Pharmacological Properties of ABT-239 [4-(2-[(2R)-2-Methylpyrrolidinyl]ethyl]-benzofuran-5-yl)benzonitrile]: II. Neuropharmacological Characterization and Broad Preclinical Efficacy in Cognition and Schizophrenia of a Potent and Selective Histamine H3 Receptor Antagonist


ABSTRACT

Acute pharmacological blockade of central histamine H3 receptors (H3Rs) enhances arousal/attention in rodents. However, there is little information available for other behavioral domains or for repeated administration using selective compounds. ABT-239 [4-(2-[(2R)-2-methylpyrrolidinyl]ethyl)-benzofuran-5-yl)benzonitrile] exemplifies such a selective, nonimidazole H3R antagonist with high affinity for rat (pKᵢ = 8.9) and human (pKᵢ = 9.5) H3Rs. Acute functional blockade of central H3Rs was demonstrated by blocking the disopropia response to the selective H3R agonist (∗R)-α-methylhistamine in mice. In cognition studies, acquisition of a five-trial, inhibitory avoidance test in rat pups was improved with ABT-239 (0.1–1.0 mg/kg), a 10- to 150-fold gain in potency, with similar efficacy, over previous antagonists such as thioperamide, ciproxifan, A-304121 [(4-((3-(4-(cyclopropylcarbonyl)phenoxy)propyl)-1-piperazinyl)-1-methyl-2-oxoethyl)-2-furamide], and A-349821 [(4′-3-((R,R)2,5-dimethyl-pyrrolidin-1-yl)-propoxy) biphenyl-4-yl)morpholin-4-yl-methanone]. Efficacy in this model was maintained for 3 to 6 h and following repeated dosing with ABT-239. Social memory was also improved in adult (0.01–0.3 mg/kg) and aged (0.3–1.0 mg/kg) rats. In schizophrenia models, ABT-239 improved gating deficits in DBA/2 mice using prepulse inhibition of startle (1.0–3.0 mg/kg) and N40 (1.0–10.0 mg/kg). Furthermore, ABT-239 (1.0 mg/kg) attenuated methamphetamine-induced hyperactivity in mice. In freely moving rat microdialysis studies, ABT-239 enhanced acetylcholine release (0.1–3.0 mg/kg) in adult rat frontal cortex and hippocampus and enhanced dopamine release in frontal cortex (3.0 mg/kg), but not striatum. In summary, broad efficacy was observed with ABT-239 across animal models such that potential clinical efficacy may extend beyond disorders such as ADHD to include Alzheimer’s disease and schizophrenia.

Impaired cognitive function is present across a broad range of neuropsychiatric diseases including attention deficit hyperactivity disorder (ADHD), schizophrenia, Alzheimer’s disease (AD), and mild cognitive impairment, the purported precursor to AD. However, improvement of cognitive function represents a complex challenge since numerous neurotransmitter systems and brain regions are implicated (Myhrer, 2003); this is largely reflected by the significant unmet medical need that currently exists for treating the various cognitive domains affected in many of these diseases (Green et al., 2001; Courtney et al., 2004).

ABBREVIATIONS: ADHD, attention deficit hyperactivity disorder; AD, Alzheimer’s disease; CNS, central nervous system; H3R, H3 receptor; A-304121, 4-[(3-[(4-((2R)-2-aminopropanoyl)-1-piperazinyl)propoxy]phenyl)(cyclopropyl)methanone; A-317920, N-[(1R)-2-[(4-((cyclopropylcarbonyl)phenoxy)propyl)-1-piperazinyl]-1-methyl-2-oxoethyl]-2-furamide; A-349821, 4′-3-((R,R)2,5-dimethyl-pyrrolidin-1-yl)-propoxy)-biphenyl-4-yl)morpholin-4-yl-methanone; ABT-239, 4-[(2-[(2R)-2-methylpyrrolidinyl]ethyl]-benzofuran-5-yl)benzonitrile; (∗R)-α-MeHA, (∗R)-α-methylhistamine; PPI, prepulse inhibition; SHR, spontaneously hypertensive rat; EEG, electroencephalogram; RID, ratio of investigation duration; ANOVA, analysis of variance; PLSD, protected least significant difference; MPH, methylphenidate; MAMPH, methamphetamine.
It is now well established that the monoamine histamine functions as an important neurotransmitter, originating from hypothalamic neurons that project widely throughout the brain to regions that include the cortex, hippocampus, amygdala, and striatum (Brown et al., 2001). In the brain, histamine exerts its activity by interacting with three separate G protein-coupled receptors: \( H_1 \), \( H_2 \), and \( H_3 \). Blockade of \( H_3 \) receptors by antihistamines induces sleepiness and cognitive deficits, the severity of which is correlated with \( H_3 \) receptor occupancy in the human brain (Tashiro et al., 2002). In contrast, increased availability of histamine is known to promote wakefulness, attention, and cognition (Hancock and Fox, 2004).

Release (Arrang et al., 1983) and synthesis (Arrang et al., 1987) of histamine is controlled by presynaptic histamine \( H_3 \) autoreceptors that are located almost exclusively in the CNS, with high densities in the cortex, hippocampus, and striatum (Druet et al., 2001). \( H_3 \)Rs are also found as heteroreceptors and, thus, regulate not only the release of histamine itself but also other neurotransmitters such as dopamine, serotonin, and acetylcholine (Blandina et al., 1996). As a result, the clinical implication of modulating \( H_3 \)Rs in neuropsychiatric diseases is an area receiving significant attention from a number of academic and industrial laboratories (for recent reviews, see Hancock and Fox, 2004; Witkin and Nelson, 2004). Previous studies have demonstrated that pharmacological blockade of \( H_3 \) receptors by early imidazole-based \( H_3 \)R antagonists such as thioperamide, cipriloxan, and cipralisint promotes attention and wakefulness and improves short-term and social memory in rodents (Ligneau et al., 1998; Fox et al., 2002a, 2003; Komater et al., 2003). However, several recently discovered properties of \( H_3 \)Rs have challenged drug discovery efforts to find antagonists suitable for clinical studies. These include: the discovery of multiple splice isoforms that are differentially expressed in the brain (Druet et al., 2001), differential isoform regulation of signaling (Lovenberg et al., 1999; Druet et al., 2001), constitutive activity of cloned and native \( H_3 \)Rs (Morisson et al., 2000), and differential pharmacological activity across species (Leurs et al., 1995). In addition, early imidazole-based antagonists such as those mentioned above, although important tools, also suffer from several drawbacks including potential interactions with cytochrome \( P_{450} \) enzymes (LaBella et al., 1992), limited selectivity versus other drug targets (Leurs et al., 1995; Fox et al., 2003), and complex pharmacology (Wulff et al., 2002).

We recently described the pharmacological properties of A-304121, A-317920 (Fox et al., 2003), and A-349821 (Ebenshade et al., 2004), nonimidazole antagonists at \( H_3 \)Rs that improved cognitive function in models of attention/impulsivity and social memory. Although these compounds were an advance over previous antagonists and proved to be valuable tools, A-304121 and A-317920 exhibited weaker binding affinity for human \( H_3 \)Rs, whereas blood brain barrier penetration in the rat was suboptimal for A-349821 (rat brain and blood levels were 372 ng/g and 677 ng/ml, respectively, 1 h following a 5 mg/kg i.v. dose, giving a brain/blood ratio of 0.54), and some cardiovascular liabilities were subsequently identified with A-349821 (Fryer et al., manuscript in preparation). ABT-239 (Fig. 1) was identified (Cowart et al., 2004) as a nonimidazole antagonist with high affinity and selectivity for both rat and human \( H_3 \)Rs and distinct drug-like properties including good oral bioavailability and excellent blood brain barrier penetration (for detailed in vitro pharmacology and pharmacokinetics, see Esbenshade et al., 2005, companion paper). We now describe in-depth the in vivo neurophysiological and behavioral profile for ABT-239.

**Materials and Methods**

**Chemicals**

ABT-239 (Fig. 1) was synthesized at Abbott Laboratories (Abbott Park, IL); nicotine, amphetamine, methylphenidate, and risperidone were purchased from Sigma-Aldrich (St. Louis, MO), and (R)-\( \alpha \)-MeHA was purchased from Tocris Cookson Inc. (Bristol, UK). Saline (0.9% w/v; Abbott Laboratories) was used as a vehicle, and drug solutions were titrated to approximately pH 6.

**Animals**

**Mice.** Male CD-1 mice were obtained from Charles River Breeding Laboratories (Portage, MI) at approximate postnatal day 70 for diphenegy studies and at 20 to 25 g body weight for general observation and methamphetamine hyperactivity studies. Male DBA/2J mice (16–20 g) for prepulse inhibition (PPI) and N40 studies were obtained from The Jackson Laboratory (Bar Harbor, ME) or Harlan (Indianapolis, IN), respectively; all mice were maintained at Abbott Laboratories facilities for at least 10 days prior to testing. Mice were housed up to 10 per large colony cage (52 cm × 28 cm × 20 cm) in a dedicated quiet room under conditions of 12 h lights on/12 h lights off (on at 6:00 AM), with food and water available ad libitum. Nestlets were provided on cage/bedding change days to reduce territorial fighting. All testing occurred during the light phase.

**Rats.** Male spontaneously hypertensive rat (SHR) pups for repeated acquisition avoidance studies were obtained from Harlan at postnatal day 7 and housed in Abbott Laboratories facilities until use on postnatal days 20 to 24 (body weights ranging from 35–50 g). Pups were housed up to 12 per cage (average of two litters) and fostered with Long-Evans lactating females (two per cage), largely to avoid the poor maternal care of SHR females and possible associated effects on brain and cognitive development (Fox et al., 2002a). Adult (350–450 g) and juvenile (75–100 g) male Sprague-Dawley rats were purchased from Elvage Janvier leGenest Saint-Ile, France.) Aged rat social recognition studies, adult (450–550 g) male Sprague-Dawley rats for EEG studies (6 months old), adult (250–350 g) male Sprague-Dawley rats for general observation studies, and adult Long-Evans rats for water maze studies (300–400 g) were obtained from Charles River Breeding Laboratories, whereas Sprague-Dawley rats for microdialysis studies (350–380 g) were purchased from Elvegage Janvier leGenest Saint-Ile, France). All rats were housed in a quiet room under conditions of 12 h lights on/12 h lights off (at 6:00 AM), with food and water available ad libitum. Rats for EEG studies were housed singly. All testing occurred during the light phase, and all experiments were conducted in accordance with Abbott Animal Care and Use Committee and National Institutes of Health Guide for Care and Use of Laboratory Animals guidelines in facilities accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care.
Functional Activity: Dipsogenia

Mice were tested for blockade of H₃R agonist-induced water intake in a dipsogenia model as previously described (Fox et al., 2002b). Briefly, each mouse was injected i.p. with either saline or ABT-239. After a period of 5 min, saline or (R)-α-MeHA (30 mg/kg) was injected i.p. on the opposite side of the abdomen, and the mice were returned to their respective home cages. After 30 min, mice were individually placed into shoe box cages with access to water at the back of the cage via a modified sipper (Fox et al., 2002b). The mice remained in these cages in a quiet room for a further 30 min, after which they were removed to their home cages once again; the amount of water consumed was then measured from the modified sippers to the nearest 0.01 ml. Sixteen mice were tested at a time for a total of 12 to 24 mice per group (lower numbers in the saline-saline control group). A satellite group of mice was treated as above and euthanized at the end of the study to measure blood and brain concentrations of ABT-239 at efficacious doses (see below for details of pharmacokinetic measurements).

Attention and Cognition Models: Five-Trial Inhibitory Avoidance

Acute Studies: Efficacy. SHR pups were trained in a five-trial, repeated acquisition avoidance response with components of attention and impulsivity as previously described (Fox et al., 2002a). We have previously demonstrated poor acquisition of this model relative to other control strains such as Wistar, Wistar-Kyoto, and Sprague-Dawley (Fox et al., 2002a). Briefly, pups were trained to avoid a mild foot shock (0.1 mA, 1-s duration) delivered upon transfer from a brightly illuminated to a darkened compartment which they normally prefer) of a computer-controlled Gemini inhibitory avoidance system (San Diego Instruments, San Diego, CA). After the first trial, the pup was removed and returned to its home cage and the transfer latency was noted. After approximately 60 s, the same pup was again placed in the brightly illuminated compartment, and the training process was repeated; a total of five trials was conducted in this manner. A criterion time of 60 s applied for the first trial and 180 s for each of the four subsequent trials. ABT-239 or saline vehicle was injected s.c. 30 min prior to the first trial. A V-212 oscilloscope (20 MHz; Hitachi, San Jose, CA) and a 100-KOhm resistor were used frequently to ensure correct calibration of the equipment in producing this relatively mild foot shock. Pups were not habituated to the avoidance apparatus before the first trial to avoid potentially confounding latent inhibitory effects. Each drug treatment was equally represented among each litter (n = 12 per group). Ciproxifan (3.0 mg/kg) was used as a positive control and the experimenter was blinded to treatment.

Acute Studies: Additional Controls. Separate groups of pups (n = 6 per group) were used to control for sensitivity to the foot shock in the five-trial, repeated acquisition avoidance test. In these experiments, pups were treated with saline vehicle or drug as before but were exposed to an inescapable foot shock that was increased gradually from approximately 0.05 to 0.4 mA and back to 0.05 mA over a period of about 30 s. The currents at which vocalization/jumping first occurred (I_max) and then ceased (I_min) were noted. To control for any potential nonspecific procedural effects in the five-trial, repeated acquisition avoidance test (e.g., noise of the door closing, repeated handling, and testing in the presence of saline vehicle or drug), pups were treated with saline or ABT-239 as for the regular acute efficacy test, but no foot shock was delivered. Transfer latencies were recorded as before.

Repeated Administration Studies. To determine whether repeated administration of ABT-239 would produce tolerance or extinction-like effects in the five-trial, repeated acquisition avoidance model, separate groups of SHR pups were administered s.c. 1.0 mg/kg ABT-239 or saline vehicle over 5 days according to the following schedule. Group 1 received saline for 4 days from postnatal days 20 to 23 and were challenged with saline 30 min prior to testing on postnatal day 24; group 2 received saline for 4 days from postnatal days 20 to 23 and were challenged with ABT-239 30 min prior to testing on P24; group 3 received ABT-239 for 4 days from postnatal days 20 to 23 and were challenged with ABT-239 30 min prior to testing on P24. Pups were weighed prior to dosing on each day.

Duration of Action Studies. To determine the duration of action of ABT-239 in the five-trial, repeated acquisition avoidance model, ABT-239 (0.1 mg/kg s.c.) was given to SHR pups at varying time points prior to testing. These included 0.5 (the standard administration time), 1.5, 3, and 6 h. Separate groups of saline-treated controls were included for each point. Methylphenidate (3.0 mg/kg), used in the clinic to treat ADHD, was given s.c. over the same time course for a reference comparison, whereas ciproxifan (3.0 mg/kg) was given s.c. at 0.5 h as a positive control for the overall study. Group sizes were n = 11 to 12, and all pups were tested for efficacy of the various treatments using the same test procedure as described above for acute studies.

Social Recognition

Adult Social Memory. Adult rats were trained in a social recognition memory test to recall prior exposure to a conspecific juvenile as previously described (Fox et al., 2003). Briefly, adult rats (350–450 g) were separated into fresh test cages and allowed to habituate for 30 min. An unfamiliar juvenile was introduced, and overall investigation duration (grooming, sniffing, and close following) was recorded for a 5-min period. Both rats were then removed to their respective holding cages. After 90 min, the adult was replaced into the test cage, the same juvenile reintroduced 30 min later, and overall investigation duration was again recorded during a second 5-min period. ABT-239 or saline vehicle was administered i.p. to the adult rat immediately after the first exposure period. To control for nonspecific effects, immediately after the second investigation period, a new, unfamiliar juvenile was introduced to the same adult rat for a third 5-min period, and overall investigation duration was recorded once again. Social memory was quantitated by determining, for each adult rat, the ratio of investigation duration (RID) of the second to the first investigation periods. Nonspecific effects were assessed by determining the RID (third to the first investigation periods) for the unfamiliar juvenile. Group sizes were n = 15 to 16.

Aged Social Memory. Aged rats were trained in a social memory test to recall prior exposure to a conspecific juvenile as described above for adult rats, but with the following changes: two 5-min investigation periods were separated by only 30 min instead of the 120 min for adult rats; ABT-239 or vehicle (0.2% hydroxypropyl-methylcellulose in distilled water) was given p.o. to the aged rat 60 min prior to the first exposure to the juvenile. An additional group of adult rats, administered vehicle as above, was also included as a control. Group sizes were n = 11 to 12 (one rat for the two lower treatment groups and one rat in the adult control group were excluded from data analysis because ratios of investigation durations for these points were outliers according to Grub’s test). Third control contact periods with unfamiliar juveniles were not assessed in aged rat studies. This study was conducted at Porsolt and Partners, Pharmacology in accordance with French legislation concerning the protection of laboratory animals and in accordance with a currently valid license for experiments on vertebrate animals.

Two-Choice Discrimination Water Maze. Adult male Long-Evans rats (Charles River Breeding Laboratories) were trained in a two-choice, visual discrimination water maze test of spatial learning and reference memory as previously described (Decker et al., 1997). This version of the water maze is known to be sensitive to age, septal lesions, and scopolamine-induced impairments and is proposed as a model that may be pertinent to cognitive deficits associated with Alzheimer’s disease. Following an initial 2-day pretraining exposure of the rats to the water maze (2-m diameter, water depth 40 cm, water temperature 24 ± 1°C) with only one stable, 10-cm diameter visible platform (designed to acclimate the rats to the water and basic procedure), rats were allowed to rest for a further 2 days before commencing 5 additional days of testing with two visible platforms of identical dimensions. Over three pairs of trials (three start locations,
rat placed immediately back into the tank on the second of each trial pair; approximately 6 min between each trial pair), rats were trained to discriminate a visible stable escape platform from an otherwise identical, visible floating platform. Escape from the water was only possible on the stable platform. The anticholinergic amnestic agent scopolamine (0.3 mg/kg) was administered i.p. 15 min before each test session on each of the 5 test days to disrupt performance and ABT-239 (1.0, 3.0 mg/kg), or vehicle was given i.p. 45 min prior to scopolamine on each test day. Spatial cognition in this task was quantified by averaging the number of errors to escape onto the correct platform across treatment for each day.

Gating and Schizophrenia-Relevant Models

PP1 of Startle. Male DBA/2 mice were tested separately in eight Hamilton Kinder startle chambers (SM100 version 4.1, Poway, CA). Each chamber contained a Plexiglas rectangle with an adjustable ceiling housed in a ventilated, sound-attenuated cubicule. The ceiling was adjusted on an individual basis to allow for adequate headroom but no rearing or extensive locomotion. The chamber was placed over an anchor plate attached to a piezoelectric disk to transduce startle responses to a computer for storage and analysis. A loudspeaker located in each chamber delivered the background noise (65 dB) and the acoustic stimuli. In addition, a constant white noise (65 dB) was maintained in the experimental room for the duration of each experiment by a white noise generator (Lafayette Instrument Co., Lafayette, IN). Mice were individually placed in the startle chamber and left undisturbed for 5 min (total session duration was approximately 22 min). Following this acclimation period, four successive 120-dB, 40-ms trials were presented. These trials were not included in analyses. Mice were then presented with five different trial types: startle pulse (120 dB for 40 ms), no stimulus, or prepulse stimulus of one of three sound levels (70, 75, or 80 dB) for 20 ms, followed 100 ms later by an acoustic startle (120 dB for 40 ms). A total of 12 trials under each condition was delivered in a random sequence, and all trials were separated by a variable intertrial interval of 5 to 25 s. Finally, this sequence ended with the presentation of four 120-dB, 40-ms trials (not included in the analysis). Three prepulse levels were selected based on the observation that this procedure maximized the ability to detect changes in PP1. In the startle-alone trials, the basic auditory startle (startle response) was measured, excluding the first and last blocks of four pulse-alone trials presented. In the prepulse plus startle trials, PP1 was calculated as a percentage score for each acoustic prepulse trial type using the formula: 100 − [(startle response for prepulse + pulse)/(startle response for pulse alone)] × 100. For all analyses, the maximum amplitude was used. Data from the no-stimulus trials are not included in the results section because the values were negligible relative to values on trials containing startle stimuli.

N40 Sensory Gating. Male DBA/2 mice were stereotaxically implanted with tripolar stainless steel wire electrode head stages (Plastics One, Inc., Roanoke, VA) for EEG recordings in the CA3 region of the hippocampus. The mice were first anesthetized with a solution of 2.8% ketamine, 0.28% xylazine, and 0.05% acepromazine (Sigma-Aldrich). Three access holes for the electrodes were made (no. 44x261) to convey EEG biosignals to differential AC EEG amplifiers (Grass Instrument Division, Astro-Med, West Warwick, RI) and allowed the mice free movement within the chambers. The EEG was amplified 1000× with a 50- to 60-Hz notch filter engaged, and high- and low-pass filters were set at 1 and 100 Hz, respectively. Hippocampal auditory evoked potentials were generated by presentation of 60 sets of 3 KHz paired tone bursts from a speaker within the recording chamber at a distance of 15 to 20 cm from the mouse. The first tone of the pair is referred to as the conditioning stimulus, and the second is referred to as the test stimulus. The durations of both the condition and test stimuli were 5 ms, with 0.5 s between stimuli and 20 s between pairs. Data acquisition software (Neuroscan, Inc., El Paso, TX) recorded EEG signals 100 ms before and for 899 ms after the initial conditioning stimulus. The software averaged the 60 paired responses into one composite-evoked response. ABT-239 (1.0, 3.0, and 10.0 mg/kg i.p.) was administered 20 to 30 min before mice were placed into the recording chambers and initiation of auditory-evoked potential recording. Nicotine, as a positive control, was administered at 0.1 and 1.0 mg/kg i.p. (free base) 5 to 7 min before recordings began. Recording of paired auditory evoked potentials continued for two 20-min sessions, each comprised of 60 paired stimuli. Each mouse was administered every treatment dose and a control vehicle treatment in a balanced order on separate days with at least 48 h between treatments. This within-subjects design allowed each mouse to serve as its own control. The hippocampal response to auditory stimuli was identified as the highest positive peak deflection in the ongoing EEG at a latency of 10 to 20 ms after the stimulus (P20), followed by lowest negative peak deflection in the ongoing EEG at 20 to 45 ms after the stimulus (N40). The difference in amplitude between P20 and N40 was calculated as the N40 amplitude in microvolts. N40 amplitudes were determined for both the averaged conditioning and test-evoked potentials, and a ratio was derived between the two responses by dividing the test amplitude by the conditioning amplitude (T/C ratio).

Methamphetamine Hyperactivity. Male CD-1 mice were injected i.p. with drug (ABT-239, 0.1, 0.3, and 1.0 mg/kg i.p.; reserpine, 0.01, 0.03, 0.1, and 0.3 mg/kg i.p.) or saline vehicle (n = 16 per group) and placed separately into one of 16 acrylic open-field environments (42 length × 42 base × 40 cm (height); Piper Plastics, Libertyville, IL) situated inside Versamax/Digiscan monitors, each equipped with 32 horizontal and 16 vertical infrared sensors (AccuScan Instruments, Inc., Columbus, OH) in a dimly illuminated test room. The mice were allowed to habituate while baseline activity data were recorded for a period of 60 min at which point they were injected with methamphetamine (1.0 mg/kg i.p; Sigma-Aldrich) and monitored for a further 90 min. Distance traveled (centimeters) was automatically recorded by computer at 1-min intervals and analyzed in 5-min bins.

Neurophysiology

Microdialysis. Pentobarbital (Narcoren; Rhone-Merieux, Lyon, France)-anesthetized (60 mg/kg i.p.) rats were mounted in a Kopf stereotactic frame and implanted with one microdialysis guide cannula (CMA/12; Axel Semrau GmbH, Essen, Germany) into the medial prefrontal cortex (AP, 2.5; ML, 0.6; DV, −0.2) and a second cannula into the hippocampus (AP, −5.5; ML, −4.5; DV, −4.5) or striatum (AP, 1.3; ML, 2.2; DV, −3.0) for a total of two cannulae per rat. Guide cannulas were secured with dental cement (Technovit 2060; Kulzer GmbH, Dormagen, Germany) and four anchor screws into to the skull. Thereafter, a CMA/12 microdialysis probe (3-mm membrane length) was slowly lowered into final position. The probe was perfused with Ringer solution (CMA/102 microdialysis pump, 1.5 μl/min) containing 147 mM NaCl, 4.0 mM KCl, and 2.4 mM CaCl2, for about 1 h. In experiments aimed at determination of microdialysate acetylcholine, 1 μM of the acetylcholinesterase inhibitor, neostigmine, was added to the Ringer solution. After surgery, each animal was transferred into a freely moving animal system (CMA/120 consisting of a plastic bowl, wire attachment, counterbalance arm, and swivel assembly connecting inlet/outlet of the probe...
with the perfusion pump). After 24 h, the probe was perfused for at least 1 h before microdialysis fractions were collected every 20 min. Six fractions before and 69 fractions after i.p. drug administration were analyzed for microdialysis levels of acetylcholine and choline or dopamine by high-performance liquid chromatography with electrochemical detection. For acetylcholine analysis, 10 μl of each dialyzed fraction was injected onto a reversed phase column (MF-8908 Acetylcine SepStik Kit; microbore column, particle size 10 μ, 530 × 1.0 mm coupled to immobilized enzyme reacter 50 × 1.0 mm, particle size 10 μ, containing acetylcholinesterase and choline oxidase; BAS, Lafayette, IN) using a refrigerated autosampler (CMA/200; Axel Semrau GmbH). The mobile phase consisted of 50 mM Na₂HPO₄, pH 8.5, and 5.5 ml KATHON preservative (Roehm and Haas, GmbH, Arnsberg, Germany). With a flow rate of the mobile phase of 0.13 ml/min (CMA/250 pump), the sample run time was less than 15 min. Acetylcholine and choline were detected by an electrochemical detector (LC-4C; BAS) with a platinum working electrode set at +500 mV versus an Ag/AgCl reference electrode. The system was calibrated by standard solutions (acetylcholine and choline) containing 1 pmol/10 μl injection. For dopamine analysis, 10 μl of each dialyzed fraction was injected onto a reverse-phase column (Nucleosil C18, particle size 3 μ, 150 × 4.0 mm; Macherey-Nagel GmbH, Duren, Germany) using a refrigerated autosampler. The mobile phase consisted of 150 mM Na₂HPO₄, pH 3.7, 1 mM Na₂EDTA, 0.23 mM sodium octylsulfate, and 4% isopropanol. With a flow rate of the mobile phase of 1.0 ml/min (CMA/250 pump), the sample run time was less than 12 min. Dopamine was detected by an electrochemical detector (LC-4C; BAS) with a glassy carbon working electrode set at +750 mV versus an Ag/AgCl reference electrode.

**Electroencephalogram Slow-Wave Activity.** EEG recording electrodes were bilaterally implanted under pentobarbital anesthesia (50 mg/kg i.p.; Abbott Laboratories) over the parietal cortex (−2.0 mm AP, 4.0 mm L). A reference electrode was placed 11 mm posterior to bregma, and a miniature connector was affixed to the skull. Implanted rats were allowed 2 weeks of recovery from the surgery before use. EEG (sampling rate 200 Hz) was recorded from previously habituated rats inside sound-attenuating chambers. Before experiments began, a flexible cable was attached to the implanted miniature connector that allowed the rats unrestricted movement during the recording sessions. Standard EEG amplifiers (Grass Instrument Division, Astro-Med) and a computer-based system (Stelate Systems, Montreal, Canada) were used to acquire and analyze data. The average EEG amplitude in microvolts was determined using fast Fourier transform analysis and was broken down into an analysis of the 1- to 4-Hz slow-wave band activity. Dose response effects on EEG were determined for i.p. administration of ABT-239 (0.1, 0.3, 1.0, and 3.0 mg/kg) and compared with saline vehicle. The treatments were administered in a random order on different days with one treatment per day and 3 days between each treatment. On one of these treatment days, the rat would receive a vehicle control treatment. This within-subjects design allowed each rat to serve as its own control. EEG recordings were begun within 5 min after injection, and recording sessions lasted for 360 min. A total of eight rats were used in these studies.

**ABT-239 Exposure Levels and Pharmacokinetic Analysis.** To evaluate blood, plasma, and brain exposure levels of ABT-239 at relevant time points in the five-trial, repeated acquisition avoidance test, pups treated with drug from the foot shock control studies and from the repeated administration studies were terminally anesthetized with 60 mg/kg Nembutal (Abbott Laboratories). Similarly, samples were obtained from adult rats immediately after social recognition testing. Blood samples were collected via cardiac puncture, and brains were then immediately removed and snap frozen on dry ice for later analysis. Timing of sampling was approximately 40 min after injection, corresponding to a time during which pups would have just completed acquisition of the test. Samples were analyzed in our Pharmacokinetic Analysis Department. Briefly, ABT-239 was selectively removed from the blood or brain homogenate using liquid-liquid extraction with a mixture of ethyl acetate and hexane (1:1, v/v) at basic pH. The samples were vortexed vigorously followed by centrifugation. The organic layer was transferred and evaporated to dryness with a gentle stream of nitrogen over low heat (−35°C). The samples were reconstituted by vortexing with mobile phase. ABT-239 and internal standard were separated from coextracted contaminants on a 5-cm × 3-mm × 3-μm Aquasil C18 column with an acetonitrile/0.1% trifluoroacetic acid (60:40, by volume) mobile phase at a flow rate of 0.35 ml/min with a 25-μl injection. ABT-239 was quantified using MRM detection, m/z 331.3 to >98.0 using a turbo spray source on a mass spectrometer (PerkinElmerSciex Instruments, Boston, MA). The limits of quantitation were approximately 50 pg/ml for blood and 750 pg/g for brain samples.

**CNS Safety: General Observation Test**

Adult mice were separated into groups of three and placed into observation cages (23 × 21 × 20 cm). Baseline core (rectal) body temperature was recorded with a rapid read digital thermometer (Model BAT-12; Physitemp Instruments Inc., Clifton, NJ). In separate experiments, mice were then injected with vehicle, ABT-239 (3, 9, 28, 45, and 90 mg/kg i.p.; 3, 9, 27, 45, and 90 mg/kg s.c.; 3, 9, 28, 45, 90, and 180 mg/kg p.o.). All mice were continuously observed for adverse behavior such as general changes in activity levels, pilorec- tion, ptosis, tremor, and seizure activity (including Straub tail, wild running, clonus, and瞳us) for the 1st h and then intermittently at 2, 3, 6, and 24 h following drug administration. All subjective observations (e.g., activity) in drug-treated mice were made with constant reference to a cage of vehicle-treated control mice. Body temperature was recorded 0.25, 0.5, 1, 2, 3, 6, and 24 h following drug administration, and a decrease of 2°C or more was considered hypothermia. In a similar experiment, adult rats were treated with ABT-239 (1, 3, 9, and 28 mg/kg i.p.) and observed as described above for mice.

**Data Analysis**

Nonparametric Kruskal-Wallis and individual Mann-Whitney U tests were used to compare performance in the repeated acquisition avoidance test. Dipsogenia, spontaneous locomotor activity, foot shock sensitivity, social memory, and microdialysis (area under curve 0–120 min) data were assessed for significance using one-way analysis of variance (ANOVA) followed by Tukey’s pair-wise comparison post hoc tests. PPI gating data were analyzed using one-way ANOVAs (for data collapsed across prepulse intensities) and two-way ANOVAs (for individual prepulse and treatment interactions) followed by Fisher’s PLSD post hoc analyses. One-way ANOVAs followed by Fisher’s PLSD post hoc tests were used to analyze two-choice water maze and methamphetamine-induced hyperlocomotion (area under curve 60–150 min) data. For N40 gating studies, a within-subjects design was employed so that each animal served as its own control; data were analyzed using one-way ANOVAs followed by Fisher’s PLSD post hoc analyses. A repeated-measures one-way ANOVA was used for statistical evaluation of fast Fourier transform data, with treatment as the repeated measure, and Fisher’s post hoc tests for comparisons between treatment groups. p < 0.05 was considered significant for all tests. All analyses were performed using Statview 5.0 or JMP 5.01 for Windows (both from SAS Institute, Cary, NC).

**Results**

**Functional Activity: Dipsogenia Model**

(R)-α-MeHA (30 mg/kg) produced a large dipsogenia response in vehicle-treated mice (ANOVA, F(10,178) = 7.883, p < 0.0001; Fig. 2). ABT-239 attenuated the dipsogenia response in a dose-dependent manner, reaching significance at 0.3 (1.1 ± 0.1 mg blood level, 92.9 ± 7.7 ng/g brain level) and 1.0 (4.7 ± 0.4 mg/ml blood level, 403.7 ± 44.1 ng/g brain level) mg/kg. By itself, ABT-239 (1.0 mg/kg) had no effect on
water intake (0.03 ± 0.02 ml for ABT-239 versus 0.03 ± 0.01 ml for saline alone). Ciproxifan (1.0 mg/kg), included as a positive control, was also fully efficacious in reversing (R)-α-MeHA-induced dipsogenia (data not shown), as previously described (Fox et al., 2002b).

**Attention and Cognition Models**

**Five-Trial Inhibitory Avoidance: Acute Efficacy.** ABT-239 at doses as low as 0.1 mg/kg robustly enhanced performance in the five-trial, repeated acquisition avoidance model in SHR pups as evidenced by the increased cumulative transfer latencies relative to vehicle-treated controls (Fig. 3A). A Kruskal-Wallis analysis performed across trials 2 through 5, considered the learning trials (Fox et al., 2002) revealed a significant treatment effect ($H = 12.690; p = 0.0129$). Planned, individual Mann-Whitney comparisons revealed significant ($p < 0.05$) differences between SHR pups treated with ABT-239 (0.1–1.0 mg/kg) and those treated with vehicle (Fig. 3B). These effects were specific to enhanced learning since ABT-239 had no significant effect [ANOVA, $F(4,54) = 1.595, p = 0.1890$] on foot shock sensitivity threshold (Fig. 4A) or on transfer latencies (Kruskal-Wallis, $H = 3.141; p = 0.6782$) at any dose when administered in the absence of foot shock (Fig. 4B). Plasma and brain exposure levels for acute dosing of ABT-239 in this model are detailed in Table 1. Of note is that 4.7 ng/ml ABT-239 in the plasma (97.7 ng/g in brain) produced full efficacy with regard to improvement over vehicle-treated controls.

**Five-Trial Inhibitory Avoidance: Repeated Dosing.** Repeated treatment with ABT-239 at the relatively high dose of 1.0 mg/kg for 5 days continued to robustly improve performance in SHR pups, as evidenced by the increased cumulative transfer latencies relative to vehicle-treated controls.
were indistinguishable for latencies for trial 1, indicating no adverse effects on extinction. A Kruskal-Wallis analysis performed across trials 2 through 5 revealed a significant treatment effect ($H = 37.648; p = 0.0002$) across administration times and groups. Planned, individual Mann-Whitney comparisons revealed significant ($p < 0.05$) differences between SHR pups treated with a low dose of ABT-239 (0.1 mg/kg) and those treated with vehicle 0.5, 1.5, and 3.0 h prior to testing (Fig. 6). SHR pups receiving the internal standard control, ciprofloxin (3.0 mg/kg), exhibited significant ($p < 0.05$) improvement at 0.5 h, whereas methylphenidate (1.0 mg/kg) was effective at 1.5 h only (a strong trend, $p < 0.06$, was observed for methylphenidate at 0.5 h) (Fig. 6).

**Social Memory: Adult Rats.** In a social recognition model (Fig. 7A), a significant effect of treatment was observed with a one-way ANOVA [$F(5,87) = 3.365$, $p = 0.008$]. Post hoc Tukey’s pair-wise comparisons revealed that vehicle-treated adult rats did not remember ($p > 0.05$) the juveniles from the first investigation period when allowed to investigate the same juveniles after a 120-min delay, as evidenced by the increased duration of investigatory behavior during the second investigation period and the corresponding RID values of around 1.0 or higher (Fig. 7A). In contrast, ABT-239 (0.01–0.1 mg/kg) significantly enhanced social memory ($p < 0.05$, Tukey’s post hoc) (Fig. 7A) when administered i.p. just after the first exposure period. This effect was specific for enhanced social memory since ABT-239 did not significantly affect [$F(5,88) = 0.645$, $p = 0.666$] normal investigational behavior when the same adult rats were subsequently exposed to separate novel juveniles (data not shown). Blood and brain exposure levels for ABT-239 in this model are shown in Table 2.

**Social Memory: Aged Rats.** When aged rats were evaluated in a social recognition model (Fig. 7B), a significant effect of treatment was observed with a one-way ANOVA [$F(4,52) = 3.355$, $p = 0.0166$]. Post hoc Tukey’s pair-wise comparisons revealed that vehicle-treated aged rats did not remember ($p > 0.05$) the juveniles from the first investigation period when allowed to investigate the same juveniles after a 30-min delay, as can be seen by the increased duration of investigatory behavior during the second investigation period and the corresponding RID values of around 1.0 or higher. This is in contrast to vehicle-treated adult control rats, which showed a significant improvement in recall compared with the vehicle-treated aged rats (Fig. 7B). ABT-239 significantly improved recall in aged rats at 30 min when administered at 0.3 and 1.0 mg/kg p.o. 60 min prior to the first exposure period (Fig. 7B), comparable with the level of adult rats after only 30 min. Blood exposure levels for ABT-239 in this model are shown in Table 2.

**Spatial Memory.** In a two-choice, spatial memory task in which rats were trained to discriminate a stable from an unstable visible escape platform over a period of 5 days, a significant effect of treatment was observed with individual

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**TABLE 1**

<table>
<thead>
<tr>
<th>Dose ABT-239</th>
<th>Plasma</th>
<th>Brain</th>
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<tbody>
<tr>
<td></td>
<td>ng/ml</td>
<td>ng/g</td>
</tr>
<tr>
<td>0.03 mg/kg s.c.</td>
<td>1.2 ± 0.1</td>
<td>28.5 ± 1.5</td>
</tr>
<tr>
<td>0.1 mg/kg s.c.</td>
<td>4.7 ± 0.4</td>
<td>97.7 ± 7.2</td>
</tr>
<tr>
<td>0.3 mg/kg s.c.</td>
<td>20.8 ± 4.2</td>
<td>338.8 ± 29.7</td>
</tr>
<tr>
<td>1.0 mg/kg s.c.</td>
<td>56.2 ± 2.6</td>
<td>1374.2 ± 119.7</td>
</tr>
</tbody>
</table>

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mg/kg) on the 5th day ($38.5 ± 11.6$ ng/g brain level 24 h after last dose) or SHR pups treated with ABT-239 (1.0 mg/kg) for all 5 days ($55.7 ± 9.2$ ng/g brain level 24 h after last dose) and those treated with vehicle for 5 days (Fig. 5D).

**Repeated Acquisition Avoidance Response: Duration of Action.** To determine duration of action and to compare directly over the same time course with methylphenidate (MHP) in this model, ABT-239 and MHP were assessed for efficacy at several time points after dosing (Fig. 6). A Kruskal-Wallis analysis performed across trials 2 through 5 revealed a significant treatment effect ($H = 37.648; p = 0.0002$) across administration times and groups. Planned, individual Mann-Whitney comparisons revealed significant ($p < 0.05$) differences between SHR pups treated with a low dose of ABT-239 (0.1 mg/kg) and those treated with vehicle 0.5, 1.5, and 3.0 h prior to testing (Fig. 6). SHR pups receiving the internal standard control, ciprofloxin (3.0 mg/kg), exhibited significant ($p < 0.05$) improvement at 0.5 h, whereas methylphenidate (1.0 mg/kg) was effective at 1.5 h only (a strong trend, $p < 0.06$, was observed for methylphenidate at 0.5 h) (Fig. 6).

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**Fig. 4.** Additional control studies for five-trial, repeated acquisition, inhibitory avoidance response in SHR pups with ABT-239. Foot shock thresholds of SHR pups examined in the same apparatus were not affected by ABT-239 (A), indicating effects in Fig. 3, A and B are specific to learning. Rat pups were dosed with antagonist 30 min before inescapable exposure to increasing currents ($I_{max}$) and subsequent vocalization, followed by decreasing current ($I_{min}$) and subsequent cessation of vocalization. Data are expressed as mean ± S.E.M. Similarly, ABT-239 did not affect transfer latencies in the absence of foot shock (B). Here, rat pups were dosed as for efficacy studies, but the precision shocker was turned off. Data are expressed as mean ± S.E.M. No statistically significant effects of treatment were observed.
ANOVAs on days 2 \( F(3,29) = 3.073, p = 0.0433 \), 3 \( F(3,29) = 8.442, p = 0.0003 \), 4 \( F(3,29) = 6.579, p = 0.0016 \), and 5 \( F(3,29) = 8.746, p = 0.0003 \). Post hoc Fisher’s analyses revealed that rats receiving vehicle + scopolamine (0.3 mg/kg) on these days exhibited a significant impairment \( (p < 0.05) \) relative to rats administered vehicle + vehicle (Fig. 8A).

Fig. 5. Effect of repeated dosing of vehicle or ABT-239 at 1.0 mg/kg on efficacy in five-trial, repeated acquisition, inhibitory avoidance response in SHR pups. SHR pups were treated with vehicle for 4 days and vehicle again on day 5 (Veh/Veh), vehicle for 4 days and ABT-239 on day 5 (Veh/ABT), or ABT-239 for all 5 days (ABT/ABT). Pups were tested 30 min after this last dose on day 5 (A, data for all trials; B, cumulative transfer latencies days 2–5). SHR pups from these same groups were tested again on day 6 in the absence of drug administration (C, data for all trials; D, cumulative transfer latencies trials 2–5). Data are expressed as mean ± S.E.M. for clarity. Statistical calculations used nonparametric analyses. *, significant \( (p < 0.05) \) effect with respect to vehicle-treated controls.

Fig. 6. Duration of action of ABT-239 at 0.1 mg/kg versus MPH at 1.0 mg/kg in the five-trial, repeated acquisition, inhibitory avoidance response in SHR pups. ABT-239 or MPH was dosed at 0.5, 1.5, 3, or 6 h prior to testing. Ciproxifan (CPX; 3.0 mg/kg) was given to separate pups 0.5 h prior to testing as an internal positive control for the overall experiment. Data are expressed as mean ± S.E.M. for clarity. Statistical calculations used nonparametric analyses. *, significant \( (p < 0.05) \) effect with respect to vehicle-treated controls at the same time point.
However, rats treated with 3.0 mg/kg ABT-239 + scopolamine, although exhibiting some impairment \((p < 0.05)\) on days 3 to 5, also demonstrated significantly improved \((p < 0.05)\) task acquisition on days 3 to 5 relative to vehicle + scopolamine-treated rats; Fig. 8A). When data were collapsed over test days 2 to 5, a significant effect of treatment \(F(3,29) = 12.420, p < 0.0001\) was observed. Rats treated with ABT-239 at 3.0 mg/kg again demonstrated a significant \((p < 0.05, \text{Fisher's post hoc})\) improvement in task acquisition over rats receiving vehicle + scopolamine, although a full reversal was not attained (Fig. 8B). These data demonstrate at least a partial reversal of a scopolamine-induced deficit and, together with efficacy data from five-trial inhibitory avoidance and social recognition, suggest efficacy across a broad range of cognitive domains.

**Gating and Schizophrenia-Relevant Models**

**Prepulse Inhibition of Startle.** ABT-239 (1.0–3.0 mg/kg i.p.) significantly improved PPI in a dose-related manner (Fig. 9A), resulting in significant effects of treatment...
F(3,60) = 6.350, p = 0.0008], prepulse level [F(2,120) = 102.104, p < 0.0001], and prepulse × treatment interaction [F(6,120) = 2.651, p = 0.0189]. Planned Fisher’s post hoc analyses revealed a significant (p < 0.05) improvement in prepulse inhibition versus vehicle for 3.0 mg/kg ABT-239 at 70 and 75 dB and at 70 dB for 1.0 mg/kg. The maximal effect obtained at a dose of 3.0 mg/kg was equivalent to that observed for risperidone (1.0 mg/kg), which was also significantly different (p < 0.05) from vehicle controls (Fig. 9A).

When data were collapsed across prepulse intensities, significant improvements (p < 0.05) in percentage of PPI were observed at 3.0 mg/kg for ABT-239 and at 1.0 mg/kg for risperidone compared with vehicle controls (Fig. 9B). Interestingly, a significant treatment effect on startle alone [F(3,60) = 6.233, p = 0.0009] was revealed to be a result of a significant depression (p < 0.05) of baseline startle amplitude by risperidone at 1.0 mg/kg (Fig. 9C). In contrast, ABT-239 did not alter baseline startle at any dose tested (Fig. 9C), indicative of a lack of sedative-like adverse effects.

N40 Gating. ABT-239 also significantly enhanced N40 gating in DBA/2 mice (Fig. 9D). A one-way ANOVA revealed a significant treatment effect [F(3,21) = 4.070, p = 0.02], and post hoc Fisher’s analyses indicated significant effects (p < 0.05) at 1.0, 3.0, and 10.0 mg/kg i.p. compared with vehicle treatments (Fig. 9D). Efficacy with ABT-239 approached that of nicotine, which was tested as a positive comparator control and produced a significant treatment effect [F(2,23) = 8.901, p = 0.0014], with post hoc Fisher’s analyses revealing significant improvement (p < 0.05) over vehicle at 1 mg/kg (Fig. 9D). Together with PPI, these data suggest that selective blockade of H₃ receptors could enhance sensory gating in schizophrenia patients.

Methamphetamine Hyperactivity. Methamphetamine causes a long-lasting hyperactivity in rodents that can be
blocked by clinically useful antipsychotic drugs. ABT-239 (0.1–1.0 mg/kg i.p.) was tested for its effects on methamphetamine-induced hyperactivity in mice. A significant effect of treatment was revealed with a one-way ANOVA for AUCs for 60 to 150 min \( F(4,75) = 6.542, p < 0.0001 \), and Fisher’s post hoc analyses revealed a significant \( (p < 0.05) \) increase in locomotor activity for methamphetamine-treated mice compared with vehicle-treated mice (Fig. 10A). ABT-239 attenuated methamphetamine-induced hyperactivity in a dose-related manner, reaching significance at 1.0 mg/kg \( (p < 0.05, \text{Fisher’s post hoc}) \). A significant effect of treatment \( F(5,58) = 4.349, p = 0.002 \) was also seen in a second study to evaluate the effect of risperidone on methamphetamine-induced hyperactivity. Risperidone (0.3 mg/kg) significantly \( (p < 0.05) \) attenuated methamphetamine-induced hyperactivity but also significantly \( (p < 0.05) \) decreased basal activity levels over the 60 min (statistical analyses performed on AUCs 0–60 min) prior to administration of methamphetamine (Fig. 10B). This is in contrast to ABT-239, which did not significantly \( (p > 0.05) \) affect activity levels at any dose prior to methamphetamine administration (Fig. 10A). These data indicate that ABT-239 may have activity for treating positive-like symptoms in schizophrenia, without the sedative liabilities of current antipsychotic drugs.

**Neurophysiology**

**Microdialysis.** Using microdialysis in conscious, unrestrained rats, a one-way ANOVA for data collapsed across 0 to 120 min revealed a significant treatment effect for acetylcholine concentration in the prefrontal cortex (Fig. 11A) \( F(4,35) = 9.031, p < 0.0001 \) and hippocampus (Fig. 11B) \( F(4,35) = 8.004, p = 0.0001 \). Subsequent Tukey’s pair-wise comparisons revealed significant increases in acetylcholine concentration compared with vehicle-treated animals in both the medial prefrontal cortex \( (p < 0.05, \text{Fig. 11A}) \) and the hippocampus \( (p < 0.05, \text{Fig. 11B}) \) following administration of 1.0 and 3.0 mg/kg i.p. ABT-239 or 3.0 mg/kg i.p. ABT-239, respectively. In a second microdialysis study, a one-way ANOVA for data collapsed across 0 to 120 min revealed a significant treatment effect for dopamine concentration in the prefrontal cortex (Fig. 11C) \( F(3,26) = 4.560, p = 0.0107 \) but, notably, not in the striatum (Fig. 11D) \( F(3,26) = 1.451, p = 0.2509 \). Subsequent Tukey’s pair-wise comparisons revealed a significant increase in dopamine concentration com-

![Fig. 10. Dose-related attenuation of methamphetamine (MAMPH)-induced hyperactivity in mice with ABT-239 (A) and risperidone (Risp) (B). MAMPH was administered 1.0 mg/kg i.p. to mice after 60 min of baseline activity recording and distance was recorded over a further 90 min in mice pretreated with vehicle (Veh-MAMPH), ABT-239 at the doses indicated (ABT-239-MAMPH), or Risp at the doses indicated (Risp-MAMPH). Small (upward pointing) arrow indicates time of drug or vehicle administration; large (downward pointing) arrow indicates time of MAMPH or vehicle administration to the same animals. Data are represented as mean ± S.E.M. Note that although the full time course is shown, AUCs were used for more rigorous statistical analyses.](image-url)
pared with vehicle-treated animals in the medial prefrontal cortex (*p* < 0.05; Fig. 11C) following administration of 3.0 mg/kg ABT-239.

**EEG Slow-Wave Activity.** ABT-239 (0.1–3 mg/kg i.p.) had no significant effect on slow-wave EEG activity across doses for up to 6 h after administration (Fig. 12) \( F(4,35) = 0.508, p = 0.7304 \) for treatment; \( F(5,35) = 49.105, p < 0.0001 \) for time; \( F(20,175) = 1.623, p < 0.0517 \) for treatment \times time interaction], confirming our previous interpretation (Fox et al., 2003) that EEG activation does not seem to be a prerequisite for cognition enhancement. This is in contrast to ciproxifan, which significantly decreases slow-wave activity in a manner similar to stimulants such as methylphenidate (Fox et al., 2003).

**CNS Safety: General Observation Tests**

ABT-239 did not induce any severe adverse effects in mice or rats at comparable doses to those effective in any of the behavioral tests. Mild to moderate tremor, hyperreactivity, and aggression were observed in mice treated with 28 mg/kg ABT-239 either i.p. or s.c. and following 46 mg/kg ABT-239 p.o. At 45 to 90 mg/kg i.p, ABT-239 induced hypothermia, seizures, and lethality (one of three mice at 45 mg/kg and three of three mice at 90 mg/kg). Following s.c. administration, ABT-239 at 45 to 90 mg/kg induced hypothermia, severe tremor, and clonic seizures (three of three mice) but no lethality up to 24 h. Following p.o. administration, ABT-239 at 90 to 180 mg/kg induced hypothermia, moderate to severe tremor, and seizure activity (three of
Clinical studies. Evidence for in vivo functional blockade of central H3Rs (Fox et al., 2002b), these data support ABT-239 that was fully efficacious, outlasting the best effect of methylphenidate. ABT-239 remained effective for at least 3 h, the lowest dose late in the brain or plasma after repeated dosing. In addition, ABT-239 was efficacious in children and adults, respectively (Fox et al., 2002b). In the present studies, acute drinking elicited by (R)-α-MeHA was completely blocked by ABT-239, which had no effect on water intake when administered alone. Since the dipsogenia response to (R)-α-MeHA is likely mediated by central H3Rs (Fox et al., 2002b), these data support ABT-239 being a functional antagonist of H3Rs in the CNS, consistent with in vitro antagonistic effects (Esbenshade et al., 2005).

**Discussion**

**Specificity and in Vivo Functional Activity at H3Rs.** ABT-239 is a potent and selective antagonist and inverse agonist at H3Rs across several species, including rodent and human (see Esbenshade et al., 2005). A balanced affinity across species is important to allow assessment of the behavioral effects of selective blockade of H3Rs in preclinical and clinical studies. Evidence for in vivo functional blockade of central H3Rs by ABT-239 was first established using a previously characterized mouse dipsogenia model (Fox et al., 2002b). In the present studies, acute drinking elicited by (R)-α-MeHA was completely blocked by ABT-239, which had no effect on water intake when administered alone. Since the dipsogenia response to (R)-α-MeHA is likely mediated by central H3Rs (Fox et al., 2002b), these data support ABT-239 being a functional antagonist of H3Rs in the CNS, consistent with in vitro antagonistic effects (Esbenshade et al., 2005).

**Efficacy and Potency across Behavioral Models Relevant for ADHD and AD.** ABT-239 was efficacious in three different behavior tests designed to assess attention/impulsivity, short-term social memory, and spatial cognition. However, potency and efficacy varied somewhat, dependent on the cognitive domain assessed. In a five-trial, repeated acquisition, inhibitory avoidance task previously linked to attention/impulsivity deficits pertinent to ADHD (Fox et al., 2002a), acute administration of ABT-239 produced a dose-dependent improvement of acquisition compared with vehicle-treated controls. Full efficacy was achieved at 0.1 mg/kg and maintained over a broad dose range, comparing favorably with methylphenidate and ABT-418, both efficacious in ADHD in children and adults, respectively (Fox et al., 2002a). Improved acquisition was also maintained after 5 days of repeated dosing of ABT-239, with no evidence of tolerance or extinction. Similarly, ABT-239 did not accumulate in the brain or plasma after repeated dosing. In addition, ABT-239 remained effective for at least 3 h, the lowest dose that was fully efficacious, outlasting the best effect of methylphenidate. ABT-239 was even more potent in a model of short-term social memory in adult rats, with full efficacy achieved at 0.01 to 0.1 mg/kg i.p. ABT-239 also improved social memory in aged rats, with robust improvement observed at 0.3 to 1.0 mg/kg p.o. (the lowest doses tested). In contrast, ABT-239 partially reversed scopolamine-induced deficits in a spatial navigation test in the water maze at the highest dose tested, 3.0 mg/kg i.p. The reasons for these apparent discrepancies in efficacious doses are not clear and may offer a clue to potential mechanisms mediating the effects of ABT-239. For example, SHR mice were used as a model for ADHD since they exhibit hyperactivity and impulsivity in novel environments, as well as impaired sustained and nonselective attention (David et al., 2003). These intrinsic cognitive impairments may be related to a reduced number of nicotinic-acetylcholine receptors in a number of brain regions including cortex, hippocampus, thalamus, and striatum, as shown in several studies in juvenile, adult, and aged SHRs (Gatu et al., 1997; Terry et al., 2000). Impaired release of dopamine from nerve terminals in the prefrontal cortex is also evident in SHRs (David et al., 2003). Since H3Rs regulate the release of both acetylcholine and dopamine, blockade of H3Rs with antagonists such as ABT-239 would be expected to improve function in either case. Indeed, ABT-239 was found to increase release of acetylcholine in frontal cortex and hippocampus of freely moving adult rats at doses ranging from 0.1 to 3.0 mg/kg; dopamine release was also enhanced but only at a higher dose of 3.0 mg/kg in the frontal cortex but not in the striatum. Although the most robust increases in neurotransmitter levels were seen at doses higher than those efficacious in five-trial inhibitory avoidance, it is likely that only subtle, localized changes are required for efficacy. In this regard, these differences may simply reflect the limited sensitivity of microdialysis techniques, which do not measure the subtle changes in synaptic cleft neurotransmitter levels.

In contrast to the five-trial inhibitory avoidance model, social recognition measures the memory of an adult rat for a conspecific juvenile to which the adult rat has been previously exposed. Social memory and social cognition in general are impaired in CNS disorders such as Alzheimer's disease and schizophrenia and have received growing attention in recent years (Pinkham et al., 2003). Although this model is dependent on the olfactory system and interconnected cortical structures, additional brain regions and neuromodulatory systems are also

### TABLE 3

<table>
<thead>
<tr>
<th>Test Model</th>
<th>Dose of ABT-239</th>
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<tbody>
<tr>
<td></td>
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<tr>
<td>Dipsogenia (adult mouse, i.p.)</td>
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</tr>
<tr>
<td>Five-trial inhibitory avoidance (SHR pups, acute, s.c.)</td>
<td>++</td>
</tr>
<tr>
<td>Social recognition (adult rats, i.p.)</td>
<td>+</td>
</tr>
<tr>
<td>Social recognition (aged rats, p.o.)</td>
<td>+</td>
</tr>
<tr>
<td>Two-choice water maze (adult rats, i.p.)</td>
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</tr>
<tr>
<td>PPI sensorimotor gating (DBA mice, i.p.)</td>
<td>+</td>
</tr>
<tr>
<td>N40 sensory gating (DBA mice, i.p.)</td>
<td>+</td>
</tr>
<tr>
<td>Methamphetamine hyperactivity (adult mice, i.p.)</td>
<td>+</td>
</tr>
<tr>
<td>Free-moving microdialysis (adult rats, i.p.)</td>
<td>+</td>
</tr>
<tr>
<td>Free-moving EEG (adult rats, i.p.)</td>
<td>–</td>
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Approximate efficacy is indicated as +, <30% effect; ++, 30 to 70% effect; ++++, > 70% effect; and –, no effect; blank indicates not determined.
involved such as the hippocampus (Kogan et al., 2000) and entorhinal cortex (Bannerman et al., 2002) as well as the neurotransmitters acetylcholine (Winslow and Camacho, 1995) and histamine (Prast et al., 1996). Given the relatively modest effects of ABT-239 on acetylcholine levels in the hippocampus relative to the frontal cortex (Fig. 11), it is likely that ABT-239 affects other neurotransmitters in addition to acetylcholine. Indeed, the apparent loss of activity in social recognition at the highest dose may reflect a negative influence of ABT-239 at other H3 heteroreceptors, producing a U-shaped dose response in this model. Taken together, it is possible that the observed improvement of social recognition memory with ABT-239 reflects enhanced acetylcholine and histamine release (for effects of ABT-239 on histamine release in rat brain cortical synaptosomes, see Esbenshade et al., 2005) in one or more of these key brain regions.

ABT-239 was least potent and least efficacious in the water maze. Hippocampal cholinergic neurotransmission plays an important role in spatial memory, which is abolished by scopolamine. The version of the test used in the present studies is dependent on the septal-hippocampal pathway, so it is possible that the modest improvement in spatial discrimination observed with ABT-239 is a result of enhanced release of acetylcholine in the hippocampus overcoming the postsynaptic inhibition of muscarinic receptors by scopolamine. The partial efficacy is reflected in part by the smaller effects of ABT-239 in acetylcholine levels in the hippocampus, as detected in the present microdialysis studies. In this regard, ABT-239 may differentially distribute within the brain, and further studies are required to ascertain if this is the case.

**Efficacy and Potency across Behavioral Models Relevant for Schizophrenia.** The effects of ABT-239 in models related to schizophrenia were assessed across three additional behavioral models. Abnormalities in sensory gating are an early clinical symptom in schizophrenia; patients often cannot filter out unimportant stimuli and have difficulty focusing their attention (Geyer and Mogbaddam, 2002). One measure of sensory gating abnormalities is PPI of startle, a response (often to an acoustic tone) that is normally inhibited when preceded by a weaker stimulus. Evident in schizophrenia patients, and reversed by some antipsychotics, PPI deficits seem to result from neurodevelopmental perturbation and are thought to reflect deficient sensorimotor gating underlying sensory flooding and cognitive fragmentation (Geyer et al., 2001). DBA/2 mice exhibit an intrinsic deficit in PPI that is also believed to be neurodevelopmentally mediated and can be reversed with antipsychotics (Browman et al., 2004). In our studies, risperidone enhanced PPI in DBA/2 mice, but at a dose that also impaired baseline startle response. In contrast, ABT-239 enhanced PPI without affecting baseline startle response. ABT-239 also normalized responses in a second gating model in DBA/2 mice, N40, a rodent model analogous to the P50 auditory-evoked response in humans. In rodents, N40 is dependent on acetylcholine release and usually recorded from the hippocampus because of its suspected role in gating (Bast and Feldon, 2003; Simosky et al., 2003). In our studies, the effect of ABT-239 was similar to that observed with nicotine. Notably, human P50 deficits in schizophrenia patients are also normalized by nicotine (Adler et al., 1993). Furthermore, the atypical antipsychotic, clozapine, also improved N40 in DBA mice, reportedly through a cholinergic mechanism (Simosky et al., 2003).

Thus, ABT-239 may be normalizing N40 sensory gating in the hippocampus through a local cholinergic action. Interestingly, although efficacy of current antipsychotics for broader cognitive symptoms in schizophrenia remains controversial (Green et al., 2001), it should be noted that clozapine (20 mg/kg s.c.) and risperidone (1.0–2.0 mg/kg) can also increase ACh levels in the rat prefrontal cortex (K. U. Drescher and G. Plotzky, unpublished data; not significant in hippocampus), which may account for their efficacy in these models.

Increased mesolimbic and mesocortical dopamine function is hypothesized to underlie the positive symptoms in schizophrenia, and current antipsychotics are believed to work through blockade of dopamine hyperactivity in these areas. Methamphetamine-induced psychomotor hyperactivity can model positive symptoms of schizophrenia in rodents, and currently used antipsychotic drugs are efficacious in blocking this hyperlocomotion. In our studies, ABT-239 blocked the psychostimulant effects of methamphetamine, but without the sedative or cataleptogenic (Zhang et al., 2005) effects of risperidone. Since H3Rs are found in high density in mesolimbic areas and regulate dopamine function (Sánchez-Lemus and Arias-Montano, 2004), effects of ABT-239 in this regard require further experimentation.

**Conclusions**

This work represents a comprehensive demonstration of broad preclinical efficacy of the H3R antagonist, ABT-239, across multiple behavioral domains and neurotransmitter systems affected in CNS disorders such as ADHD, AD, and schizophrenia (for summary, see Table 3). The availability of highly selective drugs like ABT-239 will facilitate future mechanism-related studies as well as validation of the effectiveness of this approach in humans.

**References**


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