Research report

A detailed analysis of the early context extinction deficits seen in APPswe/PS1dE9 female mice and their relevance to preclinical Alzheimer's disease

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1. Introduction

Alzheimer’s disease (AD) is an incurable age-related neurodegenerative condition, characterised by progressive decline in cognitive and physical functions, and extensive brain damage. Identifying cognitive deficits that accompany early AD is critical, as the accompanying synaptic changes can be effectively targeted by current treatments – at present AD is typically not diagnosed until brain pathology is established, and treatment relatively ineffective. We therefore examined early cognitive changes in 4-month-old mice over-expressing 2 genes responsible for AD (APPswe/PS1dE9 mouse line). Experiment 1 tested 4-month-old female APPswe/PS1dE9 mice and their wild-type littermates on 4 validated tasks involving executive, working memory, visual recognition and reversal performance. Experiment 2 examined conditioning and extinction of an auditory stimulus paired with a sucrose reinforcer. No effect of genotype was observed. A third experiment investigated whether the context extinction impairment could be attributed to an attentional deficit. One conditioning stimulus (CS) was preexposed without consequence, and then it and a second, novel auditory CS were paired with food. Preexposure produced equal retardation of conditioning of the preexposed CS in both genotypes. However, in Experiment 2, and marginally in Experiment 3, additional tests revealed evidence of a selective impairment in context extinction in transgenic mice. These data suggest that context extinction deficits precede other cognitive impairments in APPswe/PS1dE9 mice, an effect that has intriguing parallels with findings in patients with mild AD.
be the result of changes in synaptic density that precede plaque deposition [11,12]; the fact that synaptic loss is a major correlate of cognitive deterioration in human AD is consistent with this proposal [13,14]. Research into such early cognitive deficits is therefore of critical importance, because little effective treatment is available once plaque deposition is established – and yet typically AD is not diagnosed until such a point is reached [15]. Identifying cognitive markers that are characteristic of early AD could therefore be of vital importance in devising behavioural screening tests for early diagnosis. Moreover, treatments have been reported that can reverse some of the effects of synaptic changes in AD [11,12], so early diagnosis could be critical in improving the degree to which therapeutic interventions are successful.

The aim of the present study was thus to undertake a more detailed analysis of the cognitive deficits observed in young (4-month-old) doubly transgenic mice. We took as our model the APPswe/PS1dE9 mouse [16] which is the best characterised model to date. APPswe/PS1dE9 mice co-express the mutated Swedish APP gene and the exon-9 deleted variant of the PS1 gene, and show many features characteristic of the disease. Moreover, central to the requirements of the current study, they show cognitive deficits prior to amyloid deposition, as mentioned above [6,9,10]. The dominant hypothesis to date is that cognitive decline in AD is caused by soluble oligomeric forms of Aβ which are toxic to the cells, rather than by amyloid plaque deposition [17]. Consistent with this hypothesis, elevated levels of oligomeric Aβ and synaptic deficits are observed by 3.5 months of age in the cortex and hippocampus of APPswe/PS1dE9 mice [18,19] and are associated with swollen dystrophic cholinergic neurites [20], while amyloid plaques develop between 4 and 6 months of age in these mice [21]. Females were chosen on the basis that accelerated amyloid pathology is seen in females from various APP and APP/PS1 models, including the APPswe/PS1dE9 line [22], which Pistell et al. [9] speculate may be reflected in earlier onset of cognitive impairments. Furthermore, despite the fact that women are at higher risk of developing AD [23], preclinical studies usually use male subjects.

To provide an overview of the cognitive status of these animals, we first assessed 4-month-old APPswe/PS1dE9 and WT female mice in 4 validated tasks involving 8 different cognitive and non-cognitive measures: the open-field (locomotor and anxiety-related behaviours), novel object location and recognition (episodic spatial and recognition memory), spontaneous alternation (spatial working memory) and contextual fear conditioning (associative learning, memory and memory extinction). These same tests were used in our previous studies using a different APP/PS1 model, the TAPSTM mouse line [24–27]; here the key finding was that 4-month-old transgenic mice showed a major deficit in extinction of contextual fear, despite apparently intact associative learning and memory performance. One aim of the first experiment was therefore to replicate this finding in this APPswe/PS1dE9 mouse model. Experiment 2 aimed to assess the generality of this finding by replicating it in an appetitive training procedure recently described by Bonardi et al. [28], in which an auditory conditioned stimulus (CS), rather than a context, signalled the outcome (sucrose delivery). Experiment 3 attempted to evaluate different theoretical explanations of this extinction deficit. The aim was to characterise more precisely the underlying cognitive processes affected in APPswe/PS1dE9 female mice at this early stage of disease development.

2. Experiment 1: Cognitive status of 4-month-old APPswe/PS1dE9 female mice

2.1. Materials and methods

2.1.1. Animals
Breeding stock was purchased from the Jackson laboratory; all experimental animals used in the present work were bred in the University of Nottingham's transgenic animal facility. 18 experimentally naive 17-week-old female mice were used, 9 APPswe/PS1dE9 transgenic mice (mean ad libitum weight: 22.5 g; range: 20.7–25.2 g) and 9 wild-type (WT) littermates (mean ad libitum weight: 22.2 g; range: 21.7–25.0 g). All mice were housed in the same room which was maintained on a 12/12 h light cycle, with lights on at 07:00 h; the room temperature, relative humidity and air exchange were automatically controlled. Animals were group-housed (three per cage), with ad libitum access to food and water, and provided with nesting material and a play tube.

Mice were subjected to the following sequence of behavioural testing. On Day 1, mice were assessed in the spontaneous alternation task. On Day 2 they were habituated to the object test arena and their open-field behaviour was recorded, and on Day 3 they were subjected to the sequential object location and discrimination test. Acquisition, retention and extinction of contextual fear were assessed on Days 4, 5 and 6, respectively.

2.1.2. Spontaneous alternation task
The procedure used was adapted from a previously validated protocol (see [29]). The T-maze comprised 3 arms made of grey Plexiglass, each 41.5 cm long and 6 cm wide, surrounded by walls of transparent Plexiglass (15 cm high). The start box, 6 cm × 7.5 cm, was located at the bottom of the central arm. The start box and the entrance to each arm could be closed by vertical sliding doors.

On Day 1 mice were subjected to a series of 7 trials, separated by a 5-s interval. Mice were first placed in the start box with the door closed for 5 s. The door then opened and the mice chose to enter either the left or right arm. The mice were left alone in their arm of choice for 5 s before being allowed to return to the start box. If they did not freely return to the start box, they were gently pushed back, to avoid the stress of handling. Alternation rate (alternation) of arm choice was measured over the following six trials, and unpaired t-test was used to test the impact of the genotype on this variable. For each group, alternation rate was compared to chance level (50%) using one-sample t-tests, in order to determine if the mice were able to discriminate the arm not visited during the previous trial (see [30]).

2.1.3. Novel object location and discrimination test
Mice were habituated to the open-field arena (30 cm × 35 cm × 30 cm) on Day 2. They were allowed to explore the arena freely for 30 min each day; when locomotor behaviour was recorded and analysed using Ethovision Software (Noldus, Wageningen, Netherlands). The dependent variables were the total distance moved as an indicator of locomotor performance, and the percent locomotion in the centre as an index of emotional reactivity.

On Day 3 mice were subjected to the three trials of the novel object location and discrimination test, each separated by a 1-h interval (see [25]). During the first acquisition, trial mice were presented with two identical wooden blocks (pyramid or cylinders) located in adjacent corners of the arena. They were free to explore these two objects for 10 min, after which they were returned to their home cage for 1 h. The novel object location test was carried out in the second trial, in which one of the two identical objects was moved to a new location (another corner of the arena, so that the two objects were diametrically opposed); again the mice were allowed to explore the objects freely for 10 min. The animals were again returned to their home cage for 1 h, after which they underwent the novel object discrimination test (trial 3). Here one object from the previous pair, identical in shape, was replaced by a wooden block of a different shape at the same location (for example, one pyramid might be exchanged for a cylinder) and mice were again free to explore these objects for 10 min. The position of the object moved to another location or replaced by another shape was controlled between mice to avoid side preference biases. Object shapes were also counterbalanced between mice to prevent preference biases. After each trial, the whole arena and the objects were cleaned thoroughly using 20% ethanol solution to remove olfactory markers. Behaviour was videotaped. The locomotor activity in all trials was analysed using the Ethovision software, and the object exploration times were subsequently manually scored twice by an observer blind to all treatment conditions. When the two measures differed by more than 5 s, the manual scoring was repeated and the two closest values were averaged for use in statistical analyses. A preference index was calculated for each trial to determine any location preference during the acquisition (trial 1) and object location test (trial 2), and preference for the novel over the familiar shape during the object discrimination test (trial 3); these indices were expressed as the % time spent exploring the object to be displaced in trial 1, the object at the novel location in trial 2, or the novel object in trial 3.

The impact of genotype on habituation, locomotor activity, total object exploration times and preference indices in the three trials of the object tests was compared using unpaired t-tests. As in our previous work [25,31], for each group, preference indices in the object tests were compared to chance levels (50%) using one-sample t-tests, in order to determine if the mice were able to discriminate the novel location or novel object.

2.1.4. Contextual fear conditioning
Contextual fear conditioning is a form of classical conditioning in which a simple association of a conditioned stimulus (context) with an electrical foot-shock is analysed. The study was performed as described previously [24,26]. Acquisition, retention and extinction (i.e. learning that the context is no longer aversive) of contextual fear conditioning were conducted in the same chamber.
(25 cm × 25 cm × 38 cm) consisting of three grey stainless steel walls and a fourth, transparent, Perspex wall. The chamber floor was formed with stainless steel rods spaced 1.0 cm apart. A foot-shock could be administered via a shock generator connected to alternate floor bars (Campden Instruments, Loughborough, UK). Mice were observed through a camera positioned above the behaviour apparatus. Experiments were videotaped and subsequently analysed using Ethovision Software (Noldus, Wageningen, Netherlands). For the acquisition of contextual fear conditioning (Day 4), the animals were placed in the conditioning context for 60 s, and then exposed to a foot-shock (1 s, 0.4 mA) which was followed, 60 s later, by another shock; this was repeated until 6 foot-shocks had been administered during the 6-min session. The mouse was then removed from the chamber and returned to its home cage. Increasing immobility (e.g. lack of displacement, body movement, head turn and grooming) in response to the successive shocks was used as an index of acquisition of fear conditioning. The distance moved (cm) during the minute prior to administration of the first foot-shock was recorded to control for differences in the locomotor response to the new arena.

Twenty four hours later (Day 5), recent contextual memory was tested in a retention trial, during which no shocks were delivered. The animal was placed back into the test chamber for 3 min (trial 2), and total immobility time was used as an index of the retention of the aversive experience. The mouse was then removed from the chamber and returned to its home cage.

Extinction of contextual fear memory was assessed, 24 h after the retention trial, on Day 6. Mice were again exposed to the context for 3 min, and total immobility time was used as an index of residual memory. A reduction in immobility levels with repeated trials was expected if the mouse had learned, during the previous trial, that the context was no longer aversive.

Immobility in all trials was manually scored at least twice by an observer blind to the experimental groupings, and the values were averaged. When the two measures differed by more than 10 s, the manual scoring was repeated and the two closest values were averaged. Data were then expressed as percentage time spent immobile during each minute of the acquisition trial and the 3 min of the retention and extinction trials.

Four-month-old WT and APPswe/PS1dE9 mice were compared for the acquisition of contextual fear conditioning using a two-way ANOVA with repeated measures, with genotype as the between-subject factor (2 modalities) and Shocks (1 per minute, 6 modalities) as the within-subject factor. Genotype-related changes in the locomotor response to the conditioning arena were analysed using unpaired t-tests. The effects of genotype on retention and extinction of contextual fear conditioning (immobility during trials 2 and 3) were analysed using a two-way ANOVA with genotype as the between-subject factor and repeated measure over Trials (2 modalities). Extinction of the aversive memory was reflected by a reduction in the level of immobility upon repeated exposure to the context alone, and was therefore assessed by the within-subject comparison of performance on trials 2 and 3, using paired t-tests.

2.2. Results

2.2.1. Spontaneous alternation

Arm choice was recorded over 6 consecutive trials and alternation rate was calculated as the percentage of correct choices (alternation between left and right) made. The alternation rate for each experimental group is shown in Table 1. Both WT and APPswe/PS1dE9 mice alternated above chance level (p < .05 compared to 50%, Table 1), and there was no significant difference in alternation rate between the two groups (t (16) = −0.5, p = .62).

2.2.2. Novel-object location/discrimination test

During habituation to the arena, WT and APPswe/PS1dE9 did not differ in either the total distance moved (t (16) = −1.82; p = .09) (Table 1) or the percentage activity in the centre of the arena (t (16) = −1.24; p = .10) (Table 1). APPswe/PS1dE9 mice were, however, significantly more active than WT mice during the acquisition (trial 1) of the object tests (t (16) = 3.18; p = .006) (Table 1) and the object discrimination trial (t (16) = 2.10; p = .05) (Table 1). Neither group of mice showed a preference for either object during the acquisition trial (two similar objects in the arena, p > .05 compared to 50% in all cases, Table 1), and the total level of object exploration was not different between WT and APPswe/PS1dE9 mice in any of the three trials (p > .05 in all cases, see Table 1). During the novel object location/discrimination trials, the two groups of mice demonstrated a preference for the object at the new location (p > .05 compared to 50%, Table 1), and a preference for the novel object in the following trial (p > .05 compared to 50%, Table 1). There was no effect of genotype on the preference for either the novel location, or the novel object (p > .05 in both trials, see Table 1).

2.2.3. Contextual fear conditioning

2.2.3.1. Distance moved prior to first foot-shock administration. Locomotion was recorded during the 60 s of the contextual fear conditioning acquisition trial, prior to administration of a foot-shock. There were no significant differences between 4-month-old WT and APPswe/PS1dE9 female mice for this variable (240.87 cm ± 16.25 and 232.75 cm ± 14.74, respectively; t (16) = −0.37; p = .72), indicating that immobility times were not confounded by possible differences in general activity levels.

Table 1

<table>
<thead>
<tr>
<th>Modified parameters</th>
<th>Wild-type</th>
<th>APPswe/PS1dE9</th>
<th>Genotype effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spontaneous alternation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal alternation (frequency)</td>
<td>6/9</td>
<td>7/9</td>
<td>t (16) = −0.5; p = .61</td>
</tr>
<tr>
<td>% Alternation over 6 trials</td>
<td>64.81**</td>
<td>59.26**</td>
<td>t (16) = 0.73; p = .48**</td>
</tr>
<tr>
<td>Habituation to the object tests’ arena</td>
<td>86.81</td>
<td>97.36</td>
<td>t (16) = 1.82; p = .09</td>
</tr>
<tr>
<td>Distance moved (m)</td>
<td>24.39</td>
<td>29.39</td>
<td>t (16) = −1.78; p = .09</td>
</tr>
<tr>
<td>% Activity in centre</td>
<td>28.24</td>
<td>31.25</td>
<td>t (16) = −1.32; p = .20</td>
</tr>
<tr>
<td>Distance moved (m) during object tests</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acquisition</td>
<td>23.01</td>
<td>30.79</td>
<td>t (16) = −3.18; p = .006</td>
</tr>
<tr>
<td>Novel location</td>
<td>19.73</td>
<td>23.40</td>
<td>t (16) = −1.91; p = .07</td>
</tr>
<tr>
<td>Novel object</td>
<td>15.25</td>
<td>20.88</td>
<td>t (16) = −2.10; p = .05</td>
</tr>
<tr>
<td>Total object exploration time (s)</td>
<td>22.94</td>
<td>29.39</td>
<td>t (16) = −1.78; p = .09</td>
</tr>
<tr>
<td>Novel location</td>
<td>20.78</td>
<td>24.39</td>
<td>t (16) = −1.30; p = .21</td>
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<tr>
<td>Novel object</td>
<td>17.61</td>
<td>18.78</td>
<td>t (16) = −0.40; p = .59</td>
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<tr>
<td>Object preference (%)</td>
<td>50.89</td>
<td>50.89</td>
<td>t (16) = 0.02; p = .99</td>
</tr>
<tr>
<td>Novel location</td>
<td>62.09**</td>
<td>60.13**</td>
<td>t (16) = 1.61; p = .13</td>
</tr>
<tr>
<td>Novel object</td>
<td>60.40**</td>
<td>60.70**</td>
<td>t (16) = −0.09; p = .92</td>
</tr>
</tbody>
</table>

Mean ± SE: **p < .01; *p < .0001 compared to chance level (50%, one sample t-test).

2.2.3.2. Acquisition of contextual fear conditioning. Shock-induced memory was recorded during the conditioning trial as a measure of acquisition of contextual fear. As illustrated in Fig. 1A, both groups showed increasing immobility with time, and hence repeated administration of the electric shocks ([F(5,80) = 89.45; p < .0001] Fig. 1A). There was no significant overall effect of genotype (p > .98) or shock × genotype interaction (p = .27).

2.2.3.3. Retention and extinction of contextual fear-conditioning. As illustrated in Fig. 1B, both WT and APPswe/PS1dE9 mice showed a similar level of immobility at the retention trial, indicating a lack of difference in contextual fear memory performance. The ANOVA with repeated measures revealed a significant effect of trials ([F(1,16) = 7.92; p < .01] and a significant trial × genotype interaction effect ([F(1,16) = 6.03; p = .03], but no significant effect of the genotype (p = .07). During the extinction trial, 4-month-old WT mice exhibited significantly lower immobility levels than seen during the retention trial, indicating extinction of contextual fear memory. (post-hoc paired t-test: t (8) = 4.87; p = .001) (Fig. 1B), but APPswe/PS1dE9 female mice did not (post-hoc paired t-test: t (8) = 0.21; p = .84).

3. Experiment 2: Evaluation of associative learning performance in APPswe/PS1dE9 mice using an appetitively motivated task

The data obtained in Experiment 1 reveal the existence of a selective deficit in extinction of contextual fear at the age of 4 months in APPswe/PS1dE9 female mice. We also observed that transgenic mice were hyperactive during some of the trials of the sequential object location and discrimination test, but this did not interfere with cognitive performance. In order to investigate the generality of this extinction deficit, Experiment 2 examined associative learning and extinction in an appetitively motivated procedure in which the presentation of one auditory conditioned stimulus (CS+) signalled delivery of the reinforcer (a sucrose pellet), while a second cue, the CS−, was nonreinforced [28]. The conditioned response was the head entry into the food tray. The presence of the CS− allowed us to ensure that the increase in responding to the CS+ was due to being paired with the outcome, rather than a non-specific effect of sucrose being present. After this acquisition phase both cues were presented without reinforcement, so that extinction of the initial learning could also be evaluated.
which water could be delivered, but no water was provided during these experiments. Med-PC for Windows [58] controlled experimental events, and recorded the time at which events occurred with 10 ms resolution.

3.1.3. Data treatment
Responding, entries into the foodcup, was measured during each 20 s CS presentation, and also during the 20 s preCS period that immediately preceded each stimulus presentation. The measure of conditioning was then calculated as a difference score, by subtracting the rate of responding during the preCS period from the rate of responding during the CS, pooled over all trials of a particular type in the session. PreCS response rates were also analysed, pooled across the two trial types. All data were converted to responses per minute. The data were analysed using mixed ANOVA with genotype as a between subject factor, and sessions/session block and trial type as within subjects factors; significant interactions were examined with simple main effects analysis using the pooled error term. A rejection level of \( p < 0.05 \) was employed.

3.1.4. Procedure
All mice first received magazine training, in which 10 sucrose pellets were delivered according to a VT-240s schedule over a period of 40 min. This training, which is designed to familiarise the animals with pellet retrieval from the food cup, is not normally necessary with this type of apparatus, as normal animals retrieve the pellets readily. However, we included it in this first experiment to confirm that the transgenic subjects were able to retrieve the pellets as efficiently as the control animals.

3.1.4.1. Acquisition phase. The six acquisition sessions each consisted of 15 presentations of one of the auditory stimuli followed by a sucrose pellet (CS+), and 15 of the alternative auditory stimulus followed by no outcome (CS−). For five subjects in each group the clicker was followed by sucrose and the noise was nonreinforced, and for the remaining subjects the reverse was true. Each stimulus presentation was of 20 s duration, and was preceded by a 20 s preCS period during which responding was also recorded; this comprised a trial. Trials were separated by a variable duration intertrial interval (ITI) comprising a fixed 60 s plus an additional variable interval with a mean of 60 s. The different types of trial were presented in a semi-random order.

3.1.4.2. Extinction phase. The two extinction sessions were identical to those of the acquisition phase, except that each successive 10 trials was constrained to comprise five of each trial type, and data were recorded separately for each 10-trial block; in addition neither of the stimuli were reinforced during this stage.

3.2. Results

3.2.1. Acquisition phase
The group mean difference scores for responding during the CS+ and CS− are presented in Fig. 2A. The results of the ANOVA, with group, session and trial type (CS+ and CS−) as factors, revealed main effects of trial type \( F(1,15) = 34.22, \ p < 0.001 \) and session \( F(5,75) = 16.51, \ p < 0.001 \) and a significant interaction between these two factors \( F(5,75) = 14.45, \ p < 0.001 \). It is clear that subjects learned to respond at higher rates to the CS+ than to the CS−, and simple main effects performed on the session by trial type interaction revealed that the animals were responding at a higher rate to the CS+ than to the CS− on sessions 2–6 inclusive, smallest \( F(1,90) = 5.07, \ p = 0.027 \), and that there was a significant main effect of sessions for the CS+ \( F(5,150) = 30.43, \ p < 0.001 \) but not the CS− and \( F(5,150) < 1, \ p > 0.05 \). None of the effects or interactions involving group were significant [smallest \( p = 0.24 \)]. Thus both groups learned this discrimination equally effectively.

Fig. 2B shows rates of preCS responding during acquisition; it is clear that responding decreased significantly with repeated sessions \( F(5,75) = 14.62, \ p < 0.001 \) and that transgenic animals were responding at a higher rate than control subjects \( F(1,15) = 5.16, \ p = 0.038 \); there was no significant interaction between these two factors \( F < 1 \). In order to ensure that this difference in preCS responding was not compromising interpretation of the difference scores (high preCS rates would tend to depress the difference scores, and so might mask an elevation of conditioning during the course of the experiment; both were excluded from the analysis. The transgenic animals died at the start of the study, and one WT control subject was excluded according to a VT-240s schedule over a period of 40 min. This training, which is designed to familiarise the animals with pellet retrieval from the food cup, is not normally necessary with this type of apparatus, as normal animals retrieve the pellets readily. However, we included it in this first experiment to confirm that the transgenic subjects were able to retrieve the pellets as efficiently as the control animals.

3.2.2. Extinction
Responding during CS+ and CS− trials during each ten-trial block of the two extinction sessions is shown in Fig. 2C. It is clear that responding to the CS+ dropped rapidly in the two groups, and again there was little sign of a difference between them. This impression was confirmed by the results of ANOVA with group,
Although to our knowledge no prior studies have examined LI in mouse models of transgenic animals. There are independent reasons for entertaining such a proposal. LI in transgenic and control subjects. If our interpretation of the extinction deficit for obtaining associability loss. Thus, Experiment 3 compared the development of this hypothesis, the third experiment examined latent inhibition (LI; [32]). If this loss of associability were accelerated in APPswe/PS1dE9 mice, sub-

It remains to explain the extinction deficit observed in Experiment 1. One account is in terms of attention. According to several versions of associative theory the associability of a stimulus – the attention required for a stimulus to condition - can change as a function of experience. For example, if a stimulus is followed by a predictable outcome its associability falls, and new learning will take place more slowly [32]. If this loss of associability were accelerated in APPswe/PS1dE9 mice, subsequent extinction would be retarded, and levels of conditioning maintained. To test this hypothesis, the third experiment examined latent inhibition (LI; [33]); in this same appetitively motivated procedure [28], LI refers to a procedure in which a CS is preexposed without consequence, and then paired with an unconditioned stimulus (US); conditioning occurs more slowly to this preexposed cue than to a novel CS. This is attributed to a loss of associability that occurs during the preexposure phase when the CS is reliably followed by no outcome, and is the most simple procedure for obtaining associability loss. Thus, Experiment 3 compared the development of LI in transgenic and control subjects. If our interpretation of the extinction deficit in terms of associability is to be sustained, then LI should be accentuated in the transgenic animals. There are independent reasons for entertaining such a proposal. Although to our knowledge no prior studies have examined LI in mouse models of AD, attention deficits have been reported in human subjects [34]. In addition, AD is associated with decreased cholinergic activity in the basal forebrain [35], and there have been reports that cholinergic agonists can have a profound effect on LI [36]. These considerations provide additional reasons to suppose that the brain pathology accompanying AD could have an influence on LI in these animals.

4.1. Materials and methods

This experiment adopted a within-subject design. All animals were preexposed to one of the two auditory stimuli, and in a subsequent phase both of these stimuli were paired with the sucrose reinforcer. Latent inhibition would be evident as slower acquisition of the conditioned response by the preexposed than by the non preexposed CS, and we anticipated that this difference would be accentuated in the transgenic animals. Finally, both preexposed and nonpreexposed cues were, as in the previous experiment, presented in extinction, to examine whether an extinction deficit could be observed.

4.1.1. Subjects

18 experimentally naïve 18-week-old female mice were used; one transgenic animal died during preexposure; these data were excluded from the analysis. The remaining 8 APPswe/PS1dE9 transgenic mice (mean ad libitum weight: 21.8 g; range: 20.3–23.3 g) and 9 WT control mice (mean ad libitum weight: 22.1 g; range: 20.0–24.2) were housed and fed exactly as those of Experiment 2.

4.1.2. Apparatus, materials and data treatment

These were identical to those used in Experiment 2.

4.1.3. Procedure

As the transgenic animals collected food pellets as efficiently as controls during the magazine training stage of Experiment 2, no magazine training was given in the present study. Unless otherwise mentioned, all procedural details not specified below were identical to those of Experiment 2.

4.1.3.1. Preexposure

In this stage all subjects were preexposed to one of the auditory stimuli. Five of each group were preexposed to the click, and the remaining animals to the noise. Each of the seven sessions of this stage comprised 40 trials, separated by a variable duration ITI with a fixed portion of 30 s plus a variable portion of 60 s.
4.1.3.2. Conditioning phase. The six sessions of conditioning were identical to those of the previous experiment, except that both preexposed and non preexposed CSs were followed by the delivery of a sucrose pellet.

4.1.3.3. Extinction. The single session of extinction was identical to those of the previous experiment.

4.2. Results

4.2.1. Preexposure

The group mean response rates during the preexposed CS are shown in Fig. 3A; it is clear that the CSs had little effect on background responding. Consistent with this description, ANOVA with group and sessions as factors revealed no significant effects or interactions. In contrast to the previous experiment, there was no sign that preCS responding was higher in the transgenic animals than the controls — if anything they seemed to respond at a lower rate, although this was not statistically significant. The ANOVA revealed only a significant main effect of sessions [F(5,75) = 17.8, p < .001] and trial type [F(1,15) = 7.32, p = .016]. There was no main effect of group or any interaction between these two factors [F(1,15) = 1.67 p = .22 and F < 1 respectively]. As the animals had received no food in the chambers at this stage, this decline in background responding could reflect an attenuation in exploration as the training context became familiar.

4.2.2. Conditioning phase

The results from the conditioning sessions are shown in Fig. 3B. Overall response rates in the transgenic animals were slightly lower than in the control animals, but in both groups the preexposed CS supported lower levels of conditioned responding than the novel cue, and the size of the effect appeared to be similar in the two groups. ANOVA with trial type (preexposed or not), group and session as factors revealed significant main effects of session [F(5,75) = 1.78, p < .01] and trial type [F(1,15) = 7.32, p = .016]. There was no main effect of group or any interactions involving this factor [smallest p = .16]. There was a significant interaction between trial type and sessions [F(5,75) = 2.94, p = .018]. Simple main effects revealed that there was significantly lower responding on preexposed CS trials on sessions 2, 4, and 5 (p < .009 in all cases). The mean rates of preCS responding were 10.19, 7.74, 8.68, 4.31, 2.83 and 3.19 rpm for APPswe/PS1dE9 mice, and 7.87, 7.80, 7.04, 4.53, 4.11 and 2.99 rpm for WT mice for sessions 1–6 respectively. ANOVA revealed only a main effect of sessions [F(5,75) = 12.46, p < .001; all other Fs < 1]. Thus there was no sign of an elevation of LI in the transgenic animals.

4.2.3. Extinction

The results of the extinction session are shown in Fig. 3C; numerically it appears as though the latent inhibition effect persisted in the transgenic animals but not in the controls; however, this effect was not statistically significant, as ANOVA revealed no significant effects or interactions [largest F(2,30) = 1.89 p = .17]. However, in the interests of evaluating whether an extinction deficit was present in this experiment independent of any latent inhibition effect, a further ANOVA was conducted on responding to the non preexposed stimulus alone in the extinction session. This revealed no effect of group or block, but a marginally significant interaction between these two factors [F(2,30) = 2.93, p = .069]. Simple main effects revealed that the groups differed significantly in block 2, [F(2,30) = 4.10, p = .049]. This suggests that an extinction deficit might be emerging in the slightly older transgenic subjects used in the present study.

Fig. 3D shows the preCS response rates during this session. Here there appeared to be greater responding in the transgenic animal in the first training block, and this was partly confirmed by the results of an ANOVA, which revealed a marginally significant interaction between group and block, [F(2,30) = 3.19 p = .056]; nothing else was significant [largest F(2,30) = 1.02 p = .37]. Simple main effects revealed a significant difference between the groups on block 1, [F(1,45) = 4.59, p = .038], and a marginally significant effect of trial block in the transgenic [F(2,30) = 3.16, p = .057] but not the control subjects [F < 1].

5. Summary of findings

This experiment provided no evidence that latent inhibition was accentuated in the transgenic subjects. The expected significant retardation of conditioning to the preexposed stimulus was of equivalent magnitude in the two groups; nor was there a significant deficit in extinction, replicating the failure to find an effect in Experiment 2. However, in the extinction stage there was marginal indication of persistent responding to the contextual cues, as was observed in Experiment 1.

6. Discussion

The first aim of this study was to provide an overview of the cognitive status of APPswe/PS1dE9 female mice, aged about

![Graph A: Responding to the CS and the background during preexposure](image)

- **A** Responding to the CS and the background during preexposure
  - Wild-type preCS
  - APPswe/PS1dE9 preCS
  - Wild-type difference
  - APPswe/PS1dE9 difference

![Graph B: Acquisition of conditioned responding to preexposed and nonpreexposed CSs](image)

- **B** Acquisition of conditioned responding to preexposed and nonpreexposed CSs
  - Wild-type PRE
  - APPswe/PS1dE9 PRE
  - Wild-type NON
  - APPswe/PS1dE9 NON

![Graph C: Extinction of conditioned responding to exposed and nonpreexposed CSs](image)

- **C** Extinction of conditioned responding to exposed and nonpreexposed CSs
  - Wild-type PRE
  - APPswe/PS1dE9 PRE
  - Wild-type NON
  - APPswe/PS1dE9 NON

![Graph D: Extinction of preCS responding](image)

- **D** Extinction of preCS responding
  - Wild-type
  - APPswe/PS1dE9

Fig. 3. Experiment 3. Latent inhibition in 18-week-old WT and APPswe/PS1dE9 mice (A) Mean ± SE responding during the preCS periods and the CS (corrected for preCS responding) in the seven sessions of preexposure; no effect of genotype was found. (B) Mean ± SE difference scores for the preexposed and nonpreexposed CSs during the six training sessions, showing a lack of effect of genotype on latent inhibition. (C) Mean ± SE difference scores for the preexposed and non preexposed CSs during the extinction session, showing no effect of genotype. (D) Mean ± SE rates of preCS responding during the extinction session. APPswe/PS1dE9 mice show higher background responding than WT mice, particularly during the first extinction block (interaction p = .056; effect on block 1 p = .038).
4-months-old. In this respect the results of experiment 1 were consistent with other reports in this mouse model. Indeed, 8-month-old APPswe/PS1dE9 females showed intact spontaneous alternation performance but hyper-locomotion in open-field [37]. Moreover, object recognition memory, tested in the same conditions (1 h ITI), was also found intact at 4 and 6 months of age in APPswe/PS1dE9 mice [38]. One could argue that the lack of memory impairment in the object tests could be due to the relative simplicity of the tasks. For example another protocol using 5 objects, of which one or two were displaced or substituted, revealed early deficits in spatial but not recognition memory in single transgenic Tg2576 mice [39]. Another possible explanation is the use of a short 1-h ITI in the object tests, as C57BL/6 mice have been found to be able to recognize spatial changes up to 3 h after training [40,41] and novel objects up to 24 h following training [42], although the latter is not a consistent observation [43]. There is no report of object location performance in young APPswe/PS1dE9 mice which could allow us to evaluate the impact of ITI duration; however, APPswe/PS1dE9 mice aged 9–13 months show severe object recognition memory deficits both without delay between the training and memory phase [44] and with a 4 h ITI [45]. Furthermore, in another APP/PS1 model, we and others found that object recognition memory deficits emerge at about 6 months of age using a 4-h ITI [30,46], suggesting that age is the major factor in the deterioration in object recognition memory. Contextual fear memory, assessed at 24 h post acquisition, was also found intact in 4-month-old APPswe/PS1dE9 mice [47] consistent with our data, although extinction of such memory was not tested.

Experiment 1 also revealed a selective deficit in extinction of contextual fear. This emergence of this effect at a pre-pathological age is in agreement with our previous findings in another APP/PS1 line. Indeed, we found that male TASTPM mice also showed a robust deficit in contextual fear extinction from the age of 4 months. This extinction deficit preceded the onset of impairment in recent contextual memory performance [24,26] as well as the development of spatial alternation, object recognition and spatial reference memory deficits [30,46].

But although the extinction deficit was evident in the averrively motivated conditioning procedure, in Experiments 2 and 3 no sign of a difference in extinction of a discrete auditory CS was obtained in an appetitively motivated task. As noted above, this discrepancy could be due to a number of factors. One observation that might cast light on this issue was the elevated rate of preCS responding in the transgenic subjects during the acquisition phase of Experiment 2. This could reflect no more than a differential sensitivity to the novel training environment, or heightened stereotypy or impulsivity; for example in Experiment 1 the transgenic mice also showed enhanced locomotor activity during some trials of the object tests. But it also raises the possibility that, even though extinction of discrete cues was unaffected, extinction of contextual cues was impaired. More specifically, during the magazine training given in this study the contextual cues would have become associated with food; this association would gradually extinguish during the acquisition stage, when all sucrose deliveries were signalled by an auditory cue, which would have curtailed further association forming with the context. Thus the higher preCS responding could reflect slower context extinction in the transgenic animals. Evidence in favour of such an interpretation was provided by the results of Experiment 3, in which no magazine training was given, and no such difference was observed. The fact that in Experiment 3 the animals had no experience of sucrose in the chambers before the start of training means that a strong context-sucrose association could not form – meaning that no difference in preCS responding during acquisition was observed because there was no association to extinguish. This suggestion is further supported by the fact that a marginal difference in preCS responding was evident in Experiment 3 during the extinction phase – again following training in which sucrose had been experienced in the context. The suggestion is that adult APPswe/PS1dE9 females show a selective context extinction deficit in appetitive as well as in aversive tasks, and that this effect is especially evident in tasks with strong emotional component. Moreover, the fact that the effect was observed in both appetitive and aversively motivated tasks rules out simple explanations in terms of behavioural abnormalities in general activity. In the aversive task the transgenic animals were freezing more than controls, which could be interpreted as hypoactivity; but in the appetitive task the transgenic mice were showing the opposite, more responding than controls. In addition, any explanation in terms of gross behavioural changes would have to explain why no effect was observed on acquisition either in the appetitive or the aversive task.

Altogether, these data suggest that the context extinction deficit is the earliest cognitive disturbance occurring in APP/PS1 mouse models. It is therefore crucial to understand the processes underlying this extinction deficit in transgenic mice, as this could lead to the development of human behavioural screening tests for early diagnosis of AD, as well as therapeutic strategies targeting the early stages of the disease.

The fact that the extinction deficit is primarily evident with contextual stimuli is curious, but there are various possible explanations. Discrete auditory CSs of the type used in Experiments 2 and 3 are quite different from the multimodal and spatially separated cues that comprise an experimental context. Thus a difference in sensitivity of discrete and contextual cues to an extinction deficit could be attributed to differences in perceiving the CS. For example, an auditory CS is necessarily perceived throughout its presentation, whereas the visual cues comprising the context are not – a particular corner of the box might be experienced before shock delivery on some trials but not on others. Thus conditioning to a context depends more on associations between the elements of the stimulus; for example, if the animal has learned to associate all views of the experimental chamber with each other, conditioning will occur far more readily than if he perceives one aspect of the context during a training trial and a different aspect at test. Considerations of this type could explain the differential sensitivity to the two types of cue. Moreover selective differences in conditioning to contexts and discrete cues have been reported in other work – for example in animals with hippocampal damage [48]. However, it should be acknowledged that an alternative and more mundane possibility is that the difference in sensitivity is a function of modality – perhaps the primarily visual cues that make up an experimental context are more sensitive to these effects than are auditory CSs.

A further aim of this work was to analyse the nature of the extinction deficit from an associative perspective. We found no evidence that it could be attributed to attenuated associability loss in the APPswe/PS1dE9 mice, as normal latent inhibition was observed in these animals. Another possible reason why extinction might be impaired while acquisition is unaffected relates to the nature of learning in these two procedures. It is now well established that extinction is not unlearning but rather a new learning (see [49] for a recent review), because manipulations such as the passage of time can reveal the continued existence of the extinguished association. Instead it has been argued that the omission of a shock, for example, activates an affective state of relief that counteracts the fear elicited when shock is expected [50,51]. This relief becomes associated with the CS during extinction, with the result that the conditioned response is attenuated. Thus, although the learning process that is engaged during extinction is the same as that involved in acquisition of a conditioned response, the nature of the outcome and the brain areas engaged in each task are not. Some argue that the hippocampal system plays an important role in associative learning regardless of the relevance of spatial information to any aspect
of the association [52]. Moreover, it has been suggested that the medial prefrontal cortex and basolateral amygdala mediate extinction of both aversive and appetitive conditioning [53,54], while the hippocampus modulates the contextual aspects of extinction [55]. Thus, our results showing that context extinction is altered early in APPswe/PS1dE9 mice could suggest an early impairment in neural circuitry involving the medial prefrontal cortex, basolateral amygdala and hippocampus.

In summary, we found evidence of a specific cognitive abnormality in APPswe/PS1dE9 at an age at which Aβ plaque deposition has not yet developed. This manifests as a deficit in context extinction first seen in tasks with a strong emotional component, and there was also a suggestion of its presence in non-aversive tasks. Given the importance of identifying AD prior to plaque formation, this is a potentially critical finding. The fact that context extinction deficits precede other cognitive impairments has intriguing parallels with findings in patients with mild AD [56,57] and suggests that a new kind of diagnostic test might be effective in screening for early signs of AD. The fact that this specific deficit could be also detected in an appetitive procedure is of importance, in the respect that tasks of this type could be adapted far more easily for use in humans than those using aversive outcomes.

Disclosure

The authors disclose that they have no actual or potential conflicts of interest, financial or otherwise, related to the present work.

All animal procedures were carried out in accordance with the UK Animals Scientific Procedures Act and approved by the Home Office under Project Licences 40/3283 (Experiment 1) and 40/2830 (Experiments 2 and 3).

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