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ORIGINAL INVESTIGATION

AVE1625, a cannabinoid CB1 receptor antagonist, as a co-treatment with antipsychotics for schizophrenia: improvement in cognitive function and reduction of antipsychotic-side effects in rodents

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Abstract
Rationale The psychotomimetic effects of cannabis are believed to be mediated via cannabinoid CB1 receptors. Furthermore, studies have implicated CB1 receptors in the pathophysiology of schizophrenia.
Objective These studies investigated the effects of the CB1 receptor antagonist, AVE1625, in acute pharmacological and neurodevelopmental models of schizophrenia. AVE1625 was administered to rodents alone or as a co-treatment with clinically used antipsychotic drugs (APDs).
Methods The antipsychotic potential of AVE1625 was tested using psychotomimetic-induced hyperactivity and latent inhibition (LI) deficit models. The procognitive profile was assessed using hole board, novel object recognition, auditory evoked potential, and LI techniques. In addition, the side-effect profile was established by measuring catalepsy, antipsychotic-induced weight gain, plasma levels of prolactin, and anxiogenic potential.
Results AVE1625 (1, 3, and 10 mg/kg ip), reversed abnormally persistent LI induced by MK-801 or neonatal nitric oxide synthase inhibition in rodents, and improved both working and episodic memory. AVE1625 was not active in positive symptom models but importantly, it did not diminish the efficacy of APDs. It also decreased catalepsy and weight gain induced by APDs, suggesting that it may decrease APD-induced extrapyramidal side effects (EPS) and compliance. Unlike other CB1 antagonists, AVE1625 did not produce anxiogenic-like effects.
Conclusions These preclinical data suggest that AVE1625 may be useful to treat the cognitive deficits in schizophrenia and as a co-treatment with currently available antipsychotics. In addition, an improved side-effect profile was seen, with potential to ameliorate the EPS and weight gain issues with currently available treatments.

Keywords Schizophrenia · Cognition · Endocannabinoid · CB1 antagonists · Episodic memory · Working memory · Anxiety · Mouse · Rat · AVE1625

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Introduction

The "cannabinoid hypothesis" of schizophrenia was originally based on clinical findings in marijuana abusers and has been developed as one of the potential pharmacological etiologies of the disease. Consumption of a relatively large amount of cannabis in normal people can precipitate a psychotic state called 'cannabinoid psychosis', and exacerbate symptoms in patients with schizophrenia (D'Souza et al. 2004, 2005). Administration of Δ^9-tetrahydrocannbinol, the major psychoactive component of cannabis, to normal volunteers induces cognitive impairments similar to those seen in schizophrenia (D'Souza et al. 2004). Cannabis use is also associated with other cognitive deficits including detrimental impacts on sensory processing, spatial learning tasks, and short-term memory (Abel 1970; Lundqvist 2005).

Of the cannabinoid receptors, the CB1 receptor subtype has been implicated in the pathophysiology of schizophrenia. More specifically, CB1 receptors are distributed in areas of the human brain dually implicated in schizophrenia and cognition, namely the prefrontal cortex, anterior cingulate cortex, basal ganglia, and hippocampus (Rinaldi-Carmona et al. 1996). Furthermore, postmortem studies have shown increased CB1 receptor densities in the prefrontal and anterior cingulate cortex of patients with schizophrenia, as compared with normal controls (Dean et al. 2001; Ujike and Morita 2004; Zavitsanou et al. 2004). Additionally, recent findings suggest that CB1 receptor ligands may have therapeutic properties in schizophrenia (see Roser et al. 2010 for review) and other psychiatric disorders including depression (Shearman et al. 2003) and anxiety (Porter and Felder 2001). Cannabinoid agonists often induce cognitive impairments in rodents (Ferrari et al. 1999; Pamplona and Takahashi 2006), whereas the antagonism of CB1 receptors generally enhances rodent performance in many memory tasks (Lichtman 2000; Takahashi et al. 2005; Terranova et al. 1996). Based on this evidence, the first aim of these studies was to test a novel, selective and highly potent (CB1 binding affinity, Ki = 11 nM; CB1 functional potency, IC_{50} = 25 nM) CB1 receptor antagonist, AVE1625 (Borowsky et al. 2005; Herling et al. 2007), in in vivo models sensitive to clinically active antipsychotic drugs (APDs), namely psychostimulant-induced locomotor activity using dopaminergic (i.e., amphetamine) and glutamatergic (i.e., phencyclidine (PCP) or MK-801)-based psychostimulants, and amphetamine-induced disruptions of latent inhibition (LI) and auditory evoked potential (AEP) gating. AVE1625 was also tested in models of cognition using LI (MK-801 and L-NoArg models), hole board and novel object recognition tests.

The neurodevelopmental hypothesis of schizophrenia proposes that a proportion of schizophrenia is the result of an early brain insult, either prenatal or perinatal, which affects brain development leading to abnormalities which are expressed in the mature brain (Bloom 1993; Bogerts 1993; Weinberger 1987; Weinberger and Lipska 1995). To this end, we have focused on the well-reported relationship between the *N-methyl*-D-aspartic acid (NMDA) receptor and nitric oxide synthase (NOS) (Garthwaite et al. 1988; Kiss and Vizi 2001). Evidence also exists that NOS can influence brain development (Roskams et al. 1994; Sánchez-Islas and León-Olea 2004; Wu et al. 2003). NO itself is known to play a role in brain development and neuronal connectivity during the prenatal and perinatal period (for review, see Contestabile 2000) and interfering with NO production during the very early postnatal period reproduces some of the aspects of schizophrenia in adult animals (Black et al. 1999, 2002). Recently, we have also found that neonatal NOS inhibition leads to abnormally persistent LI at adulthood that appears sensitive to putative novel antipsychotics (Barak et al. 2009; Black et al. 2009). Based on these findings, an additional aim of these studies was to test whether AVE1625 was efficacious in reversing persistent LI in the L-NoArg neurodevelopmental model.

As well as efficacy, safety and more specifically, an improved side-effect profile, is an important aspect of any new treatment for schizophrenia. Therefore, AVE1625 was also profiled in models of side effects induced by clinically active antipsychotics, as well as a model of a side effect associated with CB1 receptor antagonists. More specifically, certain APDs such as olanzapine are associated with drug-induced weight gain and the linked issue of diabetes (Allison and Casey 2001; Van Gaal 2006). As CB1 receptor antagonism has been investigated for its potential to reduce food intake and subsequently to treat obesity (Di Marzo and Matias 2005; Pertwee 2006) and AVE1625 has been shown to reduce food intake and weight gain (Herling et al. 2007), the effects of AVE1625 co-treatment on olanzapine-induced weight gain were investigated. Furthermore, as there are additional antipsychotic-related side effects such as hyperprolactinemia and extrapyramidal symptoms, AVE1625 was also assessed for any increase in plasma prolactin and potential to induce catalepsy. Finally, as CB1 antagonists have been reported to induce anxiety-like or "anxiogenic" effects in both rodents and humans (see Moreira et al. 2009 for review), AVE1625 was tested in the mouse Light–dark box, a model sensitive to the anxiogenic effects of rimonabant and taranabant, two CB1 antagonists that produced anxiogenic-like effects in humans (Christensen et al. 2007; Addy et al. 2008).

In summary, the aims of this work were to evaluate potential efficacy of AVE1625 in models sensitive to clinically active APDs, with particular emphasis on cognition and assessing its utility as a co-treatment with conventional antipsychotics. AVE1625 was also assessed

for any potential mechanism-based side effects and antipsychotic-like liabilities alone, and in combination with established APDs.

Material and methods

Animals and housing

All experiments were performed in accordance with the "Guide and Care and Use of Laboratory Animals" (National Institutes of Health) and were approved by the Sanofi-Aventis Institutional Animal Care and Use Committee (studies conducted in the USA), the Animal Ethics Committee of Sanofi-Aventis (studies conducted in France), or the Institutional Animal Care and Use Committee of Tel Aviv University, Israel (studies conducted in Israel). Animals had access to food and water ad libitum (unless otherwise indicated) with a 12-h light/dark cycle (lights on at 7:00 a.m. or 7:00 p.m. in the LI studies). All testing was performed during the light (day) or dark (LI studies) cycle.

Models of antipsychotic activity

Spontaneous and PCP- or amphetamine-induced locomotion in mice and rats

Male CD-1 mice weighing 20–30 g and male Sprague–Dawley rats weighing 250–430 g were used (Charles River Laboratories, Kingston, NY). A standard automated locomotion system (Columbus Instruments, Columbus, OH) and assay were used (see Pierce and Kalivas 2007). Horizontal activity was measured by beam breaks of photocells lining the chamber exterior to the activity boxes. The activity was measured for 60 min during spontaneous locomotion tests or 90 min for the PCP- or amphetamine-induced hyperactivity assays. AVE1625 was administered either orally (per os (po)) or intraperitoneally (ip) with a 1 h pretreatment. In co-administration experiments, haloperidol or olanzapine was administered ip with a 30 min pretreatment. PCP and amphetamine were administered ip and subcutaneously (sc), respectively, with no pretreatment. Doses of AVE1625 for mice were 1, 3, and 10 mg/kg. The typical antipsychotic, haloperidol, was used in co-administration with AVE1625 (1, 3, and 10 mg/kg po) at a dose of 0.2 mg/kg ip in mice to reverse PCP-induced locomotion and at a dose of 0.3 mg/kg in rats to reverse amphetamine-induced locomotion. The atypical antipsychotic, olanzapine, was used in co-administration with AVE1625 (1, 3, and 10 mg/kg ip) at a dose of 0.3 mg/kg ip in mice to reverse PCP-induced locomotion, and at a dose of 1 mg/kg in rats to reverse amphetamine-induced locomotion.

Latent inhibition test in rats: amphetamine-, MK-801-, or neonatal L-NoArg-induced aberrations of LI

Male Wistar rats (Tel Aviv University Medical School, Israel) approximately 3 months old and weighing 350–450 g were used. Rats were tested in rodent test chambers (model 410, Campden Instruments, Loughborough, UK) with a retractable bottle, each enclosed in a ventilated sound-attenuating chamber. When the bottle was not present, a metal lid covered the hole. Licks were detected by a lickometer (model 453). The pre-exposed to-be-conditioned stimulus was a 10-s, 80-dB, 2.8-kHz tone produced by a Sonalert module (model SC 628). Shock was supplied through the floor by a shock generator (model 521/C) and a shock scrambler (model 521/S) set at 0.5 mA intensity and 1-s duration. Equipment programming and data recording were computer controlled.

Prior to the beginning of each experiment, rats were handled for approximately 2 min daily for 5 days. A 23-h water restriction schedule was initiated simultaneously with handling and continued throughout the LI experiment. On the next 5 days, rats were trained to drink in the experimental chamber for 15 min per day. Rats received water in the test apparatus in addition to their daily ration of 1 h access in the home cages. The LI procedure was conducted on days 11–14 and consisted of four stages presented 24 h apart:

Pre-exposure With the bottle removed, the pre-exposed (PE) rats received 40 tone presentations with an interstimulus interval of 40 s. The nonpre-exposed (NPE) rats were confined to the chamber for an identical period of time without receiving the tone.

Conditioning With the bottle removed, rats received two (weak conditioning) or five (strong conditioning) tone-shock pairings given 5 min apart. Shock immediately followed tone termination. Weak conditioning produces LI in nontreated controls and thus allows the demonstration of treatment-induced LI disruption. This level of conditioning was used with amphetamine which is well documented to disrupt LI (Weiner 2003). Conversely, strong conditioning prevents LI in nontreated controls and thus allows the demonstration of treatment-induced abnormally persistent LI. This level of conditioning was used with MK-801 and neonatal L-NoArg because these treatments produce abnormally persistent LI (see Barak et al. 2009; Black et al. 2009).

Lick retraining Rats were exposed to a 15-min drinking session as during the initial training. Data for rats that failed to complete 600 licks were dropped from the analysis.

Test Each rat was placed in the chamber and allowed to drink from the bottle. When the rat completed 75 licks, the

tone was presented for 5 min. The following times were recorded: time to first lick, time to complete licks 1–50, time to complete licks 51–75 (before tone onset, A period), and time to complete licks 76–100 (after tone onset, B period). Times to complete licks 76–100 were logarithmically transformed to allow parametric analysis of variance. Longer log times indicate stronger suppression of drinking. LI is defined as significantly shorter log times to complete licks 76–100 in PE rats, compared with the NPE rats.

For the pharmacological studies, amphetamine was injected 30 min prior to both pre-exposure and conditioning stages at 1 mg/kg, i.p., while MK-801 was administered only prior conditioning at 0.05 mg/kg, i.p. (−30 min). AVE1625 was administered at doses of 1, 3, or 10 mg/kg, in a volume of 1 ml/kg. Sixty minutes prior to pre-exposure and conditioning stages. Glycine was administered 30 min prior to the conditioning stage at a dose of 800 mg/kg, in a volume of 3 ml/kg. No-drug controls received the corresponding vehicle.

For the neurodevelopmental studies, neonatal treatment was conducted in Wistar rat pups (Tel Aviv University Medical School) birthed to rats mated at 3 months of age. At birth, litters were culled to ten, composing of five males and five females whenever possible. The day of birth was defined as postnatal day 0. On postnatal days 3, 4, and 5 rat pups were given sc injection of either 10 mg/kg L-NoArg or vehicle in a volume of 1 ml/kg. On day 21, the pups were weaned and housed five to a cage by sex and litter, and maintained undisturbed until 3 months of age. In each experimental group, there was no more than one rat from the same litter.

Amphetamine-induced disruption of auditory evoked potentials in the rat

All recordings were made using Spike2 software (Cambridge Electronic Design, Cambridge, UK) running on a Windows PC connected to a 1401 interface (Cambridge Electronic Design, Cambridge, UK). EEG recordings were created by connecting the skull plug to an amplifying headstage (AI 1401, Axon Instruments, Union City, CA), via a 6-channel commutator and cable (Plastics One, Roanoke, VA). The signal was then further amplified with a multichannel amplifier (Cyberamp 380, Axon Instruments, Union City, CA). Auditory stimuli were controlled by the interface via an eight channel power amplifier (SA8, Tucker Davis Technologies, Alachua, FL) connected to a patch panel (PP16, Tucker Davis Technologies), which then connected to speakers located above each cage (TDT Magnetic Speakers, Tucker Davis Technologies). During recording sessions, animals were placed inside standard mouse cages (30 × 15 × 15 cm) and rigged so that the six channel cables could connect with the rest of the apparatus outside of the cage.

Surgery Animals were anesthetized with 3% isoflurane and their scalp was shaved and prepared for surgery. An incision was made in the scalp and the skin was retracted to allow direct contact with the skull surface. The skull was cleaned with a dry Q-tip and five holes were drilled through the skull. Three holes were drilled at coordinates AP + 1 mm, ML + 1 mm (reference); AP − 4 mm, ML ± 4 mm (leads 1 and 2) relative to bregma, while an additional hole was drilled in the frontal area (ground) and another hole was drilled at the midpoint between the anterior and posterior coordinate (anchor). Screw electrodes were then lowered through the skull so that they were in direct contact with the surface of the cortex. The leads from the screws were fed through a six channel pedestal (Plastics One) and the pedestal was secured to the skull surface with dental cement (Tylok Plus, Henry Schein, Melville, NY). Once the cement had dried, the scalp was sutured and the animal was given an injection of Metacam (1 mg/kg).

AEP testing procedure After approximately 4 weeks of recovery postsurgery, animals were tested on AEP gating over five sessions, each separated by 7 days, using a within-subjects design wherein each animal was exposed to each drug treatment. The treatment conditions were vehicle + vehicle, vehicle + amphetamine (3 mg/kg), 3 mg/kg AVE1625 + amphetamine, 10-mg/kg AVE1625 + amphetamine, and 10-mg/kg AVE1625 + vehicle. Amphetamine was dissolved in 0.9% saline. The vehicle used with AVE1625 was 0.6% carboxymethylcellulose and 0.5% Tween 80 in distilled water. Both AVE1625 and vehicle were administered po. All doses of saline and amphetamine were administered ip. For all gating sessions tone pairs consisted of two 1,500 Hz, 5 V tones, separated by a 500-ms interval, with a 10-s intertrial interval separating each double pulse. Sessions consisted of 350 tone pair presentations, each tone pair separated by 10 s, and lasted approximately 60 min. On each testing day, two sessions were administered. The first session occurred immediately after administration of vehicle or AVE1625 and consisted of 360 double pulse pairs (approximately 60 min). The second session occurred immediately after injection of vehicle or 3 mg/kg amphetamine and consisted of 360 double pulse pairs (again approximately 1 h). The second session followed immediately after the first.

AEP data analysis Data were collected and analyzed using Spike2 software (CED Software, Cambridge, UK). Data were derived by smoothing raw waveforms and constructing a waveform average for each animal/channel. Then a horizontal cursor was placed at the highest and lowest (peak and trough) point of the P1/N1 wave, for both the wave occurring to the first tone (S1) and to the second tone (S2), and the value was recorded into an Excel spreadsheet. The

P1 wave was defined as the highest peak occurring between 8 to 18 ms, and the N1 wave was defined as the lowest trough occurring between 18 and 35 ms. The amplitude of the S1 waveform was calculated by subtracting the value of the trough for the N1 wave from the value of the peak of the P1 wave. This was also done for the S2 waveform. Then, a ratio was derived by dividing the value of the S2 wave from that of the S1 wave.

Models of cognition

Hole board task in rats

Male Sprague–Dawley rats (Charles River Laboratories) weighing 200–220 g were used. Rats were maintained at 85% of their normal body weight. Rat were acclimated to the hole board testing chamber (Med-Associates, St. Albans, VT), which was a ventilated, sound-attenuating cubicle, for four 10-min trials over a 2-day period 24 h prior to drug treatments. The testing chamber contains eight holes, each of which are baited with a food reward (cocoa flavored pellet). Animals that did not retrieve all eight rewards within the 10 min period were excluded from the study. Each experiment was carried out over 2 days. Animals were pseudo-randomly assigned to treatment groups such that each animal received two of the four treatments, with an even distribution of all possible treatment combinations. Each treatment group had a total of 16 animals. Following a 60-min pretreatment, the rats were placed in the testing chamber. After consuming four food rewards, the rats were removed from the chamber for 2 min and returned to their home cage. Next, rats were again placed into the chamber and allowed 10 min to retrieve and finish the remaining four rewards. Animals that did not retrieve the first four rewards within the 10-min period were excluded from the study. The number of visits to holes that they had already been to, and latency to complete the task, were noted. AVE1625 was administered at doses of 0 (n = 16), 1 (n = 15, one rat was excluded because it did not reach the criteria), 3 (n = 16), and 10 (n = 16) mg/kg ip. The atypical antipsychotic, risperidone, was used alone at 0 (n = 14, one rat was excluded), 0.01 (n = 16), 0.1 (n = 15, one rat was excluded), and 1 (n = 14, two rats were excluded) mg/kg ip and in co-administration with AVE1625 (10 mg/kg, n = 13, three rats were excluded) at doses of 0.1 mg/kg (n = 13, three rats were excluded).

Object recognition task in rats

The object recognition task was similar to that described by Ennaceur and Delacour (1988) in young rats. The apparatus consisted of a uniformly lit (100 lx) wooden enclosure (65 × 45 × 45 high cm) with a video camera positioned 160 cm above the bench. The observer was located in an adjacent room fitted with a video monitoring system. Each experiment consisted of three sessions; during the first session (context habituation), rats (n = 10–12 animals per group, Charles River Laboratories, St. Aubin-les-Elbeuf, France) were allowed 2 min to become acquainted to the apparatus. Locomotor activity was manually recorded with a precision of ±1 s. The animals were again placed in the enclosure 24 h thereafter for the second (acquisition) session, during which they were exposed to a pair of identical objects (either 7 × 3 × 8-cm metal triangles or 9 × 3 × 7-cm plastic pyramids) placed 10 cm away from the two opposite corners of the back wall. Rats were left in the enclosure for the amount of time necessary to spend at most 20 s exploring these two objects, with a limit of 3 min. Exploration of an object was defined as the rat having its head within 2 cm of the object while looking at it, sniffing it or touching it. Any rat spending less than 20 s exploring the objects was eliminated from the study (i.e., four out of 48 rats). Two different identical sets of objects were used to allow for cleaning between one rat and the next, to minimize the possibility that olfactory cues left by the preceding rat might bias the behavior of the following one. Combinations of orders of presentation and locations of objects were balanced to reduce potential biases due to spatial or objects preferences.

During the third (recall) session, rats were exposed to the familiar (i.e., presented during the acquisition session) and to the novel (i.e., never presented before) objects for 3 min, and the time spent exploring each object was recorded (precision ±1 s). Any animal spending less than 3 s exploring both objects was discarded from the study. This third session took place 24 h after the second session: At this intersession interval, previous studies demonstrated that this 24 h interval was necessary for the rat, to loose their ability to discriminate between objects, suggestive of a spontaneous long-term episodic memory impairment that is the rat spends an equal amount of time exploring the familiar object (F) and the novel object (N). A recognition index was also defined as the ratio $N/N + F$. This interval was used to evaluate a possible improvement of performance following acute treatment with AVE1625 (or vehicle (0.6% methylcellulose in distilled water) administered orally 60 min before each of the three sessions.

Liability tests

Catalepsy in rats

Male Sprague–Dawley rats (Charles River Laboratories) weighing 250–300 g were used. Each animal was placed such that their fore paws rested on a wooden dowel (1 × 18 cm) mounted horizontally 9 cm from the floor and 4 cm

from one end of a white translucent plastic box (26 × 20 × 15 cm). The amount of time each rat spent with at least one forepaw on the bar was determined, for a maximum period of 180 s. This procedure was repeated three times. White noise was used during the acclimation and test periods to minimize the effects of outside noises. AVE1625 was administered alone at a dose of 10 mg/kg ip. Haloperidol (1 mg/kg ip) and olanzapine (10 mg/kg ip) were used alone and in co-administration with AVE1625 (1, 3, and 10 mg/kg ip).

Olanzapine-induced weight gain in rats

Female Wistar rats (Charles River Laboratories) were singly housed and maintained on a high-fat diet (Modified Rodent Diet 5001 #58039 (TestDiet®, Richmond, IN; protein 16%, fat 40% (soybean oil), carbohydrate 44%). Rats were weighed daily. A baseline period (no injections) of 5 days initiated the study. Subsequently, a 14–15-day once daily dosing schedule was utilized. Dosing was performed between the hours of 10:00 am and 2:00 pm. AVE1625 was administered ip at doses of 1, 3, and 10 mg/kg and olanzapine was dosed ip at 3 mg/kg.

Prolactin levels in rats

Female Sprague–Dawley rats (Charles River Laboratories) weighing 200–250 g were used. Rats were allowed to acclimatize to the procedure room for 60 min. During this acclimation period weighing, handling and mock injections of saline were given. This procedure was repeated for 3 days prior to the study. AVE1625 was administered at 10 mg/kg ip with a 60-min pretreatment. The atypical antipsychotic, olanzapine (0.3, and 1 mg/kg), was used alone and in co-administration with AVE1625. The dopamine agonist, bromocriptine (10 mg/kg), was used in co-administration with olanzapine (1 mg/kg). Olanzapine and bromocriptine were administered ip with 60- and 75-min pretreatment times, respectively. Following dosing, blood was harvested via cardiac puncture after carbon dioxide euthanasia. Plasma samples were analyzed for prolactin concentrations using a rat prolactin standard enzyme immunoassay.

Mouse light–dark box

NMRI mice (CERJ, France) weighing 18–20 g were used for testing. The test apparatus is based on that described by Misslin et al. (1989) and consisted of two polyvinylchloride boxes (20 × 20 × 14 cm) connected by a tunnel (5 × 7 × 10 cm). One of the boxes was darkened, the other was lit by a desk lamp placed 20 cm above it. The apparatus was equipped with infrared beams capable of recording during a 4-min period. The time spent by the mouse in the lit box, was recorded.

Statistical analysis

All data were first tested for normality and equal variance (Levene's test). If the data passed these two initial tests then data were subsequently analyzed using single or multi-factor analysis of variance (ANOVA) followed by Dunnett's comparison test. If the data failed either one of these tests the data were subsequently analyzed using a nonparametric Kruskal–Wallis test followed by multiple comparisons. In all cases, the significance level was $p < 0.05$. All analyses were conducted using SAS software (Version 8.2 for Sun 4, SAS Institute Inc., Cary, NC).

Drugs

AVE1625 (Sanofi-Aventis, France) was suspended via homogenization in 60% labrasol/40% labrafil for all mouse and most rat locomotion experiments. For all other experiments, AVE1625 was emulsified in distilled water, with the addition of 0.1% Tween 80 (Sigma, St. Louis, MO). Haloperidol (Bell Medical Services, Inc., Marlborough, NJ) was dissolved in distilled water via dilution of a 5-mg/ml stock solution. In a previous study using CB1 agonist-induced hypothermia model in mice, AVE1625 attenuated the hypothermia with an $ID_{50} = 3$ mg/kg (data not shown). Based on this data, AVE1625 was tested across a dose range of 1–10 mg/kg in these studies. Risperidone (Sigma), olanzapine (Toronto Research Chemicals, Inc., North York, Ontario, Canada), MK-801 (Sigma, USA and Sigma, Rehoboth, Israel), and glycine (Sigma, Israel) were all dissolved in 0.9% saline. Phencyclidine and amphetamine (both Sigma, USA or Israel) were dissolved in distilled water or saline. *N*ω-nitro-L-arginine (L-NoArg, Sigma, Israel) was dissolved in 1N HCl, diluted with 10-mM phosphate buffered saline and titrated with 2-M Tris (7.5 pH) buffer to a final pH of 5.5. Bromocriptine (Sigma, USA) was solubilized in distilled water, with the addition of 0.1% Tween 80. All doses refer to the weight of the free base.

Results

Models of antipsychotic activity

AVE1625 had no effect on spontaneous locomotion

AVE1625 administered alone up to 10 mg/kg ip did not significantly alter spontaneous locomotion in either mice or rats, although a tendency to a reduction in activity was observed at 10 mg/kg in mice (Mean total beam breaks, mouse study—Veh $= 4,469 \pm 349$, AVE 1 mg/kg $= 4,337 \pm 742$, AVE 3 mg/kg $= 3,959 \pm 545$, AVE 10 mg/kg $= 2,911 \pm 321$, Haloperidol 0.2 mg/kg $= 1,485 \pm 166$. Rat study—Veh =

963 ± 120, AVE 1 mg/kg = 743 ± 120, AVE 3 mg/kg = 1,038 ±143, AVE 10 mg/kg = 796 ± 101, Haloperidol 0.3 mg/kg = 296 ± 10]. In both studies, haloperidol produced a significant reduction in spontaneous locomotor activity ($p < 0.05$).

AVE1625 had no effect on amphetamine- and PCP-induced locomotion

In rats, AVE1625 (1–10 mg/kg) demonstrated no significant reversal of locomotor hyperactivity induced by amphetamine. There was a significant effect of treatment ($\chi^2 = 16.2$; $p < 0.01$) reflecting the marked increase in locomotion induced by amphetamine that was unaffected by AVE1625 at doses of 1–10 mg/kg (Table 1). This was in contrast to the reversal shown by both haloperidol (0.3 mg/kg) and olanzapine (1 mg/kg) [$F(5, 42) = 19.0$; $p < 0.0001$]. Although the overall analysis for the haloperidol study failed to reach significance ($p = 0.12$) clearly haloperidol attenuate the amphetamine hyperactivity. In both the haloperidol and olanzapine studies, AVE1625 was combined with the antipsychotics to determine if the compound affected the ability of these compounds to attenuate amphetamine hyperactivity. In both studies, AVE1625 had no effect (Table 1).

In mice, AVE1625 (1–10 mg/kg) had no effect on PCP-induced hyperactivity while haloperidol significantly reversed the PCP effect (main effect of treatment: $Chi^2 = 25.8$, $p < 0.0001$) (Table 2). In combination studies with haloperidol (0.2 mg/kg) and olanzapine (0.3 mg/kg), AVE1625 (1–10 mg/kg) had no detrimental effect on the ability of these APDs to reversed PCP hyperactivity (Table 2). In the haloperidol study, haloperidol alone and in combination with AVE1625 significantly attenuated the PCP hyperactivity ($\chi^2 = 19.8$; $p = 0.001$). In the olanzapine study (Table 2), there was a similar finding with olanzapine alone and in combination with AVE1625 attenuating the PCP effect [$F(5, 40) = 16.8$; $p < 0.0001$].

In separate studies in mice, the ability of AVE1625 (1–10 mg/kg) to improve the ability of subthreshold doses of both haloperidol (0.1 mg/kg) and olanzapine (0.03 mg/kg) to reduce PCP hyperactivity was tested. In both studies, AVE1625 did not produce any beneficial effects (data not shown).

Latent inhibition

AVE1625 had no effect on amphetamine-induced disrupted LI

The experiment included 115 rats (run in three replications) divided into 12 groups ($n = 9$–10 per group) in a 2 × 2 × 3 design with main factors of pre-exposure (PE and NPE), treatment (vehicle and amphetamine), and pretreatment (vehicle, 3 and 10 mg/kg AVE1625).

Table 1 Effect of AVE1625 on amphetamine hyperactivity in rats when administered alone or in combination with antipsychotic drugs

AVE1625 alone	
Veh/Veh	1,179 ± 238
Veh/Amph	4,909 ± 1,113*
AVE 1/Amph	3,912 ± 1,144*
AVE 3/Amph	3,571 ± 665*
AVE 10/Amph	4,310 ± 642*
AVE1625 and haloperidol	
Veh/Veh/Veh	1,370 ± 350
Veh/Veh/Amph	5,265 ± 1,346*
Veh/Halo/Amph	1,543 ± 917
AVE 1/Halo/Amph	1,593 ± 675
AVE 3/Halo/Amph	1,715 ± 669
AVE 10/Halo/Amph	2,144 ± 1,056
AVE1625 and Olanzapine	
Veh/Veh/Veh	1,560 ± 231
Veh/Veh/Amph	8,913 ± 1,621*
Veh/Olanz/Amph	1,181 ± 155**
AVE 1/Olanz/Amph	1,576 ± 160**
AVE 3/Olanz/Amph	1,535 ± 309**
AVE 10/Olanz/Amph	1,659 ± 263**

*$p < 0.01$ vs. Veh/Veh or Veh/Veh/Veh groups; **$p < 0.01$ vs. Veh/Amph or Veh/Veh/Amph groups

Table 2 Effect of AVE1625 on PCP hyperactivity in mice when administered alone or in combination with antipsychotic drugs

AVE1625 alone	
Veh/Veh	1,621 ± 353
Veh/PCP	9,455 ± 2,182*
AVE 1/PCP	7,405 ± 698*
AVE 3/PCP	8,427 ± 1,726*
AVE 10/PCP	9,329 ± 1,462*
Halo/PCP	2,308 ± 620***
AVE1625 and Haloperidol	
Veh/Veh/Veh	2,132 ± 382
Veh/Veh/PCP	12,209 ± 1,899**
AVE 1/Halo/PCP	1,738 ± 474***
AVE 3/Halo/PCP	2,733 ± 783***
AVE 10/Halo/PCP	2,283 ± 777***
Veh/Halo/PCP	2,626 ± 677***
AVE1625 and Olanzapine	
Veh/Veh/Veh	1,294 ± 207
Veh/Veh/PCP	11,434 ± 1,696**
AVE 1/Olanz/PCP	2,083 ± 493***
AVE 3/Olanz/PCP	3,934 ± 755***
AVE 10/Olanz/PCP	3,111 ± 879***
Veh/Olanz/PCP	4,033 ± 968***

*$p < 0.05$ vs. Veh/Veh or Veh/Veh/Veh groups; **$p < 0.01$ vs. Veh/Veh or Veh/Veh/Veh groups; ***$p < 0.05$ vs. Veh/PCP or Veh/Veh/PCP groups

The experimental groups did not differ in their times to complete licks 51–75 before tone onset (all p values > 0.05; overall mean A period = 7.99 s). Rats treated with vehicle or AVE1625 alone at 3 and 10 mg/kg exhibited LI whereas LI was absent in rats treated with either amphetamine or AVE1625 (3 or 10 mg/kg) in combination with amphetamine (Fig. 1a).

Three-way ANOVA yielded significant main effects of pre-exposure [$F(1, 103) = 44.3$; $p < 0.0001$] and treatment [$F(1, 103) = 25.1$; $p < 0.0001$] and a significant pre-exposure × treatment interaction [$F(1, 103) = 25.6$; $p < 0.0001$]. Post hoc comparisons revealed a significant difference between the pre-exposed and nonpre-exposed groups in the vehicle–vehicle ($p < 0.01$), AVE 3 mg/kg vehicle ($p < 0.01$), and AVE 10 mg/kg vehicle ($p < 0.01$) conditions but not in the any of amphetamine conditions.

AVE1625 reversed MK-801-induced abnormally persistent LI

The experiment included 191 rats (run in four replications) in 20 groups (n = 8–10 per group) in a 2 × 2 × 5 design with main factors of pre-exposure (PE and NPE), treatment (vehicle and MK-801), and pretreatment (vehicle, 1, 3, and 10 mg/kg AVE1625; 800 mg/kg glycine). Three rats did not reach criteria during lick retraining and their data were not included in the analysis. The 20 experimental groups did not differ in their times to complete licks 51–75 before tone onset (all p values > 0.05; overall mean A period = 8.61 s).

As can be seen in Fig. 1b, vehicle-injected rats did not show LI whereas MK-801-treated rats showed LI in spite of strong conditioning. MK-801-induced persistent LI was reversed by doses of 1, 3, and 10 mg/kg of AVE1625 and glycine (Fig. 1b, glycine data not shown). AVE1625 alone had no effect on LI. Three-way ANOVA yielded significant main effects of pre-exposure [$F(1, 168) = 8.06$; $p < 0.01$], and pretreatment [$F(4, 168) = 3.44$; $p = 0.01$], and a near significant main effect of treatment [$F(1, 168) = 2.90$; $p = 0.09$]. *Post hoc* comparisons revealed a significant difference between the pre-exposed and nonpre-exposed groups following MK-801 treatment ($p < 0.001$) but not in the other treatment groups.

AVE1625 reversed L-NoArg-induced abnormally persistent LI

The experiment included 143 rats (run in three replications) divided into 16 groups (n = 7–9 per group) in a 2 × 2 × 4

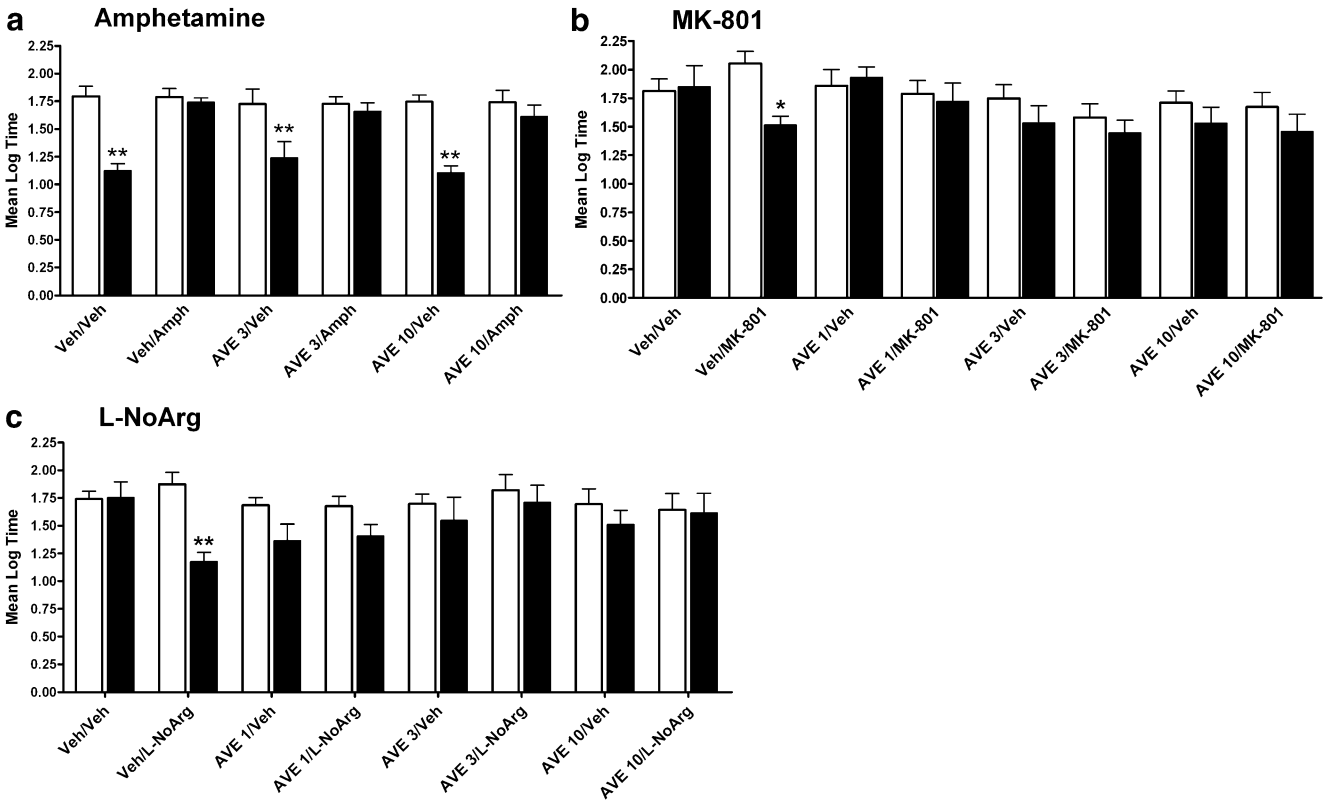

Fig. 1 The effects of AVE1625 on amphetamine-induced LI disruption (**a**), MK-801-induced LI persistence (**b**) and neonatal L-NoArg-induced LI persistence (**c**). Data represent mean (+SEM) log times to complete licks 76–100 (after tone onset) of the pre-exposed (PE; *black bars*) and nonpre-exposed (NPE; *white bars*) rats treated with amphetamine (*Veh/Amph*), MK-801 (*Veh/MK-801*), L-NoArg (*Veh/L-NoArg*), or vehicle (*Veh/Veh*), and pretreated with either AVE1625 at doses of 1, 3, or 10 mg/kg ip or vehicle. *Asterisks* indicate a significant difference between the PE and NPE groups, namely, presence of LI (*$p < 0.05$; **$p < 0.01$)

design with main factors of neonatal treatment (vehicle, L-NoArg), pre-exposure (PE and NPE), and pretreatment (vehicle, 1, 3, and 10 mg/kg AVE1625). Three rats did not reach criteria during lick retraining and their data were not included in the analysis. The 16 experimental groups did not differ in their times to complete licks 51–75 before tone onset (all $p > 0.05$; overall mean A period = 8.76 s).

LI was absent in neonatally vehicle-treated rats whereas neonatally L-NoArg-treated rats showed LI. Neonatally L-NoArg-induced abnormally persistent LI was reversed by all doses of AVE1625 (Fig. 1c). AVE1625 alone had no effect on LI when administered to neonatally vehicle-treated rats.

Three-way ANOVA yielded significant main effects of pre-exposure [$F(1, 124) = 10.61$; $p < 0.01$]. *Post hoc* comparisons revealed a significant difference between the PE and NPE groups in the neonatal L-NoArg-rats treated with vehicle ($p < 0.001$) but not in the other treatment groups.

AVE1625 had no effect on amphetamine disrupted auditory evoked potential gating

In this study, there was a significant effect of treatment on auditory gating as measured by the S2/S1 wave amplitude ratio [$F(4, 48) = 4.1$; $p < 0.01$]. Amphetamine (3 mg/kg) significantly disrupted auditory evoked potential gating (gating ratio: Veh $= 0.25 \pm 0.03$, Amphetamine $= 0.45 \pm 0.06$; $p < 0.01$ vs. Veh). AVE1625 when administered alone at 10 mg/kg ip did not affect baseline gating, nor did it affect amphetamine-induced disrupted gating at doses of 3 or 10 mg/kg (gating ratio: AVE 10 mg/kg $= 0.32 \pm 0.03$, AVE 3 mg/kg + Amphetamine $= 0.40 \pm 0.07$**, AVE 10 mg/kg + Amphetamine $= 0.45 \pm 0.05$**; **$p < 0.01$ vs. Veh). While no positive control was included in this study, previous studies in our group have demonstrated that $\alpha 7$ nicotinic agonists can reverse this disruptive effect of amphetamine on auditory evoked potential gating (Featherstone et al. 2009).

Models of cognition

AVE1625 improved performance in the hole board test

There was a significant effect of AVE1625 treatment on the number of short-term episodic memory errors following the 2-min delay [$F(3, 59) = 3.0$; $p < 0.05$] (Fig. 2). The 3 and 10 mg/kg dose of AVE1625 produced a significant decrease in the number of short-term episodic memory errors compared with the vehicle-treated animals ($p < 0.05$). Risperidone at doses of 0.01, 0.1, and 1 mg/kg had no effect on short-term episodic memory errors compared with the vehicle-treated animals (Fig. 2). The 10 mg/kg dose of AVE1625 when administered alone or in the presence of 0.1-mg/kg risperidone caused a significant decrease in the number of short-term episodic memory errors, compared with the vehicle-treated animals [$F(2, 37) = 4.8$; $p < 0.01$]. AVE1625 did not affect the latency to complete the task in either experiment whereas 1 mg/kg risperidone significantly increased this measure alone, and in combination with AVE1625 (data not shown).

AVE1625 facilitated episodic memory in the object recognition task

Under control (vehicle) conditions, rats spent an equivalent amount of time investigating the novel and the familiar object (16.0 ± 2.3 versus 15.8 ± 2.4 s), 24 h after exposure to the familiar object (Fig. 3). This indicates that rats lost their ability to discriminate between the two objects, indicative of a physiological loss of episodic memory. AVE1625 (0.3–3 mg/kg po) significantly increased the

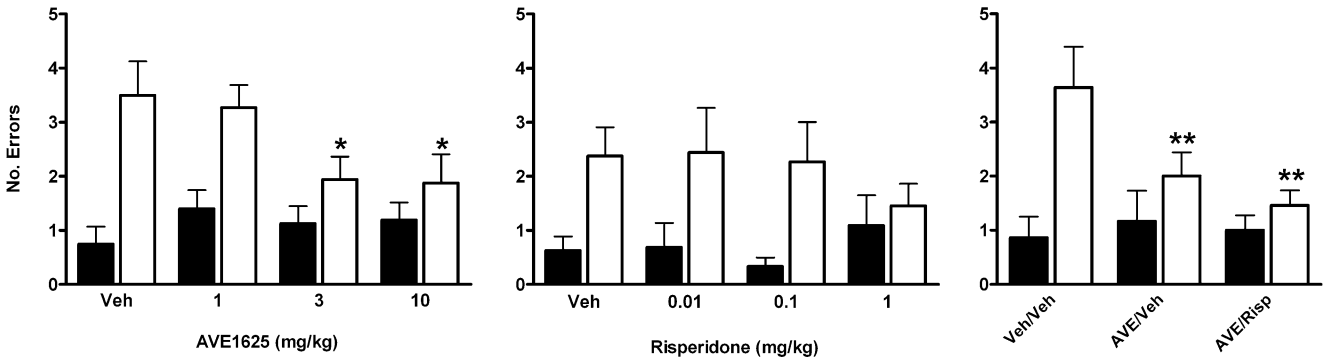

Fig. 2 AVE1625, alone and co-administered with risperidone, improved working memory in the hole board in rats. AVE1625 at 1, 3, and 10 mg/kg ip (n = 15–16 per group), risperidone at 0.01, 0.1, and 1 mg/kg ip (n = 11–16 per group), and a single dose of AVE1625 at 10 mg/kg ip alone (*AVE/Veh*) and co-administered with risperidone at 0.1 mg/kg (*AVE/Risp*; n = 13 per group). The number of total working memory errors (number of times the rat revisited a hole already visited *before* the delay, *black bar*), and the number of short-term episodic memory errors (number of times the rat revisited a hole already visited *after* the delay, *white bar*) were measured. *Asterisks* denote significant difference (*$p < 0.05$; **$p < 0.01$) from control groups (*Veh* or *Veh/Veh*). Each *bar* represents the mean + SEM

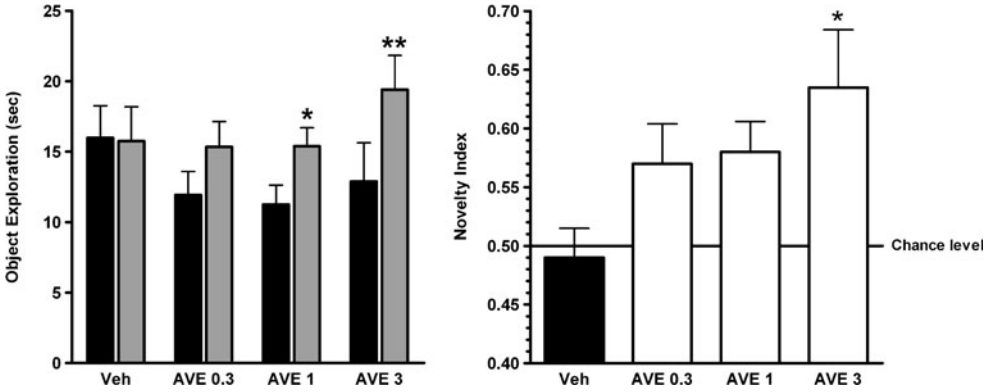

Fig. 3 Acute treatment with AVE1625 at 0.3 (*AVE 0.3*), 1 (*AVE 1*), and 3 (*AVE 3*) mg/kg, enhanced episodic memory in an object recognition task in rats. *Left figure*, each bar represents the mean (+SEM) of the time spent exploring a novel (*gray bars*) or a familiar (*black bars*) object. The interval between the acquisition and the recall session was 24 h. Post hoc analyses following a two-way analysis of variance (ANOVA): *$p < 0.05$; **$p < 0.01$, novel versus familiar object at the concerned dose of AVE1625, $N = 10$–12 rats per group after the exclusion of four rats, which did no reach the criteria of spending at least 3 s exploring objects. *Right figure*, each bar represents the mean (+SEM) of novelty index (expressed as the ratio novel object/novel + familiar object) for control animals compared with those treated by AVE1625. Post hoc analyses following one-way ANOVA, *$p < 0.05$ vs. control group (*Veh*)

amount of time preferentially spent investigating the novel object (main effect of object, $F(1, 40) = 16.48$; $p < 0.0001$; object × treatment interaction, $F(3, 40) = 2.72$; $p < 0.05$) at doses of 1 and 3 mg/kg. Additionally, the relative time spent exploring the novel object (novelty index expressed as the ratio N/N + F) was calculated for each rat (Fig. 3) and a 3 mg/kg dose of AVE1625 differed significantly from the vehicle group (treatment effect, $F(3, 40) = 3.01$; $p < 0.05$). This difference indicated that AVE1625 improved long-term visual episodic memory in a dose-dependent manner, an effect significant at 3 mg/kg.

Neither the locomotor activity recorded during the context habituation session, nor the total time spent exploring both objects during the acquisition and recall sessions, were significantly affected by AVE1625 (data not shown). Therefore, the effects of AVE1625 did not result from nonspecific effects such as sedation and/or motor effects such as ataxia.

Liability tests

AVE1625 reversed antipsychotic-induced catalepsy in rats

In the haloperidol-induced catalepsy study, there was a significant effect of treatment ($\chi^2 = 32.8$; $p < 0.0001$). Haloperidol (1 mg/kg ip) produced a significant cataleptic response and AVE1625 significantly attenuated the catalepsy at a dose of 10 mg/kg po ($p < 0.05$; Fig. 4).

In the olanzapine catalepsy study, there was no significant effect of treatment ($\chi^2 = 9.0$; $p = 0.1$). However, as illustrated in Fig. 4b, olanzapine (10 mg/kg ip) produced a cataleptic response compared with the vehicle-treated rats, and AVE1625 appeared to attenuate the catalepsy at doses of 3 and 10 mg/kg.

In both studies, AVE1625 (10 mg/kg po) did not produce any catalepsy.

AVE1625 reduced olanzapine-induced weight gain

Analysis indicated a significant main effect of treatment on weight gain [$F(5, 46) = 3.4$, $p = 0.01$]. Specifically, olanzapine (3 mg/kg ip) induced a significant ($p < 0.05$) increase in weight gain vs. saline controls during the 14-day study (Fig. 5). AVE1625 co-administration with olanzapine caused a dose-dependent attenuation of weight gain with a significant reduction at 10 mg/kg ($p < 0.05$).

AVE1625 does not affect prolactin levels or alter olanzapine-induced increases in prolactin

AVE1625 did not affect the prolactin increase produced by 0.3 and 1 mg/kg doses of olanzapine (Table 3). The effect of olanzapine was antagonized by the dopamine D_2 receptor agonist, bromocriptine (10 mg/kg ip; Table 3). In a separate study, AVE1625 had no effect on plasma prolactin levels at a dose of 10 mg/kg (Veh, 64 ± 23 ng/ml; AVE1625 10 mg/kg, 47 ± 17 ng/ml).

AVE1625 did not induce anxiogenic-like effects in mice

Oral administration of AVE1625 had no significant effect on the time spent in the lit area over the dose-range tested (10 to 100 mg/kg). However, a marked trend to a decrease in the time spent in the lit box was observed (Fig. 6). Significant decreases in time spent in the lit area were observed with the CB1 antagonists, taranabant ($\chi^2 = 9.8$; $p < 0.05$), and rimonabant ($\chi^2 = 11.4$; $p < 0.01$) at 10 mg/kg, and the

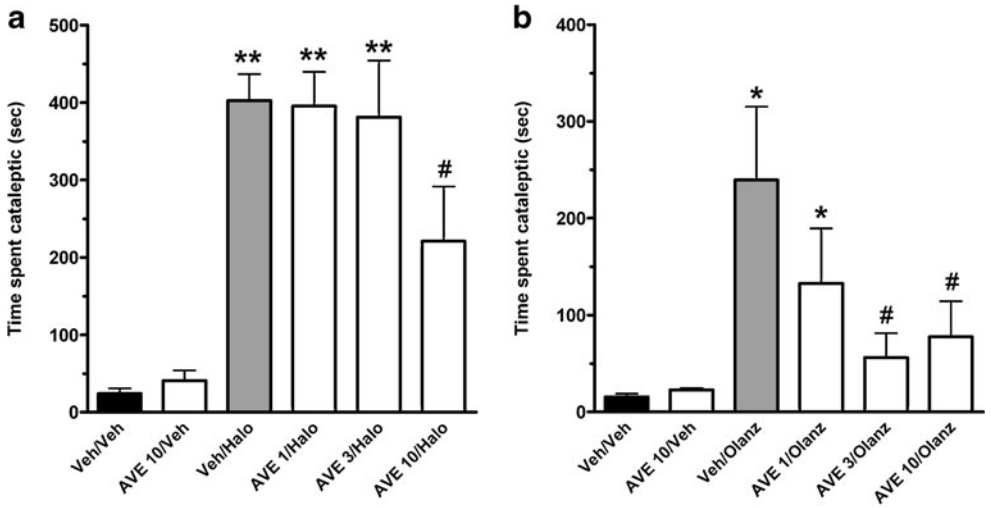

Fig. 4 The effect of AVE1625 on haloperidol (**a**) and olanzapine-induced catalepsy (**b**). AVE1625 significantly reduced haloperidol-induced catalepsy (*Veh/Halo*) at 10 mg/kg (*AVE 10/Halo)* and significantly reduced olanzapine-induced catalepsy (*Veh/Olanz*) at 3 and 10 mg/kg (AVE 3/Halo and AVE 10/Halo groups), compared with vehicle controls (*Veh/Veh*). In both studies, AVE1625 alone at 10 mg/kg (AVE, ten groups) did not induce any catalepsy. *$p < 0.05$; **$p < 0.01$ vs. respective Veh/Veh groups; #$p < 0.01$ vs. Veh/Halo or Veh/Olanz group. For both studies, data represent mean time spent cataleptic ± SEM ($n = 16$ per treatment group)

reference panicogenic compound, m-CPP (10 mg/kg; Wilcoxon, $p < 0.01$ for each study).

Discussion

The major aim of this study was to characterize the CB1 receptor antagonist, AVE1625, in models sensitive to clinically active APDs and models of APD-induced side effects. The common theme to emerge is that while AVE1625 shows activity in models of cognition relevant to schizophrenia, it failed to show activity in models of positive symptoms of the disease. As positive symptoms (hallucinations, etc.) can cause severe debilitation in patients, we would not expect AVE1625 to be successful as a monotherapy for schizophrenia. However, current therapies show little, if any, activity on the cognitive deficits associated with schizophrenia. One could thus propose that add-on or combination therapy of AVE1625 with currently available APDs would offer an attractive therapeutic strategy. Importantly, we show that AVE1625/APD co-treatment does not interfere with the ability of APDs in terms of efficacy and appears to ameliorate certain side effects of current treatments.

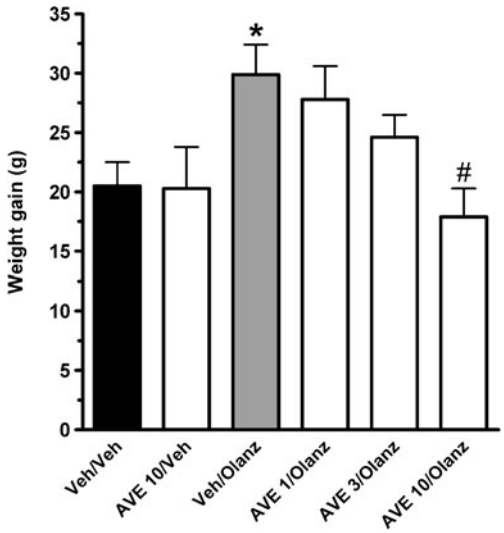

Fig. 5 The effect of co-administration of AVE1625 on olanzapine-induced weight gain in rats. Figure illustrates the effects of AVE1625 at doses of 1 (*AVE 1/Olanz*), 3 (*AVE 3/Olanz*), and 10 (*AVE 10/Olanz*) mg/kg on olanzapine-induced weight gain in rats fed a high-fat diet during a 14-day period. Olanzapine was administered at a dose of 3 mg/kg. Body weights were measured daily beginning on the day before dosing. For all groups, $n = 8$ per group. Each bar represents the mean + SEM

In the present studies, AVE1625 did not attenuate the locomotor stimulation induced by either amphetamine or PCP. This compares well with reports on other CB1 receptor antagonists indicating that acute treatment with CB1 receptor antagonists did not decrease amphetamine-induced hyperactivity or amphetamine-, apomorphine- or MK-801-disrupted PPI in rodents (Martin et al. 2002; Thiemann et al. 2008). However, it has also been shown that the CB1 antagonists can decrease amphetamine-induced hyperactivity in gerbils (Poncelet et al. 1999) and attenuate PCP-disrupted PPI in rats (Ballmaier et al. 2007). A number of studies also indicate that chronic down regulation of CB1 receptor transmission by either CB1 receptor knockout or chronic CB1 receptor antagonist administration attenuates amphetamine sensitization (Corbillé et al. 2007; Thiemann et al. 2008). Therefore, the exact role of CB1 receptor antagonists in psychostimulant

Table 3 The effect of acute treatment of AVE1625 on basal and olanzapine stimulated plasma prolactin levels (nanograms per milliliter) in the rat

Vehicle	Olanz 0.3	Olanz 0.3 + AVE	Olanz 1	Olanz 1 + AVE	Olanz 1 + Bromo
15.0 ± 4.5	175.9 ± 41.0*	116.1 ± 40.7*	471.6 ± 98.3*	426.2 ± 73.5*	11.3 ± 2.34

$N = 7–8$ per group. Olanzapine was dosed at either 0.3 (Olanz 0.3) or 1 (Olanz 1) mg/kg, AVE1625 (AVE) and bromocriptine (Bromo) were dosed at 10 mg/kg

*$p < 0.05$ vs. vehicle

hyperactivity and PPI models is far from clear, but it has been proposed that the endogenous cannabinoid system may preferentially modulate brain circuits that are involved in cognitive or motivational processes (e.g., mesocortical limbic pathways) rather than brain structures involved in motor control (e.g., nigrostriatal pathways). For example, Alonso et al. (1999) showed that the CB1 receptor antagonist, rimonabant, increased c-fos expression in key regions associated with cognitive processes, such as in prefrontal and limbic cortices, while brain structures associated with motor behavior remained unaffected. These finding suggest that CB1 receptors may exert only modest control over dopaminergic and glutamatergic pathways in nigrostriatal regions, so that the blockade of the CB1 receptors alone may not be sufficient to achieve antipsychotic activity (Martin et al. 2003). Additionally AVE1625 was tested in LI. LI is a cross-species selective attention phenomenon that is disrupted in patients with schizophrenia and rodents treated with psychostimulants, and is sensitive to APDs (Lubow 2005; Weiner 2003; Weiner and Arad 2009). AVE1625 did not attenuate amphetamine-induced disruption of LI and did not potentiate LI. Taken together with the findings from the psychostimulant LMA models, these preclinical data do not support efficacy of AVE1625 for positive symptoms. Finally, AVE1625 did not reverse amphetamine-induced disrupted gating and thus it is unlikely that such a system would show utility as a clinically relevant biomarker for central CB1 receptor antagonism. However, Hajós et al. (2008) recently demonstrated that a CB1 agonist can disrupt AEP gating in the rat and this gating disruption can be reversed by a CB1 antagonist. Thus, the relevance of CB1 agonist vs. amphetamine disrupted gating for schizophrenia is at the moment unclear.

Despite a lack of efficacy of AVE1625 in classic models of the positive symptoms of schizophrenia, AVE1625 was tested in two rodent models of cognition and results from these studies suggest that AVE1625 could potentially show activity in this domain. Specifically, AVE1625 produced procognitive effects on rats' performance in the hole board and novel object recognition tests. In a model of short-term episodic memory (hole board), AVE1625 reduced the number of errors after a delay suggesting that this drug improved the ability to "hold" information for a short period of time. This finding is supported by the ability of AVE1625 to improve working memory in the radial arm maze (Piot-Grosjean, personal communication). AVE1625 also improved performance in the novel object recognition test, presumably reflecting improved retrieval of long-term memory.

Reversal of NMDA antagonist-induced abnormally persistent LI is believed to reflect potential therapeutic activity for negative symptoms of schizophrenia and

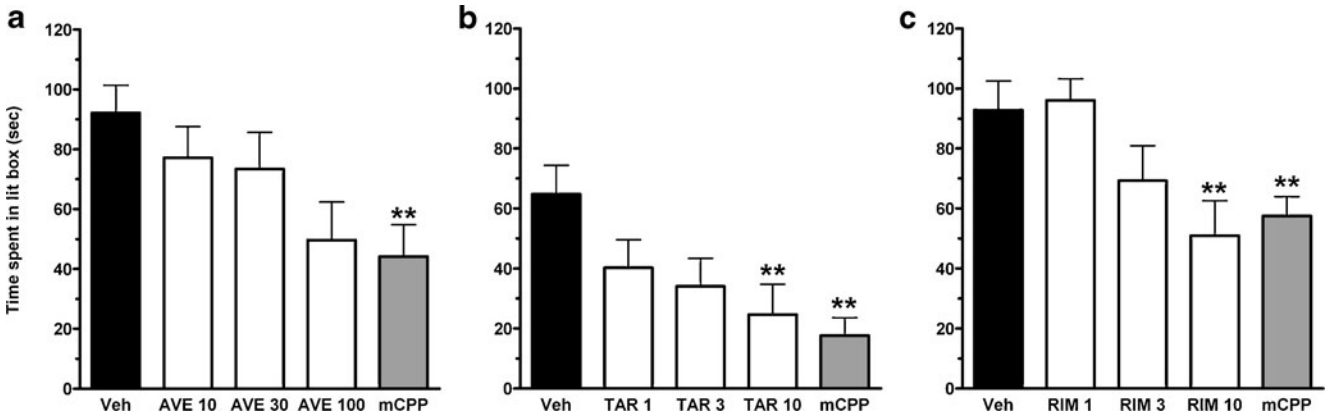

Fig. 6 AVE1625 (**a**) moderately increased anxiety levels in contrast to taranabant (**b**) and rimonabant (**c**). AVE1625 was dosed at 10 (*AVE 10*), 30 (*AVE 30*), and 100 (*AVE 100*) mg/kg. Taranabant (*TAR*) and rimonabant (*RIM*) were dosed 1, 3, and 10 mg/kg. Drugs were administered p.o. in NMRI mice (n = 15–16 per group). M-CPP was dosed at 10 mg/kg. *$p < 0.05$; **$p < 0.01$ vs. vehicle (*Veh*). Each *bar* represents the mean + SEM

cognitive impairments (see Gaisler-Salomon and Weiner 2003; Gaisler-Salomon et al. 2008). In this model, AVE1625 attenuated MK-801-induced persistent LI, therefore further supporting a potential procognitive profile for AVE1625. There is compelling evidence that CB1 receptor antagonists exert procognitive effects in animals (for a review, see Roser et al. 2010). It has been suggested that these effects may involve a modulation of glutamatergic synapses (Shen et al. 1996; Shen and Thayser 1999). CB1 receptor agonists, like NMDA receptor antagonists, may inhibit glutamatergic neurotransmission in brain regions implied in the pathogenesis of the cognitive impairment associated with schizophrenia, such as the hippocampus (Misner and Sullivan 1999; Pistis et al. 2001) or the prefrontal cortex (Auclair et al. 2000). Therefore, the activity of AVE1625 in the MK-801 model of persistent LI may be the consequence of its capacity to increase glutamate levels in brain areas involved in the modulation of cognitive processes such as the hippocampus.

In order to further support the preclinical profile of AVE1625 on acute schizophrenia models, AVE1625 was also tested in a neurodevelopmental model of schizophrenia. Specifically, AVE1625 was tested in the L-NoArg model based on postnatal manipulation of the NO system (Barak et al. 2009; Black et al. 1999, 2002, 2009). In this model, AVE1625 reversed neurodevelopmentally induced abnormally persistent LI. Previous studies using the L-NoArg model have shown that the abnormal behaviors in this model are reversed by procognitive agents and atypical antipsychotics (Barak et al. 2009; Black et al. 2009; Gaisler-Salomon and Weiner 2003; Gaisler-Salomon et al. 2008). Such activity therefore strengthens the case for AVE1625 as a potentially effective treatment in the cognitive domain of schizophrenia. The neurodevelopmental model requires no psychotomimetic challenge; therefore, demonstrating activity in a neurodevelopmental model is suggestive of activity at the neuronal level rather than just interfering with psychotomimetic activity.

Based on all these collective findings from these efficacy studies, one could suggest that AVE1625 may not be suitable as a monotherapy for schizophrenia and instead may need to be co-administered with established APDs. If this is the case, then it is important to ensure that AVE1625 does not compromise the efficacy of commonly used APDs. It is also important to check that administration of AVE1625 does not induce side effects by itself, or potentiate the known side effects of established APDs such as weight gain, hyperprolactinemia, and extrapyramidal symptoms (Casey 1997). Therefore, AVE1625 was combined with select APDs in both efficacy and side effect models. In the efficacy tests, AVE1625 did not alter the ability of APDs to alleviate psychostimulant-induced hyperactivity. We would thus predict that combination therapy of AVE1625 with an APD would not interfere with the antipsychotic action on positive symptoms in patients with schizophrenia. In the side effect models, AVE1625 did not produce any weight gain or stimulation of prolactin release when given alone. The drug was recently shown to reduce body weight in fed rats by affecting primarily lipolysis, which is increased in fat tissues, and to a lesser extent, by reducing caloric intake (Herling et al. 2007). Moreover, our current results confirm that AVE1625 is able to reduce weight gain induced by olanzapine, an effect which was explained by a combination of diminished hyperphagia, maintenance of increased energy expenditure and possibly a counteraction of impaired olanzapine-induced lipolysis (Liebig et al. 2010). While producing no catalepsy when given alone, AVE1625 reduced APD-induced catalepsy. At the moment, the neurochemical mechanism of this latter effect is not clear. It is unlikely that CB1 receptors interfere directly with the activity of dopaminergic neurons, but they can modify dopamine transmission through mechanisms involving inhibitory GABAergic striatal efferent terminals on which CB1 receptors are located (see van der Stelt and Di Marzo 2003, for a thorough review on the endocannabinoid/dopamine interaction), and this effect may explain at least in part the APD-induced catalepsy attenuation of AVE1625. It is interesting to note that stimulation of CB1 receptors has been shown to increase catalepsy produced by administration of dopamine receptor antagonists (Anderson et al. 1996). Together, these findings suggest that AVE1625 may potentially ameliorate some of the APD-induced side effects seen in patients.

Finally, as CB1 antagonists such as rimonabant and taranabant have been shown to induce mechanism-based anxiogenic-like effects in humans (see Moreira et al. 2009 for review), AVE1625 was tested in a mouse model sensitive to the anxiogenic-like effects of these CB1 antagonists. When compared with rimonabant and taranabant, AVE1625 appears to have a reduced potential to produce such effects, suggesting the potential for a therapeutic window between efficacious effects in cognition models and potential mechanism-based side effects. While this is an interesting feature, the mechanism explaining such a difference between CB1 receptor antagonists remains to be elucidated. Clearly, this hypothesis can only be tested conclusively in the clinical setting.

In conclusion, AVE1625, a CB1 antagonist, shows efficacy in models thought predictive for the cognitive aspects of schizophrenia. AVE1625 does not show potential efficacy for positive symptoms of schizophrenia alone, but does not affect the efficacy of APDs when co-administered with these drugs. Indeed co-administration of AVE1625 with APDs revealed a potential benefit with respect to APD-induced side effects such as weight gain and EPS. The effectiveness of AVE1625 compared with other established CB1 receptor antagonists in

terms of dose-effect potency is quite comparable. However, unlike other CB1 antagonists, AVE1625 does not appear to induce any anxiogenic-like effects at the efficacious dose range, at least in mice. It is therefore proposed that combination therapy with AVE1625 and APDs in patients with schizophrenia may improve cognition and reduce APD associated side effects.

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