Psychopharmacology (2015) 232:3327–3344
DOI 10.1007/s00213-015-3984-0

ORIGINAL INVESTIGATION

Dissociable effects of mGluR5 allosteric modulation on distinct forms of impulsivity in rats: interaction with NMDA receptor antagonism

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Received: 23 January 2015 / Accepted: 29 May 2015 / Published online: 12 June 2015

Abstract
Rationale Impaired *N*-methyl-D-aspartate (NMDA) receptor signalling underlies several psychiatric disorders that express high levels of impulsivity. Although synergistic interactions exist between NMDA receptors and metabotropic glutamate receptor 5 (mGluR5), the significance of this interaction for impulsivity is unknown.
Objective This study aims to investigate the effects of negative and positive allosteric mGluR5 modulation (NAM/PAM) on trait impulsivity and impulsivity evoked by NMDA receptor antagonism in rats.
Methods Motor and choice impulsivity were assessed using the five-choice serial reaction time task (5-CSRTT) and delayed-discounting task (DDT), respectively. The effects of RO4917523 and 3-[(2-methyl-1,3-thiazol-4-yl)ethynyl]pyridine (MTEP) (NAMs) and ADX47273 (PAM) were investigated in non-impulsive rats and in trait high- and low-impulsive rats. The effects of these compounds on impulsivity induced by NMDA receptor antagonism (MK801) in the 5-CSRTT were also investigated.
Results RO4917523 (0.1–1 mg/kg) decreased premature responding and increased omissions but had no effect on locomotor activity up to 0.1 mg/kg. MTEP significantly increased omissions, decreased accuracy and slowed responding but had no effect on premature responding. ADX47273 decreased premature responding at doses that had no effect on locomotor activity. MK801 increased premature responding and impaired attentional accuracy; these deficits were dose dependently rescued by ADX47273 pre-treatment. Allosteric modulation of mGluR5 had no significant effect on choice impulsivity, nor did it modulate general task performance.
Conclusions These findings demonstrate that mGluR5 allosteric modulation selectively dissociates motor and choice impulsivity. We further show that mGluR5 PAMs may have therapeutic utility in selectively targeting specific aspects of impulsivity and executive dysfunction.

Keywords 5-choice serial reaction time task · Delay discounting · Glutamate · G-Protein coupled receptors · MK801

Electronic supplementary material The online version of this article (doi:10.1007/s00213-015-3984-0) contains supplementary material, which is available to authorized users.

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Introduction

Impulsivity can be defined as a tendency to act prematurely without foresight (Dalley et al. 2011) and is a multi-faceted behavioural construct spanning several domains from impaired response inhibition to an intolerance of delayed rewards (Cardinal et al. 2004; Evenden 1999; Moeller et al. 2001a). High levels of impulsivity are symptomatic of several major neuropsychiatric disorders, including schizophrenia (Kaladjian et al. 2011; Moeller et al. 2001a), attention deficit/hyperactivity disorder (Aron and Poldrack 2005; Crunelle et al. 2013) and addiction (de Wit 2009; Ersche

et al. 2010; Hester and Garavan 2004; Lee et al. 2009; Moeller et al. 2001b). Pre-clinically, individual differences in trait impulsivity predict psychostimulant self-administration (Dalley et al. 2007; Diergaarde et al. 2008), heightened propensity for relapse (Economidou et al. 2009) and the subsequent development of compulsive cocaine self-administration (Belin et al. 2008).

Whilst much research highlights the significant involvement of the dopaminergic, serotonergic and noradrenergic systems in impulsivity (Dalley and Roiser 2012; Pattij and Vanderschuren 2008; Winstanley et al. 2006), there have been fewer studies on the role of glutamate in this area of research. Glutamate is the principal, most abundant excitatory neurotransmitter within the mammalian central nervous system and exerts its effects by activating ionotropic (iGluR) and metabotropic (mGluR) glutamate receptors (Conn and Pin 1997; Schoepp 2001). Based on sequence homology, signal transduction and electrophysiological properties, iGluRs and mGluRs have each been classified into three distinct subgroups; *N*-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and kainate iGluRs and group I (mGluR1,5), II (mGluR2,3) and III (mGluR4,6,7,8) mGluRs (Conn and Pin 1997; Nakanishi and Masu 1994; Nakanishi 1992).

Dysfunctional glutamatergic signalling has been associated with a number of neuropsychiatric disorders where impulse control deficits are prominent. For example, NMDA receptor hypofunction is postulated to contribute to the pathophysiology of schizophrenia (Deakin et al. 1989; Goff and Coyle 2001; Konradi and Heckers 2003; Lindsley et al. 2006). Thus, dysregulation of cortical glutamatergic signalling by pharmacological blockade of NMDA receptors induces schizophrenia-like symptoms in healthy volunteers and exacerbates positive and negative symptoms in schizophrenic patients (Adler et al. 1999; Krystal et al. 1994; Lahti et al. 2001; Luby 1959). In experimental animals, systemic and local administration of the NMDA receptor antagonists MK801, phencyclidine (PCP) and 3-(2-carboxypiperazine-4-yl)propyl-1-phosphoric acid (CPP) also have the common effect of increasing impulsivity in rats and mice (Agnoli and Carli 2012; Carli et al. 2004; Fletcher et al. 2011; Greco et al. 2005; Higgins et al. 2003; Paine et al. 2007).

The ubiquitous expression and global role of iGluRs in mediating fast, excitatory synaptic transmission limits their use as therapeutic targets for selectively improving impulse control deficits in humans. However, recent evidence suggests that targeting mGluRs may provide a more appropriate, subtle modulation of glutamatergic transmission. Furthermore, the heterogeneous distribution of the eight, diverse subtypes (mGluR1–8) offers an opportunity to selectively modulate glutamatergic transmission in an anatomically and functionally distinct manner (Conn and Pin 1997; Nakanishi 1992; Schoepp and Conn 2002).

In the present study, we investigated the effects of allosteric mGluR5 modulation on two distinct forms of impulsivity. We firstly investigated the effects of negative and positive allosteric mGluR5 modulation on the performance of rats on the five-choice serial reaction time task (5-CSRTT), a widely used operant task to assess sustained visual attention and impulsivity in rodents (Robbins 2002). This research was based on the rationale that mGluR5s, which are excitatory receptors and expressed widely in limbic-cortico-striatal circuitry (Romano et al. 1995; Shigemoto et al. 1993), interact synergistically with NMDA receptors (Awad et al. 2000; Campbell et al. 2004; Doherty et al. 1997; Henry et al. 2002; Homayoun and Moghaddam 2006; Kinney et al. 2003; Pisani et al. 2001). Functional coupling of NMDA and mGluR5 suggests that mGluR5s may be ideally placed to modulate impulsive behaviour sensitive to NMDA receptor transmission. To test this hypothesis, we investigated the effects of the negative allosteric modulators (NAMs), RO4917523 and 3-[(2-methyl-1,3-thiazol-4-yl)ethynyl]pyridine (MTEP), and the positive allosteric modulator (PAM), ADX47273 (de Paulis et al. 2006), on impulsivity evoked by the NMDA receptor antagonist MK801 and compared these effects with those in rats expressing trait-like impulsivity. We next investigated the specificity of allosteric mGluR5 modulation by evaluating the effects of these compounds on a delay-discounting task (DDT) to assess impulsive choice for immediate, small-magnitude rewards versus larger, but delayed rewards (Ainslie 1975; Evenden 1999).

Material and Methods

Subjects

Male Lister-hooded rats, weighing 200–250 g at the start of training, were obtained from Charles River (UK and Germany) and assessed for performance on the 5-CSRTT and DDT. A separate group of rats weighing 250–300 g was purchased from Charles River (Germany) and used for the assessment of locomotor activity. All rats were housed in groups of four, under a 12-h light/dark cycle with food and water initially available ad libitum. All rats were permitted at least 5 days acclimatisation before training on the 5-CSRTT and DDT commenced. Food restriction was initiated when body weights were at least 300 g. Body weight was then maintained at 80–85 % of free-feeding weight. All training and testing commenced between 0700 and 1200 hours, 5–6 days a week. All experimental procedures were carried out in accordance with the United Kingdom Animals (*Scientific Procedures*) Act, 1986. Studies carried out in Germany were authorised by the Local Animal Care and Use Committee in accordance with local animal care guidelines, AAALAC regulations and the USDA Animal Welfare Act.

Locomotor activity

Locomotor activity was assessed using eight Tru-Scan arena chambers (Coulbourn Instruments, Lehigh Valley, PA, USA), each with two sensing rings (Tru Scan Photo Beam sensor ring—Coulbourn Instruments, Lehigh Valley, PA, USA), detecting activity in three orthogonal planes. Mean distance travelled (cm), total rearing events and rearing time (s) were recorded.

Five-choice serial reaction time task

Twelve five-choice operant chambers (UK) and 32 five-choice operant chambers (Germany) (Med Associates Inc, St. Albans, VT, USA) enclosed in sound-attenuating, fan-ventilated cubicles were used, as described previously (Bari et al. 2008; Carli et al. 1983). Briefly, each chamber consisted of five evenly spaced apertures (2.5×2.5×4 cm) containing an LED light, set into a curved wall at the rear of the chamber. A centrally located food magazine was located on the opposite wall of the chamber, into which 45-mg reward pellets could be delivered (Sandown Scientific, UK). Infrared beams located at the entrance of each aperture and the food magazine allowed detection of nose pokes. Task parameters and data collection were controlled by 'Whisker' software (UK) (Cardinal and Aitken 2010) and Med Associates Inc. software (St. Albans, VT, USA) (Germany).

The 5-CSRTT training protocol has been described previously (Bari et al. 2008; Carli et al. 1983). Each training session consisted of 100, self-paced, trials and lasted no longer than 30 min. At later training stages, 100 trials were normally completed within 20 min. Training sessions started with the illumination of the house and magazine lights, and by the delivery of a 45-mg reward pellet (Sandown Scientific, UK). Collection of the reward initiated the first trial. A single trial consisted of an inter-trial interval (ITI), followed by the pseudo-random illumination of one of the five apertures for a fixed duration [stimulus duration (SD)]. Following stimulus detection, a nose poke to the corresponding aperture, within a fixed time interval [limited hold (LH)], was required for reward delivery. Premature responses made during the ITI, incorrect responses and responses made outside the LH (an omission) resulted in a timeout (TO) period, during which time no food was delivered, and the house light was extinguished for 5 s.

Premature responding was calculated as a percentage of completed trials (correct+incorrect+omissions). A premature response was deemed an incomplete trial and re-set the current trial. Percentage accuracy was defined as the number of correct responses divided by the sum of correct and incorrect responses. Perseveration was calculated as the number of additional responses made in the same aperture, following a correct response. Omissions were calculated in terms of the percentage of completed trials.

Animals were deemed to be trained when they completed ≥50 correct trials with ≥70 % accuracy and ≤20 % omissions (SD, 0.7 s; ITI, 5 s; and LH, 5 s). At this stage, perseverative responses (additional responses made to the same aperture following a correct response) resulted in a 5-s TO and loss of food reward.

Impulsivity screening

Screening for impulsivity consisted of three or four 'challenge' training sessions where the ITI was extended to 7 s to increase the occurrence of premature responses (Dalley et al. 2007). Each challenge session was separated by four baseline training sessions, where task parameters were restored to the training configuration (ITI, 5 s). The mean percentage of premature responses made by each rat across the challenge sessions was calculated. Rats were excluded from the study if they exhibited poor or unstable performance or failed to complete 100 trials on three of the challenge sessions. All rats were ranked, based on the mean per cent premature responses, from highly to low impulsive. The upper and lower 15th centiles of premature responders were termed high-impulsive (HI) and low-impulsive (LI) rats, respectively. The remaining rats were categorised as mid-impulsive (MI) and were used for studies involving MK801. The MK801 studies were restricted to MI rats only. This was to avoid ceiling effects on premature responding that might occur in HI rats. Furthermore, the behavioural effects of MK801 are known to be highly variable; restricting the selection of animals to the middle of the behavioural distribution was designed to generate a more homogenous cohort of animals.

Delay discounting

Thirty-two operant chambers, enclosed in sound-attenuating, fan-ventilated cubicles (Med Associates Inc, St. Albans, VT, USA) were used, as described previously (Mar and Robbins 2007; Winstanley et al. 2003). Briefly, each chamber consisted of two retractable levers located on either side of a centrally located food magazine into which 45-mg reward pellets (Sandown Scientific, UK) could be delivered. A stimulus light was located above each lever, and an infrared beam at the entrance of the food magazine detected reward collection. Task parameters and data collection were controlled by Med Associates Inc. software (St. Albans, VT, USA).

Pre-training Rats were habituated to the operant chambers for 2 days and trained under a fixed ratio-1 schedule of reinforcement (FR1). During these sessions, both levers were extended, the lever lights were illuminated and a press on either lever resulted in the delivery of a reward pellet. Rats were

required to reach a criterion of 60 lever presses (30 presses on each lever) within 60 min. A simple version of the delay-discounting task was then implemented; trials were initiated every 40 s with the illumination of the house and magazine light. Rats were required to make a nose-poke response in the food magazine within 10 s of trial initiation in order to trigger the presentation of a lever and corresponding lever light. Responding on the lever within 10 s resulted in the retraction of the lever and the lever light being extinguished, the illumination of the food magazine and the delivery of a single reward pellet. Both levers were presented an equal number of times in each session. Rats were required to reach a criterion of at least 60 successfully completed trials in 60 min.

Delay-discounting task Each training session consisted of 6 blocks of 10 trials (60 trials in total) with each trial lasting exactly 72 s. Each block began with four forced choice trials whereby the left and right lever were each presented twice in a random order. Throughout the task, responding on the right lever resulted in the immediate delivery of a single reward pellet. Responses on the left lever resulted in the delayed delivery of three reward pellets, with increasing delay across blocks from 0 s (block 1), 2 s (block 2), 4 s (block 3), 8 s (block 4), 16 s (block 5) and 32 s (block 6).

Following the completion of four forced trials, six free choice trials were introduced. As in the pre-training protocol, each trial was initiated by the illumination of the house and magazine light. Rats were required to make a nose-poke response in the food magazine within 10 s to trigger the presentation of both levers and lever lights. A failure to respond on either lever within 10 s (an omission) resulted in the retraction of both levers with all lights extinguished and an inter-trial interval (ITI) initiated before the next trial. Responding on one of the levers within 10 s resulted in the retraction of both levers with all lights extinguished. Reward delivery was preceded by the illumination of the magazine light either immediately or after the chosen delay. The length of the ITI was dependent on the choice of the immediate or delayed lever, and followed reward delivery to ensure each trial was exactly 72 s in duration.

Experiment 1: effects of RO4917523, MTEP and ADX47273 on locomotor activity

Three separate groups of rats were assessed for locomotor activity. All rats were habituated in an annex to the testing room, prior to testing. RO4917523, MTEP or ADX47273 administration occurred during the habituation period. Rats received 0.06, 0.1 or 1 mg/kg RO4917523, p.o, 2 h before testing; 3, 10 or 30 mg/kg MTEP, i.p, 15 min before testing; or 60, 80 or 100 mg/kg ADX47273, p.o, 1.5 h before testing. Rats were then placed into the locomotor activity chambers to freely explore in total darkness for 1 h.

Experiment 2: effects of RO4917523, MTEP and ADX47273 on 5-CSRTT performance

Rats that had not undergone impulsivity screening (non-selected rats), but showed stable performance on the 5-CSRT T, were used to assess the effects of (i) RO4917523 (0.03, 0.06, 0.1, 0.3 and 1 mg/kg; p.o), administered 2 h prior to behavioural assessment; (ii) MTEP (1, 3, 10 and 30 mg/kg MTEP; i.p), administered 15 min prior to testing; and (iii) ADX47273 (40, 60, 80 and 100 mg/kg ADX47273; p.o), administered 1.5 h prior to testing.

Experiment 3: effects of RO4917523, MTEP and ADX47273 on 5-CSRTT performance in HI and LI rats

Twelve HI and 12 LI rats were used to assess the effects of 0.03, 0.1 and 0.3 mg/kg RO4917523 and 40, 60, 80 and 100 mg/kg ADX47273 on baseline 5-CSRTT performance. One LI rat was excluded from the ADX47273 study due to a decline in baseline performance. The same rats were also used to assess the effects of ADX47273 on 5-CSRTT performance under a 7-s ITI. Increasing the ITI increases the occurrence of a premature response and thus increases baseline premature responding. In a separate cohort, nine HI and 11 LI trained rats were used to assess the effects of 1, 3, 10 and 30 mg/kg MTEP.

Experiment 4: effects of RO4917523, MTEP and ADX47273 pre-treatment on MK801-modulated 5-CSRTT performance

Since the behavioural response to MK801 administration, as measured on the 5-CSRTT, was variable between different cohorts of rats, two MK801 dose–response studies were carried out (Online Resource 1, Fig. S1 and Fig. S2). This ensured that the dose of MK801 was tailored to each cohort of rats. Based on the results from these studies, two different doses of MK801, 0.03 and 0.06 mg/kg, were used in these studies. In addition, the experimental design was altered from a within-subject's design to a between-subject's design to eliminate the possibility of varying effects of repeated MK801 administration. Specifically, in preliminary studies, we found that repeated injections of MK801 led to a reduced behavioural response. Therefore, a between-subject's design was adopted so that each rat received a single dose of MK801 in the study.

Sixteen MI rats were used in a within subject's design to assess the effects of 0.06 mg/kg MK801 and its vehicle (0.9 % NaCl, sterile saline) on 5-CSRTT performance following 0.03, 0.1, 0.3 and 1 mg/kg RO4917523 or 1, 3, 10 and 30 mg/kg MTEP pre-treatment. RO4917523 was administered p.o, 2 h prior to testing. MTEP was administered i.p,

15 min prior to testing. MK801 was administered s.c, 10 min before testing.

Fifty-five MI rats were tested using a between-subject's design to investigate the effects of 0.03 mg/kg MK801 and its vehicle (0.9 % NaCl, sterile saline) on 5-CSRTT performance following 80 and 100 mg/kg ADX47273 pre-treatment. Baseline performance was counter-balanced between vehicle and MK801-treated rats. MK801 was administered s.c., 10 min prior to testing.

Experiment 5: effects of RO4917523, MTEP and ADX47273 on delay discounting

A separate group of rats that showed a stable performance in the delayed-discounting task were used to assess the effects of (i) RO4917523 (0.03, 0.1 and 0.3 mg/kg; p.o; n=24), administered 2 h prior to behavioural assessment; (ii) MTEP (1, 3, 10 and 30 mg/kg MTEP; i.p; n=18), administered 15 min prior to testing; and (iii) ADX47273 (40, 60, 80 and 100 mg/kg ADX47273; p.o; n=20), administered 1.5 h prior to testing.

Drugs

Drugs were administered according to a randomised Latin square design, unless otherwise stated. RO4917523 was synthesised by Boehringer Ingelheim Pharma GmBH & Co. KG and dissolved in 10 % Tween80 (0.1 %) (*v*/*v*) and 90 % Natrosol (0.5 %) and administered p.o. The selected dose range and pre-treatment time were based on in-house pharmacokinetic (PK) data (Online Resource, Table S1). MTEP was synthesised by Boehringer Ingelheim Pharma GmBH & Co. KG, dissolved in Tween80 (10 %) (*v*/*v*) and administered i.p. The selected dose range and pre-treatment time were based on published literature (Gass et al. 2008; Varty et al. 2005). ADX47273 was synthesised by Boehringer Ingelheim Pharma GmBH & Co. KG, dissolved in 10 % Tween80 (0.1 %) (*v*/*v*) and 90 % Natrosol (0.5 %). The selected dose range and pre-treatment time were based on in-house PK data (Online Resource, Table S1). Each compound was characterised using in vitro fluorometric imaging to confirm their allosteric properties and to validate their use as tool compounds in assessing impulsivity in rats (Online Resource, Fig. S4). We confirmed that both RO4917523 and MTEP negatively modulate mGluR5, achieving an IC_{50} of 4.53 and 19.3 nM, respectively (Fig. S4a). Furthermore, we confirmed positive allosteric modulation of mGluR5 with ADX47273, achieving a maximal glutamate EC_{50} shift factor of 21.2 at 3 μM. (Fig. S4b). MK801 was purchased from Sigma Aldrich (UK and Germany), dissolved in sterile saline and adjusted to pH 7 with 1 M NaOH. The selected dose range and pre-treatment time were based on published literature (Fletcher et al. 2011).

Data analysis

Data were analysed using SPSS (version 21) and GraphPad Prism 6. Locomotor activity data are expressed as the distance travelled, rearing frequency and rearing time over a test period of 1 h. Data were analysed using one-way analysis of variance (ANOVA) followed by Dunnett's post hoc analysis where indicated by a significant main effect of dose. Behavioural data on the 5-CSRTT were analysed using repeated measures ANOVA, unless otherwise stated. In studies where baseline and evoked impulsivity was not assessed (non-selected rats), drug dose served as a within-subject's factor. In studies involving HI and LI rats, group served as a between-subject's factor and drug dose served as a within-subject's factor. A within-subject's design was used for the RO4917523 and MTEP studies in MI rats. Dunnett's and Bonferroni post hoc analyses with paired Student's t test were used where indicated by significant main effects and interactions. A between-subject's design was used for the ADX47273 study in MI rats with Bonferroni post hoc analysis for multiple comparisons. Delayed-discounting data were analysed by repeated measures ANOVA with delay and drug dose as within-subject factors. Violation of the requirement of homogeneity of variance, assessed by Mauchley's sphericity test and confirmed by chi-squared analysis was corrected using the Geisser–Greenhouse epsilon to adjust the degrees of freedom. Statistical significance was set at $p<0.05$.

Results

Experiment 1: effects of RO4917523, MTEP and ADX47273 on locomotor activity

Table 1 summarises the effects of RO4917523, MTEP and ADX47273 on locomotor activity.

RO4917523 produced a significant effect on total rearing activity ($F_{3,28}=10.5, p<0.001$), rearing time ($F_{3,28}=13.4, p<0.001$) and distance travelled ($F_{3,28}=7.26, p<0.001$). Although rearing activity and distance travelled were unaffected by lower doses of RO4917523 (0.06 and 0.1 mg/kg), a dose of 1 mg/kg significantly decreased these variables ($p<0.001$ and $p<0.05$ respectively). The time spent rearing was significantly reduced by all doses (0.06 mg/kg, $p<0.01$; 0.1 mg/kg, $p<0.05$; 1 mg/kg, $p<0.001$). MTEP treatment also had a significant decremental effect on rearing activity ($F_{3,26}=10.1, p<0.001$), rearing time ($F_{3,26}=23.6$, $p<0.001$) and distance travelled ($F_{3,26}=8.51$, $p<0.001$). These reductions reached significance at every dose tested (rearing activity: 3 and 10 mg/kg, $p<0.01$; 30 mg/kg, $p<0.001$; distance travelled: 3 and 10 mg/kg, $p<0.05$; 30 mg/kg, $p<0.001$; rearing time: 3, 10 and 30 mg/kg, $p<0.001$). Although ADX47273 significantly decreased rearing activity ($F_{3,20}=5.2$, $p<0.01$) at 60 mg/kg ($p<0.05$), neither distance travelled nor rearing time were affected by this dose. Furthermore, locomotor

Table 1 Summary of the effects of RO4917523, MTEP and ADX47273 on locomotor activity

	Dose (mg/kg)	Rearing (sum)	Distance (cm)	Rearing time (s)
RO4917523	0	157.5±14.8	5433±456.8	406.4±42.1
	0.06	116.5±20.4	5851±570.3	220.1±36.4**
	0.1	123.9±18.4	6832±548.2	252.8±51.3*
	1	36.1±6.1***	3552±454.4*	65.5±10.8***
MTEP	0	141.0±27.2	5419±429.1	427.2±58.9
	3	47.1±15.5**	3708±382.5*	122.4±27.3***
	10	40.7±9.9**	3861±506.7*	96.1±24.7***
	30	15.3±5.8***	2342±411.9***	28.1±11.4***
ADX47273	0	157.2±11.0	5428±272.9	579.1±66.87
	60	112.7±13.4*	4314±376.2	442.3±83.1
	80	122.2±3.0	4579±348.9	463.3±48.8
	100	89.5±17.1**	3777±310.3**	302.5±41.3*

Rearing activity, rearing time and distance travelled were measured. Values represent mean±SEM (n=6–8 per group). One-way ANOVA, Dunnett's *post hoc* test

*p<0.05, **p<0.01, ***p<0.001 versus vehicle control

activity was unaffected by ADX47273 at 80 mg/kg, whereas rearing activity, distance travelled ($F_{3,20}$=4.4, p<0.05) and rearing time ($F_{3,20}$=3.3, p<0.05) were reduced following 100 mg/kg ADX47273 administration (p<0.01, p<0.01 and p<0.05, respectively).

Experiment 2: effects of RO4917523, MTEP and ADX47273 on 5-CSRTT performance

As shown in Fig. 1a, RO4917523 significantly decreased premature responding on the 5-CSRTT ($F_{3,27}$=8.4, p<0.001, X^2=34, GG ε=0.55) at 0.1 mg/kg (p<0.05) and 0.3 and 1 mg/kg (p<0.01). However, at these same doses, RO4917523 also significantly increased omissions ($F_{2,22}$=43.3, p<0.001, X^2=43, GG ε=0.56) (0.1 mg/kg, p<0.05; 0.3 and 1 mg/kg, p<0.001) compared with vehicle treatment (Fig. 1b). Although the accuracy of responding was unaffected by RO4917523 (Fig. 1c), the speed of responding (Fig.1d) was significantly decreased ($F_{2,18}$=11.43, p<0.001, X^2=25, GG ε=0.44) following the administration of 0.3 and 1 mg/kg (p<0.01 and p<0.05 respectively) compared with the vehicle treated group. However, latencies to collect reward were unaffected by RO4917523.

A summary of the effects of MTEP on 5-CSRTT performance is shown in Table 2. MTEP had no significant effect on premature responding nor did it affect attentional accuracy. However, omissions were significantly increased ($F_{4,40}$=10.1, p<0.001) at 3 mg/kg (p<0.01) and higher doses (10 mg/kg, p<0.05; 30 mg/kg, p<0.001) together with slower latencies to respond ($F_{4,40}$=3.9, p<0.01) at all doses tested compared with the vehicle treated group (1 and 3 mg/kg, p<0.01; 10 and 30 mg/kg, p<0.05).

ADX47273 selectively decreased premature responding ($F_{4,44}$=3.1, p<0.05), reaching significance at 80 (p<0.05) and 100 (p<0.01) mg/kg (Fig. 2a). The observed maximal decrease in premature responding was achieved by 100 mg/kg, reducing premature responding from a mean of 14.2±2.9 % under vehicle conditions to a mean of 8.4±2.2 %. ADX47273 had no significant effect on other behavioural variables (Fig. 2b–d).

Experiment 3: effects of RO4917523, MTEP and ADX47273 on 5-CSRTT performance in HI and LI rats

The effect of RO4917523 on 5-CSRTT performance in HI and LI rats is shown in Fig. 3. ANOVA revealed a significant decrease in premature responding (main effect of dose, $F_{2,47}$=33.9, p<0.001, X^2=19, GG ε=0.71) (Fig. 3a). This effect depended on impulsivity sub-group (dose×group; $F_{2,47}$=6.0, p<0.01, X^2=19, GG ε=0.71). Thus, whilst there was an overall significant difference in premature responding between HI and LI rats (main effect of group, $F_{1,22}$=15.9, p<0.01), and specifically following vehicle and 0.03 mg/kg RO4917523 treatment (p<0.001), the contrast in premature responding between HI and LI rats was abolished at higher doses, with premature responding also being significantly reduced compared with the relative vehicle-treated controls (HI: 0.1 and 0.3 mg/kg p<0.001; LI: 0.1 and 0.3 mg/kg p<0.01). Although accuracy was unaffected (Fig. 3c), a significant increase in omissions was observed following the administration of RO4917523 (main effect of dose, $F_{2,37}$=82.6, p<0.001, X^2=43, GG ε=0.57); this effect reached significance in HI and LI rats at 0.1 and 0.3 mg/kg compared with vehicle-treated rats (p<0.001) (Fig. 3b). Omissions were also consistently significantly higher in LI rats compared with HI rats (main effect of group; $F_{1,22}$=8.0, p<0.05). The latency to respond correctly was significantly increased (main effect of dose, $F_{1,31}$=20.0,

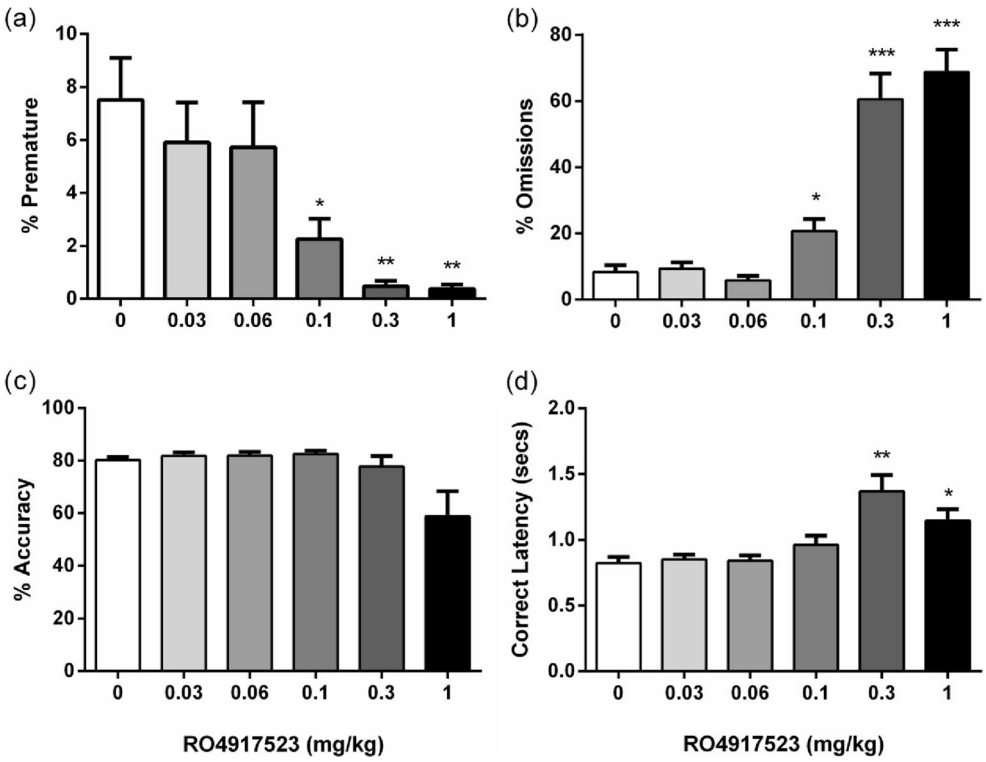

Fig. 1 Effect of RO4917523 on 5-CSRTT performance: **a** per cent premature responses, **b** per cent omissions, **c** per cent accuracy and **d** correct response latency (s). *Bars* represent means±SEM (n=11). Repeated measures one-way ANOVA, Dunnett's post hoc test. *p<0.05, **p<0.01, ***p<0.001 versus vehicle control

p<0.001, X^2=42, GG ε=0.48) following 0.1 and 0.3 mg/kg RO4917523 treatment compared with vehicle (p<0.001) and was consistently higher in LI compared with HI rats (main effect of group; $F_{1,22}$=6.6, p<0.05) (Fig. 3d). However, magazine latencies were unaffected.

As summarised in Table 3, MTEP appeared to produce biphasic effects on premature responding on the 5-CSRTT; an increase and decrease in premature responding was observed at lower and higher doses, respectively (main effect of dose, $F_{2,33}$=4.9, p<0.05, X^2=37, GG ε=0.47). Post hoc analysis revealed, however, that there was no significant difference between vehicle and any dose of MTEP. All doses of MTEP significantly reduced accuracy of responding in HI and LI rats compared with vehicle [(main effect of dose, $F_{2,29}$=3.7, p<0.05, X^2=61, GG ε=0.40) (1 mg/kg, p<0.01; 3 mg/kg, p<0.001; 10 and 30 mg/kg, p<0.05)]. Similarly, 3 and 30 mg/kg MTEP increased omissions [(main effect of dose, $F_{2,41}$=14.7, p<0.001, X^2=53, GG ε=0.57) (3 mg/kg, p<0.01; 30 mg/kg, p<0.001)]. MTEP also significantly decreased the speed of responding compared with the vehicle control group [(main effect of dose, $F_{2,42}$=5.4, p<0.01, X^2=32, GG ε=0.58) (1 mg/kg, p<0.05; 3 mg/kg, p<0.01; 30 mg/kg, p<0.001)].

The effects of ADX47273 on 5-CSRTT performance in HI and LI rats are shown in Fig. 4a–d. ADX47273 significantly decreased premature responding in HI and LI rats (main effect of dose, $F_{3,59}$=5.3, p<0.01, X^2=20, GG ε=0.70) at all doses tested (p<0.05) and had no significant effect on omissions (Fig. 4b) or accuracy (Fig. 4c). Despite correct response latencies being consistently slower in LI rats compared with HI rats (main effect of group, $F_{1,21}$=10.7, p<0.01), ADX47273 significantly increased the latency to respond correctly in both HI and LI rats (main effect of dose, $F_{4,84}$=5.1, p<0.01) (Fig. 4d) at the three highest doses tested (60 mg/kg, p<0.01; 80 mg/kg, p<0.001; and 100 mg/kg, p<0.01). Extending the ITI to 7 s robustly

Table 2 The effect of MTEP on 5-CSRTT performance (n=11) [per cent premature responses, per cent omissions, per cent accuracy and correct latency (s)]

MTEP (mg/kg)	0	1	3	10	30
Premature (%)	5.3±1.2	4±1.7	3.95±3.1	5.2±2.0	2.5±0.9
Accuracy (%)	81.4±1.8	80.8±1.1	80.8±1.6	81.1±1.7	82.3±2.2
Omissions (%)	4.1±0.8	13.8±3.3	20.6±4.0**	16.5±3.0*	30.2±5.8***
Correct latency (s)	0.842±0.05	1.079±0.09**	1.074±0.07**	1.045±0.07*	1.045±0.07*

Values represent mean±SEM. Repeated measures one-way ANOVA, Dunnett's post hoc test

*p<0.05, **p<0.01, ***p<0.001 versus vehicle control

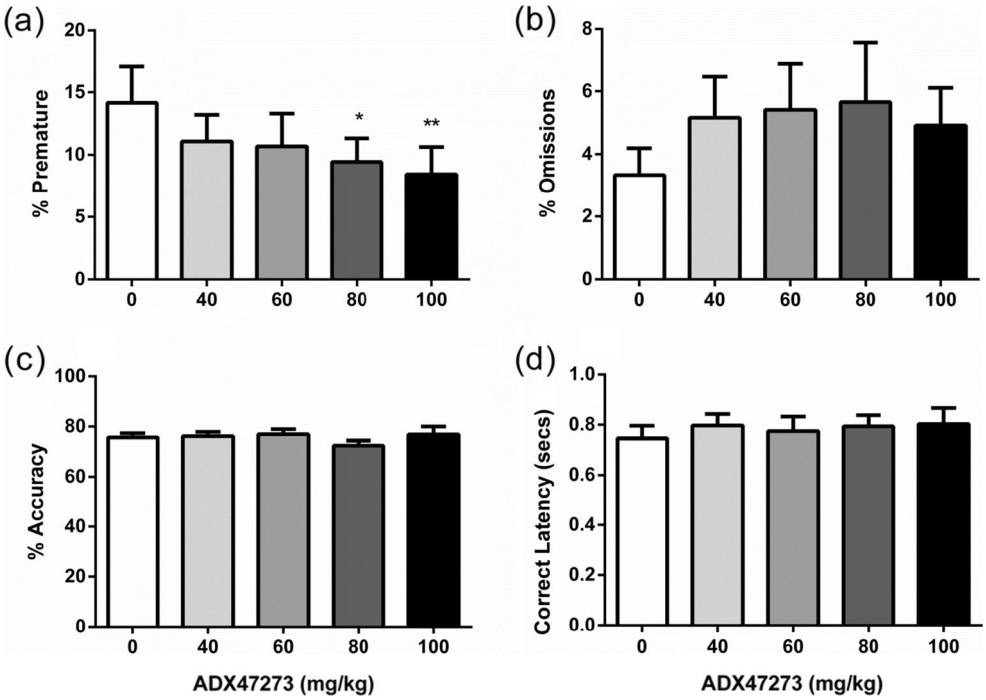

Fig. 2 Effect of ADX47273 on 5-CSRTT performance: **a** per cent premature responses, **b** per cent omissions, **c** per cent accuracy and **d** correct response latency (s). *Bars* represent mean±SEM (n=11). Repeated measures one-way ANOVA, Dunnett's post hoc test. *p<0.05, **p<0.01, versus vehicle control

increased premature responding in vehicle-treated HI and LI rats compared with the performance under a 5-s ITI (main effect of ITI; $F_{1,21}$=32.1, p<0.001) (Fig. 4e). ADX47273 decreased premature responding in both HI and LI rats (main effect of dose, $F_{2,33}$=12.6, p<0.001, X^2=35, GG ε=0.58) under a 7-s ITI, reaching significance at 60, 80, and 100 mg/kg (p<0.01, p<0.001 and p<0.01, respectively) (Fig. 4f).

Experiment 4: interactive effects of MK801 on RO4917523, MTEP and ADX47273 pre-treatment in MI rats

Based on preliminary studies, doses of 0.03 and 0.06 mg/kg were selected for the two cohorts of rats used for the MK801 studies (Supplementary Fig. 2 and Fig. 3). Both doses

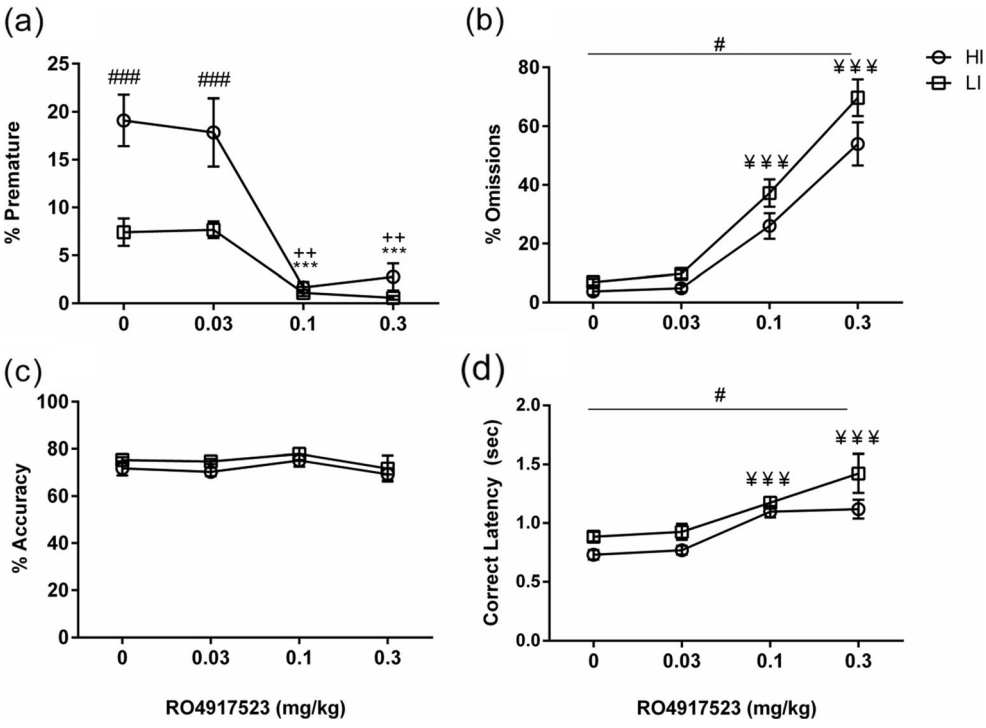

Fig. 3 Effect of RO4917523 on 5-CSRTT performance in HI (n=12) and LI (n=12) rats: **a** per cent premature responses, **b** per cent omissions, **c** per cent accuracy and **d** correct response latency (s). Data are means±SEM. Repeated measures ANOVA, Dunnett's post hoc test. $^{###}p$<0.001 HI versus LI; ***p<0.001 (HI) or ^{++}p<0.01 (LI) or $^{¥¥¥}p$<0.001 (HI and LI combined) versus relative vehicle control

Table. 3 The effect of MTEP on 5-CSRTT performance in HI (n=9) and LI (n=11) rats [per cent premature responses, per cent omissions, per cent accuracy and correct latency (s)]

MTEP (mg/kg)	0	1	3	10	30
HI					
Premature (%)	5.1±2.0	11.2±2.3	14.3±4.7	11.1±3.4	2.9±0.8
Accuracy (%)	84.8±2.5	77.3±2.0¥¥	74.2±2.3¥¥¥	66.2±8.7¥	71.8±6.1¥
Omission (%)	3.3±0.8	5.0±1.2	8.3±2.4¥¥	18.9±10.3	36.3±10.7¥¥¥
Correct latency (s)	0.565±0.232	0.684±0.051¥	0.733±0.070¥¥	0.845±0.134	0.791±0.088¥¥¥
LI	4.6±1.0	6.4±1.3	7.9±2.4	6.5±1.6	3.8±1.6
Premature (%)					
Accuracy (%)	86.3±1.1	82.2±2.0¥¥	75.9±3.4¥¥¥	70.2±7.8¥	75.7±5.2¥
Omission (%)	5.0±1.4	8.3±2.1	16.9±4.0¥¥	22.4±9.3	49.8±8.1¥¥¥
Correct latency (s)	0.652±0.041	0.722±0.067¥	0.864±0.089¥¥	0.604±0.073	1.074±0.120¥¥¥

Values represent mean±SEM. Repeated measures ANOVA, Dunnett's post hoc test

¥ p<0.05, ¥¥ p<0.01, ¥¥¥ p<0.001 HI and LI versus vehicle control

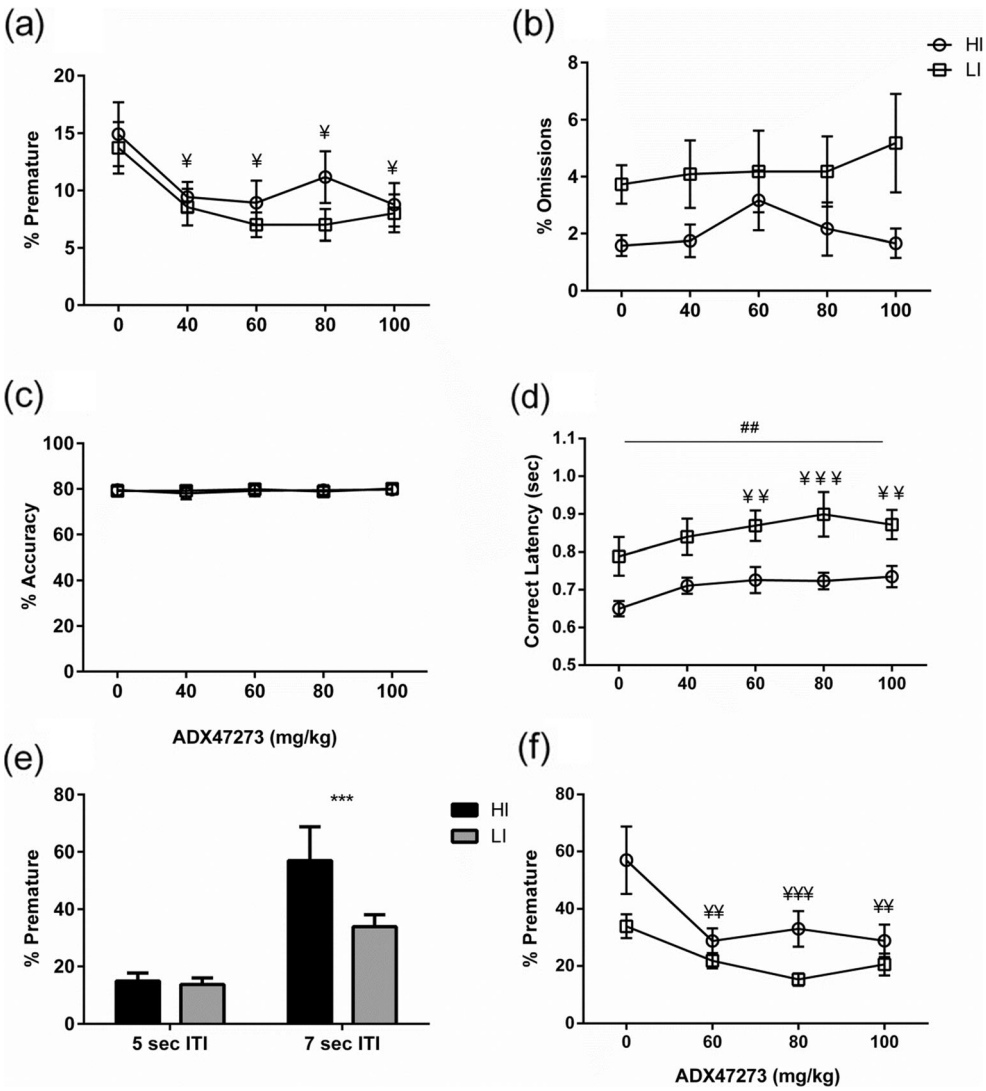

Fig. 4 Effect of ADX47273 on 5-CSRTT performance in HI (n=12) and LI (n=11) rats: **a** per cent premature responses, **b** per cent omissions, **c** per cent accuracy and **d** correct response latency (s) under a 5-s ITI. **e** Comparison of per cent premature responses in HI and LI rats at 5-s ITI versus 7-s ITI, **f** per cent premature responses under a 7-s ITI. Data represent mean±SEM. Repeated measures ANOVA, Dunnett's post hoc test. # p<0.05 HI versus LI; ¥ p<0.05, ¥¥ p<0.01, ¥¥¥ p<0.001 (HI and LI combined) versus vehicle control

increased premature responding without substantially affecting other task parameters.

The interaction of RO4917523 pre-treatment on the effects of MK801 is shown in Fig. 5. As expected, MK801 increased premature responding in MI rats (main effect of drug, $F_{1,7}$=32.5, p<0.01; Fig. 5a). In the absence of RO4917523, MK801 increased premature responding from 4.6±1.3 to 43±14.2 % (Fig. 5a). RO4917523 pre-treatment significantly modulated premature responding (main effect of dose, $F_{4,28}$=3.3, p<0.05); this effect depended on dose and the presence and absence of MK801 (dose×group interaction, $F_{4,28}$=2.9, p<0.05). Higher doses of RO4917523 (0.3 and 1 mg/kg) decreased premature responding. Post hoc analysis revealed significant differences in premature responding between vehicle- and MK801-treated rats following vehicle (p<0.01), 0.03 and 0.1 mg/kg (p<0.05) RO4917523, a contrast that was abolished following 0.3 mg/kg RO4917523 pre-treatment. In addition, premature responding following 1 mg/kg RO4917523 pre-treatment was significantly reduced when compared with the maximal response to MK801 and RO4917523 at 0.1 mg/kg (0.1 mg/kg vs 1 mg/kg, p<0.05). RO4917523 pre-treatment also significantly increased omissions (main effect of dose, $F_{4,28}$=34.0, p<0.001), latencies to respond correctly (main effect of dose, $F_{4,28}$=3.6, p<0.05) and impaired attentional accuracy (main effect of dose, $F_{4,28}$=6.4, p<0.01) at the same doses that significantly decreased premature responding. These effects were more pronounced in vehicle-treated animals, as shown by significant dose×group interactions for omissions ($F_{4,28}$=10, p<0.001), correct latencies ($F_{4,28}$=6.8, p<0.01) and accuracy ($F_{4,28}$=4, p<0.05). Furthermore, there was a significant difference in omissions and attentional accuracy between vehicle- and MK801-treated rats at the highest dose of RO4917523 tested (p<0.01 and p<0.05, respectively).

A summary of the effects of 0.06 mg/kg MK801 after MTEP administration is shown in Table 4. MK801 significantly increased premature responding (main effect of drug, $F_{1,7}$=19.6, p<0.01); in the absence of MTEP, MK801 increased premature responding from 4.25±0.77 % to 49±15.43 %. MTEP had no significant effect on this response but significantly increased omissions (main effect of dose, $F_{2,11}$=16.8, p<0.001, X^2=19, GG ε=0.4) in both MK801- and vehicle-treated rats, at all doses tested (1 mg/kg, p<0.05; 3 mg/kg, p<0.01; 10 and 30 mg/kg, p<0.001). In addition, the latency to respond correctly was significantly decreased in MK801-treated rats versus control animals (main effect of drug, $F_{1,6}$=16.9, p<0.01). MTEP also increased response latencies in both treatment groups (main effect of dose, $F_{4,24}$=13.8, p<0.001). This effect was apparent at doses of 3 mg/kg (p<0.05), 10 mg/kg (p<0.01) and 30 mg/kg (p<0.05). At the same doses, MTEP decreased attentional accuracy (main effect of dose, $F_{4,28}$=7.8, p<0.001; 3 and 10 mg/kg, p<0.001; 30 mg/kg, p<0.01).

The effects of ADX47273 pre-treatment on the response of rats to MK801 are shown in Fig. 6. In this experiment, a lower dose of 0.03 mg/kg MK801 was employed, which robustly and selectively increased premature responding (main effect, $F_{3,42}$=10.5, p<0.001) from 3.6±0.8 % to 27.5±5.7 % in the absence of ADX47273 (p<0.001). Both doses of ADX57273 (80 and 100 mg/kg, p<0.01) significantly attenuated this

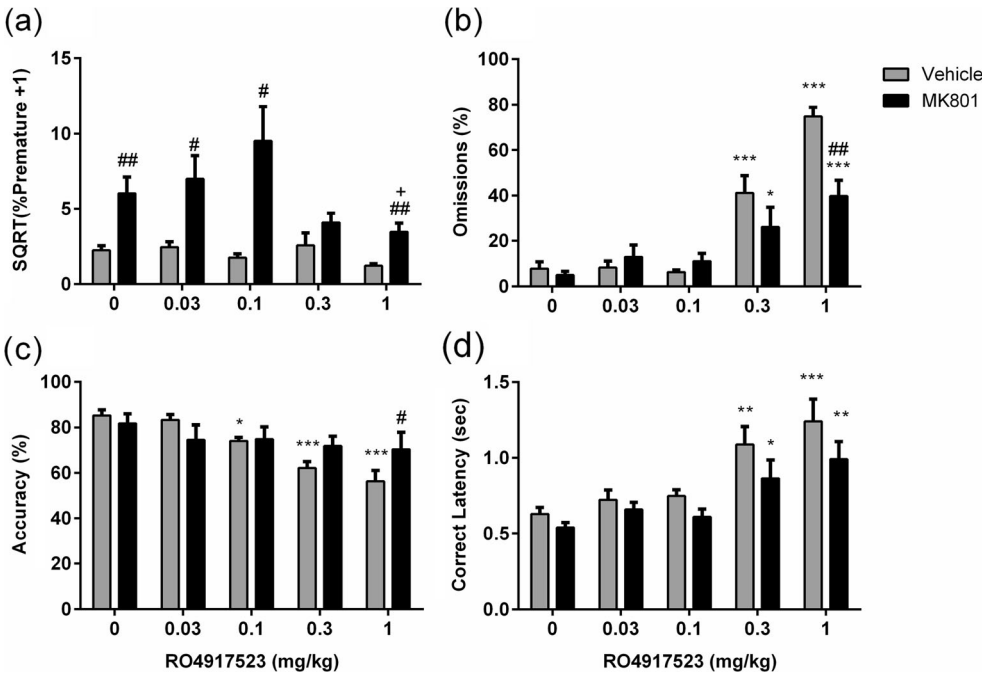

Fig. 5 Effect of MK801 (0.06 mg/kg) on 5-CSRTT performance in MI rats following RO4917523 pretreatment (n=8): **a** per cent premature responses, **b** per cent omissions, **c** per cent accuracy and **d** correct response latency (s). *Bars* represent means ±SEM. Repeated measures ANOVA, paired student's t test. $^{\#}p$<0.05, $^{\#\#}p$<0.01 vehicle versus MK801; Bonferroni post hoc test. ^{+}p<0.05 versus 0.1 mg/kg; *p<0.05, **p<0.01, ***p<0.001 versus relative vehicle control. Premature responding data were transformed [SQRT (% premature+1)] to satisfy the requirement of homogeneity of variance

Table. 4 The effect of 0.06 mg/kg MK801 on 5-CSRTT performance in MI rats (n=8) following MTEP pre-treatment [per cent premature responses, per cent omissions, per cent accuracy and correct latency]

MTEP (mg/kg)	0	1	3	10	30
Veh					
Premature (%)	4.3±0.8	6.3±2.1	6.6±1.3	4.1±1	1.5±0.6
Accuracy (%)	84.8±1.8	78.6±2.0	70.8±3.5¥¥¥	67.9±3.1¥¥¥	64.8±2.5¥¥
Omission (%)	3.8±1.4	11.4±4.5¥	17.6±5.5¥¥	27.3±6.8¥¥¥	59.5±10.9¥¥¥
Correct latency (s)	0.643±0.043	0.760±0.042	0.823±0.068¥	0.924±0.049¥¥	1.096±0.070¥
MK801					
Premature (%)	49.0±15.4	65.9±21.8	56.4±16.7	51.9±16.9	15.9±4.1
Accuracy (%)	81.0±4.5	70.4±6.0	67.5±4.7¥¥¥	69.8±4.1¥¥¥	59.3±9.8¥¥
Omission (%)	7.8±2.4	14.3±2.9¥	22.8±6.6¥¥	28.1±6.5¥¥¥	46.6±12.6¥¥¥
Correct latency (s)	0.613±0.030	0.616±0.034	0.741±0.048¥	0.695±0.038¥¥	0.726±0.115¥

Values represent mean±SEM. Repeated measures ANOVA, Dunnett's post hoc test

¥ p<0.05, ¥¥ p<0.01, ¥¥¥ p<0.001 vehicle and MK801 treated rats versus vehicle control

response such that premature responses in saline control animals were no longer different to those in animals treated with combined MK801 and ADX47273 (Fig. 6a). MK801 also significantly decreased accuracy from 76.24±1.9 to 65.05±2.36 % (main effect, $F_{3,42}$=5.3, p<0.01), a deficit that was rescued by pre-treatment with ADX47273 (80 mg/kg, p<0.05; 100 mg/kg, p<0.01) (Fig. 6c). Omissions (Fig. 6b) and correct latency (Fig. 6d) were unaffected by either MK801 alone or in the presence of ADX47273.

Experiment 5: effects of RO4917523, MTEP and ADX47273 on delay-discounting

The effects of RO4917523, MTEP and ADX47273 pre-treatment on delay-discounting performance are shown in Fig. 7. In all cases, as the delay to the larger reward increased, the choice for that reward decreased (RO4917523; main effect of delay, $F_{2,115}$=121.9, p<0.001, X^2=93, GG ε=0.49; MTEP, main effect of delay, $F_{2,37}$=106.5, p<0.001, X^2=90, GG ε=

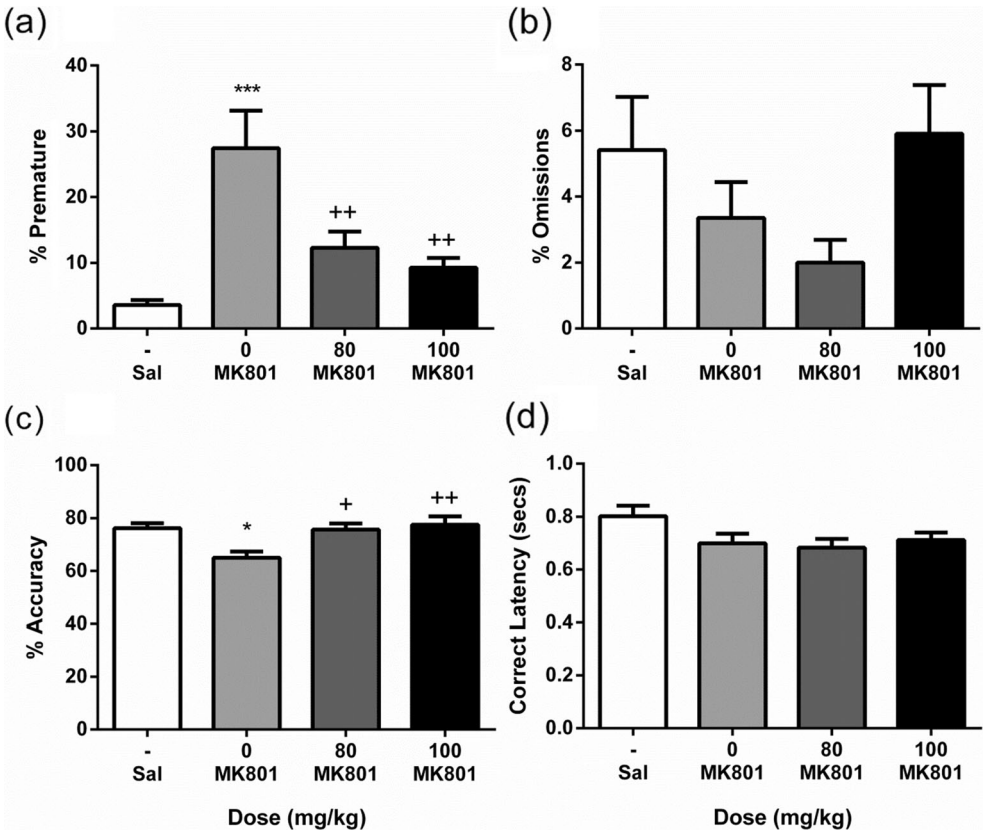

Fig. 6 Effect of MK801 (0.03 mg/kg) on 5-CSRTT performance in MI rats following ADX47273 pretreatment (n=11–12 per group): **a** per cent premature responses, **b** per cent omissions, **c** per cent accuracy and **d** correct response latency (s). *Bars* represent means±SEM. One-way ANOVA, Bonferroni post hoc test. *p<0.05, ***p<0.001 versus saline; ^{+}p<0.05, ++p<0.01 versus vehicle-MK801

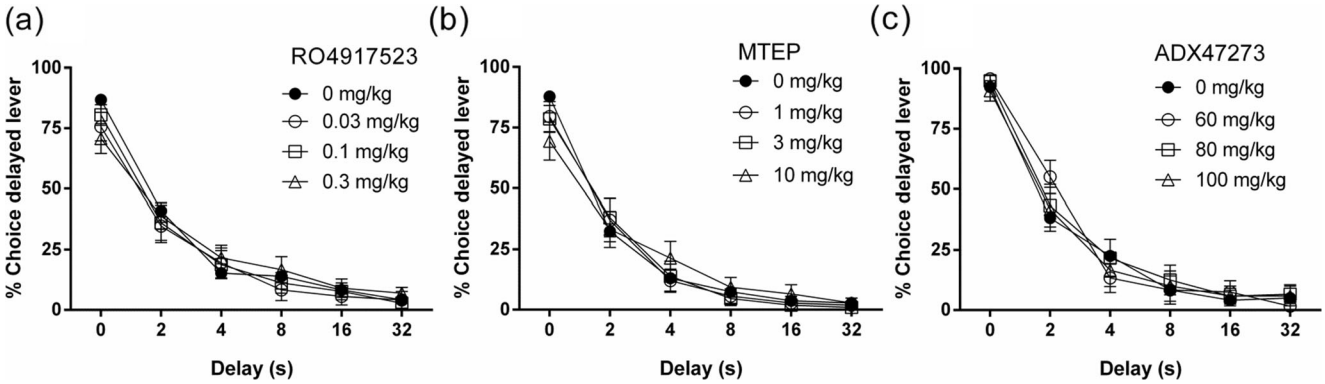

Fig. 7 Effect of **a** RO4917523 (n=24), **b** MTEP (n=18) and **c** ADX47273 (n=20) on delay discounting performance. Data represent mean (±SEM) per cent choice for the delayed reward lever

0.44; ADX47273; main effect of delay, $F_{2,37}$=158.2, p<0.001, X^2=123, GG ε=0.39). However, pre-treatment with RO4917523, MTEP or ADX47273 had no effect on choice behaviour, nor did it affect general task performance, as shown by a lack of effect on omissions.

Discussion

This study investigated the role of mGluR5 in modulating impulsive behaviour on two distinct tasks assessing 'waiting' impulsivity using the NAMs RO4917523 and MTEP, and the PAM ADX47273. Our findings indicate a prominent role of mGluR5 in suppressing responses made pre-potent by their association with reward rather than in determining response preference for immediate and delayed rewards (i.e. on temporal discounting). This distinction is theoretically important as both forms of impulsivity appear to depend on subtly different substrates of the nucleus accumbens (Dalley et al. 2011) and have been referred to collectively as exemplary of 'waiting impulsivity' in contradistinction to another form of 'stopping' impulsivity mediated by the dorsal striatum rather than the nucleus accumbens (Dalley et al. 2011, for a review).

In agreement with a previous study (Semenova and Markou 2007), negative mGluR5 allosteric modulation by RO4917523 and MTEP impaired 5-CSRTT performance. Aside from decreasing premature responding, RO4917523 administration was accompanied by an increase in omissions and response latencies and by a reduction in indices of motor function, namely in rearing and distanced moved, similar to the effects of MTEP. Thus, the effect of RO4917523 to diminish impulsivity was most likely the result of non-specific motoric and/or sedative effects. Indeed, EEG studies in rats demonstrate that mGluR5 NAMs affect distinct phases of the sleep cycle by enhancing deep sleep whilst suppressing REM sleep (Harvey et al. 2013) (Ahnaou et al. 2015). It is noteworthy that RO4921523 (0.1 mg/kg) produced a significant decrease in premature responding and increase in omissions but had no effect on response latencies, rearing behaviour or ambulation. Thus, although attentional accuracy was unaffected by RO4917523, and to some extent MTEP, the concurrent increase observed in omissions suggests that mGluR5 may play some role in attentional processing (Semenova and Markou 2007). Our findings also reveal a novel interaction between NMDA receptor antagonism and positive allosteric modulation of mGluR5 in diminishing impulsive behaviour. Thus, consistent with a cognitive enhancing influence, ADX47273 not only decreased premature responding in all groups tested, but it also attenuated the increase in impulsivity and reversed the attentional impairment produced by the acute systemic administration of MK801.

Although RO4917523 and MTEP exerted similar effects on many task parameters, the inconsistent effects on premature responding are unclear. Both compounds show high selectivity towards mGluR5, reducing the risk of off-target drug–receptor interactions (Anderson et al. 2003; Jaeschke et al. 2015). However, MTEP does exhibit a much lower affinity for mGluR5 compared with RO4917523 (Busse et al. 2004; Jaeschke et al. 2015). Despite compensatory increases in the doses of MTEP administered in the present study, MTEP failed to have any effect on impulsivity and produced a less pronounced increase in omissions and response latencies compared with RO4917523. Since MTEP had no effect on premature responding even at a presumed 100 % mGluR5 receptor occupancy (Busse et al. 2004), these findings confirm that MTEP has a lower potency to modulate mGluR5 compared with RO4917523.

Synergistic interactions between NMDA receptors and mGluR5 have been reported both electrophysiologically and behaviourally (Awad et al. 2000; Campbell et al. 2004; Henry et al. 2002; Homayoun and Moghaddam 2006; Homayoun et al. 2004; Kinney et al. 2003; Lecourtier et al. 2007; Pisani et al. 2001; Rosenbrock et al. 2010). Potentiation of NMDA-induced intracellular calcium mobilisation by mGluR5 activation has been hypothesised to underlie this interaction (Rosenbrock et al. 2010). Based on these studies, we

hypothesised that negative allosteric modulators of mGluR5 would potentiate the behavioural deficits induced by NMDA receptor antagonism. Supporting this hypothesis, MPEP (an mGluR5 antagonist) has been shown to potentiate various behaviours and behavioural deficits produced by NMDA receptor antagonism, including hyper-locomotion, impaired pre-pulse inhibition and deficits in mnemonic function (Campbell et al. 2004; Henry et al. 2002; Homayoun et al. 2004; Kinney et al. 2003). However, mGluR5 modulation has also been reported to differentially modulate cognitive and motor function in rats (Gastambide et al. 2013).

In the present study, MTEP pre-treatment failed to have any effect on 5-CSRTT performance following MK801 administration. This apparent discrepancy may reflect the differing pharmacological profiles of MPEP and negative mGluR5 allosteric modulators. Despite MPEP's high affinity for mGluR5 (Porter et al. 2005), this compound has low *in vivo* potency (Nordquist et al. 2007). Furthermore, non-specific off-target effects may underlie the behavioural discrepancies observed in the present study. Whereas both MTEP and RO4917523 show high selectivity due to their allosteric properties, (Anderson et al. 2003; Cosford et al. 2003; Jaeschke et al. 2015; Lea and Faden 2006), MPEP also affects the noradrenaline transporter (Heidbreder et al. 2003), NMDA receptors and monoamine oxidase-A (Lea and Faden 2006), which may contribute to the modulation of impulsive behaviour (Carli et al. 2004; Dalley and Roiser 2012; Pattij and Vanderschuren 2008; Winstanley et al. 2006). Alternatively, the lack of effect of MTEP on behavioural changes evoked by MK801 may have been due to a ceiling effect on premature responses. Here, it is interesting to note that low doses of RO4917523 (0.03–0.01 mg/kg, Fig. 5a), which had no effect on omissions, response latencies or two locomotor activity parameters, appeared to potentiate the effect of MK801 on premature responding. However, this effect was highly variable between animals.

Positive allosteric modulation of mGluR5 with ADX47273 dose-dependently attenuated the disruptive effects of MK801 on response inhibition and visual attention. These findings provide further support for a functional interaction between NMDA receptors and mGluR5, as discussed above, and show that positive mGluR5 allosteric modulation is sufficient to reverse behavioural and cognitive deficits associated with NMDA receptor hypofunction. Similar cognitive enhancing effects of mGluR5 PAMs have been reported in relation to behavioural flexibility, learning and memory, executive control and social cognition (Clifton et al. 2013; Darrah et al. 2008; Fowler et al. 2011; Stefani and Moghaddam 2010; Uslaner et al. 2009). Although we cannot rule out possible off-target effects of ADX47273, this would appear unlikely as the enhancing effects of ADX47273 was blocked by a selective mGluR5 antagonist (Clifton et al 2013). Furthermore, since we dosed ADX47273 orally, plasma levels were comparable to studies using intraperitoneal dosing (Schlumberger et al. 2009).

Importantly, systemic administration of ADX47273 selectively reduced baseline premature responding in all rats tested as well as impulsivity evoked by lengthening the ITI (i.e., the waiting period prior to stimulus onset). Such effects were not accompanied by global impairments in motor activity with doses as high as 80 mg/kg. Furthermore, ADX47273 did not affect the number of omissions or latencies to respond and collect food reward. Our findings are thus consistent with an earlier study showing ADX47273 to selectively reduce impulsivity when the ITI is increased and hyperactivity induced by NMDA receptor antagonism (Liu et al. 2008). However, we now extend these findings by showing that deficits in visual attention and impulse control induced by NMDA receptor antagonism can be restored by positive mGluR5 allosteric modulation.

The neural mechanisms responsible for the observed interactive effects of NMDA receptor antagonism and mGluR5 allosteric modulation on attentional control processes are unclear but may involve modulation of glutamate release in the medial prefrontal cortex (mPFC). Microdialysis studies have shown that NMDA receptor antagonists cause excessive neuronal firing (Jackson et al. 2004; Lecourtier et al. 2007), leading to increased extracellular glutamate efflux in the mPFC of freely moving rats (Adams and Moghaddam 1998; Ceglia et al. 2004; Moghaddam and Adams 1998; Moghaddam et al. 1997). It has been hypothesised that altered glutamatergic tone in the mPFC may underpin changes in impulsivity following administration of an NMDA receptor antagonist (Ceglia et al. 2004; Moghaddam and Adams 1998; Moghaddam et al. 1997; Pozzi et al. 2011). Indeed, an mGluR2/3 agonist has been shown to block both the increase in glutamate efflux in the mPFC and impulsivity resultant from NMDA receptor antagonism (Pozzi et al. 2011).

However, it remains unclear how NMDA receptor antagonists increase neuronal firing and extracellular glutamate release in the PFC, an effect described recently as 'paradoxical' (Pozzi et al. 2011). Within the PFC, there is a high density of inhibitory GABAergic interneurons that project onto and inhibit excitatory glutamatergic pyramidal neurons. A prevailing hypothesis is that increased glutamate release in the PFC depends on the inhibition of NMDA receptors predominantly expressed by GABAergic interneurons. Inhibition of GABAergic interneurons disinhibits glutamatergic neurons resulting in increased glutamate release (Moghaddam et al. 1997). Consistent with this hypothesis, intra-PFC administration of PCP and MK801 reduced extracellular levels of GABA in the PFC (Yonezawa et al. 1998). This effect depended on NMDA receptor antagonism as co-perfusion with NMDA reduced the effects of PCP and MK801 on extracellular GABA levels (Yonezawa et al. 1998). Interestingly, MK801 predominantly decreased the firing rate of

GABAergic interneurons and, at a delayed rate, increased the firing activity of glutamatergic pyramidal neurons, suggesting that inhibition of GABAergic interneurons precedes the disinhibition of glutamatergic neurons (Homayoun and Moghaddam 2007).

Positive allosteric modulation of mGluR5 by ADX47273 may partly exert its restorative effects on impaired impulse control by indirectly decreasing glutamatergic tone in the mPFC. Thus, it has been demonstrated that 3-cyano-N-(1,3-diphenyl-1H-pyrazol-5-yl)-benzamide (CDPPB), an mGluR5 PAM, attenuates MK801-induced neuronal activity and spontaneous burst firing of mPFC neurons in freely moving rats (Lecourtier et al. 2007). The behavioural effects of ADX47273 may be exerted by mGluR5-expressing GABAergic interneurons in the mPFC. Indeed, it has previously been demonstrated that activation of mGluR5 potentiates GABAergic inhibition of glutamatergic pyramidal neurons through excitation of GABAergic interneurons (Chu and Hablitz 1998). This effect may be sufficient to counteract the increase in glutamatergic tone in the mPFC following NMDA receptor blockade by reducing the excitation of glutamatergic pyramidal neurons and glutamate release in this region.

In addition to effects on glutamate and GABA function, NMDA receptor antagonists and mGluR5 modulators may interact at the level of the mesolimbic and mesocortical dopamine systems. For example, the NMDA receptor antagonists PCP and MK801 increase dopamine release in the PFC and nucleus accumbens (Adams and Moghaddam 1998; Homayoun et al. 2004). Since dopamine inputs to the nucleus accumbens are important determinants of impulsivity on this task (Cole and Robbins 1987; Van Gaalen et al. 2006), the effect of ADX47273 in reducing impulsivity may be mediated in part through effects on dopamine transmission in this region. Indeed, it has been shown that systemic administration of ADX47273 decreases dopamine release in the nucleus accumbens but not the dorsal striatum (Liu et al. 2008). However, the dependence of this effect on positive mGluR5 allosteric modulation requires further research as CDPPB was reported to have no effect on dopamine release in either the nucleus accumbens or the mPFC (Lecourtier et al. 2007). Furthermore, MPEP has been reported to potentiate the effects of MK801 on dopamine release in the PFC (Homayoun et al. 2004).

Our findings show that ADX47273 reduced premature responding on the 5-CSRTT but had no effect on the sensitivity of animals for discounting delayed, relatively large magnitude rewards. Similarly, negative mGluR5 allosteric modulation by RO4917523 and MTEP had no effect on this dissociable aspect of 'waiting' impulsivity. A recent study provides additional evidence for this distinction; a selective mGluR1 antagonist, EMQMCM, reportedly enhanced motor impulsivity in the differential reinforcement of low rates (DRL) task but attenuated choice impulsivity by increasing tolerance of delayed rewards (Sukhotina et al. 2008). Furthermore, MK801 reportedly improved choice impulsivity by appearing to decrease sensitivity to delayed reinforcement (Yates et al. 2014). This is in stark contrast to the behavioural effect that we observe on motor impulsivity in the present study. Neurally, the orbital frontal cortex (OFC) has consistently been implicated in modulating choice impulsivity. Thus, lesions to the OFC promote a shift in preference for smaller, immediate rewards, over larger delayed rewards in rats (Mar et al. 2011; Mobini et al. 2002; Rudebeck et al. 2006). An involvement of the OFC in choice impulsivity is further supported by the work of Winstanley et al. (2006), who reported increased dopamine release and molecular changes in this region during the choice phase of delayed discounting. In contrast, lesions to the mPFC reportedly had no effect on impulsive choice (Cardinal et al. 2001). However, the mPFC, but not the OFC, plays a prominent role in modulating motor impulsivity (Chudasama et al. 2003; Muir et al. 1996; Murphy et al. 2005, 2012; Pezze et al. 2009). While mGluR5 expression in the PFC has consistently been reported (Gupta et al. 2005; Romano et al. 1995; Shigemoto et al. 1993), evidence of mGluR5 expression in the mPFC or OFC sub-regions of the PFC specifically is limited. Anatomical selectivity of mGluR5 modulation towards the mPFC versus the OFC may be one possible explanation for the dissociable behavioural effects on motor and choice impulsivity observed in the present study; however, more specific sub-regional mGluR5 expression studies are required to confirm this. Collectively, these data suggest that the glutamatergic system in the OFC may be involved in regulating choice behaviour for delayed gratification; however, these effects appear to be mGluR sub-type specific.

In summary, we have demonstrated that only one form of 'waiting impulsivity' is subject to modulation by allosteric mGluR5 modulators, perhaps reflecting a distinction between 'choice' and 'motor' forms. In particular, positive allosteric modulation of mGluR5 was efficacious in decreasing not only pharmacologically evoked state impulsivity but also pre-existing trait impulsivity. In addition, we have further highlighted the prominent functional interactions that exist between mGluR5 and NMDA receptors. This study encourages the further investigation of the role of mGluR5 in impulsive behaviour and introduces the possible utility of mGluR5 PAMs as cognitive enhancers and potential therapeutic treatments for targeting specific aspects of maladaptive impulsivity.

Acknowledgements This research was supported by a Medical Research Council (MRC) grant to JWD (G0701500) and a grant from Boehringer Ingelheim Pharma GmbH & Co. KG. This work was carried out in the Behavioural and Clinical Neuroscience Institute (BCNI) at Cambridge University with joint support from the MRC (G1000183) and Wellcome Trust (093875/Z/10/Z) and at Boehringer Ingelheim Pharma GmbH & Co. KG, Germany. We thank David Theobald, Johannes Freudenreich, Peter Schorn, Alfie Wearn and Benjamin Jaehnke

for technical support and Gert Kramer, Dr. Holger Rosenbrock and Dr. Cornelia Dorner-Ciossek for helpful scientific discussions. The authors declare that the experiments performed in this manuscript followed the principles of laboratory animal care and are in compliance with the current laws of the UK and Germany.

Conflict of interest The authors declare no conflict of interest.

Sarah Isherwood, Anton Pekcec and Janet Nicholson are employees of Boehringer Ingelheim Pharma GmbH & Co. KG

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