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Differential effects of mGluR1 and mGlur5 antagonism on spatial learning in rats

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Abstract

The effects of selective mGluR1 and mGluR5 antagonists on long-term acquisition were tested in a spatial three-choice reward-finding test. Bilateral prelimbic injections of the mGluR1 antagonist, (*S*)-4-carboxyphenylglycine (4-CPG), before training sessions blocked acquisition of correct performance between sessions. Similar injections given after full training of a control group significantly impaired correct performance without causing a complete block. Pretraining injections (intraperitoneal or intravenous) of the systemically active mGluR5 antagonist, 2-methyl-6-(phenylethynyl)pyridine (MPEP), had no effect on long-term acquisition in the reward-finding task. In an open-field test, bilateral prelimbic pretest application of 4-CPG prevented normal adaptation of spontaneous exploration as seen in control animals. MPEP, on the other hand, had no effect. In conclusion, the results confirmed that mGluR1 is involved in spatial long-term acquisition and suggested an additional role in recall of acquired skills. Furthermore, it was concluded that antagonism of mGluR1 or mGluR5 had different effects both in the appetitive spatial task and in the open-field test.

Keywords: Rat; Spatial learning; Metabotropic glutamate receptor

1. Introduction

Applications of antagonists for metabotropic glutamate receptors have established that Group I receptors support the transition of spatial memory from short-term to long-term status. For instance, acquisition in a footshock-enforced spatial conditioning task has been inhibited by the Group I selective antagonist, 1-aminoindan-1,5-dicarboxylic acid (AIDA) (Nielsen et al., 1997). The same compound has inhibited long-term acquisition in a spatially cued reward-finding task (Christoffersen et al., 1999a,b). Another Group I selective antagonist, (*S*)-4-carboxyphenylglycine (4-CPG), has impeded retention of shock enforced spatial alternation in a Y-maze (Balschun and Wetzel, 1998). Before Group I-specific antagonists were available, less selective antagonism of Groups I and II by (*R*,*S*)-α-methyl-4-carboxyphenylglycine (MCPG) had demonstrated amnesic effects on long-term retention in inhibitory avoidance tasks (Bianchin et al., 1994, 2000), had impeded spatial acquisition in the water maze (Bordi et al., 1996) and had inhibited shock enforced spatial alternation learning (Riedel et al., 1994, 1995).

In parallel to such detrimental effects on spatial learning, inhibitory effects on hippocampal LTP have been reported for MCPG (Riedel and Reymann, 1996; Riedel et al., 1994), 4-CPG (Balschun et al., 1999) and AIDA (Mata et al., 2000). A coinciding involvement of Group I mGluRs in spatial learning and in hippocampal LTP is accordant with the fact that Group I mGluRs stimulate phospholipase C, which is activated during the induction of hippocampal LTP — combined with the fact that such LTP may be involved in mnemonic associations between salient stimuli and their spatial context (Morris and Frey, 1997).

Although a regulatory role of Group I mGluRs in spatial learning and in hippocampal LTP has been established, details of the involvement of the Group I subtypes (mGluR1 and mGluR5) remain to be elucidated. Today, subtype-specific antagonists are available and may be used to clarify possible differential subtype functions. 4-CPG is selective for mGluR1 over mGluR5 (Doherty et al., 1999; Lin et al., 1997; Bräuner-Osborne et al., 1998), whereas the antagonist, 2-methyl-6-(phenylethynyl)pyridine (MPEP), is highly selective for mGluR5 (Gasparini et al., 1999). Furthermore, MPEP has proven able to penetrate the blood–brain barrier

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as shown by the ability of systemically administered MPEP to inhibit activity of hippocampal CA1 neurons induced by the agonist, (*R*,*S*)-3,5-dihydrophenylglycine (DHPG) (Gasparini et al., 1999). MPEP has also been reported to counteract the excitatory effect on thalamic neurons exerted by the mGluR5 agonist, 2-chloro-5-hydroxyphenylglycine (CHPG) (Salt et al., 1999) and has proven able to inhibit footshock-enforced conditioning in rats (Schulz et al., 2001). Presently, the effects on long-term acquisition of both 4-CPG and MPEP were compared within the confines of one training task in order to elucidate a possible differential function of Subtypes 1 and 5. As a supplement to the footshock-enforced fear conditioning (Schulz et al., 2001), an appetitively reinforced spatial task was used. In this task, rats were required to visit one out of three identical alcoves for reward and to memorize the identity of the rewarding alcove in each of 20 trials per learning session (Christoffersen et al., 1998a,b).

2. Methods

2.1. Animals

Male PVG rats were housed in pairs in macrolon boxes with free access to water and food pellets in a temperature-controlled room with light on and off at 07:00 and 19:00 h daily. Experimentation began at the age of 2 months. The work was carried out according to the guidelines of the Experimental Animals Inspectorate under the Danish Government Justice Department by permission no. 1998-561-6.

2.2. Experimental procedure

A detailed description of the layout of the employed reward-finding task has been reported earlier (Christoffersen et al., 1998a,b). The task was performed in an operant chamber having three identical boxes attached to a front panel and a single box placed at the rear panel. A guillotine door covered the three front panel boxes. When open, a rat could introduce the head into each box. Behind all four boxes were drinking bottles filled with sweetened tomato juice. In their resting position, the nozzles of the bottles were out of sight and reach of the rat. However, a head entry into an alcove would trigger a motion of the bottle into the alcove if the identity of the visited box were the one designated as being "correct". In such a case, a drinking period of fixed duration ensued before the bottle was retracted and the sliding door closed. Similarly, a drinking bottle was introduced into the single box in the rear panel, triggered by a visit here. The time of entry into and the identity of an alcove were marked by the breaking of an infrared beam and fed via an A/D converter into a custom-made DASYLAB (Eurochannels, Germany) program, which also controlled the motions of the sliding door and bottles. These were pneumatically driven from a remote sound-insulated valve box. Noises caused by movements of bottles and sliding door were minimized by the use of ball bearings and slow rate controlled movements.

Three walls of the operant chamber were black, and the top was covered with a black lid to dissuade rearing. One wall was transparent, allowing visual inspection by the experimenter and also permitting the rat to form a spatial map of the position of boxes in relation to the surroundings. The experimenter was covered behind an external screen to prevent visual distractions of the rat.

Animals were allowed to familiarize with the operant chamber during a 20-min stay on the day before the first training session. In all ensuing training sessions, a 2-min adaptation period was allowed from entry into the chamber until the beginning of the first trial. At 24 h before each training session, rats were deprived of water, and immediately after each session, they were returned to the storage facility with free access to water for the next 24 h. Training sessions were therefore carried out every other day.

A trial began with the opening of the door covering the three front panel boxes. A first-choice visit to the correct one was rewarded by a 5-s drinking period before closure of the guillotine door. For each rat, the identity of the alcove that had been selected as the correct one stayed constant throughout all trials of all sessions. The designation of correct or false to each box was balanced so that each served as the correct one for one third of the specimens in both control and test groups. This was done in order to counteract possible positional preferences. Although entries into only one of the three alcoves in the front panel were rewarded, drinking bottles were placed behind all three in order to prevent identification of the correct box based on smell. After the rewarding period (5 s), a visit to the rear panel box had to be made and was rewarded by a 3-s drinking period here. The next trial began with the opening of the front panel door 60 s after the rat had last introduced the head into a front panel box.

If a rat did not visit the correct box as a first choice, all choice boxes were closed and a visit had to be made to the rear panel alcove. If this did not occur within the 60-s trial interval, it would be prolonged until the rear box had been visited. In control groups, this only happened during early trials of the first session. A session would be terminated when either 20 trials had been completed or after 1 h had elapsed. As a criterion for full training in the control group infused bilaterally in the prelimbic areas (Fig. 2A), it was required that the percentage of correctly performed trials per session remained stable within ±5% in three consecutive sessions.

When a rat had completed a training session, the operant chamber was washed with soap before a new specimen was introduced. This was done as a countermeasure against the possible use of scent trails for identification of rewarding boxes. The use of scent trails has been previously found to be unlikely in this test (Christoffersen et al., 1998a).

Spontaneous explorative locomotion was analysed in an open-field test. Here, rats were monitored by a digitised

videotracking system (Noldus, The Netherlands) in a 50 × 50-cm arena surrounded by black walls. Each animal was tracked during a 20-min observation period, and the box was washed before a new specimen was introduced.

2.3. Drug treatment

4-CPG is not known to be able to cross the blood–brain barrier and was therefore injected intracranially through microcannula implanted into the prelimbic cortex at coordinates: 3.2 mm anterior to bregma, 0.8 mm lateral to the midsaggital plane and 3.2 mm below dura (Paxinos and Watson, 1998). The locations of cannula were verified in slices through Nissl staining (Wolf, 1971) (Fig. 1). 4-CPG (Tocris Cookson, UK) was injected 2 min before each training session. A stock solution was made from 10 mg dissolved in 0.1 ml NaOH (0.5 M) supplemented with NaCl (0.9%) to 0.5 ml (pH 8), and 5 μl of this solution (0.1 mg 4-CPG) was injected. In the first series of experiments, a group of rats ($n=9$) received presession injections at the prelimbic coordinates in both hemispheres. Acquisition in the reward-finding task for this group was compared to acquisition in a control group ($n=10$) having received bilateral prelimbic saline injections of the same volume. A second control group served as test for the possible effects of bilateral prelimbic injections of vehicle. This group ($n=12$) received intraperitoneal injections of an isotonic NaCl solution (1 ml/kg).

MPEP is active in the brain after systemic injections (Gasparini et al., 1999) and could therefore be injected peripherally. In a first series of experiments, MPEP was injected intraperitoneally 20 min prior to each training session. A period of 20 min has proven effective in previous studies of a blood–brain barrier penetrating Group I antagonist, AIDA (Christoffersen et al., 1999a). MPEP hydrochloride (Tocris Cookson) was dissolved in dimethylsulfoxide (DMSO) and was diluted in 1.2% saline (one part DMSO, three parts NaCl) resulting in a stock solution of 1 mg/ml MPEP in a 0.9% NaCl solution of pH 3.1. Acquisition was observed in a group ($n=12$) receiving 1 ml/kg of this MPEP solution (1 mg/kg). The MPEP-affected group was compared to a control group ($n=11$) receiving intraperitoneal vehicle injections of the same composition, volume and pH.

In a second series of MPEP experiments, 1 mg/ml (4.35 mM) was dissolved in 0.9% saline by heating up to 40 °C; 1 ml/kg of this solution was injected (1 mg/kg iv) in a test group ($n=11$). This dose and injection route was used because it has been effective at antagonizing DHPG-induced activity of hippocampal neurons of anaesthetized rats (Gasparini et al., 1999). A control group ($n=11$) received similar intravenous volumes of saline adjusted to the same pH. Finally, in a third series of experiments using MPEP, the concentration of the injected solution was raised to 5 mg/ml by dissolving MPEP in Tween 80 in 0.9% NaCl. Furthermore, the volume injected intravenously was raised to 2 ml/kg resulting in a final concentration of 10 mg/kg given to the test group ($n=7$). A control group ($n=8$) received similar volumes of the Tween 80/NaCl vehicle.

In the open-field test, a test group ($n=6$) received bilaterally infused 4-CPG in the prelimbic cortex 2 min before introduction into the field, while a control group ($n=10$) received saline. Finally, a test group ($n=8$) received 1 mg/kg ip MPEP 20 min before the open-field test and was compared to a group ($n=8$) receiving saline intraperitoneally.

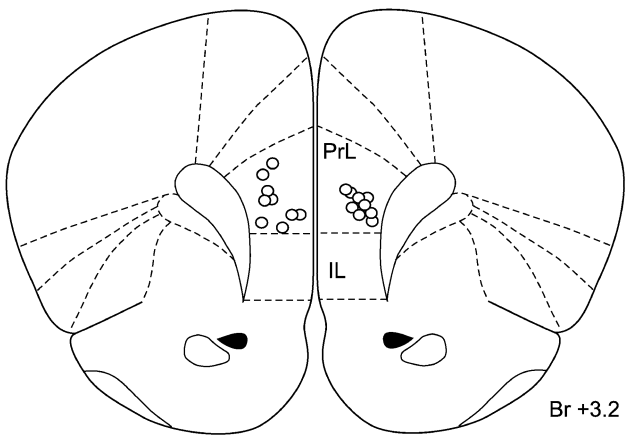

Fig. 1. Coronal section 3.2 mm anterior to bregma. Locations of the tips of cannula are marked. PrL: prelimbic cortex. IL: infralimbic cortex. (Drawing based on Paxinos and Watson, 1998.)

2.4. Data analysis and statistics

Acquisition of correct choices among the three front panel boxes was quantified by counting the number of correct first choices in a session expressed in percent of the total number of trials in each session (20). Acquisition of the procedural skill of crossing the operant chamber from the front to the rear panel after closure of the front panel door was quantified by measuring the time between closure and the introduction of the head into the rear panel box. The average "crossing time" for all trials in each session was calculated. Correct as well as false choices and the crossing time were monitored by the DASYLAB program and stored in a result file. In the open-field test, the distance moved during 20-min observation periods were separated into four consecutive periods of 5 min.

Statistical evaluations were performed by repeated-measures ANOVA — either one-factor evaluations of the effect of repeated sessions or two-factor versions testing for significant effects of different treatments throughout sessions. Significance of differences in individual sessions was calculated using Fisher's protected least significant difference (LSD) post hoc analysis. Level of significance was set at $P<.05$.

3. Results

3.1. 4-CPG: effect of bilateral prelimbic injections on acquisition

A group of rats ($n=9$) were trained in eight successive sessions under the influence of bilateral pretraining injections of 4-CPG into the prelimbic areas as outlined in Fig. 1. The average scores of correct responses are shown in Fig. 2A. In the course of the first eight sessions, no between-sessions learning appeared: Repeated-measures one-factor ANOVA showed no significant effect of session [$F(7,56)=1.7$; $P>.05$]. A control group ($n=10$) received equal volumes of vehicle injected bilaterally into the prelimbic areas. This group did express significant between-sessions acquisition (Fig. 2A) [$F(7,63)=10.5$; $P<.0001$]. Group comparison based on two-factor ANOVA showed a significant effect of treatment [$F(1,17)=40.7$; $P<.0001$] and also of the Treatment × Session interaction [$F(7,119)=8.1$; $P<.0001$]. The slopes of the acquisition curves were therefore significantly different. Post hoc analysis revealed significant differences between test and control groups in individual sessions as marked in Fig. 2A.

It was tested whether the bilateral prelimbic injections of vehicle would by themselves exert a detrimental effect on acquisition. This was particularly relevant in view of the large infused volumes. The control was performed by comparing acquisition in the first eight sessions of the group that received bilateral prelimbic vehicle (Fig. 2A) to that of a group ($n=12$) injected intraperitoneally before sessions with saline (Fig. 2C). Two-factor ANOVA showed no significant effect of treatment [$F(1,20)=3.0$; $P>.05$].

In addition to acquisition of the skill of visiting the correct front panel box, the animals had to learn the procedure of turning away from the front panel when the covering door closed, move to the single box in the rear panel and introduce the head here in order to receive reward. The time from closure of the door to head entry in the rear panel box was measured in each trial of all sessions. The average "crossing time" per trial in a session was calculated for each rat and the group averages in each session are shown with S.E.M. in Fig. 2D. The control group learned to perform the procedure increasingly fast approaching asymptotically a minimum crossing time of 6.7 ± 0.6 s. This reduction of crossing time was significant [$F(7,63)=5.6$; $P<.0001$, one-factor ANOVA]. In contrast, the group affected by 4-CPG did not display gradual task acquisition and no significant effect of session was found [$F(7,56)=1.6$; $P>.05$]. A test group versus control group comparison showed significant effects of treatment [$F(1,17)=13.8$; $P<.01$] and Treatment × Session interaction [$F(7,119)=2.6$; $P<.05$]. Significant differences in individual sessions are marked in Fig. 2D (post hoc analysis).

These results demonstrate (1) that bilateral prelimbic injections of vehicle did not inhibit between-sessions acquisition significantly and (2) that the acquisition was blocked by the applied dose of 4-CPG.

3.2. 4-CPG: effect on recall after full training

After the first eight training sessions of the bilaterally infused control group (Fig. 2A), a stable level of correct performance was approximated (mean score of correct responses in the seventh session was $78\pm4\%$ and in the

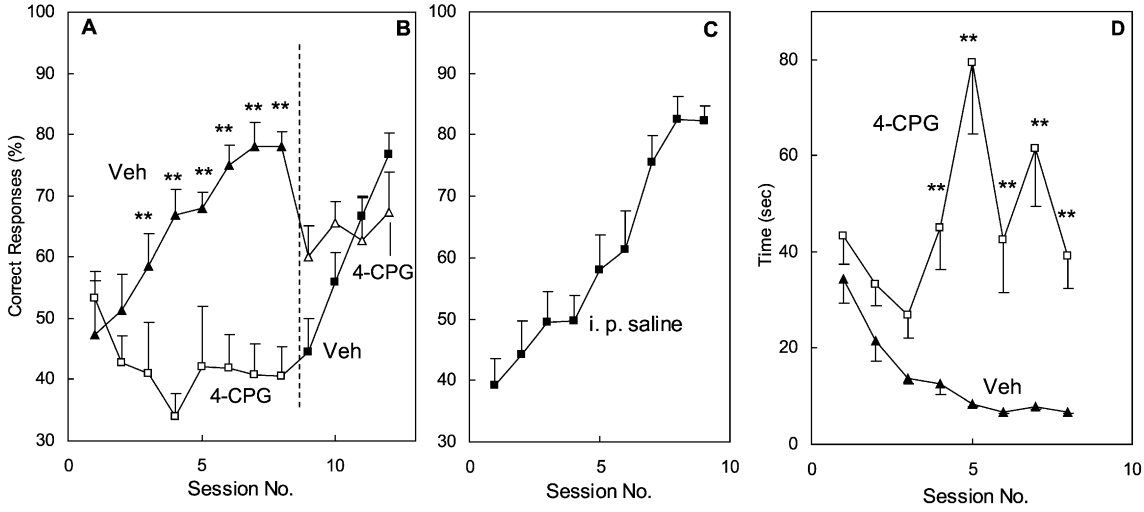

Fig. 2. Acquisition in a reward-finding task. (A) Correct choices made by initially naïve rats trained in eight sessions after bilateral prelimbic injections of either 4-CPG ($n=9$) or vehicle (Veh) ($n=10$). (B) In four subsequent sessions, the content of the injections was switched between the two groups: Specimens formerly treated with vehicle now received 4-CPG and vice versa. (C) Control experiment showing correct choices in a group ($n=12$), which had received saline intraperitoneally before training sessions. (D) "Crossing time": the development throughout sessions of the average time per trial between closure of choice boxes in the front panel of the operant chamber and head entry into the box in the rear panel. Values from a test group ($n=9$) infused bilaterally in the prelimbic area with 4-CPG compared to a control group ($n=10$) having received bilateral vehicle injections. Mean scores with S.E.M. $**P<.01$ for the marked sessions (ANOVA post hoc Fisher's LSD).

eighth session, it was 78 ± 2.5%). Nine specimens out of this control group of 10 were observed during four subsequent sessions — now under the influence of presession injections of 4-CPG rather than vehicle. The score in the first 4-CPG affected session (the ninth) fell to 60 ± 5% (Fig. 2B). The drop between Sessions 8 and 9 was statistically significant ($P < .05$; paired Student's t test). Also, when comparing the last three sessions affected by 4-CPG (10–12) to the last three vehicle sessions with nearly stable performance (6–8), a significant effect of treatment was observed [$F(1,17) = 6.0$; $P < .05$]. The results therefore showed that 4-CPG impeded expression of previously acquired correct performance.

3.3. 4-CPG: test for chronic effects on acquisition

It was tested whether the treatment in eight sessions with 4-CPG (Fig. 2A) would influence learning in subsequent sessions unaffected by the antagonist. Six specimens among the group of nine animals that had received bilateral 4-CPG in Sessions 1–8 were observed in four subsequent sessions performed after bilateral presession injections of vehicle (Fig. 2B). These animals now showed significant acquisition between Sessions 9 and 12 [$F(3,15) = 8.8$; $P < .01$]. Comparison between the last three vehicle-affected sessions (10–12) and the last three 4-CPG sessions (6–8) showed a significant effect of treatment [$F(1,13) = 35.4$; $P < .0001$].

The rate of acquisition in late vehicle affected sessions (9–12) after repeated applications of 4-CPG (Sessions 1–8) was compared to the rate in the initial vehicle sessions (1–4) of the control group. Both of these groups received bilateral vehicle injections, the only difference being the absence or presence of a prehistory of exposure to 4-CPG. Visual inspection of Fig. 2A and B indicates that the learning rate was larger during the late sessions. Indeed, the acquisition curve in these late vehicle sessions could be approximated by a regression line following the expression: $y = 10.8x + 34$, while the early vehicle sessions could be approximated by: $y = 5.6x + 42$. However, although the slope after eight 4-CPG affected sessions was higher, a Group × Session ANOVA did not reveal any significant differences between early and late vehicle sessions [$F(1,14) = 0.7$; $P > .05$]. The results therefore showed that the blocking effect of 4-CPG on long-term acquisition in eight sessions was not chronic since normal learning could take place after the treatment.

3.4. MPEP: effect on acquisition

In a first series of experiments involving MPEP, 1 mg/kg was injected intraperitoneally 20 min before each training session and correct scores were compared to those of a group submitted to intraperitoneal injections of vehicle adjusted to the same pH as the test solution (Fig. 3A). Acquisition for the MPEP group over sessions proceeded in parallel to that of the control group and no significant effect of difference in group treatments was observed [$F(1,21) = 2.4$; $P > .05$].

In order to test whether the acidic vehicle solution had impeded learning, the control group performance of Fig. 3A was compared to that of the group having received saline of neutral pH intraperitoneally (Fig. 2C). Two-factor ANOVA showed no significant effect of treatment [$F(1,21) = 1.8$; $P > .05$].

In a second set of comparisons, a test group received the same concentration of MPEP injected intravenously (1 mg/kg), while the control group received saline intra-

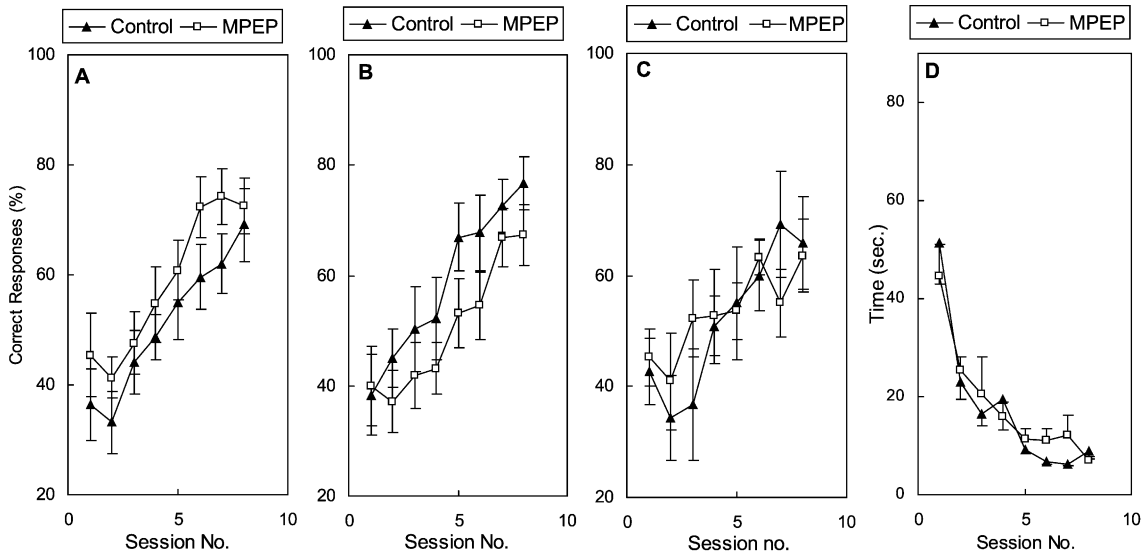

Fig. 3. Acquisition of correct choices in groups affected by MPEP. (A) The test group received intraperitoneal injections of 1 mg/kg MPEP ($n = 12$); the control group received vehicle intraperitoneally ($n = 11$). (B) A test group received intravenous injections of 1 mg/kg MPEP, while the control group was given saline intravenously ($n = 11$ in both groups). (C) The test group ($n = 7$) received 10 mg/kg iv MPEP, while the control group ($n = 8$) was given vehicle intravenously. (D) "Crossing time": average value per trial stated through sessions for a test group affected by intraperitoneal injections of MPEP ($n = 12$) compared to a control group receiving vehicle intraperitoneally ($n = 11$). Mean scores with S.E.M.

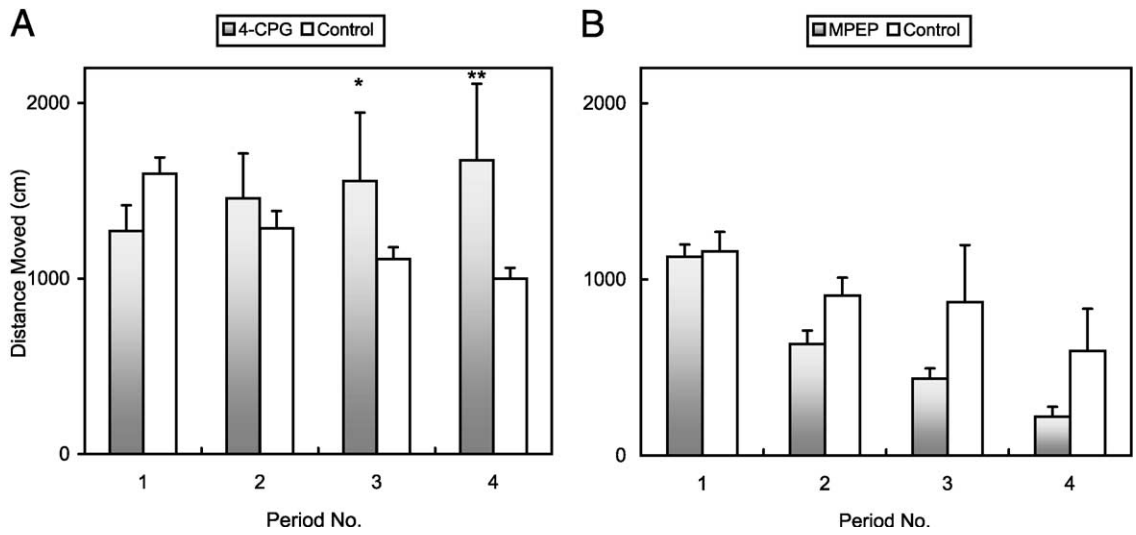

Fig. 4. Spontaneous locomotion in an open-field test during a 20-min observation period separated into four consecutive periods of 5 min. (A) Test animals ($n=6$) had received 4-CPG injections bilaterally in the prelimbic cortex while the control group ($n=10$) was bilaterally infused with vehicle. (B) Results from a test group ($n=8$) injected intraperitoneally with MPEP compared to control specimens ($n=8$) receiving vehicle intraperitoneally.

venously. The scores of correct responses are depicted in Fig. 3B. Again, the MPEP group did not show significantly different acquisition compared to controls [$F(1,20)=1.6$; $P>.05$].

Since the lack of effect of MPEP could be due to an insufficient dose, this was raised from 1 to 10 mg/kg in a third control versus test group comparison. The maximum solubility in water for MPEP is 5 mM, and the stock solution injected in the previous set of experiments (1 mg/ml) was 4.35 mM. In order to raise the concentration, MPEP was therefore dissolved in Tween 80 in 0.9% NaCl. Correct scores are compared in test and vehicle groups in Fig. 3C. Even in the presence of this high dose, there was no significant group difference [$F(1,13)=0.05$; $P>.05$].

A measure of procedural task acquisition in the form of time spent from closure of front panel boxes to entry in the rear panel box was assessed. This crossing time was measured for the group receiving MPEP intraperitoneally (1 mg/kg) and compared to the intraperitoneal vehicle group (Fig. 3D). Both groups showed reduced crossing times over sessions and no significant group differences existed [$F(1,21)=0.05$; $P>.05$]. A similar comparison of crossing times for the groups that received intravenous injections of MPEP (1 mg/kg) or vehicle (the groups included in Fig. 3B) also failed to show a significant effect of treatment, and the acquisition curves resembled those shown in Fig. 3D. The same conclusion was obtained by comparing the test group given 10 mg/kg iv to its control group (the groups of Fig. 3C).

3.5. 4-CPG: effect in the open-field test

Rats were infused with 4-CPG bilaterally in the prelimbic cortex (same dose and volume as above) 2 min before being placed in an open-field test. Here, they were observed during a 20-min period, and results were analysed in four consecutive periods of 5 min (Fig. 4A). A control group that received bilateral injections of vehicle displayed a significant reduction of spontaneous locomotion over the four observation periods [$F(3,27)=21.1$; $P<.0001$, one-factor ANOVA] in contrast to the test group [$F(3,15)=0.5$; $P>.05$]. The different development of explorative locomotion in successive periods was also expressed by a significant Treatment × Period interaction [$F(3,42)=5.1$; $P<.01$]. Post hoc analysis showed that locomotion in the two initial 5-min periods was not significantly different between groups whereas the last two periods differed as marked in Fig. 4A.

3.6. MPEP: effect in the open-field test

A group was given MPEP (1 mg/kg ip) 20 min before being tested in the open-field arena. Unlike the specimens affected by 4-CPG, these rats did show adaptive behaviour as expressed in significantly reduced spontaneous locomotion over the four observation periods [$F(3,21)=39.6$; $P<.0001$]. A control group received vehicle intraperitoneally, and no statistically significant difference due to treatment was observed between MPEP and control groups [$F(1,14)=3.4$; $P>.05$].

A direct comparison of temporal development of locomotion between the group treated with MPEP (Fig. 4B) and the 4-CPG-affected group (Fig. 4A) showed a significant effect of treatment [$F(1,12)=15.6$; $P<.05$] and Treatment × Period interaction [$F(3,36)=7.0$; $P<.001$]. These results corroborate the findings from the reward-finding task in the sense that differential behavioural effects of the two antagonists were observed.

4. Discussion

4.1. The learning task

The employed reward-finding task permits a high number of trials per session. In this work, sessions were interrupted after 20 trials, which provide a graded percentage score of correct responses per session for each rat (increments of 5%). Finely graded group scores facilitate quantitative statistical evaluation of mnemonic drug effects. Also, the high number of trials opens up for a separate analysis of short-term learning within sessions and long-term acquisition between sessions (Christoffersen et al., 1999b).

In each trial of the test, it was required that the rat paid a visit to a reward site situated opposite the three alcoves in the front panel. As a result, the rats turned away and lost sight of these boxes. This procedure was employed because previous (unpublished) experiments in which the rear panel box was not used had shown that after finding a reward in one out of the three identical boxes, rats would remain in front of that box until it was opened again after the intertrial interval. Such behaviour could be based on appetitive association between remaining in a fixed position and receiving reward. This was not the type of spatial learning desired in the test, particularly since it involved uninterrupted sensory contact with the rewarding box. Instead, by interrupting the sensory contact through the trip to the rear panel, it was attempted that correct choices should be based on a mnemonic spatial representation of the front panel and the position of its rewarding box. Use of the rear panel box was therefore employed as a countermeasure against mediated memory. A comparable arrangement with one nose-poke-operated site opposite a choice of two lever-operated sites has been used to train association between salient stimuli and their spatial context (Hampson et al., 2000).

4.2. 4-CPG: effect on correct choices of bilateral prelimbic injections

The site of injection was chosen within the prelimbic cortex because the area is believed to be implicated in the formation of long-term memory (Mulder et al., 2000; Laroche et al., 2000; Doyère et al., 1993). Bilateral injections of a high dose of 4-CPG caused a complete block of spatial long-term acquisition. This receptor selective inhibition therefore provided a new type of indication that the prelimbic cortex is essential for long-term memory formation. An inhibitory effect of ventricular injections of 4-CPG has been observed previously during Y-maze learning (Balschun and Wetzel, 1998).

Since the injected volumes were large, it could be apprehended that mechanical tissue damage might have had an amnesic effect. The possibility was, however, disproved by the control group of Fig. 2A showing a highly significant build-up of correct performance between sessions in spite of bilateral vehicle injections. Fig. 2C confirmed that this between-sessions acquisition was not significantly different from learning in rats that merely received saline intraperitoneally.

The indication of prelimbic mGluR1 receptor involvement in spatial learning implied by the present results may involve prelimbic Group I mGluR-dependent LTP. The existence of such LTP has been inferred from a facilitating effect of DHPG (Morris et al., 1999) and is supported by an inhibiting effect of MCPG (Vickery et al., 1997). Prelimbic LTP has been suggested to have a significant role in consolidation (Laroche et al., 2000) and could therefore relay 4-CPG-induced mGluR1 inhibition and the observed block of between-sessions acquisition.

4.3. 4-CPG: effect on fully trained animals

When the vehicle control group of Fig. 2A had fully acquired the coordination between locomotion and visuospatial allocentric cues required for correct performance, bilateral injections of 4-CPG had an impeding effect on expression of the skill (Fig. 2B). This result indicated that either a consolidated memory trace was impaired or recall of the acquired skill was inhibited. The latter possibility suggests that 4-CPG could have an impeding effect on working memory. The prelimbic cortex does indeed constitute a section of the prefrontal cortex that has been implicated in working memory operations. This has been indicated through the effects of lesions (Delatour and Gisquet-Verrier, 1996) and also by recordings of working memory related neural firing during mnemonic periods of delay tasks (Jung et al., 1998). The observation of an impeding effect of 4-CPG on recall of long-term acquired information has found further support in our laboratory from rats tested in the water maze. Here, fully trained rats displayed reduced memorized swimming skills after receiving bilateral prelimbic injections of 4-CPG (unpublished).

4.4. Training after treatment with 4-CPG

It was tested whether 8 days of bilateral injections with 4-CPG would affect subsequent learning. Two possible and counteracting scenarios were envisaged: (1) Repeated injections could leave some chronically inhibitory effect. (2) Although the flat performance curve in the first eight sessions of Fig. 2A indicated a block of acquisition, the sessions might facilitate later acquisition. A chronic effect was ruled out by the fact that training after eight 4-CPG affected sessions showed significant between-sessions acquisition. Facilitation was indicated by a trend towards enhanced learning rate compared to the rate of learning in a previously untreated control group. However, the trend was not statistically significant leaving the conclusion that 4-CPG completely and reversibly blocked acquisition.

4.5. MPEP: effect on acquisition of correct choices

The concentration of 1 mg/kg given systemically was chosen because it had proven efficient in a previous study at antagonizing hippocampal activity induced by the Group I mGluR agonist, DHPG (Gasparini et al., 1999). However, neither 1 nor 10 mg/kg affected long-term acquisition in contrast to the presence of effects of 1 or 6 mg/kg on hippocampal neurons (Gasparini et al., 1999). MPEP administered orally has also been found to block long-term acquisition of footshock-enforced conditioned fear (Schulz et al., 2001), and intraperitoneal injections (6 or 12 mg/kg) have attenuated conditioned taste aversion (Schachtman et al., 2001). Furthermore, bilateral MPEP infusions in the lateral amygdala have impaired long-term acquisition of footshock-enforced conditioned "freezing" responses to both spatial context and nonspatial cues (a tone) (Rodrigues et al., 2001). Conditioned "freezing" in response to context (but not tone), as well as escape from the aversive environment of the water maze, is also inhibited in mGluR5 mutant mice (Jia et al., 2002). However, in an object recognition task (Ennaceur and Delacour, 1988), MPEP injected intraperitoneally (6 mg/kg) either 30 min before or immediately after training did not affect object recognition relative to saline controls (Simonyi A and Schachtman TR, personal communication). These results (along with the presently reported) are not sufficient to pinpoint a specific mnemonic role of mGluR5.

4.6. Comparison between mnemonic effects of mGluR1 and mGluR5 antagonists

The inability of peripherally administered MPEP to affect spatial acquisition may be compared to the presence of an inhibitory effect after intraperitoneal injections of AIDA, which predominantly inhibits mGluR1. Tested in the three-choice reward-finding task, AIDA prevented between-sessions acquisition at a dose of 2 mg/kg (Christoffersen et al., 1999a). Assuming an even distribution in the animal, 2 mg/kg corresponds to about 9 μM, which may be compared to an IC_{50} for AIDA of 214 μM (Moroni et al., 1997). Under the same assumption, 10 mg/kg of MPEP corresponds to 43.5 μM compared to an IC_{50} at the human mGluR5a of 36 nM (Gasparini et al., 1999). For AIDA, the concentration was therefore 24 times below IC_{50}, while MPEP was more than 1000 times above IC_{50}. The large difference between these ratios for the mnemonically efficient AIDA and for the inefficient MPEP points towards the conclusion that the employed MPEP dose would have been high enough had the drug possessed any effect on learning in this task. In addition to the assumption of equal distribution, the ratios were calculated under the added implied premise that the injected amounts were fully dissolved. For MPEP, precipitation may occur at the injection into an intraperitoneal or intravenous environment of physiological pH, thus downsizing the ratio between dissolved compound and IC_{50} to an unknown value. However, it is still not likely that the lack of mnemonic effect can be ascribed to an insufficient concentration of dissolved MPEP within the brain, since comparable injections have affected activity in the hippocampal CA1 area (Gasparini et al., 1999) and have influenced acquisition in a punitive learning tasks (Schachtman et al., 2001).

4.7. 4-CPG and MPEP: effect on time spent between front and rear panels

The time that elapsed between closure of front panel door and a visit to the rear panel box is a parameter that expresses the degree of procedural task acquisition. The skill of running from front to rear accumulated between sessions in the two different control groups of Figs. 2D and 3D. In spite of the differences in both vehicle content and infusion route, these two curves were not significantly different and both approached asymptotically a minimum crossing time. The fact that rates of decline after bilateral prelimbic saline infusions (Fig. 2D) and after intraperitoneal injections (Fig. 3D) were nearly identical provides a second indication that the prelimbic infusions had not impeded spatial acquisition in spite of the large injected volumes.

4-CPG caused a profound impairment of the crossing skill: No learning between sessions was detected. In contrast, animals affected by MPEP acquired the skill as quickly as did the control group. It may therefore be concluded that 4-CPG inhibited acquisitions of both the spatial choices made at the front panel and the procedural skill of crossing to the rear panel, while MPEP affected neither.

4.8. 4-CPG and MPEP: effect on behaviour in the open-field test

The elevated crossing times caused by 4-CPG (Fig. 2D) could have been influenced by impeded locomotion. Spontaneous locomotion was therefore analysed in the open-field test. The results showed that during the two initial 5-min periods of the test, the bilateral prelimbic 4-CPG injections did not significantly alter the spontaneously moved distance compared to controls (Fig. 4A), and no indication of impeded locomotion was therefore obtained. However, in the course of 20 min in the open field, test and control animals adapted differently to the field. The control group reduced locomotion, and although this decline may be influenced by other factors as well, it can to some degree be viewed as the result of a learning process during which a spatial map is formed and exploration accordingly is reduced. Rats treated with 4-CPG did not display reduced locomotion in the course of the session indicating that inasmuch as spatial short-term acquisition is involved, it may have been impeded. Such an interpretation agrees with the suggestion made above that short-term memory impairment could account for the observed inhibition of correct performance of fully trained rats influenced by 4-CPG (Fig. 2B).

The time course of locomotion proceeded differently for animals receiving injections of MPEP (Fig. 4B). This group displayed reduced locomotion throughout the 20-min test period, and the reduction could not be statistically distinguished from that of the matching control group. Exploratory activity has also been found to be unchanged in mGluR5 mutant mice (Jia et al., 2002). The present results from the open-field test therefore corroborated observations from the reward-finding task in the sense that antagonism of mGluR1 and mGluR5 had differential behavioural effects.

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