Research report

Facilitative effects of an adenosine A_1/A_2 receptor blockade on spatial memory performance of rats: selective enhancement of reference memory retention during the light period

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Abstract

The present experiment was designed to examine the role of adenosine in spatial working and reference memory in rats using an 8-arm radial maze task which requires the integrity of the hippocampal formation. We investigated the effects of the unselective adenosine A_1/A_2 receptor antagonist theophylline on acquisition and retention of spatial working and reference memory. As there is evidence that brain extracellular adenosine levels vary significantly during the light–dark cycle, we tested the effects of theophylline both during the light and the dark period. Acquisition of the task was investigated for 10 consecutive days after rats received daily injections of vehicle or theophylline (15 mg/kg, intraperitoneally). Retention was tested in two nondrug sessions 7 and 14 days after completion of acquisition. The results demonstrate that in saline-treated control rats acquisition and retention of reference memory and, to a lesser extent, working memory was superior in the dark period. The results further revealed that daily administration of theophylline interacted with days to selectively enhance reference memory acquisition in the light, but not in the dark, period. In addition, reference memory retention was significantly enhanced in those rats who learned the task under theophylline treatment during the light period. Overall, the results show that in saline-treated control rats the effectiveness of acquisition and retention of spatial information in a radial maze strongly depends on the time of day. The higher levels of maze performance in the dark period might be related to a better functioning of involved brain systems in the active period of the rat. Furthermore, theophylline-induced blockade of adenosine A_1/A_2 receptors in the light, but not in the dark, period selectively enhanced reference memory acquisition and retention. Variations of brain extracellular adenosine levels during the light–dark cycle might account for the restriction of reference memory enhancing effects of theophylline to the light period. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Spatial learning; 8-arm radial maze; Light period; Dark period; Theophylline

1. Introduction

Adenosine has been established as an important modulator of neuronal activity in the central nervous system [18] acting on specific transmembrane receptors classified as A_1, A_{2A}, A_{2B} and A_3 subtypes [33]. A_1 and A_3 subtypes decrease cAMP by inhibiting adenylyl cyclase, whereas the A_{2A} and A_{2B} subtypes stimulate adenylyl cyclase and produce excitatory effects in the central nervous system [33]. The hippocampal formation, a complex of structures that intimately participate in acquisition and retention of spatial information [27] is highly enriched with A_1 receptors [38]. Also, low levels of A_{2A} receptors have been demonstrated in the hippocampus by immunohistochemistry [50] and radioligand binding [7]. There is considerable evidence that endogenous adenosine modulates the excitability of hippocampal neurons via A_1 [8,15] and A_2 [6] receptor-mediated mechanisms. Furthermore, the development and consolidation of long-term potentiation, a form of synaptic plasticity probably associated with the acquisi-

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tion and encoding of memory [36], is modulated by A_1 [3,13,58], A_{2A} [14] and A_{2B} [31] receptor-dependent mechanisms in the hippocampus. While these anatomical and electrophysiological data provide evidence for a possible involvement of adenosine in spatial learning and memory, this issue has been rarely addressed in behavioural studies. Most of the available data demonstrated a role of adenosine in passive avoidance learning and retention [9,32,40,41,43,63], while only a few data provide support for an adenosinergic involvement in spatial learning of rats in mazes [17,53,57,61].

The present experiment was designed to examine in more detail the role of adenosine in spatial working (WM) and reference (RM) memory using an 8-arm radial maze task which requires the integrity of the hippocampus and closely related areas [28,29,47]. We investigated the effects of an unselective A_1/A_2 receptor blockade by the methylxanthine theophylline [12] on acquisition and retention of spatial WM and RM in rats. An unselective adenosine A_1/A_2 antagonist was used, because little is known about the role of adenosine in spatial WM and RM in general. Furthermore, selective antagonists have a very poor water-solubility [37] requiring the use of solvents which would cause unacceptable irritation after repeated daily injections [45] during acquisition of the task used here. Recent studies revealed that brain extracellular adenosine levels significantly vary during the light–dark cycle in the basal forebrain [46], cerebral cortex [5] and in particular in the hippocampus [24,34]. Therefore we tested the effects of theophylline on maze performance both during the light and the dark period of the light–dark cycle. A different outcome of a competitive adenosine receptor blockade by theophylline [2,51] might be predicted, if extracellular adenosine levels vary with the time of day. By comparing respective controls, this approach further allows a deeper insight in basic variations of spatial learning and retention as a function of the time of day, an aspect of spatial learning which is surprisingly poorly understood at present.

2. Materials and methods

All efforts were made to minimize the suffering and the number of animals used. All experiments were conducted in accordance with the current version of the German Law on the Protection of Animals and were approved by the proper authorities.

2.1. Subjects

Male Sprague-Dawley rats (Charles-River, Sulzfeld, Germany) weighing 210–270 g at the beginning of the experiments were used. They were housed in groups up to five animals in transparent plastic cages (Type IV;

$35 \times 55 \times 10$ cm; Ebeco, Castrop-Rauxel, Germany). Ambient temperature ($20 \pm 2°C$) and humidity ($50 \pm 10\%$) were kept constant in the animal house. Standard laboratory maintenance chow (Altromin, Lage, Germany) was restricted to 12 g per animal and day. Water was available ad libitum. A 12:12-h light–dark schedule was used. One group of animals ($N = 14$) was used for experiments performed during the light period with lights on between 06:00 and 18:00 h; another group of animals ($N = 11$) was used for experiments performed in the dark period with lights on between 21:00 and 09:00 h. Rats were maintained in these conditions at least 3 weeks before the experiments to allow synchronization of all animals to the respective light–dark cycle.

2.2. Apparatus

The 8-arm radial maze was built of dark plastic and placed on the floor behind an opaque curtain. The dimensions of the maze were as follows: A central octagonal platform (42 cm in diameter) was surrounded by eight arms (60 cm long, 17 cm wide) with 25 cm high side walls. Each of the arms contained an opaque food cup at the distal end. Several extra-maze cues (tables, curtain, posters) were visible for the rat to allow allocentric orientation. During the course of all experiments the maze remained in the same position with respect to the extra-maze cues.

2.3. Drugs

Theophylline (1,3-dimethylxanthine) (Research Biochemicals International, Köln, Germany) was dissolved in 0.9% sterile NaCl solution and administered by daily intraperitoneal (i.p.) injections 20 min before onset of individual testing. Injections of sterile saline in volume of 1.5 ml/kg served as controls.

2.4. Spatial learning

2.4.1. Habituation
Animals were habituated to the maze for 5 consecutive days. First, they were made familiar with the maze together with their cage-mates over 3 days in one daily 15-min session. The day after, animals were placed individually into the maze for a 10-min session. On the last day, animals were given individually access to the maze for a 5-min session with food pellets (45 mg; Noyes) being scattered on the floor.

2.4.2. Acquisition
Acquisition of the task was tested for 10 consecutive days according to the procedure described previously [29]. Four of the eight arms were constantly baited by one pellet in a food cup. The unbaited arms contained

empty food cups. At the beginning of a trial the rat was placed at the end of a randomly chosen unbaited arm. The trial was finished when all four pellets had been retrieved. During acquisition, blocks of five trials were given per day with an inter-trial interval of ~ 60 s during which the animal was held in a separate cage. The pattern of baited arms was identical for all animals. The position of this pattern relative to the extra-maze cues was randomly varied and assigned to different animals, but remained constant for an individual rat throughout the experiments.

2.4.3. Retention

Seven and 14 days after completion of the acquisition phase, retention of the task was tested in animals without treatment in one session per day (with a block of five trials) using the same protocol as for acquisition.

2.4.4. Evaluation

Arms visits defined as entering an arm with all four paws were documented by video observation. WM and RM errors were distinguished as described earlier [28,29]. RM refers to information that remains constant over a long phase of time and impairments of RM are reflected by choices of arms that are never baited. Therefore, an RM error was defined as an entry in a never baited arm for the first time. We counted RM errors starting with the second block of trials (i.e. on day 2 of acquisition), because the position of the un-baited arms in relation to the extra-maze cues is un-known to animals on the first day of acquisition. WM refers to information that is pertinent only for a short phase of time and impairments of WM memory are indicated by re-entries in arms already visited. There-fore, a WM memory error was defined as a re-entry in a baited or never baited arm.

Furthermore, the running time from placing an animal into the maze until the end of the trial was mea-sured from each trial. As the running time varied as a function of maze performance, the ratio from running time and number of arm entries were calculated as an index for motor activity. In small percentage of re-sponses (< 5%), arm entries did not comprise the whole running distance to the food cup at the distal end of the arm. Thus, a minor bias of the running time per arm visit towards higher motor activity can not be ruled out.

2.5. Light regimen

Experiments performed in the light period of the light–dark cycle were conducted between 11:00 and 16:00 h. Brightness of illumination was ~ 1500 Lux as measured by a light sensor (FL A613-VL; Ahlborn, Holzkirchen, Germany) with a data acquisition system (Almeno 2290-8; Ahlborn, Holzkirchen, Germany). Ex-periments performed in the dark period were conducted between 16:00 and 21:00 h. Light was filtered with a longpass (cut-off 580 nm) and brightness of illumina-tion was ~ 6 Lux measured as indicated above.

2.6. Experimental procedure

In the first series of experiments, the effects of theophylline (15 mg/kg, i.p.) on acquisition and reten-tion of the spatial learning were analysed during the light period. Drug ($N = 7$) and saline groups ($N = 7$) received daily theophylline or saline injections in the acquisition phase over 10 days. Retention was tested on two retention days 7 and 14 days after completion of acquisition with one session per day with both groups untreated, respectively. The same procedure was ap-plied during a second series of experiments performed during the dark period (saline, $N = 5$; theophylline, $N = 6$).

3. Statistics

Drug effects were analysed as follows: Cumulative RM and WM errors in blocks of five trials given in one daily session during the acquisition phase of 10 days from saline- and theophylline-treated animals were compared using a two-way analysis of variance for repeated measures (ANOVA) with treatment and days as factors followed by a Tukey's t-test. The running times per arm entry (in blocks of five trials in one daily session) during acquisition were analysed accordingly. Cumulative RM and WM errors on retention days 1 and 2 (one block of five trials in one session, respec-tively) from animals which learned the task under saline or theophylline treatment were compared using a one-way ANOVA with treatment as factor, respectively. Running times per arm entry from retention day 1 and 2 were analysed accordingly.

Time of day effects were analysed as follows: Cumu-lative RM and WM errors of saline-treated rats which acquired the task during the light or dark period were compared using a two-way analysis of variance for repeated measures (ANOVA) with days and time of day as factors followed by a Tukey's t-test. Cumulative RM and WM errors from retention days 1 and 2 of saline-treated rats which acquired the task during light or dark period were compared using a one-way ANOVA with time of day as factor. Running times per arm entry from retention day 1 and 2 were analysed accordingly.

Data are presented as means $\pm$ standard errors of the mean (S.E.M.). $P < 0.05$ was considered to represent significant differences.

4. Results

4.1. Light period

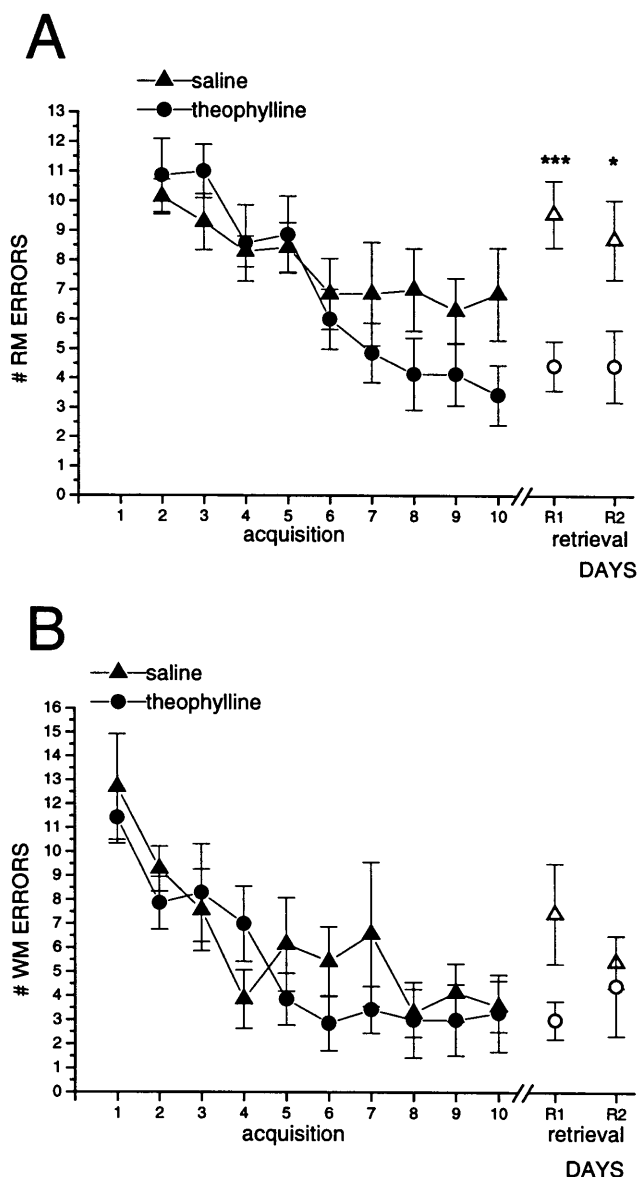During the light period, theophylline ($N = 7$; saline:

Fig. 1. Mean cumulative number of reference memory (RM) (A) and working memory (WM) (B) errors in blocks of five trials given in one daily session during the light period. RM errors are given starting with the second day of acquisition. Acquisition was tested over 10 days, retention was tested 7 (R1) and 14 (R2) days after completion of acquisition. During acquisition, groups of rats (closed symbols) received daily intraperitoneal injections of theophylline (15 mg/kg, $N = 7$) or saline (1.5 ml/kg, $N = 7$) 20 min before the onset of individual testing. A significant effect of days ($P < 0.005$) and an interaction between treatment and days ($P < 0.05$) were detected during RM acquisition (two-way ANOVA). RM and WM retention of groups (open symbols) which acquired the task under saline or theophylline treatment was tested without treatment: *$P < 0.05$, ***$P < 0.005$ (one-way ANOVA); comparison of RM and WM errors of both groups on retention days 1 (R1) and 2 (R2).

$N = 7$) had no significant effects on WM performance, but on aspects of RM performance (Fig. 1).

4.1.1. RM errors

With regard to RM errors during acquisition, ANOVA revealed no main effect of treatment ($F < 1$), but of days ($F[8, 96] = 13,2$, $P < 0.005$) and a significant interaction between treatment and days ($F[8, 96] = 2,33$; $P < 0.05$) (Fig. 1(A)). Furthermore, in both retention sessions (no drug treatment) 7 and 14 days after completion of acquisition, animals who learned the task under theophylline treatment made less errors retrieving RM information (Fig. 1(A)) (retention session 1: $F[1,12] = 13,32$; $P < 0.005$; retention session 2: $F[1,12] = 5,54$; $P < 0.05$).

4.1.2. WM errors

With respect to WM errors, ANOVA revealed no main effects of treatment ($F < 1$), an effect of days ($F[9,108] = 8,21$, $P < 0.005$) and no interaction between both factors during acquisition ($F < 1$). Furthermore, no significant differences were found during retention of WM information (Fig. 1(B)).

4.1.3. Running times

Theophylline had also pronounced effects on running times (Fig. 3(A)). There were main effects of treatment ($F[1,12] = 10,45$; $P < 0.01$) and days ($F[9,108] = 39,4$; $P < 0.005$), but no factor interactions ($F < 1$) on running times per arm visit during acquisition. No significant differences between running times were observed in the retention sessions.

4.2. Dark period

4.2.1. RM errors

During acquisition in the dark period, there were no effects of theophylline treatment on RM errors ($F < 1$), but there was an effect of days ($F[8,72] = 6,66$, $P < 0.005$) and no interaction ($F < 1$) (Fig. 2(A)). Retrieval of RM did not differ significantly between both groups.

4.2.2. WM errors

Regarding WM errors (Fig. 2(B)), ANOVA also revealed a significant effect of days ($F[9, 81] = 17,33$; $P < 0.005$), no effect of treatment ($F < 1$) and no interaction during acquisition ($F < 1$). Retrieval of WM did not differ significantly between both groups.

4.2.3. Running times

Theophylline had marked effects on running times in the dark period (Fig. 3(B)). There were main effects of treatment ($F[1,9] = 7,75$; $P < 0.05$) and days ($F[9,81] = 13,45$; $P < 0.005$) on running times per arm visit during
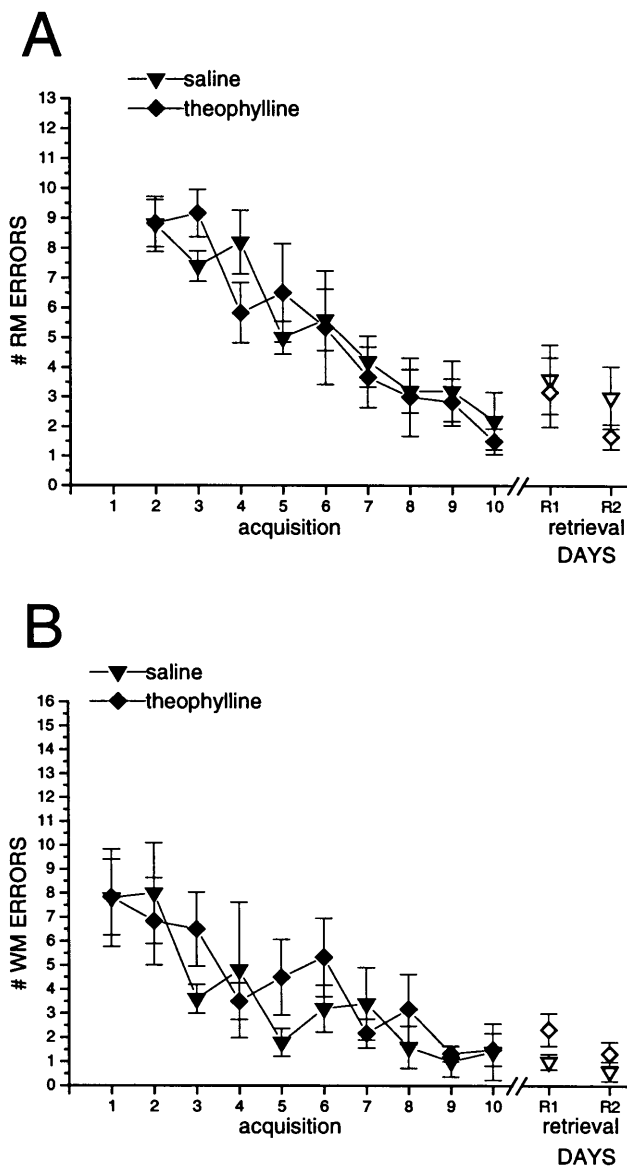
Fig. 2. Mean cumulative number of reference memory (RM) (A) and working memory (WM) (B) errors in blocks of five trials given in one daily session during the dark period. RM errors were given starting with the second day of acquisition. Acquisition was tested over 10 days, retention was tested 7 (R1) and 14 (R2) days after completion of acquisition. During acquisition, groups of rats (closed symbols) received daily intraperitoneal injections of theophylline (15 mg/kg, $N = 6$) or saline (1.5 ml/kg, $N = 5$) 20 min before the onset of individual testing. There was a significant effect of days ($P < 0.005$), no effect of treatment and no interaction during acquisition of RM and WM (two-way ANOVA). RM and WM retention of groups (open symbols) which acquired the task under saline or theophylline treatment was tested without treatment. One-way ANOVA revealed no significant difference between RM and WM errors of both groups on retention days 1 (R1) and 2 (R2).
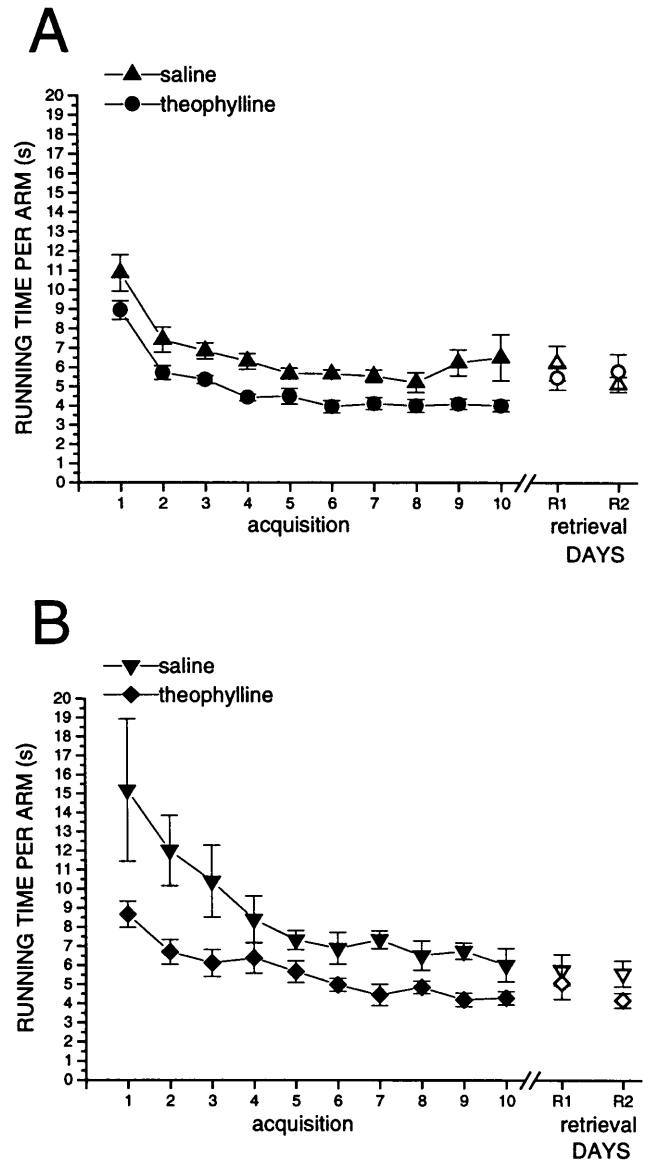
Fig. 3. Mean running times (s) per arm visit calculated from total running times and number of arm visits in blocks of five trials given in one daily session during the light period (A) and dark period (B). Acquisition (closed symbols) was tested over 10 days, RM and WM retention (open symbols) was tested 7 (R1) and 14 (R2) days after completion of acquisition. During acquisition, groups of rats (closed symbols) received daily intraperitoneal injections of theophylline (15 mg/kg, $N = 7$) or saline (1.5 ml/kg, $N = 7$) 20 min before the onset of individual testing. During acquisition in the light period (A) there were main effects of treatment ($P < 0.01$) and days ($P < 0.005$), but no factor interactions. During acquisition in the dark period (B), there were main effects of treatment ($P < 0.05$) and days ($P < 0.005$) as well as interactions between these factors ($P < 0.05$) (two-way ANOVA). RM and WM retention of groups (open symbols) which acquired the task under saline or theophylline treatment was tested without treatment. One-way ANOVA revealed no significant difference between running times per arm of both groups on retention days 1 (R1) and 2 (R2).
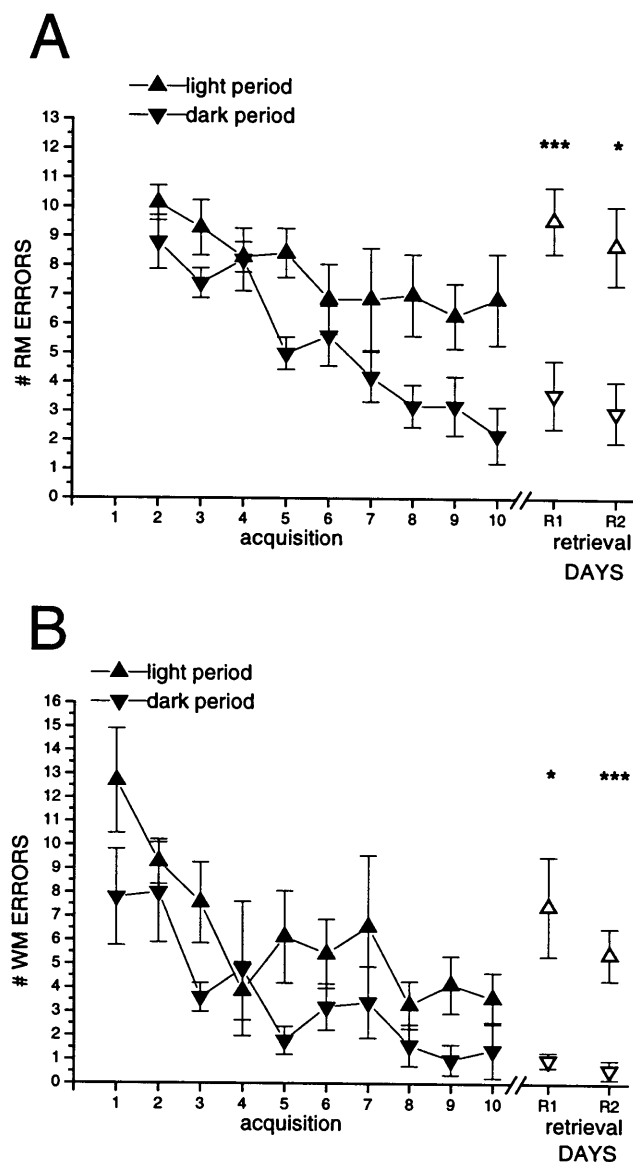
acquisition as well as interactions between these factors ($F[9,81] = 2,18$; $P < 0.05$). No significant differences between running times were observed in the retention sessions (Fig. 3(B)).

Fig. 4. Comparison of reference memory (RM) (A) and working memory (WM) (B) performance of saline groups tested either during the light ($N = 7$) or dark period ($N = 5$). RM errors were given starting with the second day of acquisition. Mean cumulative number of RM or WM errors in blocks of five trials given in one daily session are depicted. Acquisition was tested over 10 days. There were main effects of the time of day ($P < 0.05$) and days ($P < 0.005$) on RM errors during acquisition, but no interactions between these factors (A). Regarding WM performance, there was a main effect of days ($P < 0.005$), no significant effect of the time of day ($P = 0.075$) and no interaction between these factors (B) (two-way ANOVA). Retention was tested 7 (R1) and 14 (R2) days after completion of acquisition. RM and WM retention of groups (open symbols) which acquired the task under saline or theophylline treatment was tested without treatment: *$P < 0.05$, ***$P < 0.005$ (one-way ANOVA); comparison of RM and WM errors of both groups on retention days 1 (R1) and 2 (R2).

4.3. Comparison of RM and WM performance during light and dark period

4.3.1. RM errors

Direct comparison of maze performance of the respective saline groups of the dark ($N = 5$) and light period ($N = 7$) revealed main effects of the time of day ($F[1,10] = 5,13$; $P < 0.05$) and days ($F[8,85] = 12,58$; $P < 0.005$) on RM errors during acquisition, but no interactions between these factors ($F < 1,5$) (Fig. 4(A)). Furthermore, RM errors were significantly lower on both days of retention during the dark period (retention session 1: $F[1,10] = 12,85$; $P < 0.005$; retention session 2: $F[1,10] = 9,78$; $P < 0.05$) as depicted in Fig. 4(A).

4.3.2. WM errors

Regarding WM performance during acquisition, there was a main effect of days ($F[9,90] = 6,22$; $P < 0.005$) and no significant effect of the time of day, but a tendency for a difference ($F[1,10] = 3,96$; $P = 0,075$). No interaction between these factors was found ($F < 1$) (Fig. 4(B)). Furthermore, retention of WM was significantly different between on retention session 1 ($F[1,10] = 6,49$; $P < 0.05$) and retention session 2 ($F[1,10] = 12,8$; $P < 0.005$).

4.3.3. Running times

Analysis of running times during acquisition (not shown) revealed significant effects of days ($F[9,90] = 15,46$), but not of time of day ($F < 1$) and significant interactions of main factors ($F[9,90] = 2,06$; $P < 0.05$). Running times in both retention sessions were not different from one another ($F < 1$, respectively).

5. Discussion

The present study demonstrates that in saline-treated control rats acquisition and retention of RM and, to a lesser extent, WM in a spatial learning task was superior in the dark period as compared to the light period of the light-dark cycle. Furthermore, daily administration of the A_1/A_2 receptor antagonist theophylline interacted with days to selectively enhance RM acquisition in the light period leading to an enhanced RM performance in retention sessions where rats received no drug treatment.

5.1. Time of day and spatial learning

As rats are nocturnal [59], it is not surprising that maze performance of control rats was superior in the dark period. Rats made less errors during acquisition and retention of RM information in this period. Likewise, WM errors were lower during acquisition, albeit this effect reached no significance. Accordingly, WM

errors were lower in retention sessions, probably reflecting an improved WM acquisition. The maze procedure in the dark period was not performed in total darkness, but under weak illumination with red-light (> 580 nm). Analysis of spectral sensitivity obtained from a flicker photometric electroretinogram and from behavioural discrimination measurements revealed that in pigmented rats there is a low sensitivity in this wavelength range [16,26]. Thus albino rats as used in the present study might hardly perceive extra- and intra-maze visual cues for spatial navigation in the dark period and rely more on non-visual cues for navigation. There is evidence that in radial mazes visuospatial, olfactory, tactile and associative mechanisms are used by rats in parallel and co-operation for spatial learning [1,21]. Such a parallel organisation allows an animal to rely on several alternative navigational strategies and this might explain the finding that, though visuospatial information is limited in the dark period of our experiment, animals did not perform worse than in the light period.

Regarding the underlying processes which might account for the time of day differences, at least two mechanisms have to be considered. First, in the dark period, i.e. the activity period of rodents, WM and RM systems involved in acquisition and retention of spatial information might work superior resulting in higher levels of performance. In favour of this notion, daytime-dependent differences of retention of passive avoidance were abolished by lesions of the suprachiasmatic nucleus [56], the circadian pacemaker in mammals. In addition, acquisition of a multiple T-maze task was better during the dark period [25] and retention of certain types of fear conditioning vary with the time of day [60]. Alternatively, light could act as stressor during the light period or the behavioural procedure in the light period could represent sleep deprivation interfering with acquisition and retention of spatial learning. A recent study shows that RM, but not WM, was impaired in a comparable 8-arm maze task by selective deprivation of paradoxical sleep [55]. In this study, rats were given paradoxical sleep deprivation for 4 h immediately after daily training accomplished by placing rats on a small platform surrounded by water. However in our protocol, animals were placed back into the home cage after training and remained in the laboratory until final transfer to the animal house at the end of the daily experiment. It is questionable whether this procedure could already cause sleep deprivation or stress to an extent interfering with spatial memory. Accordingly, a comparative study on different sleep deprivation techniques revealed that only stressful changes in housing conditions affect spatial RM [62]. Thus a main finding of the present study is that the effectiveness of acquisition and retention of spatial information strongly depends on the time of day. Higher levels of spatial

learning and retention in the dark period might be related to a better functioning of involved brain systems during the active period of the rat. In addition, it can not be ruled out that effects of stress due to light per se or sleep deprivation account for the lower performance during the light period.

5.2. Adenosine and spatial learning

The behavioural effects of an A_1/A_2 receptor blockade by the methylxanthines caffeine and theophylline are biphasic [11,64]. In rodents, the most pronounced behaviourally stimulant effects of caffeine were produced by a dose of 15 mg/kg i.p., those of theophylline were produced in a dose range of ~ 10–32 mg/kg i.p. [10] The dose of theophylline used here was chosen accordingly to ensure prominent behavioural effects. Furthermore, the behavioural effects induced by theophylline in a dose of 15 mg/kg are likely to be mediated by an unselective adenosine A_1/A_2 receptor blockade, while higher doses of theophylline and other methylxanthines might involve additional mechanisms as inhibition of phosphodiesterases [10,39,48].

Facilitative memory effects of theophylline were observed with regard to RM and they were restricted to the light period. The stimulating effects of theophylline on motor activity seem to be unrelated to enhanced RM performance, because theophylline-induced motor hyperactivity occurred irrespective of the time of day, while RM facilitation was restricted to the light period. In the maze task used here, no tolerance to theophylline-induced motor activity was observed. In several other studies it has been shown that repeated administration of methylxanthines can lead to locomotor tolerance in open fields [19,22,48]. The lack of tolerance in the present study might be related to the nature of the task involving reward delivery. Motor activation induced by environmental or contextual cues predictive for food reward is not attenuated by drugs which inhibit locomotion [35]. Thus, one might assume that reward-directed spatial behaviour as examined in the task used here is less susceptible to locomotor tolerance. Remarkably, theophylline had no effects per se, but significantly interacted with days to enhance RM towards the end of the acquisition probably leading to the improved RM in retention sessions where no drug treatment was given. Previous studies revealed that unselective blockade of A_1/A_2 receptors [39] as well as selective blockade of A_1 [57] and A_2 [57] receptors facilitated learning and memory in passive avoidance tasks. Also, acute or chronic administration of A_1 receptor antagonists reversed scopolamine-induced deficits in water maze tasks [17] and improved spatial learning of old rats and of rats with lesions in the nucleus basalis magnocellularis [53]. In contrast, chronic treatment with the A_1 receptor antagonist 8-cyclopentyl-1,3-dipropylxanthine (CPX) did not improve,

but even impair some aspects of acquisition and retention in a water maze task [61]. Among several reasons, e.g. task, drug and species differences, which might account for this variance, most importantly a completely different dosing regimen was used in that study. Drugs were given already in a pre-treatment period of 9 days before the onset of acquisition and were administered during acquisition after the sessions and not before as done here. Thus our present data extend previous findings on cognitive enhancing effects of adenosine receptor antagonists and show that a chronic unselective A_1/A_2 receptor blockade during acquisition of a spatial learning task might enhance consolidation of RM leading to a superior retention in sessions without drug treatment. Our findings further confirm that A_1/A_2 receptors seem not to be involved in WM performance [23,44].

An improved RM performance in the light period might be due either to 'direct' actions of theophylline on learning and memory abilities per se or 'indirect' actions on attention, wakefulness and activity or both [39]. 'Direct' and 'indirect' effects are in general difficult to distinguish and our data also do not allow a clear separation. There is ample evidence for an involvement of adenosine in the modulation of wakefulness [4,46,49] and attention [39]. However, 'indirect' effects are less likely to account for the effects measured here, because in this case theophylline treatment should also improve WM which is attention demanding as well.

The hypothesis on 'direct' actions of theophylline on learning and memory systems is mainly based on findings that the hippocampus and closely related areas thought to be necessary for normal performance in maze tasks as used here [28,29,47] have a high A_1 receptor density [38] and might show moderate A_2 receptor expression as well [54]. Furthermore, adenosine has been implicated in the development of LTP, as A_1 receptor agonists impaired, while A_1 receptor antagonists facilitated the development of LTP [3,13,58]. These data provide a possible link between hippocampal A_1 receptors and effects on spatial learning induced by the A_1/A_2 receptor antagonist theophylline. However, the selective facilitation of RM, but not of WM, induced by theophylline does not support this notion. The hippocampal formation is predominantly involved in WM [27,42], but also in RM [28] in radial maze tasks as used here. Thus, the lack of effects on WM we observed argues against a hippocampal action of theophylline. Besides, the glutamatergic hippocampal-accumbens pathway is known to play a role in radial maze performance by transferring RM, but not WM, information from the hippocampus to the motor system [52]. Furthermore, there is evidence for a selective neocortical involvement in RM as lesions of the nucleus basalis magnocellularis produced a marked disruption of RM, but not of WM [30]. As glutamate

release in the nucleus accumbens is inhibited via an action of adenosine on presynaptic A_1 receptors [20] and adenosine also inhibits cortical [18] and basal forebrain cholinergic [46] neurons, these structures might be possible targets of theophylline to selectively facilitate RM by an unselective A_1/A_2 receptor blockade. Neocortical and accumbal actions underlying theophylline's effects on RM might also explain the restriction of its efficacy to the light period, however ceiling effects during the dark period could contribute to this effect as well. Using in vivo microdialysis, Huston et al. [24] found that extracellular adenosine concentrations in the striatum are higher in the dark as compared to the light period. Likewise, in the rat cerebral cortex a bimodal purine pattern was found [5]. The effects of a competitive receptor antagonist as theophylline [2] are stronger with lower adenosine concentrations, i.e. the light period, and this might explain why facilitating effects of theophylline on RM are restricted to this particular period of the light–dark cycle. In addition, the restriction of facilitative effects of theophylline to the light period, i.e. the rest period of rodents, suggests that the proposed therapeutical potential of A_1 receptor antagonists for the treatment of cognitive deficits [53,57] could be seriously limited.

The present data demonstrate an involvement of adenosine in RM acquisition and retention depending on the period of the light–dark cycle. Future analysis will be necessary to investigate further the specific role of adenosine A_1 and A_2 receptors in spatial learning and memory by selective antagonists and to analyse in detail time-of-day effects on extracellular adenosine in implicated brain areas.

Acknowledgements

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