ン酸化レベルの低下も認められる。一方，グリシンをPCP連続投与マウスの前頭前皮質に微量注入すると，CaMK Ⅱリン酸化レベルの増加とともに潜在学習障害が緩解される。また，マウスの前頭前皮質のスライスにNMDAを添加し，CaMK Ⅱの活性変化を測定すると，生理食塩水連続投与マウスの前頭前皮質のスライスではNMDA刺激によりCaMK Ⅱリン酸化レベルの増加がみられるが，PCP連続投与マウスのそれにおいては，CaMK Ⅱリン酸化レベルの増加は認められない。PCP連続投与マウスの前頭前皮質において，NMDA受容体の主要なサブユニットであるNR1の897番目のセリン残基のリン酸化レベルが低下しており，このような変化は統合失調症患者の剖検脳の報告と類似している[20]。これらの知見から，PCP連続投与は，前頭前皮質の細胞外グルタミン酸量の減少だけでなく，NMDA受容体の機能低下を惹起することにより，潜在学習障害を引き起こすことが示唆される。

統合失調症に認められる認知障害には，前頭前皮質におけるグルタミン酸作動性神経系の機能低下だけでなく，ドパミン作動性神経系の機能低下も関与していることが示唆されている[2]。マイクロダイアリシス法を用いて，高カリウム刺激による前頭前皮質における細胞外ドパミン遊離量の増加を測定すると，生理食塩水連続投与マウスで認められる細胞外ドパミン遊離量の顕著な増加が，PCP連続投与マウスでは認められない。一方，ドパミンD1受容体作動薬であるSKF81297をPCP連続投与マウスの前頭前皮質に微量注入すると，NR1のリン酸化の割合低下が改善し，潜在学習障害が緩解される。ドパミンD1受容体を刺激するとPKAが活性化される。また，NR1の897番目のセリン残基はPKAの基質である。さらに，前頭皮質におけるドパミンD1受容体を刺激すると，長期増強が亢進する電気生理学的知見がある[21]。以上の知見から，PCP連続投与マウスに認められる潜在学習障害は，前頭前皮質におけるNMDA受容体/CaMK Ⅱシグナル経路の機能低下により生じ，この機能低下はドパミン神経系の機能低下が関与していることが示唆される。統合失調症患者の前頭前皮質において，グルタミン酸作動性神経系およびドパミン作動性神経系の機能低下がそれぞれ報告されているが，本知見は，それら神経系の機能低下が関連していることを示唆している[22]。

3．PCP連続投与マウスを用いた新規治療薬の評価

上述のように，PCP連続投与マウスは行動感作，意欲低下や社会性行動の低下，潜在学習障害などの統合失調症の指標となる行動異常を示し，その行動障害が統合失調症病態仮説を支持していることから，統合失調症モデル動物として有用である。我々はこのPCP連続投与マウスを用いて，新規抗精神病薬や統合失調症の新規治療薬として可能性を持つ薬物の評価を行っている（図1）。

1．PCP連続投与マウスに認められる統合失調様症状に対するアリピプラゾールの緩解効果

アリピプラゾールは，近年開発された新規第二世代抗精神病薬であり，SDAやMARTAなど従来から臨床で用いられている治療薬とは異なる作用機序により抗精神病効果を示す[23]。ドパミンD2受容体部分作動作用を持つアリピプラゾールは，ドパミン作動性神経系機能の亢進時にはドパミンD2受容体拮抗薬として働き，一方，ドパミン作動性神経系機能の低下時にはドパミンD2受容体作動薬として働く。このように，アリピプラゾールはドパミン作動性神経系の活動に応じてドパミンD2受容体を介する神経伝達をそれ自身の固有活性レベルに安定化させることから，ドパミンシステム・スタビライザーとしての性質を有し，ドパミン作動性神経機能を調節して陽性症状や陰性症状，認知機能障害を改善する[24]。

PCPを3日間連続投与したラットに認められる社会性行動の低下を，アリピプラゾールの併用投与により緩解するという報告がある[25]。我々も陰性症状に対するアリピプラゾールの効果を強制水泳試験において見出しており，PCP連続投与マウスにアリピプラゾールを連続投与すると，PCP連続投与マウスで認められる無動状態の増強が緩解される。一方，PCP連続投与マウスは新奇物体認識試験において認知障害を示すことが報告されて

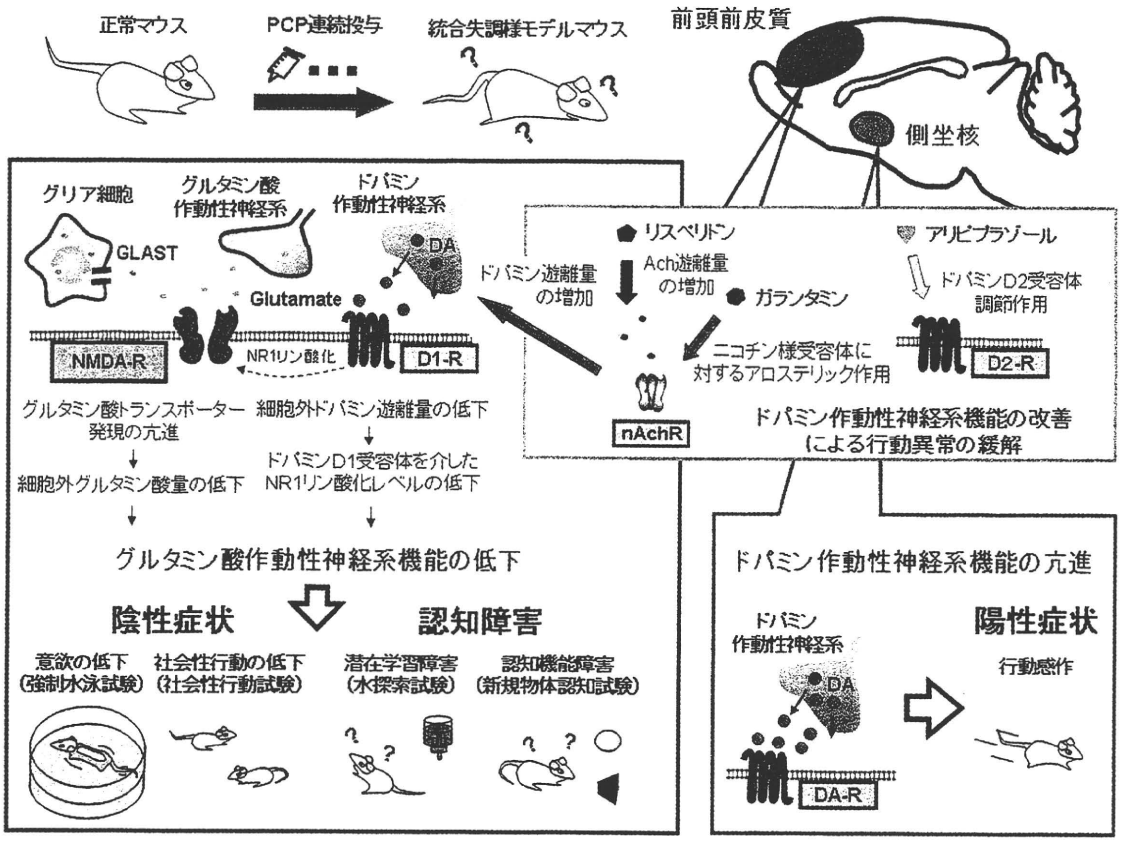

図1　PCP連続投与マウスに認められる統合失調様行動異常とその病態メカニズム，それらに対する新規治療薬（アリピプラゾール，ガランタミン）の効果

いる[26]。新奇物体認識試験は，マウスの新奇性を好むという特性を利用した試験で，マウスを二つの異なる形をした物体が設置された実験装置内に入れて，自由に探索させる（訓練試行）。訓練試行の24時間後に，片方の物体を新奇物体と置換し，装置内を自由に探索させる（保持試行）。正常なマウスでは，訓練試行における物体に対する探索時間に比べ，新奇物体に対する探索時間が増加することから，認知記憶を評価できる。PCP連続投与マウスでは，保持試行において新奇物体に対する探索時間の増加がみられず，認知障害が認められる。PCP連続投与マウスにアリピプラゾールを連続投与すると，保持試行における新奇物体に対する探索時間が増加し，PCP連続投与マウスで認められる認知障害が緩解される。

2．PCP連続投与マウスに認められる統合失調様症状に対するガランタミンの緩解効果（リスペリドンとの併用による相乗効果）

ガランタミンは，ニコチン様アセチルコリン受容体（nAchR）に対するアロステリックモデュレーターとして働き，また弱いコリンエステラーゼ阻害作用を併せ持つアルツハイマー病の治療薬である[27]。統合失調症患者の前頭前皮質において，nAchRの発現量が減少しており[28)29)]，また，ガランタミンが統合失調症患者に認められる感情鈍麻などの陰性症状や注意力低下などの認知障害の改善効果を示したとの臨床報告がある[28)29)30)]。これらの報告から，ガランタミンはnAchRを介して統合失調症状を改善していることが考えられ，

統合失調症治療薬としての可能性がある。PCPをマウスに連続投与し，休薬後にガランタミン（0.05，0.3mg/kg），リスペリドン（0.05，0.1 mg/kg）の単独またはこれらを併用（ガランタミン；0.05mg/kg，リスペリドン；0.05mg/kg）急性投与したところ，ガランタミン単独の低用量（0.05mg/kg）ではPCP連続投与マウスに認められる社会性行動の低下が緩解されないのに対し，高用量（0.3mg/kg）では緩解される。リスペリドン単独においても，低用量（0.05mg/kg）では緩解されないが，高用量（0.1mg/kg）では緩解される。また，ガランタミンとリスペリドンの低用量の併用投与により社会性行動低下が緩解され，この緩解作用はnAchR拮抗薬であるメカミラミンにより拮抗される。一方，PCPをマウスに連続投与し，休薬後に水探索試験におけるガランタミンおよびリスペリドンの効果を検討したところ，訓練試行前にガランタミン単独の低用量（0.05mg/kg）またはリスペリドン単独の低用量（0.05mg/kg）を投与しても，PCP連続投与マウスに認められる潜在学習障害は緩解されないのに対し，ガランタミンの高用量（0.3mg/kg）およびリスペリドンの高用量（0.1mg/kg）では緩解される。また，ガランタミンとリスペリドンの低用量の併用投与により潜在学習障害が緩解され，この緩解作用はメカミラミンにより拮抗される。

PCP連続投与マウスにリスペリドンおよびガランタミンの低用量を単独投与しても，細胞外ドパミン遊離量の有意な変化は認められないが，それぞれを併用投与したとき，細胞外ドパミン遊離量が著しく増加し，この遊離量の増加作用はメカミラミンの併用投与により拮抗される。PCP連続投与マウスに認められる社会性行動の低下および潜在学習障害に対するガランタミンとリスペリドンの低用量の併用投与による緩解作用は，ドパミンD1受容体拮抗薬であるSCH23390を前頭前皮質に微量注入すると拮抗される[31)32)]。リスペリドンは前頭前皮質のアセチルコリン遊離量を増加させることが報告されており[33)]，またnAchRが活性化されるとドパミンの遊離量が増加することが知られていることから[34)35)]，この相乗作用は，リスペリドンによるアセチルコリンの遊離を介した間接的nAchRの活性化とガランタミンのアロステリック作用によるnAchRの活性化により，ドパミン遊離量が増加し，ドパミンD1受容体が刺激された結果であると考えられる。

現在，臨床において統合失調症の治療にはSDAであるリスペリドンが頻用さているが，用量依存的にみられる高プロラクチン血症や錐体外路系症状などの多様な副作用の発現が問題となっている。以上の知見から，ガランタミンとの併用によりリスペリドンの使用量を下げることができ，そのような副作用の軽減が期待される。

4．DISC1 KDマウスに認められる統合失調様症状

統合失調症は家系研究，双生児研究，養子研究から遺伝要因が強く示唆されている[36)]。発症の原因となりうる脆弱性を示す遺伝子は単一ではなく，いくつかの遺伝子が報告されている[37)]。DISC1遺伝子は，スコットランドにおける精神疾患多発単一家系を対象とした遺伝学的研究により，1番染色体上に存在する統合失調症関連遺伝子として変異が発見された[38)]。この家系において，DISC1遺伝子の転座が認められ，この転座によりDISC1のC末端側の257アミノ酸が欠如し，高い確率で統合失調症が発症することが報告されている[39)]。DISC1はいくつかの機能的タンパク質（NudE-like，fasciculation and elongation protein zeta-1，Lissencephaly 1，Phosphodiesterase 4Bなど）と相互に結合し，細胞移動，神経突起の伸長などの神経発達に重要な機能を担っている[7)]。そのため，DISC1の遺伝子変異動物は，統合失調症の神経発達障害仮説に基づいた統合失調症モデル動物となることが期待される。

我々は，子宮内エレクトロポレーション法（in utero electroporation法）を用いて胎生14日目の子宮内胎児の前頭前皮質にDISC1 siRNAを導入し，神経発達過程のDISC1の発現を抑制したDISC1 KDマウスを作製した（**図2**）。統合失調症患者では，精神刺激薬に対する反応性が亢進していることが知られており[40)]，このような応答性はKDマウスでも認められ，生後56日目のKD

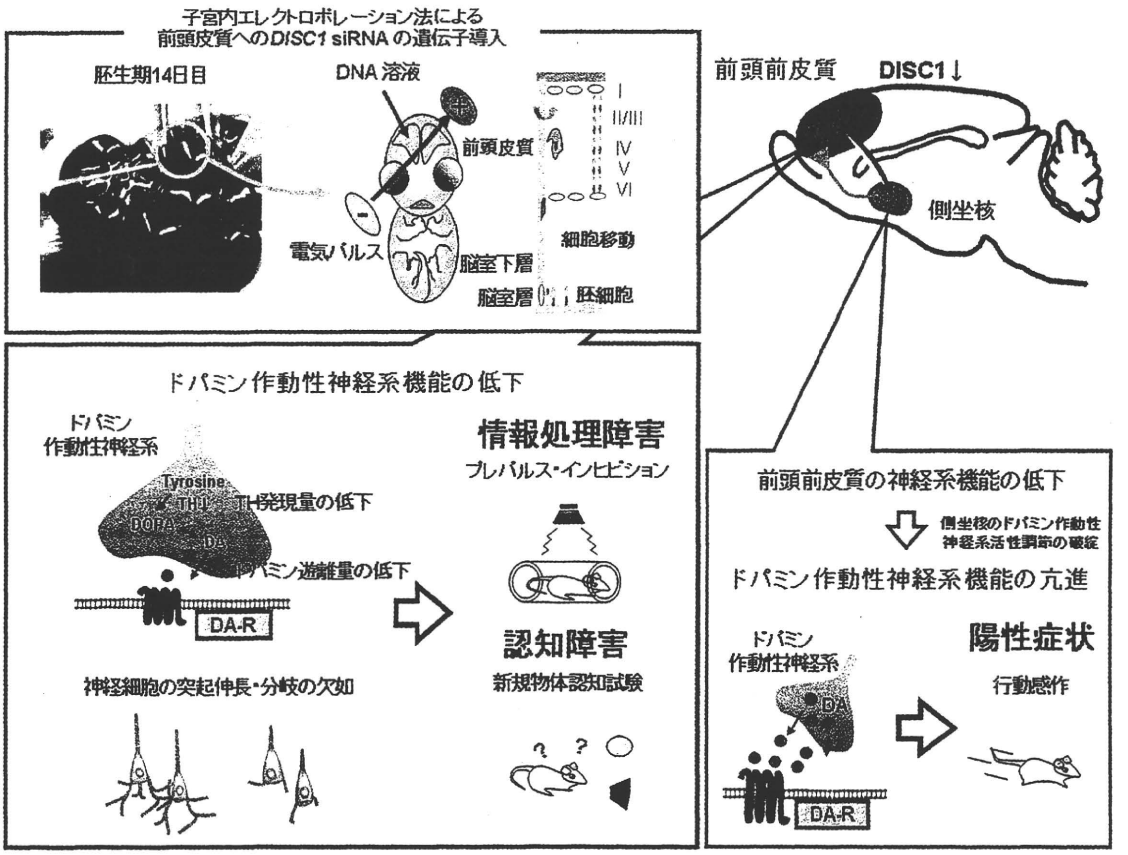

図2　DISC1 KDマウスに認められる統合失調様行動異常とその病態メカニズム

マウスに低用量のメタンフェタミンを投与すると，正常なマウスに比べて自発運動量の著しい増加が認められる。このような精神刺激薬に対する応答性の亢進は，腹側被蓋野から側坐核へ投射するドパミン作動性神経系の機能亢進により生じるものと考えられ，実際にマイクロダイアリシス法を用いて，高カリウム刺激による側坐核の細胞外ドパミン遊離量の増加を測定すると，KDマウスにおいて著しい増加が認められる。新規物体認知試験を行うと，正常なマウスに比べてKDマウスでは保持試行における新奇物体に対する探索時間の減少が認められる。情報処理障害は，統合失調症患者で認められる機能障害の一つであり，プレパルス・インヒビション（Prepulse Inhibition；PPI）で評価することができる。PPIとは，大きな音を突然聴かせると，驚愕反応を示すが，その直前にそれよりも小さな音を聴かせた後，大きな音を聴かせると驚愕反応が抑制される現象である。小動物驚愕反応測定装置を用いて，生後56日目のKDマウスのPPIを測定すると，正常なマウスに比べて驚愕反応抑制率の著しい減少が認められる。

KDマウスの前頭前皮質神経細胞を組織形態学的に検討すると，KDマウスでは神経細胞数の減少やグリオーシスといった器質的変化は認められないが，神経細胞の突起伸長や分岐の欠如が認められる。前頭前皮質におけるドパミン合成酵素のチロシンヒドロキシラーゼ（TH）の発現量を免疫組織化学的に検討すると，KDマウスでは正常なマウスに比べてTH発現量の減少が認められる。また，マイクロダイアリシス法を用いた検討において，KDマウスでは前頭前皮質における基

礎細胞外ドパミン濃度の減少が認められる。これらの知見から，DISC1機能の障害により前頭前皮質での神経発達が妨げられ，前頭前皮質のドパミン作動性神経系の機能低下が生じているものと考えられる。前頭前皮質と大脳辺縁系が神経ネットワークを介して，お互いに神経活性を調節していることが知られている[41]。KDマウスに認められる側坐核のドパミン作動性神経系の活性亢進は，側坐核のドパミン作動性神経系に対する活性調節機構が前頭前皮質の神経発達障害により破綻して生じていることが示唆される。

DISC1遺伝子を変異させたマウスの表現型解析においても，行動量の増加や強制水泳試験における無動状態の増強，PPIにおける情報処理障害など，統合失調症様の行動異常が報告されている[42)43)]。胎生期のDISC1の発現を抑制させた本KDマウスでは，成熟後に統合失調症患者に認められる病態に類似した神経系の機能異常および行動異常を示すことから，特に胎生期のDISC1の機能低下が統合失調症の発症に関与していることを強く示唆している。

5．おわりに

統合失調症の病態解明や新規治療薬開発の研究には，妥当性の高い統合失調症モデル動物を用いることが必要不可欠である。本総説では，PCP連続投与マウスおよびDISC1 KDマウスの統合失調症モデル動物としての妥当性，これらモデルを用いた新規治療薬の評価について概説した。これら統合失調様モデル動物を用いた研究成果が，統合失調症患者に福音をもたらすことを期待したい。

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ORIGINAL INVESTIGATION

Aripiprazole ameliorates phencyclidine-induced impairment of recognition memory through dopamine D_1 and serotonin 5-HT_{1A} receptors

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Abstract
Rationale Cognitive deficits, including memory impairment, are regarded as a core feature of schizophrenia. Aripiprazole, an atypical antipsychotic drug, has been shown to improve disruption of prepulse inhibition and social interaction in an animal model of schizophrenia induced by phencyclidine (PCP); however, the effects of aripiprazole on recognition memory remain to be investigated.

Objectives In this study, we examined the effect of aripiprazole on cognitive impairment in mice treated with PCP repeatedly.
Materials and methods Mice were repeatedly administered PCP at a dose of 10mg/kg for 14days, and their cognitive function was assessed using a novel-object recognition task. We investigated the therapeutic effects of aripiprazole (0.01–1.0mg/kg) and haloperidol (0.3 and 1.0mg/kg) on cognitive impairment in mice treated with PCP repeatedly.
Results Single (1.0mg/kg) and repeated (0.03 and 0.1mg/kg, for 7days) treatment with aripiprazole ameliorated PCP-induced impairment of recognition memory, although single treatment significantly decreased the total exploration time during the training session. In contrast, both single and repeated treatment with haloperidol (0.3 and 1.0mg/kg) failed to attenuate PCP-induced cognitive impairment. The ameliorating effect of aripiprazole on recognition memory in PCP-treated mice was blocked by co-treatment with a dopamine D_1 receptor antagonist, SCH23390, and a serotonin 5-HT_{1A} receptor antagonist, WAY100635; however, co-treatment with a D_2 receptor antagonist raclopride had no effect on the ameliorating effect of aripiprazole.
Conclusions These results suggest that the ameliorative effect of aripiprazole on PCP-induced memory impairment is associated with dopamine D_1 and serotonin 5-HT_{1A} receptors.

Keywords Aripiprazole · Dopamine D_1 receptor · Memory · Phencyclidine · Serotonin 5-HT_{1A} receptor

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Introduction

Schizophrenia is a devastating psychiatric disorder that impairs mental and social functioning and affects approx-

imately 1% of the population worldwide (Rössler et al. 2005). Typical symptoms can be separated into positive symptoms (e.g., hallucinations, delusions, and thought disorder), negative symptoms (e.g., deficits in social interaction, emotional expression, and motivation), and cognitive dysfunction (e.g., impaired attention/information processing, problem-solving, processing speed, verbal and visual learning, and memory and working memory) (Nuechterlein et al. 2004; Pearlson 2000). Pharmacological treatment of schizophrenia is available. First-generation (typical) antipsychotics alleviate psychotic symptoms, but lead to severe motor side effects through the blockade of dopamine D_2 receptors (Kapur et al. 2000). Second-generation (atypical) antipsychotics have improved tolerability and milder motor side effects than typical antipsychotics but induce weight gain and metabolic disturbances (Newcomer 2005). Despite appropriate treatment with either typical antipsychotics or atypical antipsychotics, schizophrenic patients continue to exhibit pronounced cognitive impairment (Keefe et al. 2007; Mishara and Goldberg 2004; Woodward et al. 2005).

Aripiprazole, 7-(4-[4-(2,3-dichlorophenyl)-1-piperazinyl] butyloxy)-3,4-dihydro- carbostycil, is a novel atypical antipsychotic drug that differs from other typical and atypical antipsychotics, improving both positive and negative symptoms of psychosis without producing extrapyramidal side effects or increases in serum prolactin (DeLeon et al. 2004; Tamminga 2002). It has been demonstrated that aripiprazole has high affinity for a large number of monoaminergic receptors, including dopamine D_2, serotonin 5-HT_{1A}, and 5-HT_{2A} receptors (Green 2004; Shapiro et al. 2003) and acts as a partial dopamine D_2 receptor agonist (Kikuchi et al. 1995), a partial 5-HT_{1A} receptor agonist (Jordan et al. 2002), and as an 5-HT_{2A} receptor antagonist (McQuade et al. 2002). These pharmacological properties may play a role in the therapeutic effects of aripiprazole. Although aripiprazole has been reported to enhance cognitive function in schizophrenia (Rivas-Vasquez 2003), the mechanism of the improving effect of aripiprazole on cognitive impairment is unclear.

Phencyclidine [1-(1-phenylcyclohexyl) piperidine hydrochloride (PCP)], a noncompetitive *N*-metyl-D-aspartate receptor antagonist, has been shown to induce schizophrenia-like psychosis, presenting as positive symptoms, negative symptoms, and cognitive deficits in humans (Javitt and Zukin 1991), which persist several weeks after withdrawal of chronic PCP use (Allen and Young 1978; Lerner and Burns 1986; Rainey and Crowder 1975). To investigate the pathophysiology of schizophrenia, an animal model of schizophrenia was established using PCP (Mouri et al. 2007a). We have previously demonstrated that repeated treatment with PCP (10mg/kg/day s.c. for 14days) induces several schizophrenia-like behavioral abnormalities, such as increased immobility in a forced swimming test (Murai et al. 2007; Noda et al. 1995, 1997, 2000), social deficits in a social interaction test (Qiao et al. 2001), impairment of latent learning in a water finding test (Mouri et al. 2007b), and associative learning impairment in cued and contextual fear conditioning tests (Enomoto et al. 2005) in mice. Moreover, it has been reported that PCP induces the disruption of sensorimotor gating in a prepulse inhibition test (Bakshi et al. 1994) and recognition memory in a novel object recognition test (Hashimoto et al. 2005); therefore, PCP-treated mice might be a useful animal model of schizophrenia.

There are a few reports suggesting the effectiveness of aripiprazole on cognitive dysfunction and negative symptoms in PCP-treated animals. For example, aripiprazole improves PCP-induced disruption of prepulse inhibition (Fejgin et al. 2007) and social interaction (Bruins Slot et al. 2005) in mice and rats, respectively; however, the effects of aripiprazole on recognition memory remain to be investigated. In this study, we examined whether aripiprazole improves PCP-induced cognitive impairment in a novel object recognition test in mice.

Materials and methods

Animals

Male ICR mice (7weeks old) were obtained from Nihon SLC (Shizuoka, Japan). The animals were housed in plastic cages and kept in a regulated environment (23 ± 1°C, 50 ± 5% humidity), with a 12/12h light–dark cycle (lights on at 09:00hours). Food (CE2; Clea Japan, Tokyo, Japan) and tap water were available ad libitum. All animal care and use were in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of Nagoya University.

Drugs

PCP hydrochloride was synthesized by the authors according to the method of Maddox et al. (1965) and was checked for purity. Aripiprazole was provided by Otsuka Pharmaceutical (Tokyo, Japan). SCH23390 hydrochloride, *S*-(−)-raclopride (+)-tartrate, haloperidol and WAY100635 were purchased from Sigma-Aldrich (St. Louis, MO, USA). The dose of each drug refers to previous reports (Noda et al. 1995; Bruins Slot et al. 2005; Kamei et al. 2006; Ito et al. 2007b).

PCP was dissolved in saline. SCH23390, raclopride, and WAY100635 was dissolved in distilled water. Aripiprazole and haloperidol were suspended in saline containing 0.1% carboxymethylcellulose (CMC) sodium salt. All drugs were administered in a volume of 0.1ml/10g body weight.

Measurement of locomotor activity

Each mouse was placed in a standard transparent rectangular rodent cage (25 × 30 × 18 high cm). Locomotor activity was then measured for 1h, using an infrared sensor (NS-AS01; Neuroscience, Tokyo, Japan) placed over the cage (Ito et al. 2007a).

Novel object recognition test

The novel object recognition test was performed according to previously reported methods (Nagai et al. 2003; Tang et al. 1999). The experimental apparatus consisted of a Plexiglas open-field box (30 × 30 × 35 high cm), the floor of which was covered in sawdust. The apparatus was located in a sound-attenuated room and illuminated with a 20-W bulb.

The procedure for the novel object recognition test consisted of three different sessions: habituation, training, and retention. Each mouse was individually habituated to the box, with 10min of exploration in the absence of objects each day for three consecutive days (habituation session, days1–3). In the training session, two different novel objects were symmetrically fixed to the floor of the box, 8cm from the walls, and each animal was allowed to explore the box for 10min (day4). The objects were a golf ball, wooden cylinders, and square pyramids, which were different in shape and color but similar in size. An animal was considered to be exploring the object when its head was facing the object or it was touching or sniffing the object. The time spent exploring each object was recorded. After training, mice were immediately returned to their home cages. In the retention sessions, the animals were placed back into the same box 24h (day5) after the training session, but with one of the familiar objects used during training replaced by a novel object. The animals were then allowed to explore freely for 5min, and the time spent exploring each object was recorded. Throughout the experiments, the objects were balanced in terms of their physical complexity and emotional neutrality. A preference index, the ratio of the amount of time spent exploring any one of the two objects (training session) or the novel object (retention session) over the total time spent exploring both objects, was used to measure cognitive function.

Drug treatment

For effect of single treatment on locomotor activity, 0.1% CMC, aripiprazole (0.1−1.0mg/kg) or haloperidol (0.3−1.0mg/kg) was orally (p.o.) administered 1h before the experiment. The number of animals included in each drug treatment was as follows: CMC (n = 12), 0.1mg/kg aripiprazole (n = 12), 0.3mg/kg aripiprazole (n = 12), 1.0mg/kg aripiprazole (n = 11) for Fig. 1b,c; CMC (n = 10), 0.3mg/kg haloperidol (n = 10), 1.0mg/kg haloperidol (n = 10) for Fig. 1d,e.

For effect of repeated treatment on locomotor activity, 0.1% CMC, aripiprazole (0.01−0.1mg/kg), or haloperidol (0.3−1.0mg/kg) was p.o. administered for 7days; the experiment was performed 24h after last treatment. Locomotor activity was recorded for 1h. The number of animals included in each drug treatment was as follows: CMC (n = 10), 0.01mg/kg aripiprazole (n = 10), 0.03mg/kg aripiprazole (n = 10), 0.1mg/kg aripiprazole (n = 10) for Fig. 2b,c; CMC (n = 10), 0.3mg/kg haloperidol (n = 10), 1.0mg/kg haloperidol (n = 10) for Fig. 2d,e.

We have previously demonstrated that repeated treatment with PCP (10mg/kg/day s.c. for 14days) induces several schizophrenia-like behavioral and neurochemical abnormalities in mice (Murai et al. 2007; Noda et al. 1995, 1997, 2000; Qiao et al. 2001; Mouri et al. 2007b; Enomoto et al. 2005). Therefore, in typical experimental conditions, mice were given the same regimen of PCP (10mg/kg, s.c., for 14days, days1–14). Five days after the last treatment with PCP, the novel object recognition test was performed, including habituation (i.e., days19–21), training (i.e., day22), and retention (i.e., day23) sessions.

To study the single effects of antipsychotics, aripiprazole (0.01−1.0mg/kg) or haloperidol (0.3−1.0mg/kg) was administered p.o. (i.e., day22) to mice that had been previously treated with PCP for 14days (day1–14). One hour after treatment with antipsychotics, the training session using the novel object recognition test was conducted. The number of animals included in each drug treatment was as follows: saline + CMC (n = 16), PCP + CMC (n = 14), PCP + 0.01mg/kg aripiprazole (n = 10), PCP + 0.03mg/kg aripiprazole (n = 9), PCP + 0.1mg/kg aripiprazole (n = 8), PCP + 0.3mg/kg aripiprazole (n = 10), PCP + 1.0mg/kg aripiprazole (n = 11) for Fig. 3b,d; saline + CMC (n = 14), PCP + CMC (n = 13), PCP + 0.3mg/kg haloperidol (n = 7), PCP + 1.0mg/kg haloperidol (n = 9) for Fig. 3c,e.

To study the subchronic effects of antipsychotics, aripiprazole (0.01–0.1mg/kg) or haloperidol (0.3–1.0mg/kg) was administered p.o. once a day for seven consecutive days (i.e., days15–21) to mice that had been previously treated with PCP for 14days (days1–14). During habituation session of the novel object recognition test (i.e., days19–21), mice were administered antipsychotics after the habituation session. One day after the last treatment with antipsychotics (i.e., day22), the training session of the novel object recognition test was conducted. The number of animals included in each drug treatment was as follows: saline + CMC (n = 11), PCP + CMC (n = 10), PCP + 0.01mg/kg aripiprazole (n = 11), PCP + 0.03mg/kg aripiprazole (n = 8), PCP + 0.1mg/kg aripiprazole (n = 8) for Fig. 4b,d; saline + CMC (n = 15), PCP + CMC (n = 14), PCP + 0.3mg/kg haloperidol (n = 8), PCP + 1.0mg/kg haloperidol (n = 9) for Fig. 4c,e.

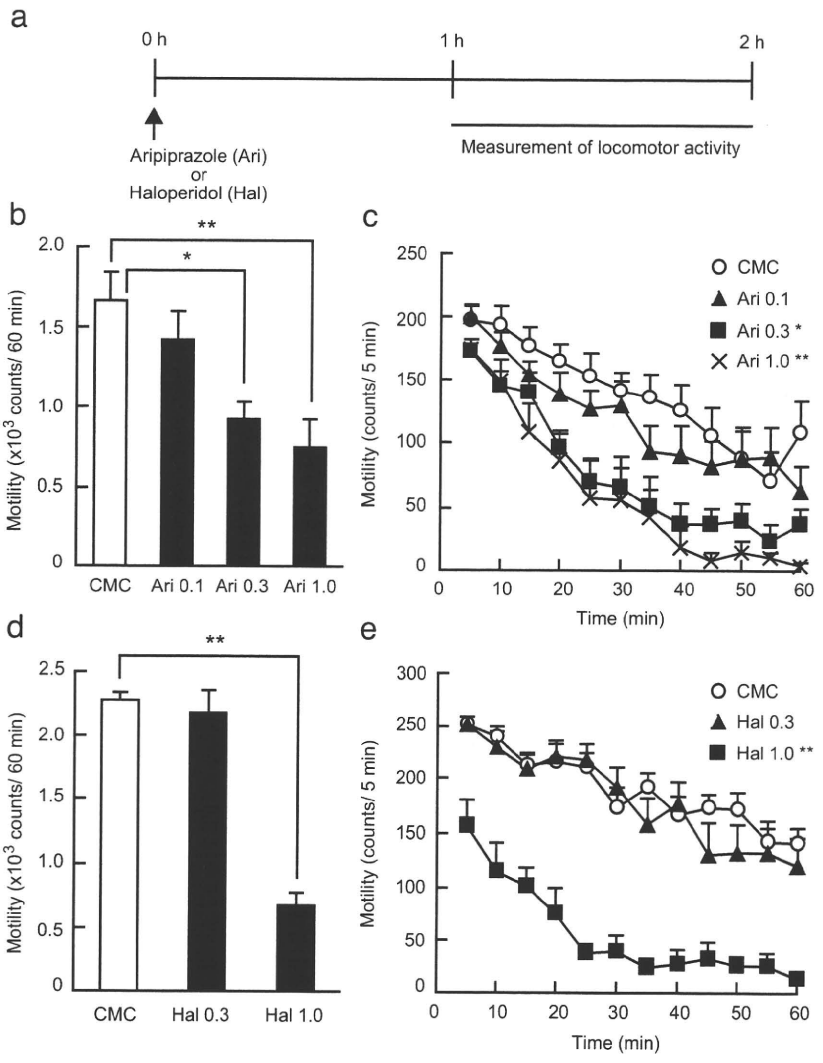


Fig. 1 Effects of single administration of aripiprazole and haloperidol on locomotor activity. **a** Experimental schedule for the measurement of locomotor activity. Mice were administered aripiprazole (*Ari*, 0.1–1.0 mg/kg, p.o.), haloperidol (*Hal*, 0.3–1.0 mg/kg, p.o.) or vehicle (0.1% *CMC*) 1 h before the measurement of locomotor activity. **b** and **c** Effect of single administration of aripiprazole on locomotor activity. **d** and **e** Effect of single administration of haloperidol on locomotor activity. **b** and **d** Total locomotor activity for 1 h. **c** and **e**: Time course of changes in locomotor activity. Values indicate the mean±SE (n=11–12). Analysis of variance: group, $F(3,43)=7.323$, $p<0.01$ for (**c**); time, $F(11,473)=58.971$, $p<0.01$ for (**c**); group × time, $F(33,473)=1.168$, $p=0.24$ for (**c**); group, $F(2,27)=46.806$, $p<0.01$ for (**e**); time, $F(11,297)=24.709$, $p<0.01$ for (**e**); group × time, $F(22,297)=1.370$, $p=0.13$ for (**e**). $*p<0.05$ and $**p<0.01$ compared with CMC group

To examine the role of dopamine D_1, D_2, and serotonin 5-HT_{1A} receptors in ameliorating the effect of aripiprazole on PCP-induced cognitive impairment, SCH23390 (0.03mg/kg), a dopamine D_1 receptor antagonist, raclopride (0.3mg/kg), a dopamine D_2 receptor antagonist, or WAY100635 (0.6mg/kg), a serotonin 5-HT_{1A} receptor antagonist, was administered intraperitoneally (i.p.) 30min after treatment with aripiprazole (0.1mg/kg, p.o.) for 7days because brain concentration of aripiprazole is the maximum at 2–3h after the oral administration, and declined $t_{1/2}$ of 1.8–2.0h in rats (Shimokawa et al. 2005). One day after the last treatment with aripiprazole and dopamine or serotonin receptor antagonists, the novel object recognition test was performed. The number of animals included in each drug treatment was as follows: saline + CMC + DW (n = 11), PCP + CMC + DW (n = 11), PCP + CMC + SCH23390 (n = 11), PCP + aripiprazole + DW (n = 11), PCP + aripiprazole + SCH23390 (n = 11) for Fig. 5b,d; saline + CMC + DW (n = 9), PCP + CMC + DW (n = 9), PCP + CMC + raclopride (n = 10), PCP + aripiprazole + DW (n = 9), PCP + aripiprazole + raclopride (n = 10) for Fig. 5c,e; saline + CMC + DW (n = 10), PCP + CMC + DW (n = 10), PCP + CMC + WAY100635 (n = 9), PCP + aripiprazole + DW (n = 10), PCP + aripiprazole + WAY100635 (n = 10) for Fig. 6.

Statistical analysis

All data were expressed as the mean±SEM. Statistical significance was determined using analysis of variance (ANOVA) with repeated measures (Figs. 1c,e, and 2c,e) or one-way (Figs. 1b,d, 2b,d, and 3–6), followed by the Bonferroni/Dunn test when F ratios were significant ($p<0.05$).

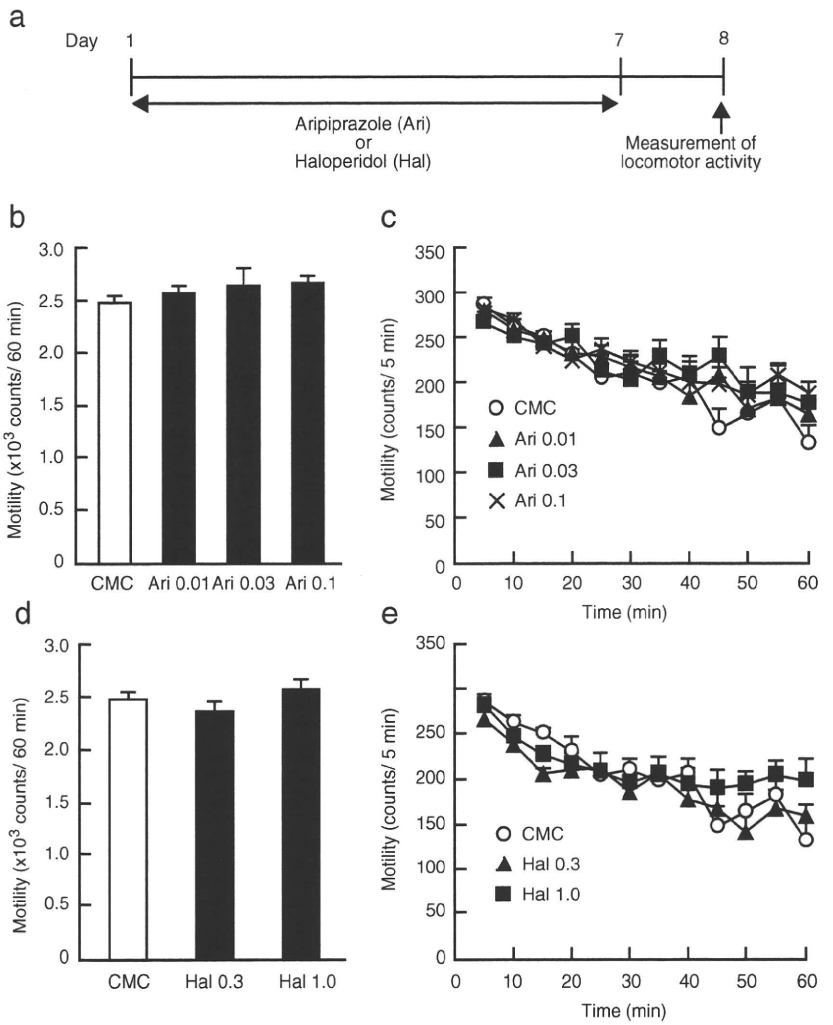


Fig. 2 Effects of repeated administration of aripiprazole and haloperidol on locomotor activity. **a** Experimental schedule for the measurement of locomotor activity. Mice were administered aripiprazole (*Ari*, 0.01–0.1 mg/kg, p.o.), haloperidol (*Hal*, 0.3–1.0 mg/kg, p.o.) or vehicle (0.1% *CMC*) for 7 days. Locomotor activity was measured 24 h after the last treatment. **b** and **c** Effect of repeated administration of aripiprazole on locomotor activity. **d** and **e** Effect of repeated administration of haloperidol on locomotor activity. **b** and **d** Total locomotor activity for 1 h. **c** and **e** Time course of changes in locomotor activity. Values indicate the mean±SE (n=10). Analysis of variance: group, $F(3,35)=0.743$, $p=0.53$ for (**c**); time, $F(11,385)=24.376$, $p<0.01$ for (**c**); group × time, $F(33,375)=1.099$, $p=0.33$ for (**c**); group, $F(2,27)=1.290$, $p=0.29$ for (**e**); time, $F(11,297)=18.444$, $p<0.01$ for (**e**); group × time, $F(22,297)=1.318$, $p=0.16$ for (**e**)

Results

Effects of administration of aripiprazole and haloperidol on locomotor activity

To explore the dose of aripiprazole and haloperidol which did not cause sedation in mice, we measured locomotor activity after oral administration of aripiprazole. Figure 1b and c shows the effect of a single administration of aripiprazole on locomotor activity in mice. Treatment with aripiprazole decreased total locomotor activity in a dose-dependent manner [$F(3,43)=7.323$, $p<0.01$, Fig. 1b]. The time course of changes in locomotor activity revealed that aripiprazole at doses of 0.3 and 1.0 mg/kg caused marked locomotor suppression 1 h after treatment [effect of group: $F(3,43)=7.323$, $p<0.01$; effect of time: $F(11,473)=58.971$, $p<0.01$; effect of interaction between group and time: $F(33,473)=1.168$, $p=0.24$ by two-way ANOVA with repeated measures, Fig. 1c]. Single treatment with haloperidol also decreased total locomotor activity in a dose-dependent manner [$F(2,27)=46.806$, $p<0.01$, Fig. 1d]. The time course of changes in locomotor activity revealed that haloperidol at the dose of 1.0 mg/kg caused marked locomotor suppression 1 h after treatment [effect of group: $F(2,27)=46.806$, $p<0.01$; effect of time: $F(11,297)=24.709$, $p<0.01$; effect of interaction between group and time: group × time, $F(22,297)=1.370$, $p=0.13$ by two-way ANOVA with repeated measures, Fig. 1e].

Effects of repeated administration of aripiprazole and haloperidol on locomotor activity were also examined. Mice were administered 0.1% CMC, aripiprazole (0.01–0.1 mg/kg, p.o.) or haloperidol (0.3–1.0 mg/kg, p.o.) was administered for 7 days. Locomotor activity was recorded 24 h after the last treatment. In contrast to the single treatment, repeated treatment with aripiprazole (0.01–0.1 mg/kg) and haloperidol (0.3–1.0 mg/kg) had no effect on the locomotor activity (Fig. 2).

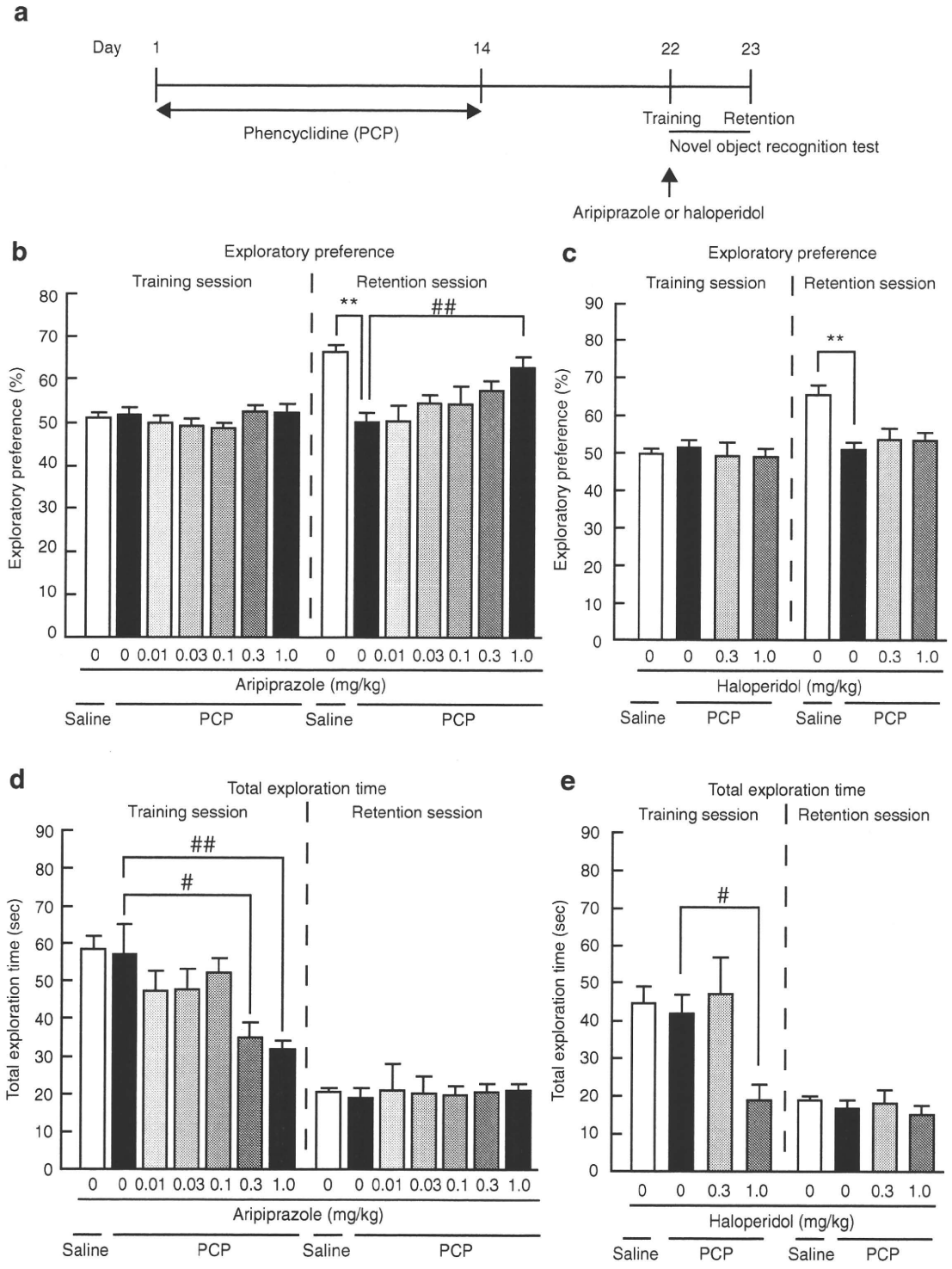


Fig. 3 Effects of single administrations of aripiprazole and haloperidol on PCP-induced cognitive impairment in novel object recognition. **a** Experimental schedule for the novel object recognition test. Eight days after withdrawal from repeated PCP (10 mg/kg, s.c., for 14 days) treatment, mice were subjected to the novel-object recognition test. Aripiprazole (0.01–1.0 mg/kg, p.o.), haloperidol (0.3–1.0 mg/kg, p.o.), or vehicle (0.1% CMC) was administered 1 h before the training session. **b** and **d** Effect of aripiprazole on PCP-induced cognitive impairment. **c** and **e** Effect of haloperidol on PCP-induced cognitive impairment. **b** and **c** Exploratory preference. **d** and **e** Total exploration time. Values indicate the mean±SE (n=8–16). Analysis of variance: $F(6,77)=0.911$, $p=0.49$ for (**b**) training; $F(6,77)=7.304$, $p<0.01$ for (**b**) retention; $F(3,39)=0.303$, $p=0.82$ for (**c**) training; $F(3,39)=8.69$, $p<0.01$ for (**c**) retention; $F(6,77)=5.009$, $p<0.01$ for (**d**) training; $F(6,77)=0.057$, $p=0.99$ for (**d**) retention; $F(3,39)=4.665$, $p<0.01$ for (**e**) training; $F(3,39)=0.600$, $p=0.62$ for (**e**) retention. $^{**}p<0.01$ compared with saline + vehicle group. $\#p<0.05$ and $\#\#p<0.01$ compared with PCP + vehicle group

Effect of PCP on performance in novel object recognition

Eight days after the last treatment with PCP (10 mg/kg, for 14 days), the novel object recognition test was performed. During habituation, no behavioral change was observed between PCP- and saline-treated mice. In the training session, PCP-treated and saline-treated mice spent equal amounts of time exploring either of the two objects (Fig. 3b,c), and thus there was no biased exploratory preference in either group of animals. In addition, total time spent in the exploration of objects in the training session did not differ between PCP- and saline-treated mice (Fig. 3d,e). These observations suggest that repeated PCP treatment has no effect on motivation, curiosity, or motor function.

When retention session was performed 24 h after the training session, the level of exploratory preference for the novel objects in the PCP-treated mice was significantly decreased compared to that in the saline-treated mice ($p<$ 0.01, Fig. 3b,c). The total exploration time did not differ between the two groups in the retention session (Fig. 3d,e). These results suggest that repeated PCP treatment for 14 days induces recognition memory impairment 8 days after the withdrawal of PCP.

Effects of single and repeated administrations of aripiprazole and haloperidol on PCP-induced cognitive impairment in novel object recognition

We examined whether PCP-induced cognitive impairment was reversed by aripiprazole, an atypical antipsychotic, or haloperidol, a typical antipsychotic, treatment. After the cessation of repeated PCP treatment (10 mg/kg s.c., for 14 days), mice were subjected to the novel object recognition test. Aripiprazole (0.01–1.0 mg/kg, p.o.) or haloperidol (0.3–1.0 mg/kg, p.o.) was acutely administered 1 h before the training session. A one-way ANOVA revealed that single treatment with aripiprazole dose-dependently improved cognitive impairment in PCP-treated mice [$F(5,56)=3.474$, $p<0.01$, Fig. 3b]. In contrast, single treatment with haloperidol had no effect on the cognitive impairment induced by repeated PCP treatment (Fig. 3c). Neither aripiprazole nor haloperidol affected the level of exploratory preference for the novel object in the training session (Fig. 3b,c). However, single treatment with aripiprazole and haloperidol decreased total exploration time in the training session of PCP-treated mice [one-way ANOVA: $F(6,77)=5.009$, $p<0.01$, Fig. 3d; $F(3,39)=4.665$, $p<0.01$, Fig. 3e]. Single treatment with aripiprazole (0.3 and 1.0 mg/kg) or haloperidol (1.0 mg/kg) significantly decreased the total exploration time in the training session of PCP-treated mice ($p<0.05$ and $p<0.01$, respectively, Fig. 3d; $p<0.05$, Fig. 3e). In the saline-treated mice, neither aripiprazole nor haloperidol affected the level of exploratory preference for the novel object in the training session (data not shown). However, single treatment with aripiprazole and haloperidol decreased total exploration time in the training session of saline-treated mice [one-way ANOVA: $F(5,66)=6.532$, $p<0.01$ for aripiprazole; $F(2,30)=22.346$, $p<0.01$ for haloperidol].

Next, we examined the effect of repeated treatment with antipsychotics on PCP-induced cognitive impairment. Aripiprazole (0.01–0.1 mg/kg) or haloperidol (0.3 and 1.0 mg/kg) was repeatedly administered p.o. for 7 days to mice that had been previously treated with PCP for 14 days. As shown in Fig. 4, repeated treatment with aripiprazole dose-dependently improved cognitive impairment in PCP-treated mice [$F(4,43)=9.166$, $p<0.01$], and a significant change was observed with doses of 0.03 and 0.1 mg/kg ($p<0.05$ and $p<0.01$, respectively, Fig. 4b). In contrast, repeated treatment with haloperidol failed to improve PCP-induced cognitive impairment (Fig. 4c). Repeated treatment with aripiprazole and haloperidol affected neither the level of exploratory preference for the novel object in the training session nor the total exploration time in either the training or retention sessions for PCP-treated mice (Fig. 4). In the saline-treated mice, repeated treatment with aripiprazole or haloperidol alone showed no effect on performance in the novel object recognition test (data not shown).

Effects of dopamine D_1 and D_2 receptor antagonists on ameliorative effect of aripiprazole against PCP-induced cognitive impairment

We have previously demonstrated that repeated PCP treatment in mice induces the dysfunction of dopamine neurotransmission in the prefrontal cortex which is necessary for the recognition memory (Mouri et al. 2007b; Nagai et al. 2007). Therefore, we investigated whether activation of dopamine receptors was involved in the ameliorating effect of aripiprazole on memory impairment in PCP-treated mice. SCH23390 (0.05 mg/kg i.p.), a dopamine D_1 receptor antagonist, or raclopride (0.3 mg/kg i.p.), a dopamine D_2 receptor antagonist, was co-administered with aripiprazole for 7 days, and the training session of the novel object recognition test was performed 1 day after the last treatment.

SCH23390 significantly blocked the ameliorating effect of aripiprazole on the impairment of exploratory preference for a novel object in PCP-treated mice ($p<0.01$, Fig. 5b), although it had no effect on PCP-induced impairment of memory retention (Fig. 5b). Treatment with SCH23390 did not affect the total exploration time in either the training or retention sessions (Fig. 5d). In contrast, treatment with raclopride had no effect on exploratory preference or total exploration time in the training and retention sessions (Fig. 5c,e).

Effect of serotonin 5-HT_{1A} receptor antagonist on ameliorative effect of aripiprazole against PCP-induced cognitive impairment

It has been reported that aripiprazole also has partial agonistic activity for serotonin 5-HT_{1A} receptors in parallel to its actions at dopamine D_2 receptors (Jordan et al. 2002); therefore, we examined whether 5-HT_{1A} receptors were involved in the ameliorative effect of aripiprazole on memory impairment in PCP-treated mice. The 5-HT_{1A} receptor antagonist WAY100635 (0.6 mg/kg, i.p.) was co-administered with aripiprazole for 7 days, and the training session of the novel object recognition test was performed 1 day after the last treatment.

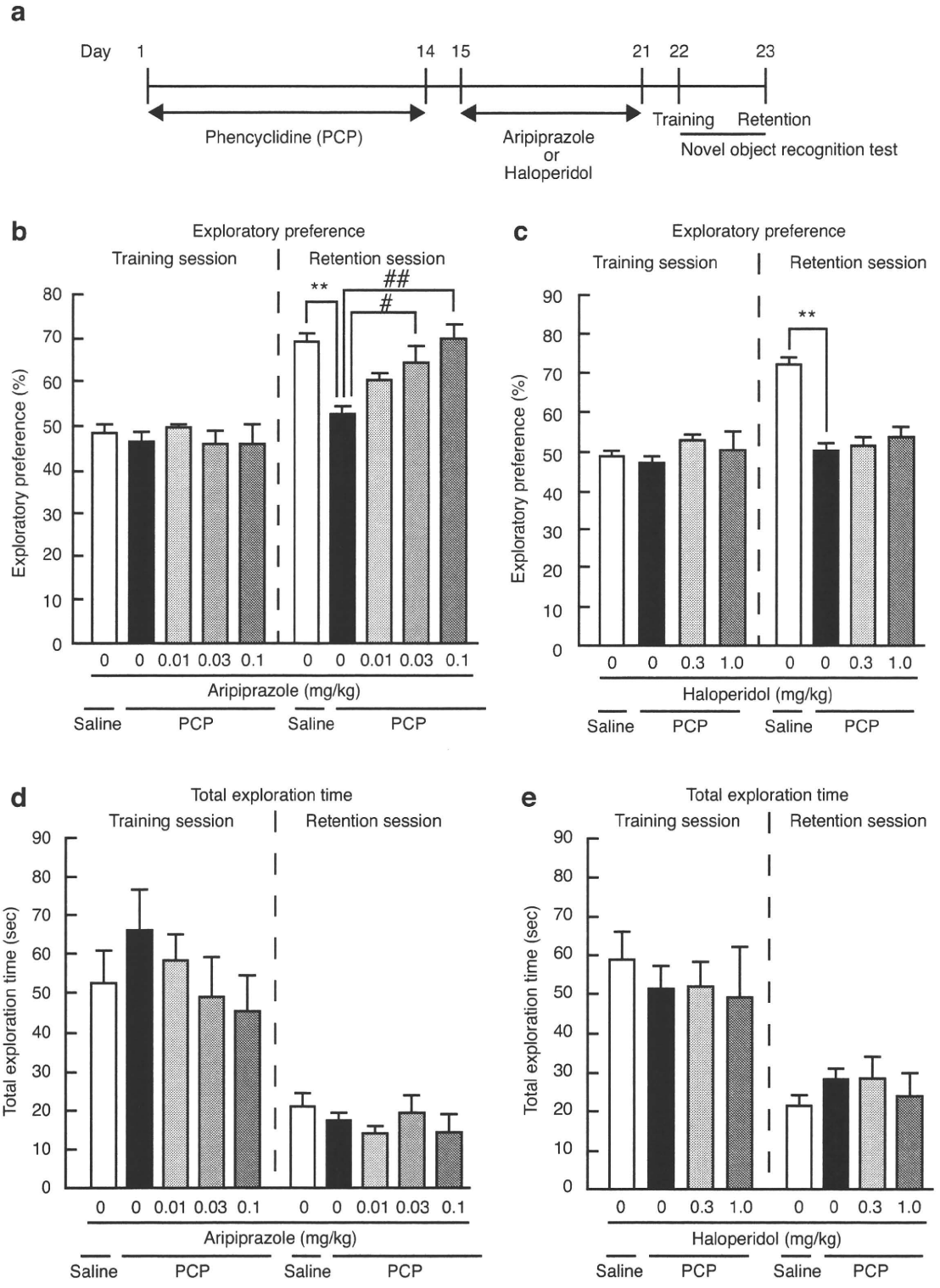
In the training session, treatment with WAY100635 alone did not affect the exploratory preference for objects in PCP-treated mice (Fig. 6b). In the retention session, WAY100635 blocked the ameliorating effect of aripiprazole on the impairment of exploratory preference for a novel object in PCP-treated mice ($p<0.01$, Fig. 6b), although it had no effect on PCP-induced impairment of memory retention (Fig. 6b). The antagonistic effect of WAY100635 on aripiprazole-induced improvement of exploratory preference in PCP-treated mice was not associated with changes in total exploration time (Fig. 6c).

Discussion

Cognitive deficits, including memory impairment, are regarded as a core feature in schizophrenia (Tamminga 2006). Recognition memory is a fundamental facet of the ability to remember and an integral component of the class of memory lost in amnesia (Aggleton and Brown 1999). The ability to discriminate familiar from novel stimuli is supported by this form of memory. To assess the effect of a novel atypical antipsychotic, aripiprazole, on cognitive dysfunction, we used a novel object recognition task, which is similar to visual recognition tests widely used in subhuman primates (Ennaceur and Delacour 1988). In the present study, an object preference index, the ratio of the amount of time spent exploring any one of the two objects (training session) or the novel object (retention session) over the total time spent exploring both object, was used to measure cognitive function. However, it is possible that the ratio misleads the results when based on a low amount a little second on average for the whole group of mice. Therefore, we also run a paired comparisons test for each group comparing the time spent on a novel with that spent on a familiar object. The saline-treated mice spent an equal time exploring the two objects (object A and object B) in the training session (Supplemental Fig. 1a), but a significantly greater time exploring the novel object (object C) versus the familiar object (object A) in the retention session (Supplemental Fig. 1b), showing that they were able to discriminate the novel object during the retention session. PCP + vehicle-treated mice also spent an equal time exploring the two objects in training session (Supplemental Fig. 1a). However, PCP + vehicle-treated mice spent equivalent times exploring the novel and the familiar objects in retention session (Supplemental Fig. 1b), whereas PCP + single aripiprazole-treated mice spent greater time exploring the novel (Supplemental Fig. 1b). These observations agree with the results using an object preference index ratio. Taken together, it is unlikely that the ratio misleads the results in this study.

In the present study, repeated PCP treatment for 14 days induced recognition memory impairment 8 days after the withdrawal of PCP, and PCP-induced cognitive impairment was ameliorated by aripiprazole, but not haloperidol. The results are consistent with a previous report that PCP-induced cognitive deficits were improved by atypical antipsychotics, such as clozapine and perospiron, but not a typical antipsychotic, haloperidol, in a novel object recognition test (Hagiwara et al. 2008; Hashimoto et al. 2005). It is possible that the doses of haloperidol (0.3–1.0 mg/kg) used in the present study is probably too high in terms of occupancy compared to that produced by aripiprazole (0.01–1.0 mg/kg) and the relative affinities for D_2 receptors (Hirose and Kikuchi 2005). Haloperidol at the dose of 0.3 mg/kg was used as the maximal dose which did not cause locomotor suppression in this study, but it had no effect on PCP-induced memory impairment. In addition, it has been demonstrated that more low doses of haloperidol (0.05–0.1 mg/kg) do not improve PCP-induced memory impairment in the novel object recognition test (Grayson et al. 2007; Hashimoto et al. 2005). Taken together, these findings suggest that aripiprazole, but not haloperidol, may be useful for the treatment of cognitive dysfunction in schizophrenia.

Fig. 4 Effects of repeated administrations of aripiprazole and haloperidol on PCP-induced cognitive impairment in novel object recognition. **a** Experimental schedule for the novel object recognition test. Aripiprazole (0.01–0.1 mg/kg, p.o.), haloperidol (0.3–1.0 mg/kg, p.o.), or vehicle (0.1% CMC) was administered for 7 days to mice that had previously been treated with saline or PCP (10 mg/kg, s.c.) for 14 days. The novel-object recognition test was performed 24 h after the last treatment with aripiprazole or saline. **b** and **d** Effect of aripiprazole on PCP-induced cognitive impairment. **c** and **e** Effect of haloperidol on PCP-induced cognitive impairment. **b** and **c** Exploratory preference. **d** and **e** Total exploration time. Values indicate the mean±SE (n=8–15). Analysis of variance: $F(4,43)=0.851$, $p=0.50$ for (**b**) training; $F(4,43)=9.166$, $p<0.01$ for (**b**) retention; $F(3,42)=1.049$, $p=0.38$ for (**c**) training; $F(3,42)=25.898$, $p<0.01$ for (**c**) retention; $F(4,43)=1.157$, $p=0.34$ for (**d**) training; $F(4,43)=1.029$, $p=0.40$ for (**d**) retention; $F(3,42)=0.305$, $p=0.82$ for (**e**) training; $F(3,42)=0.915$, $p=0.44$ for (**e**) retention. $^{**}p<0.01$ compared with saline + vehicle group. $\#p<0.05$ and $\#\#p<0.01$ compared with PCP + vehicle group



It has been reported that aripiprazole acts as a dopamine D_2 receptor antagonist in the state of excessive dopamine neurotransmission and as a dopamine D_2 receptor agonist in the state of low dopaminergic neurotransmission (Burris et al. 2002; Kikuchi et al. 1995; Inoue et al. 1996). Single treatment with aripiprazole (1.0 mg/kg) ameliorated PCP-induced impairment of recognition memory, although the treatment significantly decreased total exploration time in

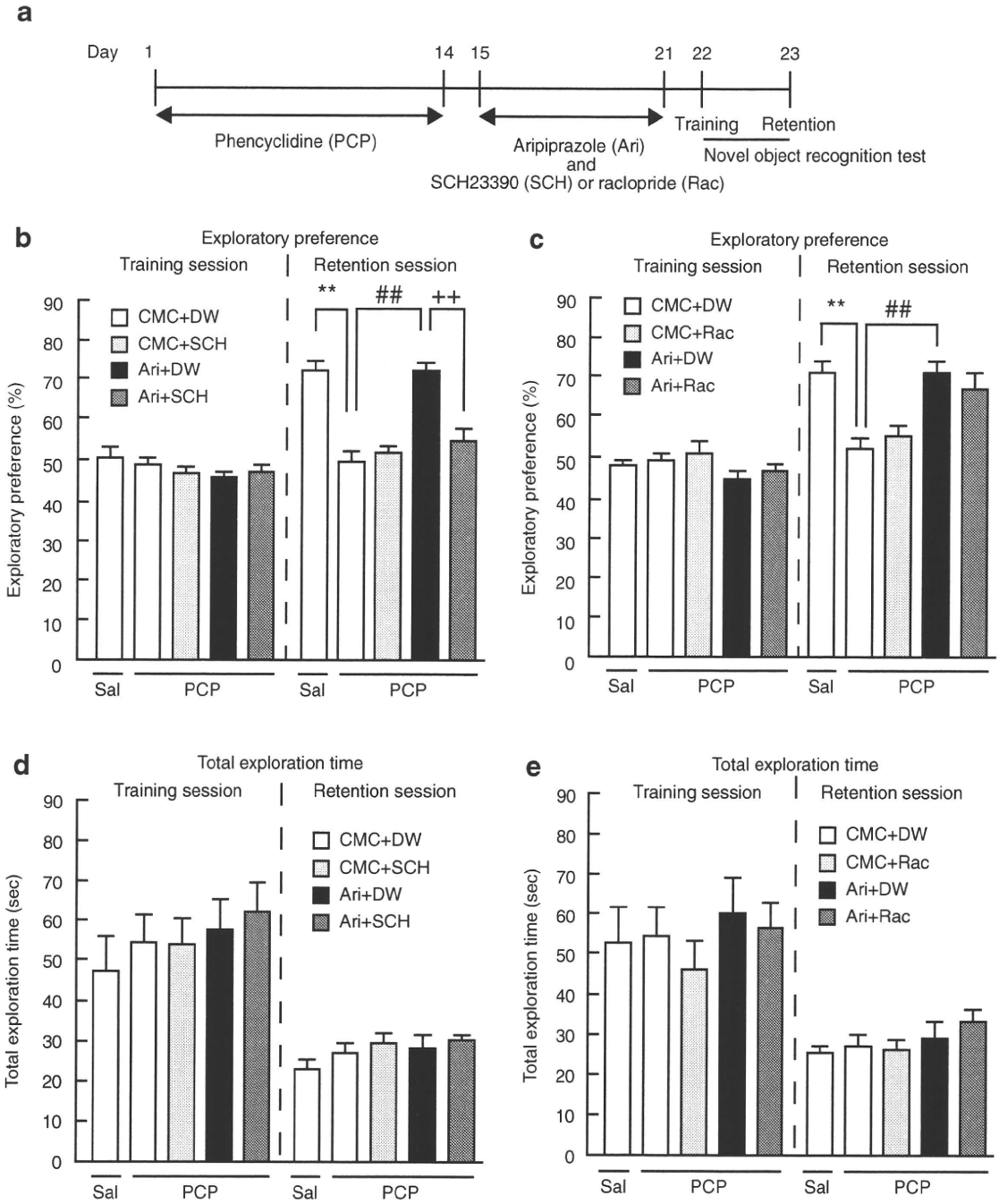


Fig. 5 Effect of dopamine D_1 and D_2 receptor antagonists on ameliorative effect of aripiprazole against PCP-induced cognitive impairment. **a** Experimental schedule for the novel object recognition test using dopamine D_1 and D_2 receptor antagonists. Aripiprazole (Ari, 0.1 mg/kg, p.o.) or vehicle (0.1% CMC) was administered for 7 days to mice that had previously been treated with saline (Sal) or PCP (10 mg/kg, s.c.) for 14 days. SCH23390 (SCH, 0.05 mg/kg, i.p.), Raclopride (Rac, 0.3 mg/kg, i.p.) or distilled water (DW) was administered 30 min after aripiprazole treatment for 7 days. The novel-object recognition test was performed 24 h after the last treatment with Ari. **b** and **d** Effect of SCH on ameliorative effect of Ari against PCP-induced cognitive impairment. **c** and **e** Effect of Rac on ameliorative effect of Ari against PCP-induced cognitive impairment. **b** and **c** Exploratory preference. **d** and **e** Total exploration time. Values indicate the mean±SE (n=9–11). Analysis of variance: $F(4,50)=0.951$, $p=0.44$ for (**b**) training; $F(4,50)=20.732$, $p<0.01$ for (**b**) retention; $F(4,42)=1.212$, $p=0.32$ for (**c**) training; $F(4,42)=8.520$, $p<0.01$ for (**c**) retention; $F(4,50)=0.527$, $p=0.72$ for (**d**) training; $F(4,50)=1.261$, $p=0.30$ for (**d**) retention. $F(4,42)=0.426$, $p=0.79$ for (**e**) training; $F(4,42)=1.210$, $p=0.32$ for (**e**) retention. $^{**}p<0.01$ compared with Sal + CMC + DW group. $^{\#\#}p<0.01$ compared with PCP + CMC + DW group. $^{++}p<0.01$ compared with PCP + Ari + DW group

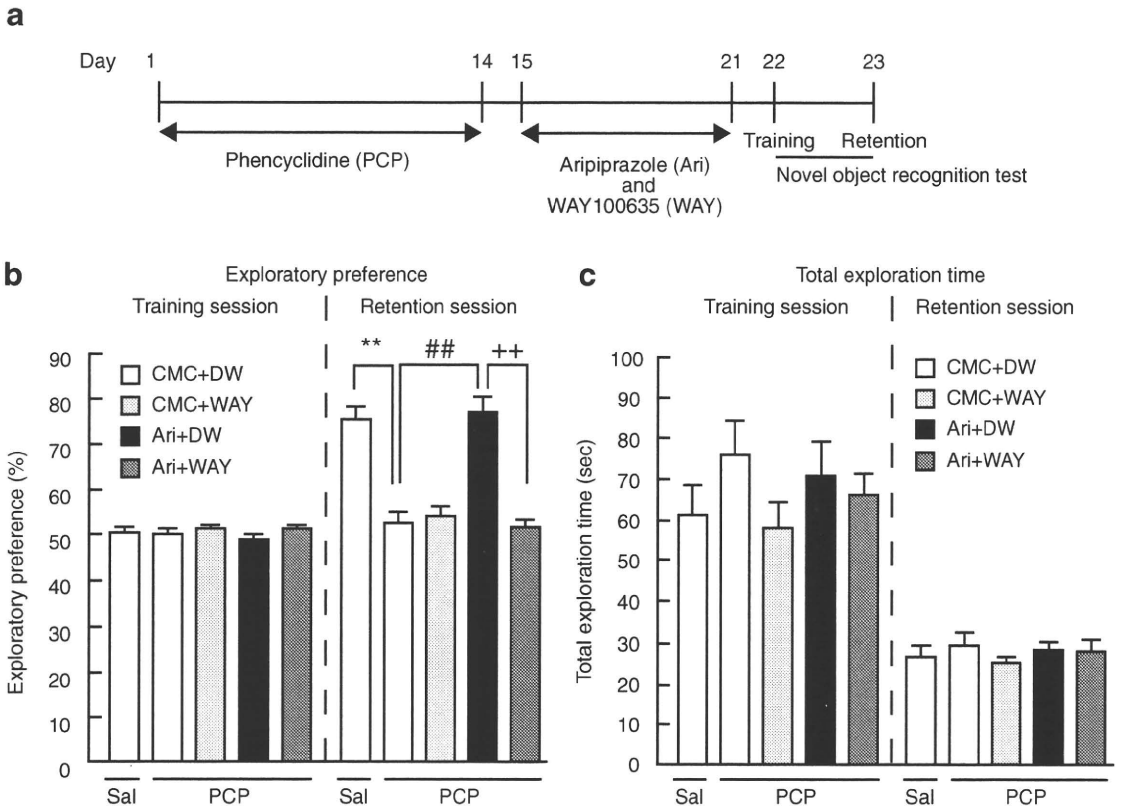


Fig. 6 Effect of serotonin 5-HT_{1A} receptor antagonist on ameliorative effect of aripiprazole against PCP-induced cognitive impairment. **a** Experimental schedule for the novel object recognition test using a serotonin 5-HT_{1A} receptor antagonist. Aripiprazole (Ari, 0.1 mg/kg, p.o.) or vehicle (0.1% CMC) was administered for 7 days to mice that had previously been treated with saline (Sal) or PCP (10 mg/kg, s.c.) for 14 days. WAY100635 (WAY, 0.6 mg/kg, i.p.) or distilled water (DW) was administered 30 min after Ari treatment for 7 days. The novel-object recognition test was performed 24 h after the last treatment with Ari. **b** Exploratory preference. **c** Total exploration time. Values indicate the mean±SE (n=9–10). Analysis of variance: $F(4,44)=0.921$, $p=0.46$ for (**b**) training; $F(4,44)=25.562$, $p<0.01$ for (**b**) retention; $F(4,44)=0.915$, $p=0.46$ for (**c**) training; $F(4,44)=0.327$, $p=0.86$ for (**c**) retention. $^{**}p<0.01$ compared with Sal + CMC + DW group. $^{\#\#}p<0.01$ compared with PCP + CMC + DW group. $^{++}p<0.01$ compared with PCP + Ari + DW group

the training session. Therefore, aripiprazole at 0.1 mg/kg was used in the repeated treatment as the maximal dose which did not cause locomotor suppression in mice.

Accumulating evidence suggests that the dopaminergic system in the prefrontal cortex is involved in cognitive function. For instance, disruption of dopamine transmission in the prefrontal cortex by infusions of dopamine D_1 receptor antagonists or by excitotoxic lesions impairs the performance of object retrieval detour tasks, as well as delayed response tasks in nonhuman primates (Sawaguchi and Goldman-Rakic 1991; Dias et al. 1996a, b). A recent study with functional magnetic resonance imaging showed that dysfunction in the prefrontal cortex of schizophrenic patients is related to cognitive impairment (Tan et al. 2007). Accordingly, cognitive impairment in schizophrenia may be associated with deficits in dopamine transmission in the prefrontal cortex. In the present study, the ameliorative effect of aripiprazole on PCP-induced cognitive impairment was prevented by a dopamine D_1 receptor antagonist, but not a dopamine D_2 receptor antagonist. Our previous study has demonstrated that stimulation with dopamine D_1 receptors is necessary for long-term retention of recognition memory in the prefrontal cortex (Kamei et al. 2006; Nagai et al. 2007). Taken together, these findings indicated that dopamine D_1 receptor in the prefrontal cortex may play a critical role in the ameliorative effect of aripiprazole on PCP-induced cognitive impairment.

Although aripiprazole has high affinity for dopamine D_2 receptors, a dopamine D_2 receptor antagonist had no effect on the ameliorative effect of aripiprazole on PCP-induced cognitive impairment. One possible reason for this discrepancy is that, the ability of dopamine D_1 receptor stimulation to improve cognition is due to a particular cellular localization in cortical networks: It has been demonstrated that dopamine D_1 receptors preferentially localize to non-pyramidal neurons, while dopamine D_2 receptors localize to both nonpyramidal and pyramidal cells in the prefrontal cortex of rats (Vincent et al. 1995). The other possible reason is that the ameliorative effect of aripiprazole on PCP-induced cognitive impairment may be involved in

receptors other than the dopamine D_2 receptor, since aripiprazole interacts with not only dopamine D_2 receptor, but also a large number of biogenic amine receptors (Shapiro et al. 2003). However, we cannot exclude the possibility that a part of ameliorative effect of aripiprazole on PCP-induced cognitive impairment is through dopamine D_2 receptor. Further studies are needed by using other dopamine D_2 antagonists or dopamine D_2 receptor knockout mice.

Accumulating evidence has suggested that serotonin 5-HT_{1A} receptors are an important target for cognitive dysfunction in schizophrenia (Bantick et al. 2001; Meltzer 1999). The density of 5-HT_{1A} receptor binding is altered in the hippocampus and cerebral cortex of the postmortem brain of schizophrenic patients (Burnet et al. 1996; Gurevich and Joyce 1997; Joyce et al. 1993; Lopez-Figueroa et al. 2004). Adjunctive treatment with tandospirone, a selective 5-HT_{1A} receptor agonist, is associated with improvements in some types of memory function as well as the cognitive performance of schizophrenic patients (Sumiyoshi et al. 2001a, b). Preclinical studies on the action of aripiprazole at 5-HT_{1A} receptors have shown partial agonist activity in vitro and in vivo (Jordan et al. 2002; Shapiro et al. 2003; Stark et al. 2007). In the present study, 5-HT_{1A} receptor antagonist blocked the ameliorating effect of aripiprazole on cognitive impairment in PCP-treated mice. Therefore, these results supported that atypical antipsychotic drugs, such as aripiprazole, clozapine, ziprasidone, and quetiapine, which have 5-HT_{1A} receptor agonist activity, are useful for cognitive impairment in schizophrenia (Jordan et al. 2002; Newman-Tancredi et al. 2001; Rollema et al. 2000; Sprouse et al. 1999)

The mechanisms by which aripiprazole ameliorates PCP-induced cognitive dysfunction through serotonin 5-HT_{1A} and dopamine D_1 receptors remain to be determined; however, it is known that the activation of 5-HT_{1A} receptors in the prefrontal cortex enhances the activity of dopaminergic neurons in the ventral tegmental area and mesocortical dopamine release (Díaz-Mataix et al. 2005). Aripiprazole increases the release of dopamine in the prefrontal cortex of rats and mice through the activation of 5-HT_{1A} receptors (Bortolozzi et al. 2007; Li et al. 2004; Zocchi et al. 2005). Recently, we have also observed that microinjection of 5-HT_{1A} receptor antagonist into the prefrontal cortex blocked the ameliorating effect of aripiprazole on cognitive impairment in PCP-treated mice (unpublished data). Accordingly, it is likely that stimulation of 5-HT_{1A} receptors in the prefrontal cortex induces dopamine D_1 receptor activation through the mesocortical dopaminergic pathway, which is involved in the ameliorating effect of aripiprazole on PCP-induced cognitive dysfunction.

Since aripiprazole has the 5-HT_{2A} receptor antagonistic activity displayed by atypical antipsychotics, such as clozapine, olanzapine, and risperidone (McQuade et al. 2002), involvement of 5-HT_{2A} receptors in the ameliorating effect of aripiprazole remains to be determined. However, it has been reported that 5-HT_{2A} receptor blockade increases dopamine release in the prefrontal cortex by atypical antipsychotics, and the increase of dopamine release is partly or totally antagonized by 5-HT_{1A} antagonist and by a defect of the 5-HT_{1A} receptor gene (Ichikawa et al. 2001; Díaz-Mataix et al. 2005). Therefore, it is likely that atypical antipsychotics through 5-HT_{2A} blockade, regardless of intrinsic 5-HT_{1A} affinity, may promote the ability of 5-HT_{1A} receptor stimulation to increase dopamine release.

In conclusion, we demonstrated that repeated PCP treatment impaired the recognition memory of novel objects. Single treatment with aripiprazole (1.0 mg/kg) ameliorated PCP-induced impairment of recognition memory, although it significantly decreased the total exploration time in the training session. Repeated treatment with aripiprazole at doses of 0.03 and 0.1 mg/kg for 7 days showed a significant ameliorating effect on PCP-induced impairment of recognition memory without affecting the total exploration time in training and retention sessions. In contrast, both single and repeated treatment with haloperidol (0.3 and 1.0 mg/kg) failed to reverse PCP-induced cognitive impairment. The ameliorating effect of aripiprazole on recognition memory in PCP-treated mice was blocked by dopamine D_1 and serotonin 5-HT_{1A} receptor antagonists; however, dopamine D_2 receptor antagonist had no effect on the ameliorating effect of aripiprazole. These results suggest that the ameliorative effect of aripiprazole on PCP-induced cognitive impairment is associated with dopamine D_1 and serotonin 5-HT_{1A} receptors.

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