The effect of mutant ubiquitin on proteasome function in relation to neurodegenerative disease
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Alzheimer-associated mutant ubiquitin
impairs spatial reference memory

In preparation

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Abstract

UBB$^{+1}$ is a mutant ubiquitin which accumulates in the hallmarks of tauopathies, including Alzheimer’s disease. Transgenic mice expressing high levels of neuronal UBB$^{+1}$ exhibit moderately decreased proteasome activity and spatial reference memory deficits at 9 months of age. In the present study, we characterized the behavioral phenotype of male UBB$^{+1}$ transgenic mice at different ages. We found that UBB$^{+1}$ transgenic mice showed an age-related functional decline similar to wild-type litter mates, without displaying gross neurological abnormalities or alterations in procedural (motor-) learning and motor coordination at 3, 9, 15 and 21-24 months of age. At 15 months of age, spatial learning was not affected during the acquisition of the Morris watermaze. However, spatial reference memory during the probe trial was initially impaired in the transgenic mice, this deficit was eliminated after intense training. We conclude that the previously reported spatial reference memory deficits of UBB$^{+1}$ transgenic mice persist, but do not aggravate during aging. In addition, our results demonstrate that spatial reference memory formation depends on intact forebrain proteasome activity.

Introduction

The ubiquitin-proteasome system (UPS) is the main regulated pathway for intracellular protein turnover and is essential for maintaining cellular homeostasis. Substrate proteins are selectively targeted for proteolytic degradation by covalent attachment of a chain of ubiquitin moieties, ultimately resulting in degradation of the substrate by the 26S proteasome (Glickman and Ciechanover, 2002). Besides tagging proteins for degradation, protein modification with one or more ubiquitins is involved in many other cellular processes e.g. endocytosis, DNA repair and transcriptional regulation (Welchman et al., 2005; Mukhopadhyay and Riezman, 2007). In the adult nervous system, the UPS also plays an important role in synaptic plasticity and learning and memory formation (DiAntonio and Hicke, 2004). This was first found in Aplysia, where the deubiquitinating enzyme Ap-Uch as well as 26S proteasome function proved critical for inducing long-term facilitation (Hegde et al., 1997; Chain et al., 1999). In rat, inhibiting hippocampal proteasome activity blocks long-term memory formation in an inhibitory avoidance task (Lopez Salon et al., 2001). More recently, a role in cognitive function for deubiquitinating enzymes belonging to the class of ubiquitin C-terminal hydrolases is also described in mouse; Uch-l1 is essential for normal synaptic function (Gong et al., 2006) and both Uch-l1 and Uch-l3 are required for memory formation (Wood et al., 2005; Gong et al., 2006). Also in several other genetically manipulated mouse models with defective components of the UPS, learning and memory is affected (reviewed by (van Tijn et al., 2008)).

As ubiquitin-dependent protein degradation plays an important role in neuronal devel-
opment, as well as in maintenance of the adult nervous system, it is not surprising that malfunctioning of the UPS is implicated in the pathogenesis of neurodegenerative disease. This is reflected by the ubiquitin-positive protein aggregates present in the hallmarks of many neurological disorders, including Alzheimer’s disease (AD) and Parkinson’s disease (PD) (Ciechanover and Brundin, 2003). In addition, mutations in UPS related enzymes are proposed to be causative for forms of familial PD (Kitada et al., 1998; Leroy et al., 1998) and several disease-related proteins, including amyloid-β and tau in AD, can diminish proteasomal activity (Gregori et al., 1995; Keck et al., 2003; Lopez Salon et al., 2003).

Another indication for UPS involvement in disease pathogenesis is the accumulation of a mutant ubiquitin (UBB +1) in the neuropathological hallmarks of tauopathies, including AD, and in Huntington’s disease (van Leeuwen et al., 1998; Fischer et al., 2003; De Pril et al., 2004). We previously reported that UBB +1 is a ubiquitin-fusion degradation substrate for proteasomal degradation (Lindsten et al., 2002). However, after exceeding a threshold level of expression, UBB +1 acts as inhibitor of the 26S proteasome (van Tijn et al., 2007). We therefore proposed that accumulation of UBB +1 in human brain is an endogenous marker for proteasomal dysfunction (Fischer et al., 2003).

We recently developed UBB +1 transgenic mouse lines with varying levels of neuronal UBB +1 expression to further study the properties of UBB +1 in vivo (Chapter 3). In the transgenic line 3413, high levels of postnatal UBB +1 expression led to neuronal accumulation of the UBB +1 protein mainly in the cerebral cortex, hippocampus and striatum and resulted in a chronic low-level reduction of cortical chymotryptic proteasome activity in vivo, leading to the accumulation of ubiquitinated proteins (Chapter 3). In addition, these mice exhibited cognitive defects in spatial memory at 9 months of age; male 3413 transgenic mice showed intact spatial learning, but were significantly impaired in spatial memory retention in the Morris watermaze task. Also in a fear conditioning paradigm, 9-months-old 3413 transgenic mice exhibited decreased context-related memory, whereas cued memory was unaffected (Chapter 3).

In the present study, we further characterized the effects of chronic low-level neuronal UPS inhibition on gross neurological functioning, motor coordination and procedural motor learning in male UBB +1 3413 transgenic mice and their wild-type littermates, aged 3, 9, 15 and 21-24 months. To study the persistence of the recently reported explicit memory dysfunction in these mice at 9 months of age, we assessed spatial learning and memory in the Morris watermaze paradigm at the age of 15 months.

Materials and Methods

Transgenic mice
The previously described transgenic UBB +1 mouse line 3413 (Chapter 3) expresses human
UBB$^{+1}$ cDNA under control of the murine CamKIIα promoter and was maintained on a C57/Bl6 background by breeding hemizygous mice males to wild-type mice females. Mice were genotyped on DNA isolated from ear-snips (Chapter 3) and kept in group housing on a 12/12 h light-dark cycle with food and water ad libitum in specific pathogen free conditions (Nicklas et al., 2002). Behavioral experiments were performed in the light phase. Mice were housed solitarily one week before behavioral testing commenced. All experimental mice used in the present study were male, the experimenter was blind to the genotype of the mice. Animal experiments were performed conforming to national animal welfare law and under guidance of the animal welfare committee of the Royal Netherlands Academy of Arts and Sciences.

**General neurological behavior assessment**

General neurological reflexes were tested (Rogers et al., 1997) using the righting reflex, by observing if a mouse returned to a normal posture standing on four paws after being flipped over onto its backside (scored as present/not present). The corneal reflex was measured by observing an eye-lid blinking response after gently touching the cornea with the tip of a cotton swab (scored as present/not present). The reaching reflex was measured by lifting the mouse by the base of the tail to approximately 15 cm above a firm surface. The ability to extend the forelimbs reaching towards the surface was scored (present/not present) (Rogers et al., 1997). Neuromuscular strength was measured with a hanging wire test by placing a mouse on a stainless steel wire cage grid suspended approximately 30 cm in the air above soft bedding material. After the mouse firmly gripped the cage wires, the cage lid was slowly turned upside down and the latency to fall (s) was recorded, with a maximum duration of 60 s (protocol described in (Crawley, 1999)). To monitor hind limb escape extension mice were lifted by the base of the tail and the hind limb response, normally extension in an outward v-shape, was scored (present/not present) (as described in (Lewis et al., 2000)).

**Rotarod**

An accelerating rotarod (model 47600, Ugo Basile Biological Research Apparatus, Italy) was used to measure motor-learning and coordination. The rotarod had a grooved rotating beam (diameter 3 cm) raised 16 cm above a platform, which was divided into five sections for five mice to be tested simultaneously. When falling from the rotating beam, the latency to fall (s) was recorded electronically. Mice were allowed to familiarize with the beam for two 180 s trials with the rod rotating at a constant speed of 4 rpm on day 0. Starting the next day, mice were subjected to four 300 s trials per day for three consecutive days (days 1-3) with an intertrial interval of ~15 min. Over the 300 s, the rotating
beam accelerated from 4 rpm to 40 rpm. Mice remaining on the beam during the full 300 s of the task were taken from the rotarod and given the maximum score. Mice were trained for another three consecutive days following the same protocol one week later (days 8-10). The 3 and 15 months old mice were first subjected to watermaze training before being tested on the rotarod. Analysis of the data was done using WinDAS 2004 software (v 3.0.145, Ugo Basile, Italy).

Watermaze
Mice were handled for four days before the experiment commenced. The watermaze consisted of a circular pool of 1.22 m in diameter filled with water at 26 ± 1°C, made opaque by addition of white non-toxic latex paint. Training was performed as described previously in Chapter 3. Briefly, training commenced with a 120 s free-swim trial on day 1. Hidden platform training was conducted for four consecutive days (4 trials per day, ~30 min inter-trial interval). Mice were allowed to search for a hidden circular platform (11 cm diameter) for 60 s. The platform location remained constant during the trials (NW), the inlet position was chosen pseudorandomly (N, E, S, W) every trial. Memory retention was tested three days after acquisition training in a 60 s probe trial, in which the hidden platform was removed. Inlet position was chosen in the opposite quadrant of the former platform position. Directly following the first probe trial a visual platform test was performed, with both the platform location and the inlet location pseudorandomised. Visual training consisted of three consecutive trials wherein the animals had to locate a clearly visible platform. Mice not able to find the visual platform were excluded from the final analysis (2 transgenic, 3 wild-type mice). A second series of acquisition trials, followed by a second probe trial, commenced three weeks after the first probe trial, following the same procedures as during the first acquisition phase. Directly following the second probe trial a third four-day acquisition phase commenced, followed by a third probe trial. The testing series were concluded by performing a second visual platform test following the third probe trial, similar to the first visual platform test. All trials were monitored by a camera, and recorded and analyzed using a computerized tracking system (Ethovision, Noldus, The Netherlands).

Data analysis
Analysis of bodyweight and hanging wire latencies were assessed with a Student’s t-test or Mann-Whitney when the data was not normally distributed. Effects of age and genotype interactions on bodyweight and hanging wire latencies were analyzed with univariate ANOVA. For rotarod data analysis, the latencies to fall were averaged for every subject over the four trials per testing day. Latencies for wild-type vs. transgenic mice were com-
pared per day with a Students t-test. To analyze the overall genotype and learning effects, a repeated measures ANOVA was performed with averaged day latencies for all six training days as a repeated within-subjects measure and genotype as a between-subjects factor. Additionally, repeated measures ANOVA was performed separately for days 1-3 and days 8-10.

For analysis of the watermaze acquisition trials, the latencies to find the hidden platform location were averaged per acquisition day. Swimming speed was averaged over all four days of acquisition. For the probe trials, swimming speed was calculated separately. In all cases, swimming speed did not significantly differ between transgenic and wild-type mice. Statistical analysis was performed by planned comparisons between wild-type and transgenic mice per day for the acquisition and visual tasks using a Students t-test. For learning effects during the acquisition phase and visual tasks, repeated measures ANOVA was performed with day as repeated within-subject measure and genotype as a between-subjects factor. For the probe trial scores (percentage of time spent in each quadrant), the time spent per quadrants was tested per quadrant per genotype against the expected value of 25% with a non-parametric one-sample Wilcoxon signed ranks test. All results are expressed as mean +/- S.E.M. and were considered significant when p<0.05. Statistical analysis was performed using SPSS for Windows (version 12.0.1).

Results

General neurological phenotype assessment
UBB+1 transgenic mice over-express an aberrant form of ubiquitin in forebrain neurons, mainly located in the hippocampus, cortex and striatum (Chapter 3). These mice do not suffer from overt neuropathology in the form of tangles, amyloid plaques or activated neuroglia (Chapter 3). In this study, we examined if the 3413 UBB+1 transgenic mice showed gross phenotypic abnormalities in spontaneous home-cage behavior, including hyperactivity, anxiety and aggression. We did not detect any overt deviation from normal behavior in the 3413 transgenic mice up to 24 months of age. Body weight increased significantly during aging (p<0.001), however without differing between male wild-type and 3413 transgenic mice at any age (Table 1). The righting reflex was present in the wild-type as well as the 3413 transgenic mice at all ages tested (Table 1). Also the reaching reflex and the corneal reflex were present and comparable in wild-type and 3413 transgenic mice at all ages (Table 1). To test the neuromuscular function we examined the escape extension. Normally, when lifted by the tail, the hind limbs of the mouse extend in an outward “v-shape” (Lewis et al., 2000). Correct escape extension of the hind limbs was present up to 15 months of age without differences between the wild-type and transgenic mice. In the 21-24 month old mice, all the 3413 transgenic mice and the majority of the
wild-type mice (86%) displayed poor escape extension, keeping the hind limbs close to their body (Table 1). Neuromuscular strength was assessed with the hanging wire test (Crawley, 1999). The latency to fall (with a maximum of 60 s) did not significantly differ between the wild-type and transgenic mice at any age, although a general decrease in performance was observed during aging (p<0.001). Also, no significant genotype or age*genotype interactions were present (Table 1).

Motor coordination and motor-learning

An accelerating rotarod task was used to assess motor coordination and motor-learning skills. We tested naive male 3413 transgenic and age-matched wild-type mice at the ages of 3, 9, and 15 months. The first training-week consisted of four trials per day during three consecutive days (days 1-3) to assess basic motor skills and procedural motor-learning capacities. A second comparable training session took place one week later (days 8-10). At the age of 3 months, both wild-type (n=9) and 3413 transgenic mice (n=6) performed equally well (fig. 1A). Repeated measures ANOVA over all training days revealed a significant effect of training day (p=0.014), indicating that both wild-type and 3413 transgenic mice improved their motor skills, without showing an effect of genotype or day*genotype interaction. When the first and second training week (days 1-3 and days 8-10) were analyzed as separate cohorts, during days 1-3 not only a significant effect of day was present (p<0.01), but also a significant interaction of day*genotype (p=0.049). This interaction between day and genotype was not significant for days 8-10.

Table 1  Results of general neurological tests

<table>
<thead>
<tr>
<th>Test</th>
<th>3 months</th>
<th>9 months</th>
<th>15 months</th>
<th>24 months</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>wt</td>
<td>tg</td>
<td>wt</td>
<td>tg</td>
</tr>
<tr>
<td>n</td>
<td>11</td>
<td>7</td>
<td>14</td>
<td>7</td>
</tr>
<tr>
<td>weight (g)(^a)</td>
<td>27.5±0.5</td>
<td>27.3±1.0</td>
<td>35.6±1.4</td>
<td>33.9±0.6</td>
</tr>
<tr>
<td>righting reflex</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>forelimb placing</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>eye lid reflex escape</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>escape ext. hanging</td>
<td>outward</td>
<td>outward</td>
<td>outward</td>
<td>outward</td>
</tr>
<tr>
<td>wire (s)(^a)</td>
<td>60.0</td>
<td>60.0</td>
<td>50.6±5.6</td>
<td>60.0</td>
</tr>
</tbody>
</table>

* data are represented as average ± S.E.M.; +: present
At 9 months of age, the average latencies to fall did not differ at any day between 3413 transgenic mice (n=12) and wild-type mice (n=13) (fig. 1B). A significant effect of training day was present over days 1-10 as well as over days 1-3 (p<0.001). This effect of training day was not present over days 8-10, indicating that the mice had reached a plateau performance on this task after the first week of training. No additional significant effects were present, further confirming that 3413 transgenic and wild-type mice performed equally well at this age.

Figure 1  3413 transgenic mice exhibit normal rotarod performance. Motor coordination and motor-learning were tested on an accelerating rotarod in naive 3413 transgenic mice and wild-type control mice at 3, 9 and 15 months of age. Latency to fall (s) from the rod were recorded electronically, with a maximum of 300 s. A-B: At 3 months of age (A) and at 9 months of age (B) motor skills are comparable between wild-type and transgenic mice. C: At 15 months of age, the 3413 transgenic mice perform significantly less than wild-type mice on training day 8 (p=0.032) and day 9 (p=0.021). D: When the training period is extended for the 15 months old mice, no additional significant differences between wild-type and 3413 transgenic mice are present up to day 38. All data are averages ± S.E.M. * p<0.05.
At 15 months of age, the 3413 transgenic mice performed significantly inferior to age-matched wild-type mice on day 8 and day 9 in the second week of training (p=0.032, p=0.021 respectively, fig. 1C). A learning effect was present over days 1-3 and over all training days (effect of day; p<0.001), without an effect of genotype or day*genotype interaction. However, repeated measures ANOVA for days 8-10 showed a significant effect of genotype (p=0.038), without an effect of training day. These data suggest that at 15 months of age, the motor-learning skills of 3413 transgenic and wild-type mice were identical, but the plateau performance levels were significantly lower in the UBB\textsuperscript{3} transgenic mice. To investigate if this decreased motor performance persisted over time, we extended rotarod training to a total of 18 days (3 training days per week, 6 weeks total). The results, depicted in figure 1D, show that the performance of the 3413 transgenic mice remained inferior to wild-type mice, even after saturated learning (fig. 1D). However, this effect did not reach significance for any individual timepoint after day 9, nor did repeated measures ANOVA for days 1-18 reveal a significant effect of genotype or day*genotype interaction.

**Spatial learning and memory**

We recently reported that 3413 transgenic mice exhibit spatial reference memory deficits in the Morris watermaze and in a fear conditioning paradigm at 9 months of age (Chapter 3). Here, we analyzed if spatial reference memory further declined during aging. Therefore, we tested naïve 3413 transgenic mice (n=8) and wild-type littermates (n=10) in the watermaze at 15 months of age. The hidden platform position remained constant in the NW quadrant during acquisition. During acquisition days 1-4, transgenic and wild-type mice performed indistinguishable on a day-by-day basis, without significant effects of day, genotype or interaction between these factors (fig. 2A). The absence of an effect of day suggested that both wild-type and transgenic mice did not learn the task properly. The results of the 60 s probe trial to assess spatial memory retention indeed demonstrated that neither group showed a significant preference for the NW quadrant (fig. 2B), the transgenic mice even spent significantly less time in the former platform quadrant NW (p=0.008 for NW, fig. 2B). The average distance to the former platform position did not differ significantly between wild-type and transgenic mice (fig. 3A, probe trial 1). In the visual task, directly following the probe trial, both groups performed also sub-optimal, as no learning effect was present (fig. 4A). Although the 3413 transgenic mice tended to perform less than the wild-type mice, this effect was not statistically significant.

Aged C57Bl/6 mice show declined spatial reference memory, expressed as a reduced preference for the former platform quadrant than young mice (Bach et al., 1999; von Bohlen und Halbach et al., 2006). This could possibly account for the poor performance displayed by the wild-type and transgenic mice at 15 months of age after one week of acqui-
**Figure 2** 3413 transgenic mice show impaired performance in the Morris watermaze. Spatial performance at 15 months of age was assessed in the Morris watermaze. A: Mean escape latencies (s) to find the hidden platform in the NW quadrant do not differ between wild-type and 3413 transgenic mice during days 1-4 of acquisition. B: The mean time spent in every quadrant of the maze is plotted as percentage of the total time of the 60 s probe trial after the first week of acquisition training. Neither the wild-type mice or the 3413 transgenic mice significantly prefer the former platform quadrant NW. C: Mean escape latencies (s) to find the hidden platform are plotted for the second week of acquisition training, with the wild-type and transgenic mice performing equally well during the second acquisition phase. D: The percentage of time spent in each quadrant of the maze during the second probe trial. The wild-type mice show a significant preference for the former platform quadrant NW during the second 60 s probe trial, whereas the 3413 transgenic mice do not show a preference for the NW quadrant. E: Mean escape latencies (s) to find the hidden platform plotted for the third week of acquisition, no significant differences are present between wild-type and 3413 transgenic mice. F: The percentage of time spent in each quadrant of the maze during the third probe trial, both the wild-type and 3413 transgenic mice show a significant preference for the former platform quadrant NW. All data are averages ± S.E.M. * p<0.05.
CHAPTER V

sition. Therefore, we extended the training period to improve acquisition of the watermaze task. Three weeks after the first probe trial, two additional sessions of acquisition trials (days 5-8 and days 9-12) were conducted in a similar fashion as days 1-4, each followed by a probe trial. During this prolonged acquisition, mice showed decreasing escape latencies over all acquisition days (days 1-12, effect of day, p<0.01), however this effect did not reach significance if the acquisition sessions were analyzed separately for days 5-8 (fig. 2C) or days 9-12 (fig. 2E). Furthermore, no effects of genotype or day*genotype interactions were present or significant differences between the groups on a day-by-day basis.

In the second probe trial, following the first week of extended acquisition, wild-type mice demonstrated a significant preference for the NW quadrant during the probe trial (p=0.01, fig. 2D), indicating the establishment of spatial reference memory. Also, the time spent in the opposite quadrant (SE) was significantly lower than the expected 25% chance level (p=0.002, fig. 2D). In contrast, the 3413 transgenic mice did not prefer the former hidden platform location in the NW quadrant, but rather showed a significant preference for the NE quadrant (p=0.016, fig. 2D). This effect was also observed as a trend in the transgenic mice during the first probe trial. The average distance to the former platform position was lower for the wild-type mice, however this effect was not significant (p=0.109, fig. 3, probe trial 2). These results indicated that the wild-type mice were capable of remembering the hidden platform position after four days of extended acquisition, whereas the transgenic mice were not, although the 3413 transgenic mice did have an increased preference for this quadrant compared to the first probe trial. In the final probe trial following acquisition days 9-12, both the wild-type and transgenic mice showed a

Figure 3  Distance to the former platform position and average distance moved. A: The mean distance (cm) to the former platform position in the NW quadrant is plotted per probe trial. B: The average total distance moved (cm) is plotted for probe trial 1 to 3. For each trial, the distances to do not differ significantly between wild-type and 3413 transgenic mice. All data are averages ± S.E.M.
significant preference for the former platform quadrant NW (p=0.004 and p=0.031 for wild-type and transgenic mice respectively, fig. 2F). The distance to the former platform position did not differ between the groups (fig. 3, probe trial 3). The observed minor motor-deficit on the rotarod task in the transgenic mice at the age of 15 months (fig. 1C, day 8 and 9) might also affect the swimming performance of these mice in the watermaze. However, swimming speed (not shown) and average distance moved (fig. 3B) did not differ significantly between the transgenic and wild-type mice during any of the probe trials. These results indicated that the 3413 transgenic mice eventually were capable of optimal performance on the watermaze task after prolonged training. Finally, a second visual test was performed, in which both groups performed equally well and improved their performance compared to the first visual test (fig. 4B).

Discussion

In this study we examined neurological functions, coordinated movement and cognitive performance in 3413 UBB+1 transgenic mice. At 15 months of age, UBB+1 transgenic mice display a delay in the formation of hippocampus-related spatial reference memory in the Morris watermaze task. Initial deficits were compensated for by intense training. This observation corroborates our previous results showing impaired hippocampus-dependent spatial memory at 9 months of age (Chapter 3). This cognitive deficit is not accompanied by an overt neurological phenotype. Also, procedural motor-learning and motor skills are
unimpaired in the 3413 transgenic mice at the ages of 3, 9 and 15 months. The lack of an obvious aggravation of the cognitive phenotype during aging is corroborated by the apparent equal levels of expression of the UBB\(^{+1}\) transgene during aging of the mouse (Chapter 3).

We measured motor skills with an accelerating rotarod, a test suitable to assess motor-learning and coordination in mice (Rustay et al., 2003). The cerebellum plays a central role in general motor coordination (Hikosaka et al., 2002), and in rotarod performance in C57Bl/6 mice (Goddyn et al., 2006). Additional brain regions, e.g. the striatum and motor cortex, are also implicated in rotarod motor-learning in mice (Costa et al., 2004). In the 3413 transgenic line, UBB\(^{+1}\) is expressed under the CamKII\(\alpha\) promoter, giving rise to UBB\(^{+1}\) protein expression in neurons mainly in the hippocampus, cortex and the striatum. However, the cerebellum and brainstem are devoid of UBB\(^{+1}\) (Chapter 3). Therefore, we did not expect to observe severe motor deficits. Indeed, rotarod performance was not significantly decreased in the 3413 transgenic mice at 3 or 9 months. At 15 months, the performance of the transgenic mice appeared to be modestly decreased compared to wild-type mice, although this effect reached significance only on day 8 and 9. We did detect a general age-related decline in performance, a similar effect was observed in other rotarod studies conducted in C57Bl/6 mice (Fetsko et al., 2005; Serradj and Jamon, 2007).

We did not observe a clear aggravated decline in watermaze performance of the UBB\(^{+1}\) transgenic mice between 9 and 15 months of age. However, a direct comparison of data was not feasible due to the extended acquisition required for the wild-type control mice to learn the task at 15 months of age compared to 9 months of age. This decreased performance in aged wild-type mice corresponds to results obtained in other studies showing an age-dependent decline in spatial memory in C57Bl/6 mice (Bach et al., 1999; von Bohlen und Halbach et al., 2006). Another confounding factor was the preference for the NE quadrant displayed by the 3413 transgenic mice during the first two probe trials. Possible factors contributing to this erroneous behavior could be unanticipated additional visual, auditory or olfactory cues in the testing area during the probe trials which preferentially affected the transgenic mice. It has been reported previously that extended training improves spatial memory retention in mice (e.g. (Nicolle et al., 2003)). In genetically manipulated mice, showing a spatial memory deficit on earlier probe trials, extended training can ultimately result in performance indistinguishable from wild-type mice (Gordon et al., 1996; Costa et al., 2003). We also corroborate in our study that extended training improved spatial memory in the wild-type mice and further training restored the performance of the transgenic mice to wild-type levels during the third probe trial. The transgenic mice did not perform inferior to the wild-type mice during acquisition of the task, indicating that spatial learning is intact. This is also supported by the observation that throughout probe trial 1 to probe trial 3, the percentage of time spent in the NW target quadrant increased in the wild-type mice as well as the 3413 transgenic mice.
Cognitive decline is the most salient and earliest clinical feature of AD (Walsh and Selkoe, 2004), and is also manifest in a substantial percentage of PD patients (Emre, 2003). This is reflected in many transgenic mouse models for AD, wherein AD-related neuropathology is accompanied by a decline in cognitive function, including deficits in spatial reference memory (Hsiao-Ashe, 2001). Transgenic models of PD are mainly characterized by dopaminergic changes in the brain, motor dysfunction and decline in procedural memory (Fleming and Chesselet, 2006), however age-dependent cognitive decline has also been described in a PD mouse model carrying a mutation in α-synuclein (Freichel et al., 2007). Our recent findings of hippocampus-dependent explicit memory deficiencies in 9 months-old UBB⁺¹ 3413 transgenic mice (Chapter 3) are extended in the present study to 15 months of age. The UPS inhibitory properties of UBB⁺¹ induced a modest decrease in the chymotryptic activity of the 26S proteasome in cortex homogenates of these mice (Chapter 3), suggesting that a life-long inhibition of the proteasome is related to the inferior cognitive performance. Inhibition of the UPS could thus be an underlying mechanism in AD and PD contributing to the decline of cognitive functions. Indeed, changes in components of the UPS machinery are found in brains of AD and PD patients (Keller et al., 2000; Lopez Salon et al., 2000; McNaught and Jenner, 2001).

However, the molecular mechanisms of the observed behavioral phenotype are not yet unravelled. We did not observe altered levels of (ubiquitinated) synaptic proteins in cortex homogenates of transgenic mice using proteomic analysis in previous experiments (Chapter 3). This does not rule out the possibility that subtle changes in the levels of these relatively low-abundance proteins were not detected using this setup (Garbis et al., 2005). Analyzing expression of (known) UPS substrates locally at the synapse might allow discovering the mechanism responsible for chronic proteasome inhibition-induced memory deficits. Besides proteolytic degradation, local protein synthesis is also required for long-term synaptic modulation (Pfeiffer and Huber, 2006). In rat hippocampal slices, the balance between protein synthesis and protein degradation determines long-term synaptic strength (Fonseca et al., 2006). The low-level neuronal proteasome inhibition in the UBB⁺¹ transgenic mice might disturb this delicate balance, and thus hamper long-term memory formation. Inhibition of the proteasome also impairs protein synthesis in neuronal cell lines (Ding et al., 2006), adding an additional layer of complexity to the mechanism behind UBB⁺¹ mediated cognitive decline.

In conclusion, our results point to a role for the UPS in establishment of spatial reference memory, as proteasome dysfunction induced by UBB⁺¹ expression abrogates this process in UBB⁺¹ transgenic mice. These observations provide further evidence that intact forebrain proteasome function is essential for cognitive function.
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