

Research report

Cognitive decline, neuromotor and behavioural disturbances in a mouse model for fragile-X-associated tremor/ataxia syndrome (FXTAS)

Debby Van Dam^a, Vanessa Errijgers^b, R. Frank Kooy^b, Rob Willemsen^c,
Edwin Mientjes^c, Ben A. Oostra^c, Peter Paul De Deyn^{a,d,*}

^a Laboratory of Neurochemistry and Behaviour, Institute Born-Bunge, Department of Biomedical Sciences, University of Antwerp, Universiteitsplein 1, B-2610 Wilrijk, Belgium

^b Department of Medical Genetics, University of Antwerp, Universiteitsplein 1, B-2610 Wilrijk, Belgium

^c Department of Clinical Genetics, Erasmus MC, Dr. Molewaterplein 50, 3015 GE Rotterdam, The Netherlands

^d Department of Neurology/Memory Clinic, Middelheim Hospital, ZINA, Lindendreef 1, B-2020 Antwerp, Belgium

Received 13 January 2005; received in revised form 14 March 2005; accepted 18 March 2005

Available online 3 May 2005

Abstract

Carriers of premutation alleles (55–200 CGG repeats) of the fragile X mental retardation 1 (*FMRI*) gene are spared the major neurodevelopmental symptomatology of fragile X syndrome patients carrying a full mutation (>200 repeats). In a proportion of premutation carriers, the repeat expansion is associated with a specific neurological profile involving intention tremor, ataxia, intellectual decline compatible with dementia syndrome, Parkinsonism and autonomic dysfunction at older age, commonly referred to as fragile-X-associated tremor/ataxia syndrome (FXTAS). Typical CNS changes include hyperintense signals on T2 weighted magnetic resonance images and the presence of ubiquitin-positive intranuclear neuronal inclusions. A knock-in mouse model with a (CGG)₉₈ repeat in the premutation range has been generated and shown to exhibit elevated *Fmr1* mRNA levels and ubiquitin-positive intranuclear neuronal inclusions, suggesting it may be a valid model for the human disease. Given the specific clinical profile of FXTAS patients, the expanded CGG repeat model was assessed for cognitive, behavioural and neuromotor performance at different ages (20, 52 and 72 weeks). The Morris water maze task exposed age-dependent decline of visual-spatial memory. Open field recordings revealed decreased exploration of the centre of the arena in the oldest group of expanded CGG repeat mice, potentially reflecting increased anxiety. Neuromotor tasks primarily showed decline of performance on the accelerating rotarod with age in the premutation carriers but not in control littermates. The age-dependent cognitive decline and neuromotor disturbances may be related to the progressive cognitive and behavioural difficulties observed in FXTAS patients.

© 2005 Elsevier B.V. All rights reserved.

Keywords: CGG repeat; Premutation; Morris water maze; Neuromotor performance; Anxiety

1. Introduction

Fragile X syndrome, the most frequent form of hereditary mental retardation, is mostly caused by an expansion of a CGG repeat in the 5' untranslated region of the *FMRI* gene [17]. Whereas, in the normal population, the CGG repeat is polymorphic with a length ranging from 5 to 55 units, fragile X patients display >200 hypermethylated CGG units (i.e.

full mutation) causing transcriptional silencing of the *FMRI* gene and consequently lack the fragile X mental retardation protein (FMRP) [13]. Individuals with the fragile X premutation have expanded repeat lengths varying from 55 to 200 CGG units. While not affected with the fragile X syndrome, females carrying a premutation may transmit the repeat unstably and have affected offspring. Dependent on the length of the premutation, elevated levels of *FMRI* RNA and at the same time slightly decreased levels of protein are synthesised [9,10,15]. Recently, it appeared that a subgroup of premutation carriers in their 50s and older are confronted with a variety of neurological symptoms, referred to as fragile-

* Corresponding author. Tel.: +32 3 820 26 20; fax: +32 3 820 26 18.

E-mail addresses: peter.dedeyn@ua.ac.be, dedeyn@skynet.be (P.P. De Deyn).

X-associated tremor/ataxia syndrome (FXTAS) [7–9]. Patients may develop progressive intention tremor and ataxia often accompanied by gradually increasing cognitive and behavioural complications including memory loss, executive function deficits, dementia, anxiety and reclusive or irritable behaviour. Additionally, more variable features may include Parkinsonism, peripheral neuropathy, lower limb proximal weakness and autonomic dysfunction (impotence, urinary and bowel incontinence).

Typical FXTAS-related CNS alterations consist of hyperintensity on T2 weighted magnetic resonance images in cerebellar white matter and middle cerebellar peduncles [2]. Post mortem neuropathological evaluation revealed ubiquitin-positive intranuclear inclusions in neurons and astrocytes throughout cortex and brainstem, with highest density in hippocampus, and Purkinje cell dropout with associated gliosis (Bergman gliosis) [6].

A premutation CGG repeat knock-in mouse model was generated, which showed CGG repeat instability [1]. These mice were consequently employed as a model for FXTAS. In analogy with human patients, ubiquitin-positive intranuclear inclusions were detected in the brains of expanded CGG repeat mice [19]. The subcellular localization of these inclusions was comparable with those of human patients, though dissimilarities in affected brain regions and cell types were observed. In mice, inclusions were restricted to neurons and absent in astrocytes. Inclusions were most abundant in specific brain regions, including colliculus inferior, pontine nucleus, parafascicular thalamic nucleus, 10th cerebellar lobule and vestibular nucleus, but contrastingly, inclusions were barely detectable in the hippocampus. Inclusions increased in size and number with ageing of the expanded CGG repeat mice. Cell loss and gliosis, however, were not observed irrespective of the ages examined.

The premutation animals showed no obvious signs of disease or abnormal behaviour. To examine whether mice with an expanded CGG repeat showed evidence of neurological dysfunction, expanded CGG repeat and wild-type (WT) animals were subjected to an elaborate cognitive, behavioural and neuromotor assessment at different ages.

2. Materials and methods

2.1. Mouse model

The generation of the expanded CGG repeat knock-in mice was described previously [1]. Briefly, the endogenous mouse (CGG)₈ repeat was replaced by a human (CGG)₉₈ repeat, which is in the premutation range. Repeats were mildly unstable during intergenerational transmission. The mice used for this study were of a mixed C57BL/6J × FVB/N background, and the repeats were between 106 and 123 CGG units in size. Animals were housed in mixed genotype groups in standard mouse cages under conventional laboratory conditions with food and water available ad libitum, constant room temperature and humidity, and a 12/12 h light–dark cycle. Behavioural testing was performed on separate groups of naïve male

expanded CGG repeat mice and male WT littermates at the ages of 20 ($n = 10$ for expanded CGG repeat, and $n = 5$ for WT mice) and 52 weeks ($n = 9$ for expanded CGG repeat, and $n = 6$ for WT mice). The latter group was re-assessed for the non-cognitive tasks and subjected to two additional neuromotor tasks and recording of cage activity profiles at the age of 72 weeks. Experimenters were blinded as to the genetic status of the animals. All experiments were carried out in compliance to the European Communities Council Directive (86/609/EEC) and the Animal Ethics Committee of the University of Antwerp approved all protocols.

2.2. Cognitive performance assessment

2.2.1. Hidden-platform Morris water maze test

The experimental set-up for the Morris water maze (MWM) task consisted of a circular pool (diameter: 150 cm, height: 30 cm) filled with opacified water kept at 25 °C. A round Perspex platform (diameter 15 cm) was placed 1 cm below water surface at a fixed position in one of the quadrants. Acquisition training consisted of eight trial blocks of four daily trials commencing at four different positions from the border of the maze in a semi-random order and with a 15 min intertrial interval. If the platform was not reached within 120 s, the mouse was placed on the platform during 15 s before being returned to its home cage. Swimming trajectories were recorded using a computerized video-tracking system (Chromotrack, San Diego Instruments, USA). Four days after finishing the acquisition phase, a probe or retention trial was performed; the platform was removed from the maze, and animals were allowed to swim freely for 100 s. Spatial accuracy was expressed as the percentage of time spent in each quadrant of the MWM, and the number of crossings through the previous platform position.

2.2.2. Passive avoidance learning

Passive avoidance learning was assessed in a step-through box during the dark phase of the animal's activity cycle. The step-through box consisted of a first, brightly lit compartment, connected with the second, dark compartment by means of a sliding door. A mouse was put in the illuminated compartment and after 5 s the sliding door was opened. Upon complete entry into the dark compartment (four-paw criterion), the animal received a slight foot shock (0.3 mA, 1 s). Exactly 24 h later, the escape latency to re-enter the dark compartment was timed up to 300 s, and the percentage of animals not reaching this criterion was compared between experimental groups.

2.3. Assessment of exploration and activity

2.3.1. Open field activity

Open field behaviour was recorded in a brightly lit 50 cm × 50 cm arena during the dark phase of the animal's activity cycle. Mice always started from the same corner of the arena and were allowed 1 min of adaptation before the 10 min recording period commenced. A computerized video-tracking system (Chromotrack, San Diego Instruments, USA) was used to record trajectories and calculate path length and number of entries in the centre circle (diameter 25 cm) or the 7 cm × 7 cm corners of the arena.

2.3.2. Cage activity

After 1 h of adaptation in the laboratory room where experiments were to be conducted, ambulatory cage activity was measured in solitary housed animals using standard transparent mouse

cages (22.5 cm × 16.7 cm × 14 cm; length × width × height). Methods and interpretation of activity profiles were previously described by our group [18]. Cages were placed between three infrared sensors (two perpendicular to and one parallel with the length of the cage) in closed cabinets accommodated with electricity-driven ventilation fans to keep optimum temperature, and lights to imitate the animals' 12/12 h light–dark cycle. The number of beam interruptions in a 23 h period was counted using a microprocessor counter interfaced with a computer. Cage activity recordings were carried out in the 72-week-old group.

2.4. Neuromotor assessment

2.4.1. Accelerating rotarod

Equilibrium and motor co-ordination were tested on an accelerating rotarod apparatus (Ugo Basile, Italy). After two adaptation trials of a maximum of 2 min each at a constant speed (4 rpm), each mouse was placed on the rotating rod for four test trials during which the rotation speed gradually increased from 4 to 40 rpm (intertrial interval: 1 min). The time an animal could stay on the rod was timed up to a maximum of 5 min.

2.4.2. Wire suspension test

For the wire suspension test of grip strength and endurance, the front paws of the mouse were positioned on a horizontal steel wire (0.6 mm thick) suspended at a height of 46 cm above tabletop. Test parameters were latency to the first fall and the number of falls during the 2 min assessment period.

2.4.3. Stationary beam test

The test setting consisted of a wooden beam (diameter: 25 mm, length 110 cm) covered with a layer of masking tape to provide a firmer grip. The beam was divided into 11 segments and placed at a height of 38 cm above a cushioned floor. A piece of cardboard was inserted at each end to prevent the mice from escaping. Testing commenced by placing an animal in the middle of the beam. The number of segments crossed (four-paw criterion), the latencies before falling, and the number of falls were measured for four trials with a cut-off period of 1 min per trial and an intertrial interval of 10 min. The stationary beam test was performed at the age of 72 weeks.

2.4.4. Gait test

Gait characteristics (stride and track width) were analysed by applying ink to the animals' hind paws and letting them walk on a strip of paper, down a brightly lit alley (4.5 cm wide, 40 cm long), towards a dark goal box. The 72-week-old groups were subjected to the gait test.

2.5. Statistics

Significance of differences between mean scores of path length, escape latency and swim speed during MWM acquisition, and latencies on the accelerating rotarod, were assessed with two-way analysis of variance with correction for repeated measures (RM-ANOVA). Genotype and trial (block) were considered as sources of variation. Spatial acuity during probe trial was assessed with two-way ANOVA. Two-way RM-ANOVA was also used to assess age-dependent effects on rotarod performance. Two-tailed Student's *t*-test (*t*-test) was used for comparison between pairs of means, and

differences between proportions were analysed with a Fisher exact test. Tukey post hoc multiple comparisons was employed to analyse age-dependent differences within genotypes for specific parameters. Statistical analysis was performed with Sigmasat software (SPSS Inc., Erkrath, Germany) with the level of probability set at 95%.

3. Results

3.1. Cognitive performance assessment

Visual-spatial learning and memory was assessed in the MWM paradigm, whereas the passive avoidance task was employed to study primarily non-spatial learning. Mice of both age groups improved their performance during the MWM acquisition phase as indicated by significant effects of trial block on path length and escape latency. At the age of 20 weeks, factor trial block significantly influenced path length (two-way RM-ANOVA; $F_{7,84} = 9.955$, $P < 0.001$) and escape latency ($F_{7,84} = 7.487$, $P < 0.001$). Similar results were obtained for the 52-week-old animals (path length: $F_{7,77} = 5.089$, $P < 0.001$; escape latency: $F_{7,77} = 3.003$, $P = 0.008$).

Two-way RM-ANOVA did not show a significant effect of genotype on path length and escape latency at the age of 20 weeks ($P = 0.612$ and $P = 0.886$, respectively, data not shown). Although the interaction genotype × trial block revealed a significant effect on both variables (path length: $F_{7,84} = 2.992$, $P = 0.007$; escape latency: $F_{7,84} = 2.607$, $P = 0.017$), post hoc comparison was unable to show significant differences between genotypes on specific training days. Swim speed was not affected by genotype, neither by the interaction genotype × trial block ($P = 0.407$ and $P = 0.446$, respectively; data not shown). A probe trial confirmed the equivalent learning capacities of 20-week-old expanded CGG repeat and WT mice. The spatial search pattern in expanded CGG repeat mice was not different from the WT group (two-way ANOVA; effect genotype × quadrant: $P = 0.981$, data not shown). Moreover, the number of entries through target was similar in expanded CGG repeat and WT mice (*t*-test; $P = 0.829$), as was total path length ($P = 0.960$) (Table 1).

At the age of 52 weeks, the acquisition curves suggested impairment of visual-spatial learning and memory abilities in the expanded CGG repeat mice (Fig. 1A and B). When considering escape latency, expanded CGG repeat mice performed significantly worse compared to the WT group ($F_{1,77} = 5.603$, $P = 0.037$; Fig. 1B). Statistical analysis revealed a strong trend towards a significant effect of genotype on path length (two-way RM-ANOVA; $F_{1,77} = 4.766$, $P = 0.052$; Fig. 1A). These differences were not attributable to lower swim speed in the expanded CGG repeat group ($P = 0.858$; data not shown). The interaction genotype × trial affected neither path length ($P = 0.784$), nor escape latency ($P = 0.387$) or swim speed ($P = 0.707$). Although two-way ANOVA failed to show significant differences in

Table 1

Morris water maze probe trial, passive avoidance learning, open field behaviour and performance on wire suspension test in wild-type (WT) and expanded CGG repeat (CGG) mice at different ages

	20 weeks		52 weeks		72 weeks	
	WT	CGG	WT	CGG	WT	CGG
Morris water maze probe^a						
No. of entries through target	3.4 ± 1.2	3.9 ± 1.5	4.5 ± 1.0	0.7 ± 0.5**	N/A	N/A
Path length (cm)	1961 ± 309	1978 ± 181	1935 ± 74	1747 ± 379		
Passive avoidance^b						
Latency to re-enter (s)	98 ± 22	87 ± 17	95 ± 13	91 ± 15	N/A	N/A
Animals not reaching criterion (%)	20	30	50	33		
Open field test^c						
Total path length (cm)	2133 ± 364	3223 ± 396	3923 ± 172	3394 ± 367	2897 ± 192	2116 ± 336
No. of entries corners	45 ± 6	64 ± 6	80 ± 5	71 ± 7	57 ± 2	47 ± 6
No. of entries centre	28 ± 4	39 ± 7	41 ± 3	30 ± 5	40 ± 6	18 ± 7*
Path length centre	287 ± 44	408 ± 83	428 ± 31	316 ± 60	429 ± 65	192 ± 77*
% path length centre	14 ± 1	12 ± 1	11 ± 1	9 ± 1	15 ± 2	8 ± 2*
Wire suspension test^d						
Latency to first fall	103 ± 11	81 ± 16	34 ± 18	27 ± 4	74 ± 18	77 ± 17
No. of falls	0.4 ± 0.2	1.3 ± 0.4	3.8 ± 1.1	8.7 ± 1.1*	2.8 ± 1.0	2.8 ± 1.2

Data are mean ± S.E.M. Statistical significance was determined by two-tailed Student's *t*-test: N/A = not applicable.

a 20 weeks: WT (*n* = 5), CGG (*n* = 9); 52 weeks: WT (*n* = 6), CGG (*n* = 7).

b 20 weeks: WT (*n* = 5), CGG (*n* = 10); 52 weeks: WT (*n* = 6), CGG (*n* = 9).

c 20 weeks: WT (*n* = 5), CGG (*n* = 10); 52 weeks: WT (*n* = 6), CGG (*n* = 9); 72 weeks: WT (*n* = 6), CGG (*n* = 8).

d 20 weeks: WT (*n* = 5), CGG (*n* = 10); 52 weeks: WT (*n* = 6), CGG (*n* = 9); 72 weeks: WT (*n* = 6), CGG (*n* = 9).

* *P* < 0.05.

** *P* < 0.01.

the percentage of time spent in the different quadrants of the MWM (effect genotype × quadrant; *P* = 0.806), impaired visual-spatial learning and memory in expanded CGG repeat mice was equally notable during probe trial. A significantly lower number of entries through target indicated decreased spatial accuracy in the expanded CGG repeat group (*t*-test; *P* = 0.005), which could not be ascribed to decreased swim speed, hence total path length during probe trial (*P* = 0.661) (Table 1).

Neither at the age of 20 weeks, nor at 52 weeks, passive avoidance learning was affected in the expanded CGG repeat model. No significant differences between genotypes in latency to re-enter the dark compartment (*t*-test; 20 weeks, *P* = 0.696; 52 weeks, *P* = 0.847) and percentage of animals not reaching criterion (Fisher exact; 20 weeks, *P* = 1.000; 52 weeks, *P* = 0.344) were observed (Table 1).

3.2. Activity and exploration assessment

Exploratory behaviour was registered in an open field arena, while the comparison of cage activity profiles enabled us to investigate possible differences in 23 h patterns of horizontal activity. At the age of 20 weeks, as well as in the 52-week-old mice, open field behaviour did not differ between expanded CGG repeat and WT mice (Table 1). At the age of 72 weeks, however, expanded CGG repeat mice travelled a significantly shorter distance (*t*-test; *P* = 0.044) and entered a significantly lower number of times in the centre circle of the open field arena (*P* = 0.045). Reduction of these parameters was not attributable to an overall lower activity/exploration

level (total path length: *P* = 0.091) as the percentage of path length spent in the centre circle was also significantly reduced in expanded CGG repeat mice (*P* = 0.016).

Cage activity profiles recorded at the age of 72 weeks, showed no differences between genotypes (two-way RM-ANOVA; factor genotype: *P* = 0.908, factor genotype × time: *P* = 0.959; Fig. 2).

3.3. Neuromotor assessment

Neuromotor performance and equilibrium were analysed initially with an accelerating rotarod apparatus. Comparison of rotarod performance revealed no major differences between expanded CGG repeat and WT mice in any age group tested. However, considering each genotype separately, a significant decrease in performance with age was eminent in the expanded CGG repeat mice (two-way RM-ANOVA; *F*_{2,75} = 7.317, *P* = 0.003), but not in the WT groups (*P* = 0.263). Post hoc comparison indicated significantly reduced latency (time spent on rod) in the 52-week and 72-week-old group compared to the 20-week-old expanded CGG repeat mice (Tukey; *P* = 0.002 and *P* = 0.042, respectively; Fig. 3).

The wire suspension test, which assesses grip strength and endurance, showed no differences at the age of 20 weeks. In 52-week-old animals, latency to the first fall did not differ between genotypes, but expanded CGG repeat mice fell significantly more compared to WT mice (*t*-test; *P* = 0.007). However, when these animals were re-tested at age 72 weeks, performance did not differ between genotypes (Table 1).

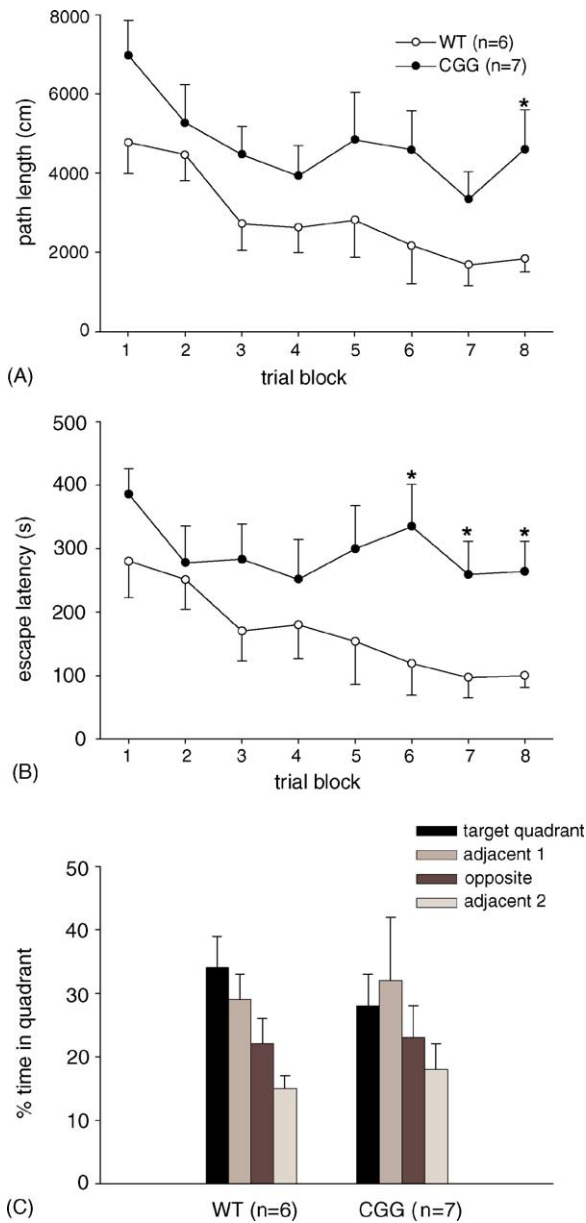


Fig. 1. Morris water maze performance in expanded CGG repeat (CGG) mice (closed symbols) and wild-type (WT) mice (open symbols) at age 52 weeks. On learning curves representing (A) path length and (B) escape latency each data point depicts mean (\pm S.E.M.) summed results of four daily trials. (C) Mean percentage (\pm S.E.M.) of time spent in each quadrant of the Morris water maze during probe trial. Asterisks indicate significance of difference between CGG repeat and WT values (post hoc two-tailed Student's *t*-test; * $P < 0.05$).

Additional neuromotor tasks to evaluate putative ataxia at the age of 72 weeks consisted of the stationary beam task and the gait test. Latter was not able to distinguish between expanded CGG repeat and WT mice (Table 2), whereas the stationary beam test showed a significantly lower number of segments crossed by expanded CGG repeat mice ($P = 0.045$; Table 2). Other parameters measured during the stationary beam task did not vary between genotypes (Table 2).

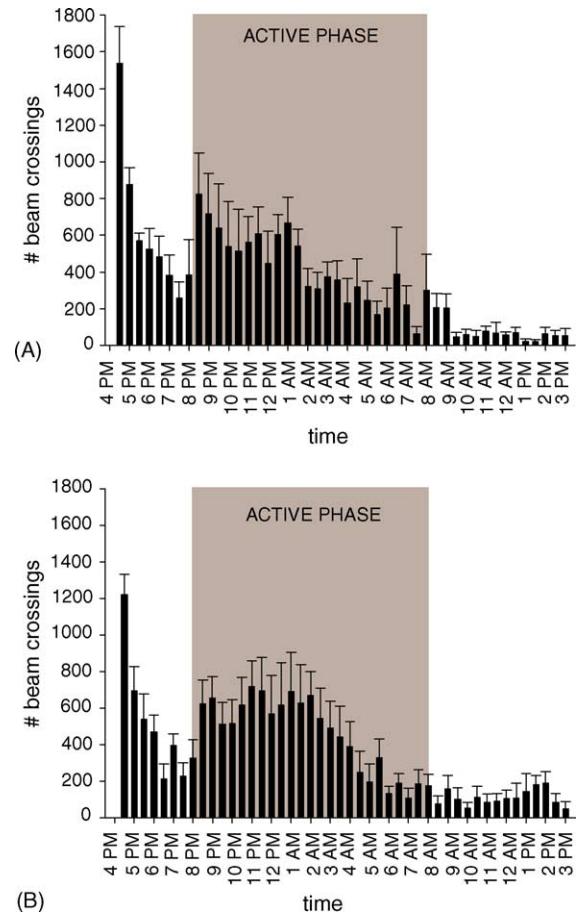


Fig. 2. Cage activity profiles in (A) wild-type ($n = 5$) and (B) expanded CGG repeat (CGG) mice ($n = 10$) at the age of 72 weeks. Bars depict mean (\pm S.E.M.) summed number of beam crossings during the preceding 30 min of recording. Shaded background represents the dark, i.e. active phase of the animals' 12/12 h light–dark cycle.

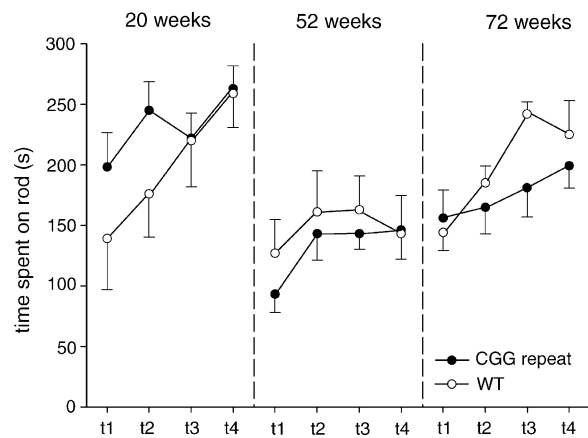


Fig. 3. Age-dependent performance on the accelerating rotarod apparatus during four trials in expanded CGG repeat (CGG, closed symbols) and wild-type mice (WT, open symbols). Group sizes were: CGG ($n = 10$), WT ($n = 5$) at the age of 20 weeks; CGG ($n = 9$), WT ($n = 6$) at the ages of 52 and 72 weeks. Each data point represents mean (\pm S.E.M.) latency spent on the accelerating rod during four trials (t1–t4).

Table 2

Additional neuromotor tasks at the age of 72 weeks in expanded CGG repeat (CGG) and wild-type (WT) mice

	WT	CGG
Stationary beam test ^a		
Total no. of segments crossed	25 ± 12	3 ± 2*
Total no. of falls	0.5 ± 0.2	0.9 ± 0.3
Total latency	213 ± 12	203 ± 12
Gait test ^a		
Stride maximum left	8.31 ± 0.20	8.30 ± 0.39
Stride maximum right	7.90 ± 0.22	8.44 ± 0.42
Stride median left	6.95 ± 0.34	6.99 ± 0.35
Stride median right	6.71 ± 0.26	7.08 ± 0.31
Track width maximum	2.86 ± 0.14	2.89 ± 0.09
Track width median	2.44 ± 0.11	2.55 ± 0.07

Data are mean ± S.E.M. Statistical significance was determined by two-tailed Student's *t*-test with **P* < 0.05.

^a WT (*n* = 6), CGG (*n* = 9).

4. Discussion

The cognitive and behavioural characteristics of the knock-in mouse model for FXTAS were evaluated using an extensively validated test battery, consisting of the MWM and passive avoidance learning tasks, an open field exploration test and evaluation of neuromotor performance. Pathologically, the presence of neuronal intranuclear inclusions has been described in both FXTAS patients [6], and the CGG repeat mice [19]. Whether the formation of inclusions underlies the clinical symptoms remains to be resolved, and formed the rationale for the present study.

The MWM test is a widely used laboratory tool to investigate visual-spatial learning and memory in rodents [5]. Memory acquisition is reflected in learning curves of escape latency and path length, whereas the probe trial assesses storage and retrieval of spatial information. At the age of 20 weeks, expanded CGG repeat mice performed equally well on this task as WT mice. Expanded CGG repeat mice aged 52 weeks, however, displayed obviously deviant learning curves and inferior spatial accuracy during probe trial compared to the control group. A relative consistent pattern of cognitive impairment, including memory problems and executive function deficits, in some individuals gradually progressing to dementia, was reported in FXTAS patients [7,9]. Abnormalities in the MWM, nevertheless, were not a priori expected in the expanded CGG repeat model. The integrity of the hippocampal formation was originally described as imperative for the hidden-platform MWM task in rodents [11,12]. However, in the expanded CGG repeat mouse model, hippocampal involvement is seemingly restricted: less than 1% of the hippocampal neurons were shown to exhibit inclusions around the age of 52 weeks [19]. Two reasons might explain this discrepancy. First, the presence of inclusions might not be related or even protective to cell damage, as has been postulated for inclusions in patients with hereditary ataxia [20]. Second, brain regions other than the hippocampal formation have been implicated in MWM learning, including the mam-

illary bodies [3,5,14,16], a brain structure with a high percentage of inclusions (22% of neurons affected) in the mouse model [19].

In the youngest group, the formation of intranuclear inclusions is only at its initial stage, and in most brain regions examined, the amount of inclusion-positive neurons is less than 1% [19]. With aging, the inclusions increase both in size and number [19], not only correlating with the progressive character of the human FXTAS syndrome, but also with the age-dependent decline of visual-spatial learning in the expanded CGG repeat mice here presented.

Passive avoidance learning was affected at neither of the ages tested (20 and 52 weeks), suggesting that the expanded CGG premutation induces cognitive defects disturbing some (MWM), but not all forms of learning and memory. It remains to be elucidated whether passive avoidance learning, which is mostly based upon non-spatial abilities, will perhaps exhibit impairment in a more advanced stage of the disorder. Open field behaviour did not differ between expanded CGG repeat and WT mice at the ages of 20 and 52 weeks. When re-tested at the age of 72 weeks, however, the latter group displayed evidence of altered open field exploration. The significantly lower number of entries in the centre circle and the decrease in the percentage of path length in the centre might indicate increased anxiety in the expanded CGG repeat mice at this age [4]. Increased anxiety, rather than decreased exploration/activity is suggested by these observations since no significant difference in total path length was noted and the comparison of cage activity profiles confirmed that expanded CGG repeat and WT mice did not differ in general activity levels. However, more specific tests to assess anxiety, like the elevated plus-maze or a holeboard paradigm, would allow further appraisal of this presumption of increased anxiety. FXTAS patients tend to display a variety of psychological symptoms, including anxiety [7,9]. The presence of inclusions in cortical areas (mainly frontal and cingulate cortex) at the age of 72 weeks might be related to the increased anxiety in the CGG repeat model. Since the two major clinical criteria proposed for definite FXTAS are intention tremor and gait ataxia [7], the expanded CGG repeat model was subjected to a variety of neuromotor tasks. Although at first sight the accelerating rotarod failed to show differences in performance between WT and expanded CGG repeat, a significant deterioration between weeks 20 and 52 in CGG repeat mice was observed when specifically considering the effect of age on rotarod performance within each genotype. Decline of motor performance coincides with the increase of inclusion-positive neurons in CGG repeat brain, specifically in the 10th lobule of the posterior cerebellum [19]. At the age of 52 weeks, some differences in wire suspension performance were observed, but the same expanded CGG repeat mice performed indistinguishable from WT when re-tested at the age of 72 weeks. It is therefore not fully clear whether wire suspension is affected in the expanded CGG mice. Besides grip strength and endurance, a major motivational factor is implicated in the performance of this task,

which might explain these observations. The gait test showed no ataxic disturbances in the 72-week-old expanded CGG repeat mice, whereas the stationary beam task suggested some neuromotor deficits, though it has to be taken into consideration that the underlying cause of the decreased number of segments crossed on the stationary beam task might also be related to the increased anxiety as observed in the open field test.

These data clearly indicate an age-dependent decline of visual-spatial learning capacities, a potential increase of anxiety levels, and mild neuromotor disturbances in the expanded CGG repeat mouse model. On average, the first inclusions appear in mouse brain around the age of 30 weeks, which might explain why no behavioural alterations were observed in our youngest age group. With increase of inclusion size and number, cognitive and behavioural alterations became evident. The whole of our observations in the mouse model seem compatible with the progressive character of decline of cognitive function and the appearance of behavioural problems in FXTAS patients. The FXTAS mouse model facilitates molecular studies from onset of symptoms, through disease progression, until final stage of disease, whereas human post mortem brain only allows end stage studies. In addition, this expanded CGG repeat model offers new opportunities in understanding RNA gain-of-function effect in the pathogenesis of FXTAS.

Acknowledgements

This work was supported by the Belgian Fund for Scientific Research-Flanders (FWO, grant G.0038.05), agreement between Institute Born-Bunge and the University of Antwerp, Medical Research Foundation Antwerp, Neuroresearch Antwerp, the FRAXA Research Foundation, NIH grant (BAO) and the National Fragile X Foundation (R.W.). We would like to thank M. Hoogeveen-Westerveld and I. Nieuwenhuizen for technical assistance.

References

- [1] Bontekoe CJ, Bakker CE, Nieuwenhuizen IM, van der Linde H, Lans H, de Lange D, et al. Instability of a (CGG)₉₈ repeat in the Fmr1 promoter. *Hum Mol Genet* 2001;10:1693–9.
- [2] Brunberg JA, Jacquemont S, Hagerman RJ, Berry-Kravis EM, Grigsby J, Leehey MA, et al. Fragile X premutation carriers: characteristic MR imaging findings of adult male patients with progressive cerebellar and cognitive dysfunction. *Am J Neuroradiol* 2002;23:1757–66.
- [3] Conejo NM, Gonzalez-Pardo H, Vallejo G, Arias JL. Involvement of the mammillary bodies in spatial working memory revealed by cytochrome oxidase activity. *Brain Res* 2004;1011:107–14.
- [4] Crawley JN, Paylor R. A proposed test battery and constellations of specific behavioral paradigms to investigate the behavioral phenotypes of transgenic and knockout mice. *Horm Behav* 1997;31:197–211.
- [5] D'Hooge R, De Deyn PP. Applications of the Morris water maze in the study of learning and memory. *Brain Res Rev* 2001;36:60–90.
- [6] Greco CM, Hagerman RJ, Tassone F, Chudley AE, Del Bigio MR, Jacquemont S, et al. Neuronal intranuclear inclusions in a new cerebellar tremor/ataxia syndrome among fragile X carriers. *Brain* 2002;125:1760–71.
- [7] Hagerman PJ, Hagerman RJ. Fragile X-associated tremor/ataxia syndrome (FXTAS). *Ment Retard Dev Disabil Res Rev* 2004;10:25–30.
- [8] Hagerman RJ, Leehey M, Heinrichs W, Tassone F, Wilson R, Hills J, et al. Intention tremor, Parkinsonism, and generalized brain atrophy in male carriers of fragile X. *Neurology* 2001;57:127–30.
- [9] Jacquemont S, Hagerman RJ, Leehey M, Grigsby J, Zhang L, Brunberg JA, et al. Fragile X premutation tremor/ataxia syndrome: molecular, clinical, and neuroimaging correlates. *Am J Hum Genet* 2003;72:869–78.
- [10] Kenneson A, Zhang F, Hagedorn CH, Warren ST. Reduced FMRP and increased FMR1 transcription is proportionally associated with CGG repeat number in intermediate-length and premutation carriers. *Hum Mol Genet* 2001;10:1449–54.
- [11] Logue SF, Paylor R, Wehner JM. Hippocampal lesions cause learning deficits in inbred mice in the Morris water maze and conditioned-fear task. *Behav Neurosci* 1997;111:104–13.
- [12] Morris RGM, Garrud P, Rawlins JNP, O'Keefe J. Place navigation impaired in rats with hippocampal lesions. *Nature* 1982;297:681–3.
- [13] Pieretti M, Zhang F, Fu Y-H, Warren ST, Oostra BA, Caskey CT, et al. Absence of expression of the FMR-1 gene in fragile X syndrome. *Cell* 1991;66:817–22.
- [14] Sziklas V, Petrides M. Selectivity of the spatial learning deficit after lesions of the mammillary region in rats. *Hippocampus* 2000;10:325–8.
- [15] Tassone F, Hagerman RJ, Taylor AK, Gane LW, Godfrey TE, Hagerman PJ. Elevated levels of FMR1 mRNA in carrier males: a new mechanism of involvement in the Fragile-X syndrome. *Am J Hum Genet* 2000;66:6–15.
- [16] Vann SD, Aggleton JP. Evidence of a spatial encoding deficit in rats with lesions of the mammillary bodies or mammillothalamic tract. *J Neurosci* 2003;23:3506–14.
- [17] Verkerk AJ, Pieretti M, Sutcliffe JS, Fu YH, Kuhl DP, Pizzuti A, et al. Identification of a gene (FMR-1) containing a CGG repeat coincident with a breakpoint cluster region exhibiting length variation in fragile X syndrome. *Cell* 1991;65:905–14.
- [18] Vloeberghs E, Van Dam D, Engelborghs S, Nagels G, Staufenbiel M, De Deyn PP. Altered circadian locomotor activity in APP23 mice: a model for BPSD disturbances. *Eur J Neurosci* 2004;20:2757–66.
- [19] Willemsen R, Hoogeveen-Westerveld M, Reis S, Holstege J, Severijnen LA, Nieuwenhuizen IM, et al. The FMR1 CGG repeat mouse displays ubiquitin-positive intranuclear neuronal inclusions; implications for the cerebellar tremor/ataxia syndrome. *Hum Mol Genet* 2003;12:949–59.
- [20] Zoghbi HY, Botas J. Mouse and fly models of neurodegeneration. *Trends Genet* 2002;18:463–71.