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# Improved spatial recognition memory in mice lacking adenosine $A_{2A}$ receptors

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**Abstract**

Adenosine receptors play an important role in learning and memory as their antagonists have been found to facilitate learning and memory in various tasks in rodents. However, few studies have examined the effect of adenosine $A_{2A}$ receptor deficiency on cognition. In the present study, we therefore used the Y-maze, a simple two-trial recognition test to measure spatial recognition memory in mice lacking adenosine $A_{2A}$ receptors. The results showed that adenosine $A_{2A}$ receptor knockout mice had a higher percentage of novel arm visits as first choice than wild-type CD1 mice. Moreover, these mice showed longer duration of visits in the novel arm when compared with controls, suggesting that the lack of adenosine $A_{2A}$ receptors improved spatial recognition memory. On the other hand, mice lacking the adenosine $A_{2A}$ receptors had low scores in the number of arm visits, suggesting that they were hypoactive. In conclusion, these data suggest the involvement of adenosine receptors in modulating spatial recognition memory in mice, consistent with earlier findings using adenosine receptor antagonists.


*Keywords:* Adenosine; Knockout; Wild type; Y-maze

## Introduction

Adenosine, a neuromodulator in the CNS (Cunha, 2001), modulates central neurotransmission through four G-protein-coupled receptor subtypes which are termed $A_1$, $A_{2A}$, $A_{2B}$, and $A_3$ receptors (Ralevic and Burnstock, 1998). The effects of adenosine are mediated through activation of high affinity $A_1$ and $A_{2A}$ receptors, which are probably of physiological importance, and of low affinity $A_{2B}$ receptors, which might be relevant in pathological conditions. The $A_3$ receptor, though a high affinity receptor in humans, has a low density in most tissues.

The highest density of adenosine $A_{2A}$ receptors in the brain is found in the basal ganglia, including striatum, nucleus accumbens and olfactory tubercle, and hippocampus, with lower levels found in the cortex and midbrain. In addition, the adenosine $A_{2A}$ receptor is also found in heart, lung, kidney, liver, vasculature, and platelets (Dixon et al., 1996; Ledent et al., 1997).

The purinergic system including adenosine modulates second messenger systems, neurotransmitters, energy metabolism, and different behaviors, such as sleep, motor activity, cognition, memory, aggressive behavior, and social interaction (Machado-Vieira et al., 2002). Adenosine plays an important role in learning and memory (Zarrindast and Shafaghi, 1994; Ohno and Watanabe, 1996) by regulating synaptic transmission, neuronal excitability (Sebastiao and Ribeiro, 2000; Kaplan and Abel, 2003), and neuronal synaptic plasticity including processes such as hippocampal long-term potentiation (LTP) (de Mendonca and Ribeiro, 1994; Kessey et al., 1997; Tabata et al., 2001) and long-term depression (LTD) (de Mendonca et al., 1997; de Mendonca and Ribeiro, 1997) which presumably underlies certain forms of learning and memory (Errington et al., 1987; Collingridge and Bliss, 1995).

Previous studies have furthermore demonstrated that adenosine receptor agonists disrupt learning and memory in rats and

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mice (Normile and Barraco, 1991; Normile et al., 1994; Zarrindast and Shafaghi, 1994; Ohno and Watanabe, 1996), while adenosine receptor antagonists facilitate learning and memory in the passive avoidance task (Nehlig et al., 1992; Suzuki et al., 1993; Kopf et al., 1999; Pereira et al., 2002) and maze task (Angelucci et al., 1999; Hauber and Bareiss, 2001; Angelucci et al., 2002) in rodents. While most of these studies were done with adenosine $A_1$ receptor agonists, less is known about the effect of adenosine $A_{2A}$ receptor deficiency on cognition. Recently, Pereira et al. found that stimulation of adenosine $A_{2A}$ receptors in the posterior cingulate cortex impaired memory retrieval in rats for an one-trial inhibitory avoidance task (Pereira et al., 2005).

At least two lines of mice have been developed that lack adenosine $A_{2A}$ receptors ($A_{2A}R^{-/-}$) (Ledent et al., 1997; Chen et al., 1999). These animals were reported to be generally healthy and breeding normally. Dopamine $D_1$ and $D_2$ receptor binding was normal in these mice, although locomotor hyperactivity induced by treatment with amphetamine or cocaine was reduced (Chen et al., 1999). Their behavior was furthermore characterized by higher anxiety and aggression, lower exploratory behavior, and higher nociceptive threshold than normal mice (Ledent et al., 1997).

In addition, we recently showed disrupted prepulse inhibition of acoustic startle and reduced startle habituation in these mice (Wang et al., 2003). In the present study, we investigated whether there are differences in spatial recognition memory between adenosine $A_{2A}$ knockout mice and wild-type mice using the Y-maze.

The Y-maze is a simple two-trial recognition test for measuring spatial recognition memory. It is based on the innate tendency of rodents to explore novel environments (Dellu et al., 2000). This paradigm avoids the effects of punishment (such as electric shock) or reward (such as food) that is commonly used in other paradigms and may have non-specific effects on the results. In addition, it does not require learning of a rule, thus, it is useful for studying memory in rodents, and in particular for the study of genetic influences on the response to novelty and recognition processes (Dellu et al., 1992, 2000; Conrad et al., 1997; Martin et al., 2003).

## Materials and methods

### *Animals*

Male $A_{2A}R^{-/-}$ of 8 weeks of age ($n = 12$) were obtained from a breeding colony at the Department of Pharmacology, Monash University, Melbourne (Snell et al., 2000a). Both null and wild-type ($A_{2A}R^{+/+}$, $n = 9$) mice weighed between 32 and 44 g at the start of the experiments and were kept at the Mental Health Research Institute animal facility (temperature controlled 22 ± 1°C; 12:12-h light:dark cycle) in groups of 2–4 animals in standard plastic mouse boxes with pellet food and tap water available ad libitum. The animals were regularly handled and weighed before behavioral experiments. All experiments were approved by the Animal Experimentation Ethics Committee of the Department of Pathology, University of Melbourne.

### *Behavioral apparatus and method*

The Y-maze was made of green–blue painted timber and consisted of three arms with an angle of 120° between each two arms. Each arm was 8 cm × 30 cm × 15 cm (width × length × height). The three identical arms were randomly designated: Start arm, in which the mouse started to explore (always open), Novel arm, which was blocked at the 1st trial, but open at the 2nd trial, and Other arm (always open).

The maze was placed in a sound attenuated room with dim illumination. The floor of the maze was covered with sawdust, which was mixed after each individual trial in order to eliminate olfactory stimuli. Visual cues were placed on the walls of the maze, and the observer was always in the same position at least 3 m from the maze.

The Y-maze test consisted of two trials separated by an intertrial interval (ITI) to assess spatial recognition memory. The first trial was 10-min duration and allowed the mouse to explore only two arms (Start arm and Other arm) of the maze, with the third arm (Novel arm) being blocked. After a certain ITI (1 h, 2 h, or 4 h), the second trial (retention) was conducted, during which all three arms were accessible and novelty vs. familiarity was analyzed through comparing behavior in all three arms. For the second trial, the mouse was placed back in the maze in the same starting arm, with free access to all three arms for 5 min. Using a ceiling-mounted CCD camera, all trials were recorded on a VCR. Video recordings were later analyzed using the Noldus Ethovision video tracking system, enabling the number of entries, time, and distance spent in each arm to be analyzed. Data were also expressed as percentage of total time and distance spent in arms every 30 s and during the total 5min (Akwa et al., 2001).

On the second trial, we also assessed which of the arms was entered first as another reflection of recognizing the novel arm—discrimination memory (Dellu et al., 1992, 2000; Conrad et al., 1997).

Because retention in the Y-maze test does not last longer than a few hours, this task can be assessed several times in the same animal (Dellu et al., 2000). All mice were therefore tested in the Y-maze three times using different ITI (1 h, 2 h, and 4 h). In the first week, the mice were tested with a 1-h ITI. In the second week, the ITI was 4 h, and, after analysis of the first two tests, it was decided to test the animals also with an ITI of 2 h in the fourth week.

### *Statistical analysis*

Data were expressed as (1) the percentage of novel arm as first choice (as discrimination memory measure); (2) percentage of the amount of time spent in novel arm compared to the other arms minute by minute in the 5-min retention phase (as spatial recognition memory measure); (3) the number of visits in each arm and total distance in the novel arm in the 5-min retention test as a locomotor activity index. Percentage scores were used to compensate for the difference in locomotor activity between genotypes. Data were expressed as mean ± standard error of the

mean (SEM) and analyzed using the SPSS statistical software package (version 10). Percentages of the first choices in the novel arm were compared to chance (50%) with the Chi-squared test ($\chi^2$). Differences between groups were considered significant at $P < 0.05$. Differences between groups for percentage time spent in the different arms and for number of arm visits and distance moved were assessed with analysis of variance (ANOVA) with repeated measures where appropriate. Genotype ($A_{2A}R^{+/+}$ or $A_{2A}R^{-/-}$) was the between-group factor, whereas arm (Novel, Start, Other) and ITI were within-group factors. One-way ANOVA was used to further analyze the difference between the three arms. Finally, post hoc between-group comparisons were completed with Fisher's Least Significant Difference test (LSD).

## Results

### *The percentage of novel arm selection as first choice*

As shown in the Fig. 1, after a 1-h ITI, all $A_{2A}R^{-/-}$ mice entered the novel arm as the first choice (100%) during the retention trial. In contrast, the percentage of novel arm as first choice in the wild-type $A_{2A}R^{+/+}$ mice was only 78% which was not significantly different from chance (50%) ($\chi^2 = 2.78$, $P = 0.096$).

After a 2-h ITI, both $A_{2A}R^{+/+}$ ($\chi^2 = 5.44$, $P = 0.02$) and $A_{2A}R^{-/-}$ ($\chi^2 = 5.33$, $P = 0.02$) mice chose the novel arm as the first choice with significant higher percentages than chance (50%). The percentage of novel arm as first choice in the wild-type $A_{2A}R^{+/+}$ mice was 89% while $A_{2A}R^{-/-}$ mice was 83%.

After a 4-h ITI, neither $A_{2A}R^{+/+}$ nor $A_{2A}R^{-/-}$ mice appeared to be able to discriminate between the novel and the other arms reflected by the finding that percentages of the novel arm as the first choice were decreased to random chance levels.

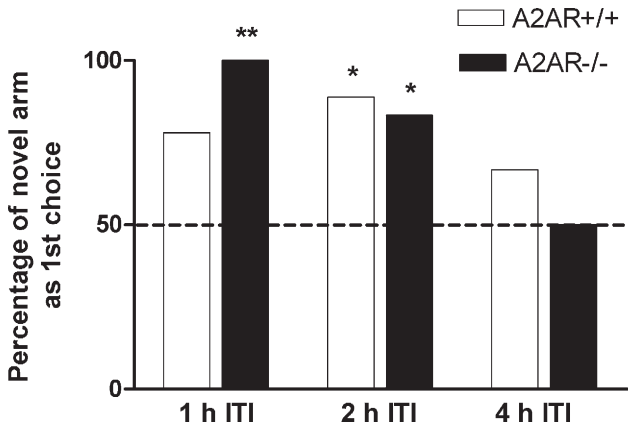


Fig. 1. Percentage of the first choice in the novel arm with different intertrial intervals (ITI) in adenosine $A_{2A}$ receptor knockout ($A_{2A}R^{-/-}$) and wild-type ($A_{2A}R^{+/+}$) mice. The dotted line represents the chance level (50%). The percentage of novel arm as first choice was significantly higher above chance in the $A_{2A}R^{-/-}$ (ITI = 1 h, 2 h) and the $A_{2A}R^{+/+}$ mice (ITI = 2 h). $A_{2A}R^{-/-}$ mice showed a better performance than wild-type mice when the ITI was 1 h. $^*P < 0.05$, $^{**}P < 0.01$ for difference in percentage of novel arm as 1st choice compared with the chance level on ITIs.

### *Total duration of arm visits in the 5-min retention test*

Total duration of visits in the novel arm was significantly higher than in the start and other arms in both $A_{2A}R^{+/+}$ and $A_{2A}R^{-/-}$ mice after 1-h, 2-h, and 4-h ITI (main effect of arm: $F(2,38) = 27.4$, $P < 0.001$).

As shown in Fig. 2A, $A_{2A}R^{-/-}$ mice spent more time in the novel arm than in the other arms after the 1-h ITI ($F(2,33) = 53.4$, $P < 0.001$). Post hoc analysis with LSD showed the novel arm percentage to be significantly higher than the start arm percentage ($P < 0.001$) and the other arm percentage ($P < 0.001$). $A_{2A}R^{-/-}$ mice also spent more time in the novel arm than in the other arms after a 2-h ITI ($F(2,33) = 6.5$, $P = 0.004$; LSD: novel arm vs. the start arm: $P = 0.002$; novel arm vs. the other arm: $P = 0.011$), and after a 4-h ITI ($F(2,33) = 3.4$, $P = 0.045$; LSD: novel arm vs. the start arm: $P = 0.017$; novel arm vs. the other arm: $P = 0.069$).

Similar to $A_{2A}R^{-/-}$ mice, $A_{2A}R^{+/+}$ control mice spent more time in the novel arm than in the other arms after a 1-h ITI ($F(2,24) = 4.9$, $P = 0.017$; LSD: novel arm vs. start arm: $P = 0.008$; novel arm vs. other arm: $P = 0.024$), after a 2-h ITI ($F(2,24) = 5.5$, $P = 0.011$; LSD: novel arm vs. start arm: $P = 0.005$; novel arm vs. other arm: $P = 0.019$), but not after a 4-h ITI ($F(2,24) = 2.3$, $P = 0.12$; LSD: novel arm vs. start arm: $P = 0.121$; novel arm vs. other arm: $P = 0.055$) (Fig. 2B). Notably, $A_{2A}R^{-/-}$ mice showed significantly increased total duration of visits in the novel arm when compared with $A_{2A}R^{+/+}$ control mice (main effect of genotype $F(1,19) = 28.1$, $P < 0.001$).

### *Percentage of duration of arm visits in the 5-min retention test*

Expressing duration of visits as percentage of total produced comparable results as the total duration of visits (Figs. 2C and D), reflected by higher percentages in the novel arm than in the two familiar arms (main effect of arm $F(2,38) = 24.0$, $P < 0.001$).

As shown in Fig. 2C, $A_{2A}R^{-/-}$ mice showed a higher percentage of duration of visits in novel arm than in the other arms after a 1-h ITI ($F(2,33) = 55.5$, $P < 0.001$). Again, post hoc analysis with LSD showed higher percentage time in the novel arm vs. the start arm ($P < 0.001$) and vs. the other arm ($P < 0.001$). $A_{2A}R^{-/-}$ mice also showed higher percentage time in the novel arm than in the other arms after a 2-h ITI ($F(2,33) = 6.6$, $P = 0.004$; LSD: novel arm vs. start arm: $P = 0.002$; novel arm vs. other arm: $P = 0.01$), and after a 4-h ITI ($F(2,33) = 3.5$, $P = 0.042$; LSD: novel arm vs. start arm: $P = 0.016$; novel arm vs. other arm: $P = 0.062$).

In contrast to $A_{2A}R^{-/-}$ mice, $A_{2A}R^{+/+}$ control mice showed higher percentage of duration of visits in novel arm compared to the other arms only after a 2-h ITI ($F(2,24) = 5.5$, $P = 0.011$; LSD: novel arm vs. start arm: $P = 0.004$; novel arm vs. other arm: $P = 0.019$) (Fig. 2D).

### *The number of arm visits in the 5-min retention test*

As shown in Figs. 3A and B, ANOVA on total number of arm entries revealed a significant difference among the three

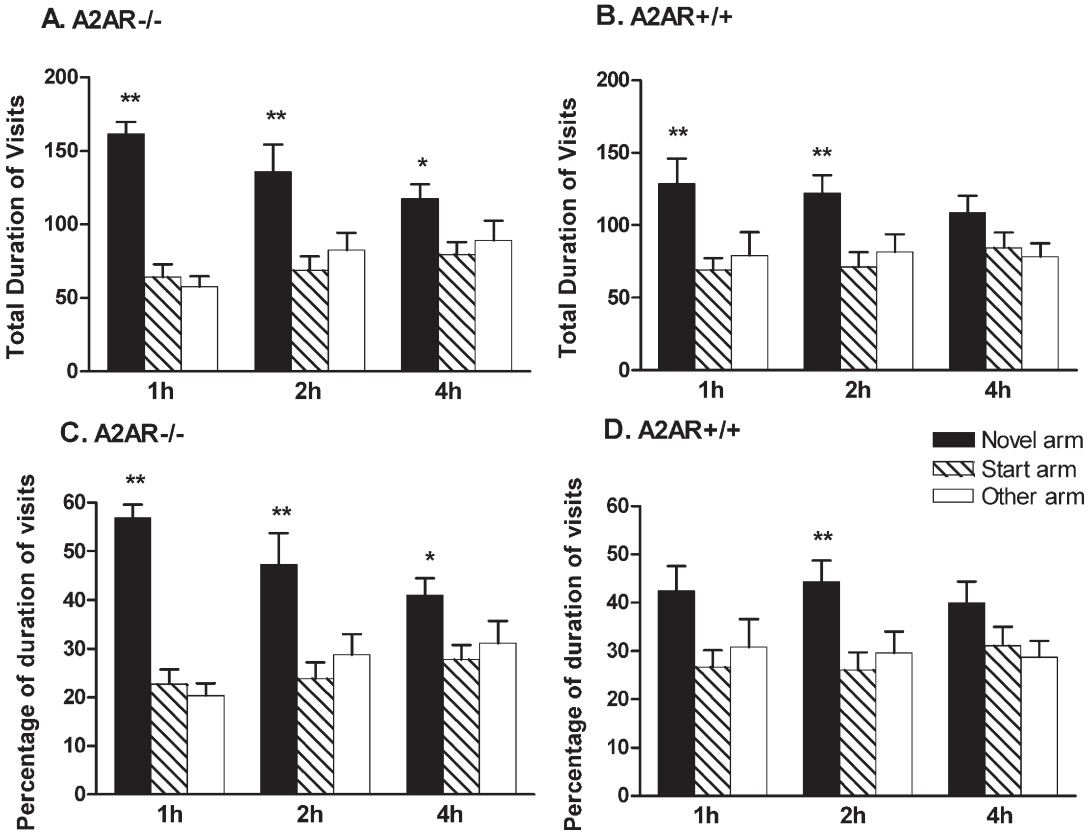


Fig. 2. Total duration and percentage of duration for mice visiting the novel, start, and other arms in the Y-maze. Panel A shows that the mean total duration for $A_{2A}R^{-/-}$ mice to explore the novel arm was significantly higher than the start and other arms with a 1-h, 2-h, and 4-h ITI. Panel B shows that the mean duration for $A_{2A}R^{+/+}$ mice exploring the novel arm was significantly higher than the start and other arms with an ITI of 1 h and 2 h. $A_{2A}R^{-/-}$ mice showed increased performance when compared with the $A_{2A}R^{+/+}$ mice on ITIs ($F(1,19) = 28.1$, $P < 0.001$). Panel C shows the percentage of duration for $A_{2A}R^{-/-}$ mice to visit the novel arm was higher than the start and other arms after a 1-h, 2-h, and 4-h ITI. Panel D shows the percentage of duration for $A_{2A}R^{+/+}$ mice visiting the novel arm was higher than the start and other arms after 2-h ITI. $^*P < 0.05$, $^{**}P < 0.01$ for difference in performance of mice in the novel arm vs. the start and other arms.

arms in both genotypes (main effect of arm $F(2,38) = 21.1$, $P < 0.001$).

A higher number of arm entries into the novel arm compared to the other arms was found in the $A_{2A}R^{-/-}$ mice after a 1-h ITI ($F(2,33) = 3.7$, $P = 0.036$; LSD: the novel arm vs. the start arm: $P = 0.051$; vs. the other arm: $P = 0.015$) (Fig. 3A). However, no such difference was found in the $A_{2A}R^{+/+}$ control mice ($F(2,24) = 1.7$, $P = 0.204$) (Fig. 3B).

$A_{2A}R^{-/-}$ mice showed a reduced locomotor activity reflected by lower overall number of visits in the three arms when compared with the $A_{2A}R^{+/+}$ mice (Fig. 3B) (main effect of genotype $F(1,19) = 19.6$, $P < 0.001$). This genotype difference was particularly present at longer ITIs (genotype × ITI interaction $F(2,38) = 3.9$, $P = 0.029$).

*Percentage of number of arm visits in the 5-min retention test*

$A_{2A}R^{-/-}$ mice showed a similar percentage of number of visits in three arms as $A_{2A}R^{+/+}$ mice did. Both genotypes presented higher scores in the novel arm than in the other two arms (main effect of arm $F(2,38) = 28.8$, $P < 0.001$) (Figs. 3C and D).

$A_{2A}R^{-/-}$ mice showed a higher percentage of number of visits in the novel arm than in the other two arms after 1-h ITI ($F(2,33) = 16.8$, $P < 0.001$; LSD: novel arm vs. start arm: $P < 0.001$; novel arm vs. other arm: $P < 0.001$) and after 2-h ITI ($F(2,33) = 8.4$, $P = 0.001$; LSD: the novel arm vs. the start arm: $P = 0.009$; novel arm vs. other arm: $P < 0.001$) (Fig. 3C). However, $A_{2A}R^{+/+}$ mice showed a higher percentage of number of visits in the novel arm only after a 1-h ITI ($F(2,24) = 5.5$, $P = 0.011$; LSD: novel arm vs. start arm: $P = 0.014$; novel arm vs. other arm: $P = 0.006$) (Fig. 3D).

*Total distance in the novel arm in the first 1 min and total 5 min in retention test*

ANOVA on the total distance moved in the novel arm after a 1-h ITI showed higher scores in the first 1 min in $A_{2A}R^{-/-}$ than $A_{2A}R^{+/+}$ mice, but this difference decreased over time (Fig. 4). In contrast, $A_{2A}R^{-/-}$ mice showed lower distance moved in the novel arm compared to $A_{2A}R^{+/+}$ mice after the 2-h and 4-h ITI. Thus, there was a significant interaction of ITI and genotype ($F(2,38) = 3.7$, $P = 0.034$). The total distance moved in the novel arm decreased with

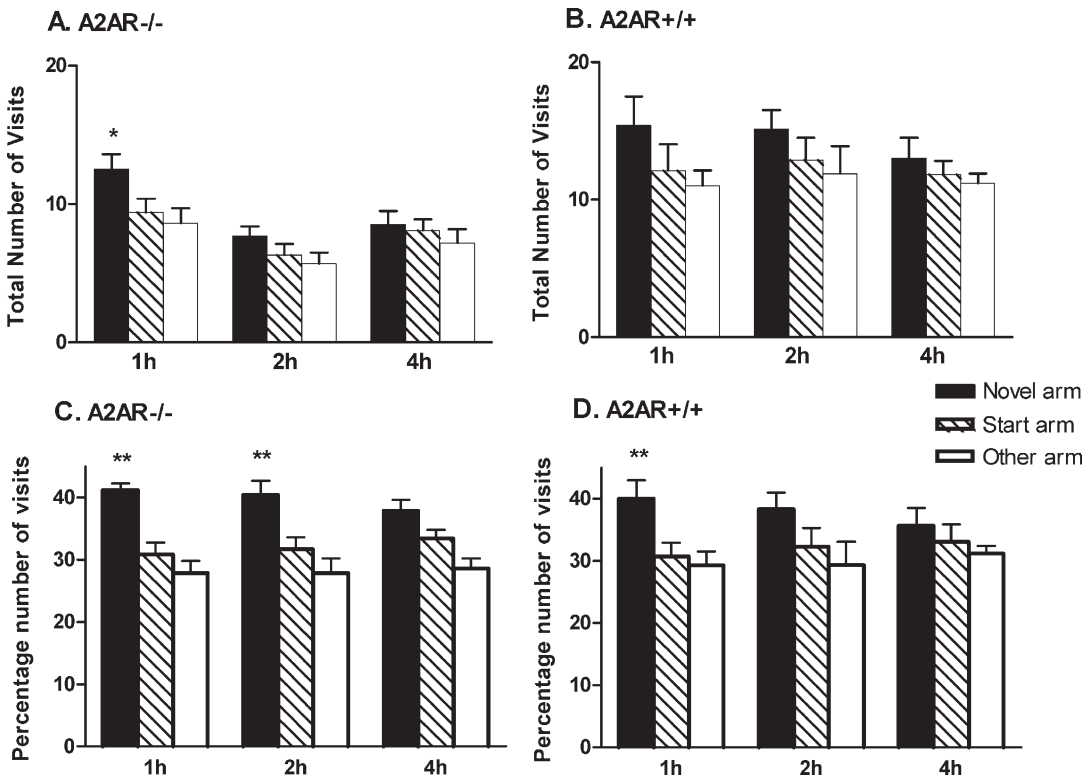


Fig. 3. Number of arm visits and percentage of number of arm visits for mice in the Y-maze. Panel A shows the number of novel, start and other arms entered by $A_{2A}$ $R^{-/-}$ mice. Scores for the novel arm were significantly higher than in other two arms after a 1-h ITI. Panel B shows the number of novel, start and other arm entries by $A_{2A}R^{+/+}$ mice. $A_{2A}$ $R^{-/-}$ mice showed a lower number of visits than $A_{2A}R^{+/+}$ mice ($F(1,19) = 19.6$, $P < 0.001$). Panel C shows that the percentage number of arm entries into the novel arm was higher than in the start and other arms in $A_{2A}$ $R^{-/-}$ mice at 1-h and 2-h ITIs. Panel D shows the percentage number of arm entries into the novel arm was higher than in the start and other arms in $A_{2A}R^{+/+}$ mice after 1-h ITI. $^{*}P < 0.05$, $^{**}P < 0.001$ for difference in performance of mice in the novel arm vs. in the start and other arms.

ITI in both genotypes (main effect of ITI: $F(2,38) = 5.3$, $P = 0.009$).

## Discussion

The results represent the first assessment of the effect of adenosine $A_{2A}$ receptor deficiency on spatial recognition memory. Our findings demonstrate that mice lacking adenosine $A_{2A}$ receptors showed improved spatial recognition memory in the Y-maze. However, these animals also displayed reduced locomotor activity.

According to Dellu et al. (1992, 2000), the two-trial Y-maze task is a specific and sensitive test of spatial recognition memory in rodents. Our data support this view by showing that there were always significant arm effects on either absolute or percentage measures of total duration of visits and number of visits during the retention test. For both genotypes, the time mice spent in, and the frequency mice entered, were higher for the unfamiliar novel arm than for the familiar start and other arms after a 1-h and 2-h ITI. This suggested that mice are highly sensitive to their spatial and contextual environment. Moreover, our data are consistent with previous findings in CD1 mice which demonstrated a very high level of novelty exploration (Dellu et al., 2000).

### *Improved discrimination and spatial recognition memory in ($A_{2A}R^{-/-}$) mice*

Firstly, the first choice for novel arm seems to be testing discrimination memory. In the retention test, mice have to make a choice between the novel arm (unfamiliar) and the other arm (familiar) when they are released from the start arm in the Y-maze. Adenosine $A_{2A}$ receptors knockout ($A_{2A}R^{-/-}$) mice showed a higher score in discrimination memory than wild-type mice after a 1-h ITI. However, this higher performance declined with the ITI.

Secondly, two measures of exploratory behavior (inspective and inquisitive) should be separately analyzed according to Dellu et al. (1992, 2000). Inspective exploratory behavior is reflected in the duration spent in arms. However, the number of arm visits is an index of inquisitive behavior and can measure locomotor activity as well. In our data, $A_{2A}R^{-/-}$ mice showed increases in inspective exploratory behavior compared to $A_{2A}R^{+/+}$ mice, suggesting that loss of adenosine $A_{2A}$ receptors enhanced spatial recognition memory in mice.

With respect to inquisitive behavior, $A_{2A}R^{-/-}$ mice showed a similar behavior as $A_{2A}R^{+/+}$ mice, reflected by the similar percentage of number of arm entries compared to $A_{2A}R^{+/+}$ mice. Thus, it can be assumed that lacking adenosine $A_{2A}$ receptors did not influence inquisitive exploratory behavior in mice. That these

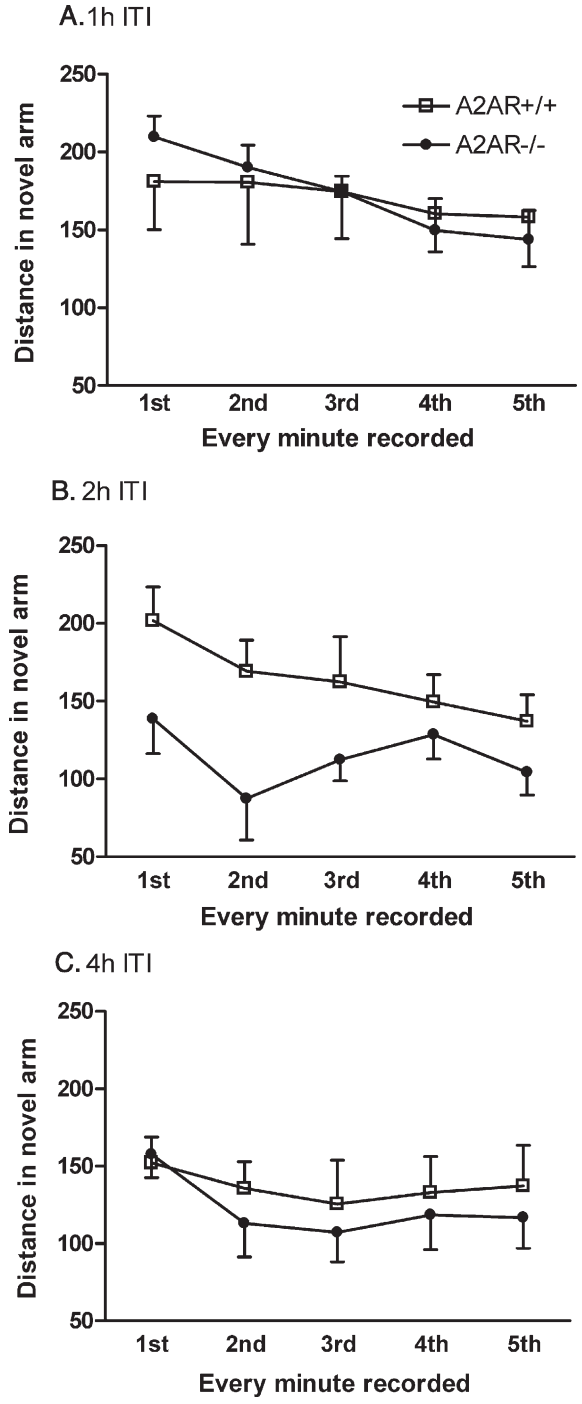


Fig. 4. Distance moved in the novel arm expressed per minute during the retention test after a 1-h ITI (A), 2-h ITI (B), and 4-h ITI (C). $A_{2A}R^{-/-}$ mice showed a higher score for distance moved during the first 1 min than $A_{2A}R^{+/+}$ mice after a 1-h ITI, however, a lower score for distance moved in the novel arm than $A_{2A}R^{+/+}$ mice after a 2-h and 4-h ITI.

two measures here do not completely parallel each other is consistent with the observations of Conrad et al. (1997). Additionally, it has been pointed out that drugs such as amphetamine may affect these two measures in opposite ways (Dellu et al., 1992).

There is ample evidence for the modulatory role of adenosine in memory in the hippocampus, olfactory bulb and striatum (de Mendonca and Ribeiro, 1994; Kessey et al., 1997; de Mendonca and Ribeiro, 2000; Tabata et al., 2001). Interestingly, several studies have demonstrated antagonists at adenosine receptors as potential cognitive enhancers. For instance, treatment with adenosine receptor antagonists facilitated retention of and prevented scopolamine-induced amnesia in a passive avoidance task in rodents (Pitsikas and Borsini, 1997; Jin et al., 2000). Caffeine and the selective adenosine $A_{2A}$ receptor antagonists SCH 58261 and DMPX enhanced memory consolidation and reversed the detrimental effect induced by dizolcipine in a model of working memory in mice (Fraser et al., 1997). In humans, the improvement of cognition by caffeine has been attributed to its antagonism of adenosine receptors (Fredholm, 1995).

Recently, Prediger and colleagues showed that blockade of adenosine $A_{2A}$ receptors enhances short-term social memory in rats (Prediger and Takahashi, 2005; Prediger et al., 2005a,b). However, Gimenez-Llort et al. found that mice lacking adenosine $A_1$ receptors have normal spatial learning and plasticity in the CA1 region of the hippocampus, although they habituate more slowly (Gimenez-Llort et al., 2005).

Although the administration of adenosine $A_{2A}$ receptor antagonists does not completely mimic the inactivation of the gene encoding adenosine $A_{2A}$ receptors, their modifications involve the dopaminergic pathway (Dassesse et al., 2001). Antagonistic interactions between adenosine $A_{2A}$ receptors and dopamine $D_2$ receptors have been reported (Ferre et al., 1996; Johansson et al., 1997). Adenosine $A_{2A}$ receptor antagonists mainly act by enhancing dopamine D2 receptor signaling in the striatum (Tanganelli et al., 2004). Blockade of adenosine $A_{2A}$ receptors resulted in a reversible decrease of the excitatory postsynaptic potential in the hippocampus (Kessey and Mogul, 1998).

The present study demonstrated the involvement of adenosine receptors in modulating spatial recognition memory in mice. It is consistent with the previous findings of adenosine $A_{2A}$ receptor antagonists facilitating memory in some tasks.

### *Reduced locomotor activity in $A_{2A}R^{-/-}$ mice*

In mice lacking adenosine $A_{2A}$ receptors, locomotor activity was decreased when compared with the $A_{2A}R^{+/+}$ control mice as shown by the low number of visits in all three arms. This is in line with previous studies which suggested that lacking adenosine $A_{2A}$ receptors caused hypoactivity in mice (Ledent et al., 1997). Reduced locomotor activity in $A_{2A}R^{-/-}$ mice was also reflected by the lower distance moved in the novel arm. Although $A_{2A}R^{-/-}$ mice showed high scores in total duration visits in novel arms, they had generally lower scores for distance moved in the novel arm than $A_{2A}R^{+/+}$ mice, especially after the first minute.

Adenosine $A_{2A}$ receptors are particularly abundant in the striatum (Schiffmann and Vanderhaeghen, 1993; Schiffmann et al., 1991) and contribute to the function of the cortico-basal ganglia–cortical loop. The lack of adenosine $A_{2A}$ receptors leads to decreased extracellular dopamine concentrations and increased extracellular glutamate concentrations in the striatum (Dassesse et al., 2001). The adenosine $A_{2A}$ receptor deficiency-

induced altered locomotor activity may be partly through a functional hypodopaminergic state in the basal ganglia. In addition, the glutamatergic system may contribute to this hypoactivity as well. Snell et al. (2000b) also found that glutamatergic neurotransmission within certain neural pathways involved in autonomic and motor control was altered in the brains of $A_{2A}R^{-/-}$ mice. Moreover, endogenous adenosine is a potent neuromodulator at motor nerve endings (Pereira et al., 2000). Therefore, adenosine receptor deficiency may lead to a reduced locomotor activity.

The reduced locomotor activity in mice lacking adenosine $A_{2A}$ receptors was in accordance with Ledent et al. (1997)'s finding. Since the low anxiety may influence the present results, we used the percentage as the memory performance to avoid the difference in locomotor activity between genotypes and found that lacking adenosine $A_{2A}$ receptors improved spatial memory in the Y-maze.

In a previous study (Wang et al., 2003), we showed that adenosine $A_{2A}$ receptors, most likely in the nucleus accumbens, are involved in prepulse inhibition of acoustic startle. In the present study, our findings provided behavioral evidence for the role of adenosine $A_{2A}$ receptors in modulation of spatial recognition memory.

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