session (one sample t-test, $P=0.0105$) or above chance set at 50% (one sample t-test, $P=0.0257$). However, a significant difference between vehicle- and $A\beta_{(25-35)}$-injected mice in the exploratory preference for the novel object was observed during the retention session [Kruskal–Wallis, $H=11.78$, d.f. = 5, $P=0.0379$; Dunnett, $P=0.0251$], indicating impairment of visual recognition memory. The administration of selegiline (3 mg/kg) alone significantly ameliorated $A\beta_{(25-35)}$-induced impairment of the exploratory behavior in the retention session, but this effect was not seen with donepezil [Kruskal–Wallis, $H=11.78$, d.f. = 5, $P=0.0379$; Dunnett, $P=0.0069$] (Fig. 2D).

3.1.3. Contextual fear conditioning task

In the preconditioning phase, mice hardly showed a freezing response. There were no differences in basal levels of freezing response among all the groups (Fig. 2E). In the retention test, the vehicle-injected mice showed a marked contextual freezing response 24 h after fear conditioning (Fig. 2F), whereas the $A\beta_{(25-35)}$-injected mice presented less freezing responses in the contextual tests [Kruskal–Wallis, $H=17.43$, d.f. = 5, $P=0.0037$; Dunnett, $P=0.0005$]. The performance of $A\beta_{(25-35)}$-injected mice was completely restored by treatment with donepezil (0.1 mg/kg) or selegiline (3 mg/kg) [Kruskal–Wallis, $H=17.43$, d.f. = 5, $P=0.0037$; Dunnett, $P=0.0159$ (donepezil), $P=0.0053$ (selegiline)] (Fig. 2F). Since the low doses of donepezil (0.05 mg/kg) and selegiline (1 mg/kg) failed to improve $A\beta_{(25-35)}$-induced cognitive impairment, their conditions were used in all subsequent experiments. In the conditioning phase, there was no difference in the levels of flinching, running and jumping responses or vocalization by a foot shock among all the groups (data not shown), indicating no changes in nociceptive response, because we excluded the animals that did not represent normal nociceptive response in the conditioning phase from the contextual fear conditioning test.

3.2. Effects of co-administration of selegiline and donepezil on $A\beta_{(25-35)}$-induced memory impairment

We investigated whether co-administration of low-dose selegiline and donepezil attenuated $A\beta_{(25-35)}$-induced cognitive impairment.

In the Y-maze test, $A\beta_{(25-35)}$-induced impairment of alternation behavior was significantly improved by combined administration of donepezil (0.05 mg/kg) and selegiline (1 mg/kg) [Kruskal–Wallis, $H=47.36$, d.f. = 4, $P<0.0001$; Dunnett, $P<0.0001$], at doses that were not effective individually (Fig. 3A). The number of arm entries was not changed by any treatments (data not shown).

In the novel object recognition test, there were no significant differences in exploratory preference for two objects (the training session), or total exploratory time (the training and retention sessions), among all the groups (data not shown). The combined administration of donepezil (0.05 mg/kg) and selegiline (1 mg/kg) significantly improved $A\beta_{(25-35)}$-induced impairment of visual recognition memory [Kruskal–Wallis, $H=12.25$, d.f. = 4, $P=0.0156$; Dunnett, $P=0.0209$] (Fig. 3B).

In the contextual fear conditioning test, there were no differences in basal levels of freezing response among all the groups (data not shown). The combined administration of donepezil (0.05 mg/kg) and selegiline (1 mg/kg) significantly improved $A\beta_{(25-35)}$-induced impairment of the contextual freezing response [Kruskal–Wallis, $H=17.08$, d.f. = 4, $P=0.0019$; Dunnett, $P=0.008$] (Fig. 3C). In the conditioning phase, there was no difference in the levels of flinching, running and jumping responses or vocalization by a foot shock among all the groups (data not shown), indicating no changes in nociceptive response, because we excluded the animals that did not represent normal nociceptive response in the conditioning phase from the contextual fear conditioning test.

3.3. Antagonistic effects of scopolamine and haloperidol against the synergistic effect of selegiline and donepezil on $A\beta_{(25-35)}$-induced cognitive impairment

To determine whether the improving effect of co-administration of selegiline and donepezil on $A\beta_{(25-35)}$-induced cognitive impairment is mediated via muscarinic and/or dopamine receptors, we examined its antagonism by a muscarinic receptor antagonist scopolamine and a dopamine receptor antagonist haloperidol. We preliminarily confirmed that administration of scopolamine (0.1 mg/kg) and haloperidol (0.1 mg/kg) alone had no effect on the cognitive impairment in $A\beta_{(25-35)}$-injected mice in all behavioral tests, while in the contextual fear conditioning test, haloperidol (0.1 mg/kg)-treated, $A\beta_{(25-35)}$-injected mice did not represent less freezing responses compared to vehicle-injected mice during the retention session (data not shown). Therefore, we evaluated antagonistic effect of haloperidol at the dose of 0.03 mg/kg that did not change freezing responses in $A\beta_{(25-35)}$-injected mice in the contextual fear conditioning test.

Pre-administration of scopolamine (0.1 mg/kg) or haloperidol (0.1 mg/kg) significantly antagonized the improving effect of co-administration of selegiline and donepezil on $A\beta_{(25-35)}$-induced impairment of spontaneous alternation in the Y-maze task [Kruskal–Wallis, $H=23.37$, d.f. = 4, $P<0.0001$; Dunnett, $P=0.0111$ (scopolamine), $P=0.0495$ (haloperidol)] and novel object recognition test [Kruskal–Wallis, $H=16.30$, d.f. = 4, $P=0.0026$; Dunnett, $P=0.0027$ (scopolamine), $P=0.0243$ (haloperidol)] (Fig. 4A and B). In the novel object recognition test, there were no significant differences in exploratory preference for two objects (the training session), or total exploratory time (the training and retention sessions), among all the groups (data not shown). These results indicate that all groups of mice have the same levels of motivation, curiosity and motor activity.

In the contextual fear conditioning tests, the improving effects of co-administration of selegiline and donepezil on $A\beta_{(25-35)}$-induced cognitive impairment were significantly antagonized by both scopolamine (0.1 mg/kg) and haloperidol (0.03 mg/kg) [Kruskal–Wallis, $H=18.93$, d.f. = 4, $P=0.0008$; Dunnett, $P=0.0138$ (scopolamine), $P=0.0069$ (haloperidol)] (Fig. 4C). There were no differences in basal levels of freezing response among all the groups (data not shown), indicating no changes in motor function. In the conditioning phase, there was no difference in the levels of flinching, running and jumping responses or vocalization by a foot shock among all the groups (data not shown), indicating no changes in nociceptive response, because we excluded the animals that did not represent normal nociceptive response in the conditioning phase from the contextual fear conditioning test.

4. Discussion

A number of studies have demonstrated that acute or continuous injections of Aβ into the brain cause neurodegeneration and impairment of learning and memory [20,40]. $A\beta_{(25-35)}$ containing the 11-amino acid sequence (25–35) of Aβ is neurotoxic *in vitro* [43] and *in vivo* [20], and its neurotoxicity may more likely mimic the oligomeric Aβ which is believed to be a key factor influencing cognitive function in AD [22]. A single i.c.v. injection of $A\beta_{(25-35)}$ induces marked deficiencies in both short- and long-term memory in mice, and increases deposition and dissemination of Aβ in the cortex and hippocampus of mice, which is consistent with the clinicopathological picture of AD [20,23]. In the present study, we found that $A\beta_{(25-35)}$-injected mice showed impairments of spatial working memory in the Y-maze test, visual recognition memory in the novel object recognition test, and associative fear memory in the contextual fear

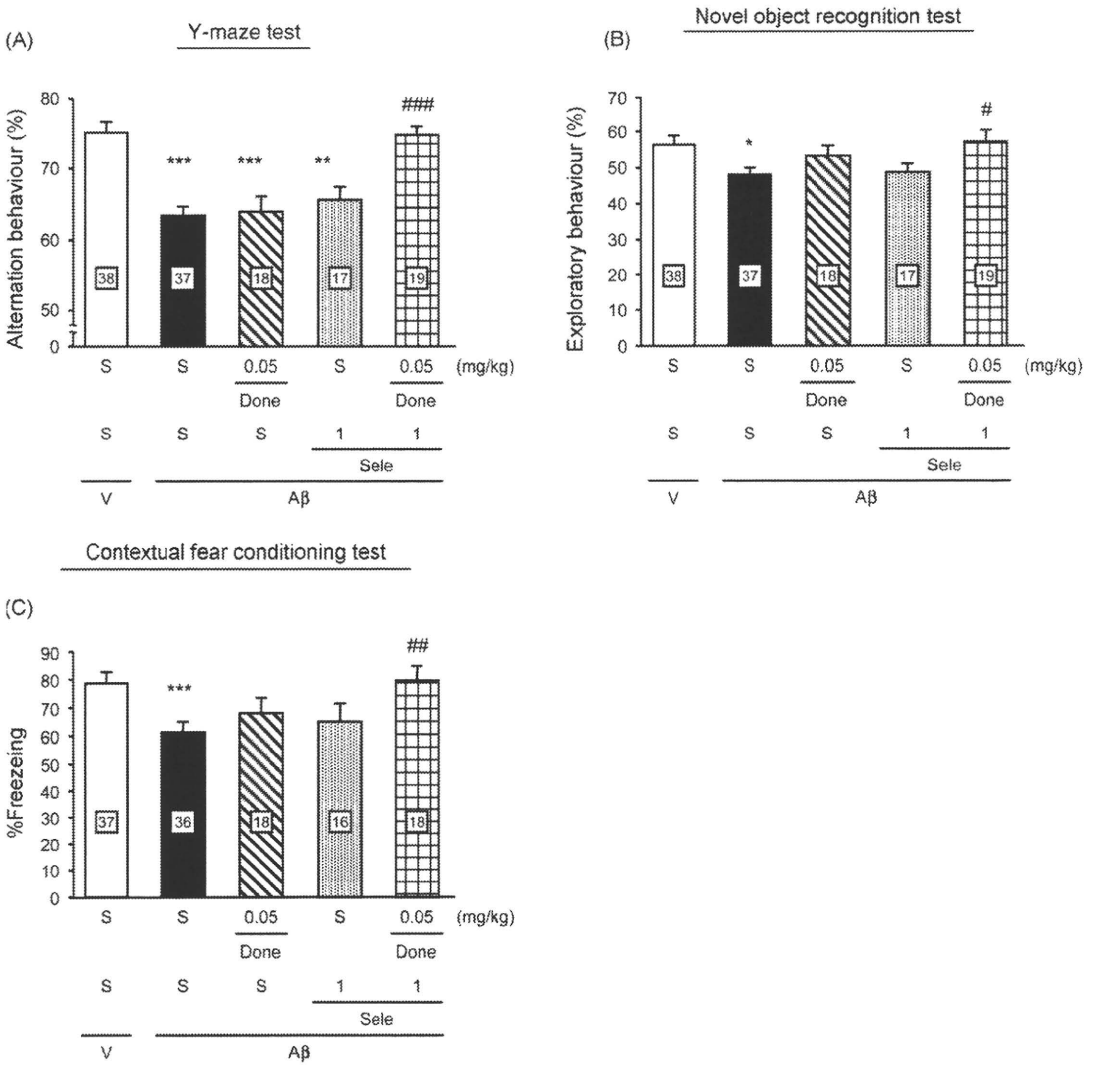

Fig. 3. Effects of co-administration of selegiline and donepezil on $A\beta_{(25-35)}$-induced memory impairment. Six, seven and nine days after i.c.v. injection of $A\beta_{(25-35)}$, the mice were subcutaneously administered donepezil (0.05 mg/kg), selegiline (1 mg/kg) or saline 30 min before each behavioral test. Panels A, B and C show the result of alternation behavior (A) in the Y-maze test, retention trial (B) in the novel objective test, and retention session (C) in the contextual fear conditioning test, respectively. S, saline; V, vehicle (distilled water); Aβ, $A\beta_{(25-35)}$; Done, donepezil; Sele, selegiline. Values represent means ± S.E.M. The number of mice used in each group is shown in the column. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ vs. saline-treated, vehicle-injected mice. $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ vs. saline-treated, $A\beta_{(25-35)}$-injected mice.

conditioning test, which are known to require the control function of the hippocampus. $A\beta_{(25-35)}$-injected mice did not show any significant differences in motivation and movements, as evidenced by the number of arm entries in the Y-maze test, exploratory preference found during the training session, the total amount of time spent exploring two objects in the novel object recognition test, and the freezing time during the preconditioning phase in the contextual fear conditioning test. From these results, it is likely that impairment of performance in the $A\beta_{(25-35)}$-injected mice is due to learning and memory deficits associated with hippocampal functions.

The mechanism of memory impairment in the $A\beta_{(25-35)}$-infused mice is still unknown. However, previous reports [20,38] have demonstrated that histological examination of Cresyl violet-stained brain sections indicates a moderate but significant cell loss within the frontoparietal cortex and the hippocampal formation of mice treated with aged $A\beta_{(25-35)}$ (9 nmol) and that examination of Congo red-stained sections in the same animals exhibits the presence of numerous amyloid deposits throughout these brain areas. Although we did not perform histochemical experiments in the $A\beta_{(25-35)}$-injected mice in the present study, we consider the $A\beta_{(25-35)}$-injected mice as the animal model of AD in the incipient stage.

Single administration of donepezil at 3 mg/kg improved memory impairment induced by $A\beta_{(25-35)}$ in the Y-maze and contextual fear conditioning tests (Fig. 2). Our findings were consistent with previous reports that donepezil significantly improves alternation deficits in Y-maze and impairment of memory in step-through type passive avoidance tests in the $A\beta_{(25-35)}$-injected mice [21] and deficits of spatial learning in a water T-maze, and contextual and cued memory in fear conditioning tests in the Tg2576 transgenic mouse, which overexpresses human amyloid precursor protein linked to AD [5]. Another AchEI, tacrine, recovers memory impairment induced by i.c.v. injection of $A\beta_{(25-35)}$ [20]. Therefore, it is suggested that $A\beta_{(25-35)}$ induces hypofunction of the cholinergic system in the hippocampus.

The hippocampal formation plays a central role in learning and memory in the mammalian brain. The hippocampus also receives dopaminergic input, particularly from the ventral tegmental area [24]. A functional role of the hippocampal dopaminergic system has been indicated by behavioral studies

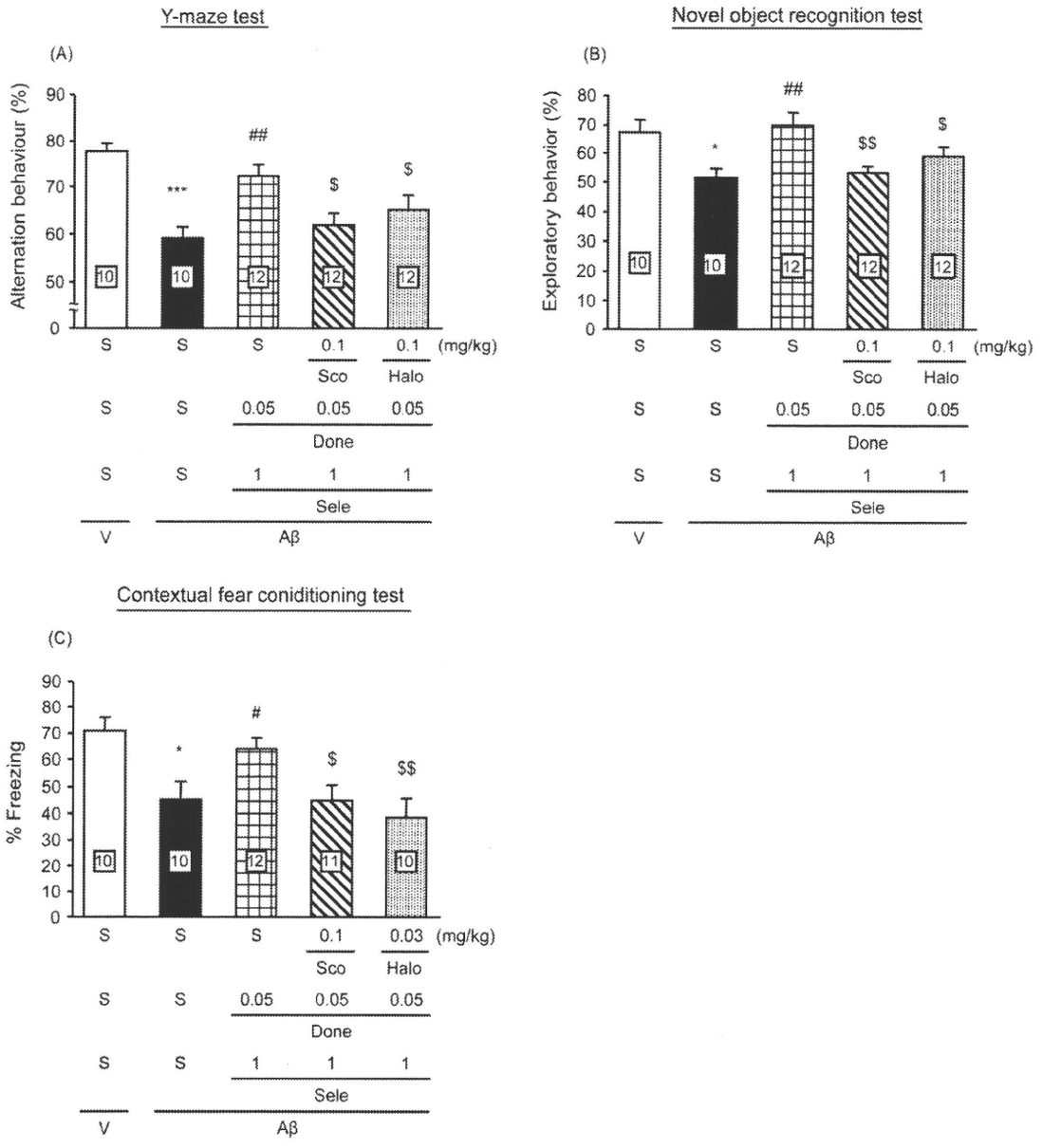

Fig. 4. Antagonistic effects of scopolamine and haloperidol against the synergistic cognition-improving effect of co-administration of selegiline and donepezil in $A\beta_{(25-35)}$-injected mice. Six, seven and nine days after i.c.v. injection of $A\beta_{(25-35)}$, the mice were subcutaneously administered donepezil, selegiline or saline 30 min before each behavioral test. The mice were subcutaneously administered scopolamine, haloperidol or saline 45 and 60 min before each behavioral test, respectively. Panels A, B and C show the result of alternation behavior (A) in the Y-maze test, retention trial (B) in the novel objective test, and retention session (C) in the contextual fear conditioning test, respectively. S, saline; V, vehicle (distilled water); Aβ, $A\beta_{(25-35)}$; Done, donepezil; Sele, selegiline; Sco, scopolamine; Halo, haloperidol. Values represent means ± S.E.M. The number of mice used in each group is shown in the column. $^{*}P<0.05$, $^{***}P<0.001$ vs. (saline + saline + saline)-treated, vehicle-injected mice. $^{\#}P<0.05$, $^{\#\#}P<0.01$ vs. (saline + saline + saline)-treated, $A\beta_{(25-35)}$-injected mice. $^{\$}P<0.05$, $^{\$\$}P<0.01$ vs. (Done + Sele + saline)-treated, $A\beta_{(25-35)}$-injected mice.

demonstrating enhancement of positive reinforcement learning, visual discrimination, and passive avoidance behavior after intrahippocampal injections of dopamine receptor agonists, as well as impairment of spatial navigation after depletion of hippocampal dopamine [19]. Thus, the dopaminergic system is implicated in cognitive processes in a variety of brain regions, including the hippocampus. Monoamine oxidase B is localized in various regions of the human brain including the hippocampus. In the present study, single administration of selegiline also improved memory impairment induced by $A\beta_{(25-35)}$ in the Y-maze, novel object recognition and contextual fear conditioning tests. These effects might be mediated by the increased level of dopamine in the hippocampus. In several clinical trials, selegiline improved episodic memory and learning in patients with AD [35].

Co-administration of selegiline and donepezil at subthreshold doses significantly ameliorated memory impairment in $A\beta_{(25-35)}$-injected mice in all of the behavioral tests, which was consistent with the finding that selegiline and tacrine improve performance in scopolamine + *p*-chlorophenylalanine-treated rats in a water maze task [6]. It is considered that the interaction of selegiline and donepezil is synergistic in nature, because the acting sites are different between both drugs.

In the present study, synergistic effects of co-administration of selegiline and donepezil on memory impairment induced by $A\beta_{(25-35)}$ were antagonized by pretreatment with dopamine receptor antagonist haloperidol, as well as muscarinic receptor antagonist scopolamine. These findings indicate that the dopaminergic–cholinergic interaction is partly involved in the synergistic effects of selegiline and donepezil. Pathological abnor-

malities in monoaminergic innervations in the forebrain of AD patients are known to exist in addition to abnormal cholinergic innervations. Previous studies have reported that: (1) the forebrain dopaminergic and cholinergic systems in humans are related to cognitive function [27]; (2) increases in hippocampal levels of dopamine and acetylcholine are associated with the learning process [41]; and (3) dopamine modulates acetylcholine release at cholinergic [11] and glutamatergic [42] synapses in the hippocampus. Selegiline can enhance dopaminergic neurotransmission due to its monoamine oxidase B inhibitory action. Shimazu et al. [30] have shown that selegiline increases acetylcholine release in the frontal cortex, and that such an effect is mimicked by dopamine D1 receptor agonists and blocked by dopamine D1 receptor antagonists. Thus, it is possible that selegiline enhances the level of dopamine in the hippocampus, followed by increasing the level of acetylcholine in the hippocampus, and remission of memory impairment. It is unlikely that the synergistic effects of co-administration of selegiline and donepezil or tacrine on memory impairment are due to pharmacokinetic mechanisms related to metabolism by cytochrome P450 (CYP), because donepezil, tacrine and selegiline are mainly metabolized through CYP2D6/3A4, CYP1A2 and CYP2B6, respectively [14,28].

It is reported that donepezil interacts with the sigma 1 receptor [16] and its anti-amnesic effects against $A\beta_{(25-35)}$-induced toxicity involve its sigma 1 agonistic property as well as cholinergic agonistic property [21]. Furthermore, haloperidol, used as dopamine receptor antagonist in this study, also has affinity for sigma 1 receptor. Therefore, it is possible that sigma 1 receptor is involved in the synergistic effects of co-administration of selegiline and donepezil in $A\beta_{(25-35)}$-injected mice and further investigation would be needed into this point.

Oxidative stress plays an important role in AD, and is induced by several processes related to Aβ, including toxic inflammatory responses [39]. One major index of oxidative stress is the level of glutathione (GSH). The GSH system is responsible for removing hydrogen peroxide from mitochondria and the cytosol, and therefore, constitutes an important protective mechanism for minimizing oxidative damage during energy metabolism. Reduction in GSH levels has been observed in specific regions of the central nervous system affected by AD [10]. Furthermore, $A\beta_{(25-35)}$ used in the present study are known to deplete endogenous GSH levels in neurons and astrocytes in a calcium-dependent manner [1]. In our preliminary experiment, we found that i.c.v. injection of $A\beta_{(25-35)}$ caused a reduction in GSH levels in the frontal cortex and hippocampus in mice, and co-administration of selegiline and donepezil tended to alleviate the $A\beta_{(25-35)}$-induced reduction in GSH level in the frontal cortex (data not shown). Selegiline has been reported to produce a significant increase in GSH levels and activities of superoxide dismutase (SOD) 1 and SOD2 in mesencephalic slice cultures [34]. Donepezil has been also reported to attenuate $A\beta_{(25-35)}$-induced toxicity in PC12 cells [31]. Therefore, neuroprotective action through antioxidant effects induced by co-administration of selegiline and donepezil may be involved in amelioration of cognitive deficits.

In conclusion, selegiline, as well as donepezil, improved memory impairment in $A\beta_{(25-35)}$-injected mice. Co-administration of selegiline and donepezil, at doses that do not exert efficacy individually, ameliorated memory impairment induced by $A\beta_{(25-35)}$ in a battery of learning and memory behavioral tests. These results suggest that selegiline can synergistically potentiate the improving effects of donepezil on the memory and cognitive deficits, and that the synergistic effects may be partly mediated through both the cholinergic and dopaminergic systems. Thus, selegiline may be a new drug for therapy of AD, in combination with AchEIs.

Acknowledgements

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The effects of acute treatment with paroxetine, amitriptyline, and placebo on driving performance and cognitive function in healthy Japanese subjects: A double-blind crossover trial

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Objective To assess the effects of antidepressants on driving performance from a different methodological viewpoint in light of the recent traffic accidents.
Methods In this double-blinded, 3-way crossover trial, 17 healthy males received acute doses of 10 mg paroxetine, 25 mg amitriptyline, and placebo. The subjects were administered three driving tasks—road tracking, car following, and harsh braking—performed using a driving simulator and three cognitive tasks—Wisconsin Card Sorting Test, Continuous Performance Test, and N-back test at baseline and at 1 h and 4 h post-dosing. The Stanford Sleepiness Scale scores were also assessed.
Results At 4 h post-dosing, amitriptyline significantly impaired road-tracking and car-following performance, reduced driver vigilance, and caused subjective somnolence. Paroxetine impaired neither driving performance nor cognitive function.
Conclusions Acute doses of amitriptyline significantly impaired driving performance in the context of driving on crowded urban roads at relatively low speeds. This setting is important with respect to skills necessary for daily driving and may be difficult to measure in actual driving tests. This simulator-based study replicated the results of previous studies and could be considered complementary to them. This method may enable easy and safe screening of the driving hazard potential of drugs.

KEY WORDS — antidepressants; paroxetine; amitriptyline; driving performance; cognitive function

INTRODUCTION

Most of the currently available antidepressants have similar therapeutic efficacies, regardless of whether they are selective serotonin reuptake inhibitors (SSRIs) or tricyclic antidepressants (TCAs) (Anderson, 2000). The choice of an antidepressant is therefore largely determined by its side effects and the tolerability profile of an individual. According to their pharmacological profiles, antidepressants can impair cognition. Although continuous antidepressant therapy is required for patients with recurrent depressive disorders to prevent relapse (Geddes *et al.*, 2003) and improve their social and occupational lives, the unpleasant side effects could force them to discontinue treatment (Nemeroff, 2003) and may impair their daily activities, including driving in a motorized society.

Epidemiological data indicate that compared to nonusers, TCA users are twice as likely to be involved in traffic accidents (Leveille *et al.*, 1994; Ray *et al.*, 1992). Given the cross-sectional nature of these studies, no study has clarified the causal relationship

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between antidepressants and traffic accidents. Various effects of antidepressants on driving performance were recently evaluated in healthy subjects (Kerr *et al.*, 1996; Ridout and Hindmarch, 2001; Ridout *et al.*, 2003; Robbe and O'Hanlon, 1995; Wingen *et al.*, 2005) and depressed patients (Brunnauer *et al.*, 2006; Wingen *et al.*, 2006). Most of these recent studies used actual driving tests to measure driving performance. In these tests, the driving tasks were designed to reproduce real-life situations; however, these tasks addressed only certain aspects of driving because of the inherent safety risks and measurement limits of actual driving tests. Meanwhile, rear-end collisions account for nearly 30% of all traffic accidents in both Japan (National Police Agency Transportation Authority, 2007) and the United States (National Highway Traffic Safety Administration, U. S. Department of Transportation, 2007). It is imperative that a driver's ability to maintain a contextually appropriate following distance be reviewed to avoid rear-end collisions (Brookhuis *et al.*, 1994). Previous car-following tests (Brookhuis *et al.*, 1994; Ramaekers *et al.*, 1995; Ramaekers *et al.*, 2002) focused on the perception of speed deceleration of a leading car traveling at relatively high speeds and on safe following distance. The car-following performance in the context of driving at relatively low speeds on crowded urban roads has not been fully examined thus far and may be difficult to evaluate in actual driving tests.

Car driving is a complex task requiring many cognitive processes, including perception, attention, learning, memory, decision making, and action control. Therefore, the effects of antidepressants should be evaluated in terms of not only the driving performance but also each cognitive function. Previous studies used conventional tasks such as the critical flicker-fusion frequency task, divided attention task, and choice reaction time task for assessing the cognitive function. Such studies are prone to yielding varying results, depending on the type of cognitive task utilized. Therefore, it is important to employ widely used tasks that are more complicated than conventional tasks. The Wisconsin Card Sorting Test (WCST), Continuous Performance Test (CPT), and N-back test are examples of the desired complicated tasks, and the neural correlates of these tasks involve broad cortical areas, particularly the frontal cortex, which is related to driving skills. However, the effects of antidepressants on these three tasks have not yet been fully elucidated.

Thus, the aim of the present study was to evaluate the influences of acute paroxetine or amitriptyline treatment on driving performance, particularly in the context of the recent traffic accidents. We used simulator scenarios to examine the car-following performance in the context of driving on crowded urban roads at relatively low speeds. In addition, other established driving performance variables were also measured in the simulator scenario. Furthermore, the cognitive functions of subjects were evaluated by the WCST, CPT, and N-back test.

METHODS

Subjects

The study recruited 17 healthy male volunteers aged 30–42 years (mean ± SD, 35.8 ± 3.3 years). Only male subjects were included in the study because the changes in hormone levels occurring during the menstrual cycle can substantially affect cognition in healthy women (Hampson, 1990; Maki *et al.*, 2002; Phillips and Sherwin, 1992). All subjects had held a driving license for at least 10 years and drove a car daily for a minimum of 5000 km per year. All participants were drug-free prior to the study. Health interviews and the Structured Clinical Interview for DSM-IV (SCID) conducted at the time of the study indicated that none of the participants had any physical or psychiatric disorders. The study was approved by the ethics committee of the Nagoya University School of Medicine, and written informed consent was obtained from each subject prior to participation.

Study design

The study used a randomized, double-blind, placebo-controlled, 3-way crossover design. The subjects received acute doses of 10 mg paroxetine, 25 mg amitriptyline, and matched placebo in three different treatment sessions. The doses selected were based on generally recommended clinical starting dose, and minimizing possible risks of side effects, such as nausea and vomiting, which could confound the results. There was a washout period of at least 7 days between the treatment sessions, and the medications and placebo were presented as identical capsules.

Testing procedure

The subjects received substantial training in driving and cognitive tests 1 or 2 weeks prior to first testing; in order to minimize the learning effects, the subjects were trained until they reached the plateau level. On

each test day, the participants arrived at the laboratory at 9:00 AM and filled out self-rating questionnaires. Under baseline conditions, the driving tests started at 9:30 AM and lasted for approximately 15 min, while the cognitive tests started at 10:00 AM and took approximately 30 min. After the baseline assessment, each subject was administered one of the three drugs. The assessments of driving skills and cognitive function were repeated at 1 h and 4 h post-dosing.

Furthermore, the subjects were prohibited from consuming alcohol or caffeinated beverages for 12 h before testing and were directed to sleep adequately on the eve of testing. On the test days, the subjects were also prohibited from ingesting caffeine, supplement drinks, chewing gum, or candies to stay awake since these substances could exert a stimulating effect on their performance. During the intervals between the test batteries, the subjects were given light tasks to prevent short naps.

Driving performance

We divided the daily driving skills associated with traffic accidents into three tasks. A driving simulator (Toyota Central R&D Labs, Inc., Nagakute, Japan) was used to test the driving performance; the same simulator was used in a previous functional magnetic resonance imaging study to determine the neural substrates of driving skills (Uchiyama *et al.*, 2003). This simulator software was run on a personal computer (PC) (Windows XP) equipped with a steering wheel, accelerator, and brake pedal system (SIDEWINDER; Microsoft). Images from the PC were projected onto a 1620×1220 mm^2 screen via an LCD projector (TH-LB30NT; Panasonic, Osaka, Japan). While watching the driving scenes on the screen, the subjects controlled the speed and position of their car by manipulating the steering wheel, accelerator, and brake pedal. The driving simulation was conducted in a dark, sound-attenuated room.

Road-tracking test

The gently winding 2-lane road with no other traffic continued throughout the test duration of 5 min. The subjects were instructed to drive at a constant speed of 100 km/h and stabilize the vehicle in the center of the left lane. The lateral position of the vehicle (in cm) from the right edge of the left lane was recorded every 10 ms. The standard deviation of the lateral position (SDLP; in cm), which indicates weaving, was taken as a performance measure. This test is based on a road-tracking test developed previously (O'Hanlon, 1984; O'Hanlon *et al.*, 1982).

Car-following test

The test included a straight 2-lane road with no other traffic, except for a single preceding car. When the preceding car decelerated, its brake lights came on. As the preceding car accelerated (to 60 km/h) or decelerated (to 40 km/h), the subject was required to maintain the distance between the cars as close to 5 m as possible. The car-following distance (m) was recorded every 10 ms, and performance was measured as the coefficient of variation (CV) obtained by dividing the standard deviation of the distance between the cars by the mean value (Uchiyama *et al.*, 2003). Therefore, a smaller distance CV value (DCV) indicated better performance. The test duration was 5 min.

Harsh-braking test

The test included a straight 2-lane road with no traffic, but with humanoid models on either side of the left lane. The humanoid models randomly ran onto the road as the subject's car approached. The subject was instructed to maintain a constant speed of 50 km/h and to avoid hitting the humanoid models by harsh braking as quickly as possible. As described previously (Hindmarch *et al.*, 1983; Ridout and Hindmarch, 2001), the brake reaction time (BRT; in ms) was used as a measure of the cognitive psychomotor performance, including attention efficiency. Each test consisted of 7 BRT trials over a 5-min period, and the mean BRT was calculated from these results.

Cognitive function

The cognitive test battery consisted of 3 tasks performed on a PC by manipulating a computer mouse or numeric keypad.

WCST

The WCST (Heaton, 1981) was used to measure the executive function, for example, abstract reasoning ability or the ability to shift cognitive strategies in response to changing environmental contingencies. A modified computerized version of the WCST (Kashima *et al.*, 1987) was administered, and the test lasted until such time as 48 cards were sorted. In this study, performance was measured by the following indices: category achievement (CA), perseverative

errors of Nelson (PEN), and difficulty of maintaining set (DMS).

CPT

The CPT was used to measure sustained attention or vigilance. We used the CPT-Identical Pairs version (CPT-IP) software, as described previously (Cornblatt *et al.*, 1988). A series of 4-digit stimuli were presented for a period of 50 ms, with an interstimulus interval (ISI) of 950 ms. Each complete task consisted of 150 trials of which 30 were target trials requiring a response. In this study, performance was measured by the signal detection index d-prime (d′), a measure of discriminability computed from "hits" and "false alarms."

N-back test

The N-back test was used to measure working memory. We used a working memory task software that requires subjects to update their mental set continually while responding to previously seen stimuli (i.e., numbers); the details thereof have been described previously (Callicott *et al.*, 2000; Callicott *et al.*, 2003). The stimulus duration was 0.4 s, and the ISI was 1.4 s; each test comprised 14 trials. The subjects responded to the stimuli by using the numeric keypad of the PC. In the present study, a 2-back condition was used, and performance was measured as the percentage of correct responses (accuracy, %).

Subjective measurements—Stanford Sleepiness Scale and adverse events

The Stanford Sleepiness Scale (SSS) is a 7-point, self-reporting measure with proven sensitivity in several studies (Hoddes *et al.*, 1973) and examines the level of alertness of an individual. The subjects were instructed to evaluate themselves on this scale before the initiation of the test battery at baseline and at 1 h and 4 h post-dosing. In addition, the adverse events spontaneously reported by the subjects or elicited by a nonleading question were recorded.

Statistical analyses

None of the outcome variables of the driving tests, cognitive tests, and subjective scales, except for BRT (harsh-braking test) and d′ (CPT), showed normal distribution. In order to compare the conditions following the administration of the 3 drugs, the differences between the baseline values and the 1-h and 4-h post-dosing values were analyzed. The non-normally distributed variables were analyzed by the nonparametric Friedman's χ^2 *r*-test. In the case of a significant treatment effect, a post-hoc analysis was performed by Wilcoxon signed-rank test (nonparametric) with Bonferroni's correction. The BRT and d′ data were normally distributed, and the differences between the baseline values and the 1-h and 4-h post-dosing values were analyzed using repeated measures analysis of variance (ANOVA). In the case of a significant treatment effect, a post-hoc analysis was done using the Bonferroni test for multiple comparisons. To clarify the correlations between driving performance and cognitive function, single regression analyses were conducted by the Spearman rank-order correlation (nonparametric); however, BRT and d′ were analyzed by the Pearson product-moment correlation. All statistical tests were conducted using SPSS version 11 for Windows (SPSS Japan Inc., Tokyo, Japan).

RESULTS

Missing data

In the road-tracking test, three subjects administered amitriptyline failed to complete the test at 4 h post-dosing as they were sliding off the track. These subjects were not factored into the relevant statistical analyses. Because of technical malfunctions, three other subjects with incomplete data records were not factored into the statistical analyses of the N-back test. There were no missing data for the other driving and cognitive tests.

Driving performance

Summaries of results of the driving and cognitive tests are provided in Table 1 (a and b). Friedman's χ^2 *r*-test revealed a statistically significant effect of treatment on the differences between the baseline and 4-h post-dosing SDLP ($\chi^2 = 12.0$, $df = 2$, $p = 0.0025$) and DCV ($\chi^2 = 8.82$, $df = 2$, $p = 0.0121$) values. The post-hoc test demonstrated that the SDLP was significantly greater under the amitriptyline condition than under the other two conditions ($p < 0.05$ vs. placebo, $p < 0.01$ vs. paroxetine), and the DCV was significantly greater under the amitriptyline condition than under the paroxetine condition ($p < 0.01$). Repeated measures ANOVA showed no statistically significant differences in BRT among the three conditions. The results of the SDLP and DCV are

Table 1. Summary of the results of driving tests, cognitive tests, and subjective measurements in healthy male subjects enrolled in a crossover trial of paroxetine, amitriptyline and placebo ($N = 17$)

		Mean (SD)		
Measure	Test time	Placebo	Paroxetine 10 mg	Amitriptyline 25 mg
(a) Driving test				
SDLP* (cm)	Baseline	37.4 (7.81)	41.8 (10.68)	38.9 (10.84)
	1 h	37.2 (7.66)	38.9 (9.00)	38.9 (8.55)
	4 h	36.9 (8.45)	38.9 (10.11)	51.3 (12.67)**
DCV	Baseline	23.6 (10.11)	26.8 (10.50)	25.3 (8.10)
	1 h	24.4 (10.92)	28.5 (16.21)	26.8 (13.21)
	4 h	25.7 (12.67)	27.2 (9.46)	36.1 (19.16)***
BRT (ms)	Baseline	547.7 (42.76)	551.1 (69.40)	557.9 (58.30)
	1 h	546.1 (64.16)	542.3 (60.49)	557.8 (66.90)
	4 h	549.7 (55.61)	553.4 (48.75)	573.7 (52.72)
(b) Cognitive tests				
WCST				
CA	Baseline	6.1 (0.43)	5.8 (0.66)	5.9 (0.43)
	1 h	5.9 (0.56)	5.9 (0.33)	5.6 (0.80)
	4 h	5.9 (0.43)	5.9 (0.60)	5.5 (0.87)***
PEN	Baseline	0.4 (0.79)	0.9 (1.93)	1.1 (1.34)
	1 h	0.8 (2.19)	0.7 (1.10)	1.6 (2.74)
	4 h	0.6 (1.28)	0.5 (1.28)	1.1 (2.60)
DMS	Baseline	0.1 (0.24)	0.2 (0.56)	0.2 (0.39)
	1 h	0.1 (0.24)	0.1 (0.24)	0.4 (0.71)
	4 h	0.0 (0.0)	0.1 (0.24)	0.4 (0.61)
CPT (d')	Baseline	3.0 (0.87)	2.7 (0.91)	2.9 (0.80)
	1 h	3.1 (0.78)	3.1 (0.83)	3.0 (0.79)
	4 h	3.3 (0.69)	3.0 (0.92)	2.6 (0.89)***
N-back test* (accuracy, %)	Baseline	90.3 (19.93)	88.8 (16.98)	87.76 (12.35)
	1 h	91.8 (14.52)	86.7 (16.78)	86.7 (14.25)
	4 h	88.3 (14.72)	81.1 (17.85)	86.2 (16.45)
(c) Subjective measurement				
SSS	Baseline	1.9 (0.70)	1.7 (0.59)	1.9 (0.56)
	1 h	2.1 (0.66)	2.1 (0.70)	2.1 (0.78)
	4 h	2.2 (0.64)	2.3 (0.69)	4.6 (0.86)****

SDLP, Standard deviation of lateral position; DCV, Distance coefficient of variation; BRT, Brake reaction time; WCST, Wisconsin Card Sorting Test; CA, Category achievement; PEN, Perseverative errors of Nelson; DMS, Difficulty of maintaining set; CPT, Continuous Performance Test; SSS, Stanford Sleepiness Scale.0

*$N = 14$.

**$p < 0.01$ overall treatment effect between the three groups (difference between 4 h and baseline); ***$p < 0.05$ overall treatment effect between the three groups (difference between 4 h and baseline); ****$p < 0.001$ overall treatment effect between the three groups (difference between 4 h and baseline).

presented in Figures 1 and 2. No subject suffered from simulator sickness during the experiment.

Cognitive function

In the CPT, repeated measures ANOVA revealed a statistically significant effect of treatment on the difference between the baseline and 4-h post-dosing d′ values ($F = 4.79$, $df = 2$, $p = 0.015$). The post-hoc test demonstrated that d′ was significantly decreased under the amitriptyline condition when compared with the placebo condition ($p < 0.05$). The CPT results are presented in Figure 3. In the WCST, Friedman's χ^2 r-test revealed a statistically significant effect of treatment on the difference between baseline and 4-h post-dosing CA values ($\chi^2 = 6.54$, $df = 2$, $p = 0.038$); however, the post-hoc test did not show significant differences among the three groups. For the remaining cognitive measurements, no statistically significant effects of treatment were observed.

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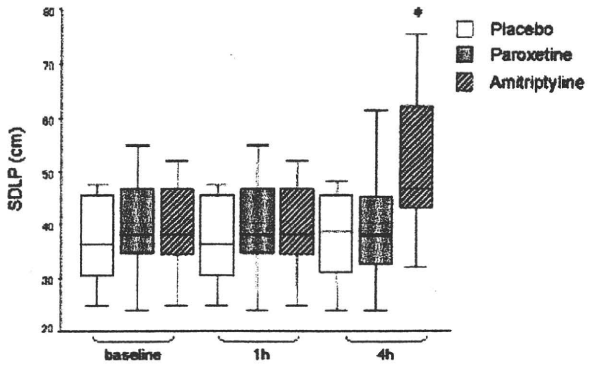

Figure 1. Box-and-whisker plot of the SDLP at baseline and at 1 h post dose and 4 h post-dosing in the crossover treatment with paroxetine, amitriptyline, and placebo ($N = 14$). Boxes indicate the interquartile ranges, with medians designated by the horizontal line. The differences between the baseline and the 4-h post-dosing values under the three conditions were compared. There was a significant effect of treatment ($^*p = 0.0025$). The post-hoc test demonstrated that the SDLP was significantly greater under the amitriptyline condition than under the placebo condition ($p < 0.05$) and the paroxetine condition ($p < 0.01$)

Subjective measurements

A summary of results of the SSS is shown in Table 1-c. Friedman's χ^2 *r*-test showed that there was a significant effect of treatment on the difference between the baseline and 4-h post-dosing SSS scores ($\chi^2 = 31.3$, $df = 2$, $p < 0.001$). Post-hoc tests clarified that alertness was significantly decreased under the amitriptyline condition when compared with the paroxetine ($p < 0.01$) and placebo ($p < 0.01$) conditions. A summary of adverse events is provided in Table 2.

Correlations among driving performance, cognitive function, and subjective assessments

Single regression analyses revealed significant correlations between driving performance and cognitive function. The significant correlations are outlined in Table 3.

DISCUSSION

The results of the present study demonstrated that 4 h after taking a single 25-mg dose of amitriptyline, there was significant impairment of DCV and of the established driving performance variable SDLP in the context of driving on crowded urban roads at relatively low speeds. Vigilance in the CPT and subjective somnolence in the SSS were also significantly impaired at 4 h after amitriptyline dosing. In contrast, acute doses of paroxetine or placebo did not significantly impair driving performance or cognitive function.

Although most of the present results are consistent with those of prior studies (Ramaekers, 2003; Ridout and Hindmarch, 2001; Ridout *et al.*, 2003; Wingen *et al.*, 2005), the present study was conducted from a different methodological viewpoint. The car-following performance is important with respect

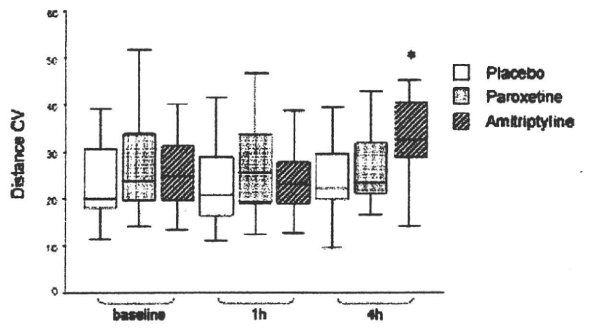

Figure 2. Box-and-whisker plot of the DCV at baseline and at 1 h and 4 h post-dosing in the crossover treatment with paroxetine, amitriptyline, and placebo ($N = 17$). Boxes indicate the interquartile ranges, with medians designated by the horizontal line. The four outlier values (1.5- to 3-fold of the interquartile range) and four extreme values (>3-fold of the interquartile range) have been omitted from the figure, but these values were included in the statistical analysis. The differences between the baseline and 4-h post-dosing values under the three conditions were compared. There was a significant effect of treatment ($^*p = 0.012$). The post-hoc test demonstrated that the DCV was significantly greater under the amitriptyline condition than under the paroxetine condition ($p < 0.01$)

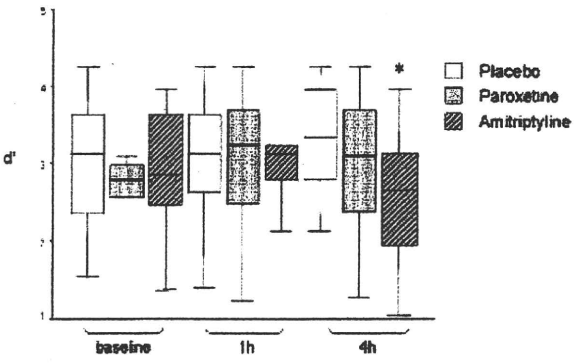

Figure 3. Box-and-whisker plot of the CPT (d′) at baseline and at 1 h post dose and 4 h post dose of crossover treatment with paroxetine, amitriptyline, and placebo ($N = 17$). Boxes indicate the interquartile ranges, with medians designated by the horizontal line. The seven outlier values (1.5- to 3-fold of the interquartile range) and two extreme values (>3-fold of the interquartile range) have been omitted from the figure, but these values were included in the statistic analysis. The differences between the baseline and 4-h post-dosing values under the three conditions were compared. There was a significant effect of treatment ($^*p = 0.015$). The post-hoc test demonstrated that the d′ was significantly decreased under the amitriptyline condition than under the placebo condition ($p < 0.05$)

Table 2. Adverse events in healthy male subjects enrolled in a crossover trial of paroxetine, amitriptyline and placebo, *N* (%) (*N* = 17)

Adverse event	Placebo	Paroxetine 10 mg	Amitriptyline 25 mg
Somnolence	0 (0)	2 (11.8)	16 (94.1)
Dizziness, light headedness	0 (0)	0 (0)	5 (29.4)
Stuffy head	1 (5.9)	1 (5.9)	11 (64.7)
Nausea	0 (0)	0 (0)	1 (5.9)
Dry mouth	0 (0)	0 (0)	3 (17.6)
Fatigue	0 (0)	0 (0)	5 (29.4)
Uncomfortable feeling in the head	0 (0)	0 (0)	1 (5.9)

to skills necessary for daily driving on crowded urban roads. The following distance is important for avoiding car crashes (Brookhuis *et al.*, 1994); however, it was hitherto difficult to measure the following distance in actual driving tests and the same setting was not investigated in previous simulator driving tests. Therefore, our simulator test may be considered complementary to actual driving tests. In addition, the results of the present 5-min simulator road-tracking test were similar to those of previous actual driving tests, which may require more time and higher expenditure.

The present simulator scenarios were different from actual driving tests in terms of course configuration and driving settings. Therefore, it is difficult to compare the parameters between simulator testing and actual driving. The gently winding road resulted in difficulties in stabilizing the vehicle in the center of the road, thereby yielding considerably higher SDLP values than actual driving tests, even under the placebo condition. The non-normal distributions of the driving variables could be attributed to the small sample size, complicated by the inability of some subjects to complete the task and by some outliers related to differences in the drug metabolizing capacities of subjects. Although the effects of antidepressants on the DCV were similar to those on the SDLP, no significant differences in BRT were observed among the three conditions. It was assumed that the other driving tasks were more complex than the harsh-braking task and might have therefore caused the significant differences. In the present study, there were no significant differences in executive function and working memory performance between baseline and post-dosing values. Although amitriptyline administration has been repeatedly associated with negative effects (Hindmarch *et al.*, 1983; Kerr *et al.*, 1996; Richardson *et al.*, 1994; van Laar *et al.*, 2002), the variables measured in the WCST and N-back test could be considered to have high SD values that may have potentially influenced the present outcome.

Driving skills comprise many basic cognitive and psychomotor elements, and the simultaneous application of these functions is required for safe driving. Regression analyses revealed that the negative effects of antidepressants on driving performance were associated with diminished sustained attention, executive impairment, and increased somnolence, although the low correlation coefficients warrant further investigations. Previous findings that are consistent with ours also show that somnolence or sedation is the most important cause of driving impairment in patients treated with antidepressants (Ramaekers, 2003).

Differences in the pharmacological properties of SSRIs and TCAs may provide a reasonable explanation for our results. Amitriptyline, unlike paroxetine, has strong antagonistic effects on cholinergic, adrenergic (α1), and histaminergic (H1) receptors, causing cognitive impairment, balance disturbance, and sedation, respectively. These common characteristics of TCAs may impair driving performance (Hindmarch *et al.*, 1983; Robbe and O'Hanlon, 1995; van Laar *et al.*, 1995; Wingen *et al.*, 2005). In the present study, amitriptyline did not significantly impair driving performance and cognitive function at 1 h post-dosing; this is not consistent with the previous results obtained using amitriptyline. However, most of the previous studies administered 25 mg amitriptyline 2 or 3 times daily. Although several studies showed impaired performance 1–2 h after a single administration of 25 mg or less amitriptyline (Bye *et al.*, 1978), the results obtained at the low doses varied with the tasks or subjects' ages (Crome and Newman, 1978; Kinirons *et al.*, 1993; Nathan *et al.*, 2000; Ogura *et al.*, 1983; Peck *et al.*, 1979; Tiller, 1990). The absence of amitriptyline effects at 1 h post-dosing could be chiefly due to the present tests employing single low doses. Furthermore, the low sensitivity of the driving simulator to the drug effects might also have contributed to the absence of amitriptyline effects at 1 h post-dosing.

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Table 3. Correlation between driving tests, cognitive tests, and subjective assessments

Measure	Driving test		
	SDLP (cm)	DCV	BRT (ms)
	r	*r*	*r*
Cognitive tests			
WCST			
CA	−0.23*	−0.17**	−0.053
PEN	0.13	0.21*	0.017
DMS	−0.012	0.20**	−0.092
CPT (d')	−0.28*	−0.20**	−0.094
N-back test (accuracy, %)	0.035	−0.067	0.019
Subjective measurement			
SSS	0.14	0.25*	0.14

SDLP, Standard deviation of lateral position; DCV, Distance coefficient of variation; BRT, Brake reaction time; WCST, Wisconsin Card Sorting Test; CA, Category achievement; PEN, Perseverative errors of Nelson; DMS, Difficulty of maintaining set; CPT, Continuous Performance Test; SSS, Stanford Sleepiness Scale.
*$p < 0.01$; **$p < 0.05$.

The present study has some potential limitations. First, participation was restricted to healthy adult volunteers. Neither elderly nor depressed patients were studied; their responses to antidepressant treatment could widely differ from those of healthy, younger adults. Second, we can extrapolate the results of our study only to patients receiving initial administration since our treatments were restricted to acute dosing. Third, the validity and sensitivity of the driving simulator need to be considered. A 5-min testing scenario may be inadequate for a behavioral test. In the harsh-breaking test, breaking to avoid hitting seven people crossing the road within 5 min might have alerted the subjects, thereby overcoming the drug-induced sedation. In the car-following test, subjects can choose different car-following distances under different conditions (Brookhuis *et al.*, 1994). The authors are aiming to improve the driving simulator and testing conditions in future studies to reflect real driving conditions in cooperation with Toyota Central R&D Labs., Inc. Finally, the authors' methodology could be useful to evaluate driving skills, particularly under hazardous conditions without real driving; however, it is necessary to significantly increase the sensitivity and reliability of the driving simulator. Since females constitute a major proportion of depressed patients, future investigations should include female subjects after adjusting for their menstrual cycles.

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特別寄稿

165-172

統合失調症の病態と新薬開発の動向

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抄録：これまでに臨床導入されてきた統合失調症の治療薬は，1970年代に提唱されたdopamine（DA）仮説を根幹としており，chlorpromazineやhaloperidolなどの第一世代抗精神病薬（FGA）に代表される。FGAは主にD_2受容体遮断作用を有し，陽性症状を改善する効果に優れている一方で，副作用である錐体外路症状（EPS）が避けられず，陰性症状，認知機能障害に対する効果も十分ではない。その後，錐体外路系副作用が弱い抗精神病薬としてrisperidoneを初めとする第二世代抗精神病薬（SGA）が開発され，陽性症状のみならず，陰性症状や認知機能障害にもある程度の改善効果を有することから，その恩恵に与る患者は増加していった。しかし，臨床効果が向上してきたとはいえ治療抵抗例は未だ数多く存在し，既存の抗精神病薬とは異なる機序の治療薬の開発が望まれている。

近年，DA仮説に因らない新たな薬理学的特徴を持つ治療薬が開発されつつあり，中でもglutamate作動性神経系，gamma-aminobutyric acid（GABA）作動性神経系，nicotinic acetylcholine（nACh）作動性神経系，neuropeptide関連因子などを介する新たな抗精神病薬が注目されている。本稿では，統合失調症の病態仮説に基づき開発されてきた抗精神病薬の開発経緯と新規治療薬の開発動向について概説する。

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Key words：schizophrenia, first generation antipsychotics（FGA）, second generation antipsychotics（SGA）, dopamine hypothesis, dopamine-serotonin hypothesis, glutamate hypothesis

1．はじめに

これまでの基礎と臨床の研究報告から数多くの病態仮説が提唱され[1)]，それに伴い様々な新規抗精神病薬の開発が進められてきた。1952年にchlorpromazineが統合失調症の治療に用いられるようになり，D_2受容体遮断作用を主体とする第一世代抗精神病薬（FGA）が世に広められた[2)]。一方で，強力なD_2受容体遮断作用をもつ薬剤は，錐体外路症状（EPS）や循環器系の副作用の発現頻度が高く，服薬持続が困難となる症例も出現し

The perspective of the novel artipsychotics and the pathophysiology of the schizophrenia

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た。このような背景の中で，clozapine の登場を契機に EPS などの副作用が少なく，陽性症状改善作用のみならず，陰性症状，認知機能障害の改善作用を兼ね備えた非定型抗精神病薬の概念が生まれ[3]，serotonin-dopamine antagonist（SDA）や multi-acting receptor targeted antipsychotics（MARTA），dopamine system stabilizer（DSS）が相次いで登場し，いわゆる第二世代の抗精神病薬（SGA）が世に送り出された。しかし，臨床上の利点があるとは言え，一向に治療抵抗性例の克服に繋がる糸口が見えず，既存の抗精神病薬とは異なる機序の治療薬の開発が切に望まれている。近年では，DA 仮説に因らない新たな分子機序を有する新規抗精神病薬の開発が試みられており，その方向性は glutamate 作動性神経系，gamma-aminobutyric acid（GABA）作動性神経系，nicotinic acetylcholine（nACh）作動性神経系，neuropeptide 関連因子など多彩なものである。

本稿では，現在までに提唱されている統合失調症の病態仮説に基づき，抗精神病薬開発の経緯と新規治療薬開発動向について概説する。

2．DA 仮説と dopamine-serotonin 仮説

1．DA 仮説と FGA

統合失調症の薬物療法は，1952 年に chlorpromazine が臨床導入され，複数の臨床試験によりその症状改善効果が実証されたことに始まった。その後，高力価型の haloperidol をはじめとする抗精神病薬が相次いで開発・導入され，統合失調症の薬物療法が急速に発展した。抗精神病薬の臨床力価と脳内 D_2 受容体遮断作用が相関することが見出され，統合失調症では脳内 DA 伝達系が亢進しているという DA 仮説が提唱されるようになった[2]。この仮説は，覚醒剤（amphetamine，methamphetamine など）や cocaine などの DA 作動薬が統合失調症の陽性症状様の幻覚・妄想状態を惹起すること，統合失調症患者および覚醒剤精神病患者において，症状が治まった状態で少量（健常者には精神変調を誘発しない程度）の DA 作動薬を投与すると，精神運動症状が再燃することからも支持されている[4]。しかし，D_2 受容体拮抗作用を主体とする FGA は脳内すべての DA 作動性神経系を抑制するために，陽性症状には効果が認められる一方で，EPS や循環器系副作用などが問題となり，また，陰性症状や認知機能障害に対する効果も不十分であった。

2．Dopamine-serotonin 仮説と SGA

1980 年代から FGA による薬物治療上の問題点を克服するため，抗精神病作用に関与する中脳辺縁系 DA 作動性神経系に対してより選択的であり，EPS をはじめとする副作用が生じにくく，陰性症状に対する効果が優れている新規抗精神病薬の開発が進められてきた。1961 年に開発された clozapine は，優れた陽性症状改善作用に加え EPS を生じないだけでなく，陰性症状改善作用を有し，他の抗精神病薬に治療抵抗性の症例にも有効性が高かった[5]。いわゆる非定型性の概念がここに誕生し，SGA 開発の礎を築いた。抗精神病薬の非定型性を説明する最も重要な薬理学的特性の一つには，D_2 受容体遮断作用に比べ，5-HT_{2A} 受容体遮断作用が相対的に強い性質にあると考えられている（dopamine-serotonin 仮説）[6]。これは，5-HT_{2A} 受容体遮断作用により黒質線条体ドパミン作動性神経終末部の DA 遊離量を増加させることで EPS の発現を減弱し，前頭前皮質（PFC）や海馬において DA の遊離を亢進させることで陰性症状および認知機能障害を改善すると考えられている。Paul Janssen はこの薬理学的特徴を追究し，1985 年に risperidone を開発して統合失調症の薬物治療に大きな変革をもたらした。その薬理学的特徴から SDA に分類されており，risperidone に続き perospirone，blonanserin（AD-5423）および MARTA に属する quetiapine，olanzapine が国内で既に承認され，lurasidone，asenapine，zipracidone などが現在開発中である。

Blonanserin は 2008 年 4 月 22 日に上市され，perospirone に次ぐ国産 2 番目の SDA である。陽性症状と陰性症状の改善，体重増加や耐糖能異常，EPS などの副作用発現頻度が低いことに加えて認知機能障害の改善効果が期待されている。Blonanserin は D_2 受容体に対して clozapine よりもはるかに強く，haloperidol と同程度の高親和性

を有する[7]。5-HT_{2A}受容体に対しても高親和性を示すが，ドパミンD_2受容体の遮断作用の方が強いため，dopamine-serotonin antagonist（DSA）とも称される。Haloperidolとの二重盲検比較試験[8]において，blonanserinは最終全般改善度における改善率（中等度改善以上）が有意に高く，陽性症状，陰性症状に対して優れた改善効果が認められている。EPSの発現率が有意に低かったことから新規抗精神病薬の特性を十分兼ね備えていることが実証されている。その後のrisperidoneとの比較試験[9]においてもblonanserinの非劣性が確認されたことから，統合失調症の薬物治療の新戦力として十分期待できる。

Lurasidone（SM-13496）はperospironeの後継に位置するSDAであり，D_2受容体と5-HT_{2A}に対する親和性が高く，SGAとしての特徴を備えている。抗不安薬のtandospironeと構造が類似していることから5-HT_{1A}受容体親和性を有し，不安および抑うつ症状に対しても効果が期待されている[10]。米国では1995年から臨床試験に入り，第Ⅱ相試験において優れた抗精神病作用と副作用発現率の低さが確認された。2007年には第Ⅲ相試験に着手し，2010年を目標に上市予定である。わが国では第Ⅱ相試験を終え，第Ⅲ相試験が開始されたところである。国産のSDAが海外市場に流通するのは初めてのことであり，今後の臨床成績に期待がかかる。

Asenapine（ORG5222）は四環系構造をしたSDAであり，D_1，D_2，5-HT_2受容体に対して高親和性を有する[11]。比較的強いα_1受容体阻害作用を有し，この作用が陽性症状の抑制に関与していることが示唆されている。抗コリン作用がないため，口渇や便秘などの副作用は少ない。国内外の臨床試験では，risperidoneと比較して陽性症状に対しては同等の効果を示し，陰性症状に対してはasenapineの方が優るという結果が示されている[10]。米国では統合失調症と双極性障害ですでに承認申請中であり，欧州では第Ⅲ相試験が実施されている。わが国では，臨床試験において比較的高い改善率を示したが，悪化率が高く，興奮・易刺激性，不眠，不安，焦燥などの副作用のため1996年に開発が中止となった。2007年12月には急性期統合失調症患者を対象とした海外臨床試験で，placeboと比較してPANSS得点が有意に改善したことから，高い効果と良好な認容性が確認され，実用化への期待が高まっている[12]。

Zipracidone（CP-88059）はSDAの中でも5-HT_{2A}/D_2親和性比が最も高く，様々なDA，5-HT受容体サブタイプにも強力に作用することから，EPSや遅発性ジスキネジアなどの副作用が少ないとされている[13]。特徴的な薬理作用として，5-HT，norepinephrine（NE）再取り込み阻害作用を有しているが，両作用がどのような薬効を反映するのか注目されている。国内外の臨床試験ではPANSS症状評価において陽性症状，陰性症状の改善度がplaceboと比較して有意に優れ，EPSの発現率が少なかった[14]。2005年までに70カ国以上で承認されており，日本でも後期第Ⅱ相試験が進められている。

3．DA受容体部分作動薬

SDA，MARTAがD_2受容体拮抗薬であるのに対し，aripiprazoleはDA作動性神経伝達が過活動状態の場合には拮抗作用を示し，DA作動性神経伝達が低下している場合には刺激作用を発揮して陽性症状，陰性症状を改善することから，DSSとも称される[15]。国内外の臨床試験において，陽性症状，陰性症状に対して改善作用が認められ，EPS，内分泌代謝異常，QTc延長，体重増加などの有害事象が少なく，有効性と認容性に優れていることが確認されている[16]。2003年にKaneら[5]によって改訂されたエキスパートコンセンサスガイドラインでは，初発エピソードおよび急性増悪期に対する第一選択薬として推奨されている。Aripiplazoleの成功を機に，新たなDA受容体部分作動薬の開発に期待が高まり，bifeprunox（DU-127090），ACR-16が開発中である[17]。Bifeprunoxは海外において第Ⅲ相試験まで開発が進められているが，被験者の死亡とbifeprunoxとの因果関係に関する追加データを要求されており，承認にはまだまだ時間がかかりそうである。日本ではbifeprunoxの開発に着手していないが，今後の臨床試験において成果が挙がり有効性が確固たるものとなれば，治療抵抗例に対する新たな

治療戦略として期待される。

3．グルタミン酸仮説

1．Glutamate hypofunction 仮説

1970年代初頭，米国にて精神異常発現薬であるphencyclidine（PCP）が乱用され，統合失調症類似の症状を呈するPCP精神病の入院患者数が増大した。抗精神病薬に抵抗性であり，DA仮説の根拠ともなる覚醒剤精神病で認められる幻覚，妄想に加え，陰性症状様の意欲減退，感情鈍麻なども認められることから，統合失調症の広範な臨床症状を反映するモデルとして考えられている[18]。PCPは非競合的NMDA受容体拮抗薬であり，NMDA受容体のチャネル内部に存在するPCP結合部位に作用してイオンチャネルを遮断し，グルタミン酸作動性神経系の機能を抑制する。したがって，NMDA受容体機能の低下が陽性症状，陰性症状および認知機能障害の発現に関与していることが示唆されている。Ketamineやdizocilpine（MK-801）などのNMDA受容体遮断薬も統合失調症様の精神障害をきたすことからも，glutamate hypofunction仮説が支持されている[18]。

2．NMDA受容体機能促進薬

NMDA受容体拮抗薬により統合失調症様の陽性症状，陰性症状および認知機能障害が惹起されることから[18]，NMDA受容体機能を亢進させる薬剤の開発により，既存の抗精神病薬に治療抵抗性を示す陰性症状および認知機能障害にも有効性が期待されている[19]。NMDA受容体は複数のサブユニットから構成されており，イオンチャネルを形成している。興奮性神経伝達物質であるglutamateの結合部位のほか，glycine結合部位，PCP結合部位，ポリアミン結合部位，亜鉛結合部位，マグネシウム結合部位などが存在し，中でもNMDA受容体促進系のglycine結合部位が治療標的として注目されている[20]。

Glycineは1988年のWaziriによる報告[21]において，統合失調症の治療薬としての可能性が見出されている。その後，D-serine，D-alanineが開発され，それぞれの臨床試験において，既存の抗精神病薬と併用した場合に陽性症状，陰性症状および認知機能障害の改善効果が認められている[22]。しかし，これら薬剤は脳内移行性が低く，抗精神病作用を発揮するのに高用量を必要とするため経口摂取は現実的ではない。腎障害の惹起，抑制性glycine受容体に対する作用などの問題のために臨床への応用は困難である[23]。

D-cycloserineはNMDA受容体glycine結合部位に対する部分作動薬であり，従来，細胞壁ペプチドグリカン生合成阻害作用を持つために，抗結核薬として開発された。脳移行性が比較的高いが，作動薬としても拮抗薬としても働く部分作動薬であるために用量の設定が難しいとされている。統合失調症を対象とした臨床試験において，低用量（50mg/日）を抗精神病薬と併用した場合に陰性症状の改善が認められたという結果が多数報告されているが，clozapineとの併用では陰性症状を悪化させ[24]，高用量（250mg/日）では症状を悪化させ，期待した効果は得られていない。

3．Glycine transporter type 1（Gly T1）阻害薬

Glycine結合部位の作動薬は脳内移行性が悪く，治療には高用量を要する。これらの問題を克服するため，血液脳関門透過性が高く，シナプス間隙におけるglycine濃度を上昇させるための戦略として，glycine transporter阻害薬の開発が進められている[25]。N-methyl-glycine（sarcosine）はglycine transporter type 1（Gly T1）阻害薬であり，臨床試験において既存の抗精神病薬への付加療法において陽性症状，陰性症状に加え認知機能障害の改善効果が認られており，有害事象もplaceboと比較して有意な差はなかった[26]。SSR-504734は前臨床試験までの結果が公表されており[27]，臨床試験の実施が待ち望まれている。JNJ-17305600は第Ⅰ相試験に着手されているが，有害事象のために開発を中断している。Gly T1阻害薬は既存薬との付加的併用療法のみならず，単剤による治療効果も十分に期待できると考えられており，多くのGly T1阻害薬が合成されている。これら薬剤はNMDA受容体機能を促進し，DA神経伝達の是正を図る新規抗精神病薬として注目されており，今後の臨床成果が大いに期待されて

いる。

4．AMPA受容体作動薬

AMPA受容体はnon-NMDA受容体の1つであり，glutamate神経伝達を制御していることから抗精神病薬の治療標的となっている。CX516はAMPA受容体を賦活するampakineの1つであり，チャネルの開口を制御して長期増強（LTP）や学習，記憶などの認知機能の改善効果が期待されている。臨床試験において，clozapineとの併用で陰性症状および認知機能の改善が認められている[28]。しかし，他の非定型抗精神病薬との併用ではPANSS，SANS，GASによる評価はplaceboと比較して差はなく[29]，単剤使用においても有効性が確認されていない[30]。米国ではアルツハイマー病や注意欠陥多動性障害に対する第Ⅱ相試験が行われているが，統合失調症治療薬としての開発は進展することはないと考えられている。

5．Glutamate hyperfunction仮説

Glutamate hypofunction仮説[23]に相反して，NMDA受容体拮抗薬がPFCおよび側坐核におけるglutamateとDAの遊離量を増加させることが報告された[31]。Glutamateの遊離を抑制するmetabotropic glutamate receptor（mGluR）作動薬がPCP誘発性精神症状を改善することから，統合失調症ではglutamate作動性神経系の機能亢進が関与しているというglutamate hyperfunction仮説が提唱された[32]。その後の研究で，NMDA受容体の遮断作用がGABA作動性神経系を介するglutamate作動性神経系の抑制機構を解除し，その結果glutamateの遊離が促進して機能亢進状態に至るとの報告がなされている[33]。

6．mGlu2/3R作動薬

mGluRは8つのサブタイプが存在し[34]，統合失調症に関与するのはmGluR2/3とmGluR5であると考えられており，両受容体を創薬標的として新規抗精神病薬の研究，開発が実施されている。mGlu2/3Rは前脳部に多く発現し，シナプス前部の自己受容体としてglutamate遊離を制御している。mGlu2/3R作動薬はglutamate作動性神経系の過活動状態を是正することで抗精神病作用を発揮すると考えられる。

1998年，mGlu2/3R作動薬であるLY354740は，非臨床試験においてPCPにより惹起される異常行動を改善したことから，glutamate hyperfunction仮説を推進する立役者となった[31]。米国では抗不安薬として第Ⅱ相試験が実施されている。その後，脳移行性を改善したプロドラッグであるLY404039の開発を経て[35]，生体内利用率をさらに向上させたLY2140023が臨床開発されている。統合失調症を対象とした二重盲検比較試験の結果，4週間にわたるLY2140023投与により対照薬のolanzapineと同様に陽性症状，陰性症状がplaceboと比較して有意に改善された[36]。プロラクチン濃度の上昇，EPSの発現，体重増加などの副作用はplaceboと差は無かった。現在では後期第Ⅱ相試験が実施されており，今後も大いに注目していきたい。

7．mGlu5R allosteric modulator

mGlu5Rは前脳部，中脳部のシナプス後部に存在し，統合失調症をはじめ，パーキンソン病，薬物依存などの精神障害に関与すると考えられている[37]。mGlu5R作動薬はNMDA受容体拮抗薬などにより惹起される異常行動を改善することが見出されており，統合失調症治療薬となる可能性が示唆される。CDPPBはmGlu5RのallostericmodulatorであるとともにmGlu5Rのallosteric modulatorであり，非臨床試験の結果が多数報告され[38]，臨床試験の実施が待ち望まれる。

4．ニコチン性アセチルコリン受容体作動薬

脳内acetylcholine（ACh）作動性神経系は認知機能に重要な役割を果たしていること，非定型抗精神病薬が統合失調症における認知機能障害を改善すること，非臨床試験で内側前頭前皮質におけるAChの遊離を有意に増大させるという知見から，AChesterase阻害薬などのアルツハイマー型認知症治療薬が統合失調症における認知機能障害に臨床応用できるかどうか検討されている[39]。しかし，世界各国でアルツハイマー型認知症治療薬として発売されているAChesterase阻害薬の

donepezilを用いた臨床試験において，明らかな認知機能の改善効果は認められていない[39]。一方，統合失調症の脳内では，ニコチン性アセチルコリン受容体数が減少しており，認知機能障害との関連が注目されている[40]。

GalantamineはACh esterase阻害作用に加え，nicotinic ACh receptor（nAChR）アロステリック作用も有し，海外では既にdonepezil同様，アルツハイマー型認知症治療薬として承認されており[41]，わが国では大規模第Ⅲ相試験が行われている。統合失調症における認知機能障害に対する臨床試験の結果，risperidoneへの上乗せにより，placeboと比較して有意に認知機能が改善された[42]。これは認知機能に関与しているnAChR（特にα7サブタイプ）に対してアゴニストとして作用することが統合失調症の認知機能障害に関与していることを示唆している[40]。マウスを用いた非臨床試験において，galantamineの単剤およびrisperidoneとの併用投与は，PCPにより誘発される認知機能障害を改善することが報告されている[43]。

5．おわりに

統合失調症の病態生理は完全には解明されておらず，これまで様々な病態仮説に基づいて治療薬の開発が行われてきた。DA仮説[2]，dopamine-serotonin仮説[6]に基づき開発されたFGAやSGAは，少なからず統合失調症の病態には神経伝達系の異常が関与し，それを是正することで症状を改善することを印象付けた。一方で，FGAやSGAでは十分な効果が得られない治療抵抗性の統合失調症が存在し，これら治療抵抗例の克服を実現する治療薬の開発が切に望まれている。これら治療抵抗例は陰性症状や認知機能障害を伴うことが多く，SGAの使用により若干の改善が見られる症例があるが十分とは言えない。認知機能障害は患者の生活の質（QOL）を大きく損なう要因の一つであることから，治療上の大きな課題となっている。したがって，統合失調症の治療薬開発の指標として陽性症状，陰性症状よりもむしろ認知機能障害に焦点が当てられている。例えば，米国ではMATRICSプロジェクトと呼ばれる臨床評価法と治療法を探索する研究が開始されており[44]，新規治療薬の開発構想や既存の治療法が見直される可能性も十分ある。

新規抗精神病薬の開発動向として，従来の抗精神病薬とは異なる病態仮説に基づいた薬理学的特性を持つ治療薬の開発が手掛けられ，そのうちのいくつかは仮説検証的な創薬において実用化に向けて着実に臨床成果を挙げている[24]。近い将来，統合失調症の薬物治療は新たな局面を迎えるものと考え，今後の新規治療薬の開発にも大いに注目していきたい。

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