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Research report

Mood and male sexual behaviour in the APP23 model of Alzheimer's disease

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Abstract

Alzheimer's disease is characterised by both cognitive deterioration and the development of a wide range of neuropsychiatric disturbances, among which affective disturbances, stereotyped behaviour, dietary hyperactivity and changes in sexual behaviour. The transgenic APP23 mouse models Alzheimer's disease and has shown to be a unique tool in the study of this condition. APP23 mice develop, next to the age-dependent cognitive decline, also a range of behavioural problems, such as circadian activity disturbances and increased aggression, in analogy with the dementing patients. The present study aimed to investigate whether this model also develops mood disturbances and changes in sexual behaviour. Using two behavioural despair paradigms and the sucrose preference test, we did not find evidence for the development of depression-related behaviours. A sophisticated protocol was neither able to unravel changes in male sexual behaviour between APP23 and WT mice. The present study nevertheless provides evidence that the APP23 mice are more anxious and fearful in comparison with control littermates, which opens perspectives to future treatment studies.

Keywords: Alzheimer's disease; APP23 model; Transgenic mouse model; Depressive behaviour; Anxiety; Sexual behaviour

1. Introduction

Alzheimer's disease (AD) is characterised by both cognitive deterioration and the development of a wide range of neuropsychiatric disturbances. Reisberg et al. [27] classified these various behavioural disturbances into seven categories; delusions, hallucinations, activity disturbances, aggression and agitation, diurnal rhythm disturbances, depression, anxiety and phobia, and clustered them under the name of behavioural and psychological signs and symptoms of dementia (BPSD). Besides these, dementing patients also present with other behavioural problems, such as stereotyped behaviour, dietary hyperactivity and changes in sexual behaviour [10]. Anxiety and depressive behaviour seem to cluster in one neuropsychiatric sub-syndrome of affective behaviours [1]. Whilst depression has been the focus of a number of studies in people with dementia (for review see Ref. [34]), far less attention has been paid to anxiety and phobia. However, anxiety symptoms are common, with a prevalence in excess of 30% [15,23]. A secondary accompanying manifestation to depression includes disturbances affecting sexual

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behaviour. The most frequent sexual disorder is sexual indifference, however a small fraction of the dementing population remains sexually active and controversially shows hypersexuality, often considered as inappropriate [12,28]. Hyperactive sexual behaviour is rare, and might in fact express affective needs [11].

APP23 transgenic mice harbour the Swedish double mutation, known to cause early-onset familial AD in humans. The inserted transgenic construct leads to a seven-fold overexpression of the human amyloid precursor protein (APP), in comparison with the endogenous, murine APP. The APP23 model has shown to be a unique tool in the study of AD (for review see Ref. [38]). Especially fascinating is the fact that – next to the age-dependent cognitive decline [37] – the transgenic mice also develop a range of behavioural problems in analogy with the dementing patients, such as circadian activity disturbances [41] and increased aggression [39]. Although the evaluation of mood-related disturbances in animal models is not straightforward, several paradigms to investigate anxiety and depression-related symptoms in rodents have been developed. The present study aims to investigate whether the APP23 model develops mood disturbances and changes in sexual behaviour in addition to the other behavioural problems.

2. Materials and methods

2.1. Transgenic mouse model

Sturchler-Pierrat et al. [30] have generated transgenic APP23 mice in a hybrid C57BL/6 × DBA2 background. They have introduced a genetic construct, i.e. the neuron-specific murine Thy-1.2 promoter, followed by human APP 751 cDNA encoding the Swedish double mutation (K670N/M671L), which is known to cause early onset familial AD. To provide a genetically homogeneous (isogenic) line, the engineered transgenic mice were backcrossed to the C57BL/6J strain for at least 20 generations. Genotypes (i.e. the presence or absence of the transgenic construct) were identified by polymerase chain reaction as previously described [40].

Male heterozygous APP23 mice and wild-type (WT) control littermates were bred by crossing heterozygous APP23 males with control inbred C57BL/6J females. Young (6 weeks old) stimulus C57BL/6J females for the sexual behaviour experiments were purchased from Charles River laboratories (Brussel, Belgium). All animals were group-housed in standard mouse cages (38.2 cm × 22 cm × 15 cm), unless otherwise specified for a particular behavioural assessment. Conventional laboratory conditions – constant room temperature (22 ± 2 °C), humidity level (55 ± 5%), 12 h light:12 h dark cycle (lights on at 8 a.m.) – were maintained and food and water were available *ad libitum*.

All experiments were approved by the local Animal Ethics Committee and performed in accordance with the European Communities Council Directive (86/609/EEC).

2.2. Depression-related tests

Depression-related symptoms in rodents can be evaluated by means of stress models. In these, a mouse is faced with a stressful, inescapable situation that has been suggested to engender a state of behavioural despair, akin to the hopelessness manifest in clinical depression. The Porsolt forced swim test (FST) measures the time spent swimming versus the time spent floating in a tall cylinder filled with water [26]. We used a glass 5 L cylinder (27 × 16 cm; height × diameter), filled to a depth of 15 cm with water maintained at 25 ± 2 °C. The observation period typically lasted 6 min, during which cumulative immobility was scored by the experimenter. Immobility was scored; "when a mouse ceased struggling and remained floating motionless in the water, making only those movements necessary to keep its head above the water" [29].

A behavioural despair test with considerable face value similarity is the tail suspension test (TST), in which we suspended mice by the tip of their tail to a rod with adhesive tape at 60 cm above tabletop. The observer recorded cumulative immobility time versus the time that they struggled during a 6 min observation period. Immobility was defined as "hanging passively and completely motionless" [3].

A core symptom of depression is anhedonia, defined as the decreased capacity to experience pleasure of any sort [2]. This feature is modelled in rodents as a decrease in responsiveness to rewards, such as a sweet sucrose solution. For our sucrose preference test, mice were isolated in small cages (22.5 cm × 16.7 cm × 14 cm; length × width × height). They each had free access to two drinking bottles, one filled with 250 mL tap water and the other with a 2% sucrose solution. Prior to testing, there was a 48 h adaptation period to habituate to the different types of fluid. The mice were subsequently deprived from food and liquids for 3 h. During the next 24 h, free consumption of water and 2% sucrose solution took place, in the presence of *ad libitum* food. Fluid intake was measured afterwards by weighing the drinking bottles. Sucrose preference was calculated as the ratio of the sucrose solution intake over the total fluid intake.

We subjected mice of three age groups; 3 (WT $n = 12$; APP23 $n = 7$), 6 (WT $n = 14$; APP23 $n = 15$) and 12 (WT $n = 8$; APP23 $n = 7$) months, to each of these depression-related tests. We excluded six mice from the TST analysis, because they crawled up their tail during the experiment rendering accurate scoring impossible. One mouse was excluded from the sucrose preference analysis because there was leakage from his drinking bottle during the experiment.

2.3. Elevated plus-maze

To evaluate anxiety, we used the elevated plus-maze apparatus for use with mice, which consisted of four cross-shaped arms, of which two were brightly lit and open, and the other two dark and enclosed (30 cm × 5 cm × 15 cm; length × width × height of the enclosed arms). The maze itself was elevated 60 cm from the floor and mice were always placed in the central area (5 cm × 5 cm), facing the left enclosed arm. All mouse movements were tracked by camera for 5 min and registered with the Ethovision system (Noldus, Netherlands). The number of entries, latencies to the first arm entry, the duration of the time spent in open and closed arms, the number of faecal boli, the total distance moved, velocity and the number of rearings were the parameters measured. Different naive groups of mice were subjected to the elevated plus-maze at the ages of 3 (WT $n = 23$; APP23 $n = 24$), 6 (WT $n = 30$; APP23 $n = 16$) and 12 (WT $n = 26$; APP23 $n = 20$) months.

2.4. Conditioned fear test

Context- and cue-dependent fear conditioning was studied as previously described [36]. An aversive, unconditioned stimulus (US) (an electric shock), is paired with two conditioned stimuli (CS) (the context, i.e. the experimental environment, and the cue, i.e. a tone) to elicit a freezing response, a reliable measure of fear in rodents [5]. A freezing score was calculated by expressing the number of observed freezing bouts as the percentage of the maximum possible number of bouts in each of the five trial blocks (pre-US, post-US, context, pre-CS, CS). A naive group of 6-month-old mice was subjected to the conditioned fear test (WT $n = 23$; APP23 $n = 14$).

2.5. Sexual behaviour test

For the observation of male sexual behaviour, we used pair-tests with 3-month-old ovariectomized and hormonally primed stimulus C57BL/6J females. Ovariectomy was performed 1 month before testing under general anaesthesia (Nembutal, Sanofi, Belgium). Hormonal priming consisted of two subcutaneous 0.10 mL injections with β-estradiol 3-benzoate dissolved in sesame oil (30 μg at 48 h and 15 μg at 24 h before tests) and one subcutaneous 0.10 mL injection with progesterone dissolved in sesame oil (500 μg at 4 h before tests). Males, as well as stimulus females, were group housed and placed on a reversed day/night-rhythm (lights on at 11.30 p.m., lights off at