Metabotropic Glutamate Receptors in Spatial and Nonspatial Learning in Rats Studied by Means of Agonist and Antagonist Application

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Abstract

We examined the effects of both the metabotropic glutamate receptor (mGluR) antagonist MCPG and the agonist tADA in two behavioral paradigms in rats: (1) brightness discrimination and (2) spatial alternation. Compounds were applied intracerebroventricularly at different times, either 30 min prior to training or immediately after training, and rats were tested for retention 24 hr later in the same paradigms. Both MCPG and tADA caused amnesia in the spatial alternation test, when applied pretraining, but no effect was obtained in the brightness discrimination paradigm. Drug-induced amnesia was shown not to be attributable to state-dependent effects of MCPG or tADA. Moreover, the memory inhibiting effect of MCPG was dose dependent, with a low dose (20 mM/5 ml) having no effect on learning and memory and a 10 times higher concentration (200 mM/5 ml) causing complete amnesia. Application of both saline and MCPG immediately post-training prevented memory formation, which may be attributable to an interference by the injection procedure with the process of memory formation. The mGluR agonist tADA, however, facilitated memory formation in the spatial alternation task, when injected immediately after training.

Post-training application of the compounds had no effect on retention in the brightness discrimination task. On the basis of these data we conclude that (1) mGluRs are of particular importance for spatial learning and play no role in visual discrimination; (2) both the block and the activation of mGluRs inhibit spatial learning, suggesting that saturated activation prevents further modulation of mGluRs, which may be required during learning or memory formation; and (3) mGluR agonist tADA may be memory facilitating when applied after training, thus enhancing the establishment of the memory trace.

Introduction

Learning is the modification of behavior by experience, and memory is the retention of that modification. As learning and memory have become accessible to study on cellular and molecular neuronal levels, a variety of mechanisms of neuronal plasticity have now been identified that are thought to contribute to different forms of learning. One idea that received wide recognition was the concept of modified synapses as expressed by Hebb (1949). The hypothesis of "intensified use" (Eccles 1964) supposes that repeated activation of certain pathways leads to a strengthening of synaptic effectiveness. A typical example of such a mechanism is the phenomenon of long-term potentiation (LTP), first described by Bliss and Lomo in 1973. Because LTP is mainly studied at glutamatergic synapses, much emphasis has been placed on research investigating the role of gluta-

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mate receptors in learning and memory. To date, three major classes of glutamate receptors are well defined: (1) AMPA (*D,L*- α -amino-3-hydroxy-5-methyl-4-isoxazolone propionate)/Kainate receptors, which form channel pores mainly for passage of sodium ions, (2) NMDA (*N*-methyl-*D*-aspartate) receptors, which are coincidence detectors permeable to both sodium and a considerable amount of calcium ions, and (3) metabotropic glutamate receptors (mGluRs), which are coupled to G proteins activating a variety of second-messenger cascades. Specific antagonists for AMPA and NMDA receptors have been available for a long time now, and the role of these glutamate receptors in learning and memory is well established (Morris et al. 1986, 1990; Izquierdo 1994).

At present, eight different subtypes of mGluRs have been cloned (for review, see Pin and Duvoisin 1995) that can be further subclassified on the basis of their amino acid sequence similarities and the second-messenger systems that they act on. Class I mGluRs (mGluR1 and mGluR5) are linked via G protein to phospholipase C, thus hydrolyzing phosphoinositol (PI)-bisphosphate into diacylglycerol (DAG) and PI-trisphosphate (IP₃; Sladeczek et al. 1985; Nicoletti et al. 1986). Class II and III mGluRs (mGluR2, mGluR3, mGluR4, mGluR6, mGluR7, and mGluR8) are negatively coupled to adenylyl cyclase (Schoepp et al. 1992; Schoepp and Conn 1993). Some kinds of glutamate analogs, namely quisqualate, ibotenate, 1-aminocyclopentane-1,3-dicarboxic acid (ACPD), (2*S*, 1 + *S*, 2 + *S*)-carboxycyclopropyl glycine (L-CCG I), and (2*S*, 1 + *R*, 2 + *R*, 3 + *R*)-2-(2,3-dicarboxycyclopropyl) glycine (DCG-IV), appear to be selective agonists for mGluRs in the mammalian central nervous system (Monaghan et al. 1989; Sugiyama et al. 1989; Ishida et al. 1990 1993a,b; Nakagawa et al. 1990; Schoepp et al. 1990; Porter et al. 1992; Shinozaki and Ishida 1992). Specific antagonists, however, have been lacking. Recently, two antagonists have been described, 2-amino-3-phosphonopropionate (AP3) and (*R,S*)- α -methyl-4-carboxyphenylglycine (MCPG; Eaton et al. 1993), the latter belonging to a whole family of phenylglycine derivatives with different pharmacological profiles against mGluR subtypes (Watkins and Collingridge 1994). Unfortunately, these compounds are not subtype specific, and some express both agonistic and antagonistic capacity on different mGluRs. We concentrated on MCPG, because we have shown previously that MCPG antagonizes agonist-induced PI-hydrolysis in hippocampal slices (Riedel et al.

1995a) but has no effect on class II and III mGluR-induced alterations in second messengers (Riedel et al. 1995a). In cerebrocortical slices, however, MCPG also competitively antagonized the depressant effect of ACPD on forskolin-stimulated cAMP production (Eaton et al. 1993). Recently, another compound, trans-azetidine-2,4-dicarboxylic acid (tADA), was described as a potent and selective agonist of mGluRs coupled to phospholipase C (Favaron et al. 1993; Kozikowski et al. 1993). We therefore compared both the antagonist MCPG and the agonist tADA in their ability to alter learning and memory processes. Given the specificity of both compounds, we assume that drug-induced behavioral changes will be the result of a block/activation of class I mGluRs.

Recent investigations provide consistent evidence for a role of mGluRs in LTP. Antagonists, namely AP3 and MCPG, have been repeatedly shown to block late phases of LTP in CA1 in vitro (Bashir et al. 1993; Behnisch and Reymann 1993; Bortolotto et al. 1994; for contrary results, see Chinestra et al. 1993; Manzoni et al. 1994), and in the dentate gyrus in vivo (Riedel and Reymann 1993; Richter-Levin et al. 1994; Manahan-Vaughan and Reymann 1995; Riedel et al. 1995a). In urethane anesthetized rats, however, MCPG failed to block dentate gyrus LTP (Bordi and Ugolini 1995), suggesting changes in sensitivity owing to the anesthetic status of the animals. Furthermore, mGluR agonists like ACPD and tADA facilitate LTP induction both in vitro (Otani and Ben-Ari 1991; Behnisch and Reymann 1993) and in vivo (Riedel et al. 1994c 1995b; Manahan-Vaughan et al. 1996). Given that LTP is suggested to be a substrate for some forms of learning and memory, these data imply that mGluRs may play a pivotal role in behavior and that mGluR antagonists may block memory formation, whereas mGluR agonists might facilitate learning under certain conditions.

In a previous study we provided evidence that the mGluR antagonist MCPG causes amnesia (Riedel et al. 1994a) in rats tested in a newly developed shock-motivated spatial alternation paradigm in a Y-maze (Riedel et al. 1994b). Acquisition was not completely blocked in either the spatial alternation task (Riedel et al. 1994a) or the Morris water maze task (Richter-Levin et al. 1994), but MCPG, applied pretraining, clearly inhibited retention in both paradigms. Similarly, intracerebroventricular (i.c.v.) injections of the specific mGluR agonist ACPD in subtoxic doses attenuated acquisition of a spatial version of the Morris water maze

(Pettit et al. 1994). No probe trials were performed to analyze retention in drug-treated animals. In contrast, Bianchin and co-workers (1994) reported that post-training intrahippocampal injection of MCPG blocks and of ACPD facilitates retention of a step-down inhibitory avoidance task, indicating that there may be differences in drug effects depending on the time point of application. This hypothesis was further supported by the observation that MCPG effectively blocks only when given immediately after, not 180 min after, training (Bianchin et al. 1994). Drug infusion into the olfactory bulb has indicated that, whereas MCPG does not affect pheromonal memory formation in female mice, ACPD and other agonists effectively facilitate the formation of a specific olfactory memory (Kaba et al. 1994).

Further evidence for the importance of mGluRs in behavior has been provided by work on mutant mice lacking mGluR1 (Aiba et al. 1994a,b; Conquet et al. 1994). These mice have severe motor coordination deficits, and thus learning impairments are difficult to interpret. However, the studies indicate that mGluRs contribute to normal behavior. Thus, behavioral investigations using pharmacological treatments of mGluRs should carefully control for side effects and ataxia.

In this study we have investigated further the involvement of mGluRs in learning and memory in rats by means of drug application. MCPG and tADA were chosen for comparison and injected i.c.v. into animals that were trained either in a brightness discrimination task or in a spatial alternation paradigm.

Experiment 1

If one is interested in the relationship between hippocampal LTP and learning and memory, it is important to analyze different behavioral procedures. Morris and co-workers (1986) provided compelling evidence that inhibition of NMDA receptors by i.c.v. injection of 2-amino-5-phosphonopentanoate (AP5) blocked both LTP in the dentate gyrus and spatial learning as assessed in the Morris water maze task. Interestingly, AP5 did not block visual discrimination learning, suggesting that NMDA receptor activation may be crucial for spatial as opposed to other learning tasks.

In this experiment we intended to determine whether mGluRs, in contrast to NMDA receptors, might be also involved in visual discrimination learning.

Materials and Methods

ANIMALS

Forty-eight male Wistar rats, which weighed 270–320 grams at the time of surgery, were used in this experiment. They were housed individually under acclimatized laboratory conditions with free access to water and food, maintained under 12:12 hr light–dark cycle (light on at 6 AM; light off at 6 PM). Test animals were randomly assigned to the various groups, and two different injection regimes were applied.

In experiment 1A, animals were i.c.v. injected with the compounds 30 min prior to training [saline, $n = 15$; MCPGh (read MCPG high, because a lower concentration was used in spatial alternation as well), $n = 19$], whereas in experiment 1B injection was performed immediately after training (saline, $n = 7$; MCPGh, $n = 7$).

SURGERY

For implantation of cannulae, animals were anesthetized with nembutal (40 mg/kg, i.p.) and mounted in a stereotaxic frame. The skin was carefully removed, and the skull exposed. A hole was drilled into the skull for unilateral insertion of a guide cannula into the lateral ventricle according to stereotaxic coordinates relative to Bregma (caudally, 0.8; laterally, 1.6; depth, 3.6 mm). The guide cannula was then lowered into the ventricle and cemented to the head with dental acrylic. Animals were transferred back to their home cages for recovery, which lasted 5–7 days.

HISTOLOGY

After finishing the experimental procedures, animals were killed by an overdose of nembutal, and histological analysis was conducted to confirm the position of the cannula in the ventricle. Only animals that had the cannula inserted into the ap-

appropriate location were chosen for further analysis of the data.

DRUGS AND DRUG APPLICATION

The mGluR antagonist MCPG (racemic form, Tocris, UK) was applied in a concentration that has been shown in physiological experiments to affect expression of LTP in the dentate gyrus in vivo (Riedel et al. 1994a, 1995a). MCPG was dissolved in saline (0.9%) prior to use, and a total volume of 5 μ l was injected within 5 min. The concentration of MCPG was 200 mM in 5 μ l, referred to as MCPGh, because in a later series (experiment 3) a second concentration that was 10 times lower (referred to as MCPGl) was introduced. MCPGh may cause a drug concentration in the brain of 500 μ M given a uniform distribution and a brain volume of 2 ml. Saline injection served as control.

In experiment 1A, substances were i.c.v. injected 30 min prior to training with a flow rate of 1 μ l/min by means of a microsyringe and an injector fitting into the guide cannula. Animals remained freely moving in their home cages during injection and for 30 min afterwards.

In experiment 1B, substances were injected immediately post-training. Animals were removed from the test apparatus, placed in their home cages, and given a 5- μ l injection with a flow rate of 1 μ l/min.

APPARATUS

The tasks were performed in a computer-controlled Y-maze as originally described by Ott and co-workers (1972). Briefly, the Y-maze consisted of three equal arms (30 $\times$ 15 $\times$ 15 cm L $\times$ W $\times$ H) equipped with a grid floor of stainless steel bars (4.5 mm diam., spaced 1.5 cm apart center to center), wired for electric shock from a constant source. Walls and lids were made of red polyvinylchloride. On the end of each arm, small light bulbs (15 W) were installed, covered with opaque glass.

BRIGHTNESS DISCRIMINATION TASK

HABITUATION

Rats were placed in the maze. Habituation, during which animals moved freely in the test ap-

paratus, lasted for 5 min prior to training (day 1) and prior to retention test (day 2). This period of equilibration was important for the information gained on the behavioral and motivational effects of applied drugs during exploration of a novel environment. We measured (1) the number of entries in left arms of the maze, (2) the number of entries in right arms of the maze, and (3) the number of fecal boli.

TRAINING

At the beginning of each trial, the goal box was illuminated and a footshock was delivered to the start box. Rats had to escape from footshock (0.7–1.3 mA, depending on individual sensitivity) in the start alley of the Y-maze by running into one of the other arms. The entry into the dark arm was also punished by footshock and counted as an error; the animal had to learn to escape into the illuminated arm. When the animal had escaped into the goal box, shock was terminated and the light remained on for another 20 sec. Rats remained in the goal arm for the 60-sec intertrial interval; between-trial movement was extremely rare, and those animals were discarded from the experiment. For the next trial, the former goal box served as the start box, thus avoiding intertrial handling of the test animals. Again, one illuminated arm served as goal box and shock was applied in the start and the second dark (wrong) arm. A total of 30 trials were performed both during training and retention with an intertrial interval of 60 sec. The side of illumination was changed after three consecutive runs, and a correct performance required three right followed by three left turns. Other parameters were also recorded: first, the shock intensity applied during the session, which differed between individuals; second, the reaction time (time of escape from the start box after onset of the shock); and third, total trial time (time required to escape into the goal box). These parameters may provide information about drug side effects on motor coordination and sensory systems.

RETENTION

Retention was tested 24 hr after training in the same test apparatus, and the same behavioral procedure was applied as on day 1 during training.

Again, 30 trials were performed. The reduction in the number of errors compared with day 1 (training) was expressed as an index of retention (% savings), calculated as

$$\% \text{ savings} = \frac{\text{training errors} - \text{retention errors}}{\text{training errors}} \times 100$$

Results

EXPERIMENT 1A—MCPG/SALINE INJECTION 30-MIN BEFORE TRAINING

HABITUATION

Pretraining injection of MCPGh caused no alteration in habituation. Table 1 shows that controls and MCPGh-treated animals made comparable numbers of left and right entries during the habituation period; similarly, fecal boli did not differ between groups. MCPGh has then no effects on behavioral or motivational reactions to novelty in rats.

TRAINING AND RETENTION TEST

Rats injected with saline showed rapid acquisition of the new behavior. ANOVA revealed no difference between groups but a significant difference between days [$F(1,32) = 28.21, P < 0.01$]. The number of errors (all given as means $\pm$ S.E.M.) progressively decreased over trials. During training, controls made 7.9 ± 1.0 errors, and this number was significantly reduced on day 2 to 5.7 ± 1.0 (paired t -test: $t = 2.26, P < 0.05$). Similarly, the number of errors in the MCPGh group was 9.2 ± 0.7 and 4.9 ± 0.9 on day 1 and day 2, respectively (paired t -test: $t = 5.46, P < 0.01$). The learn-

ing curves of the groups are shown in Figure 1 for both controls (Fig. 1A) and MCPGh group (Fig. 1B). Pretraining injection did not significantly affect learning on day 1 (training), and rats quickly acquired a low level of errors during training, which was maintained on day 2 (retention test), indicating normal learning in the MCPGh group. The curves of both groups showed a considerable degree of overlap. We did not find a significant group by day or group by block interaction. Accordingly, the percent savings in both groups reached similar levels (Fig. 1C) with no difference between groups.

To test for MCPG-induced alterations in sensory mechanisms or coordination, we compared shock intensities, reaction time (RT; time to escape from the start box), and total trial times (TT; time required to enter the goal box) as summarized in Figure 2, A and B. No considerable effect involving groups was found, indicating that the drug caused neither sensory nor motor deficits. These data confirm results obtained during the habituation period suggesting that MCPG application caused no side effects that could affect the learning paradigm.

EXPERIMENT 1B—MCPG/SALINE INJECTION IMMEDIATELY AFTER TRAINING

HABITUATION

All animals underwent habituation before any drug treatment, no differences between groups were observed.

TRAINING AND RETENTION TEST

As indicated above, animals were trained in a drug-free state (Fig. 3A, control; Fig. 3B, MCPGh),

Table 1: Habituation in the Y-maze after pretraining injection of saline or MCPGh

Group	n	Left	Right	FB
Control	15	9.9 ± 1.1	9.3 ± 1.1	0.3 ± 0.2
MCPGh	21	10.6 ± 1.0	9.9 ± 1.4	0.4 ± 0.2

Animals were exposed to the test chamber for 5 min to equilibrate in the novel environment. Number of entries into left and right arms and, in addition, number of fecal boli (FB) were counted as measures for activity as well as emotional status of the rats. Values are expressed as means $\pm$ S.E.M.; n = number of animals per group.

Figure 1: Effects of saline and MCPGh on learning and retention of a shock-motivated brightness discrimination task. Both saline (A) and MCPGh (B) were injected 30 min prior to training (day 1 = d1). Thirty trials per session (day 1 and day 2 = d2) were performed. The errors (%) made in blocks of five trials are compared. Data are expressed as mean $\pm$ S.E.M. As indicated by the learning curves, MCPGh-treated rats performed slightly worse on day 1. On day 2 (retention test), both groups performed at a low level of errors, indicating normal retention. This is confirmed by calculating the percent savings (for details, see Materials and methods) shown in C, where no difference between groups occurred.

and ANOVA revealed no group difference in acquisition. Both groups rapidly improved the performance as indicated by a progressive decline in the number of errors per block of five trials on day 1. MCPGh injection after training did not affect retention of the brightness discrimination task. Groups significantly reduced the number of errors from day 1 to day 2 (means $\pm$ S.E.M.; controls: day 1, 10.1 ± 0.9 ; day 2, 4.9 ± 1.0 ; paired *t*-test; $t = 4.12$, $P < 0.01$; MCPGh: day 1, 9.1 ± 1.7 ; day 2, 4.6 ± 1.3 ; paired *t*-test, $t = 3.67$, $P < 0.01$). Similarly, percent savings in both groups (Fig. 3C) were not significantly different.

Discussion

The results of experiment 1 provide evidence that the mGluR antagonist MCPGh, given in a concentration that interferes with the expression of LTP (Riedel et al. 1994a, 1995a), does not alter either acquisition or retention of a brightness discrimination task. Assessment of possible drug-induced side effects showed that MCPGh does not affect general sensitivity or motor coordination, although such effects are seen in mGluR1 mutant mice (Aiba et al. 1994a,b; Conquet et al. 1994). The lack of effect of MCPGh suggests either (1) that

Figure 2: Effects of saline and MCPGh on shock intensity (A), reaction time (RT in B) and total trial time (TT in B) in the brightness discrimination task. Neither shock intensities nor times to perform the task were different between groups. Data are expressed as mean $\pm$ S.E.M. (d1) Day 1; (d2) day 2.

Figure 3: Effects of saline and MCPGh on retention of a brightness discrimination task. Both compounds were injected i.c.v. immediately after training. Controls (A) and MCPG-treated animals (B) performed similarly during acquisition (day 1) and retention (day 2) showing no retention deficit. This result was confirmed by the retention index (% savings) given in C. Data are expressed as mean $\pm$ s.e.m. (d1) Day 1; (d2) day 2.

mGluR activation is not required during normal behavior and that the motor deficits in mutant mice should be ascribed to developmental disturbances or (2) that motor disturbances in mutant mice occur because of a lack of cerebellar mGluR1, and i.c.v. injection of MCPG does not reach the cerebellum.

The failure to detect any deficit in MCPGh-treated animals suggests that mGluR activation in general is not required to learn and/or remember a brightness discrimination task. Similarly, AP5 treatment has no effect on visual discrimination learning in rats (Morris et al. 1986).

Experiment 2

On the basis of the data obtained in experiment 1 we have suggested that mGluRs are not involved in brightness discrimination learning. Experiment 2 explores this proposal by investigating the effects of the mGluR agonist tADA on brightness discrimination.

Materials and Methods

ANIMALS

Thirty-seven male Wistar rats, which weighed 270–320 grams at the time of surgery, were used in this experiment. Test animals were randomly assigned to the various groups, and two different injection regimes were applied.

In experiment 2A, animals were i.c.v. injected

with the compounds 30 min prior to training (saline, $n = 10$; tADA, $n = 9$), whereas in experiment 2B, injection was performed immediately after training (saline, $n = 9$; tADA, $n = 9$).

SURGERY AND HISTOLOGY

Implantation of cannulae and histological examinations were performed as described in the preceding experiment. Only animals with cannulae in the appropriate location were chosen for data analysis.

DRUGS AND DRUG APPLICATION

In this series of experiments, the mGluR agonist tADA (racemic form, Calbiochem, USA) was applied in a concentration that has been shown to facilitate LTP in freely moving rats in vivo (Riedel et al. 1994c, 1995b; Manahan-Vaughan et al. 1996). tADA was dissolved in saline (0.9%) prior to use, and a total volume of 5 μ l was injected into the ventricle of test animals within 5 min. The dose of tADA was 20 mM in 5 μ l, which may cause a brain concentration of 50 μ M given a uniform distribution and a brain volume of 2 ml. Saline injection served as control.

In experiment 2A, substances were injected 30 min prior to training, and in experiment 2B, immediately after training.

APPARATUS AND BRIGHTNESS DISCRIMINATION TASK

The same apparatus and behavioral test was used as described in experiment 1.

Results

EXPERIMENT 2A—tADA/SALINE INJECTION 30 MIN BEFORE TRAINING

HABITUATION

Habituation performance (Table 2) assessed over 5 min following pretraining injection indicated a significant reduction in exploration to novelty in the tADA group, measured as entries into left and right arms of the maze [$F(1,17)=10.56$, $P<0.01$]. Post hoc unpaired t -test revealed a significant reduction only in left entries ($t=2.56$, $P<0.05$; asterisk (*) in Table 2).

TRAINING AND RETENTION TEST

Pretraining injection of tADA did not affect acquisition and retention in the shock-motivated brightness discrimination task compared with saline injected controls (Fig. 4A, controls; Fig. 4B, tADA). Both groups started at chance level on the first block of training trials (day 1) and subsequently reduced the number of errors to a low level that is maintained during retention test (means $\pm$ S.E.M.; controls: day 1, 10.1 ± 0.9 ; day 2, 5.0 ± 0.75 ; tADA: day 1, 9.9 ± 1.2 ; day 2, 5.2 ± 1.2). No significant group differences were found, but there was a highly significant reduction in errors between days [$F(1,17)=41.11$, $P<0.01$]. Subsequent within-group comparisons by means of paired t -tests showed significant reductions in errors in both groups on day 2 (controls: $t=4.83$, $P<0.01$; tADA: $t=4.26$, $P<0.01$). As would be expected, the savings were similar in both groups (Fig. 4C). Comparison of the shock intensities used (Fig. 5A) found no significant group difference, indicating that tADA-treated animals showed

no changes in sensitivity to footshock. Likewise, both reaction (RT) and total trial times (TT; Fig. 5B) were comparable in both groups. Thus, the drug-induced effects observed during habituation did not influence performance in the brightness discrimination task.

EXPERIMENT 2B—tADA/SALINE INJECTION IMMEDIATELY AFTER TRAINING

HABITUATION

All animals were tested drug free, and no significant differences in habituation were observed.

TRAINING AND RETENTION TEST

The learning curves of both groups (still drug free) were similar on day 1 (Fig. 6A, controls; Fig. 6B, tADA). Post-training injection of tADA did not affect performance of the brightness discrimination task on day 2, compared with controls. The performance of both groups was similar on day 2, and the statistical comparison indicated no difference between groups but a significant difference between days [$F(1,16)=20.22$, $P<0.01$]. Post hoc within-group comparisons revealed significantly reduced numbers of errors for both groups on day 2 (controls: day 1, 10.3 ± 0.8 ; day 2, 5.7 ± 0.9 ; $t \leq 3.14$, $P<0.01$; tADA: day 1, 11.3 ± 1.0 ; day 2, 6.6 ± 1.2 ; $t=3.22$, $P<0.01$). Percent savings were similar in controls and tADA-treated animals. We therefore conclude that neither pre- nor post-training application of tADA affects retention of a shock-reinforced brightness discrimination task.

Discussion

The experiments performed under MCPGh treatment suggested that mGluRs may not be in-

Table 2: Habituation in the Y-maze after pretraining injection of saline or tADA

Group	<i>n</i>	Left	Right	FB
Control	10	11.6 ± 1.6	10.0 ± 1.3	0.3 ± 0.2
tADA	9	$7.5 \pm 0.8^*$	7.6 ± 0.8	0.7 ± 0.4

Animals were exposed to the test chamber for 5 min to equilibrate in the novel environment. Number of entries into left and right arms and, in addition, number of fecal boli (FB) were counted as measures for activity as well as emotional status of the rats. Values are expressed as means $\pm$ S.E.M.; *n* = number of animals per group. Animals treated with tADA showed a significant reduction in activity during habituation (*). (For details, see text.)

Figure 4: Injection of saline and tADA did not affect brightness discrimination learning. Substances were applied 30 min prior to training. Thirty trials were performed during acquisition (day 1 = d1) and retention test (day 2 = d2) comprised to blocks of five trials. Data are expressed as mean $\pm$ S.E.M. Controls (A) and tADA-treated rats (B) progressively reduced the number of errors during training and maintained this low level during retention test, suggesting normal memory in both groups. Similarly, the percent savings (C) were equal for both groups.

involved in brightness discrimination learning. The results of experiment 2 confirm this suggestion, showing that activation of mGluRs either before or after training has no influence on either learning or retention of this visual task. Although tADA slightly reduced exploratory activity during habituation, this appears to be unimportant for performance in the task.

The specificity of tADA for class I mGluRs suggests in particular that mGluRs coupled to phospholipase C do not play a part in visual discrimination learning. Further experiments using drugs selectively acting on class II and III mGluRs are required to show whether mGluRs in general, or class I mGluRs in particular, are not involved in such forms of learning.

Experiment 3

The preceding experiments have indicated that both the mGluR agonist tADA and the antagonist MCPG, in concentrations that affect LTP, have no influence on brightness discrimination learning, whether injected before or after training.

The second part of this investigation addresses the question of whether spatial learning is sensitive to drugs acting on mGluRs. We have recently established a spatial alternation paradigm, performed in the same test apparatus (Riedel et al. 1994b). Learning of this task has been shown to be sensitive to the anticholinergic compound scopolamine and the NMDA receptor antagonist AP5 (Riedel et al. 1994b). Because MCPG has been

Figure 5: Injection of tADA did not produce any side effects as revealed by measurement of shock intensity (A), reaction time (RT in B), and total trial time (TT in B) in the brightness discrimination task. Data are expressed as mean $\pm$ S.E.M. (d1) Day 1; (d2) day 2.

Figure 6: Effects of saline and tADA on retention of a brightness discrimination task, when injected immediately after training. Both saline (A) and tADA-treated animals (B) performed similarly during acquisition (day 1 = d1) and retention test (day 2 = d2) showing no retention deficit. This result was confirmed by the retention index (% savings) given in C. Data are expressed as mean $\pm$ S.E.M.

shown to block hippocampal LTP *in vivo* (Riedel et al. 1995a) and the hippocampus is believed to be involved in spatial learning (O'Keefe and Nadel 1978), we were particularly interested to see whether the mGluR antagonist MCPG, which did not affect brightness discrimination, might disrupt this form of spatial learning.

Materials and Methods

ANIMALS

Sixty-two male Wistar rats weighing 270–320 grams at the time of surgery were used in this experiment and were randomly assigned to the various test groups.

Experiment 3A examined the effects of MCPG on learning and retention of a spatial alternation task when applied 30 min prior to training. Five different groups were compared: (A) controls ($n=8$), injected with 5 ml of saline (0.9%) 30 min prior to training (day 1); (B) MCPGh ($n=8$), injected 30 min prior to training (day 1); (C) state-dependency 1 ($n=8$), injected with MCPGh 30 min prior to retention test (day 2); (D) state-dependency 2 ($n=9$), injected with MCPGh 30 min prior to training (day 1) and prior to retention test (day 2); (E) MCPGl ($n=10$), injected 30 min prior to training (day 1). We had to control for state dependency, because MCPGh significantly impaired retention in the spatial alternation task, and we thus had to exclude possible drug-induced side effects on performance (Overton 1984; Poling and Cross 1993).

In experiment 3B, post-training injection of

MCPGh ($n=9$) and saline ($n=10$) were compared.

SURGERY AND HISTOLOGY

Implantation of cannulae and histology was performed as described in detail in experiment 1. Only animals with cannulae in the appropriate location were chosen for data analysis.

DRUGS AND DRUG APPLICATION

MCPG (racemic form, Tocris, UK) was dissolved in saline (0.9%) prior to use and injected in a total volume of 5 μ l per animal. Two different concentrations of MCPG were applied: MCPGh was 200 mM in 5 μ l and MCPGl was 20 mM in 5 μ l. Thus, the overall drug concentration in the brain may be 500 μ M and 50 μ M, respectively. The lower MCPG dose was introduced, because MCPGh caused severe retention deficits. Saline served as control.

In experiment 3A we applied the compounds 30 min prior to training, and as we observed a retention deficit, we went on to control for state dependency by adding two further groups (see Animals). Experiment 3B analyzed the effects of MCPGh on spatial alternation learning when applied immediately after training.

APPARATUS

The task was performed in the same Y-maze as described in detail in experiment 1. No intramaze cues were provided.

SPATIAL ALTERNATION TASK

The procedure of this spatial alternation task has been described in detail elsewhere (Riedel et al. 1994b).

HABITUATION

Rats were placed in one arm of the maze and allowed to habituate for 5 min prior to both training and the retention test. We measured the number of entries in either left or right arms of the maze, as well as the number of fecal boli.

TRAINING

At the beginning of the first trial, a footshock from a constant source (0.7–1.3 mA depending on individual sensitivity) was delivered to the start box (arm 1). The animal had to escape into the “right” alley (arm 2) to avoid further footshocks. Entry into the “left” alley (arm 3) was counted as an error and punished by footshock as long as the animal escaped into the goal box (arm 2). Reentering the start box was also punished by footshock until the test animal had escaped into the goal box. In the next trial, the former goal box (arm 2) served as start box, thus avoiding intertrial handling, and the animal had to escape the footshock by running into the left alley (arm 1), and so on. A wrong choice again was punished by footshock. The intertrial interval (time between the end of, for instance, trial one and onset of trial 2) was set at 60 sec. During this period, animals remained in the goal box; animals that moved between the alleys during the intertrial interval were discarded ($n = 3$). A total of 20 trials were performed in which the animal alternated between arm 1 and 2 (phase 1). Then, the animal was removed by hand and replaced into alley 3 (phase 2). A second phase of 20 trials were performed with arms 3 and 1 as start/goal boxes. The design was chosen for the following reason: During the first phase (trials 1–20), animals had to alternate between arm 1 and 2. A high percentage of correct choices could be achieved simply by learning to avoid arm 3 rather than by learning spatial alternation. In phase 2, therefore, we placed the animals in arm 3, which was punished in all trials of phase 1. Thus, rats could not escape successfully simply by avoiding one particular arm. As indicated by the data, the change in start boxes leads

to a drop in performance back to chance levels followed by a progressive decline in the number of errors during phase 2. In a pilot study (data not shown), we included a third phase in which the rats were also placed in arm 2 for another 20 trials. Performance in this phase was similar to that of phase 2, and because data were not different, we conducted two-phase experiments only.

RETENTION

Twenty-four hours after training, a retention test was performed in the same apparatus, using the same behavioral procedure as during training. Again, a total of 40 trials in two phases were conducted, and the reduction in the number of errors between training and retention test was calculated as percent savings and used as an index of retention.

Results

EXPERIMENT 3A—MCPG/SALINE INJECTION 30 MIN BEFORE TRAINING

HABITUATION

In none of the five groups were any abnormalities observed during habituation. Entries in left and right alleys as well as the number of fecal boli did not differ significantly between groups (see Table 1).

TRAINING AND RETENTION TEST

Saline-injected rats showed rapid acquisition during phase 1 of training (Fig. 7A, blocks 1–4) as indicated by a decline in the number of errors per block of five trials. In phase 2, they again start at chance level and subsequently improve (Fig. 7A, blocks 5–8). A similar behavior was observed in all other groups (Fig. 7B–E). During retention test, the control (Fig. 7A) and MCPGI group (Fig. 7E) showed clear reduction in the number of errors (mean $\pm$ S.E.M.; saline: day 1, 13.1 ± 1.5 ; day 2, 8.33 ± 0.8 ; MCPGI: day 1, 12.5 ± 1.1 ; day 2, 9.6 ± 1.2). In the MCPGh group (Fig. 7B), the number of errors on day 1 and 2 were similar (means $\pm$ S.E.M.; day 1, 12.6 ± 1.8 ; day 2, 12.3 ± 1.5),

Figure 7: (See facing page for legend.)

Table 3: Statistical group comparison of the effects of MCPG on percent savings in spatial alternation learning as revealed by an unpaired *t*-test

	MCPGh pretraining	MCPGh pretraining and preretention
Controls	$t = 3.0, P < 0.01$	$t = 2.46, P < 0.05$
MCPGh preretention	$t = 2.77, P < 0.01$	$t = 2.23, P < 0.05$
MCPGI	$t = 2.66, P < 0.05$	$t = 2.08, P < 0.05$

As indicated, both groups injected with MCPGh 30 min prior to training were significantly different from the three other groups, as revealed by an unpaired *t*-test.

indicating amnesia. When tested for state-dependent drug effects, we found that animals injected with MCPGh prior to retention (group C; Fig. 7C) showed normal acquisition and retention (means $\pm$ S.E.M.; day 1, 13.0 ± 1.2 ; day 2, 8.8 ± 1.1). However, animals injected with MCPGh 30 min prior to training and prior to retention (group D; Fig. 7D) were amnesic (means $\pm$ S.E.M.; day 1, 11.9 ± 1.3 ; day 2, 11.2 ± 1.4). These data confirm that the deficit in the MCPGh pretraining group is not the result of state-dependent drug effects.

An overall ANOVA revealed no significant differences between groups but a significant effect of days [$F(1,38) = 21.21, P < 0.01$]. Moreover, a significant group by day interaction was obtained [$F(4,38) = 4.36, P < 0.01$]. Subsequent post hoc paired *t*-test confirmed that only the control group ($t = 4.16, P < 0.01$), the MCPGI group ($t = 3.18, P < 0.01$), and the MCPGh group injected before retention ($t = 3.29, P < 0.01$) significantly decreased the number of errors on day 2.

Calculation of the retention index supported these findings. As summarized in Figure 7F, the two groups that were i.c.v. injected with MCPGh prior to training showed no retention. ANOVA on the percent savings revealed significant group differences [$F(4,38) = 4.03, P < 0.01$], and group comparison by an unpaired *t*-test confirmed significant

differences between both MCPGh before training and the remaining three groups (Table 3).

EXPERIMENT 3B—MCPGH/SALINE INJECTION IMMEDIATELY AFTER TRAINING

HABITUATION

All animals showed normal exploration.

TRAINING AND RETENTION TEST

As observed for the habituation period, all animals performed similarly during drug-free training, showing a progressive decline in the number of errors (Fig. 8A,B). During the retention test, however, errors were only minimally reduced in both groups compared with training (means $\pm$ S.E.M.; controls: day 1, 13.8 ± 1.2 , day 2, 11.9 ± 1.5 ; MCPGh: day 1, 14.4 ± 1.9 ; day 2, 12.7 ± 1.5), and neither a group nor a day effect was revealed by ANOVA. These data suggest that post-training injection of both saline and MCPGh inhibits memory formation. As further indicated by percent savings (Fig. 8C), no significant difference was found be-

Figure 7: Spatial alternation learning in the Y-maze is blocked by pretraining-injected MCPG in a dose-dependent manner. Two concentrations of MCPG were used: MCPGh (high) = 200 mm/5 ml; MCPGI (low) = 20 mm/5 ml. Data are summarized as blocks of five trials and errors (%) per block are indicated comparing day 1 (d1) and day 2 (d2). Controls (A) injected with saline 30 min prior to training showed rapid acquisition and good retention of the task. In contrast, MCPGh (B) applied prior to training produced amnesia as confirmed by percent savings close to zero (bar B in F). Thus, two state-dependency controls were performed, in which MCPGh was applied 30 min prior to retention test (C) or prior to both training and retention test (D). Group C showed no retention deficit (bar C in F), but group D was similar to the MCPG pretraining group (bar D in F), strongly indicating that the MCPG-induced retention deficit is not attributable to state-dependent effects of the drug. MCPGI (E) applied prior to training, however, caused no learning deficit (bar E in F). (F) The savings for the five individual groups were calculated; significant differences were marked by asterisks (*), and values are shown in Table 3. Data were expressed as mean $\pm$ S.E.M.

Figure 8: Spatial alternation learning is influenced by post-training injection of both saline and MCPGh. The course of the learning curves of controls (A) and MCPGh-treated animals (B) is very similar on day 1 (d1) and day 2 (d2). Both groups showed no reduction in the number of errors on day 2, suggesting amnesic effects of either the substances or the injection procedure. No group difference was found for percent savings (C). Data were expressed as mean $\pm$ S.E.M.

tween groups and the retention index is clearly reduced compared with animals injected prior to training with saline (Fig. 7F).

Discussion

The results of experiment 3 provide compelling evidence that the mGluR antagonist MCPG blocks retention of spatial alternation when applied prior to training. Moreover, the data suggest that the action of MCPG is concentration dependent, because MCPGI caused no deficit and MCPGh caused amnesia. This is a particularly interesting observation if compared with data obtained from LTP *in vivo* showing that MCPGI selectively blocks late LTP and MCPGh inhibits both early and late stages of LTP (Riedel et al. 1995a). The behavioral results thus imply that it is the inhibition of early LTP that may lead to retention deficits. In agreement with this hypothesis, AP5, which also blocks early and late LTP, has been shown to cause amnesia of spatial alternation learning (Riedel et al. 1994b; Walker and Gould 1994).

Because we have excluded possible drug-induced side effects and spatial alternation is believed to depend on intact hippocampal physiology, it seems likely that blockade of hippocampal mGluRs may have been responsible for the memory deficit. Given that MCPG selectively blocks class I mGluRs in the hippocampus (Riedel et al. 1995a), these data may be taken as evidence for a role of mGluR1 and mGluR5 in spatial rather than discrimination learning.

Interestingly, post-training application of both

saline and MCPGh caused amnesia as well. Similar saline effects have been reported for on-trial avoidance learning in chicks (Rosenzweig et al. 1993). A plausible explanation is that the injection procedure itself may have blocked memory formation. Clearly, effects of post-training injection of MCPGh can not be assessed with this method.

Experiment 4

In addition to some nootropic agents acting on glutamate receptors of the AMPA type, which have been shown to facilitate both learning and LTP induction (Granger et al. 1993; Stäubli et al. 1994), the mGluR agonists ACPD and tADA have been shown to facilitate LTP *in vitro* and *in vivo*, respectively (Behnisch and Reymann 1993; Riedel et al. 1994c, 1995b). Because mGluR stimulation activates a variety of second-messenger cascades and therefore induces long-lasting changes, mGluR agonists may also facilitate or prolong memory. In agreement with the hypothesis that LTP may be a substrate for learning and memory, MCPG has been shown to block both spatial learning and LTP (Richter-Levin et al. 1994; Riedel et al. 1994a). Recent investigations have provided evidence that the mGluR agonists ACPD and L-CCG I improve memory of inhibitory avoidance learning in rats (Bianchin et al. 1994) and formation of an olfactory memory block in female mice (Kaba et al. 1994). This series of experiments therefore examined the effect of the mGluR agonist tADA in a spatial alternation paradigm, to see whether tADA might facilitate learning.

Materials and Methods

ANIMALS

Fifty-one male wistar rats weighing 270–320 grams at the time of surgery were used in experiment 4. Animals were housed individually in acclimatized rooms with free access to water and food. They were randomly assigned to the various test groups.

Experiment 4A analyzed the effect of tADA on acquisition and retention of a spatial alternation task when i.c.v. injected 30 min prior to training. Three different groups were tested: (A) injection of 5 μ l of saline ($n=10$); (B) injection of 5 μ l of tADA ($n=10$); (C) control for state dependency, because we observed an effect in the tADA pre-training group (B). Animals of group C received i.c.v. injection of 5 μ l of tADA 30 min prior to training and prior to retention ($n=10$).

In experiment 4B, saline ($n=12$) and tADA ($n=9$) were applied immediately after training.

SURGERY AND HISTOLOGY

The implantation of the cannulae was performed as described in experiment 1. After behavioral testing, brains were examined histologically. Animals with cannulae not located in the appropriate place were discarded from data analysis.

DRUGS AND DRUG APPLICATION

tADA was dissolved and injected as described in experiment 2.

APPARATUS AND SPATIAL ALTERNATION TASK

Behavioral testing was performed in the Y-maze described in experiment 1. Spatial alternation was performed as in experiment 3.

Results

EXPERIMENT 4A—tADA/SALINE INJECTION 30 MIN BEFORE TRAINING

HABITUATION

Animals injected with tADA 30 min prior to training again showed a reduction in exploration

in the Y-maze, as observed in experiment 2. The number of entries into left and right arms was reduced, the number of fecal boli remained unchanged.

TRAINING AND RETENTION TEST

Figure 9 summarizes the results obtained from this series of experiments. All three groups [Fig. 9A, controls; Fig. 9B, tADA; Fig. 9C, tADAsd (state dependency)] rapidly acquired the task and progressively reduced the number of errors on day 1. During the retention test 24 hr later, both tADA groups were found to be amnesic, showing the same number of errors as on day 1 (means $\pm$ S.E.M.: tADA; day 1, 14.4 ± 1.1 ; day 2, 13.1 ± 1.4 ; tADAsd: day 1, 12.5 ± 0.8 ; day 2, 12.6 ± 1.3). Controls, on the other hand, clearly showed improved performance during the retention test (means $\pm$ S.E.M.; day 1, 13.6 ± 0.8 ; day 2, 8.2 ± 0.8). Statistical comparison failed to reveal a difference between groups, but a significant group by day interaction was found [$F(2,27)=7.59$, $P<0.01$]. Post hoc within group comparison confirmed that only controls significantly reduced the number of errors on day 2 (paired t -test: $t=5.11$, $P<0.01$). Between-group comparison applying an unpaired t -test revealed a significant difference between control and tADA ($t=3.61$, $P<0.01$) as well as between control and tADAsd groups ($t=2.77$, $P<0.01$).

Calculation of percent savings (Fig. 9D) showed that controls clearly improved between training and retention, whereas both tADA groups were amnesic. ANOVA revealed a significant difference between groups [$F(2,27)=9.8$, $P<0.01$], and subsequent between-group comparison confirmed group differences between controls and tADA and controls and tADAsd ($t=3.39$, $P<0.01$ and $t=4.16$, $P<0.01$, respectively). These data provide compelling evidence that pretraining i.c.v. application of the mGluR agonist tADA causes amnesia, and this effect is not attributable to unspecific side effects of the drug.

EXPERIMENT 4B—tADA/SALINE INJECTION IMMEDIATELY AFTER TRAINING

HABITUATION

No difference in exploratory behavior was found between groups.

Figure 9: Spatial alternation learning in the Y-maze is blocked by pretraining i.c.v. injection of tADA. Data are summarized as blocks of five trials and errors (%) per block are shown comparing day 1 (d1) and day 2 (d2) in each group. Means $\pm$ S.E.M. Saline injected controls (A) showed rapid acquisition during training as indicated by a progressive reduction of the number of errors. During retention test, controls performed significantly better than on day 1 and thus have high percent savings (D, NaCl). Pretraining injection of tADA (B) did not affect acquisition but prevented retention tested 24 hr later. This is confirmed by percent savings that were close to zero (D, tADA). Similarly, animals tested for state dependency (C, tADA sd, injection of tADA prior to training and retention test) revealed normal learning on day 1, but failed to show any retention. Thus, the memory inhibition is not attributable to side effects of tADA. As indicated by asterisks (*) in D, significant differences between controls and both tADA groups were obtained.

TRAINING AND RETENTION TEST

Both groups showed normal acquisition on day 1 (Fig. 10A, controls; Fig. 10B, tADA). During retention test, saline-injected controls performed as on day 1, showing no reduction in the number of errors (means $\pm$ S.E.M.; day 1, 12.2 ± 1.1 ; day 2, 12.3 ± 1.4). This is in agreement with the results reported in experiment 3B where post-training injection of saline also resulted in amnesia. In contrast, animals posttraining injected with tADA clearly reduced the number of errors on day 2 (means $\pm$ S.E.M.; day 1, 14.2 ± 1.4 ; day 2, 10.5 ± 1.4), suggesting that the drug facilitated memory formation. A significant group-by-day interaction was observed [$F(1,19) = 4.7$, $P < 0.05$], and post hoc within-group comparisons confirmed a significant reduction in the number of errors in the tADA

group (paired t -test: $t = 2.26$, $P < 0.05$) but not in controls. Similarly, the percent savings (Fig. 10C) were close to zero in controls and clearly enhanced in tADA-exposed animals [$F(1,19) = 8.08$, $P < 0.05$].

Discussion

On the basis that pretetanus-applied tADA facilitates in vivo LTP (Riedel et al. 1994c, 1995b; Manahan-Vaughan et al. 1996), we predicted that application of tADA prior to training might facilitate learning. The results reported in experiment 4A, however, indicate the opposite. Pretraining injection of tADA blocked, rather than facilitated,

Figure 10: tADA enhances memory formation in a spatial alternation paradigm when injected immediately after training. Data are summarized in blocks of five trials, and errors (%) per block are shown comparing day 1 (d1) and day 2 (d2). Means $\pm$ S.E.M. Rapid acquisition indicated by a progressive reduction in the number of errors is obvious in both controls (A) and tADA-treated rats (B). On day 2, however, tADA-injected rats performed significantly better than controls, also indicated by higher percent savings (C).

memory formation in the spatial alternation task. However, tADA facilitated memory formation when applied immediately after training (experiment 4B). Although the amnesic effect of post-training-injected saline is somewhat unexpected and its interpretation remains unclear, we believe the tADA effect to be genuine for the following reasons: (1) Post-training injection of saline was a replication of experiment 3B and yielded similar results. (2) MCPGh injection after training had no effect compared with controls, suggesting that the facilitation in the tADA group may be the result of the selective activation of mGluRs, possibly those of class I. Because the task is believed to be hippocampus dependent, it is likely that it might be the activation of those mGluRs located in the hippocampal formation that facilitated spatial memory formation.

General Discussion

SPECIFICITY OF THE DRUGS

The work described here clearly indicates that mGluRs are involved in some forms of learning and memory (Table 4). Compared with other glutamate receptor subtypes, the lack of specific compounds acting on mGluRs has long hindered investigation of this interesting issue. The selective mGluR agonist ACPD was unsuitable because it was found to be toxic *in vivo*, resulting in severe neuronal apoptosis and cell death (Sacaan and Schoepp 1992; McDonald et al. 1993). We have investigated mGluR function in learning and memory using the recently available mGluR agonist tADA, which appears to be specific for class I mGluRs (Favaron et al. 1993; Riedel et al. 1995b). We have not observed any cell damage in our his-

Table 4: Summary of the effects of mGluR antagonist MCPG and agonist tADA on retention of a spatial and nonspatial task

Learning paradigm	Pretraining		Post-training	
	MCPG	tADA	MCPG	tADA
Brightness discrimination	no effect	no effect	no effect	no effect
Spatial alternation	amnesia	amnesia	no effect	enhancement

Neither drug affects brightness discrimination learning, but both substances cause amnesia in a spatial alternation paradigm when applied prior to training, and agonist injection immediately after training may be memory enhancing for spatial alternation.

tological material. Furthermore, tADA has the advantage of distinguishing between mGluR classes, because it stimulates PI hydrolysis but does not depress forskolin-stimulated cAMP formation in either hippocampal slices (Manahan-Vaughan et al. 1996) or mixed cortical cell cultures (Bruno et al. 1995). However, in cell lines expressing human mGluR2, tADA acted as a weak agonist (Knöpfel et al. 1995). The effects of tADA injections could therefore be interpreted as mediated via those mGluRs that are coupled to PI hydrolysis. We also used MCPG, which is a selective mGluR antagonist (Eaton et al. 1993). Although we have provided evidence for MCPG being a competitive class I mGluR antagonist in the hippocampus (Riedel et al. 1995a), others have demonstrated that MCPG also acts on other subtypes in cortical brain structures (Eaton et al. 1993). Because alterations in memory formation were found only in the spatial alternation paradigm (see Table 4 for summary), which critically involves hippocampal function, we suggest that the behavioral deficits/enhancements documented here are specifically attributable to blockade/activation of class I mGluRs.

GENERAL EFFECTS OF THE COMPOUNDS

Three recent reports on inbred mice lacking mGluR1 have consistently shown severe behavioral deficits (Aiba et al. 1994a,b; Conquet et al. 1994). These animals show impaired motor coordination and sensory sensitivity, thus making interpretation of learning deficits rather difficult. However, the studies provide evidence that at least mGluR1 is important for development and normal behavior and further suggest that, when investigating mGluR function with pharmacological tools, care must be taken to control for side effects of the drugs. Hence, we have analyzed drug-induced alterations occurring during adaptation to the test apparatus. Right and left entries were counted during habituation and taken as an index of arousal and activity. Furthermore, the number of fecal boli was monitored as a measure for drug-induced changes in emotionality. MCPG caused no alteration in performance during habituation (Table 1). Neither activity nor emotional parameters differed from controls. In tADA-treated rats, however, we observed a reduction in activity (Table 2), which may be owing to activation of class I mGluRs.

During training and retention of the brightness discrimination task, we also monitored the shock intensities as well as reaction times and total trial times required to escape the start chamber and to arrive in the goal box. These parameters reflect the sensitivity of sensory systems. Again, MCPG- or tADA-exposed rats failed to show any alteration in shock intensities or in times required to perform the trials (Fig. 2), suggesting that neither activation nor inhibition of mGluRs induces deficits in sensory transduction that could account for the learning deficit in spatial alternation. This is in line with reports showing that i.c.v. injection of MCPG or ACPD causes deficits in spatial learning but does not lead to gross behavioral abnormalities (Pettit et al. 1994; Richter-Levin et al. 1994).

We cannot exclude, however, the possibility that other side effects may occur, but it seems unlikely that such effects influence the learning of the tasks. Our data on the brightness discrimination task support this hypothesis in that they clearly show that neither drug causes a learning deficit, independent of the time of application (before or after training).

DIFFERENCES IN COMPLEXITY BETWEEN TASKS

One possible account of the differential sensitivity of the two behavioral tasks is that one task might be more complex than the other. It might, for example, be that complex tasks require mGluR activation, whereas the simple tasks do not (or vice versa). We explored this possibility by comparing the learning curves of the first 20 training trials of untreated rats subjected to brightness discrimination ($n = 14$) or spatial alternation learning ($n = 14$). Statistical analysis revealed no group difference and no group by block interaction [$F(1,26) = 0.289$, $P > 0.05$ and $F(1,26) = 0.57$, $P > 0.05$, respectively]. These data suggest that both paradigms are equally difficult, and the hypothesis that mGluRs may be involved only in complex/simple task seems unlikely.

MGLURS IN BRIGHTNESS DISCRIMINATION

A number of recent reports have indicated that mGluRs play a role in a variety of behavioral paradigms in mammals, namely the spatial water maze task (Pettit et al. 1994; Richter-Levin et al. 1994), spatial alternation learning in the Y-maze

(Riedel et al. 1994a), olfactory learning (Kaba et al. 1994), and inhibitory avoidance (Bianchin et al. 1994). Similarly, amnesic effects have been reported after blockade of mGluR in passive avoidance in chicks (Hölscher 1994; Rickard and Ng 1995). These data might suggest that mGluRs may be involved in learning in general.

We provide evidence, however, that neither MCPG nor tADA affect brightness discrimination learning when applied before training or immediately after training. These results thus support the notion that mGluRs do not act as modulators in learning and memory in an unspecific manner. Given the specificity of tADA, which acts on class I mGluRs rather than on classes II and III, our results demonstrate that class I mGluRs are not involved in brightness discrimination learning. Further support on this issue is provided by our data on MCPG application, because MCPG also acts preferably on class I mGluRs (Hayashi et al. 1994; Riedel et al. 1995a). It is not surprising that MCPG does not affect formation of an olfactory memory (Kaba et al. 1994), because, in the accessory olfactory system of mice, mGluR2 is predominantly expressed at the dendrites of granule cells. Accordingly, MCPG, which appears not to act on this subtype, has no effect on the formation of an olfactory memory, although mGluR2 activation seems to be critically involved (Kaba et al. 1994). Because we have not examined compounds specifically acting on class II and III mGluRs, we can not exclude the possibility of a learning-related activation of these mGluR subtypes. Further investigations using more specific drugs are required to address this question.

Paralleling the involvement of class I mGluRs, NMDA receptors have been shown to be not involved in discrimination learning (Morris et al. 1986) but may be activated during spatial learning situations (Morris et al. 1986; Riedel et al. 1994b).

mGluRs IN SPATIAL ALTERNATION

It has been shown previously that blockade of NMDA receptors by means of AP5 disrupts spatial but not visual discrimination learning (Morris et al. 1986, 1990). Our data on brightness discrimination learning support the notion that learning and retention of visual tasks does not require class I mGluR activation. In contrast, spatial alternation learning appears to depend on the activation of class I mGluRs, because both MCPG and tADA pre-

training injection caused amnesia of the task. This is in agreement with reports providing evidence that both the mGluR antagonist MCPG and the agonist ACPD block spatial learning in the water maze (Pettit et al. 1994; Richter-Levin et al. 1994). Richter-Levin and colleagues (1994) have acutely applied MCPG into the ventricle prior to giving rats spatial training in the water maze. This procedure was performed on 4 consecutive days. Escape latencies to reach the hidden platform during training did not differ for MCPG-treated animals compared with controls, but a significant difference was obtained in the transfer test, when no platform was available on day 5 and the distance traveled in different quadrants was monitored. Whereas controls spent ~50% of the total distance in the correct quadrant, MCPG injected rats fell to chance levels (~25%), indicating complete amnesia.

Animals exposed to ACPD prior to training in the water maze showed a significant increase in escape latency during acquisition in the water maze (Pettit et al. 1994). Whether this increase may have caused a retention deficit remains unclear, because no transfer test was performed. However, if animals of the ACPD group were tested under drug-free conditions on subsequent days, they progressively increased their performance up to control levels. These data clearly indicate that ACPD substantially disrupts acquisition in the water maze. In contrast, we observed no acquisition deficit in spatial alternation after tADA treatment, a difference that could be attributable to several reasons. First, the specificity of the two compounds is different and it seems possible that the acquisition deficit of ACPD-exposed animals is the result of an activation of class II rather than class I mGluRs. Second, differences between tasks could account for the discrepancy. Third, it is possible that ACPD has toxic effects, leading to the deficit. Finally, in the water maze task, three trials per day were given, whereas we gave our animals 40 acquisition trials, which could be regarded as mass training, thus masking some drug-induced effects that may have become obvious under a different training regime. The resolution of this issue awaits further experiments.

The effect on spatial tasks of post-training injection of mGluR antagonists or agonists has not been reported previously. Only one investigation, which used a one-trial step-down avoidance task with rats (Bianchin et al. 1994), reported on post-training application of ACPD and MCPG, but in

contrast to our study, both substances were injected intrahippocampally. ACPD enhanced memory formation and MCPG resulted in amnesia (Bianchin et al. 1994), suggesting that both compounds have interfered with the process of memory consolidation. We found that saline injection immediately after training caused amnesia, indicating either that sodium chloride may block memory formation or that the injection process interacts with the formation of memory. Similarly, MCPG injected after training resulted in amnesia. In conjunction with the saline data, this is further evidence for the idea that the injection process itself may have led to a disruption of the memory process and that neither saline nor MCPG affect memory formation. Hence, the results on tADA showing an improvement of memory when applied after training appear to be owing to a genuine effect of tADA. However, the results are clear only with respect to amnesic controls. Further actions of MCPG that we cannot rule out have to be assessed with different behavioral approaches.

Finally, the question arises: How could mGluR activation prior to training prevent memory formation and post-training stimulation of mGluRs facilitate retention? In cellular terms, one interpretation is that tADA may cause a saturated and non-specific activation of all class I mGluRs and that this saturated activation hinders further learning-dependent modulation of these receptors. On this basis we hypothesize that tADA injection may alter the signal-to-noise ratio of neurons in that pre-training injection increases the noise rather than the signal, whereas post-training application increases the signal rather than the noise. Spontaneous neuronal activation is supposed to constitute a basic excitation (noise). In training, glutamate release increases the activation level at those mGluRs that were use-dependently activated. This activation results in an increase of the signal-to-noise ratio, but only for specific mGluRs. Post-training injection of tADA may be capable of amplifying this signal-to-noise ratio n -fold. In contrast, pretraining infusion of tADA nonspecifically increases the noise level and subsequent learning-dependent activation of mGluRs is unable to generate a signal.

Physiological experiments on hippocampal slices have provided evidence that activation of mGluRs by ACPD reduces the net input into the dentate gyrus from the entorhinal cortex (Desai and Conn 1991; Baskys et al. 1993). In contrast, mGluR activation in CA3 increases the amplitude

of responses of mossy fiber–CA3 synapses (Desai and Conn 1991), and for CA1, both increases and decreases in neuronal excitation in the presence of ACPD have been described (Baskys and Malenka 1991a,b; Desai et al. 1992). These data suggest that stimulation of mGluRs may indeed set the signal-to-noise ratio in the hippocampal circuit.

CONCLUSIONS

In summary, our data confirm earlier work indicating a role of mGluRs in learning and memory. Given the specificity of MCPG and tADA, it is reasonable to argue that class I mGluRs appear to be particularly important for spatial learning conditions and not for visual discrimination paradigms. Interestingly, pretraining application of tADA, the mGluR agonist, caused amnesia, but post-training injection enhanced retention. This result indicates that an unspecific saturated activation of class I mGluRs prevents spatial learning, whereas a pharmacological activation of mGluRs shortly after training may enhance the signal-to-noise ratio, thus leading to better performance during retention. Further investigations should examine the role of particular mGluR subtypes in specific brain areas.

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