

The cognitive capacities of the dopamine D1 mutant rat

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Abstract

The importance of dopamine as a neurotransmitter has been studied extensively. However, the selective role of dopamine D1 receptor (D1) in cognition is yet to be clarified. The rat model with a D1 mutation offers an intriguing possibility which the pharmacological model can not provide due to the lack of selective D1 (ant)agonists.

In the present study, the mutant rats were tested with a battery of cognitive tests. Despite the absence of any gross impairment in locomotor activity, the animals showed deficits in most of the spatial memory tasks replicating the previous studies with D1/D5 antagonists. This impairment was most pronounced in cognitively more demanding tasks such as the response based (egocentric) Morris Water Maze test.

However, the results from non spatial memory tasks (object recognition, passive avoidance) revealed no major deficit in the D1 mutant rats. The mutant animals could discriminate novel object as well as the controls did. Moreover, they showed superior performance in passive avoidance test. It can be speculated that either the tests involve memory functions which are not D1 dependent or they failed to challenge the D1 related cognitive function strongly enough. This question can only be answered when more tests which require a higher level of cognitive capacities (such as set shifting) are performed along with molecular work, which will elucidate the possible compensatory mechanism underlying the D1 mutation in the current study.

Keywords: dopamine, DA, dopamine D1 receptor, D1, D1R, cognition, memory, rat, genetic, mutant.

Abbreviations: DA: dopamine; D1R: dopamine D1 receptor, NMDAR: N-methyl-D-aspartate receptor, PFC: prefrontal cortex, PPI: prepulse inhibition; LTP: Long term potentiation.

1. Introduction

Nearly three decades ago, Brozoski and his colleagues showed the importance of dopamine (DA) on cognition in their study with monkeys (Brozoski et al., 1979). The effect of DA depletion in prefrontal cortex (PFC) on working memory performance was as drastic as the complete removal of PFC region itself. Since then a large number of studies confirmed the importance of DA on working memory and cognition in general (Nieoullon, 2002; Meyer-Lindenberg et al., 2005; Collins et al., 2000). Moreover, it has been shown that dysregulation of dopaminergic function is closely associated with the cognitive impairment in several major brain disorders such as Parkinson's disease and schizophrenia (Knable and Weinberger, 1997; Koerts et al., 2007).

However, it has been difficult to elucidate how exactly DA influence cognitive performance. This is mainly due to the fact that DA has a complicated function and can not be defined as a mere excitatory or inhibitory neurotransmitter such as glutamate or GABA. DA works in a rather subtle way and is involved in modulating the working of other neurotransmitters through G protein coupled signaling cascades (Schoffeleer et al., 2000).

This complexity can be achieved since DA exerts its influence via five subtypes of receptors. Among them, the D1 receptor family (encompassing the D1R and D5R) seems to be the most important one in cognition. Firstly, they are the most abundant form of DA receptors in PFC whose activity is closely related to working memory load as revealed in a neuroimaging study (Okubo et al., 1997). Secondly, the blockade of D1 family and not D2 family (encompassing the D2R, D3R and D4R) impaired a memory guided task in primates including humans (Arnsten et al., 1994; Muller et al., 1998) and radial maze performance in rats (Tinsley et al., 2001). In line with this, D1 polymorphisms have been associated with executive function impairment in schizophrenics (Sawaguchi and Goldman-Rakic, 1994) and attention disorder in school age children (Misener et al., 2004).

One of the most important functions of the D1 family receptors in PFC is inhibition of irrelevant stimuli. Stimulation of dopaminergic neurons leads to activation in GABAergic interneurons (Zhou and Hablitz, 1999), which themselves exerts inhibitory action. D1 family receptor agonists reduce the cross talk between pyramidal neurons and limit the recurrence of activation which leads to the sharpening of information flow. On the other hand, they facilitate N-methyl-D-aspartate receptors and enhance excitatory post synaptic potentials in response to external stimuli and enable the converging of information from different areas in association cortex (Seamans et al., 2001). This close cooperation between the D1 receptor family and NMDARs is very crucial in synaptic plasticity. D1 receptor family signaling increases Ca^{2+} currents and make pyramidal cells more receptive to the inputs from NMDARs (Chen et al., 2004). However, when Ca^{2+} signaling becomes too intense, the D1 receptor family attenuates the Ca^{2+} level (Surmeier et al., 1995). By this modulation, these dopamine receptors can influence short and long term synaptic plasticity, gene expression and consequently neuroadaptation and memory.

When the D1 receptor family is compromised either by pharmacological interventions or genetic manipulation, it can be predicted that memory functions become impaired. Firstly, the signal to noise ratio would become lower because of the inefficient filtration of irrelevant information and lack of the facilitation of meaningful input. Secondly, the reduced capacity of synaptic plasticity will degenerate long term memory formation. Importantly, the sustained level of DA activity via the D1 receptor family in PFC is crucial in making mnemonic links

between external stimuli and rewards. Hence, when these receptors are dysfunctional, it becomes difficult to learn a task as well as to remember the solution. In addition, this impairment would not be limited to very short term processes such as working memory, but would most likely also include other forms of learning and memory.

By far, most of studies have used pharmacological manipulation to study the function of the D1 receptor family. However, as already mentioned above, the lack of selective (ant)agonist for the D1R or the D5R has hampered progress considerably. Genetic models have been suggested as an alternative. In a study done with D1 knockout mice (Granado et al., 2007), they found that the mice had significantly lower level of LTP. However, when a D1/D5 antagonist was applied, this did not reduce the LTP level further, suggesting that the role of D5 is not as crucial as D1 in LTP, which is important in memory and learning. Recently, researchers from the University of Nijmegen and the Hubrecht laboratory in Utrecht have developed a D1R mutant rat using the ENU mutagenesis technique (Smits et al., 2006). The mutant rats have a single point mutation in the third transmembrane domain of the D1Rs, exchanging a nonpolar isoleucine for a polar serine presumably rendering them dysfunctional, whereas the D5 receptor is intact. Several pilot studies done on the mutant rats indicate the compromised nature of the dopaminergic system. Prepulse inhibition (PPI) is often used to assess the sensory gating function in animals and humans. The mutant rats showed attenuated baseline PPI as well as a reduced response to dopamine agonists such as amphetamine and apomorphine (unpublished data). Importantly, the D1R mutant rats showed no behavioral response to the selective D1/D5 receptor antagonist SCH23390. Moreover, the normal increase in Arc and FGF-2 mRNA levels in the striatum after the administration of cocaine was completely absent (unpublished data). Taken together, the data imply that these animals are, at least in a functional sense, D1R knock out rats.

This mutant rat model offers us an intriguing possibility to elucidate the role of D1 receptors on cognition which pharmacological approaches could not yet offer. In addition, rats are more suitable for cognitive studies than mice due to their higher level of intelligence and therefore can be used to test various aspects of cognition with relative ease. Importantly, there is evidence that the D1/D2 balance show pronounced differences between rats and mice. Thus, whereas D1 agonists show weak inhibition and D2 agonists strong inhibition of PPI in rats (Geyer et al., 2001), the reverse seems to be the case in most mice strains (Ralph and Caine, 2005). Since D2 agonists also reduce PPI in humans (Braff et al., 2001) it seems that rats and humans are indeed more similar than mice and humans in this respect.

To assess cognition in more than one way is important to reveal the selective influence of D1 receptor function. It is well known that compensatory mechanisms occurring in mutant and knockout animals can have a strong influence on the performance which can occlude possible genotype effect. On the other hand it should be realised that humans with a point mutation in the genes which code for molecules in DA pathway have altered cognitive performance in spite of such compensatory mechanisms (Caldu et al., 2007; Misener et al., 2004).

In the present study, the animals were tested in spatial memory assessments (Morris Water Maze, object location recognition, spontaneous alteration) and non spatial models (object recognition, passive avoidance conditioning).

Although it can be easily hypothesized that the mutant animals will show deficit in most of the tasks considering the crucial role of D1 receptor on cognition, it was predicted that the extent of deficit will depend on the nature of the tasks.

Both the PFC and the hippocampus play an important role in spatial learning. It has been reported, however, that there exists a functional dissociation between them as well. While the hippocampus is more involved in spatial tasks with short or no delay, PFC lesioning led to the selective deficit in long term memory tasks or non spatial memory tasks such as behavioural flexibility measurement (Sloan et al., 2006). Therefore, we hypothesized that the mutant animals will show more severe deficit in the object discrimination task than in its spatial variation, since the PFC contains much more D1Rs than the hippocampus.

The data concerning the role of D1Rs in passive avoidance learning is largely inconsistent. Studies with pharmacological approaches mostly found impairment (Kabai et al., 2004; Ichihara et al., 1988) while a study in which D1 knockout mice were used showed an intact performance and even prolonged extinction period (El-Ghundi et al., 2001). Hence, no clear hypothesis was made here.

Further it is expected that the impairment would be more pronounced in cognitively more demanding tasks. For this purpose, next to the standard Morris Water Maze setup, the animals were tested in a modified version where the integration of information across trials and response based strategy become important and therefore relies on PFC function more heavily. Considering the role of D1Rs in PFC, we hypothesized that the animals would show more clear impairment in this modified test even if they could successfully learn the original task.

2. Methods

2.1. Animals and housing

The subjects were male and female Wistar rats at the age of two to three months. The animals were divided into 2 groups according to the date of birth. The first group consists of 8 homozygous animals and the same number of wild types. The gender was evenly distributed. Due to the small number of male rats born, the second group of animals consisted of females only. There were 9 homozygous animals in the group and 7 wild type controls. Both group underwent the locomotor activity test before other tests. After that, the first batch of animals was tested in spontaneous alteration, object recognition and Morris Maze (egocentric). The second group was assessed in object recognition (spatial change), Morris Maze (allocentric) and passive avoidance.

The tip of the tail of each animal was collected and was used for genotyping in Hubrecht lab in Utrecht before the experiment started. The genotyping was done once more with the samples taken from sacrificed animal after all the experiments were over. Although the genotypes of the animals were known to the experimenter, nearly all the measurements (with the exception of spontaneous alteration, which has little room for ambiguity) were done with automated systems, thereby minimizing the observer bias.

The animals were housed in groups of two or three in a standard Macrolon® cage (Type 2) with cage enrichments and had free access to water and food (standard chow pellets). They were handled for 3 minutes per day for 3 consecutive days. 24 hours before each experiment, the animals were singly housed and transferred to the experiment room at least 30 minutes before the experiment started. Since more than one experiment was done with the same group, the animals were housed again in groups after an experiment and the next test began at least after one week interval to minimize possible distress by the experiment itself and regrouping process. To avoid confounding effect of aversive tests, Morris Water Maze and passive

avoidance tests were done as the last test that the animals had to perform before being sacrificed.

Importantly, the animals were housed under a reversed day night schedule (light on at 9 pm and off at 9 pm) and all the experiments were done during the dark phase (between 10 am till 5 pm).

All experiments and use of animals were approved by the animal research committee of the State of Hamburg (permit 08/07) and every effort was made to minimize the discomfort of the subjects during the experiment.

2.2. Procedures

2.2.1. Locomotor activity

Before being assessed for their cognitive capacities, the animals were first tested for any gross motor impairment. The locomotor activity was measured within the automated activity cage developed by TSE (TSE, Germany). The size of each box was 45 * 45 cm, equipped with two rows of in total 32 infrared beams, which were evenly placed (space between beams: 15 mm) measuring both horizontal activity and rearing. Three different aspects of activity were analyzed: 1) activity ratio between centre area and peripheral area (the centre area was defined as the area between 13th and 20th beam in XY axis). 2) Horizontal activity 3) vertical activity (rearing). Activity was recorded when an infrared light beam was interrupted. Each animal was placed in the centre of the box and motor activity was recorded in the dark room for one hour. Four boxes were used for the experiments and the equipments were thoroughly cleaned with 10 percent ethanol solution before each use. At the end of each experiment, the number of droppings was recorded to measure anxiety level. All the measurements and analyses were done with the Labmaster® software developed by the same company.

2.2.2. Spontaneous alteration

Spontaneous alteration test is considered to measure spatial memory at a relatively basal level. The idea behind the test is that when an animal has explored one area (one arm), if given a choice next time, it will choose to explore a less familiar area (the other arm) and this alternating behaviour occurs when the animal remembers which arm it investigated previously. It has been reported in a few studies (Myhrer, 2003) that dopamine antagonist can impair this alternating behaviour. However, it is not clear if it is an indication of poor spatial memory or pronounced perseveration.

The animals were tested in T shaped maze (each of the three arms was 12 cm in width and 80 cm in length) with three guillotine doors blocking the start area and both arms respectively as described previously (Isseroff, 1980). First the animal was introduced in the start area for 20 seconds then all the doors were removed so the animal could make a choice between each arm. When the animal made a choice, the guillotine door blocked the access to the other arm and the animal returned to the start area (in case when this did not occur spontaneously, the animal was guided gently by hand to the start arm). The animal was confined again there for 20 seconds, before the doors were lifted. In total 7 trials were made and the total number of alterations was recorded. An alteration was scored when the animal did not choose the same arm consecutively. For instance, when the arm entry was left-right-right-left-left-right, the total alteration number was 3. After the trials were over with all animals, each animal was introduced again into the maze and allowed 10 minutes of free roaming and the total arm

entries was recorded to investigate if attenuated explorative behaviour in mutant rats could account for a possible biased arm choice in the test.

2.2.3. Morris Water Maze

Since designed by Richard Morris in 1984, the water maze paradigm has been extensively used to assess the spatial memory in rodents. Although rats are superb swimmers, they are very willing to escape the pool by locating a platform, which is normally hidden under the water surface. The escape latency decreases rapidly over time as the animal learns the relation between external cues provided and the location of the hidden platform. Not surprisingly, lesioning the hippocampal area where spatial memory is processed impairs the performance dramatically. Lesioning of the PFC, on the other hand, does not impair the performance in the traditional setup of the water maze (de Bruin et al., 2001). However, the animals with PFC lesions showed drastic deficit when response based strategy (when the platform as well as the starting location varied) was required. Despite the varying platform and starting positions, the relation between the two locations was kept constant. The platform was always located on the left side (for the half of the group) or right (the rest) side of the start location.

The water maze was a circular pool (diameter: 140 cm) filled with transparent lukewarm water (26° Celsius). A circular platform (diameter: 10 cm) made of transparent Plexiglas was placed 2 cm beneath the water surface. The animals were gently introduced into the maze from four different sides: North, East, South and West. The platform areas were also divided into four areas: Northeast, Southeast, Southwest, and Northwest. And the platform was placed in the centre of each quadrant. The centre area was defined as the inner circle surrounded by all the platform locations. The peripheral area was the outer circle (ca. 15 cm from the wall of the maze). Three intra maze cues (a plastic box (15x10cm), a paper towel cylinder (7x20cm), and a metal board (10x40cm) were placed on the edge of the maze. On the walls around the maze, extra maze cues were given as well; a plastic basin (diameter 40cm) and a golden coloured star-shaped origami (ca.50 cm width and height). The computer and other experimental equipment in the room possibly served as additional extra cues for the animals. All the cues including the equipments location in the room were kept constant during both allocentric and egocentric versions of the test. All the trials were recorded and tracked with Ethovision XT (Noldus, the Netherlands). Because tracking problems occurred frequently during the egocentric trials, only escape latency data analysis was possible for this test. Latency, swimming velocity and relative time spent in the central area of the maze could be analyzed in the allocentric experiment, however. After each trial, the animals were dried with towels and were returned to their home cages. There were two infrared lamps (100 W) next to the home cages so the animals could be kept warm. The water temperature was checked every one hour and the water was partially replaced to ensure a constant temperature. When the animal did not locate the platform within 3 minutes, the animal was gently guided by hand to the platform and was left there for additional 30 seconds before being retrieved to the home cage.

2.2.3.1. Allocentric test

The animals were released from four different sides while the platform was always kept at the Northeast position. The animals went through four trials per day for four days with inter-trial period of 2-3 minutes. After the third trial on the last training day, the animals were tested in the maze without the hidden platform (probe trial) and the relative time spent in the target quadrant was measured.

2.2.3.2. Egocentric test

During egocentric trials, the platform locations changed constantly as well, but in accord with the start location of the animals. Half of the animals were trained to locate the platform on the right side from the start location (e.g. When released from North, the platform was hidden in the location Southwest). The rest of the group were trained to find the platform on the left side from where they are released. (e.g. When released from North, the platform was hidden in the location Southeast). Due to the increased difficulty of this test compared to the allocentric version reported previously (de Bruin et al., 2001), the animals were trained four trials per day for five days (one day more than in allocentric test).

2.2.4. Object Recognition

This test exploits the natural inquisitiveness of rats. When given a choice, the animal will explore a less familiar object more than an already known item. It has been shown that the animals not only remember the objects but also the locations of each object as well. So when a familiar object was moved to a new location, it will spend more time in investigating the item.

To make the comparison between the two versions of this test easily, the experimental setups were kept constant here as in the case of Morris Water Maze. A circular shaped open field (diameter 80 cm) made of dark grey coloured plastic served as the arena of the test. Three intra maze cues were placed on the edge of the maze to offer spatial cues: a paper towel cylinder (7x20cm), a metal board (10x40cm) and a polygonal shaped Plexiglas plate (ca. 60cm). The objects used were tested in the pilot experiment to ensure there was no significant preference for one object. All objects were of similar size and were all painted with a blue spray paint in advance. The objects were; a glass flask with a plastic lid filled with crystal violet solution, a glass yogurt bottle with a metal lid with the same dark coloured liquid and a metal tin. To exclude the confounding effect of remaining odour, three identical copies of each object were prepared and they were all thoroughly cleaned with 70 % ethanol solution before and after each trial. The time the animal spent with each object was recorded by Ethovision XT (Noldus, the Netherlands). The interaction was defined as when the animal's snout was within 1 cm of each object. The discrimination index was calculated as the ratio between the exploration time difference of the novel object and the familiar object divided by the total exploration time.

2.2.4.1. Object Recognition (original)

The rat was introduced in the middle of the arena and was allowed to explore the area for five minutes (without objects) before being returned to the home cage. Two objects (a glass flask and a metal tin) were in the meanwhile placed in the middle of the arena 8 cm apart from each other. The animal was again placed in the arena, this time in the middle of the two objects. After two hours, the animals were again introduced to two objects. This time the metal tin was replaced with a glass yogurt bottle and the glass flask was substituted with a copy of it.

2.2.4.2. Object Recognition (spatial change)

The protocol was nearly the same as the original task. Instead of two different objects, however, the animals were given a chance to explore two same items (glass flasks). During the test trial, the two objects were replaced with identical copies. One object was kept at the

old location (in the centre), the other object was moved nearer to the edge of the arena (5 cm from the edge).

2.2.5. Passive Avoidance

This test is based on fear conditioning in rats. The test apparatus (TSE, Germany) has two separate chambers which are separated by an automated guillotine door. One chamber is brightly lit by a lamp above, and when an animal is placed there, it will escape to the adjacent dark room immediately. During the training session, first the animals were habituated for 30 seconds to the light chamber before the guillotine door opened. All animals nearly immediately escaped to the dark room. Since the escape latency mostly did not exceed five seconds, this latency was not included in the analysis. When the animal moved to the dark room, the guillotine door was closed automatically and the animal received a short duration of phasic electric shock (0.7 mA) for five seconds through the metal grid on the floor of the apparatus. To avoid fear conditioning with handling, the animal was retrieved 30 seconds after the shock was given. After 24 hours, the animals were placed again in the light room for 30 seconds before the door to the dark chamber was opened. The latency before the animal moved to the dark compartment was recorded.

After two hours (26 hrs after the conditioning), the animals were tested again. This time, however, the animal was placed in the dark compartment. It was reasoned that when a more intense fear conditioning was formed in association with the dark room, the animal would escape that compartment more quickly. The latency until the animal escaped to the light room was measured again.

2.3. Data Analysis

Since the main aim of the study was to reveal any genotype difference, one way ANOVA was used as the standard statistics. Due to the relative small size of the groups, the equal variances assumption violation was checked by Levin's homogeneity test, which revealed no significance. Gender*Genotype interaction was not investigated due to the small number of animals in each category. Data from Morris Water maze were analyzed by repeated measurement ANOVA to reveal the interaction between the trials and genotype, which in turn reflects the different learning curve between the groups. The homogeneity of variances was checked with Mauchly's Test of Sphericity here which turned out to be significant. The variance in homozygous group was larger than the one in the control animals. However, since only two groups were compared, this violation was not taken into account.

3. Results

3.1 Locomotor activity

Three different aspects of locomotor activity were assessed: total distance travelled, activity in the centre area (data not shown), and rearing activity (data now shown). There was no significant genotype or gender effect (Fig 1). However, there was a tendency that females showed more rearing behaviour than males ($F(1, 31) = 4.01, p = .055$) (Fig.2). The number of defecation did not differ significantly, either (data not shown).

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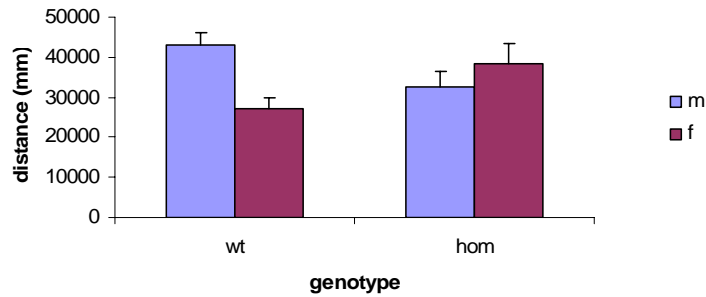


Fig 1: Distance covered during 1 hour of exploration in the open field. The horizontal activity in the open field was measured. There was no significant difference between the groups. (wt: wild type, hom: D1 homozygous, m: male, f: female)

3.2 Spontaneous alteration

There was no significant genotype or gender effect on the number of alterations each group made in the T maze (Fig.2). In addition, the groups did not differ in the number of arm entries during the 7 minutes of free exploration in the maze in a separate protocol.

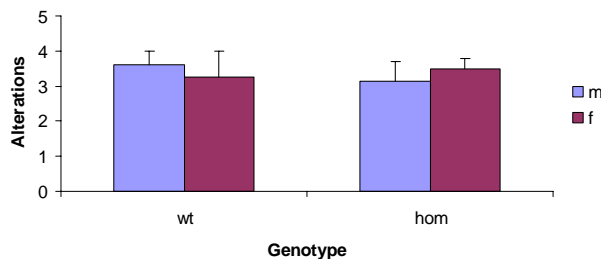


Fig 2: Number of alterations in T maze during 7 trials. There was no significant difference between the groups. (wt: wild type, hom: D1 homozygous, m: male, f: female)

3.3 Morris Water Maze (allocentric)

The ANOVA revealed a genotype main effect ($F(1, 15) = 6.94, p < 0.01$). The wild type animals located the platform more quickly than the D1 mutant rats (Fig.3). There was no interaction between genotype and trials, however. The wild types reached the escape latency of approximately 20 seconds at the end of the second day and maintained this throughout the rest of trials. Additionally, there was a significant difference between the two groups in velocity and the relative time spent in the central area of the maze during the training. ($F(1, 15) = 26.57, p < .01$, $F(1, 15) = 15.67, p < .01$ respectively). The mutant animals swam more slowly and spent more time in the peripheral area than in the centre (Fig. 4). However, the escape latency decreased steadily in the mutant group as well so that in the last trial, the latency did not differ significantly between the groups. More importantly, in the probe trial after the final training, both groups spent approximately equal amount of time in the target area (data not shown).

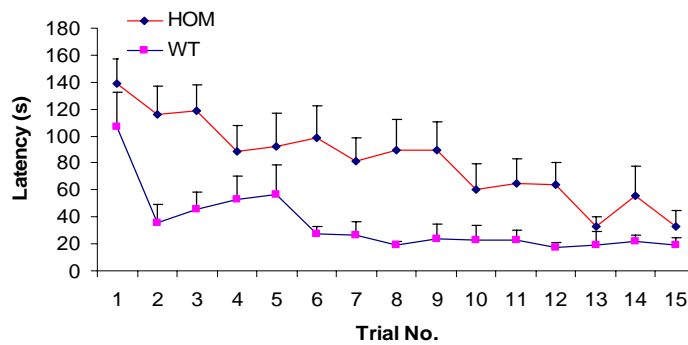


Fig 3: Escape latency in the Morris water maze (allocentric)

The wild types escaped the maze more quickly than the homozygous animals. There was no overall significant interaction between trials and genotype, however. (wt: wild type, hom: D1 homozygous)

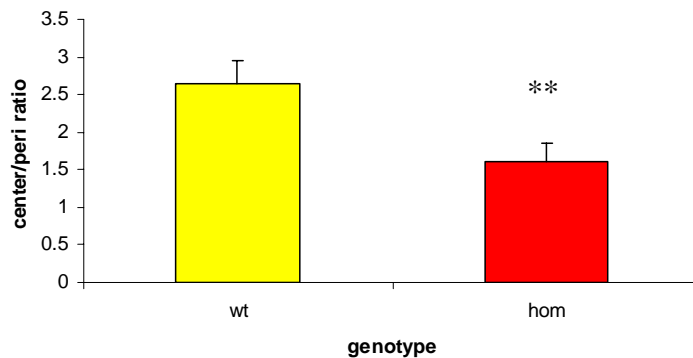


Fig 4: The ratio between the time spent in the centre area and peripheral area.

The D1 mutants spent more time in the edge of the maze rather than exploring the possible platform locations in the centre. ** $p < 0.01$ (wt: wild type, hom: D1 homozygous)

3.4 Morris Water Maze (egocentric)

There was a significant genotype main effect. ($F(1, 15) = 11.69, p < .001$). There was no significant gender effect. The wild types located the platform more quickly than the other group (Fig.5). More importantly, an interaction was found between genotype and trials ($F(19, 301) = 2.63, p < .001$). While there was virtually no trial effect in the homozygous group, the escape latency in the other group steadily decreased as seen by the trend lines in Fig.6. Due to the frequent tracking problems, velocity and other variables could not be calculated. However, the animals showed similar pattern of swimming as seen in the allocentric version of the water maze. The wild types swam more often across the platform and by doing so came across with the possible platform locations (Fig.6). In contrast to this, the homozygous animals mostly swam along the edges of the pool and thereby missing the chance to locate the platform.

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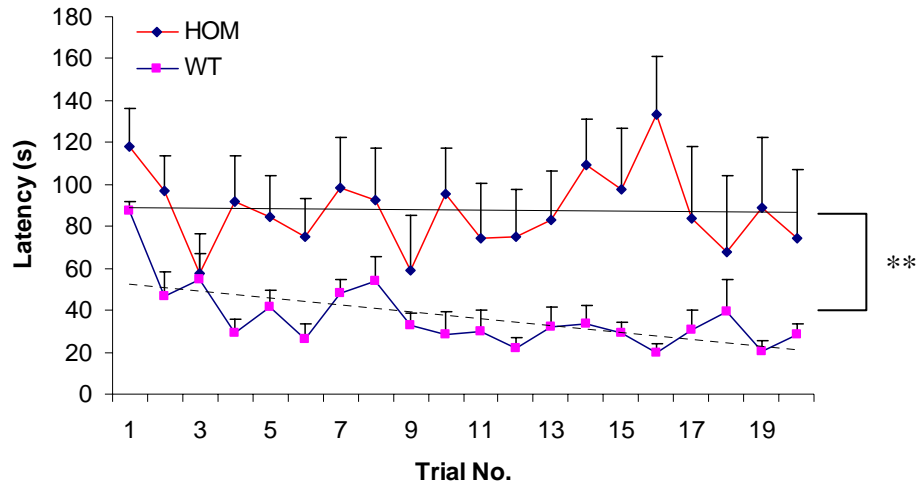


Fig 5: Morris water maze (egocentric)

There was a main effect of genotype on escape latency. The trendline shows that there was virtually no task learning in the mutant rats. ** $p < 0.01$ (wt: wild type, hom: D1 homozygous)

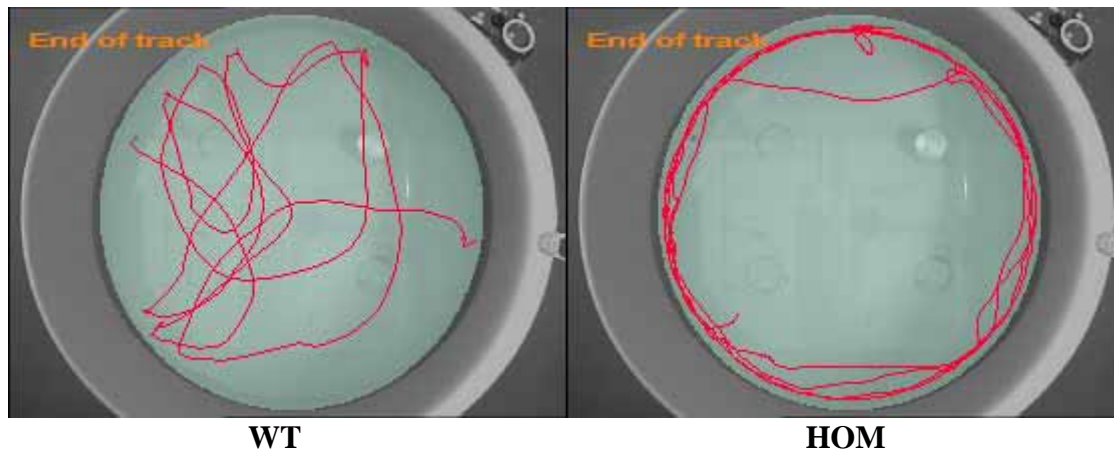


Fig 6: Captured images from Ethovision® video tracking during the second day of training.

While the wild types explored the central area (possible platform locations), the mutant rats swam more often along the edge of the pool. (wt: wild type, hom: D1 homozygous)

3.5 Object Recognition

There was no significant difference between the groups regarding the novel object discrimination. Although females performed slightly better, the difference did not reach significance (Fig 7).

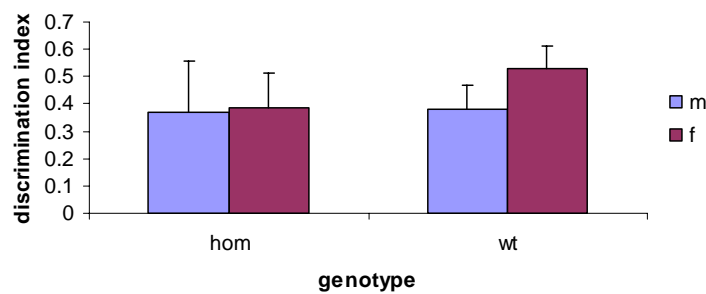


Fig 7: Object recognition.

The discrimination index was calculated as the ratio between the exploration time difference of the novel object and the familiar object divided by the total exploration time. There was no significant main effect of genotype or gender. (wt: wild type, hom: D1 homozygous, m: male, f: female)

3.6 Object Recognition (spatial change)

The wild types showed significantly better performance than the mutant rats ($F(1, 15) = 6.67$, $p < .05$). They spent more time in exploring the familiar object in a novel location thereby showing a better memory for location change (Fig.8).

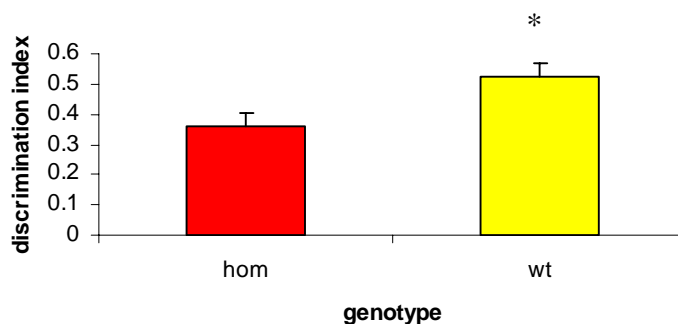


Fig 8: Object recognition (spatial change)

The discrimination index was calculated in the same way as in Fig 7. The novel object was substituted as the familiar object in a novel location in this version. There was a significant genotype main effect. The wild types significantly spent more time with the object at a novel location. * $p < .05$ (wt: wild type, hom: D1 homozygous)

In both object recognition tests, the total distance which animals moved in the arena did not differ significantly between the groups (data not shown).

3.7 Passive Avoidance

There was a significant genotype difference both in latency and reversal latency ($F(1, 15) = 8.59$, $p < 0.01$) (Fig 8). The mutant rats stayed in the bright chamber longer than the controls before entering the dark room after conditioning. However, there was no significant difference when the animals were placed in the dark chamber and the escape latency was measured at the second test trial which took place after 2 hour after the first test trial

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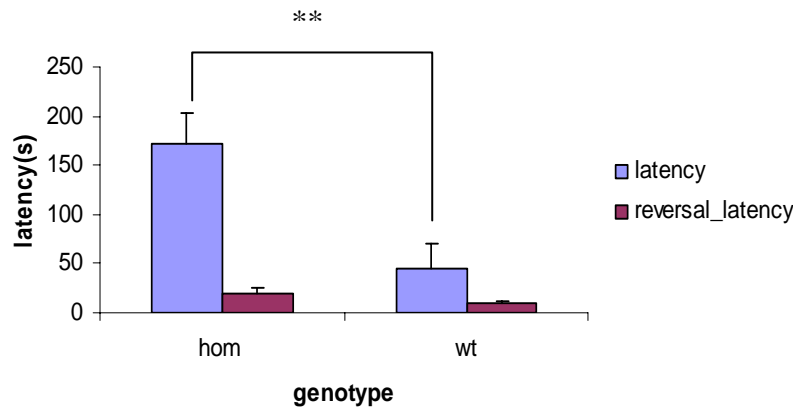


Fig 8: Passive avoidance

The animals were given electric foot shock when they entered the dark chamber from the light one. After 24 hours, the animals were placed again in the light chamber and the time spent there (latency) before entering the dark room was recorded. The homozygous group showed higher level of latency. After the test session, the animals were then placed in the dark chamber, and the escape time was again recorded (reversal latency), there was no significant difference between the groups here. $**p < 0.01$ (wt: wild type, hom: D1 homozygous)

4. Discussion

The results above show that the experiments can be categorised in three different sets: [I] Tests in which the mutant group showed inferior performance (Morris water maze in both versions, Object recognition with spatial change); [II] tests in which the groups did not differ significantly (Locomotor activity, spontaneous alteration, object recognition) and [III] one test where the mutant group performed better than controls (Passive avoidance).

Preserved functions

In a sense, it is not very surprising that the mutant rats performed normally in a several tests. Although the genetic approach is often compared to the pharmacological manipulation to draw a conclusion concerning the role of a specific receptor, they are different from each other in some aspects. Blocking a receptor by an antagonist often leads to more acute and drastic change in animals than genetic manipulation since it hardly involves any compensatory mechanism. The locomotor dysfunction observed after applying D1 antagonist in several studies (Chipkin et al., 1988; Iorio et al., 1983) was absent in D1 knockout mice (Smith et al., 1998). The reason might be the longer period in which the genetically manipulated animals can compensate for the functional loss of the receptor. In the case of ENU mutagenesis, the consequences can be even more subtle since it often involves just a single base change. Although the results of immunoassay strongly suggests that the mutation occurred in the D1 rats led to a loss in binding potential of the receptor, still it is a matter of speculation since it is possible that dopamine itself has a different affinity for D1 receptor than the compound used in the assay. On the other hand, considering there was virtually no early gene expression after cocaine administration (which acts by increasing the extracellular levels of dopamine) in the mutant rats, it is likely that dopamine also do not bind to the D1 receptor. Rather it seems more likely that compensatory mechanisms (for instance in the D2 receptors) may have reduced the possible impact of the mutation on motor behaviour.

In addition to the normal behaviour in the open field boxes the D1 mutant showed a non-significantly trend towards a decrease in rearing compared to the controls. Distance analysis data from other test (e.g. object recognition) also suggests that the locomotor dysfunction was not different. Also when allowed 7 minutes of free roaming in the T maze which was used in

the spontaneous alteration test, the total arm entries did not differ from each other (data not shown). Hence, it seems likely that the observed differences in the learning and memory tasks are not due to differences in motor performance, but rather reflect genuine cognitive deficits.

Spatial memory deficit

As mentioned in the introduction, the role of D1 receptor in cognition, especially spatial memory has been reported in many studies. D1 KO mice were impaired in the standard Morris water maze task (Smith et al., 1998; El-Ghundi et al., 1999) and D1 blockade by SCH23390 led to a similar deficit (O'Carroll et al., 2006).

The first spatial test in the present study was the spontaneous alteration which is supposed to assess spatial memory at a very basal level. The reason that the homozygous group did not differ from the control might be that the task was not demanding enough to challenge D1 function suitably. Although cortical D1Rs have a crucial role in maintaining information 'online', the deficit by D1 blockade is more drastic when retrieval and integration of information becomes necessary as well. In a study by Seamans and his colleagues (Seamans et al., 1998), the animals given D1 antagonist showed unimpaired performance in a foraging task when the task did not require long term memory, while a deficit was observed when a delay of 30 minutes was introduced. Although 20 seconds of delay was introduced before each new trial in the current study, maybe a longer delay or a maze with more arms is necessary to challenge the cortical – hippocampus circuit sufficiently.

The spatial memory deficit was strongly present in both versions of Morris water maze. In the allocentric test, the mutant rats eventually learned the task, but it took them nearly all 4 days of training, while the wild types learned the task by the end of second day. Poor swimming skill as suggested by the significantly slow velocity can be a reason for this. However, the velocity did not differ significantly during the first trials. A more likely explanation is the use of different swimming strategies. The mutant rats spent more time around the perimeter of the pool thereby missing the chance to accidentally bump into the platform. They therefore stayed in the pool longer, which may have influenced their motivation and physical condition which subsequently could have led to the reduction in swim speed.

As predicted, the animals showed more severe deficit in the egocentric version of the task. While the mutant animals managed to learn the allocentric task, there was no significant trial main effect in the egocentric test. Both versions of water maze require reference memory as well as working memory (Baldi et al., 2005). However, integration of spatial memory with other aspects of cognition becomes more crucial in the egocentric version for several reasons. Firstly, the animals have to 'inhibit' their natural tendency to rely on visual cues during the task. Normally animals rely both on the external and internal cues and this need of active suppression one strategy might have taxed the PFC function more strongly (Ridderinkhof et al., 2004). Secondly, the response based strategy (egocentric) requires information across the trials much more so than in the allocentric test. It has been reported that a lesion in PFC leads to a deficit in the response based strategy but not in the place learning task (allocentric) (de Bruin et al., 2001). Thirdly, to solve the task successfully, the animal should keep the starting position 'online' during the trial whereas it is possible to refer to the external cues during the trial constantly in the allocentric version. This feature of working memory-keeping the necessary information in workspace- is often attributed to the one of most important functions of PFC (De Pisapia et al., 2007; Rypma et al., 2002).

The results from object recognition test were not in line with the hypothesis, however. The impairment was present in the spatial change test rather than in the original version of the task. Considering the dissociation between spatial memory and non spatial memory in relation to D1Rs function reported in some studies (Dere et al., 2007), this result is rather puzzling.

First, it can be speculated that the original object recognition test did not challenge the cognitive features sufficiently as in the case of spontaneous alteration. In some studies, the effect of D1 blockade or stimulation was more clearly manifested after a longer period of the delay (Hotte et al., 2005). Moreover, with both tasks, the discrimination index was rather high (around 0.4 to 0.5) indicating that all animals showed excellent recognition. It may therefore be worthwhile to investigate longer delays as well. On the other hand, a few studies showed that D1R blocking leads to deficits more in spatial memory tasks than other types of tasks (Floresco and Phillips, 2001). In one neuroimaging study, D1/D2 agonist increased PFC activity during a spatial memory task and decreased it during the phase which required object memory (Gibbs and D'Esposito, 2006).

It is possible that despite the extra maze cues during the spatial change discrimination task the spatial change has been quite subtle hence more difficult to memorize. In the studies where both versions have been tried, the discrimination index was lower in the spatial change discrimination task, reflecting the accentuated difficulty of the task (Ennaceur et al., 1997; Dere et al., 2007). It should be noted, however, that the index did not differ to a great extent in the current study across the two conditions.

Intact emotional memory

The result from passive avoidance test is dramatically at odds with the result of other experiments in the current study. Considering that the test is often used to test cognitive functions along with other measurements, it is surprising that the mutant group actually seemed to show better performance.

However, it can not be excluded as a possibility that the superior performance does not actually reflect stronger fear conditioning or better memory in the mutant rats. If the fear conditioning was accentuated in D1 rats, the animals should have escaped the dark chamber more promptly than control animals, since they should associate the dark chamber with the shock more strongly. However, this did not occur. If anything, the homozygous rats stayed in the dark room longer than the control, though the difference was not significant. It is possible that the animals just could not move as fast as the controls. However, since the mutant rats did not show any motor dysfunction in other tests, this is rather unlikely.

One possible explanation might be, that D1 mutant suffer from 'mental slowness' (in humans often referred to as psychomotor retardation). Although it could not be quantified, the mutant rats showed a few seconds of immobility in the beginning of nearly every test. When released into the water maze, for instance, the animal floated for a few seconds before starting to swim vigorously. When placed in the passive avoidance box, the animal showed a kind of 'freezing' behaviour for 2 to 3 seconds before exploring the area. Since this occurred even before any foot shock was given, it is unlikely that this freezing behaviour is due to fear conditioning. In addition, the two groups did not differ when the number of defecation was measured in the locomotor activity test implying that their anxiety level was not drastically different from each other. In a study with D1 knockout mice, the animals showed a decreased level of locomotor activity in the beginning and the activity level increased over time so the groups did not differ significantly in the end (Smith et al., 1998). It might be interesting to use the

active avoidance set up to test this possibility. If the prolonged latency in the passive avoidance test was due to the 'mental slowness', the animals will show inferior performance in the active avoidance test since it requires a very quickly selected response. This slowness is reminiscent of psychomotor retardation in patients suffering from Parkinson's disease or schizophrenia, whose cognitive deficit is often related to reduced dopaminergic activity in the frontostriatal pathway. Therefore, it sounds plausible that the compromised D1R function evoked a similar state in the mutant animals

Although it is difficult to explain this 'mental slowness', several possibilities can be mentioned. Considering the role of the basal ganglia (where D1 receptors are abundant) it might be that the D1 animals have a difficulty in choosing appropriate alternative among many (Ragozzino, 2002) hence slower at responding to the environment.

Alternatively, the animals might be inflexible to the change of the environment. All the animals from each group were exposed to the dark chamber eventually without electric shock during the test trial. The higher reversal latency therefore can reflect the ineffective and inflexible strategy of the mutant rats. In line with this possibility, a passive avoidance study with D1 knockout mice showed that the animals have a difficulty in forgetting the painful stimuli. The freezing behaviour and lengthened latency perseverated even after 90 days of the single conditioning session (El-Ghundi et al., 2001). The role of D1Rs in flexibility has been reported in many studies where set shifting paradigms were used. It will be interesting to test the hypothesis by testing the animals in attentional set shifting tasks such as the rodent version of Wisconsin Card Sorting Test (Joel et al., 1997).

The last alternative explanation is that the emotional memory is acquired and consolidated via D1 independent pathway. Firstly, fear conditioning activates different brain areas than other memory tasks. It can be speculated that fear related memory relies more heavily on the function of other neurotransmitters or other DA receptors whose function might have been sensitized or strengthened due to the dysfunctional D1Rs in the mutant rats. Floresco and Magyar suggested in their extensive review paper that although D1R plays an important role in working memory in general, the involvement of D2R and D4R is more pronounced in aversive learning tasks (Floresco and Magyar, 2006).

Limitations and Future studies

Taken all the data together, it can be concluded that there was no gross impairment in the D1 rats regarding locomotor activity and explorative behaviour. Even in the cognitive tests, the animals did not reveal severe deficit. However, the deficits became more pronounced when the task became cognitively more demanding as in the case of Morris Maze water maze egocentric version or object discrimination test with a spatial memory component.

However as revealed in the passive avoidance test, it was at times not clear which mechanism is underlying the impaired or enhanced performance in the mutant rats. The results of the current paper could, therefore, be interpreted with more robustness if more tests could have been done.

For instance, the battery of tests used in the present study includes a few spatial memory tasks but the number of measurements which assessed non spatial memory was very limited. To assess the role of D1 in cognition to a full extent, it will be necessary to assess their cognitive ability with other tasks, especially those challenging non spatial memory. In addition, the

measurements used here mostly either involves negative incentive (electric shock, being in the water) or no reward component at all. Since D1 plays an important role not only in cognition but also in motivation, it will be interesting to conduct a memory task where positive reinforcement plays an important role (e.g. radial maze). Most importantly, if it is true that the cognitive deficits in the mutant rats can be revealed better by more challenging tasks, it will be beneficial to test the animals in a more complex test which challenges attention, memory, and set shifting flexibility together. The difficulty of tests can be manipulated also by introducing more than one level of delays or including a switching component in the temporal order of the tests. For instance, one might switch the chamber where electric shock is given after they learned the task and repeat the training and test to see if they can unlearn the previous association. D1 animals might show inferior performance here due to the switching part.

In addition to behavioural studies, additional research is also necessary to investigate the precise nature of the D1 deficit. In principle there are several different possibilities. First of all, dopamine might not bind to the D1 receptor, due to the point mutation. Autoradiography studies using [³H]SCH23390 seem to support this possibility, though an antagonist and the endogenous ligand do not necessarily bind to the same binding pocket. A binding studies using [³H]dopamine in the presence of a D2 antagonist to mask the binding for this receptor, could be performed to investigate this. A second possibility is that the D1 receptor is not properly coupled to its second messenger system. In most cases, the D1 receptor is positively linked to adenylate cyclase through a G_s protein. If this is true, one would expect a reduction in the production of cAMP after dopamine (agonist) administration. Although this has not been investigated in the rat brain, some preliminary experiments *in vitro*, using cells that express the mutated D1 receptor showed that dopamine can induce an increase in cAMP production (unpublished data). Another possibility is that due to the point mutation, the receptor has lost (some of) its potential to dimerize. In this respect, it is interesting to note that a recent paper showed that D1 and D2 receptor can dimerize, thereby producing a new secondary messenger pathway, since the D1-D2 dimer is coupled to G_q/11 (Rashid et al., 2007), leading to phospholipase C activation and an increase in intracellular Ca²⁺. Additional research will be needed to investigate whether the D1 mutant rats also have deficits in this pathway.

The different results obtained in the present study compared to the pharmacological approaches strongly suggest that there is a complicated compensatory mechanism ongoing in the mutant animals. Although this is criticized as a main caveat in genetic animal models, it should be noted at the same time that this offers an important insight into the possible treatment of modelled disorders. Indeed, Zambrowicz and Sands argued in their paper that the effects of the 100 best selling pharmacological agents in the market could have been predicted from the relevant genetic animal models (Zambrowicz and Sands, 2003).

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