Deficits in object-in-place but not relative recency performance in the APPswe/PS1dE9 mouse model of Alzheimer's disease: Implications for object recognition.

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Abstract

Performance was examined on three variants of the spontaneous object recognition (SOR) task, in 5-month old APPswe/PS1dE9 mice and wild-type littermate controls. A deficit was observed in an *object-in-place* (OIP) task, in which mice are preexposed to four different objects in specific locations, and then at test two of the objects swap locations (Experiment 2). Typically more exploration is seen of the objects which have switched location, which is taken as evidence of a *retrieval-generated priming* mechanism. However, no significant transgenic deficit was found in a *relative recency* (RR) task (Experiment 1), in which mice are exposed to two different objects in two separate sample phases, and then tested with both objects. Typically more exploration of the first-presented object is observed, which is taken as evidence of a *self-generated priming* mechanism. Nor was there any impairment in the simplest variant, the *spontaneous object recognition* (SOR) task, in which mice are preexposed to one object and then tested with the familiar and a novel object. This was true regardless of whether the sample-test interval was 5 minutes (Experiment 1) or 24 hours (Experiments 1 and 2). It is argued that SOR performance depends on retrieval-generated priming as well as self-generated priming, and our preliminary evidence suggests that the retrieval-generated priming process is especially impaired in these young transgenic animals.

1. Introduction

Alzheimer's disease (AD) is the most common senile dementia, and its prevalence in western society is a major economic and social challenge. A problem in treatment of AD is the difficulty of diagnosis; in its early stages it is hard to distinguish from normal aging or Mild Cognitive Impairment (MCI), which does not always progress into AD. Thus current drug therapies are not optimally effective because they are administered only once clear clinical symptoms are manifest [1]. But the neuropathological changes underlying AD begin many years before symptoms emerge [2], meaning early intervention is possible. Increasing importance is thus being placed on gaining a better understanding of the biomarkers and cognitive changes that characterise preclinical AD [3], to facilitate early detection and give a better idea of how and when to administer treatment. This has been addressed in part through the use of genetically modified mice which over-express one or more of three genes implicated in familial AD, and display both its neuropathological symptoms as well as its characteristic cognitive degeneration. Although imperfect analogues of human AD, these models provide a valuable shortcut for identifying potential early cognitive symptoms, and are regarded by many as a fundamental tool in understanding AD [4].

The aim of our research has been to identify early cognitive signs of AD in one specific genetic model, the double-transgenic APPswe/PS1dE9 mouse. This may be the best-characterised transgenic model of AD to date, co-expressing the mutated Swedish APP gene and also the exon-9 deleted variant of the PS1 gene [5]. Elevated levels of oligomeric Aβ in the cortex and hippocampus have been observed at 3.5 months of age in these mice; these changes are accompanied by synaptic deficits [6, 7], and are also closely associated with swollen dystrophic cholinergic neurites [8]. Although the amyloid plaques characteristic of AD have been reported at 4 months of age in these mice, it is only from 6 months that they are consistently observed [9]. Aβ deposition is paralleled by progressive degeneration of monaminergic [10, 11] and striatal [12] neurons, and neuroinflammatory reactions [13] which mirror human AD pathology. These animals also recapitulate the age-related cognitive decline characteristic of AD [14], which is thought to depend on these neuropathological changes.

The neuropathology that develops in AD in general, and in this mouse model in particular, is well specified. But precisely *which* aspects of this brain pathology underlie the cognitive deficits that are such a central feature of AD is still under debate [15]. In this particular mouse line, some findings

are consistent with the view that the degree of cognitive impairment is related to the level of plaque deposition [14]; but other work has cast doubt on this suggestion. First, cognitive deficits have been observed *before* plaque deposition in these animals [16, 17, 18]. This suggests that Aβ deposition is unrelated to cognitive decline, a conclusion supported by the fact that spatial learning deficits in older mice are correlated not with plaque load but with levels of soluble amyloid [19]. There have also been reports that manipulations that increase plaque levels *improve* performance on a spatial memory task [17]. These findings all point to the suggestion that the cognitive markers characterising the early stages of AD stem from the increased levels of soluble oligomeric Aβ which precede plaque formation rather than the plaques themselves. For example, high levels of Aβ produce local synaptic abnormalities and breakage of neuronal branches [20], and impair long-term potentiation [21, 22]; soluble Aβ is also synaptotoxic, producing a reduction in synaptic density that occurs even when plaques are absent [23]. Evidence like this has led some to argue that these Aβ-induced changes in synaptic function underlie the cognitive deterioration [24]. This interpretation is supported by the fact that cognitive decline correlates with synaptic loss in human AD [25]. Our approach has thus been based on the assumption that elevated Aβ is likely to be responsible for the earliest impairments in cognition seen in AD. Thus we have focussed on examining cognitive ability at about 4-5 months of age in these mice - by which point levels of oligomeric Aβ are elevated, but substantial plaque deposition has not yet occurred.

We concentrated on one specific component of recognition memory, the perception of familiarity [26], as a potential early cognitive symptom. A subset of patients with MCI show selective impairments in visual recognition memory - a task that relies on familiarity judgements - and distinctive patterns of grey matter loss similar to those seen in AD [27]. Thus it has been suggested that visual recognition deficits might be a diagnostic marker of the early stages of AD [28, 29]. In non-human animals this type of memory is assessed in the spontaneous object recognition task (SOR), which exploits the observation that rodents will preferentially explore a novel object in preference to one that is familiar [30, 31]. Animals are exposed to a pair of identical, junk objects, and then after a retention interval returned to the apparatus, where one of the preexposed objects has been replaced with a novel item. Selective exploration of the novel object is taken as evidence that the preexposed object is recognised as familiar. This widely-used task has revealed deficits in a wide range of different

transgenic models of AD [32], and impairments are routinely observed in older APPswe/PS1dE9 mice [33, 34, 35, 36, 37; but see 38]. SOR deficits are also occasionally reported in these mice at 6-7 months of age [34, 39, 40, 41], but never in animals younger than 6 months [40, 42]. As elevated levels of A β are present from around 3.5 months of age in this strain [6, 7], it is difficult to explain the SOR deficits in terms of this factor. However, a more detailed theoretical analysis suggests that this assessment might be misleading.

Although a number of different theoretical accounts of SOR performance have been proposed [43 44], as a starting point we focus on one, which is based on *SOP* (Sometimes Opponent Process). This is an influential theory of associative learning [45] that has proved effective in predicting and explaining a wide variety of learning phenomena [46, 47, 48]. Because SOP is unique in explaining associative learning through a specific conceptualisation of memory, it has also been successfully applied to recognition memory [49, 50, 51, 52, 53]. SOP asserts that any stimulus may be regarded as a set of elements. These are normally inactive, but stimulus presentation probabilistically activates a subset of its elements into a state of primary activation termed *A1*. *A1* is of limited capacity, and elements in this state decay rapidly into a secondary, *A2* activation state, and thence more slowly to the inactive state. These activation states differ in critical ways. First, it is typically assumed that an element in *A1* elicits more vigorous responding than one in *A2*. Second, once an element has reached the *A2* state it must become inactive again before it can re-enter *A1* - no direct transition from *A2* to *A1* is possible. This creates a refractory period during which a second presentation of a stimulus will not create as strong a response as the first, because many of its elements are 'waiting' to decay into the inactive state - meaning fewer are available for recruitment into *A1*. This transient ability of stimulus presentation to reduce the impact of subsequent presentations is termed *self-generated priming*. Stimulus elements may also reach *A2* via *retrieval-generated priming*: if two stimuli co-occur in *A1* an association forms between them, such that presentation of one is able to activate the representation of the other - *and this activation puts its elements directly into A2*. Thus when the predicted stimulus actually occurs, fewer of its elements are available to enter *A1* and the response to the stimulus is reduced.

According to this analysis both self- and retrieval-generated priming may contribute to performance on the SOR task [52]. Initial presentation of the preexposed object will prime its elements into *A1*, from where they decay into *A2*. If the test occurs before the preexposed object's

elements have returned to the inactive state, re-presentation of the object will produce less *A1* activity than a completely novel item, and result in less exploration of the familiar item through self-generated priming. But in addition, during initial preexposure associations may form between the preexposed item and the surrounding context. At test these contextual cues can prime some of the preexposed object's elements directly into *A2*, which *also* reduces exploration via retrieval-generated priming.

Experimental evidence has been provided in support of this proposal that retrieval-generated priming can contribute to SOR performance. In a series of studies with rats, Whitt et al. [54] exposed rats to two objects *P* and *Q; P* was presented in context *X* and *Q* in context *Y* (*X* and *Y* were either other objects, or patterned inserts placed round the perimeter of the experimental arena). Then the rats experienced a presentation of *X*, and were then immediately tested with *P* and *Q* in the absence of either *X* or *Y*. The rationale was that, during the sample phase, *X*→*P* and *Y*→*Q* associations would form, giving *X* the capacity to prime *P*, and *Y* to prime *Q*. The subsequent presentation of *X* would thus produce selective retrieval-generated priming of *P*, so that in the test that immediately followed, the elements of *P* would be placed in the *A2* state, resulting in selective exploration of *Q*. This is what was observed.

The fact that, in terms of this analysis, SOR may be multiply determined raises the possibility that one of the mechanisms underlying it could be impaired, but SOR performance overall could appear unaffected if the other mechanism remains intact and can mediate performance to a sufficient level. The purpose of this work was thus to explore the possibility that one of these two mechanisms underlying SOR performance might be impaired in the younger animals, even if SOR performance is not. Thus we tested performance of 5-month-old APPswe/PS1dE9 mice on tasks that independently assess the self- and retrieval-generated priming processes.

2. Experiment 1

Experiment 1 employed a relative recency (RR) task, which establishes if animals can discriminate objects on the basis of how recently they have been encountered, and provides a relatively pure measure of self-generated priming (see Figure 1). The animal is first allowed to explore object, *B* and then a different object, *A*, in the same apparatus. After a delay, the animal receives a test with both objects [51, 55]. Animals typically show a preference for object *B*, the object

encountered earlier in the series. According to SOP this task is primarily a measure of self-generated priming: as both the more recent *A* and the less recent *B* are presented before test, their elements should have all been primed into *A1*, and started to decay into *A2*; however, because *B* is encountered first, by the test *B*'s elements will have had more of a chance to return to the inactive state, and so be ready to enter *A1* and elicit a strong response, than those of *A*. Critically, as both items have been encountered in the test apparatus, both have had the same opportunity to become associated with the surrounding context and hence suffer retrieval-generated priming. Thus differences in exploration of *A* and *B* are more obviously attributed to the self-generated priming process. We conducted this RR task in 5 month old APPswe/PS1dE9 and their wild-type littermates, and then examined performance on the SOR task to confirm no deficit was evident, as the previous literature suggests. We first conducted the SOR task with a 5-min retention interval between the sample and test; as no deficit was found, we went on to employ a more difficult version of the task in which the retention interval was 24-hours in duration.

2.1. Materials and Methods

2.1.1. Animals

All experimental animals were bred in the University of Nottingham's transgenic animal facility from breeding stock purchased from the Jackson laboratory. Experiment 1 employed 15 experimentally naïve male mice, 8 APPswe/PS1dE9 transgenic mice and 7 wild-type littermates (Groups APP/PS1 and WT respectively). Testing began when they were approximately 20 weeks old and lasted about three weeks. All mice were housed in the same room, which was maintained on a 12/12 hour light cycle, with lights on at 07:00 hours; the room temperature, relative humidity and air exchange were automatically controlled. Animals were group-housed with *ad libitum* access to food and water, and provided with nesting material and a play tube.

2.1.2. Apparatus and Stimuli

The experiments were conducted in a rectangular arena with walls and floors of white translucent plastic (length × width × height: 60 cm × 40 cm × 45 cm), located in a quiet, brightly lit room. A camera was suspended from a frame 90.0 cm above the centre of the arena, flanked by two

LED spotlights 22 cm apart, producing a floor-level illumination of 50 lux. The camera view (~ 45° arc) included the entire floor and the lower part of the four walls. The trajectory of the animals' heads was tracked by Any-maze software (Version 4.5; Stoelting, Wood Dale, Illinois). Four copies of each of ten assorted junk objects (*i*) - (*x*) served as stimuli (see supplementary materials); copies of a specific object were randomly selected from this 4-object pool for any sample or test phase requiring that object. The RR task employed *i* & *ii*, and *iiii* & *iv,* the SOR (5-min) task *v* & *vi*, and the SOR (24-hour) task *vii* & *viii*, and *ix* & *x*. A square zone of length 9.5 cm was defined around each object in the arena, allowing *exploration time* - the duration of time the mouse's head was within the active zone for a particular object - to be computed. The zone size was chosen to be large enough to include a perimeter of between about 2 to 3.5 cm around the various objects, and the objects themselves were constructed on the basis of pilot work establishing the mice did not show much tendency to climb or sit on them. Visual inspection confirmed that the time the mice spent with their head in the active zone normally reflected the mouse orienting toward the object, and so we adopted the automated measure of time in the active zone as a relatively uncontaminated measure of exploration behaviour.

2.1.3. Procedure

2.1.3.1. Preexposure. Before the start of training each mouse was habituated to the empty arena. In each of seven sessions the mouse was placed in the centre of the apparatus and allowed to explore for 5 min. The floor and walls of the apparatus were cleaned with diluted alcohol before each mouse was placed in the arena.

2.1.3.2. General procedures. Both tasks involved 5-minute sample phases - two in the RR task and one in the SOR task - and a 3-minute test phase (see Figure 1). In the sample phases mice were exposed to two copies of the same object, and in the test to two different objects, *A* and *B*; *A* was the most recently experienced object in the RR task and the preexposed object in the SOR task*; B* was the less recently experienced object in the RR task, and the novel object in the SOR task. At the start of each phase the mouse was placed in the arena centre facing the gap between the two objects; on its removal the objects, walls and floor were cleaned with diluted alcohol.

In order to avoid ceiling effects, in both Experiments 1 and 2 animals received two repetitions of each task unless discrimination between *B* and *A* was very strong in the first repetition (power > .85). Thus in the present study all mice received two repetitions of the RR task, followed by

one repetition of the SOR 5-min task, and finally two of the more difficult SOR 24-hour task, giving a total of five repetitions. In each repetition objects could be placed in two of the four zones, which were situated in opposite corners of the arena. The first repetition employed the bottom left and top right corners as the two active zones, the second the top left and bottom right, and the position of the active zones continued to alternate in the remaining three task repetitions. Within each repetition stimulus identity and position were counterbalanced: thus, for example, in the first RR repetition (roughly) half the mice in each group had object *i* as *A* and *ii* as *B*, and the remainder the opposite; then all were tested with *i* at top left and *ii* at bottom right, so object identity and position were counterbalanced across *A/B* and genotype.

2.1.3.3. RR: Mice were exposed to two copies of *B* in the first sample phase, and 24 hours later to two copies of *A* in the second sample phase. The test with *A* and *B* occurred approximately five minutes after the second sample phase.

2.1.3.4. SOR 5-min: In the sample phase each mouse was exposed to two copies of *A*, and then after approximately five minutes was tested with *A* and *B*.

2.1.3.5. SOR 24-hour: This task was identical to the 5-minute version except that the test phase occurred approximately 24 hours after the sample phase.

2.2. Results

2.2.1. Data Treatment

Exploration time was computed in 1-minute bins for each of the objects in each phase for each mouse. Data from the sample phases were summed across the entire phase and all objects. Data from the test phase were computed separately for *A* and *B* in three 1-minute bins[1]. Data were analysed using mixed ANOVAs, and significant two-way interactions explored with simple main effects analysis using the pooled error term. η_p^2 was reported for significant effects and interactions.

2.2.2. RR Results

[1] Raw exploration rates were used as the primary measure, rather than the more usual discrimination ratio, because they provide a more direct index of behaviour and do not mask potential differences in baseline exploration.

Exploration was calculated separately for the first and second sample phases, to assess potential differences in exploration of *A* and *B*; for example, if *B* were explored *less* during preexposure it might be explored more at test simply because it was less familiar than *A*, rather than because it was less recent. Group mean exploration of *B* was, for Groups APP/PS1 and WT respectively, 25.98 and 22.98s; the corresponding means for *A* were 19.95 and 20.10s. ANOVA with group (APP/PS1/WT), sample (B/A) and repetition as factors revealed significant interactions between repetition and both group, $F(1, 13) = 4.92$, $p = .045$, $MSe = 115.05$, $\eta_p^2 = .27$, and sample, $F(1, 13) = 7.35$, $p = .018$, $MSe = 71.95$, $\eta_p^2 = .36$. Exploration of the Repetition * Group interaction revealed that Group APP/PS1 explored more in the first repetition (25.98s) than in the second (20.03s), $F(1, 13) = 4.80$, $p = .047$, $MSe = 115.05$, whereas Group WT did not (with means of 20.13 s and 22.95 s respectively), $F < 1$. Exploration of the Repetition * Sample interaction revealed more exploration of *B* (27.35s) than of *A* (18.69s) in repetition 1, $F(1, 13) = 14.95$, $p < .001$, $MSe = 78.95$, but not in repetition 2 (with means of 21.61s and 21,37s respectively), $F < 1$. However, the higher exploration of *B* in repetition 1 would if anything reduce, not enhance, the size of any relative recency effect.

The results of the test are presented in Figure 2 (upper panel) as *difference scores* -- time spent exploring *B* minus time spent exploring *A* -- in each minute of test (separate exploration times for *B* and *A* are presented in Table 1). In the first two minutes the less recent *B* was explored more than the more recent *A* in both groups, but by the third minute this effect had dissipated in the transgenic animals. However, ANOVA with group (APP/PS1/WT), object (*A/B*), repetition and minute as factors revealed only a significant effect of object, $F(1, 13) = 7.26$, $p = .018$, $MSe = 24.39$, $\eta_p^2 = .36$; there was no effect of group, $F(1, 13) = 2.77$, $p = .12$, $MSe = 24.56$, and no interaction between these two factors, $F(1, 13) = 1.01$, $p = .33$, $MSe = 24.39$; nothing else was significant, largest $F(2, 26) = 2.12$, $p = .14$, $MSe = 15.92$ for the effect of minute. An additional analysis performed on the data for minute 3 revealed only a main effect of group, $F(1, 13) = 4.76$, $p = .048$, $MSe = 16.12$, $\eta_p^2 = .27$; neither the Group x Object interaction, $F(1, 13) = 2.66$, $p = .127$, $MSe = 33.15$, nor anything else was significant, largest , $F(1, 13) = 2.67$, $p = .126$, $MSe = 22.62$. Thus there was no statistical evidence for the apparent attenuation of performance in the transgenic animals in the last minute of the test.

2.2.3. SOR 5-min Results

The mean exploration time during the sample phase was 16.28 s in Group APP/PS1 and 24.61 s in Group WT - somewhat lower in the transgenic mice. ANOVA with group (APP/PS1/WT) as a factor showed this to be significant, $F(1, 13) = 5.49$, $p = .036$, $MSe = 94.46$, $\eta_p^2 = .30$. The lower exploration in the transgenic mice would, if anything, increase the likelihood of seeing a deficit at test - yet this was not observed. The test difference scores are shown in Figure 2 (centre panel), and it is evident that both groups explored the novel *B* more than the familiar *A*; ANOVA with group (APP/PS1/WT), object (A/B) and minute as factors revealed a significant effect of object $F(1, 13) = 11.86$, $p = .004$, $MSe = 16.35$, $\eta_p^2 = .48$ which did not interact with group $F < 1$. There was also a significant interaction between object and minute, $F(2, 26) = 6.55$, $p = .005$, $MSe = 17.52$, $\eta_p^2 = .34$, and the effect of object was significant in minutes 1 and 2, $F(1, 39) = 19.78$, $p < .001$, $MSe = 17.13$, and $F(1, 39) = 4.17$, $p = .048$, but not in minute 3, $F < 1$. Nothing else was significant, largest $F(1, 13) = 4.06$, $p = .065$, $MSe = 10.89$. Thus the tendency to preferentially explore the novel *B* was present only in the first two minutes of the test.

2.2.4. SOR 24-hour Results

The mean exploration time during the sample phase was 15.00 s in Group APP/PS1 and 26.29 s in Group WT - again lower in the transgenic mice - and ANOVA with group (APP/PS1/WT) and repetition (1/2) as factors revealed that this was significant, $F(1, 13) = 11.49$, $p = .005$, $MSe = 165.71$, $\eta_p^2 = .47$; there was no effect or interaction involving repetition, largest $F(1, 13) = 1.16$, $p = .30$, $MSe = 72.87$. The test difference scores are shown in Figure 2 (lower panel). Although the preference for the novel *B* was modest it was consistent across the test, and again there was no sign of a deficit in the transgenic mice, despite their lower levels of exploration in the sample phase. ANOVA with group APP/PS1, object (*A/B*), repetition (1/2) and minute as factors revealed a significant effect of object $F(1, 13) = 9.31$, $p = .009$, $MSe = 10.15$, $\eta_p^2 = .42$ which did not interact with group $F < 1$. There was also a significant effect of group, $F(1, 13) = 6.77$, $p = .022$, $MSe = 21.81$, $\eta_p^2 = .34$, again revealing lower levels of exploration in the APP/PS1 animals; nothing else was significant, largest $F(1, 13) = 3.21$, $p = .096$, $MSe = 4.65$.

2.3 Discussion

The results of this first experiment replicated the results of previous studies, showing no deficit in an SOR task in 5-month-old APPswe/PS1dE9 mice. This was true regardless of whether the retention interval was 5 minutes or 24 hours, despite the fact that these tasks varied substantially in difficulty. The novel result was that there was no significant transgenic impairment in the RR task, which we have argued is a relatively pure measure of self-generated priming. If self-generated priming is the primary contributor to performance on the SOR task, then the suggestion is that this process is intact in the transgenic animals.

However, the argument that RR depends solely on self-generated priming assumes that the degree to which the context is associated with *A* and *B* is equated, because they are both presented in the context for the same amount of time during the sample phases. However, in the second sample phase in which *A* is preexposed, the context is presented without *B*, which could result in some extinction of the context→*B* association. This could reduce the degree to which the context primes *B* on test, increasing the tendency of the animals to explore this object: thus a component of RR performance could be explained in terms of retrieval-generated priming. Moreover, one might expect that the contribution of retrieval-generated priming would become increasingly evident with time, as the longer the test continues, the more elements from *A* will have decayed from A2 back to inactive, making the difference in the self-generated priming of *A* and *B* increasingly small. In this respect it is intriguing that the apparent deficit in transgenic performance was evident only at the end of the test - something which might be taken to indicate a deficit in retrieval-generated priming in these animals. Experiment 2 directly examined this possibility.

3. Experiment 2

Experiment 2 employed an object-in-place (OIP) task [56], which has been argued to provide a pure measure of the retrieval-generated priming process [52]. In the sample phase four different items were presented in an array; in the subsequent test, two of the items remained in their original positions while the remaining two items exchanged locations. Exploration of the items that have changed location is typically greater than exploration of those that have not [51, 56]. According to

SOP, this is due to retrieval-generated priming: during preexposure, associations may form between the local features of the arena and the objects that are placed there, so at test those items presented in the preexposure location will be primed by the contextual cues that still surround them; in contrast the moved items, being tested in a location with which they have not been associated, will not be primed in this way. Thus greater exploration of the moved items at test is attributed to the fact that they suffer less retrieval-generated priming than their static counterparts. Critically all test items are equally familiar, and have been experienced equally recently - so any differential exploration cannot be attributed to differences in self-generated priming. In addition, all items are presented at test in equally familiar locations. After the animals had been tested on this task they were again tested on the SOR task, but in this study only the more difficult, 24-hour version was employed.

3.1. Materials and Methods

All aspects of the method that are not specified were identical to that of the previous experiment.

3.1.1. Animals

Experiment 2 employed 15 experimentally naïve male mice, 7 APPswe/PS1dE9 transgenic mice and 8 wild-type (WT) littermates. Testing began when they were approximately 21 weeks old and lasted about two weeks. All mice were housed and maintained exactly as in the previous experiment.

3.1.2. Apparatus and Stimuli

The same apparatus was used as in the last experiment, but with the addition of wall inserts to increase the distinctiveness of the local features of the context. These inserts were made from medium density fibreboard lined with linoleum, and each one covered the whole of one of the shorter walls, and half of both longer walls, of the arena; thus two inserts covered the entire arena wall. These were 45.0 cm high, and when inside the arena reduced the floor space to 42.0 cm x 32.0 cm. Two different patterns were used, one on each side of the arena: *Mb,* a mosaic of 2.3 cm^2 blue squares whose edges were 45° from horizontal, and *Dw,* a mosaic of white 272-cm^2 squares whose edges were 90° from horizontal, with a black, 16-cm^2 square superimposed at each point where four white

squares met (see Figure 2). The same objects were used as in the previous experiment: the OIP task employed objects *i*, *ii*, *iii* & *iv*, and *vii*, *viii*, *ix* & *x*, and the SOR task objects *v* and *vi*.

3.1.3. Procedure

3.1.3.1. Preexposure. Identical to that of the previous experiment except that the preexposure sessions were of 10-min duration, and in the seventh, final session the context inserts were present.

3.1.3.2. OIP. During the sample phase each mouse was exposed to four different objects, *A*, *A'*, *B* and *B'*, one in each zones. As this task involved four objects rather than two, the duration of the sample phase was doubled to 10 minutes. The test phase was identical to the sample phase except that two of the objects, *B* and *B'*, were transposed (see Table 1); the duration of the test was 4 minutes. Animals received two repetitions of this task. Stimulus identity and position were counterbalanced across object and genotype (see Table 1). Thus, for example, for 4 transgenic and 4 wild-type mice *B* and *B'* were objects *i* and *ii* in the top left and bottom right corners during the sample phase, and for the remaining mice *B* and *B'* were objects *iii* and *iv* in the top right and bottom left corners. The context inserts were present throughout the sample and test phases.

3.1.3.3. SOR 24-hour: All mice then received one repetition of the SOR 24-hr task, conducted exactly as in the previous experiment.

3.2. Results

3.2.1. OIP Results The mean exploration time in Group APP/PS1 was 107.16 s, and for Group WT 100.52 s. ANOVA with group and repetition as factors revealed no effect of group, $F(1, 13) = 1.31$, $p = .27$, $MSe = 50.27$, but a significant effect of repetition, $F(1, 13) = 87.38$, $p < .001$, $MSe = 282.34$, $\eta_p^2 = .87$, as exploration levels were considerably higher in repetition 1 (123.86s) than in repetition 2 (83.37s); there was no Repetition x Group interaction, $F < 1$. The results from the test are shown in Figure 3 (upper panel). In the wild type mice there was numerically more exploration of the displaced objects *B* and *B'* than of *A* and *A'* throughout the test, whereas for the transgenic mice the results were far more variable, with *less* exploration of the displaced objects in minutes 2 and 4. ANOVA with group (APP/PS1/WT), object (A,A'/B,B'), repetition and minute as factors revealed significant effects of object, $F(1, 13) = 6.99$, $p = .02$, $MSe = 30.34$, $\eta_p^2 = .35$, and also of repetition, $F(1, 13) = 21.68$, p

< .001, *MSe* = 37.87, η_p^2 = .63, and minute, *F*(1, 13) = 6.44, *p* = .001, *MSe* = 10.10, η_p^2 = .33; exploration rates continued to be higher in repetition 1 (11.06s) than in repetition 2 (7.57s). Critically there was a significant Group x Object x Minute interaction, *F*(3, 18) = 3.25, *p* = .032, *MSe* = 19.22, η_p^2 = .20, suggesting that the groups might differ in their ability to perform on the task over the course of the test. To explore this interaction further, two-way ANOVAs were performed on the data from each group, with object and minute as factors. In Group APP/PS1 this revealed nothing significant, largest *F*(3, 18) = 2.68, *p* = .078, *MSe* = 13.94, η_p^2 = .31 for the effect of minute; there was no sign of an effect of object, *F* < 1. A parallel analysis conducted on the data from Group WT revealed a highly significant effect of object, *F*(1, 13) = 49.18, *p* < .001, *MSe* = 5.86, η_p^2 = .88, and also of minute, *F*(3, 18) = 5.89, *p* = .004, *MSe* = 6.80, η_p^2 = .46; the interaction was not significant, *F*(3, 18) = 1.34, *p* = .29, *MSe* = 15.88. As an alternative means of exploring the interaction we conducted an additional ANOVA on the difference scores, with group and minute as factors. This revealed a significant interaction between minute and group, *F*(3, 39) = 3.25, *p* = .032, *MSe* = 30.34, η_p^2 = .2; the main effects of group and object were not significant, *F*(1, 39) = 2.45, *p* = .140, *MSe* = 30.34 and *F* < 1 respectively. Simple main effects analysis performed on the interaction revealed that Group WT showed superior performance on minutes 2 and 4, *F*(1, 52) = 5.97, *p* = .018, *MSe* = 22.00 and *F*(1, 52) = 5.30, *p* = .025, *MSe* = 22.00, but not on minutes 1 and 3, *Fs* < 1.

3.2.2. SOR 24-hour Results The mean exploration time during the preexposure phase was 22.31 s for Group APP/PS1 and 19.28 s for Group WT, and these scores did not differ, *F* < 1. The results of the test are shown in Figure 3 (lower panel); exploration of the novel object was higher in both groups throughout the test, and although there was a tendency for accuracy to decline in the transgenic mice over the course of the test, this proved not to be significant. ANOVA with group (APP/PS1/WT), object (B/A) and minute as factors revealed a significant main effect of object, *F*(1, 13) = 26.76, *p* < .001, *MSe* = 6.91, η_p^2 = .67, but there were no significant effects or interactions involving group, largest *F*(1, 13) = 1.10, *p* = .34, *MSe* = 6.20, and nothing else was significant, largest *F*(1, 13) = 1.65, *p* = .21, *MSe* = 9.84.

3.3. β–Amyloid Pathology

To confirm the presence of β–Amyloid pathology in male APPswe/PS1dE9 mice of this age, we examined the brains from a different cohort of 4.5-month old male APPswe/PS1dE9. Brains were post fixed in 4% paraformaldehyde for 6h and kept in 70% ethanol overnight before being embedded in paraffin wax on a tissue embedding station (Leica TP1020). Immunostaining was carried out using standard procedures at room temperature on 7µm-thick coronal sections. Briefly, all the solutions were freshly prepared using PBS + 1% Tween 80, except DAB solution that was prepared in distilled water. The tissue was re-hydrated in consecutive rinses in xylene, 100% ethanol, 70% ethanol and distilled water. Antigen retrieval was performed by incubation in 10mM EDTA pH 6.0 for 20 min at 95°C, followed by incubation in formic acid for 1 min. Tissue was then blocked in 5% normal horse serum, incubated in mouse monoclonal anti- β -amyloid antibody (1:2000, A5213 Sigma Aldrich, St. Louis, MO, USA) for 1h followed by 1 h incubation with anti-mouse secondary antibody (1:200; Vector Laboratories Inc. Burlingame, CA). After washing, sections were incubated with Vectastain Elite ABC kit (Vector Laboratories Inc. Burlingame, CA) and labelled with DAB peroxidase substrate (Vector Laboratories, Burlingame, CA) according to manufacturer's instructions. To reveal histological morphology, sections were then counterstained with haematoxylin (purplish-blue nuclear stain) and eosin (pink cytoplasmic stain) and mounted with DPX-mount media. Digital focused photo-scanning images were acquired using a Hamamatsu NanoZoomer-XR with TDI camera technology. Figure 4 shows illustrative examples of amyloid-β42 staining generated from brain tissue from 4.5 month old male mice APPswe/PS1dE9, and it is evident that although amyloid pathology had begun to emerge, it was slight and largely confined to cortex and hippocampus.

3.4 Discussion

In this experiment a deficit in performance on the OIP task was observed in the transgenic mice: the wild type animals showed a consistent preference for exploring the displaced objects throughout testing whereas the transgenic animals did not, performing significantly worse than their wild type counterparts on minutes 2 and 4 of the 4-minute test. Once again these same transgenic animals showed normal performance in the SOR task. In combination with the results of Experiment 1, the results are interpretable in terms of SOP if it is assumed that the transgenic animals have a deficit in retrieval-generated, but not self-generated, priming.

But before this conclusion is accepted, alternative interpretations of these results should be considered. First, this task differed from the SOR and RR tasks in that it required the animals to form an association between the contextual inserts and the objects. If the transgenic animals had some difficulty processing distal visual cues which, for example, rendered them unable to see the contexts clearly while they were exploring the objects, this could provide an alternative explanation of these results. However, we are not aware of any evidence suggesting that visual deficits are responsible for impaired performance in these animals. For example, Jardanhazi-Kurutz et al. [34] demonstrated that 4.5-month old APPswe/PS1dE9 mice were impaired in a spatial learning task in the Morris water maze - yet performed as well as wild types locating the platform when it was visible. But this raises a second possibility - that the present results could be interpreted as the spatial learning deficit that we know can be present in transgenic mice of this age. However, the extent to which this should be viewed as an alternative interpretation depends on the specific interpretation of spatial learning that is adopted. Some have argued that performance on spatial tasks can be explained in terms of associative learning processes just like those underlying retrieval-generated priming [57]. If this is the case, then impairments in both spatial learning and the OIP task studied here may be interpreted as a failure of retrieval-generated priming.

On a more procedural note, we attempted to make the three tasks comparable: in all versions the test locations were equally familiar, and the identity and location of novel and familiar items counterbalanced. But there were other differences between them; for example, the SOR task involved preexposure of one item, the RR task of two items, and the OIP task of four; an alternative interpretation is thus that a deficit emerges with an increase in the total number of items to be explored. While this is logically possible, it would imply that some evidence of a deficit should have been observed in the RR task -- but although there was a numerical tendency for transgenic mice to perform worse at the end of the RR test, this was not significant. Moreover, a similar nonsignificant tendency for a decline in performance in the transgenic mice was evident in the SOR task in Experiment 2. In addition, solution of the OIP task does not require the mice to encode all four objects - the reason we employed four rather than two was to ensure that all test locations were equated in familiarity. Finally, although the OIP task requires more objects to be explored in the same sample phase, we doubled the length of preexposure in this experiment to accommodate this. These

arguments notwithstanding, further experimental work would be necessary to definitely rule out such alternative interpretations.

4. General Discussion

Two experiments examined the performance of 5-month old APPswe/PS1dE9 mice on three variants of the SOR task. Although SOR performance is typically unaffected in this model at this age, it was argued that according to one specific model of recognition memory, SOP [45], performance on SOR depends on two independent cognitive mechanisms: self-generated and retrieval-generated priming [52]. Thus even if one of these mechanisms were impaired, SOR performance could appear unaffected provided the other remained intact enough to support accurate performance. This appeared to be the case. In Experiment 2 we examined performance on the OIP task, which we argue provides a pure measure of retrieval-generated priming. The transgenic mice performed significantly worse than the wild types on two of the four minutes of the test, and overall - in contrast to the wild type animals - showed no significant preference for the displaced objects. In contrast, in Experiment 1 mice of the same age were not significantly impaired on a relative recency task, supposedly a measure of self-generated priming [52]. In both experiments the transgenic mice performed normally on the SOR task, mirroring previous work using animals of this age [40, 42]. These results, although preliminary, are consistent with the suggestion that the mice suffer a selective deficit in retrieval- but not self-generated priming at 5 months of age, and that it is only at 6 months of age that the self-generated priming condition is also affected, and hence a net impairment in SOR observed. In fact the suggestion that the self- and retrieval generated priming mechanisms may be dissociated is not without precedent. Recent work on the GluA1 knockout mouse - in which the GluA1 subunit of the AMPA receptor, an important mediator of synaptic plasticity in the hippocampus, is deleted - has revealed a pattern complementary to that reported here, a deficit in self- but not retrieval-generated priming. Specifically, these mice showed an impairment on SOR and RR tasks, but performed normally on an SOR task variant that relied on retrieval-generated priming [58].

This argument implies that young transgenic animals perform accurately on the SOR task using *only* self-generated priming: because elements of the stimulus presented during the sample phase have not all decayed from the *A2* state by test, they will not all be available to enter *A1* and

elicit a strong exploratory response. But while assuming decay from *A2* is incomplete after 5 minutes is plausible, it is less so when the sample-test interval is 24 hours. With such a long delay it seems natural to attribute SOR performance to retrieval-generated priming, as this depends on the formation of associations which do not decay appreciably with the passage of time. So if retrieval-generated priming were impaired in the transgenic mice, how could they perform accurately on the 24-hour version of the task? One possibility is that there are two types of association that can support this retrieval-generated priming mechanism, only one of which is affected in the transgenic animals. We appealed solely to formation of an association between the preexposed object and the surrounding context, which is precisely what was tapped by the OIP task; but it might also be the case that associations form *among the elements of the object itself* [59]. If elements of each object were inter-associated in this manner, when a mouse begins to explore a familiar object the associations among its elements could result in retrieval-generated priming of the rest of the object. This would ensure that *all* the object's elements can be primed into *A2* reducing exploration. This process could also mediate accurate SOR performance (although it would have no effect in the OIP task as all items are equally familiar, and so the possibility of such intra-object associations forming is equated). If the transgenic mice were less able to form associations between the object and the surrounding context than among the object's elements themselves, this could explain why the transgenic animals showed a deficit in OIP but not the 24-hour SOR task. For example, different elements of the same object are more likely to be experienced in close temporal proximity than are the object and the contextual cues that surround it - and imposing a delay between events makes them more difficult to associate [60]. Interestingly, deficits in taste aversion learning - in which ingestion of a flavoured liquid is followed some time later with illness - have been reported in 2-5 month old APPswe/PS1dE9 mice [18, 61]. Thus maybe a difficulty in associating temporally distant events is an early cognitive manifestation of AD in this mouse model. Alternatively, contextual cues may have a spatial component - so that associating the object with the context involves associating elements in different dimensions (visual and spatial), in a way that associating elements of the same object may not. If transgenic mice were poorer at forming associations between visual and spatial cues, this could explain the results. Indeed, something very similar was reported by Swainson et al. [62], in a longitudinal study in which a battery of cognitive tests was performed on participants with either mild AD, questionable dementia or depression, as well as healthy controls. The test that best discriminated those with AD from other

participants was a '*visuo-spatial paired associative learning task*' in which participants had to encode where in a display a particular shape had been presented. Poor performance in this task was also evident in a subgroup of those with questionable dementia, and correlated with their subsequent cognitive deterioration - suggesting the task might be a useful diagnostic indicator of incipient AD.

These results may also be accommodated by other accounts of recognition memory, which attempt to make sense of dissociations in recognition performance evident in lesion studies. For example, Brown and Aggleton [63] proposed that recognition tasks can be divided into tasks such as SOR and RR, which require that only one item be remembered at a time and depend on perirhinal cortex, and those like OIP which rely on memory with a spatial or associative component, are hippocampal-dependent, and often involve the rearrangement or re-pairing of familiar items. A related approach has been taken by Saksida, Bussey and colleages [64], who argued that tasks like SOR and RR are in part supported by memory for stimulus conjunctions (as opposed to individual stimulus features), and mediated by the perirhinal cortex; in contrast tasks like OIP, which require representation of the object and the temporal or spatial aspects of the context, are more dependent on the hippocampus. These dissociations imply that the deficits observed in our mice are likely to stem from a selective disturbance in hippocampal function. In fact there is good evidence that neuropathological changes begin emerge in both cortex and hippocampus in the APPswe/PS1dE9 mouse at the age at which this study was conducted [9, 17]. This is an observation we confirmed in our own animals, showing that at 4.5 months of age the handful of plaques that are evident are confined to cortex and hippocampus. However we are not aware of any evidence that hippocampus is affected *before* the perirhinal cortex, as such models might predict on the basis of the results we have presented.

If our results may be taken to demonstrate a selective deficit in the OIP task in transgenic animals in the pre-plaque stage of the disease, this would suggest that performance on this task might be a diagnostic marker for the early stages of AD. There are other findings from the human literature that are also consistent with this suggestion. Several authors have reported selective deficits in AD patients on tasks which require the association of different aspects of a stimulus - termed *memory binding* [62, 65, 66, 67], and one particular study by Parra et al. [67] described results that closely mirrored our findings. Participants were presented with two visual stimuli with various colours

(C) and shapes (S). In different variants of the task these two stimuli differed either in colour (C_1S_1 & C_2S_1) or shape (C_1S_1 and C_1S_2) or both (C_1S_1 & C_2S_2) - the *binding* condition. In the former two conditions the participants with tested with the original items, but with the originally discriminating feature replaced by a novel one - i.e. C_1S_1 & $\boldsymbol{C_3}S_1$ or C_1S_1 & $C_1\boldsymbol{S_3}$, meaning they had to discriminate items with familiar and novel features - a parallel of the SOR task. In contrast, in the binding condition they were tested either with the sample items, or with items in which the two features of the sample items were rearranged (C_1S_2 & C_2S_1), meaning they had to discriminate between equally familiar features in either familiar or unfamiliar combinations - a parallel of the OIP task. They found a selective deficit in the binding task in patients with a form of familial AD linked to a specific mutation, and also in their asymptomatic relatives who were carriers of the mutation, relative to healthy non-carrier controls. Reports of this type encourage the suggestion that our results are not confined to the mouse model in which they were found, but may also have parallels in experiments using human participants, lending them translational value.

We are not aware of any other studies conducted on this particular transgenic model of AD which examine performance on recognition tasks more complex than the standard SOR task. However a recent series of studies by Davis and colleagues [68, 69] evaluated performance of a triple-transgenic model of AD, which has tau pathology as well as APP and PS1 mutations, on several variants of the SOR task. They found that younger mice performed normally on SOR, RR and OIP tasks, but showed a relatively selective impairment in a '*what-where-which*' task, in which the mice were preexposed to the same pair of objects in the transposed positions in two distinctive contexts (Figure 5), before being tested with two replicas of one of the objects in one of the contexts. Correct performance required the mouse to selectively explore the object that had not been experienced in that position in that context, and the authors interpreted this in terms of an episodic-like memory impairment. One interpretation is that these results represent a conflict of data, in that the young double-transgenic mice were impaired on the simpler task whereas the triple-transgenic mice were not. However, even within the class of APPswe/PS1 transgenic models there can be substantial variation in the pathology expressed (for example, in the amount of Aβ deposited, and the structure and appearance of the plaques [70]. In addition one marked difference was that their version of the OIP task was simpler than the one we employed, involving preexposure of two different objects and then testing two identical copies of the same object (Figure 5). Any such procedural differences could

have been responsible for the difference in results, and further work would be needed to resolve these issues.

We interpreted our findings within the framework of a general model of learning and memory, SOP [45] which, although primarily used in the context of animal behaviour, can be applied more generally. In this sense work of this type may provide a novel perspective on cognitive phenomena from that offered by theories developed solely to explain human cognition. As we have seen, SOP offers a comprehensive account of the processes underlying recognition memory, interpreting them in terms of more fundamental principles of associative learning. It also interprets the SOR, RR and OIP tasks we employed within this same framework. As these tasks may be regarded as respectively tapping the *what*, *when* and *where* components that characterise episodic memory [71], SOP may also offer a new perspective on this important memory phenomenon. Interpreting both animal and human findings in terms of this associative learning model could therefore yield new insights into the early cognitive deficits of AD, as well as underpinning the translational work that is inevitable for successful drug development.

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 Licence 40/3444.

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Figure Legends

Figure 1: Schematic of the spontaneous obect recognition (SOR), Relative Recency (RR) and Object-in-Place (OIP) tasks. A, B, A' and B' refer to various junk objects. For further details see text.

Figure 2: Experiment 1: Group mean difference scores (exploration of B minus exploration of A) in each minute of test in the Relative Recency (upper panel), Spontaneous Object Recognition 5-min (centre panel) and Spontaneous Object Recognition 24-hr (lower panel) tasks. Error bars show standard error of the mean.

Figure 3: Experiment 2: Group mean difference scores (exploration of B/B' minus exploration of A/A') in each minute of test in the Object-in-Place task (upper panel), and corresponding scores (exploration of B minus exploration of A) for the Spontaneous Object Recognition 24-hr task (lower panel). Error bars show standard error of the mean.

Figure 4: Schematic of the What-Where-Which and Object-in-Place tasks employed by Davis et al. (2013). A, B, A' and B' refer to various junk objects. For further details see text.

Figure 5: Some representative samples of b-amyloid 42 staining generated from brain tissue of 4.5-month old male APPswe/PS1dE9 mice.

Figure(s)

Figure 1: Schematic of the spontaneous obect recognition (SOR), Relative Recency (RR) and Object-in-Place (OIP) tasks. A, B, A' and B' refer to various junk objects. For further details see text.

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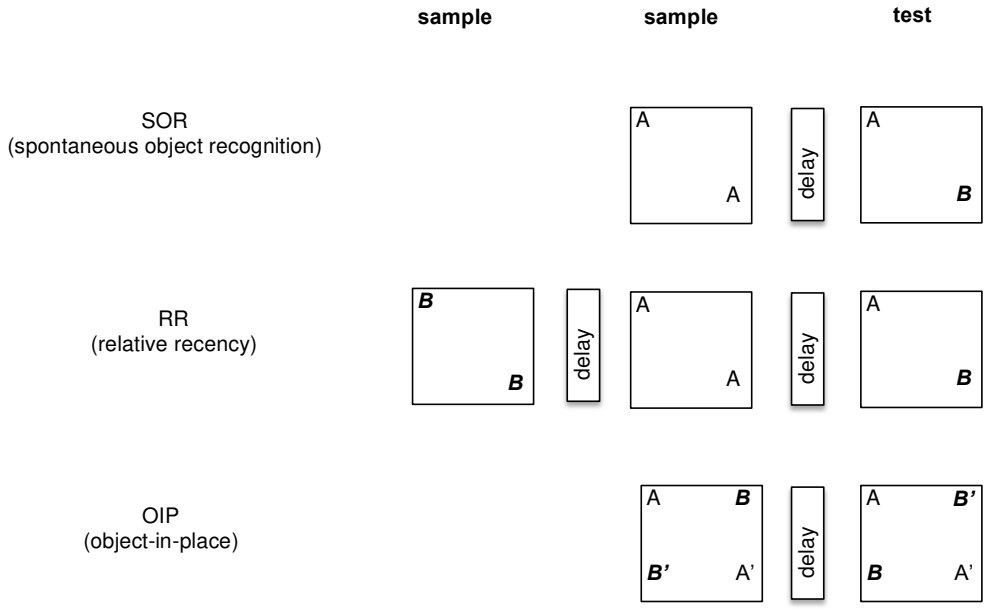


Figure 2: Experiment 1: Group mean difference scores (exploration of B minus exploration of A) in each minute of test in the Relative Recency (upper panel), Spontaneous Object Recognition 5-min (centre panel) and Spontaneous Object Recognition 24-hr (lower panel) tasks. Error bars show standard error of the mean.

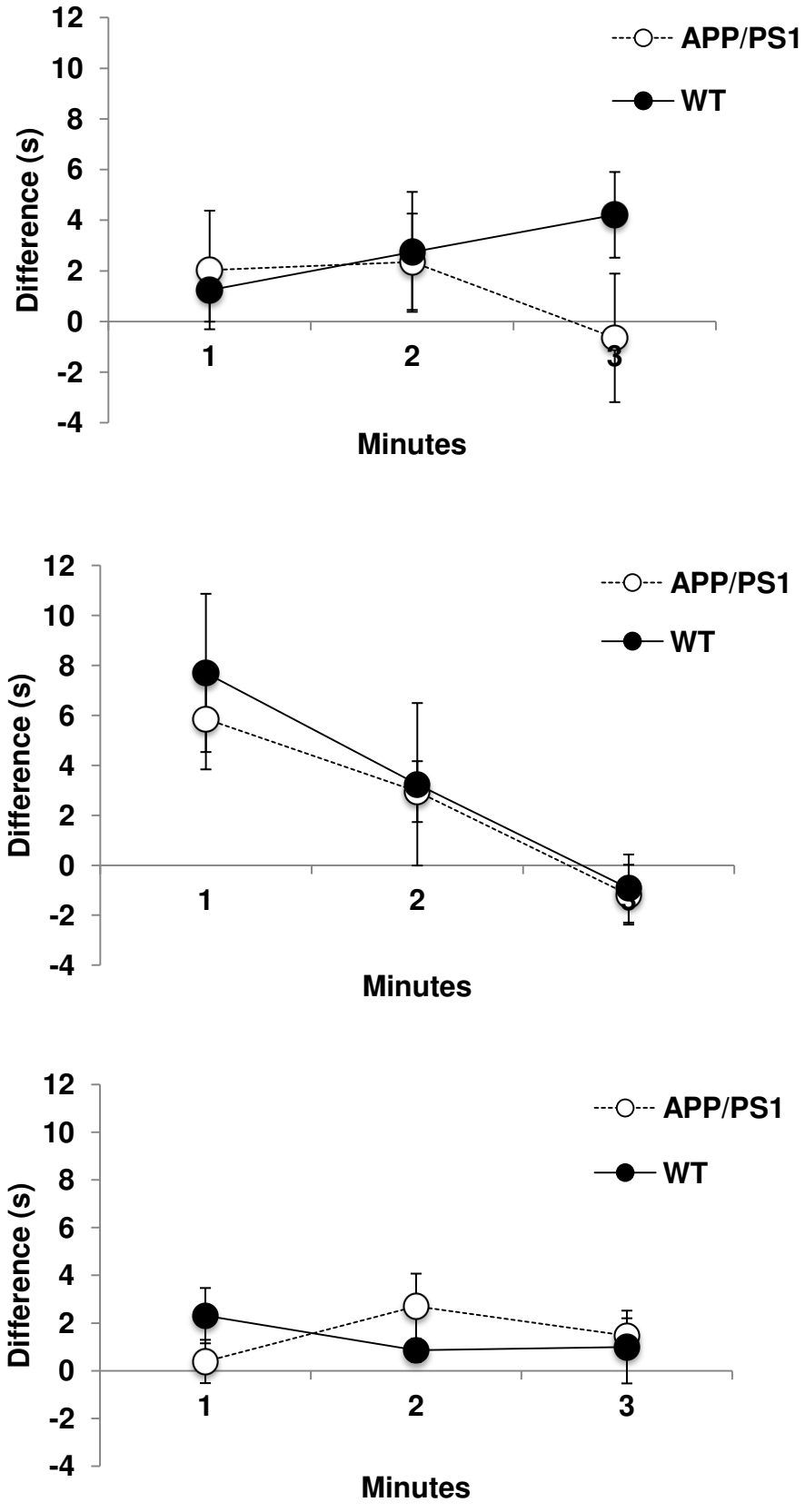
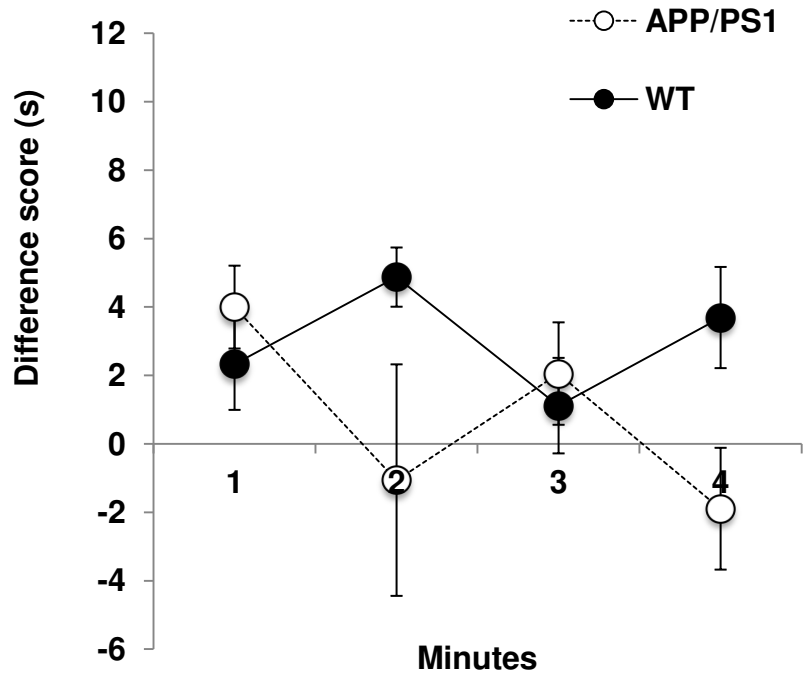


Figure 3: Experiment 2: Group mean difference scores (exploration of B/B' minus exploration of A/A') in each minute of test in the Object-in-Place task (upper panel), and corresponding scores (exploration of B minus exploration of A) for the Spontaneous Object Recognition 24-hr task (lower panel). Error bars show standard error of the mean.



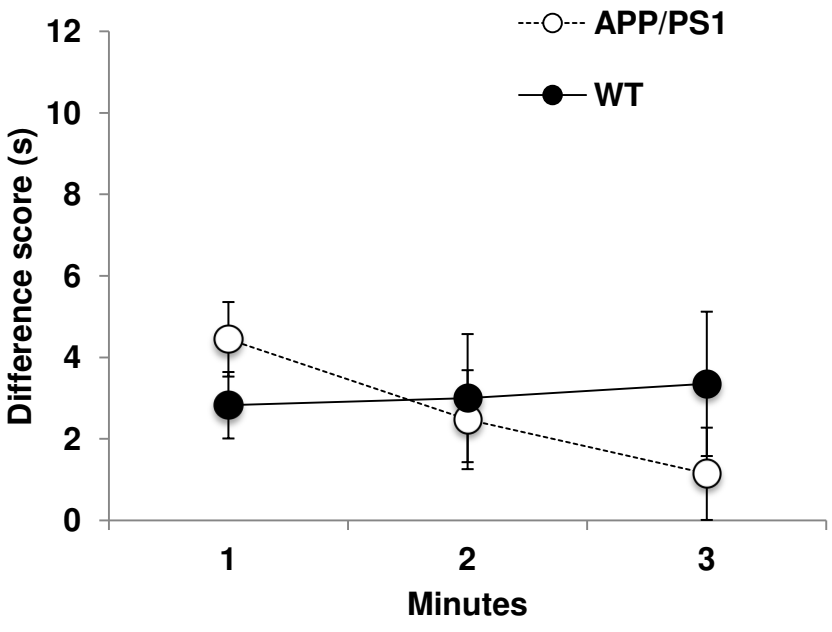


Figure 4: Some representative samples of b-amyloid 42 staining generated from brain tissue of 4.5-month old male APPswe/PS1dE9 mice.

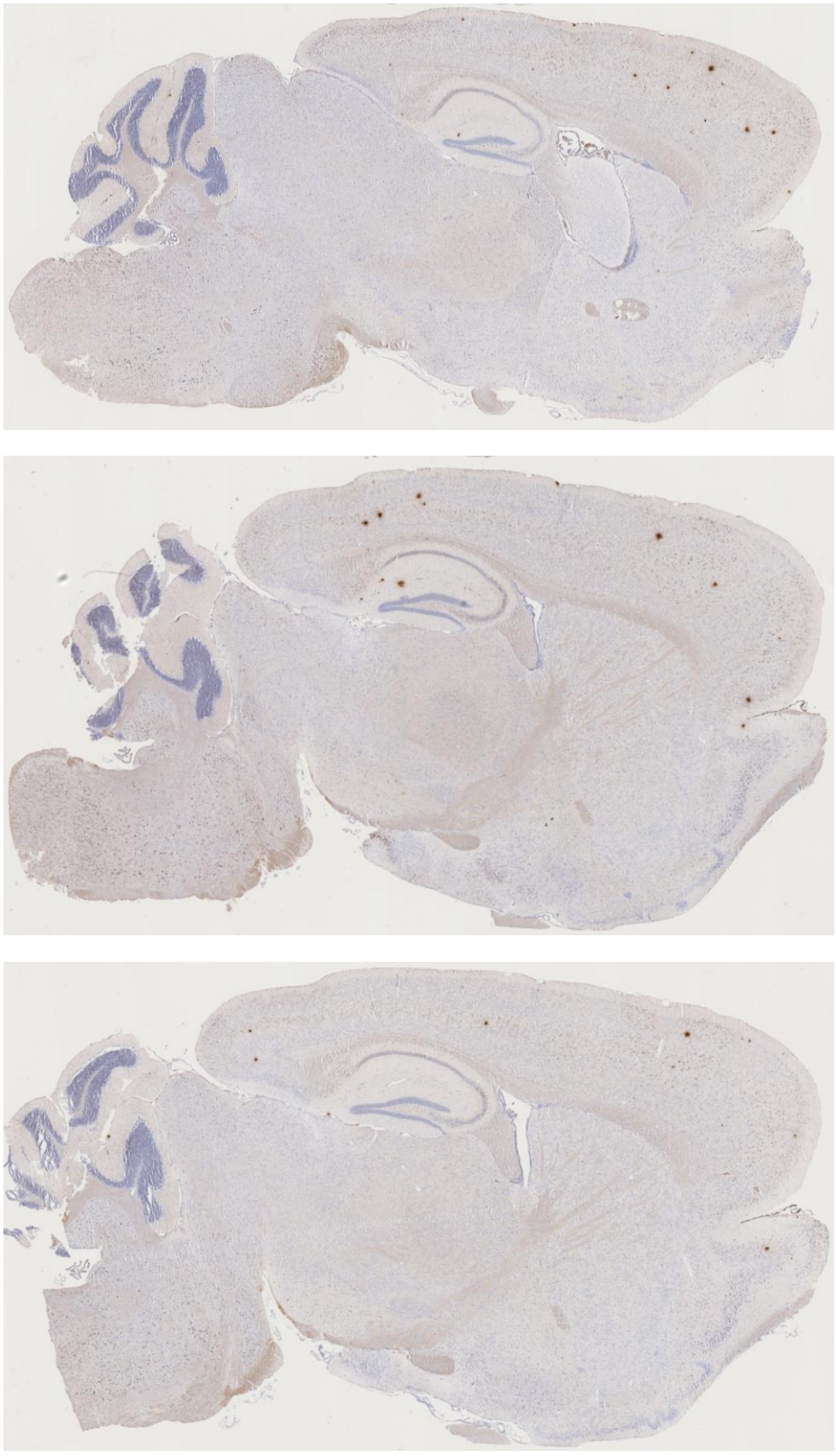


Figure 5: Schematic of the What-Where-Which and Object-in-Place tasks employed by Davis et al. (2013). A, B, A' and B' refer to various junk objects. For further details see text.

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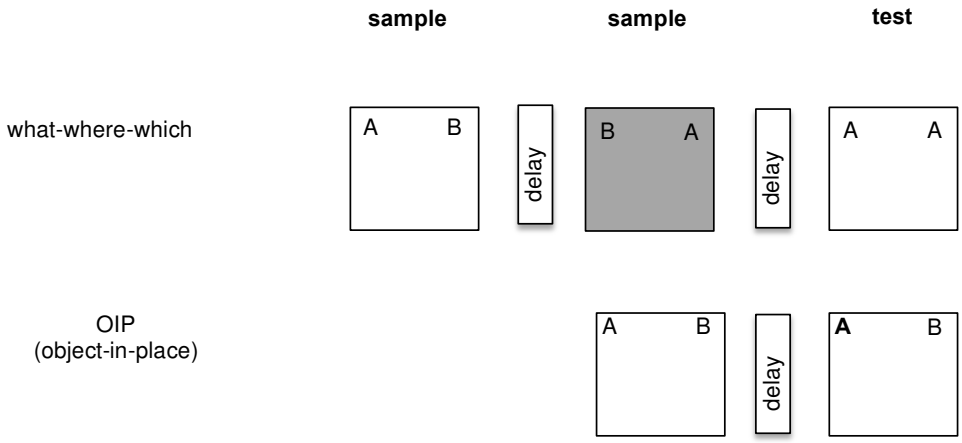

Table(s)

Table 1: Group mean time (sec) exploring the novel (B) and familiar (A) objects in each replication of the Relative Recency (RR), Spontaneous Object Recognition (SOR) 5-min and 24-hour tasks in each minute of the test of Experiment 1.

Minute		1		2		3	
RR		B	A	B	A	B	A
Rep 1	APP/PS1	5.9	3.6	7.0	5.6	6.1	7.0
	WT	5.3	3.8	6.5	5.5	10.1	3.4
Rep 2	APP/PS1	5.5	3.7	6.6	3.2	3.4	3.8
	WT	6.0	5.0	9.6	5.2	8.7	7.0
SOR 5-min							
	APP/PS1	8.3	2.5	6.4	3.5	4.4	5.6
	WT	11.5	3.8	8.1	4.9	4.9	5.8
SOR 24-hr							
	APP/PS1	2.7	2.5	5.1	3.2	3.7	4.2
	WT	6.2	4.6	6.2	5.7	6.0	4.4
	APP/PS1	2.6	2.0	5.5	2.0	4.8	1.3
	WT	6.3	3.2	4.7	3.5	5.4	4.9

Table 2: Group mean time (sec) exploring the displaced (B) and static (A), or novel (B) and familiar (A), objects in each replication of the Object-in-Place (OIP) and Spontaneous Object Recognition (SOR) 24-hour tasks in each minute of the test of Experiment 2.

Minute		1		2		3		4	
OIP		B	A	B	A	B	A	B	A
Rep 1	APP/PS1	14.2	9.6	12.1	14.1	13.6	8.3	11.2	13.0
	WT	12.8	12.2	12.0	7.2	12.4	9.8	13.1	9.7
Rep 2	APP/PS1	12.1	8.7	8.1	8.3	5.5	6.7	6.3	8.4
	WT	11.7	7.6	10.5	5.4	6.1	6.4	9.1	5.2
SOR 24-hr									
	APP/PS1	7.3	2.9	5.5	3.0	4.0	2.9		
	WT	5.6	2.7	7.1	4.1	5.3	1.9		

Supplementary Material

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