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Antagonism at metabotropic glutamate 5 receptors inhibits nicotine- and cocaine-taking behaviours and prevents nicotine-triggered relapse to nicotine-seeking

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Abstract

Previous studies in metabotropic glutamate 5 receptor (mGlu5 receptor) deficient mice have indicated the importance of this receptor in the self-administration of cocaine and locomotor sensitisation to this stimulant. Both ionotropic and metabotropic receptors have been implicated in drug-seeking and drug-taking behaviours, but the specific role of each subtype of metabotropic glutamate receptors (mGlu receptors) is still unknown. In the present series of experiments we further investigated the role of mGlu5 receptors on nicotine, cocaine- and food-taking behaviour. We also investigated the effects of the mGlu5 receptor antagonist MPEP (2-methyl-6-(phenylethynyl)pyridine) on the acute locomotor activating effects of nicotine, the expression of sensitisation to its repeated, intermittent administration, and nicotine-triggered relapse to nicotine-seeking behaviour. The results indicate that MPEP treatment reduced nicotine-induced drug-seeking behaviour in a model of nicotine-triggered relapse to nicotine-seeking. Furthermore, MPEP decreased both nicotine and cocaine self-administration without affecting food self-administration under similar schedules of reinforcement. Finally, MPEP reduced both the acute locomotor stimulant effects of nicotine as well as the expression of behavioural sensitisation to its repeated administration. Although the intravenous administration of MPEP at 1 and 10 mg/kg transiently reduced spontaneous locomotor activity during the first 25 min post-administration, we also demonstrated that performance on the accelerating rotarod was not affected when MPEP was given 5 and 30 min prior to the test. Altogether, the present findings strengthen the hypothesis that selective antagonism at mGlu5 receptors may be a new potential pharmacotherapeutic approach for the treatment of drug dependence and addiction.

Keywords: Self-administration; Nicotine; Cocaine; Food; Metabotropic glutamate receptors; MPEP

1. Introduction

Previous studies in metabotropic glutamate 5 receptor (mGlu5 receptor) deficient mice have indicated the importance of this receptor in the self-administration of cocaine and cocaine-induced hyper-locomotion (Chiamulera et al., 2001). The role of mGlu5 receptors in the self-administration of drugs other than cocaine has been recently investigated. For example, MPEP reduces nicotine self-administration in rats and mice without affecting food self-administration (Paterson et al., 2003). In contrast, MPEP does not affect the effects of nicotine on brain stimulation reward in rats (Harrison et al., 2002). Furthermore, Popik and Wrobel (2002) demonstrated that MPEP can attenuate both the acquisition and expression of morphine-induced conditioned place preference without impairing locomotor activity and acquisition of learning and memory retrieval (Popik and Wrobel, 2002). In contrast, a lack of effect of MPEP in reducing the expression of conditioned place preference produced by nicotine, D-amphetamine, ethanol, and morphine is shown in another study (McGeehan and Olive, 2003). Finally, the oral administration of MPEP does

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not affect D-amphetamine-induced locomotor activity in mice (Spooren et al., 2000).

The present series of experiments aimed to clarify the importance of mGlu5 receptors in a range of behavioural paradigms that reflect different aspects of addiction. Furthermore nicotine, cocaine and natural reinforcers were studied to provide clarification of the relative contribution of mGlu5 receptors in the mediation of their effects.

Accordingly, the effects of the selective mGlu5 receptor antagonist MPEP were investigated on intravenous nicotine self-administration and nicotine-triggered relapse to nicotine-seeking behaviour. To test whether MPEP effects would generalise to other drugs of abuse or natural reinforcers, the mGlu5 receptor antagonist was also tested for its effects on cocaine and food self-administration. In addition, MPEP was tested on the acute locomotor stimulant effects of nicotine and on the expression of behavioural sensitisation to nicotine. Finally, to clarify that MPEP was not causing non-selective behavioural disruption its effects on motor coordination using the Rotarod test were also examined.

2. Materials and methods

2.1. Self-administration studies

2.1.1. Animals

Male Wistar rats (Charles River, Germany) were individually housed in a temperature-controlled environment with lights on from 0600 to 1800 h. Water was continuously available and animals were maintained at a constant body weight (240–260 g). All experiments were performed under a Project License obtained according to Italian law (art. 7, Legislative Decree no. 116, 27 January 1992), which acknowledged the European Directive 86/609/EEC.

2.1.2. Apparatus

Sixteen operant chambers were used to perform the self-administration experiments. Each chamber (24 cm wide×22 cm deep×20 cm high) (Coulbourn Instruments, Leigh Valley, USA) was equipped with two levers 4 cm wide, 12.5 cm apart and 2 cm from the grid floor. A food magazine was situated in an opening in the panel between the two levers, 1 cm above the floor. A 2-W white houselight was located 26 cm above the food magazine. An additional light (4 W) was located on the ceiling of the food magazine (CS light). Each chamber was also provided with a Sonalert device (2.9 kHz, 70 dB) and was contained within a sound-isolated box equipped with a ventilating fan that supplied background white noise. For drug self-administration experiments, outside each box, an infusion pump (Model A-99Z, Razel Scientific Instruments, USA) was connected via a tygon tubing (Norton Plastics Performance, Akron, USA) to a single channel liquid swivel (Instech Laboratories, Plymouth Meeting, USA) mounted on the top of the chamber. This swivel was connected to the implanted catheter via another length of PVC tubing protected by a metal spring. Each chamber was also provided with a food dispenser that was connected to the food magazine by a PVC tube.

2.1.3. Computer control and data collection

Data acquisition and operant-schedule parameters were controlled by a Med-PC software (Med Associates, USA) running on two PC-microcomputers connected with the chambers via interface modules (Med Associates).

2.1.4. Experimental procedures

2.1.4.1. Self-administration training. All rats were initially trained to lever press to obtain food reward. Once the session was started the house light was illuminated. The procedure was started on a fixed ratio 1 (FR1) schedule of reinforcement during which each right (or 'active') lever press produced the delivery of a 45-mg sugar pellet (Bioserve, French Town, USA), the simultaneous illumination of the stimulus light in the food magazine for 1 s, and extinction of the house light for 60 s (see below). Presses on the left 'inactive' lever had no programmed consequences. Both lever presses and reinforcement deliveries were recorded. Each reward delivery was followed by a 60-s time-out period during which pressing on both levers was recorded, but had no scheduled consequence. Moreover, lever pressing during the time-out period would reset the time-out itself, in order to 'teach' rats to respond only in the presence of the house light, which signaled reward availability. Each session terminated once rats had obtained 100 pellets or after 2 h had elapsed, whichever event occurred first.

When animals reached the 100-pellet criterion, the response requirement was increased to 2 (FR2). Before being prepared for self-administration with catheter implantation, rats' responding was stabilized. The criterion for stable responding under this schedule was when the ratio between right-lever presses and number of reinforcements was less or equal to 4 (rats took five to seven sessions to reach this criterion). This ratio represents a measure of the selectivity of rats' responding, so that the smaller the ratio, the higher the level of responding specifically directed towards obtaining food reinforcement. Once this criterion was met a final 1-h session was run before the animals underwent surgery.

2.1.4.2. Intravenous catheterisation. Catheters consisted of a guide cannula (C313G 5UP; PlasticsOne, VA, USA) bent into a rounded 90° curve and connected to a silastic tubing (0.30×0.64 mm, Degania Silicone, UK). The junction between the catheter and the tubing was protected by a thicker piece of silastic tubing, approximately 5 cm long (0.70 mm×1.65 mm, Degania Silicone, UK). A drop of silicon rubber was placed at a distance of 3.5 cm from the tip of the silastic tubing, and was used to secure the catheter

to the jugular vein. All the catheters were checked for any leakage before being implanted, by flushing with sterile saline.

2.1.4.3. Surgery. Rats were anaesthetized with medetomidine (0.1 mg/kg, intramuscular (i.m.); Domitor, Pfizer, USA) followed by a combination of tiletamine and zolazepam (40 mg/kg i.m.: Zoletil, Virbac, France). They were then implanted with a silastic catheter in the right jugular vein, and the cannula was fixed onto the skull with tissue adhesive (3M Animal Care Products, St. Paul, USA) and dental acrylic cement (Stratford-Cookson, NY, USA).

Animals received one bolus injection of 0.25 ml of an antibiotic suspension of terramicine (Terramicina long acting, Pfizer) providing 72 h of protection. Animals were then injected i.v. with 0.1 ml of a solution containing 4 IU/ml heparin (Liquemin, Roche, Milano, Italy). This treatment was repeated twice a day for 5 days after surgery (recovery period). After recovery, rats were assigned to separate food, cocaine or nicotine self-administration groups.

2.1.4.4. Nicotine self-administration. Nicotine self-administration was initiated under an FR1 schedule of reinforcement. Each right or "active" lever press led to a 100-μl infusion of nicotine (0.03 mg/kg/infusion) delivered over a 1-s period, the extinction of the house light (60 s), and illumination of the CS light in the food magazine (1 s), as well as 1-s sounding of the Sonalert device. Each infusion was followed by a 60-s time-out during which responses were recorded, but had no scheduled consequences. The time-out length was chosen to prevent nicotine intoxication. Each session lasted until rats had received 25 infusions of nicotine or 3 h had elapsed, whichever event occurred first. When the 25-infusion criterion was met, the response requirement for nicotine infusion was increased to FR2. Under this schedule two presses were necessary to lead to the series of events described above. For the FR2 schedule of reinforcement, each session was terminated after a maximum of 2 h. This phase of training lasted until rats reached at least 12 infusions of nicotine over the 2-h session for at least three consecutive days. The animals were then placed on an FR2 schedule in 1-h sessions. Responding was considered stable when the number of responses on the active-lever did not vary more than 20% between three consecutive sessions (this took between 11 and 15 sessions).

2.1.4.5. Cocaine self-administration. After the recovery period, cocaine self-administration was initiated under an FR1 schedule of reinforcement. Each right or 'active' lever press led to a 100-μl infusion of cocaine (0.5 mg/kg/infusion) delivered over 5 s, the extinction of the house light (60 s), and illumination of the CS light in the food magazine (1 s), as well as 1-s sounding of a Sonalert device (70Hz). Each infusion was followed by a 60-s time-out during which responses were recorded, but had no scheduled consequences. Each session lasted until rats had received 25 infusions of cocaine or 3 h had elapsed, whichever occurred first. When the 25-infusion criterion was met, the response requirement was increased to FR2. Each session lasted for 2 h. Once animals had reached stable responding under this schedule, the length of the session was decreased to 1 h. An animal was considered to have achieved stable responding when it made a similar number of responses on the active-lever for three consecutive days (±10%; between 11 and 15 sessions).

2.1.4.6. Food self-administration. Rats were trained to respond on an FR2 schedule (1 h session, 60 s time-out) for delivery of sugar pellets as described above.

2.1.4.7. Pharmacological treatments. Rats were treated with either saline (1 ml/kg) or MPEP (10 mg/ml/kg) administered intravenously (i.v.), 30 min before the start of the session. Groups were established by matching performance during acquisition and baseline responding in all cases. In the saline and MPEP groups, the numbers of animals assigned to each group were 7 and 6 for nicotine; 8 and 10 for cocaine and 8 and 6 for food, respectively.

2.1.4.8. Reinstatement phase. Nicotine-seeking behaviour was investigated 24 h after the last nicotine self-administration session by using the protocol described in Andreoli et al. (2003). Briefly, rats (n=8) showing stable nicotine self-administration were exposed to a multiple 'relapse schedule' consisting of two components. First, a 30-min extinction phase during which animals were allowed to lever press under an FR1 schedule. However, this instrumental response did not produce an infusion of nicotine as during training, but only the conditioned stimuli previously presented with nicotine self-administration. This stage of the test session was termed the 'cue-component'. At the end of the 'cue-component', a non-contingent subcutaneous (s.c.) injection of nicotine (0.15 mg/kg) was administered to rats. Lever presses on the lever that previously led to nicotine infusion were then measured during the following 90-min period. This second stage of the test session was termed the 'nicotine-priming component' and again lever pressing had no consequences but, unlike the cue-component, no cues were presented contingent upon lever presses. MPEP (0, 1, 3, 10 mg/kg i.v.) was administered, following a Latin square design, to rats 5 min before the start of the test session. Two nicotine self-administration sessions intervened each reinstatement test to normalise responding.

2.1.5. Drugs

Nicotine bitartrate (Sigma, St. Louis, USA) used during self-administration sessions was dissolved in heparinised saline and the pH adjusted to 7.4 with NaOH. Nicotine unit doses were expressed as mg of free base/kg of body weight/infusion. Nicotine bitartrate (Sigma) used during the locomotor activity tests and for priming during the reinstatement phase was dissolved in physiological saline and the pH

adjusted to 7.4 with NaOH. The nicotine doses of 0.4 and 0.15 mg/kg were expressed as mg of free base. In this case nicotine was dissolved in physiological saline and given to rats subcutaneously in a volume of 1 ml/kg. Cocaine hydrochloride (Sigma) used during self-administration sessions was dissolved in heparinised saline and the pH adjusted to 7.4 with NaOH. MPEP hydrochloride (Tocris Cookson, UK) was dissolved in physiological saline and given to rats intravenously in a volume of 1 ml/kg.

2.1.6. Statistical analyses

Self-administration data were analyzed by using one-way analysis of variance (ANOVA) with repeated measurements. Inter-reinforcement time (I.R.T.) data were square root transformed before conducting statistical analyses in order to preserve homogeneity of data between groups. The comparisons of treatment groups and controls between means were assessed with the Student's *t*-test. Reinstatement of responding (either cue or nicotine primed) was analyzed by using a one-way ANOVA with Dose as the between subject factor. For active-lever responding recorded during the 'nicotine-priming component' all groups were compared using an overall two-way ANOVA with repeated measures with Dose (×5, including the group saline/saline, i.e. the group receiving saline i.v. as control of MPEP and saline s.c. as control of nicotine) as the between-subject factor and responding in each time bin (every 30 min) as the within-subject factor (Time×3). Furthermore, planned comparisons (MPEP doses versus saline/saline and saline/nicotine) were made using the PLSD Fisher's test using the total responding during the cue and drug (nicotine) primed phases to further indicate differences between dose groups. Statistical significance was set at a probability level of $P<0.05$ for all analyses.

2.2. Locomotor activity studies

2.2.1. Animals

Male Wistar rats (Charles River, Germany) weighing 250–360 g were used. Rats were housed five per cage (except for locomotor sensitisation experiments in which they were housed singly) in a temperature-controlled room (20–22 °C) with a 12-h light–dark cycle (7:00–19:00 light on). All animals had food ad libitum and free access to water.

2.2.2. Apparatus

Locomotor activity was recorded by using sixteen computerized AccuScan Versamax (AccuScan, Columbus, USA) units. Each monitor consisted of a clear Plexiglas cage (40 cm wide×40 cm deep×32 cm high) equipped with 32 infrared light beam sensors located at 1.3 cm above the floor. Photo beam interruptions were monitored in 5-min periods or bins from each monitor and analyzed by an AccuScan Analyzer software connected to an IBM-compatible computer.

2.2.3. Experimental procedures

2.2.3.1. Effect of MPEP on acute nicotine-induced locomotor activity. On the day of the test, rats were moved from their housing room to the experimental room lit with dim light and were allowed to habituate to the novel environment. Forty-five minutes later, rats were treated with MPEP (0, 1, 10 mg/kg i.v.) (n=10 rats/group) and immediately placed in the AccuScan locomotor apparatus. Their locomotor activity was measured for a 30-min period (habituation). The animals were then taken out of their experimental cage, treated subcutaneously with saline, put back in the AccuScan apparatus and observed for an

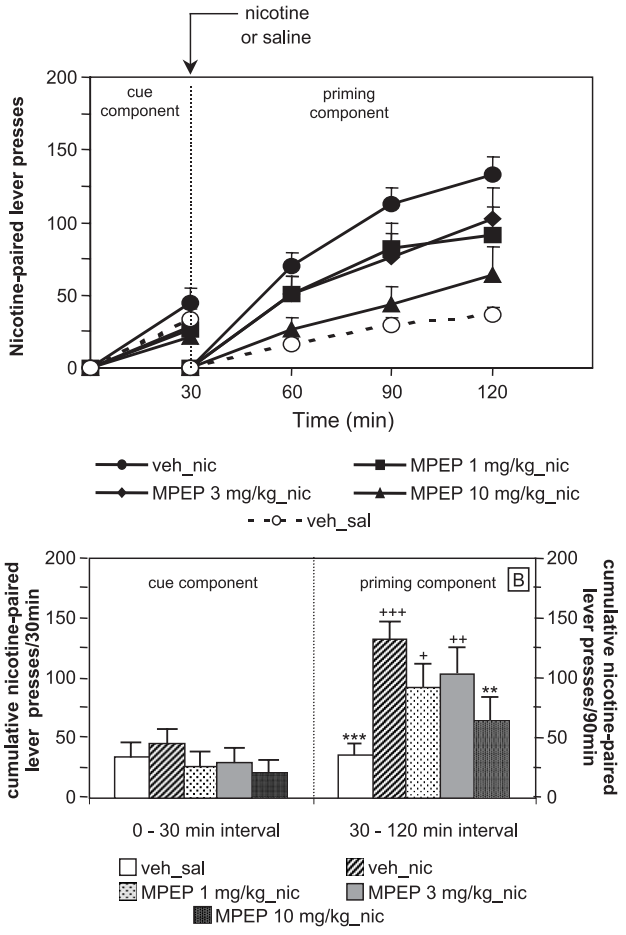

Fig. 1. Effect of MPEP on nicotine-triggered relapse to nicotine-seeking behaviour. MPEP (1, 3 and 10 mg/kg i.v.) or vehicle was given 5 min before the beginning of the session. During the 'cue-component', rats were exposed to the conditioned stimuli contingent upon responding. A priming injection of nicotine 0.15 mg/kg or saline was given s.c. at the end of the 'cue-component'. During the 90-min 'nicotine-priming component', no cues or nicotine injection were presented on responding on the levers. Upper panel: Pattern of cumulative nicotine-paired lever presses measured over 30-min intervals during both cue- and priming-components. Statistical analysis is reported in the results section. Lower panel: Histograms represent the mean±S.E.M. number of nicotine-paired lever presses during the 30-min cue- and 90-min nicotine-priming components. $^{**}P<0.01$, $^{***}P<0.001$ vs. veh_nic and $^{+}P<0.05$, $^{++}P<0.01$ and $^{+++}P<0.001$ vs. veh_sal according to the post-hoc PLSD Fisher's test; n=8 rats/group.

additional 30-min period. Animals were then treated with nicotine (0.4 mg/kg, s.c.) or saline and further observed for an additional 90-min period.

2.2.3.2. Effect of MPEP on the expression of sensitisation to the locomotor activating effects of nicotine. On Day 1, 36 rats were kept for 45 min in the experimental room to habituate to their new environment; they were then injected i.v. with saline (1 ml/kg) and immediately placed in the AccuScan apparatus. Locomotor activity was monitored for a 30-min period (habituation) and animals were then removed from the AccuScan apparatus; they subsequently received a challenge injection of either saline (groups A and B, n=9 rats/group) or nicotine (0.4 mg/kg s.c.) (groups C and D, n=9 rats/group) and put back in the AccuScan apparatus. Locomotor activity was measured for an additional 90-min period. From Days 2 to 4 rats were given the same treatment and locomotor activity was measured according to the same design in order to develop locomotor sensitisation to nicotine. After each session, animals were returned to their housing room. On Day 5, animals were first treated with MPEP (10 mg/kg i.v.) (group D) or saline (groups A, B and C) and subsequently tested in the AccuScan apparatus for 30-min. At the end of that period, rats were removed from the AccuScan apparatus and treated with a s.c. injection of either nicotine (groups B, C and D) or saline (group A); they were then placed back in the AccuScan apparatus and locomotor activity was monitored for an additional 90-min period.

2.2.3.3. Effect of MPEP on motor coordination. Thirty rats were trained on the accelerating rotarod (4–40 rpm over 270 s; Ugo Basile, Italy) twice daily for two consecutive days. On the test day (day 3), rats were treated i.v. with MPEP (0, 1, 10 mg/kg) (n=10 rats/group). Rats were then repeatedly tested for their endurance performance on the rotarod 5, 30 and 60 min after these treatments. Rotarod latencies were measured with a 300-s cut-off time.

2.2.4. Statistical analyses

All experimental data were analyzed by using two-way ANOVAs with a main factor of drug treatment and a repeated measures factor of time. The differences between individual means were assessed with the post-hoc PLSD Fisher's test. The differences between total activity means were assessed with the post-hoc Dunnett's test. Statistical significance was set at a probability level of $P<0.05$ for all tests.

3. Results

3.1. Effect of MPEP (1, 3, 10 mg/kg i.v.) on nicotine-triggered relapse to nicotine-seeking behaviour

MPEP (1, 3 and 10 mg/kg i.v.) was administered 5 min before starting the relapse session. Responding was measured throughout the session up to 120 min (Fig. 1). MPEP (1, 3 and 10 mg/kg i.v.) slightly modified responding during the first 30-min cue-component approaching significance [$F(4,35)=2.5$, $P=0.06$]. Furthermore, MPEP did not affect inactive-lever presses during the first 30-min cue-component [$F(4,35)=1.08$, $P=0.38$]. In contrast, the acute systemic administration of nicotine (0.15 mg/kg s.c.) produced a significant increase in nicotine-paired lever presses in the vehicle/nicotine group compared with the vehicle/saline control group. An overall 5×3 ANOVA with repeated measures analysis over time for absolute numbers of lever presses during each time interval revealed significant main

Table 1
Effects of MPEP 10 mg/kg i.v. on nicotine, cocaine and food self-administration

	Reinforcements	Inter-reinforcements time (sqrt I.R.T.)	Active lever presses	Inactive lever presses
Nicotine				
Baseline	23.7±2.7	1.73±0.14	82.7±5.9	2.2±1.1
Vehicle	25.4±5.1	1.90±0.47	89.9±17.4	0
Baseline	19.9±2.3	1.81±0.23	67.9±10.4	0.4±0.2
MPEP 10 mg/kg i.v.	5.3±2.7[a]	4.67±1.42[b]	14.2±7.5[a]	0
Cocaine				
Baseline	16.4±0.7	1.84±0.06	53.6±6.2	2.7±1.1
Vehicle	17.7±1.3	1.87±0.07	78.4±15.1	2.1±1.4
Baseline	17.8±1.0	1.85±0.06	64.9±7.8	1.5±0.7
MPEP 10 mg/kg i.v.	7.1±1.9[c]	3.04±0.47[b]	19.0±4.8[c]	2.5±2.1
Food				
Baseline	55.6±1.6	1.06±0.03	180.3±17.4	5.8±2.1
Vehicle	56.7±0.7	1.03±0.01	202.9±34.9	1.2±0.8
Baseline	57.2±0.5	1.02±0.01	165.3±17.2	3.4±1.9
MPEP 10 mg/kg i.v.	55.0±1.6	1.05±0.02	184.3±23.0	0.2±0.2

[a] $P<0.01$ for MPEP treated groups vs. vehicle treated group, Student's t-test.
[b] $P<0.05$ for MPEP treated groups vs. vehicle treated group, Student's t-test.
[c] $P<0.001$ for MPEP treated groups vs. vehicle treated group, Student's t-test.

effects of treatment on nicotine-paired lever presses [$F(4,35)=5.82$, $P<0.001$] and time [3 bins of 30 min each; $F(2,70)=21.64$, $P<0.001$] as well as a significant treatment×time interaction [$F(8,70)=6.89$, $P<0.001$] ($n=8$ rats/group). MPEP produced a significant reduction in nicotine-enhanced active lever presses compared with the vehicle/nicotine group. Fig. 1 (upper panel) shows the pattern of cumulative responding during the relapse session over time. Statistical analysis of cumulative responding during the 'nicotine-priming component' confirmed the ability of MPEP to prevent relapse [one-way ANOVA $F(4,35)=4.96$, $P<0.01$]. The post-hoc PLSD Fisher's test showed a significant reduction in the cumulative number of nicotine-paired lever presses at the 10 mg/kg dose ($P<0.01$ vs. veh-nic; Fig. 1, lower panel). No significant changes in the number of inactive-lever presses were seen during the priming-component [$F(4,35)=0.97$, $P=0.44$].

3.2. Effect of MPEP (10 mg/kg i.v.) on stable maintenance of nicotine, cocaine and food self-administration

All rats readily acquired stable baseline of nicotine, cocaine or food self-administration approximately 15 days after starting training. Rats were assigned to either MPEP or saline pre-treatment groups on the basis of their baseline responding, so that the number of reinforcements achieved and number of lever presses during the last three sessions before the test day were near identical in both groups, as demonstrated by a Student's t-test (active-lever presses: $P=0.187$, $P=0.265$ and $P=0.530$; reinforcements: $P=0.286$, $P=0.293$ and $P=0.364$, respectively for nicotine, cocaine and food). Table 1 summarises the effects of MPEP treatment on different parameters measured, i.e. number of active and inactive-lever presses, number of reinforcers and square-root transformed inter-reinforcement times (I.R.T.). Pre-treatment with MPEP significantly reduced the number of active-lever presses in the case of nicotine and cocaine ($P<0.01$ and $P<0.001$ vs. vehicle group, respectively), but the active-lever presses for food were not modified ($P=0.67$) (Table 1 and Fig. 2). Similarly, the number of reinforcements for nicotine and cocaine were significantly reduced ($P<0.01$ and $P<0.01$, respectively), whereas no significant difference was shown for food reinforcement ($P=0.25$; Table 1). On the other hand, the square-root transformed I.R.T. data showed significant increases for both nicotine and cocaine ($P<0.05$), while no significant differences in square root I.R.T. were seen for food self-administration ($P=0.24$). Inactive-lever presses were not significantly different for nicotine, cocaine or food.

3.3. Effect of MPEP (1 and 10 mg/kg i.v.) on acute nicotine-induced locomotor activity

MPEP caused a significant reduction in spontaneous locomotor activity during the 30-min habituation period as revealed by an overall 3×6 ANOVA, with a significant main effect of treatment [Vehicle, MPEP 1 mg/kg and MPEP 10 mg/kg i.v.; $F(2,27)=36.72$, $P<0.001$] and a repeated measures factor of time (six bins of 5 min each) [$F(5,179)=47.96$, $P<0.0001$] as well as a significant dose×time interaction [$F[10,179]=19.81$, $P<0.0001$] (Fig. 3). However, the post-hoc PLSD Fisher's test showed that 25 and 30 min after drug treatment, locomotor activity in both MPEP groups was similar to the vehicle group (Fig. 3, upper panel). Statistical analysis of total horizontal-activity during the first 30-min period (Fig. 3, lower panel) confirmed the significant effect of MPEP treatment on locomotor activity [$F(2,27)=36.72$, post-hoc PLSD Fisher's test $p<0.001$ vs. vehicle]. Following a saline injection, locomotor activity was similar in all experimental groups indicating no conditioned or non-specific consequences of the injection procedure itself. An overall ANOVA revealed

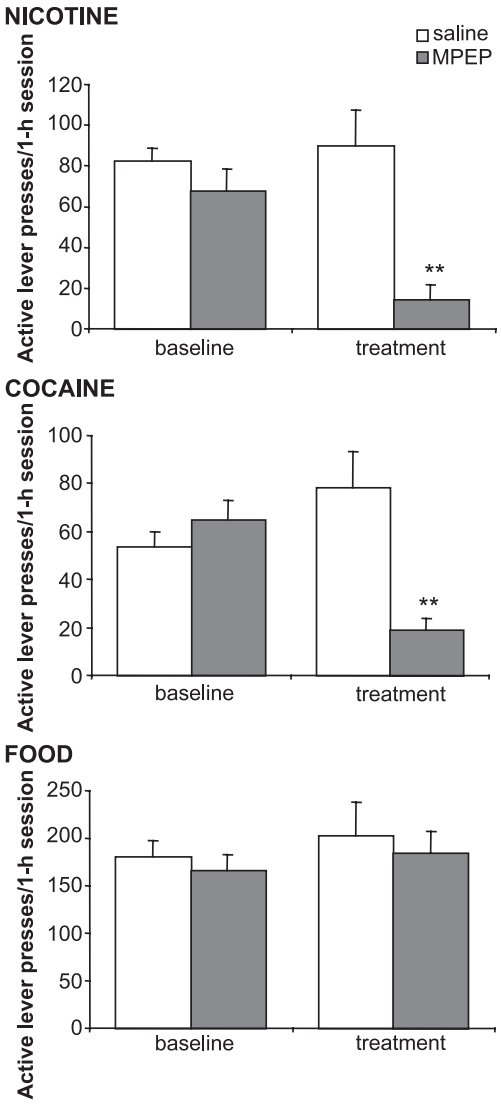

Fig. 2. Effect of MPEP on nicotine-, cocaine- and food-taking behaviour. MPEP (10 mg/kg i.v.) was administered 30 min before the beginning of the self-administration session. Data are expressed as the number (mean± S.E.M.) of active-lever presses in a 1-h session. Baseline values (mean± S.E.M.) represent the last three sessions of self-administration. ***$P<0.001$ vs. saline according to Student's t-test; n=6-8 rats/group.

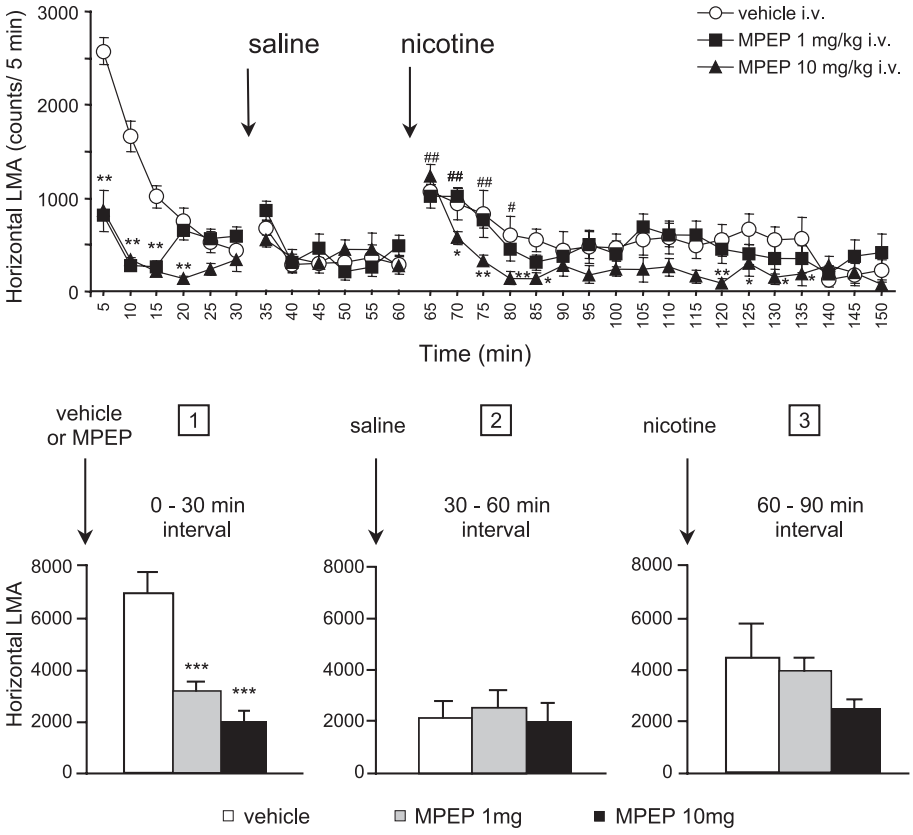

Fig. 3. Time-dependent effect of MPEP (1, 10 mg/kg i.v.) on the acute locomotor activating effects of nicotine (0.4 mg/kg s.c.). The day of the test, rats were moved from the housing room to the experimental room and were allowed to habituate to their novel environment. Forty-five minutes later, rats were treated with MPEP (1, 10 mg/kg) or saline and immediately placed in the AccuScan chambers. Their locomotor activity was measured for a 30-min period (habituation). The animals were then treated subcutaneously with saline, put back into the AccuScan apparatus and observed for an additional 30-min period. Rats were then injected with nicotine (0.4 mg/kg, s.c.) and observed for an additional period of 90 min. Upper panel: Each time point represents the mean±S.E.M. horizontal locomotor activity counts per 5-min periods. *P<0.05, **P<0.01 comparing MPEP treated groups vs. Veh group according to the post-hoc PLSD Fisher's test. $^{\#}P$<0.05, $^{\#\#}P$<0.01 comparing time points after nicotine treatment vs. pre-nicotine time points within Veh group according to the post-hoc PLSD Fisher's test; n=10 rats/group. Lower panel: Histograms represent the total horizontal locomotion during (1) the 30-min period following MPEP or vehicle treatment; (2) the 30-min period following saline treatment, and (3) the 30-min period following nicotine treatment. ***P<0.001 vs. Veh according to the ANOVA with post-hoc PLSD Fisher's test.

no significant main effect of saline treatment [F(2,27)=0.28, P=0.76] and no significant treatment×time interaction [F(10,179)=1.31, P=0.23], but a significant effect of time [six bins of 5 min each; F(5,179)=6.60, P<0.001]. The total horizontal-activity was not different between groups as shown by a one-way ANOVA [F(2,27)=0.28, P=0.76] (Fig. 3, lower panel). The acute administration of nicotine (0.4 mg/kg s.c.) produced a significant increase in horizontal-activity counts compared with the activity recorded over the prior 30 min period (following saline administration). A 2×6 ANOVA revealed main significant effects of nicotine treatment [F(1,18)=6.12, P<0.05] and time [F(5,119]=5.84, P<0.001], but no significant treatment×time interaction [F(5,119)=1.64, P=0.16]. Post-hoc analyses confirmed a significant increase in locomotor activity induced by nicotine (compared to saline) at 5, 10, 15 (P<0.01) and 20 min (P<0.05) after the challenge with nicotine. The administration of MPEP (10 mg/kg i.v.) significantly reduced the acute effects of nicotine on locomotor activity. The overall 3×18 ANOVA showed main significant effects of MPEP treatment [F(2,27)=4.61, P<0.05] and time [F(17,539)=10.40, P<0.001], but no significant treatment×time interaction [F(34,539)=1.12, P=0.30]. Post-hoc analyses confirmed a significant reduction in the acute locomotor effects of nicotine in the group treated with MPEP 10 mg/kg (see Fig. 3, upper panel). However, analysis revealed that the total horizontal-activity during the 30-min period following nicotine injection (Fig. 3, lower panel) was reduced approaching significance in the MPEP 10 mg/kg group in comparison with the vehicle group [one-way ANOVA F(2,27)=2.64, P=0.09]. The dose of 10 mg/kg was therefore chosen to evaluate the effect of MPEP on the expression of sensitisation to the psychostimulant effects of nicotine.

3.4. Effect of MPEP (10 mg/kg i.v.) on the expression of sensitisation to the locomotor activating effects of nicotine

MPEP 10 mg/kg i.v. was given to nicotine-experienced rats (group D) immediately prior to the test session

occurring on Day 5 during which a saline challenge and subsequently a nicotine challenge were given to rats (groups B, C, D) (Fig. 4). As seen in the previous experiment, spontaneous locomotor activity during the 30-min habituation period to the locomotor activity device was reduced in group D (MPEP 10 mg/kg i.v.). A 4×6 ANOVA revealed significant main effects of treatment [$F(3,32)=7.10$, $P<0.001$] and time (six bins of 5 min each) [$F(5,215)=166.21$, $P<0.0001$], but no significant treatment×time interaction [$F(15,215)=1.62$, $P=0.07$] (Fig. 4, upper panel). The post-hoc PLSD Fisher's test confirmed that the reduction of spontaneous locomotor activity produced by MPEP ($P<0.01$ vs. group A) was transient. Locomotor activity in MPEP-treated rats was again similar to group A (saline control) 20 min after treatment (Fig. 4, upper panel). At the end of the habituation period, a s.c. injection of saline was given to all rats. A 4×6 ANOVA showed a near significant effect of MPEP treatment [$F(3,32)=2.52$, $P=0.08$], but a significant effect of time [$F(5,215)=21.3$, $P<0.001$]. There was no significant treatment×time interaction [$F(15,215)=1.65$, $P=0.06$]. Interestingly, the PLSD Fisher post-hoc analysis revealed a significant increase in locomotor activity 5 min after saline injection in group C, which was treated with nicotine in the previous days. In contrast, a significant reduction in locomotor activity vs. group A was observed in MPEP-treated rats sensitized to nicotine (group D) 5 and 10 min following saline injection (Fig. 4, upper panel).

A challenge injection of nicotine produced an acute locomotor activating profile in saline-treated rats (group B). The same challenge injection of nicotine in nicotine-experienced rats produced a sensitised locomotor response that was blocked by the prior administration of MPEP (10 mg/kg i.v.) (Fig. 4). An overall ANOVA with a main factor of both treatments (groups A, B, C and D) and a repeated measures factor of time (18 bins of 5 min each) revealed main significant effects of treatment [$F(3,32)=13.88$, $P<0.001$] and time [$F(17,647)=25.81$, $P<0.001$] as well as a significant treatment×time interaction [$F(51,647)=2.77$, $P<0.001$]. Post-hoc analyses confirmed (1) a significant expression of behavioural sensitisation to the repeated, intermittent administration of nicotine (nic-

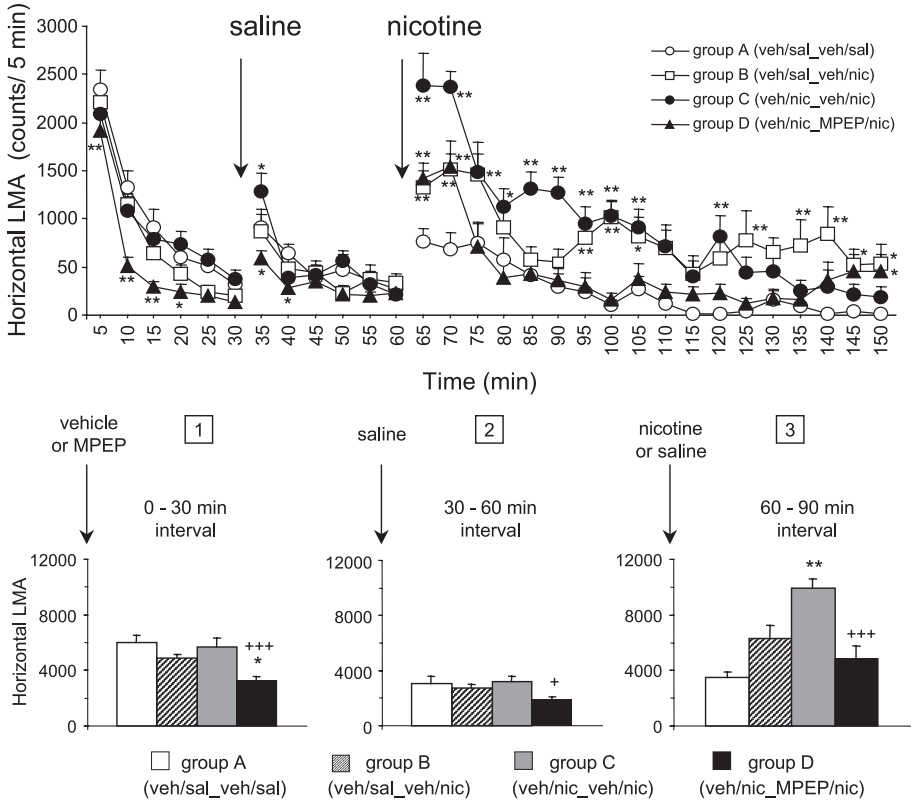

Fig. 4. Effect of MPEP (10 mg/kg i.v.) on the expression of sensitisation to the repeated, intermittent administration of nicotine (0.4 mg/kg s.c.). The animals were treated with either vehicle (i.v.)/saline (s.c.) or vehicle (i.v.)/nicotine (0.4 mg/kg s.c.) for four consecutive days during which they were kept in their housing room and brought to the experimental room to receive respective treatments and be exposed to the Accuscan apparatus. On Day 5, animals were first treated with MPEP (0, 10 mg/kg i.v.) and locomotor activity was assessed for a 30-min period; they were then injected with saline s.c. and locomotor activity patterns were recorded for an additional 30-min period. Rats were subsequently injected s.c. with either nicotine (0.4 mg/kg) or saline (only group A) and locomotor activity was observed for an additional period of 90 min. Upper panel: Each time point represents the mean±S.E.M. horizontal locomotor activity counts per 5-min periods. *$P<0.05$, **$P<0.01$ vs. group A (veh/sal_veh/sal) according to the post-hoc PLSD Fisher's test; $n=9$ rats/group. Lower panel: Histograms represent the total horizontal locomotor during (1) the 30-min period following MPEP or vehicle i.v. treatment; (2) the 30-min period following saline treatment, and (3) the 30-min period following nicotine treatment. *$P<0.05$, **$P<0.01$ vs. group B (veh/sal_veh/nic); $^{+}P<0.05$; $^{+++}P<0.001$ vs. group C (veh/nic_veh/nic) according to the post-hoc PLSD Fisher's test.

otine pre-treated group C vs. group B; $P<0.01$); and (2) MPEP-induced blockade of the expression of sensitisation to nicotine in nicotine pre-treated animals (group D vs. group C, $P<0.01$). The analysis of cumulative values (Fig. 4, lower panel, no. 3) showed that both groups B and C showed a significant increase in locomotion versus group A (both $P<0.001$) and more importantly indicated that locomotion in group C was significantly increased versus group B ($P<0.01$, PLSD Fisher's test) confirming that group C was sensitised to nicotine. Furthermore, the MPEP-treated group (group D) was not significantly different from group A treated with saline and group B treated with acute administration of nicotine ($P=0.21$ and $P=0.17$ respectively, post-hoc PLSD Fisher's test) whereas it was significantly reduced in comparison with group C ($P<0.0001$, PLSD Fisher's test) confirming the MPEP-induced blockade of the expression of sensitisation. Fig. 5 shows some examples of horizontal-locomotor activity patterns of four representative animals that underwent the nicotine sensitisation protocol as assessed by the AccuScan LMA apparatus.

3.5. Effect of MPEP (1 and 10 mg/kg i.v.) on motor coordination

Rotarod latencies measured during the last training session (pre-treatment session) were not significantly different in the three experimental groups. The time spent on the rotarod were 134±14, 144±20 and 151±17 s (means± S.E.M.) for vehicle-, MPEP 1 and MPEP 10 mg/kg-treated groups, respectively [$F(2,27)=0.28$ $P=0.76$]. As shown in Fig. 6, MPEP 1 mg/kg did not affect endurance performance. Interestingly, 60 min after the treatment, MPEP 10 mg/kg produced a significant increase in the time spent on the rotarod in comparison with vehicle group at the same post-treatment time [F dose $(2,29)=0.013$, F time

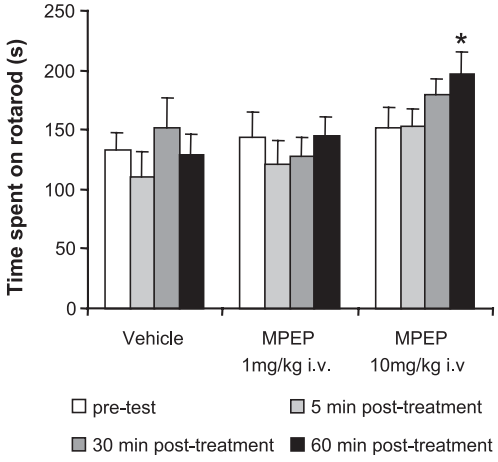

Fig. 6. Effect of MPEP on motor coordination as assessed by the Rotarod test. Rotarod performance was assessed 5, 30 and 60 min after treatment with MPEP (0, 1, 10 mg/kg i.v.). *$P<0.05$ vs. vehicle group at the same time according to the post-hoc Dunnett's test; $n=10$ rats/group.

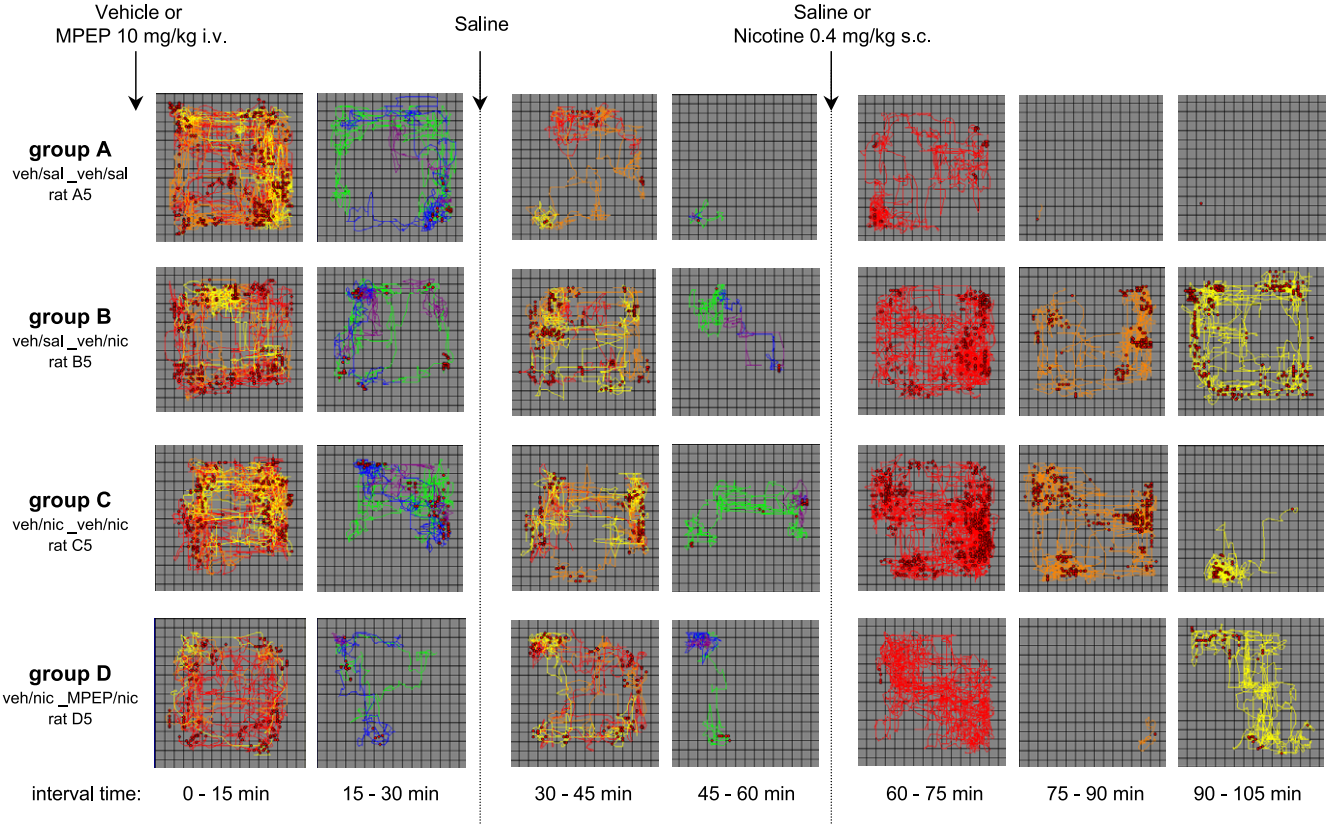

Fig. 5. Representative patterns of horizontal locomotor activity of one animal in each of the four experimental conditions of the nicotine sensitisation study. The patterns represent locomotor activity recorded during 15-min interval by using the AccuScan Versaplot software.

(2,89)=0.066, F dose×time (4,89)=0.576; followed by the post-hoc Dunnett's test, $P<0.01$ MPEP 10 mg/kg vs. veh at 60 min]. A post-hoc analysis did not show any significant effect comparing the performances at different times between the groups.

4. Discussion

The results of the present study indicate that MPEP treatment reduced nicotine-induced drug-seeking behaviour in a model of nicotine-triggered relapse to nicotine-seeking. Furthermore, MPEP decreased both nicotine and cocaine self-administration without affecting food self-administration under similar schedules of reinforcement. Finally, MPEP reduced both the acute locomotor stimulant effects of nicotine as well as the expression of behavioural sensitisation to its repeated administration. However, these effects of MPEP cannot be attributed to any non-specific motor disruption. In fact, although the intravenous administration of MPEP at 1 and 10 mg/kg transiently reduced spontaneous locomotor activity during the first 25 min post-administration, we also demonstrated that performance on the accelerating rotarod was not affected when MPEP was given 5 and 30 min prior to testing.

4.1. Role of mGlu5 receptors in drug-related behaviours

An involvement of mGlu5 receptors in drug-related behaviours was originally suggested by a study showing that repeated cocaine administration produced a significant elevation in mGlu5 receptors mRNA in the shell of the nucleus accumbens and the dorsal striatum three weeks after cessation of treatment (Ghasemzadeh et al., 1999). More recently, a series of experiments carried out in the same laboratory showed a decrease in mGlu5 receptor protein levels (Swanson et al., 2001), using a similar sensitisation paradigm. Altogether these data suggest that repeated cocaine administration not only produces an increase in mGlu5 receptor mRNA, but also blocks at some level the transcription of this message, thus ultimately leading to decreased expression of mGlu5 receptor protein levels. Furthermore, there is a growing body of evidence pointing towards a role of group I mGlu receptors (incorporating mGlu5 receptors) in mediating the reinforcing and motivational effects of cocaine and arguably, nicotine (Harrison et al., 2002; McGeehan and Olive, 2003; Paterson et al., 2003). More specifically, mGlu5 receptors seem to be involved in the mediation of long-term neuroadaptations that occur after repeated cocaine administration (Ghasemzadeh et al., 1999; Swanson et al., 2001). Moreover, mGlu5 receptor knockout mice were unresponsive to the stimulant and primary reinforcing effects of cocaine (Chiamulera et al., 2001).

An exact understanding of how MPEP exerts the effects highlighted above remains elusive. Since we observed that MPEP initially reduced spontaneous LMA, one could argue that MPEP's attenuating effect on nicotine and cocaine self-administration is due to impairment in locomotor behaviour rather than a direct effect on the rewarding effects of these drugs. This hypothesis, however, can be discarded for three reasons. First, if the decreased responding for nicotine or cocaine after MPEP was due to an impairment in locomotor activity, the drug should also have affected food self-administration, which was not changed by pre-treatment with MPEP. Second, although the experiment on acute nicotine-induced locomotor activity showed that MPEP decreased spontaneous locomotor activity, this effect disappeared within approximately 25 min. In the present set of experiments MPEP was always given 30 min before the session so that an effect on motor behaviour can be ruled out. Thirdly, experiments on the rotarod showed that MPEP (1 and 10 mg/kg i.v.) did not affect performance when given 5, 30 and 60 min before the experiment, thus demonstrating that the mGlu5 receptor antagonist did not produce motor impairment at the doses and pretreatment times used in the present experiments. Finally, although there is a difference in response following MPEP administration between spontaneous horizontal activity and Rotarod we do not consider this difference important in the interpretation of our findings. One possible reason for the observed difference is the novelty of the Accuscan activity chambers (unlike the Rotarod to which animals were habituated). This issue of novelty to explain MPEP's on spontaneous activity is perhaps relevant to the acute and sensitised measure of nicotine's locomotor effects. However in these experiments long pre-treatment periods for MPEP were included in the protocol, with identical pre-nicotine baseline activity observed before nicotine challenge to ensure that the attenuating effects of MPEP on acute nicotine challenge (in either sensitised or non-sensitised rats) did not relate to MPEP's action per se.

Perhaps of greater importance in consideration of the relevance of MPEP-like drugs for the treatment of substance dependence, are our experiments showing that MPEP decreased behavioural sensitisation to nicotine. These results suggest that mGlu5 receptors play an important role in the expression of behavioural sensitisation to psychostimulants, in agreement with studies using glutamatergic modulators performed in other laboratories investigating cocaine-induced sensitisation (Jeziorski et al., 1994; Karler et al., 1991; Stewart and Druhan, 1993; Wolf et al., 1994).

4.2. Interactions between mGlu receptors and dopamine

Experimental evidence indicates that the nucleus accumbens is critical for primary and in particular conditioned reward-related learning. However, these reward-related processes subserved by the nucleus accumbens presumably depend also upon its glutamatergic afferents (modulated by mGlu5 receptors) originating from limbic and cortical areas, especially the amygdala, the anterior cingulate and pre-

limbic cortices as well as the hippocampal formation. Electrophysiological (Floresco et al., 2001), neurochemical (Blaha et al., 1997; Floresco et al., 1998) and behavioural studies (Pap and Bradberry, 1995; Wu et al., 1987) have shown that these cortical afferents interact with dopamine (DA) in the nucleus accumbens. Among the mGlu receptor family, both mGlu5 and mGlu3 receptor mRNAs are expressed most abundantly in the nucleus accumbens (Testa et al., 1994), as well as the prefrontal cortex and hippocampus (Romano et al., 1995; Shigemoto et al., 1993). Thus, a possible mechanism of action of MPEP could be related to the neuroanatomical localisation of mGlu5 receptors and their interaction with brain reward circuitries. In fact, a recent study demonstrated that MPEP antagonises the influence of mGlu5 receptors on the activity of the ventral striatopallidal pathway (Diaz-Cabiale et al., 2002), an important motivational circuit for the expression of reward-related behaviours (Lu et al., 1999). Excitatory amino acids and mesolimbic dopaminergic terminals form synapses on single nucleus accumbens neurons and nucleus accumbens output neurons express both DA and mGlu5 receptors (Sesack and Pickel, 1990,1992; Steardo et al., 1994; Testa et al., 1998; Vezina and Kim, 1999). A number of in vitro and in vivo studies have shown that agonists at glutamate receptors increase extracellular levels of DA in the nucleus accumbens (Imperato et al., 1990; Jones et al., 1987; Youngren et al., 1993). Thus, one might hypothesise that MPEP decreased cocaine and nicotine self-administration through the modulation of glutamate and/or DA transmission in the mesocorticolimbic pathway. This hypothesis, however, must be tested in future experiments.

The temporary MPEP-induced reduction of spontaneous locomotor activity (although not relevant to the effect of MPEP on self-administration) observed in the present study could also be related to a glutamatergic modulation of dopaminergic transmission. Increasing evidence suggests that glutamatergic agents produce their effects on locomotion by interacting with DA neurotransmission (Arnt, 1981; Boldry and Uretsky, 1988; Burns et al., 1994; Donzanti and Uretsky, 1983; Hamilton et al., 1986; Svensson et al., 1994). Behaviourally relevant interactions between DA and glutamate can be inferred from the observation that infusions of glutamate receptor antagonists into the nucleus accumbens attenuate both the locomotor and dopamine activating effects of psychostimulants (Kaddis et al., 1993; Kelley and Throne, 1992; Moghaddam and Bolinao, 1994; Pap and Bradberry, 1995; Pulvirenti et al., 1989, 1991).

4.3. Anxiolytic effects of MPEP

Recent preclinical studies have shown that MPEP has potent anxiolytic-like as well as antidepressant-like effects (Brodkin et al., 2002; Pilc et al., 2002). It has also been reported that MPEP may exert its anxiolytic and antidepressant actions by blocking the norepinephrine transporter (Heidbreder et al., 2003). It could then be argued that the effect of MPEP on nicotine and cocaine self-administration was due to the anxiolytic properties of this compound and not to a specific effect on cocaine and nicotine reinforcement. However, converging evidence from earlier studies has shown that norepinephrine reuptake inhibitors such as desipramine or imipramine have no effect on cocaine self-administration (Tella, 1995) and do not substitute for cocaine in monkeys (Lamb and Griffiths, 1990; Woolverton, 1987). Furthermore, we have recently shown that while MPEP dose-dependently decreased ethanol self-administration, the norepinephrine transporter inhibitor imipramine had no effect (Marcon et al., 2003). The latter findings thus suggest that the effect of MPEP in this case was specific for the rewarding properties of ethanol, and that this effect was due specifically to a blockade of mGlu5 receptors.

4.4. Lack of effect of MPEP on food-self-administration

It has been argued above that MPEP might decrease nicotine and cocaine self-administration via a blockade (or modulation) of nicotine- or cocaine-induced enhancement in glutamate transmission. If this hypothesis has credence it could explain the lack of effect on food reward since no sensitised glutamate overflow seems to be observed under the latter conditions. Hence glutamate antagonism into the nucleus accumbens through blockade of mGlu5 receptors could decrease such an effect. One may thus suggest that because of glutamate's modulatory actions, inhibition of glutamate transmission in the nucleus accumbens would not affect normal (basal) dopaminergic transmission, such as that involved in feeding behaviour. In support of this hypothesis, recent studies have shown that a challenge of cocaine 21 days after withdrawal can increase the levels of glutamate in the core subregion of the nucleus accumbens of sensitised, but not non-sensitised rats (Pierce et al., 1996). These results indicate that repeated cocaine administration increases excitatory amino acid transmission in the nucleus accumbens only in rats that developed behavioural sensitisation. Therefore, one may suggest that a blockade of this abnormally enhanced glutamate transmission through antagonism at mGlu5 receptors present in high concentrations in the nucleus accumbens may decrease nicotine and cocaine reward and other psychostimulants actions (see also Edgar et al., 1997; Reid et al., 1997; Reid and Berger, 1996). Another point to be taken into account is the different neural basis underlying drug or natural reward reinforcement. Whereas both dopamine and glutamate have been clearly involved in mediating the reinforcing properties of most drugs of abuse—and in particular psychostimulants—the evidence is not as clear for food-seeking and taking behaviours. Similarly, data from our laboratory have previously shown that MPEP, while decreasing oral ethanol self-administration and intake, did not affect sucrose self-administration at the same MPEP doses in mice (Marcon et al., 2003).

5. Conclusion

The present results clearly showed an involvement of mGlu5 receptors in nicotine taking behaviour and in the reinstatement of nicotine-seeking behaviour. In addition, antagonism at mGlu5 receptors also reduced significantly cocaine-taking behaviour. The effects observed in the present study are likely to be mediated via complex glutamate-dopamine interactions in the mesocorticolimbic system where glutamatergic afferents from subcortical/cortical areas and dopaminergic afferents from the ventral tegmental area converge. Further studies will be required to unravel those mechanisms. Altogether, selective antagonism at mGlu5 receptors may represent a potential new target for the pharmacotherapy of drug abuse and addiction.

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