

Fig. 4. The effects of chronic treatment with haloperidol (0.75 mg/kg/d, HAL-HAL), aripiprazole (1.5 mg/kg/d, HAL-ARI), or vehicle (HAL-VEH), preceded by chronic treatment with HAL (0.75 mg/kg/d) or VEH (VEH-VEH), on the locomotor response to methamphetamine (1.0 mg/kg i.p. injection, MAP). Either HAL or VEH was administered via an implanted minipump for 14 days and then the minipump was exchanged for a second minipump, by which drugs were administered for an additional 14 days. Locomotor tests were performed on the seventh day following the cessation of the entire 28-day treatment period. In (A), following MAP injection of each group, locomotor activity increased, peaked about 40 min after the MAP injection, and then decreased with time. In (B), the total locomotor response was defined as the total locomotor activity measured for 60 min after MAP injection. The HAL-HAL group and the HAL-VEH group both exhibited significantly higher levels of activity than the VEH-VEH group, whereas there was no significant difference in the activity levels between the VEH-VEH and the HAL-ARI groups (one-way ANOVA with Bonferroni's tests; $F_3 = 5.64$; $P < .01$ [VEH-VEH vs HAL-HAL]; $P < .05$ [VEH-VEH vs HAL-VEH]; $P = 1.00$ [VEH-VEH vs HAL-ARI]). Although there was no significant difference among the HAL-HAL, HAL-VEH, and HAL-ARI groups (one-way ANOVA; $F_2 = 2.97$; $P = .073$), visual inspection of this figure reveals that the total locomotion for the 3 groups could be arranged in the following descending order of magnitude: HAL-HAL > HAL-VEH > HAL-ARI. Analysis of variance with a polynomial contrast (ie, the linear component) revealed a significant linear trend across these 3 groups ($F_1 = 5.49$; $P < .05$). In the post hoc analyses of total locomotor activity, the HAL-ARI group showed significantly less activity than the HAL-HAL group, whereas there was no such significant difference between the HAL-HAL group and the HAL-VEH group (Fisher's least significant difference tests; $P < .05$ [HAL-HAL vs HAL-ARI]; $P = .565$ [HAL-HAL vs HAL-VEH]). The rank ordering of groups in terms of the total locomotor response to MAP was HAL-HAL > HAL-VEH > HAL-ARI > VEH-VEH. $N = 8$ in each group. Error bars indicate the SEM.