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Summary

Several lines of evidence point to the involvement of adenosine in the regulation of important central mechanisms such as cognition, arousal, aggression and anxiety. In order to elucidate the involvement of the adenosine A1 receptor (A1AR) in spatial learning and the control of exploratory behaviour, we assessed A1AR knockout mice (A1AR-/-) and their wild type littermates (A1AR+/+) in a place navigation task in the water maze and in a battery of forced and free exploration tests. In the water maze, A1AR-/- mice showed normal escape latencies and were indistinguishable from controls with respect to measures of spatial performance during both training and probe trial. But despite normal performance they showed increased wall hugging, most prominently after the relocation of the goal platform for reversal training. Quantitative analysis of strategy choices indicated that wall hugging was increased mainly at the expense of chaining and passive floating, whereas the frequency of trials characterized as direct swims or focal searching was normal in A1AR-/- mice. These results indicate intact spatial cognition, but mildly altered emotional reactions to the water maze environment. In line with this interpretation, A1AR-/- mice showed normal levels and patterns of activity, but a mild increase of some measures of anxiety in our battery of forced and free exploration paradigms. These results are in line with findings published using a genetically similar line, but demonstrate that the magnitude of the changes and the range of affected behavioural measures may vary considerably depending on the environmental conditions during testing.

Key words: adenosine, adenosine-receptor, anxiety, memory, behaviour, mice, environment

Introduction

The adenosine A1 receptor is one of four known subtypes of adenosine receptors: A1, A2A, A2B and A3 [17]. A1 receptor sites are heterogeneously distributed throughout the CNS with highest levels in the hippocampus, cerebellar cortex, cerebral cortex, striatum, parts of the thalamus, and nucleus accumbens [10]. Adenosine is thought to be involved in the regulation of important central mechanisms such as cognition [25], arousal [27], aggression [26] and anxiety [11]. Benzodiazepines, the most widely used anxiolytic drugs, are known to decrease the adenosine A1 receptor binding in vivo [20] and interfere with adenosine uptake [1]. Adenosine and its analogues have been shown to reverse the diazepam-induced activation of exploratory behaviour in mice [2, 5]. Carbamazepine, which is an effective mood stabilizer and successfully used in the treatment of bipolar disorder, has been suggested to interact with adenosine A1 and A2 receptors [12]. However, a gene association study of the A1 adenosine receptor and bipolar affective disorder did not yield any significant linkage [7]. Both, A1 and A2 receptors are involved in modulating spontaneous locomotor activity [24]. The A2a adenosine receptor knockout mouse has been proposed to serve as a model for anxiety [7] and shows increased aggressiveness [22]. In order to elucidate the involvement of the adenosine A1 receptor (A1AR) in spatial learning and the control of exploratory behaviour, we assessed A1AR knockout mice (A1AR-/-) and their wild type littermates (A1AR+/+) in a place navigation task in the water maze and in a battery of forced and free exploration tests. During the course of our study observations have been reported pointing to increased anxiety and aggressiveness in another mouse line lacking the A1 adenosine receptor [15].

Material and Methods

Animals

A1AR knockout mice (A1AR) and their wild type littermate controls (A1AR+/+) were from a subcolony of the original strain generated by Sun et al. and maintained at the NIH [30]. Genotyping was done by PCR on tail DNA using A1AR and neo-R-specific primers as previously described [30]. Mice have always been reproduced by heterozygous crossing. Therefore, the genetic background of the animals was a mix of J129 and C57BL/6.

Housing and handling

All behavioural procedures were approved by Swiss animal welfare authorities. One week before the experimental period, animals were transferred to standard single mouse cages and maintained at a 12:12 h inverted cycle with lights on between 8 p.m. and 8. a.m. Standard mouse chow, water and nesting material were available ad libitum. The mice were tested in sets of 11 to 32 individuals between 8 a.m. and 8 p.m. Only one type of experiment was done on the same day and the home cage rack was brought to the test room at least 30 min before each experiment and dry surfaces of apparatus were thoroughly cleaned with 70 % ethanol before releasing the animal. Behavioural analysis began at the age of 12 weeks and ended at the age of 16 weeks, tests were done in the following order: water maze, open-field, O-maze, dark-light box, and emergence/novel object test.

Water maze

Apparatus and procedures

Spatial reference memory and reversal. We adapted the original procedure [23] for the use with mice. A round white poly-propylene swim tank with a diameter of 150 cm, was filled with water (temperature 24-26° C, depth 15 cm) and made opaque by addition of 1 l of milk. The white quadratic goal platform (14x14 cm) was hidden 0.5 cm below water. Salient distant cues such as wall-mounted laboratory shelves, cupboards and various posters were available in the indirectly illuminated room (4 40W bulbs, 12 lux). Animals performed 30 training trials, 6 per day with intertrial intervals of 30-60 min and pseudo randomly varying starting positions. To minimize handling, they were transferred to the pool using a white plastic cup. After they had reached the platform and stayed on it for five seconds they were allowed to climb onto

a wire mesh grid and transferred to their cage without further handling. During the first 18 trials (acquisition phase) the hidden platform was held in the same position and then moved to the opposite quadrant for the remaining 12 trials (reversal phase). The first trial of the reversal phase served as probe trial to test for spatial retention. The whole sample was run in four subsets, each with different target quadrants.

Data analysis

Video tracking. Animals were video-tracked at 4.2 Hz and 256x256 pixel spatial resolution using a Noldus EthoVision 1.96 system (Noldus Information Technology, Wageningen NL, www.noldus.com). Raw data were then transferred to public domain software Wintrack 2.4 (www.dpwolfer.ch/wintrack) for further analysis [31].

Training performance. Escape performance during training was assessed by calculating escape latency, time spent floating (episodes of immobility or decelerations with speed minimum < 0.06 m/s), and swim speed (excluding floating episodes). Spatial aspects of behaviour were evaluated using cumulative search error (sum of distances to target measured at 1 s intervals minus value that would be obtained for an ideal direct swim) [13], Whishaw's error (% path outside a 0.16 m wide corridor connecting release point and goal), and path efficiency (% path during which speed vector component toward goal is 75 % or more). Wall oriented behaviour was quantified by determination of % time spent in a 10 cm wide wall zone and number of wall contacts. Additional specialized parameters were needed to categorize trials according to strategy (Fig. 2).

Probe trial. Spatial retention was assessed using % time in quadrant, number of annulus crossings, and time in a circular target zone comprising 12.5 % of the pool surface. For each of these parameters, the trained quadrant was compared with the average of the left and right adjacent quadrant. The opposite quadrant was not considered because it contained the new goal. Further measures of spatial selectivity were: proximity (average distance to trained target) [13], polar error (average of angle between two lines connecting the pool center to the subject and trained target, respectively).

Exploration tests

Apparatus and procedures

Open-field. The round open-field arena had a diameter of 150 cm, a white plastic floor, and 35 cm high sidewalls made of white polypropylene. Illumination was by indirect diffuse room light (4 40W bulbs, 12 lux). Each subject was released near the wall and observed for 10 min. The same procedure was repeated the following day, resulting in a total observation time of 20 min [31].

Light-dark box. A 20x30 cm transparent Perspex chamber (20 cm high) was illuminated by direct room light (500 lux) and connected by an 7.5x7.5 cm aperture to a 20x15x20 cm polyvinyl-chloride dark box. Each subject was released in the middle of the lit compartment and observed for 5 min [5]. Rearings and grooming were recorded using the keyboard event-recorder provided by the video-tracking system.

O-maze. A 5.5 cm wide annular runway was constructed using grey plastic. It had an outer diameter of 46 cm and was placed inside the above open-field arena 40 cm above the floor [21, 28]. Two opposing 90° sectors were protected by 16 cm high inner and outer walls of grey polyvinyl-chloride (height 16 cm). Animals were released in one of the protected sectors and observed for 10 min. Exploratory head dips were recorded using the keyboard event-recorder provided by the video-tracking system.

Emergence test. Procedure was modified after Dulawa et al. [9]. Frames of non-reflective aluminum (37 cm high) were used to partition the above open-field into four square 50x50 cm arenas, allowing for concurrent observation of 4 animals. Each arena had a 12x8x4 cm plastic home box with an aperture of 8x4 cm, positioned in a corner at 5 cm from the nearest walls, with the aperture facing away from the wall. 24 h prior to testing, a thoroughly cleaned home box was placed in the home cage of each test subject. The next day, test subjects and home boxes were introduced into the arenas and observed for 30 min.

Novel object test. Procedure was modified after Dulawa et al. [9]. Arenas were the same as for the emergence test, but without the home box. The novel object was a 12x4 cm semi-transparent 50 ml Falcon tube positioned vertically in the center of the arena. Each subject was observed for 30 min in the empty, cleaned arena. Then, the novel object was introduced and observation continued for another 30.

Data analysis

Video tracking. The same system was used as for the watermaze experiment. In addition to xy position, the system recorded object area and the status of defined event recorder keys on the keyboard.

Behavioural measures common to all tests. For the analysis of *horizontal activity*, recorded tracks were segmented into three motion states according to criteria modified from [8]. (i) Progression episodes which corresponded to bouts of long-distance locomotion were defined by a threshold for average velocity (8.5 cm/s in the open-field, emergence and novel object tests; 4.0 cm/s on the O-maze and in the light-dark box) and total distance moved (5 and 3 cm, respectively). Rapid decelerations (deeper than 15 and 8 cm/s, respectively) were subtracted and classified as scanning (see below). (ii) Resting episodes were periods lasting 2 s or longer with smoothed speed values (averaging frame 0.5 s) below the system noise level of 2.5 cm/s. Resting episodes included periods of immobility as well as grooming which caused cursor movements at or near the system noise level. (iii) The remaining time was classified as scanning episodes which correlated with exploratory behaviours such as brief stopping, sniffing, establishing snout contact with the substrate or an object, looking around, stretch attend postures, rearing or leaning against the wall. Because the tracking system also monitored apparent subject area, *vertical activity* could estimated by counting reductions of subject area deeper than 250 mm^2 while the animal was not progressing. To assess approach-avoidance behaviours, dwell time as well as activity measures were broken down into *three zones*. While the most avoided and hence most aversive parts of the arena were defined as exploration zone, the most preferred parts were defined as home zone. The remainder of the arena formed the intermediate zone. The specific implementation of these three zones in each test was based on the retrospective quantification of preferences in a large number of subjects (1000-3500 mice depending on the paradigm). To allow comparison of zones irrespective of their size, an index of zone preference was calculated using the formula 100% x(100-C)/[x(100-C)+C(100-x)], where x = % time spent in the zone and C = % of arena surface occupied by the zone. According to this formula, an index value of 0 indicated complete avoidance of the zone and a value of 100 maximal preference. Irrespective of zone size, score of 50 % would be obtained by a randomly moving animal.

Open-field. The exploration zone was a circular central field comprising 50 % of the arena surface and a 7 cm wide wall zone constituted the home zone. To obtain an index of stereotypic movement, visits to a set of 5x5 tiles were scanned for repeating sequences, whereby each repetition incremented the stereotypy count of all involved tiles. Extraction of movements leading toward or away from the arena center was based on two criteria: a speed > 5 cm/s and a movement component perpendicular to the wall of >50 %. A speed ratio was calculated by dividing the average speed of centrifugal movements by the average speed of centripetal movements.

Light-dark box. We determined the latency to enter and the time spent inside the dark compartment, and the number of transitions between the compartments. In addition, we compared different zones within the illuminated compartment. The home and intermediate zones were 10 cm wide and located next to the aperture of the dark compartment and at the opposite end, respectively. The remaining central segment was the least attractive area and thus defined as exploration zone. Emergences during which the subject did not dare to enter the illuminated compartment with all four paws were counted as aborted excursions.

O-maze. An intermediate zone comprising four 30° segments at the ends of the protection walls separated the two 50° wide protected home zones and the two 70° wide unprotected exploration zones. With these boundaries, the system detected entries to the exploration zone only when the animal moved into it with all four paws and the animals could not perform exploratory head dips while registered to the home zone. We determined total number of entries into protected sectors as well as the ratio of entries to unprotected zones over entries to unprotected + protected zones. Head dips were classified as protected or unprotected based on the concurrently determined position of the animal's gravity center in the intermediate or exploration zone, respectively.

Emergence test. We determined the latency to exit and the time spent inside the home box, and the number of transitions between home box and arena. Within the visible arena, the 40x40 cm center field constituted the exploration zone and a 18x22 cm home zone surrounded the home box, including the arena corner located next to it. Measures of stereotypy were calculated following the same logic as in the open-field. Aborted excursions were defined and counted as in the light-dark box.

Novel object test. A 5 cm wide corridor along the wall formed the home zone. The circular exploration zone of 18 cm diameter was located in the arena center where the object was introduced, while the surrounding space was defined as intermediate zone. Behavioural measures were as for the emergence test. For measures relevant to object investigation (time in zone, scanning time, number of scanning episodes, cumulative scanning distance, vertical activity) we attempted to extract object-related components by extrapolating object un-related components from the surrounding intermediate zone and subtracting them from the values measured in the exploration/object zone. This method brought corrected measures close to zero in absence of the object.

Statistics

Raw frequencies of water maze strategies were compared using Chi-square tests. Zone preference measures were compared against chance values using one-sample t-tests. All other effects were explored using ANOVA because of its superior flexibility. When multiple comparisons had to be performed, p-values were corrected according to the Bonferroni method. Where possible, main effects were verified using non-parametric tests. ANOVA designs included genotype as main between subject factor. Gender was included as additional factor to assess gender dependency of effects. These comparisons are not reported, however, because no gender dependencies were found. Learning and habituation effects were assessed using repeated designs including time as within subject factor. Units of time were blocks of two trials in the water-maze, bins of 5 min in the open-field and O-maze, and bins of 10 min in the emergence and novel object tests. Comparisons of motion states and/or zones in exploration tests were done by repeated ANOVA including motion state and or zone as within subject factor. Similarly, spatial preferences during water-maze probe trials were examined using repeated ANOVA with place as within subject factors, comparing the trained quadrant with the average of the two adjacent quadrants. Statview 5.0 for Windows (SAS Institute Inc., Cary NC, USA, www.statview.com) was used for all statistical calculations.

Results

Water maze

Training performance. Statistics of training performance and probe trial measures are given in table 1. Both A1AR-/- and A1AR+/+ learned to locate the hidden platform as evidenced by decreasing escape latencies (Fig. 1A). After relocation of the platform for reversal training, escape latencies increased markedly (Fig. 1A) in both groups, confirming that the animals had adapted their escape strategy to the specific platform position. Both groups were able to adapt to the new goal position. Overall, escape latencies of A1AR-/- and A1AR+/+ mice were statistically indistinguishable. Measures designed to quantify spatial performance more specifically, such as Gallagher search error [13] and Whishaw error did not reveal group differences either. A1AR-/- mice were also indistinguishable from controls with respect to path efficiency, a measure recently introduced in our laboratory [31]. Floating time and swim speed were normal as well. Despite preserved escape performance, A1AR-/- mice did not behave in the same way as controls, however. ANOVA analysis indicated that time spent near the wall (Fig. 1B) and number of wall approaches (Fig. 1C) were significantly increased in A1AR-/- mice. During acquisition, this difference was modest. After relocation of the platform for reversal learning, controls showed a further decrease of wall hugging. In A1AR-/- mice, by contrast, the time spent near the wall increased and became highly variable (Fig. 1B). While some mutant individuals showed slightly increased degree of wall hugging compared to the end of acquisition, others responded to the change of the platform position with a marked increase in wall hugging that was evident to direct observation.

Probe trial. Both mutants and controls showed normal spatial retention as evidenced by significantly more time spent in the former goal quadrant than in adjacent quadrants (table 1). Also according to more stringent measures such as zone time (Fig. 1D) and annulus crossings there were no differences between A1AR-/- and A1AR+/+. The proximity measure [13] and polar error [31] were also not affected by the mutation.

Quantification of strategy choice. Subtle differences of strategy choice in the water maze do not necessarily affect escape or retention performance in the watermaze and therefore main remain undetected by the measures presented above. Therefore we extended the analysis and categorized trials according to the predominant swim

strategy. This permitted to compare the frequencies of 7 commonly observed and readily identifiable swim strategies among A1AR-/- and +/+ mice (Fig. 2). In the Chi-square analysis, frequencies of strategies were statistically indistinguishable during acquisition, despite a somewhat higher frequency of trials classified as wall hugging. In the reversal period, however, we found that the distribution of frequencies was significantly different in A1AR-/- mice, with wall hugging trials increased mainly at the expense of floating and chaining. Strategies that are demanding with respect to processing of spatial information (direct swims, focal searching) were not affected by this change and accounted together for 50 % of trials in both genotypes.

Exploratory behaviour

We tested A1AR-/- in the open-field, light-dark box, and on the elevated O-maze. These three paradigms are known as forced exploration tests because the animals are confronted with an entirely new environment. This analysis was complemented by testing in the emergence and novel object tests, two paradigms that belong to the category of free exploration paradigms because they present the animals with a true choice between familiar and unfamiliar areas. Statistical analysis of these tests is summarized in table 1 while graphs of selected measures are shown in Fig. 3.

Locomotor activity

A normal overall level of locomotor activity as judged from total distance moved was observed in A1AR-/- mice. Differential analysis of motion states (see methods for details on their definition) revealed normal patterns in all 5 tests: there were no differences with respect to time spent with resting, progressive locomotion, and small movements in a speed range typically correlated with exploratory behaviours such as rearing, leaning, looking around, and sniffing. During locomotion A1AR-/- mice showed normal velocity in all tests. Measures of stereotypic movement, calculated in the open-field and emergence test, were in the normal range.

Measures of anxiety-like behaviour

The zone preference measure indicated that both groups strongly avoided the central exploration zone of the open-field while strongly preferring the wall zone (Fig. 3A). The avoidance of the open-field center appeared slightly stronger in the A1AR-/- mice, but this trend was not statistically significant. Both groups showed a similar hesitance to enter the center field as judged from the ratio of centrifugal over centripetal speed. In the light-dark box, both genotypes preferred the dark compartment to a similar degree (Fig. 3B). On the O-maze, both genotypes similarly

displayed a strong avoidance of the open sectors (Fig. 3C,E), but A1AR-/- mice showed a border-line reduction of the number of exploratory head dips and a very low number of unprotected dips (Fig. 3D). In the emergence test, relatively little time was spent in the home box by both groups (Fig. 3F), which is in line with data from Dulawa [9]. A borderline trend of A1AR-/- mice was observed to spend more time in their home boxes. Their increased reluctance to leave the home box in order to explore the unfamiliar arena was clearly evident from the fact that A1AR-/- mice aborted their emergences three-times as often than controls before leaving the box with all four paws (Fig. 3G). In the novel object test, both groups had zone preference scores near chance level for the zone containing the novel object (Fig. 3H), indicating approximately equal tendencies to explore and avoid the object. The total time spent investigating the object was unaltered in A1AR-/- mice as compared with controls (Fig. 3I), as well as their cumulative horizontal and vertical object exploration activity, and the number of object investigation episodes.

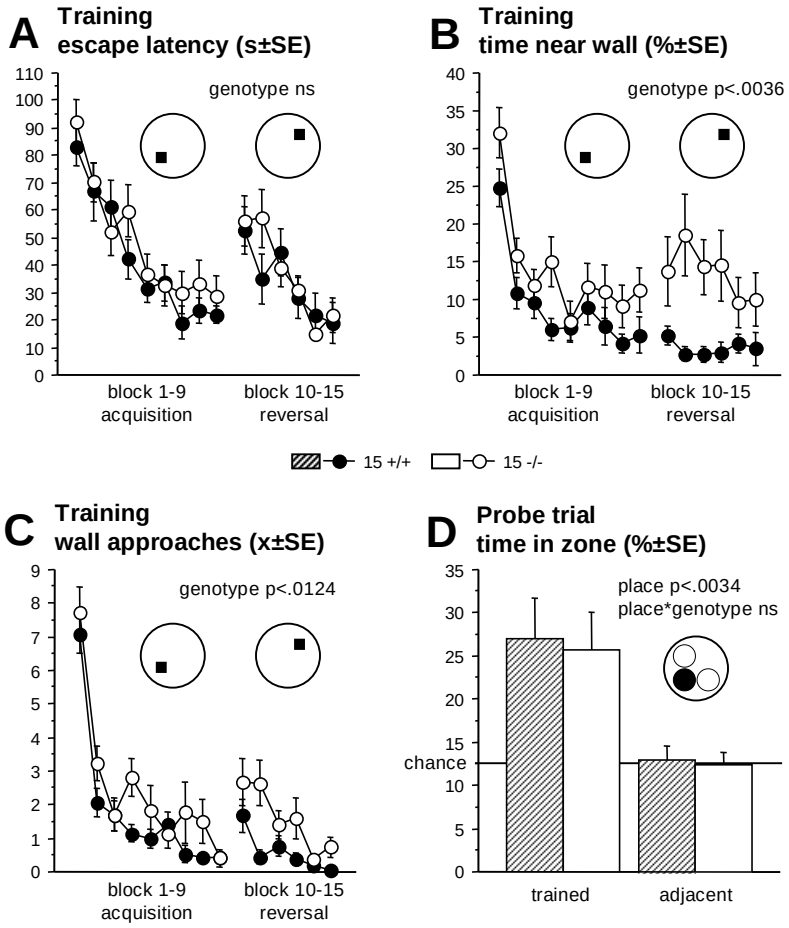
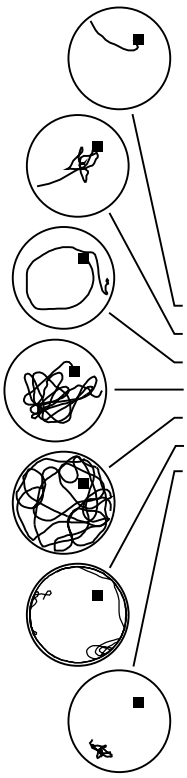


Fig. 1. Training performance (A-C) and spatial retention (D) in the watermaze. Panels A-C show time course of escape latency, time near wall, and number of wall approaches (escape attempts), respectively. Points represent group mean and SE for pairs of subsequent trials during the acquisition and reversal phase of training. Panel D illustrates spatial retention during the probe trial (first 60 s of the first reversal trial). % time spent in a circular zone (12.5% of pool surface) around the trained target, compared against the average of control zones located in the adjacent quadrants.



Strategy choice in the water maze

	Acquisition training trials 1-18 $\chi^2 = 9.1$ DF = 6 n.s.			Reversal training trials 19-30 $\chi^2 = 20.2$ DF = 6 p<.0025		
	-/-	+/+	χ^2	-/-	+/+	χ^2
direct swims[2]	15.9%	19.6%	1.0	20.0%	20.6%	0.0
focal searching[3]	24.1%	28.5%	1.0	31.7%	30.0%	0.1
chaining[4]	15.2%	14.4%	0.1	16.1%	22.8%	2.1
scanning[5]	16.7%	18.1%	0.2	16.7%	16.7%	0.0
random swimming[6]	11.1%	8.5%	0.9	4.4%	3.9%	0.1
wall hugging[7]	11.1%	5.2%	5.8	9.4%	0.6%	14.2
floating[1]	5.9%	5.6%	0.0	1.7%	5.6%	3.8

Statistics are based on raw frequencies, table cells show relative frequencies and the contribution of each category to the test statistic. Each individual trial was assigned to the first of 7 exclusive categories that met the requred criteria:

[1] floating time >30 s

[2] search error <3 m*s

[3] average distance to gravity center of path <0.35 m

[4] >1.4 serial visits per m swum to 12 annuli positioned at correct distance from wall

[5] usage of <50% of pool surface

[6] usage of >50% pool surface but no wall preference

[7] preference for 0.1 m wide wall zone

Fig. 2. Quantification of strategy choice during the acquisition and reversal phase of watermaze training. A1AR-/- mice showed a significantly higher frequency of thigmotaxis-dominated trials during the reversal phase. A weak, non insignificant trend in the same direction was already present during acquisition.

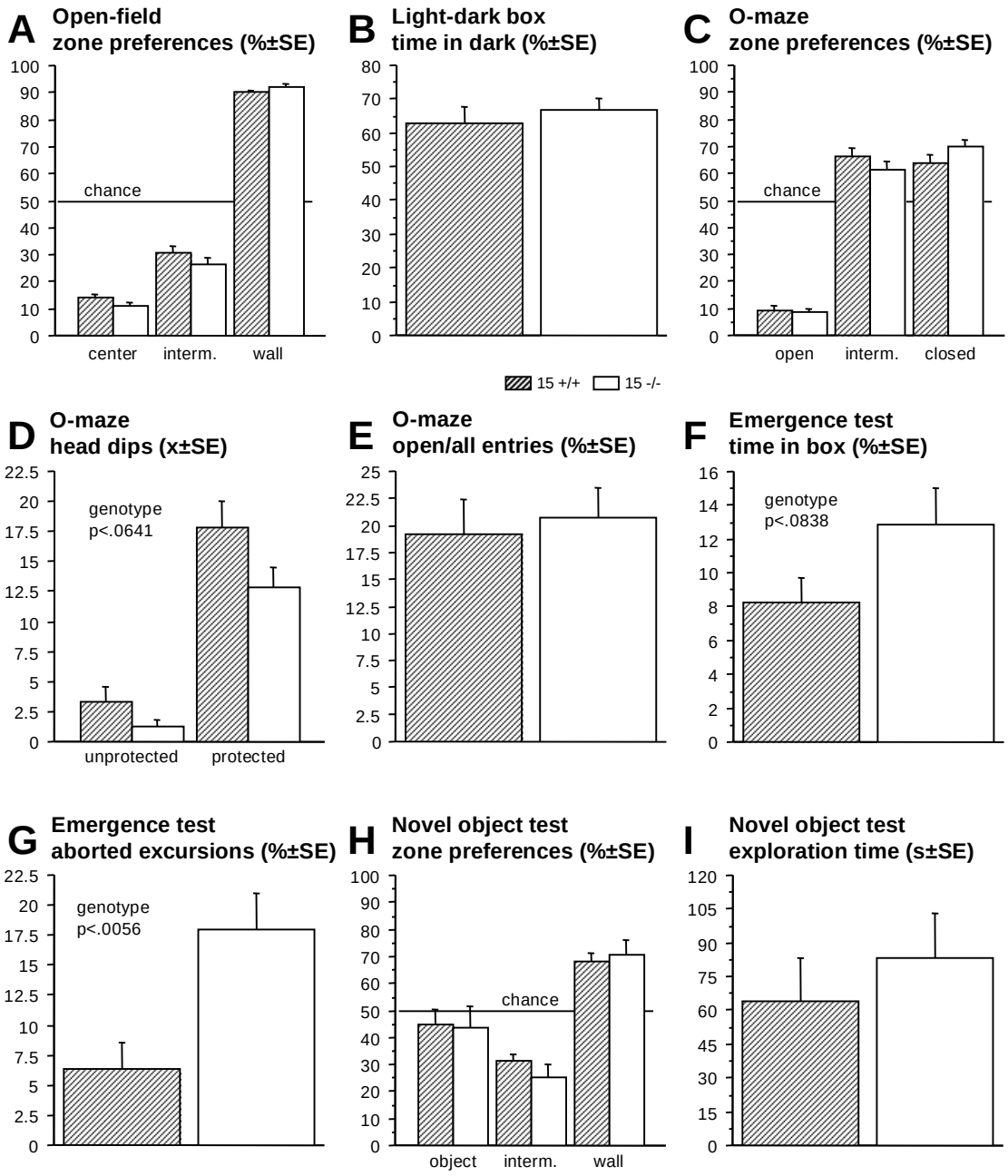


Fig. 3. Behavior of A1AR-/- in five exploration tests. Because time course of the measures shown was not affected by genotype, panels A-G show group averages + SE for the entire duration of each test. H and I represent the session during with the novel object was present. Preference scores shown in A, C, and H are corrected for zone size, bringing chance level to 50% independently of zone size (see Methods for details).

Discussion

In our study, the water maze testing showed no deficit of the adenosine A1 receptor knockout (A1AR-/-) mice in spatial memory. This finding is in agreement with similar observations of Gimenez-Llort et al. [15] who found no differences in place learning and working memory in adenosine A1 receptor knockout mice. The observations in the A1AR-/- mice thus confirm previous findings, which failed to demonstrate a role of endogenous adenosine in working memory processes [16]. However, our mutants showed mildly increased wall hugging from the very beginning, which seemed to exacerbate during reversal. This abnormal reaction to the test environment and in particular to the dislocation of the platform may be interpreted as evidence for emotional instability of A1AR-/- mice. Because this instability had only a mild effect on their behaviour during the early acquisition phase, A1AR-/- mice had the same chance to hit the platform as control mice and could learn normally according to their intact ability to process and remember spatial information. Thus, mild changes of emotional behaviour can occur in the watermaze without significantly affecting learning performance per se.

A1 agonists have been shown to exert anxiolytic like actions pointing to a role of the adenosine 1 receptor in the regulation of anxiety [11,18]. This predicts anxiogenic changes in mice with a genetic inactivation of the A1AR. A1AR-/- mice in our study did show behavioural changes in this direction, but they were modest. Despite extended analysis, we found only relatively minor differences between A1AR-/- and A1AR+/+ mice in anxiety related measures in forced (open-field, light-dark box, O-maze) as well as free (emergence test, novel object test) exploration paradigms. This result is at variance with the description of another adenosine A_1 receptor knockout mouse strain which has been reported to display a clear decrease in exploratory behaviour, higher latency to enter the light compartment in the dark light box experiments, and a higher preference for the enclosed arms of the elevated plus maze [15,19].

Several factors may contribute to the behavioural differences between the two lines of A1AR-/- mice. At the genetic and biochemical level, the two lines are equivalent, both carrying a constitutive mutation and lacking any detectable A1AR expression [30,19]. However, phenotypic effects of a knockout often depend on the genetic background of the mouse strain carrying the null mutation [29], and some confounding factors may be related to the 129 substrain genes flanking the target locus, which are present in the mutant animal and not in the corresponding wild type mice [14]. Both lines of A1AR-/-

mice were generated using 129-derived ES cell-lines and by subsequently mating male chimeras with C57BL/6 females. Despite this similar design, the two populations used for behavioural testing may not be entirely equivalent with respect to genetic background. First, the two ES-cell lines were not identical. Second, both lines were propagated by brother-sister mating for a few generations beyond the F2 generation, so genetic drift may have occurred. Thus, we cannot exclude that genetic background has contributed to the behavioural differences between the two lines of A1AR-/- mice. On the other hand, it has been estimated that only 20-40% of the total variation of anxiety-like behaviours in mice may be attributed to genetic mechanisms [3]. That environmental conditions strongly affect the expression of anxiety-like traits has been demonstrated recently in a comparative study [4] which analyzed the same set of mouse lines in three different labs under what appeared to be rigorously equated environmental conditions, but still found strong discrepancies between the labs. The A1AR-/- line presented here and the one analyzed by Gimenez-Llort et al. [15] were raised and housed under different conditions and behavioural testing procedures were not equated between the two labs. As a consequence, it is likely that the different environmental conditions have contributed substantially to the behavioural discrepancies between the two lines. Also, Gimenez-Llort et al. [15] tested only male mice, while our study included both males and females. However, we found no evidence for a gender-dependence of the mutation effect in our data. So, we would have obtained similar results by analyzing only males.

In conclusion, our study confirms that the A1AR, while dispensable for spatial learning, plays a role in the regulation of emotional behaviour. However, the behavioural changes observed in our experiments with A1AR-/- mice differed from previously published studies both with respect to the magnitude of the changes and the range of behavioural measures that were affected. Taken together, the studies demonstrate that it is often necessary to analyze a mutation under a range of different environmental (and genetic background) conditions in order to obtain a full picture of the resulting phenotypic changes, especially if emotional behaviours are affected.

Acknowledgements

This work was supported in part by the KFN, *Kommission zur Förderung von Nachwuchswissenschaftlerinnen* of the Free University of Berlin as well as the Swiss National Science Foundation, the Hartmann-Müller Foundation and the NCCR Neural Plasticity and Repair.

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