Altered object exploration but not temporal order memory retrieval in an object recognition test following treatment of rats with the group II metabotropic glutamate receptor agonist LY379268

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Abstract

Temporal order memory refers to the ability to distinguish past experiences in the order that they occurred. Temporal order memory for objects is often tested in rodents using spontaneous object recognition paradigms. The circuitry mediating memory in these tests is distributed and involves ionotropic glutamate receptors in the perirhinal cortex and medial prefrontal cortex. It is unknown what role, if any, metabotropic glutamate receptors have in temporal order memory for objects. The present experiment examined the role of metabotropic glutamate receptors in temporal memory retrieval using the group II metabotropic glutamate receptor selective agonist LY379268. Rats were trained on a temporal memory test with three phases: two sample phases (60 min between them) in which rats explored two novel objects and a test phase (60 min after the second sample phase) which included a copy of each object previously encountered. Under these conditions, we confirmed that rats showed a significant exploratory preference for the object presented during the first sample phase. In a second experiment, we found that LY379268 (0.3, 1.0, or 3.0 mg/kg; i.p.; 30 min before the test phase) had no effect on temporal memory retrieval but dose-dependently reduced time spent exploring the objects. Our results show that enhancing mGluR2 activity under conditions when TM is intact does not influence memory retrieval.

Keywords

Medial prefrontal cortex; Metabotropic glutamate receptor; Spontaneous object recognition; Cognition; Delayed recency discrimination

1. Introduction

Temporal order memory (TM) refers to memory for the order of experiences in time. In humans, TM is impaired in mental illnesses including schizophrenia and Alzheimer's disease [1–4]. Thus, understanding the neural mechanisms underlying TM may enable the development of novel therapies for these disorders. In rodents, TM is often studied using spontaneous tests with either objects [5–7] or spatial locations as stimuli [8,9], or the

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ordered sampling of odors [10]. Spontaneous memory tests are attractive for examining cognition as extended training is not necessary and reinforcing stimuli such as food or shock are not used [11–13]. The object-based TM test requires rats or mice to discriminate between objects based on visual and tactile information and are known to depend critically upon the perirhinal cortex (PRh) [5,7]. The medial prefrontal cortex (mPFC) is also necessary for the integration of object-related information with temporal information, allowing the subject to determine the relative recency of exposure to an object [5–7].

The involvement of glutamate receptors in spontaneous object recognition tests, including those related to TM, is the subject of intense investigation. Ionotropic glutamate receptors are involved in object-based TM as administration of AMPA receptor antagonists into the PRh or mPFC impair encoding and retrieval while NMDA receptor antagonists impair encoding [14]. To date, little information regarding the potential role of metabotropic glutamate receptors (mGluRs) in TM has been reported. Metabotropic GluRs are classified into three groups (group I: mGluR1, mGluR5; group II: mGluR2, mGluR3; group III: mGluR4, mGluR6, mGluR7, mGluR8). Group II mGluRs, which have been examined as drug targets for schizophrenia, are coupled to inhibitory G proteins which decrease adenylyl cyclase activity to reduce cAMP levels. Subsequent activation of K⁺ channels and inhibition of voltage-gated Ca²⁺ channels prevent cell depolarization and vesicle fusion, ultimately reducing glutamatergic activity [15–17]. Changes in dopamine and serotonin efflux in the mPFC of freely moving rats have also been reported following systemic administration of group II mGluR agonists [16,18–20]. Thus, group II mGluRs may play a role in regulating cortical neurochemical activity during cognitive tasks such as recognition memory and could serve as novel therapeutic targets for improving the temporal memory deficits in schizophrenia and Alzheimer's disease [15,21]. This hypothesis is supported by data showing that group I and II metabotropic glutamate receptor antagonists administered together impair the acquisition of object memory in a test without a temporal component [22]. Administration of the group II mGluR antagonist LY341495 after the sample phase also impaired object recognition when a 1 h delay was used [23]. Using a 4 h delay between the sample and test phases, rats treated with 1 mg/kg of the potent group II mGluR agonist LY379268 ((-)-2-oxa-4-aminobicyclo[3.1.0]hexane-4,6-dicarboxylate) [16] showed significant object recognition memory when treated before the sample phase while untreated rats did not show significant memory [24].

In the present study, we tested the effect of LY379268 on TM retrieval in rats using a spontaneous object-based paradigm. Given the localization of group II mGluRs in the mPFC [16,17,25] and the role of the mPFC in TM [5–7], we hypothesized LY379268 to affect TM retrieval, although specifying whether LY379268 would facilitate or impair memory retrieval was difficult a priori given the conflicting reports of the effect of group II mGluR agonists on cognition in rodent behavioral assays [15–17,21,24].

2. Materials and methods

2.1. Subjects

Adult male Long–Evans hooded rats (Charles River Laboratories, Quebec, Canada) were used in the experiments. Rats were given at least 7 days to acclimate to the vivarium before

any procedures were conducted. Eight rats were used in experiment one and 13 were used in experiment two. Rats were pair housed in Plexiglas cages with food and water available *ad libitum*. Lighting in the vivarium was controlled automatically on a 12:12 h light/dark cycle (lights on at 07:00) and all handling and experimentation occurred in the light phase.

Experiments were conducted in accordance with the Canadian Council on Animal Care and approved by the University of Saskatchewan Animal Research Ethics Board.

2.2. Apparatus

The apparatus used in both experiments was an open topped square arena (60 cm × 60 cm × 60 cm) made of white, corrugated plastic with a piece of Velcro 10 cm from each corner to hold the objects in place. A camera fixed to the ceiling recorded the rats' activity on a personal computer. Objects used consisted of a variety of Lego shapes, household items, and ceramic figures.

2.3. Behavioral testing

Behavioral testing procedures followed those typical of our laboratory [5,26–28]. Rats were handled for 5 days prior to habituation. Each rat received 3 habituation sessions where they were placed in an empty test arena for 10 min. These sessions occurred 24 h apart with the final habituation 24 or 48 h before the first test. There was one 10 min habituation session before each subsequent week and no rat experienced the same object pair twice. Between habituation sessions and each phase of the test, the arena and all objects were thoroughly cleaned with 40% ethanol and paper towels to eliminate odor cues.

The delayed recency discrimination test (Fig. 1A; [5,6,12]) was used to assess TM in both experiments. It consists of three four minute phases: sample 1, sample 2, and test, with a delay of 1 h between each phase. During the sample phases, the rats were exposed to two copies of a novel object (sample 1: object A1 and A2; sample 2: object B1 and B2), and duplicate copies of one object from each previous phase was presented during the test phase (object A3 and B3). In experiment one, rats were tested twice to evaluate the use of repeated testing; in experiment two, a second group of naïve rats were tested following four treatments in a counterbalanced order: saline, 0.3 mg/kg, 1.0 mg/kg, and 3.0 mg/kg LY379268. These doses were chosen based on those used in previous work [16,24]. In order to examine TM retrieval, the injection (i.p.) was administered 30 min before the test phase. Treatments were administered one week apart with one habituation session the day before treatment, repeated for four weeks in total.

2.4. Data analysis

Object exploration was scored by hand using stopwatches by one experimenter for experiment one and two experimenters for experiment two; all were blind to the treatments. Object exploration was scored when the rat had its nose directed toward the object at a distance of 2 cm or less [5,26,27]. Time spent climbing an object with the rat's face directed away from the object was not counted as exploration. Total object exploration time during the sample phase was analyzed in both experiments. Exploration of each object during the first two minutes and four minutes of the test phase was analyzed because previous studies suggest that novel object preference is reduced as the test trial length increases [29,30,7] but

see [26]. Test phase data is presented as a discrimination ratio (DR), calculated as [(old familiar time – new familiar time)/total exploration time]. Higher DRs indicate a greater preference for exploration of the old familiar object from sample phase 1 [5].

Statistics were conducted using SPSS Version 19.0. For each treatment, one sample *t*-tests were computed to assess whether performance was greater than chance (i.e., 0). Repeated measures ANOVA was used to determine the effect of drug treatment on DR as well as to examine the effect of drug treatment on total object exploration times. Newman–Keuls post hoc tests were used to follow up significant effects in the ANOVA. For all statistical analyses, *p* values of less than 0.05 were considered significant.

3. Results

3.1. Experiment one: the delayed recency discrimination test is a viable measure of TM

Rats tested using the delayed recency discrimination procedure demonstrated TM (Fig. 1C). The mean DRs for the first test was 0.47 ± 0.07 for the first two minutes and 0.36 ± 0.08 for all four minutes of the test phase. The mean DRs for the second test with a different object pair were 0.26 ± 0.12 for the first two minutes and 0.25 ± 0.12 for all four minutes of the test phase. Thus, greater exploration was seen for the old familiar object, as expected [5,6]. DRs for both weeks were significantly different from chance (i.e., 0; $p < 0.001$), indicating that the rats preferentially explored the old familiar object. Total exploration times were similar to those described below for experiment 2.

3.2. Experiment two: LY379268 failed to alter TM but altered object exploration time

All groups demonstrated significant memory regardless of treatment (Fig. 2A). There was no significant effect of drug treatment on TM for the first two minutes ($F(3,36) = 2.48$, $p = 0.077$) or four minutes of the test phase ($F(3,36) = 0.72$, $p = 0.46$). The DR for each treatment group was significantly greater than 0 for the two and four minute data (statistics not shown; $p < 0.05$) which indicates a greater than chance bias for exploration of the old familiar object.

Exploration time during the test phase (4 min) was significantly affected by treatment (Fig. 2B; $F(3,36) = 4.54$, $p = 0.008$). Exploration time decreased with increasing dose of LY379268. A post hoc analysis revealed a significant difference between the saline treatment and the 3.0 mg/kg treatment ($p < 0.05$). Exploration of the objects also differed significantly among the phases of the test ($F(2,24) = 8.20$, $p = 0.02$). No difference was observed between sample 1 and sample 2, but both were significantly different from the test phase (post hoc analysis, $p < 0.05$). The total exploration times during the test phase also decreased among weeks (Fig. 2C; $F(3,36) = 7.00$, $p < 0.01$). Post hoc analysis indicated a significant decrease between week 1 and week 4, as well as week 2 and week 4.

4. Discussion

The present experiments yielded two main results. In experiments one and two, we confirmed the reliability of the delayed recency discrimination procedure as a measure of TM using repeated testing with different object pairs. Object exploration times and

discrimination ratios were similar to previous reports [5–7]. Exploration of the old familiar object was higher during the first two minutes of the test trial (Figs. 1C and 2A), as has been reported for spontaneous object recognition tests previously [7,29,30]. We also confirmed that a within subjects design with at least four separate tests can be used [5]. However, total object exploration decreased with each subsequent week of testing (Fig. 2D) which could be due to residual familiarity of the rats for the test [5,12]. Total exploration in the test phase was also reduced relative to the sample phases (Fig. 2C). Reduced exploration in the test phase is not unexpected as object exploration in rats is related to the novelty of the objects, with novel or less familiar objects receiving greater exploration [5,12]. During the test phase, the subjects are exposed to two familiar objects and as there is no training or otherwise extrinsic motivation to encourage further investigation, exploration naturally decreases.

In experiment two, we failed to observe a significant effect of LY379268 on TM (Fig. 2A). The drug significantly reduced total object exploration at the highest dose (Fig. 2B), consistent with its well established inhibitory effect on locomotor activity [16]. While the discrimination ratio was lower during the first two minutes of the test trial for rats treated with 0.3 and 1.0 mg/kg of LY379368, those differences failed to reach significance and were not observed when all four minutes of the test trial were considered (Fig. 2A). All groups also showed a significant preference for exploring the old familiar object during the test phase regardless of the portion analyzed.

Previous work examining the effects of group II mGluR agonists on cognition have been inconsistent, particularly when studies conducted with humans are compared to those conducted using rodents [17]. In normal rodents, a number of studies have found impaired cognition following treatment with group II mGluR agonists [31–34], effects which may relate to the use of reinforcement in the cognitive paradigms [17]. The spontaneous object-based TM paradigm used in the present experiments does not involve the use of reinforcement; therefore, it may be more similar to tasks typically used in humans [5,11,17]. In the future, examining the effects of LY379268 on a TM task designed such that memory of the control animals is poor may reveal effects as previous results with object recognition have shown enhanced memory following LY379268 treatment (1 mg/kg) under such conditions [24]. Extending the delay between the sample trials and the test trial may produce a suitably poor TM in control rats [35].

In contrast to the findings from normal rodents, group II mGluR agonists have been found to reverse the cognitive impairments in rodent models of psychiatric disorders. LY379268 improves object recognition following social isolation during adolescence [24] or acute MK-801 treatment in adulthood [36]. In combination with low doses of clozapine and lurasidone, LY379268 also reversed the disruptive effects of subchronic phencyclidine on object recognition [37]. Group II agonists also have inconsistent effects on the working memory impairments observed following administration phencyclidine [33,38]. Thus, establishing the effects of group II mGluR agonists on the deficits in TM in rodent models of psychiatric disorders [39,40] will be an important direction for future research.

5. Conclusions

The delayed recency discrimination procedure produces consistent results when objects are used to examine TM. LY379268 failed to affect TM in untreated rats with intact memory thus suggesting that group II mGluRs are not involved in TM under normal circumstances. Future research examining the effects of group II mGluR manipulations during conditions where TM is compromised may reveal significant effects.

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HIGHLIGHTS

- The group II metabotropic glutamate receptor agonist LY379268 was examined in a temporal order memory test.
- No effects of the drug were found on memory when it was administered before the test phase.
- Higher doses of the drug decreased exploration time of the objects.
- Object exploration times were also reduced with repeated testing regardless of treatment.

Fig. 1. Description and characteristics of the delayed recency discrimination test of temporal order memory. (A) Cartoon illustrating an overhead view of the open field during the test. Duplicate copies of the object were used in the test phase. The delay between the successive phases of the test was 1 h. (B) Schematic of the treatment schedule for experiment two. Either saline or LY379268 (0.3, 1.0, or 3.0 mg/kg) was injected 30 min before the test phase. (C) Average performance of the rats ($n = 8$) during the two tests conducted in experiment one. Object preference was assessed for the first two minutes of the test trial or the entire four minutes. Memory is expressed as a discrimination ratio.

Fig. 2.

Effects of LY379268 on temporal order memory as assessed by the delayed recency discrimination test ($n = 13$). (A) Memory for the old familiar object is expressed as a discrimination ratio taken from exploration times during either first two minutes of the test trial or all four minutes of the test trial. (B) Total exploration time of the objects during the test phase for each treatment. (C) Total object exploration for each phase of the test. Data is averaged across all four tests. (D) Total object exploration for each week of testing. Data is averaged for all three phases and all treatments. *Denotes a significant difference among the groups indicated ($p < 0.05$).