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AZD9272 and AZD2066: Selective and Highly Central Nervous System Penetrant mGluR5 Antagonists Characterized by Their Discriminative Effects

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ABSTRACT

The metabotropic glutamate receptor 5 (mGluR5) antagonists fenobam, MPEP (2-methyl-6-(phenylethynyl)pyridine), and MTEP (3-[(2-methyl-1,3-thiazol-4-yl)ethynyl]pyridine) were previously shown to not cause *N*-methyl-D-aspartate antagonist–like psychoactive effects in phencyclidine (PCP) drug discrimination studies, but to cause MTEP-like discrimination in rats, suggesting that the psychoactive and psychotomimetic effects reported with fenobam in humans were likely mediated by mGluR5 antagonist mechanisms. The present study was designed to characterize AZD9272 (3-fluoro-5-(3-(5-fluoropyridin-2-yl)-1,2,4-oxadiazol5-yl) benzonitrile) and AZD2066 [4-(5-{(1*R*)-1-[5-(3-chlorophenyl)isoxazol-3-yl]ethoxy}-4-methyl-4*H*-1,2,4-triazol-3-yl)pyridine], two mGluR5 antagonists taken to clinical development for analgesia. AZD9272 was evaluated in several groups of rats trained to discriminate cocaine, PCP, chlordiazepoxide, (−)-Δ^9-tetrahydrocannabinol [(−)-Δ^9-THC], or MTEP from no drug. AZD9272 shared discriminative properties with MTEP only. The discriminative half-life was 3.23 hours for MTEP and 21.93 hours for AZD9272 in rats trained to discriminate MTEP from no drug. Other rats were successfully trained to discriminate AZD9272 from no drug. Due to the long duration of action of AZD9272, discrimination training was conducted every other day. AZD9272 caused a dose-dependent increase in AZD9272-appropriate responding. PCP did not cause AZD9272-appropriate responding, whereas MTEP, fenobam, and the mGluR5 antagonist AZD2066 did. The discriminative half-life of AZD9272 was 24.3 hours in rats trained to discriminate AZD9272 from no drug. It is concluded that the discriminative effects of AZD9272 and AZD2066 are similar to those of previously investigated mGluR5 antagonists and dissimilar to those of cocaine, PCP, chlordiazepoxide, and (−)-Δ^9-THC. The discriminative half-life of AZD9272 is approximately 7-fold longer than for MTEP. These data support and extend previous findings suggesting that mGluR5 antagonism causes psychoactive effects selectively mediated by mGluR5 mechanisms.

Introduction

Ligand-gated ionotropic glutamate receptors [*N*-methyl-D-aspartate (NMDA), AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid), and kainate] and G protein–coupled metabotropic glutamate receptors (mGluR; Conn and Pin, 1997) are the most abundant excitatory amino acid receptors, having major excitatory roles in the central nervous system (CNS; Monaghan et al., 1989).

Attenuating glutamate transmission has been proposed to treat cerebral ischemia, epilepsy, anxiety, pain, and depression, and anticonvulsant potential of NMDA antagonists (Hayes and Balster, 1985; Chapman and Meldrum, 1989; Chapman et al., 1991a) and the AMPA antagonist NBQX [2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(f)quinoxaline; Chapman et al., 1991b; Swedberg et al., 1995] has been demonstrated.

NMDA antagonism can cause vivid human psychoactivity and hallucinations (Aniline and Pitts, 1982), discriminative effects in primates (Nicholson et al., 2007) and rats (Willetts and Balster, 1988; Koek et al., 1990; Swedberg et al., 1995, 2014), self-administration in animals (Nicholson et al., 2007; Swedberg et al., 2014), and human drug abuse (Bey and Patel, 2007), whereas AMPA antagonism caused no phencyclidine (PCP)-like discriminative effects (Swedberg et al., 1995).

Multiple mGluR receptor subtypes are localized in forebrain and limbic regions in rats (Spooren et al., 2001) and primates (Muly et al., 2003), thereby providing several drug discovery targets (Conn and Pin, 1997) and the potential for CNS side effects. mGluR5 antagonism is implicated in several CNS functions (Schoepp, 2001) and disorders, including anxiety, depression, pain, and Parkinson's disease (Spooren et al.,

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ABBREVIATIONS: AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; AZD2066, 4-(5-{(1*R*)-1-[5-(3-chlorophenyl)isoxazol-3-yl]ethoxy}-4-methyl-4H-1,2,4-triazol-3-yl)pyridine; AZD9272, 3-fluoro-5-(3-(5-fluoropyridin-2-yl)-1,2,4-oxadiazol-5-yl)benzonitrile; CNS, central nervous system; D, drug; McN-3377/fenobam, *N*-(3-chlorophenyl)-*N′*-(4,5-dihydro-1-methyl-4-oxo-1*H*-imidazole-2-yl)urea; FR, fixed ratio; HPβCD, hydroxypropyl-β-cyclodextrin; mGluR, metabotropic glutamate receptor; MK-801, [(5*S*,10*R*)-(+)-5-Methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5,10-imine maleate; MPEP, 2-methyl-6-(phenylethynyl)pyridine; MTEP, 3-[(2-methyl-1,3-thiazol-4-yl)ethynyl]pyridine; N, no drug; NBQX, 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(f)quinoxaline; NMDA, *N*-methyl-D-aspartate; PCP, phencyclidine; SIB-1893, (*E*)-2-methyl-6-styryl-pyridine; $t_{1/2}$, half-life; (−)-Δ^9-THC, (−)-Δ^9-tetrahydrocannabinol.

2001), and in non-CNS functions such as gastroesophageal reflux disease (Keywood et al., 2009). In addition to being anxiolytic in humans (Pecknold et al., 1980, 1982; Lapierre and Oyewumi, 1982), the mGluR5 antagonist fenobam or McN-3377 [*N*-(3-chlorophenyl)-*N*′-(4,5-dihydro-1-methyl-4-oxo-1*H*-imidazole-2-yl)urea] (Porter et al., 2005) caused psychostimulant (Itil et al., 1978) and hallucinatory (Friedmann et al., 1980) effects. Further, recent mGluR5 antagonists MPEP [2-methyl-6-(phenylethynyl)pyridine; Gasparini et al., 1999] and SIB-1893 [(*E*)-2-methyl-6-styryl-pyridine; Varney et al., 1999] also inhibit NMDA receptor activity (O'Leary et al., 2000; Movsesyan et al., 2001), suggesting the possibility of NMDA-mediated psychoactive effects.

We recently showed that fenobam and MPEP caused partial PCP-like discriminative effects, whereas the more selective mGluR5 antagonist MTEP (3-[(2-methyl-1,3-thiazol-4-yl)ethynyl]pyridine; Busse et al., 2004) did not (Swedberg et al., 2014).

We also showed that rats can discriminate MTEP from no drug, and that fenobam and MPEP caused full MTEP-like discriminative effects, whereas PCP and several other psychoactive drugs did not (Swedberg et al., 2014).

The mGluR5 antagonist AZD9272 [3-fluoro-5-(3-(5-fluoropyridin-2-yl)-1,2,4-oxadiazol-5-yl)benzonitrile; Fig. 1] was highly potent in vitro, reached maximal concentrations 3–8 hours after oral administration to rats, yielded a brain-to-plasma ratio close to 1, and caused higher binding in the forebrain than the hindbrain or cerebellum (Raboisson et al., 2012a).

Consistent with rats, in nonhuman primates AZD9272 showed high brain penetration and regional distribution concordant with known mGluR5 CNS receptor distribution (Andersson et al., 2013). Human positron emission tomography data showed saturable mGluR5 receptor binding, an estimated plasma concentration corresponding to 50% occupancy, and a 4-fold higher receptor density in the ventral striatum than in the cerebellum (Kågedal et al., 2012).

AZD9272, targeted for pain, anxiety, and gastroesophageal reflux disease, was not analgesic in humans (Kalliomäki et al., 2013) but caused dose-limiting anxiety and hallucinations (Ståhle et al., 2012). AZD2066 [4-(5-{(1*R*)-1-[5-(3-chlorophenyl) isoxazol-3-yl]ethoxy}-4-methyl-4H-1,2,4-triazol-3-yl)pyridine; Fig. 2; Kågedal et al., 2013], another selective and brain-penetrant mGluR5 antagonist active against transient lower esophageal sphincter relaxations and reflux episodes in humans (Rohof et al., 2012), with brain receptor occupancy levels similar to AZD9272, caused adverse CNS events similar to AZD9272 (Raboisson et al., 2012b; Rohof et al., 2012; Ståhle et al., 2012).

To proactively assess abuse potential (Swedberg, 2013), AZD9272 was evaluated in several drug discrimination assays using known psychoactive drugs in addition to MTEP and PCP (as described above). Cocaine, an abused psychostimulant, causes discriminative effects in several species

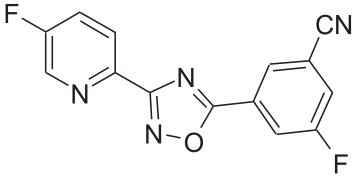

Fig. 1. AZD9272.

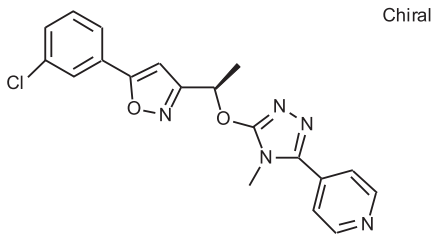

Fig. 2. AZD2066.

(e.g., Swedberg and Järbe, 1986; Nader et al., 1997; Kleven and Koek, 1998), including humans (e.g., Johanson et al., 2006; Lile et al., 2011). The benzodiazepine chlordiazepoxide (Rall, 1990), psychostimulant and sedative-anxiolytic in humans, causes discriminative effects in animals (Järbe and Swedberg, 1998; Mirza et al., 2006), and benzodiazepines cause discriminative, subjective effects and liking in humans (Griffiths et al., 1984; Ferrara et al., 1999; Carter et al., 2007). The cannabinoid agonist (−)-Δ^9-tetrahydrocannabinol [(−)-Δ^9-THC] is psychoactive in humans (D'Souza et al., 2004) and animals (Wiley, 1999).

To increase assay sensitivity (Colpaert et al., 1980) to detect potentially weak NMDA antagonist effects previously suggested with MPEP and fenobam (Swedberg et al., 2014), the PCP training dose was lowered compared with previous studies (Swedberg et al., 1995, 2014).

Other rats, trained to discriminate AZD9272 from no drug, were tested with PCP, MTEP, fenobam, and AZD2066. Discriminative half-lives ($t_{1/2}$) of MTEP and AZD9272 were determined in MTEP discrimination, and the AZD9272 $t_{1/2}$ was further determined in AZD9272 discrimination.

Materials and Methods

All animal experiments were performed in accordance with the guidelines of The Swedish National Board for Laboratory Animals under a protocol approved by the Ethical Committee of Southern Stockholm, Sweden. Studies were carried out in accordance with the Declaration of Helsinki and with the *Guide for the Care and Use of Laboratory Animals* as adopted and promulgated by the US National Institutes of Health.

Subjects

Approximately 48 male Wistar rats (Scanbur BK AB, Sollentuna, Sweden, and Møllegaard, Ry, Denmark) weighing 240–250 g at the beginning of the experiments were housed in pairs, or group housed up to 8 rats per cage, in a colony room with water accessible at all times and lights on between 6:00 AM and 6:00 PM; by restricting access to food, animals were kept at approximately 80% of free feeding weight.

Apparatus

Sixteen operant chambers enclosed in sound attenuating cubicles with exhaust fans providing "white noise" and equipped with two response levers, cue lights, a house light, and a food magazine were used (Med Associates Inc., St. Albans, VT). Food pellets used were 45-mg Dustless Precision Pellets (Bio-Serv, Frenchtown, NJ). Experiments were run and data collected by a PC with DRDIWin PC Software (Ellegaard Systems A/S, Faaborg, Denmark) and LOIS DRDI software (AstraZeneca R&D, IS, Södertälje, Sweden).

Discrimination Training and Testing

Training and testing procedures have been described earlier (Swedberg et al., 2014). In brief, an autoshaping procedure was used to train rats to approach the response levers and learn that food pellets were available. Before any drug administration, the rats were trained to press either response lever (left or right) once [a fixed ratio (FR) 1 schedule] to produce a food pellet. The production of one food pellet constitutes the completion of one trial. After learning that pressing any of the two levers would produce food, a "drug" lever and a "no-drug" lever were assigned to each rat in a balanced fashion. From this point on, each animal received either an injection of the training drug ("drug") or no injection ("no-drug"), prior to training sessions, and was required to press the lever appropriate to the pretreatment received ("correct lever") to produce a food pellet. The absence of a vehicle-alone injection during no-drug training sessions has been tested and validated using food-reinforced drug discrimination operant schedules of reinforcement in our laboratories for many years (e.g., Swedberg et al., 1995, 2014). Developing and applying the procedure in an industrial setting, we found this was 1) more cost effective, and 2) more ethical, since the number of injections can be reduced by approximately 50%, which is quite substantial considering that rats can be trained and tested 5 days per week for up to 1.5 or in some cases 2 years, and that multiple experiments are run each day. There are inbuilt controls for this procedure: acquisition test sessions are run prior to accepting a rat into the test phase (as described below) and with each dose-response curve generated, including the training drug, the appropriate "vehicle-alone" injection is administered.

Animals were run on a single-alternation schedule with an increase in the FR response requirement every other day until an FR10 was reached. Presses on the incorrect lever had no programmed consequences other than to reset the FR value on the correct lever, thus requiring the rats to emit 10 correct responses consecutively to obtain a food pellet. At FR10, a double-alternation schedule was introduced so that pairs of two consecutive drug (D) and no-drug (N) sessions alternated (D, D, N, N, D, D, N, N, etc.). A session lasted until 50 food pellets had been earned (equaling 50 trials) or until 15 minutes had elapsed, whichever occurred first. Rats were then trained to reach a criterion of 90% correct responding with no more than nine responses on the incorrect lever prior to the first reinforcement in each session, for eight consecutive sessions. The two sessions immediately following the eighth criterion session were acquisition test sessions in which the training conditions (D and N) were tested and 10 consecutive responses on either lever would produce a food pellet (i.e., both levers were "correct"), and the same performance criteria were applied.

During testing, animals were run according to a single-alternation schedule (D, N, D, N, etc.), and tests were interspersed between the training sessions (D, N; formally: D, T, N, D, T, N, T, D, N, T, etc.). Test sessions were identical to training sessions, except that during tests, both levers were correct and 10 consecutive presses on any of the levers would produce a food pellet. Test sessions were typically run on Tuesdays and Fridays, provided that the animals performed according to the criterion on the training days. If training day performance fell below the criteria for any rat on a single training day, the upcoming test was postponed for that rat, and it was tested again only after completing two consecutive training sessions during which criteria were met. During testing, the appropriate vehicle of each drug was tested as the no-drug condition.

Several groups of male Wistar rats were trained to discriminate cocaine (3.4 mg/kg i.p., 15 minutes), PCP (1.6 mg/kg i.p., 30 minutes), chlordiazepoxide (10 mg/kg i.p., 30 minutes), (−)-Δ^9-THC (3 mg/kg i.p., 20 minutes), MTEP (2 mg/kg i.p., 30 minutes), or AZD9272 (1.6 mg/kg p.o., 60 minutes) from no drug.

Data Analysis

Drug discrimination results are expressed as the mean (±S.E.M.) of the individual percentages of drug responding during drug and no drug sessions, respectively, in rats completing at least 10 trials. Rates of responding are expressed as the mean (±S.E.M.) number of responses per second in all rats tested. ED_{50} values for the discriminative effects were determined as the dose producing 50% training drug–appropriate responding. Response rate effects are shown as a percentage of vehicle control rates. ED_{50} and discriminative half-lives ($t_{1/2}$) with 95% confidence limits were calculated using analysis of variance and nonlinear regression techniques (GraphPad Prism v.6; GraphPad Software, Inc., La Jolla, CA) (Snedecor and Cochran, 1967). X values were transformed (X = log X) and estimates of Y = 50 were made with constraints of Y (percentage of drug responding) curves set to 100 (top) and 0 (bottom). SigmaPlot for Windows version 12.5 (Systat Software Inc., Chicago, IL) was used for graphics.

Drugs and doses producing less than 20% training drug–appropriate responding are considered not to produce discriminative effects similar to those of the training drug. Drugs and doses producing between 20 and 80% training drug–appropriate responding are considered to produce discriminative effects partly similar to those of the training drug. Drugs and doses causing 80% or more training drug–appropriate responding are considered to fully share the discriminative effects of the training drug; and drugs producing full training drug-like discriminative effects and having no significant effects (<50% reduction relative to vehicle alone) on response rates are considered to produce discriminative effects identical to those of the training drug.

Drugs. Cocaine (Lipomed AG, Arlesheim, Switzerland), PCP (Lipomed), and chlordiazepoxide HCl (Sigma-Aldrich, St. Louis, MO), were dissolved in saline. MTEP (AstraZeneca R&D, Mölndal, Sweden) was dissolved in 20% hydroxypropyl-β-cyclodextrin (HPβCD) and 80% water. AZD9272 (AstraZeneca R&D) was given as a microsuspension (xanthan gum 0.25%, docusate sodium 0.06%, and purified water) or as a nanosuspension (1.7 mg/ml polyvinylpyrrolidone K 30, 0.34 mg/ml sodium laurylsulfate, 25 mg/ml glycerol). AZD2066 (AstraZeneca R&D) was dissolved in 10% HPβCD in sterile water. (−)-Δ^9-THC (100 mg/ml in ethanol; Lipomed) was pipetted into vials and exposed to a slow flow of nitrogen gas to evaporate the ethanol. A mixture of polyethylene glycol and Tween 80 was added to the (−)-Δ^9-THC such that in the planned final solution, the concentration of polyethylene glycol and Tween 80 reached 3–5%. Fenobam was dissolved in 40% HPβCD in sterile water. Fenobam was administered at a volume of 10 ml/kg, AZD9272 and AZD2066 were administered at a volume of 5 ml/kg, and all other drugs were given at 2 ml/kg. Doses given refer to the weight of the base. The following routes of administration and pretreatment times were used: cocaine (i.p., 15 minutes), PCP (i.p., 15 minutes), chlordiazepoxide (i.p., 30 minutes), (−)-Δ^9-THC (i.p., 20 minutes), MTEP (i.p., 30 minutes), AZD9272 (p.o., 60 minutes; p.o., 30 minutes in cocaine study), fenobam (p.o., 30 minutes), and AZD2066 (p.o., 60 minutes). In the MTEP drug discrimination time-course study, MTEP was administered i.p. at 5–240 minutes and AZD9272 p.o. at 30–1440 minutes. In the AZD9272 time-course study, AZD9272 was administered p.o. at 240–2400 minutes.

Dose Selection. Training doses were based on previous studies using mGluR5 antagonists and PCP (Swedberg et al., 1995, 2014) and other drug discrimination studies conducted in our laboratories over the years, published and unpublished. All compounds used, including AZD9272 and AZD2066, have undergone extensive testing in many primary behavioral pharmacology models, such as analgesia testing, and safety pharmacology screening and behavioral models, such as a rat version of the Irwin screen (Irwin, 1968) and locomotor activity testing, and the dose ranges cover low to high ends of effective doses, and above.

Results

Rats were successfully trained to discriminate cocaine (3.4 mg/kg i.p., 15 minutes), PCP (1.6 mg/kg i.p., 30 mintues), chlordiazepoxide (10 mg/kg i.p., 30minutes), (−)-Δ^9-THC (3 mg/kg

TABLE 1
Maximal % drug responding ED_{50} and confidence limits, and maximal effects on response rates in rats trained to discriminate cocaine, PCP, chlordiazepoxide, (−)-Δ^9-THC, or MTEP from no drug

Compound	Max % Drug Responding					Max Response Rate Effects				
	At Dose	Mean	S.E.M.	ED_{50} (95% CL)	Rats Responding (Rats Tested)	At Dose	Mean	S.E.M.	% of Veh	Rats Responding (Rats Tested)
	mg/kg					*mg/kg*				
Cocaine	10.19	99.95	0.03	0.46 (0.29–0.73)	8 (8)	10.19	2.17	0.20	107	8 (8)
AZD9272	28.42	17.73	18.06	<50%	6 (6)	8.53	0.80	0.17	43	6 (6)
PCP	1.59	99.58	0.26	0.6 (0.44–0.82)	8 (8)	0.50	2.24	0.09	114	8 (8)
AZD9272	8.53	16.41	16.41	<50%	6 (6)	2.84	1.44	0.19	70	6 (6)
Chlordiazepoxide	18.83	100.0	0.00	2.2 (0.94–5.16)	8 (8)	18.83	0.96	0.29	74	8 (8)
AZD9272	0.85	16.73	18.16	<50%	6 (6)	28.42	1.02	0.11	69	6 (6)
(−)-Δ^9-THC	2.99	99.98	0.03	1.01 (0.22–4.53)	8 (8)	2.99	2.16	0.35	123	8 (8)
AZD9272	8.53	37.39	20.90	<50%	6 (6)	8.53	1.21	0.25	71	6 (6)
MTEP	2.00	99.01	0.69	0.42 (0.34–0.53)	8 (8)	2.00	1.58	0.11	97	8 (8)
AZD9272	8.53	99.98	0.03	0.11 (0.02–0.69)	8 (8)	8.53	1.63	0.10	96	8 (8)

CL, confidence limit; Veh, vehicle.

i.p., 20 minutes), MTEP (2 mg/kg i.p., 30 minutes), or AZD9272 (1.6 mg/kg p.o., 60 minutes) from no drug.

Rats trained to discriminate AZD9272 from no drug were initially trained Monday through Friday, and as four of the eight rats completed the criteria and proceeded into testing, it became apparent after testing a range of doses in these rats that a dose-response curve would not be reliably generated. Notably, vehicle-alone testing generated >90% AZD9272-appropriate responding in three of the four rats. Based on these initial findings and the extended $t_{1/2}$ of AZD9272 found in other in-house studies, the decision was made to continue training on an every-other-day basis to allow drug clearance. Under this training regimen, training improved and the acquisition of AZD9272 discrimination was successfully achieved within approximately 50 FR10 sessions (data not shown).

For compounds causing 50% or greater drug-appropriate responding, ED_{50} values with confidence limits are shown in Tables 1 and 2, as are the maximal percentages of drug-appropriate responding for all drugs tested. In cases where noteworthy effects on response rates occurred, these are mentioned in the text, whereas maximal effects on response rates for all compounds are given in Tables 1 and 2. In cases where no effects on response rates occurred, reference to these data is given in connection with references to percentage of drug appropriate-responding dose-response curves.

Dose-Response Studies. Cocaine caused a dose-dependent increase in cocaine-appropriate responding (Fig. 3, A and B). AZD9272 caused no cocaine-appropriate responding (Fig. 3A) and caused a non–dose-dependent reduction in response rates at higher doses (Fig. 3B).

PCP caused a dose-dependent increase in PCP-appropriate responding (Fig. 3, C and D). AZD9272 caused no PCP-appropriate responding (Fig. 3C) and caused a slight reduction in response rates (Fig. 3D).

Chlordiazepoxide caused a dose-dependent increase in chlordiazepoxide- appropriate responding (Fig. 3E), and caused a slight decrease in rates of responding at the highest dose (Fig. 3F). AZD9272 caused no chlordiazepoxide-appropriate responding (Fig. 3E) and a slight reduction in response rates (Fig. 3F).

(−)-Δ^9-THC caused a dose-dependent increase in (−)-Δ^9-THC–appropriate responding (Fig. 4, A and B). AZD9272 caused partial (−)-Δ^9-THC–appropriate responding (Fig. 4, A and B).

To ensure that a suboptimal test time had not been chosen, AZD9272 was also tested at 15 minutes and 4 hours (chlordiazepoxide discrimination), and at 15 minutes and 6 hours [(−)-Δ^9-THC discrimination]. AZD9272 caused a maximum of less than 1% at both time points in the chlordiazepoxide study, and approximately 33% (−)-Δ^9-THC–appropriate responding at 15 minutes and 16% at 6 hours (data not shown).

MTEP caused a dose-dependent increase in MTEP-appropriate responding, and AZD9272 caused dose-dependent and full generalization to MTEP (Fig. 4, C and D).

Rats were readily trained to discriminate AZD9272 from no drug when training was conducted 3 days per week. AZD9272 caused a dose-dependent increase in AZD9272-appropriate

TABLE 2
Maximal % AZD9272 responding ED_{50} and confidence limits, and maximal effects on response rates, in rats trained to discriminate AZD9272 from no drug

Compound	Max % Drug Responding					Max Response Rate Effects				
	At Dose	Mean	S.E.M.	ED_{50} (95% CL)	Rats Responding (Rats Tested)	At Dose	Mean	S.E.M.	% of Veh	Rats Responding (Rats Tested)
	mg/kg					*mg/kg*				
AZD9272	0.28	87.52	12.03	0.21 (0.03–1.76)	8 (8)	0.28	1.89	0.24	117	8 (8)
PCP	0.28	17.16	16.57	<50%	6 (6)	1.59	1.88	0.26	121	6 (6)
MTEP	2.00	50.04[a]	22.14	1.74 (1.13–2.67)	6 (6)	2.00	1.58	0.19	86	6 (6)
Fenobam	8.00	99.96	0.04	1.50 (0.17–12.81)	6 (6)	0.80	1.45	0.19	96	6 (6)
AZD2066	3.82	83.37	16.59	0.46 (0.09–2.35)	6 (6)	3.82	1.72	0.23	96	6 (6)

CL, confidence limit; Veh, vehicle.
[a]Note that the 2.0 mg/kg dose was the highest dose tested. See *Discussion* for further details.

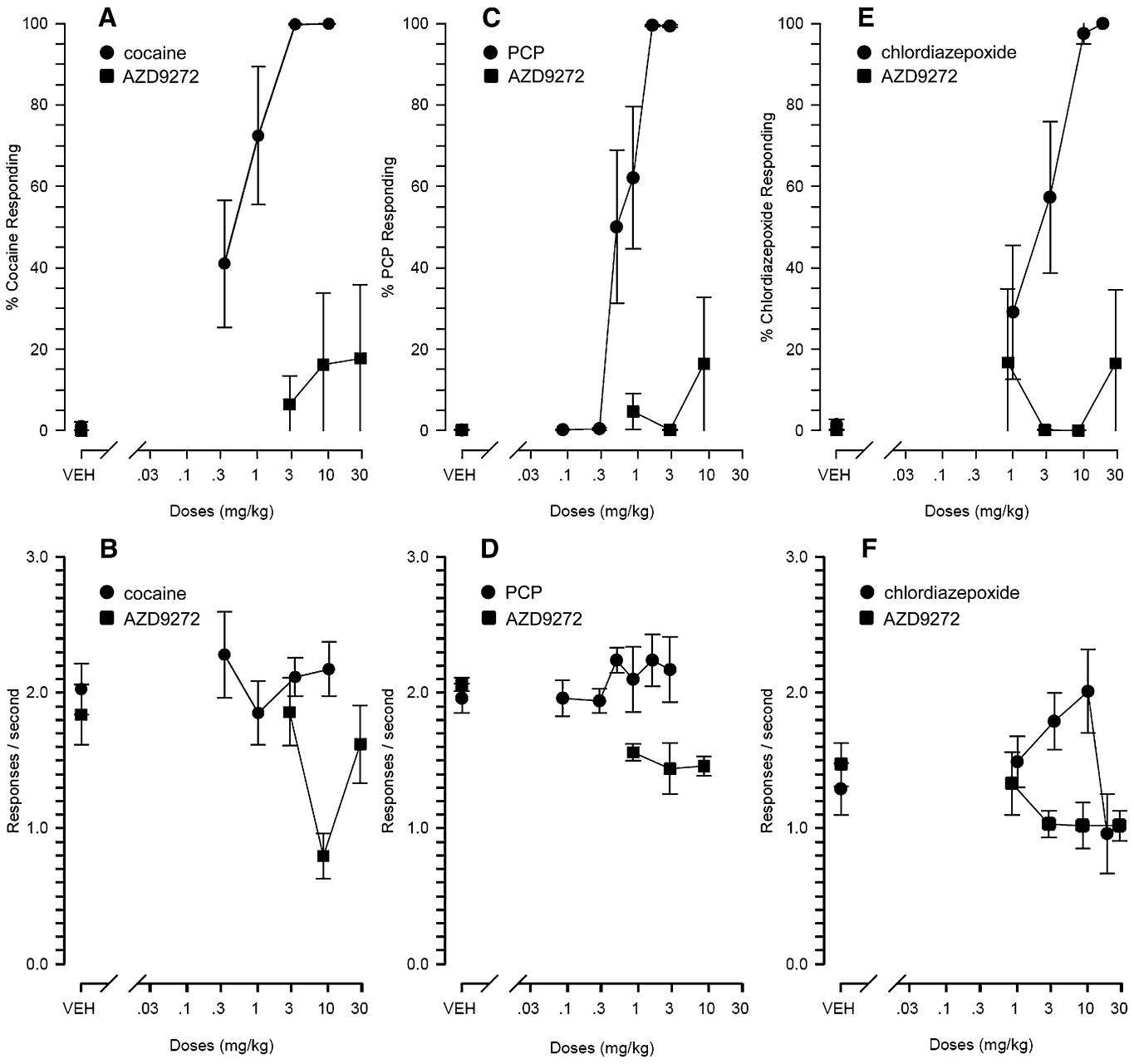

Fig. 3. Percentage of cocaine-, PCP-, or chlordiazepoxide-appropriate responding (mean ± S.E.M.) (A, C, and E) and responses per second (mean ± S.E.M.) (B, D, and F) in male Wistar rats trained to discriminate cocaine (3.4 mg/kg, 15 minutes after i.p. administration) from no drug (A and B), PCP (1.6 mg/kg, 15 minutes after i.p. administration) from no drug (C and D), or chlordiazepoxide (10 mg/kg, 30 minutes after i.p. administration) from no drug (E and F). ●, cocaine, PCP, or chlordiazepoxide; ■, AZD9272 (60 minutes after p.o. administration). VEH, vehicle.

responding (Figs. 5 and 6), whereas PCP (Fig. 5, A and B) caused no AZD9272-appropriate responding.

MTEP caused partial AZD9272-appropriate responding (Fig. 5, C and D) in the dose range investigated. However, higher MTEP doses would most likely have caused a higher degree of AZD9272 responding (see *Discussion*). Fenobam (Fig. 6, A and B) and AZD2066 (Fig. 6, C and D) caused full and dose-dependent AZD9272-appropriate responding.

Time-Course Studies. MTEP at 2.0 mg/kg caused full MTEP-appropriate responding up to 2 hours after administration and then a decline to 33.46% at 4 hours after dose (Fig. 7A), yielding a $t_{1/2}$ of 3.23 hours (Table 3), and there were no effects on response rates (Fig. 7B). The first time point at which MTEP caused >90% MTEP-appropriate responding was at 5 minutes after dose, which was also the first time point tested (Fig. 7A).

AZD9272 at 2.84 mg/kg caused greater than 80% and typically more than 99% MTEP-appropriate responding up to 20 hours after dose, with a decline to approximately 20% at 24 hours after dose (Fig. 7A), yielding a $t_{1/2}$ of 21.93 hours (Table 3), and caused no systematic effects on response rates (Fig. 7B). The first time point at which AZD9272 caused >90% MTEP-appropriate responding was at 30 minutes after dose, which was also the first time point tested (Fig. 7A).

The time course of AZD9272 at 2.84 mg/kg was also investigated in rats discriminating AZD9272 from no drug

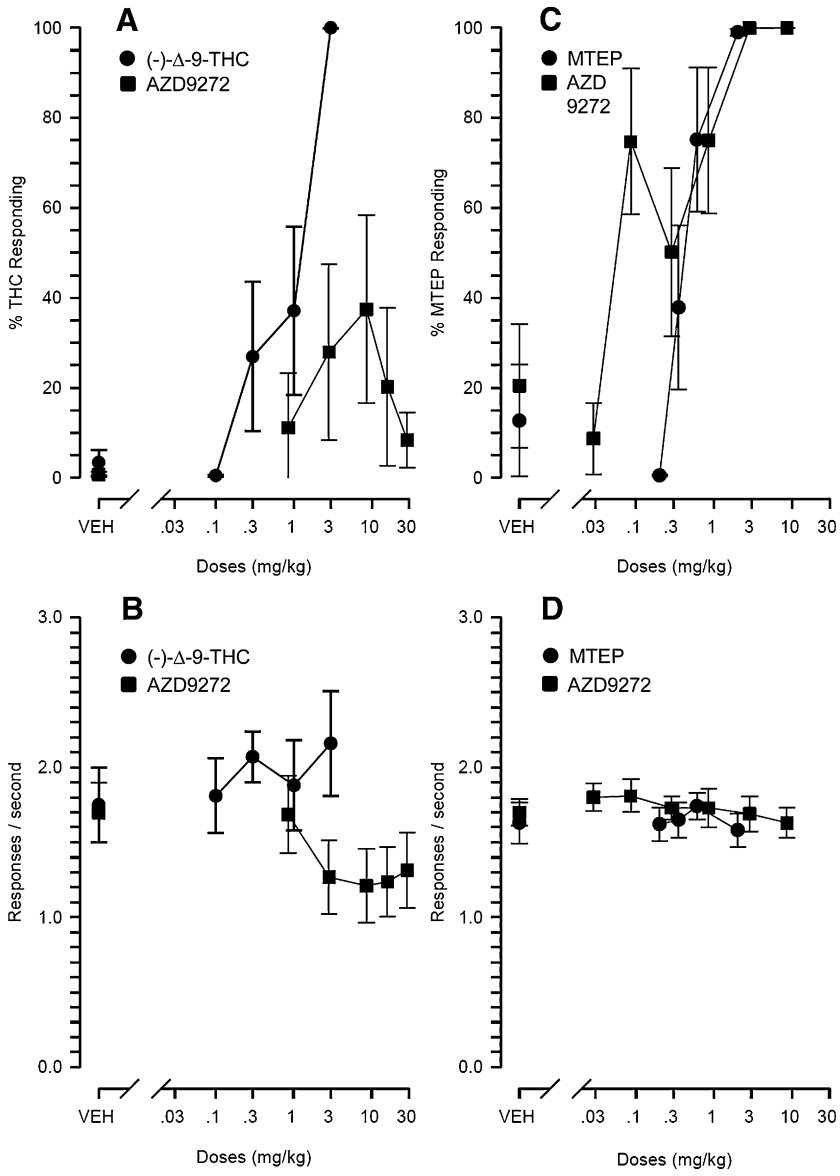

Fig. 4. Percentage of (−)-Δ^9-THC– or MTEP-appropriate responding (mean ± S.E.M.) (A and C) and responses per second (mean ± S.E.M.) (B and D) in male Wistar rats trained to discriminate (−)-Δ^9-THC (3.0 mg/kg, 20 minutes after i.p. administration) from no drug (A and B) or MTEP (2.0 mg/kg, 30 minutes after i.p. administration) from no drug (C and D). ●, (−)-Δ^9-THC or MTEP; ■, AZD9272 (60 minutes after p.o. administration). VEH, vehicle.

showing a duration of effect ranging from more than 90 to approximately 80 and 70% at 4, 6, and 20 hours, respectively. The duration of effect declined to approximately 50% at 24 hours, and to less than 20% at 40 hours after dose (Fig. 8A), yielding a $t_{1/2}$ of 24.30 hours (Table 3) with no effects on response rates (Fig. 8B). The first time point at which AZD9272 caused >90% AZD9272-appropriate responding was at 4 hours after dose, which was also the first time point tested (Fig. 8A).

Discussion

The present data show that AZD9272 does not share discriminative effects with drugs of abuse of the cocaine, benzodiazepine, or NMDA antagonist types. A partial (−)-Δ^9-THC–like effect was found, the relevance of which is not entirely clear at present. AZD9272 caused a partial but dose-dependent increase in (−)-Δ^9-THC–appropriate responding, with a maximal effect of 37.39%, and then a decrease with no dose-dependent effects on response rates. It has been shown that activation of cannabinoid CB1 receptors reduces glutamate release in rat cerebellar purkinje cells (Lévénés et al., 1998) and in the mouse forebrain (Domenici et al., 2006), suggesting that the discriminative effects of (−)-Δ^9-THC, or the components thereof, may be mediated at least partly by reducing glutamate release via the CB1 receptor.

AZD9272, by its antagonist effects on glutamate receptors, may produce part or a subset of a more complex discriminative stimulus caused by (−)-Δ^9-THC, or possibly a downstream common functional effect. Conversely, in Swedberg et al. (2014), (−)-Δ^9-THC caused a maximum of 16.67% MTEP-appropriate responding at the lowest dose tested (0.1 mg/kg), not different from vehicle alone, and at higher doses of (−)-Δ^9-THC, MTEP-appropriate responding decreased further, as did response rates. The lack of MTEP-like discriminative effects of (−)-Δ^9-THC, below and at doses substantially reducing response rates, could likely be attributable to a more selective discriminative stimulus effect produced by MTEP than by (−)-Δ^9-THC. These types of findings are referred to as asymmetric generalization and have been reported for various pharmacologies and species (Ator and Griffiths, 1983; Kelley

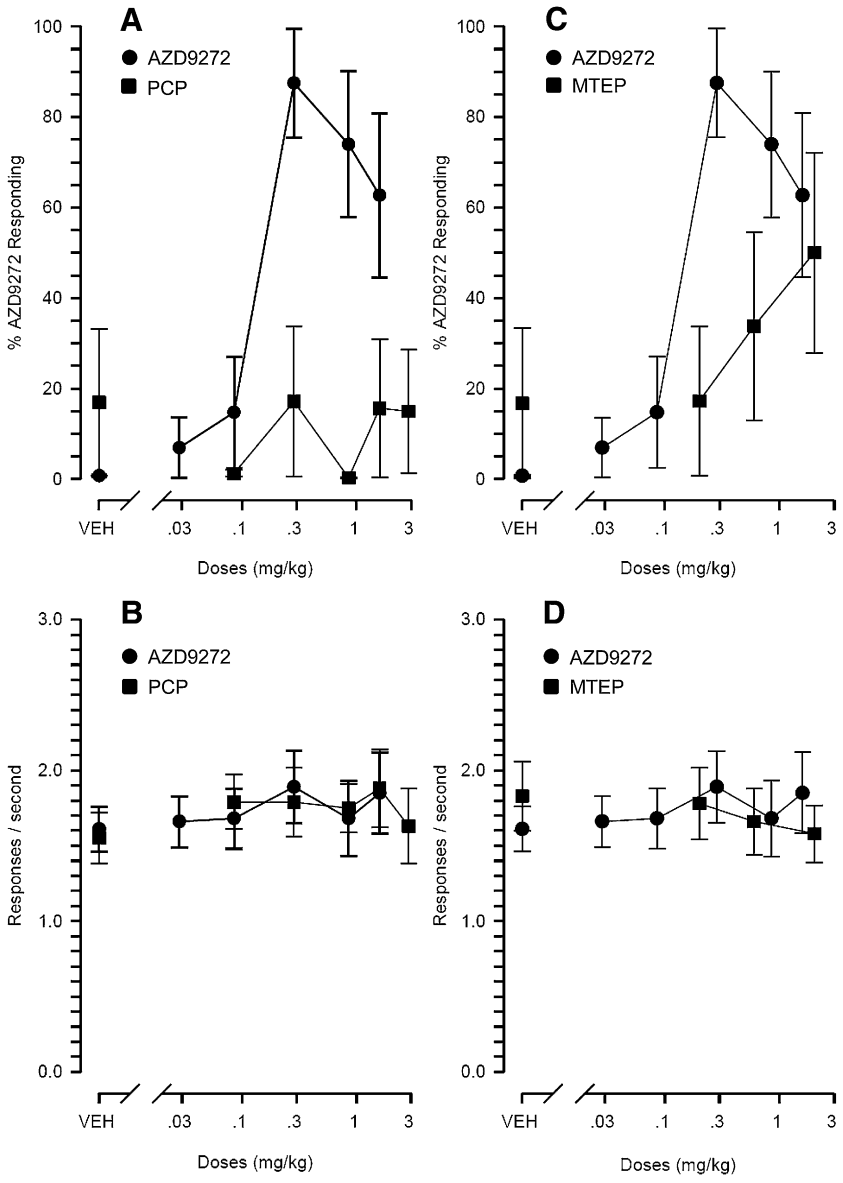

Fig. 5. Percentage of AZD9272-appropriate responding (mean ± S.E.M.) (A and C) and responses per second (mean ± S.E.M.) (B and D) in male Wistar rats trained to discriminate AZD9272 (1.6 mg/kg, 60 minutes after p.o. administration) from no drug. (A and B) ●, AZD9272 (60 minutes after p.o. administration); ■, PCP (15 minutes after i.p. administration). (C and D) ●, AZD9272 (60 minutes after p.o. administration); ■, MTEP (15 minutes after i.p. administration). VEH, vehicle.

et al., 1995; Bondareva et al., 2002). Further studies are needed to better understand the interactions between CB1 and mGluR5 receptors, and as it has been shown that mGluR receptors drive the endocannabinoid system in the hippocampus (Varma et al., 2001), the implications of this finding for the psychoactive effects produced by mGluR5 antagonists need further study.

We have previously shown that MTEP was readily established as a discriminative stimulus in rats, that the mGluR5 antagonists fenobam, MPEP, and MTEP were of similar potencies and efficacies, and that (+)-MK-801 [(5*S*,10*R*)-(+)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5,10-imine maleate] and memantine may cause at most partial MTEP-like discriminative effects (Swedberg et al., 2014).

The present findings extend the body of evidence suggesting that the lack of clear similarities of mGluR5 antagonists to known drugs of abuse indicates that mGluR5 antagonists may produce their own psychoactive profile. Consistent with reports of psychotomimetic effects of mGluR5 antagonists in humans (see *Introduction*) and with our previous findings (Swedberg et al., 2014), it can be concluded that mGluR5 antagonists can cause psychotomimetic effects, and that these effects are different from those caused by NMDA antagonists and are likely mediated by different mechanisms.

To further explore the possible involvement of NMDA antagonism in mediating the psychoactive effects of mGluR5 antagonists, the sensitivity of the drug discrimination method was exploited. It has been shown that training conditions can be modified in various ways to alter the sensitivity. For example, the fentanyl generalization gradients flattened and the ED_{50} values decreased with lower fentanyl training doses (Colpaert et al., 1980). Establishing a discrimination between two sedative/hypnotic drugs that would generalize to each other in a standard drug versus no-drug discrimination task was not only shown to be possible, but the outcome in terms of generalization of drugs and doses was also changed (Järbe and Swedberg, 1998). When training pigeons to discriminate between two drugs of different classes (cocaine and morphine),

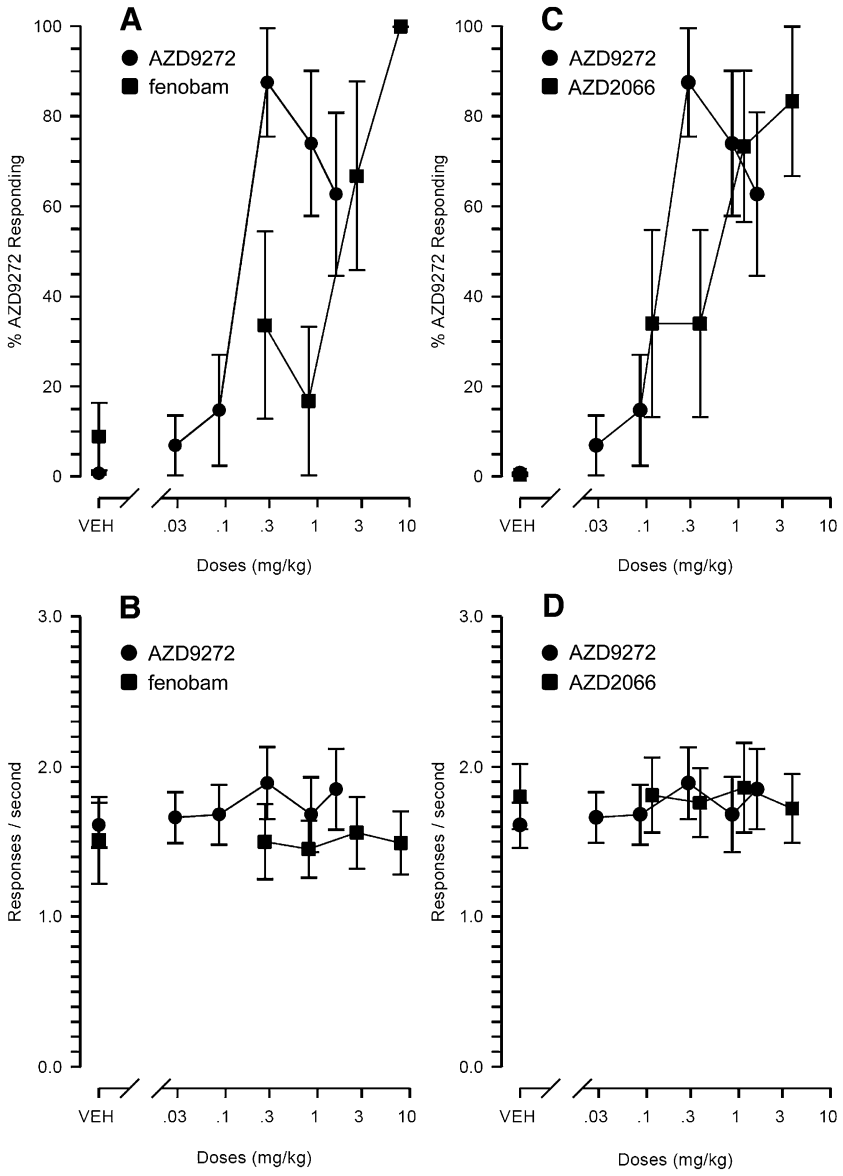

Fig. 6. Percentage of AZD9272-appropriate responding (mean ± S.E.M.) (A and C) and responses per second (mean ± S.E.M.) (B and D) in male Wistar rats trained to discriminate AZD9272 (1.6 mg/kg, 60 minutes after p.o. administration) from no drug. (A and B) ●, AZD9272 (60 minutes after p.o. administration); ■, fenobam (30 minutes after p.o. administration). (C and D) ●, AZD9272 (60 minutes after p.o. administration); ■, AZD2066 (60 minutes after p.o. administration). VEH, vehicle.

it was shown that the training doses of the two drugs were important for the shape of the generalization gradients (Swedberg and Järbe, 1985), and when a third choice (saline) was introduced into the task, the pattern of responding changed not only to the training drugs but also to novel test drugs (Swedberg and Järbe, 1986). Based on the dynamics of the drug discrimination task described earlier, it was hypothesized that lowering the training dose of PCP would make the assay more sensitive, evidenced by a lowering of the ED_{50} for PCP, and thereby detect any weak NMDA-antagonist properties heretofore undetected in mGluR5 antagonists with the potential to interact, directly or indirectly, with NMDA-antagonist mechanisms to cause psychoactive effects.

Therefore, in an effort to increase the sensitivity of the PCP discrimination, the training dose in the present study was lowered in comparison with previous studies. In the present study, 1.6 mg/kg was used as the training dose as compared with 2.8 mg/kg (Swedberg et al., 2014) and 3.0 mg/kg (Swedberg et al., 1995). Comparing the three studies, the ED_{50} values of PCP decreased with PCP-training dose, 1.75 mg/kg (Swedberg et al., 1995), 1.55 mg/kg (Swedberg et al., 2014), and 0.6 mg/kg (present study), demonstrating a leftward shift of the PCP dose-response curve, most likely attributable to an increased sensitivity to NMDA-antagonist effects. Despite the increased sensitivity of the assay, AZD9272 in the present study did not cause PCP-appropriate responding to any greater extent than did MTEP in a previous study using the higher PCP training dose (2.8 mg/kg; Swedberg et al., 2014), further supporting the conclusion that the discriminative effects of mGluR5 antagonists are selective and appear not to depend on NMDA receptors.

The present data show that, as with MTEP (Swedberg et al., 2014), the mGluR5 antagonist AZD9272 could also be established as a discriminative stimulus when training days were separated by drug-free days to allow for the elimination of the compound. The 50% maximum responding caused by MTEP is most likely attributable to an insufficient increase in the dose range tested. The monotonic increase in AZD9272-appropriate responding, previous results with mGluR5 antagonists in drug discrimination studies (Swedberg et al.,

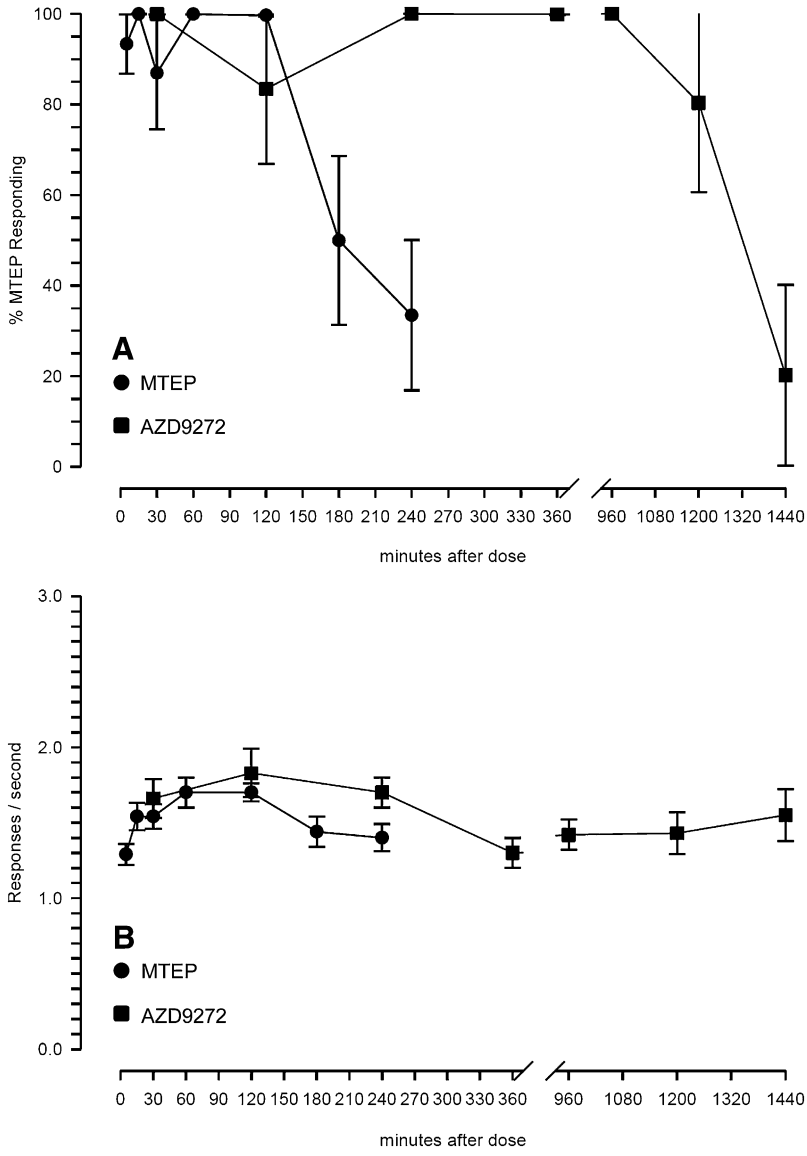

Fig. 7. Percentage of MTEP-appropriate responding (mean ± S.E.M.) (A) and responses per second (mean ± S.E.M.) (B) in male Wistar rats trained to discriminate MTEP (2.0 mg/kg, 30 minutes after i.p. administration) from no drug. ●, MTEP (p.o. administration); ■, AZD9272 (after p.o. administration).

2014), and the AZD9272 generalization to MTEP and the fenobam generalization to AZD9272 in the present study suggest that with higher MTEP doses, higher AZD9272-appropriate responding would be expected. The time course of the discriminative effects of MTEP and AZD9272 was assessed in rats discriminating MTEP from no drug, showing a 7-fold longer $t_{1/2}$ of AZD9272 (21.93 hours) compared with MTEP (3.23 hours). The long discriminative half-life of AZD9272 was confirmed in the AZD9272 discrimination (24.30 hours). Indeed, the present data demonstrated a very long duration of action of AZD9272 in both the MTEP and AZD9272 discrimination assays, consistent with t_{max} values of 3–5 hours in rats (Raboisson et al., 2012a) and a duration of action in excess of 9 hours after a 3-mg dose in humans

TABLE 3
Discriminative half-life of MTEP and AZD9272 in rats trained to discriminate MTEP or AZD9272 from no drug

Compound and Dose	Drug Discrimination	Discriminative $t_{1/2}$					
		Hours (95% CL)			Minutes (95% CL)		
		Mean	Lower	Upper	Mean	Lower	Upper
mg/kg							
MTEP (2.00)	MTEP	3.23	2.76	3.78	194	165.8	227.0
AZD9272 (2.84)	MTEP	21.93	20.75	23.18	1316	1245	1391
AZD9272 (2.84)	AZD9272	24.30	17.42	33.90	1458	1045	2034

CL, confidence limit.

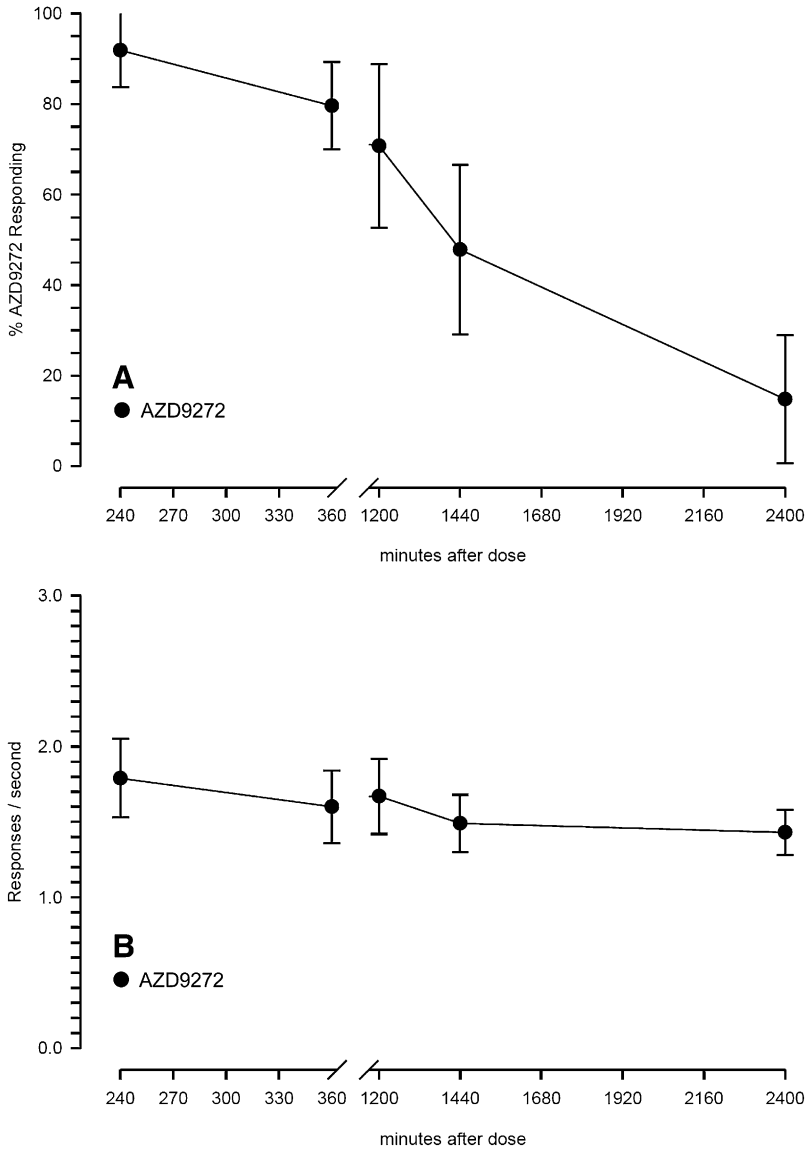

Fig. 8. Percentage of AZD9272-appropriate responding (mean ± S.E.M.) (A) and responses per second (mean ± S.E.M.) (B) in male Wistar rats trained to discriminate AZD9272 (1.6 mg/kg, 60 minutes after p.o. administration) from no drug. ●, AZD9272 (after p.o. administration).

(Kågedal et al., 2012). However, it should be noted that the E_{max} for the discriminative effects of AZD9272 occurred at 30 minutes, which was indeed the first time point studied, thereby greatly preceding the pharmacokinetic t_{max} of 3–5 hours observed in the rat (Raboisson et al., 2012a). This result demonstrates that the E_{max} of the discriminative effect is a function of the rising part of the dose/concentration response curve for its initiation and not the pharmacokinetic t_{max}.

In conclusion, these data show that the discriminative effects of AZD9272 and AZD2066 are mGluR5 antagonist–mediated and, together with previous findings with other mGluR5 antagonists (Swedberg et al., 2014), demonstrate that NMDA-antagonist mechanisms are not involved in mediating these effects, but that the discriminative and psychoactive properties of mGluR5 antagonists represent a class effect.

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Authorship Contributions

Participated in research design: Swedberg, Raboisson.
Conducted experiments: Swedberg.
Performed data analysis: Swedberg.
Wrote or contributed to the writing of the manuscript: Swedberg.

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