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Research report

Impaired Pavlovian cued fear conditioning in Tg2576 mice expressing a human mutant amyloid precursor protein gene

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Abstract

The processing of emotional and/or fear-related events is abnormal in patients with Alzheimer's disease. AD is accompanied by a number of neuropathological features, one of which is the deposition of amyloid plaques. The main aim of the present study was to examine the effects of a human amyloid precursor protein mutation on both the acquisition and expression of fear conditioning in Tg2576 mice. Sixteen-month-old, but not 4-month-old, transgenic mice showed aberrations in post-shock freezing during training. In a retention test carried out 24 h after training, Tg2576 mice showed comparable levels of conditioned fear elicited by contextual cues. However, freezing elicited by a tone conditioned stimulus was impaired in 16-month-old but not 4-month-old Tg2576 mice. The results are discussed with reference to the role of cue competition (overshadowing) in revealing fear conditioning deficits in Tg2576 mice.

Keywords: Alzheimer's disease; Fear conditioning; Tg2576; Context; Cued; Amyloid

1. Introduction

One of the clinical features of Alzheimer's disease is a change in emotional behaviour [1]. Whilst there has been some controversy in the literature surrounding the onset of emotional changes in AD, it is most commonly viewed as a late onset symptom and is taken to reflect the probable progression of pathology within the amygdala [10,23]. Pavlovian fear conditioning, in which an auditory cue and/or context is paired with foot shock, has been used extensively in animal studies to explore the neural substrates of emotion. Two brain areas, in particular, have become closely associated with fear conditioning to either contextual and/or discrete auditory cues. Impairments in freezing elicited by both contextual and auditory CS's often accompanies cell loss in amygdala [17]. In contrast, cell loss in the hippocampus is associated with a more selective deficit in contextual fear conditioning ([26], but see [19,25]).

Context and cued fear conditioning paradigms have been used to examine learning in mice that express an autosomal dominant form of an Alzheimer amyloid precursor protein mutation. Gerlai et al. [11] reported that 11-month-old female mice expressing an amyloid precursor protein mutation (APP^{V717F}) showed a complex pattern of fear conditioning deficits. Wild type and transgenic mice showed comparable levels of grooming and tail rattling elicited by either contextual cues or a tone CS that was paired with foot shock. However, mutant mice showed a deficit in conditioned freezing elicited by a tone CS and also a (non-significant) trend for reduced freezing elicited by the context.

Tg2576 mice express a human Swedish APP mutation and show age-dependent impairments on a number of spatial learning tasks [4,5,14,16,28]. These deficits have been interpreted as reflecting impaired hippocampal function (e.g. [4]). Studies of fear conditioning in Tg2576 mice have provided additional support for this conclusion. More specifically, under certain conditions, mutant mice display impaired Pavlovian contextual fear conditioning but normal auditory

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conditioning [5,6]. This pattern of results is clearly consistent with other evidence that spatial navigation is disrupted in mutant mice and supports the hypothesis that hippocampal processing is aberrant in aged Tg2576 mice. However, none of the studies using Tg2576 mice have provided an assessment of the performance of these mice during conditioning. Therefore, the purpose of the present study was simply to determine whether Tg2576 mice display aberrant conditioned and/or unconditioned behaviour to contextual or auditory events during conditioning. Two experiments are reported with old (16-month-old) and young (3–4-month-old) Tg2576 mice, respectively, and examined conditioned and unconditioned changes in locomotor and freezing behaviours during training and following a 24 h retention interval.

2. Method

2.1. Subjects

Male Tg ($HuAPP_{695}SWE$)2576 mice in a hybrid strain of C57Bl/6j with SJL were housed in mixed genotype litter groups of two to six animals. Two cohorts of Tg2576 mice and their littermate controls underwent fear conditioning. In each experiment the Tg2576 mice were compared to littermate controls so that age and background strain were comparable. No more than one wild type and one transgenic animal from an individual litter were included in the experimental cohorts to avoid problems of pseudoreplication. Breeding and other details regarding the maintenance of the colony were as described previously (see [4]). In Experiment 1, the subjects were 9, 16-month-old Tg2576 mice and 13 wild type littermate controls. In Experiment 2, the subjects were 8, 3, 4-month-old Tg2576 mice and eight wild type littermate controls. During the experiments, the animals were housed in littermate groups on a 12 h light-dark cycle. All testing was conducted during the light phase of this cycle. The mice were provided with ad lib access to food and water throughout the experiment. The animals were maintained in full compliance with Home Office (UK) guidelines.

2.2. Apparatus

The experimental apparatus consisted of two Coulbourn conditioning chambers (Coulbourn Instruments, Allentown, PA, USA), which measured 18 cm wide by 17 cm deep by 21 cm high. The sides of the chambers were made from aluminium panels that slid into rails, thus allowing the inclusion of a speaker in place of one of the top panels. The front and back of the chamber was made of clear Perspex which allowed video recording of the animal whilst it was in the chamber, via a black and white camera mounted on the inside of the rear panel of the sound attenuating box. The front Perspex panel folded downwards to allow access. The roof of the chamber was made of aluminium and housed an infrared activity monitor (Coulbourn Instruments; Model H24-61MC; set to mouse sensitivity) positioned above a hole in the roof panel. The activity monitor recorded the change in position of the subjects' infrared body heat signature. The infrared monitor was capable of detecting both lateral and vertical (rearing) movements (see www.coulbourn.com for further information). This method was used to provide a measure of locomotor activity and to provide an independent means of validating the changes in freezing behaviour. A Pearson's correlation coefficient produced for the freezing and activity measures recorded from the context and tone test stages showed that the two measures were closely negatively correlated with each other in both experiments. Experiment 1: context test, Pearson's $r = -0.81$ ($r^2 = 0.64$, $P < 0.04$); tone test Pearson's $r = -0.73$ ($r^2 = 0.53$, $P < 0.05$); Experiment 2: context test Pearson's $r = -0.72$ ($r^2 = 0.57$, $P < 0.05$); tone test Pearson's $r = -0.68$ ($r^2 = 0.51$, $P < 0.05$). The infrared activity measure therefore provided an independent assessment of changes in locomotor responses that were sensitive to changes in behaviour brought about by the conditioning contingencies.

The chamber was constantly illuminated by a single bulb positioned on the top panel of the wall opposite the speaker. The speaker was connected to a Coulbourn integrated tone generator and volume control. The (5 kHz) tone volume was set at 75 dB (A scale) in each of the two chambers. The floor was a Coulbourn Instruments modular shock floor composed of 5 mm steel bars placed 5 mm apart. The floor grids were connected to a Coulbourn precision regulated animal shocker (model number H13–16). The shock amplitude was set at 0.4 mA for 2 s. The chambers were housed in sound attenuating boxes made of white melamine (dimensions 70 cm wide by 50 cm deep by 50 cm high) with a fold-down door at the front. An electric fan set in to the right hand wall ventilated the sound attenuating boxes. The conditioning chambers were linked to a PC computer via a Coulbourn Habitest Universal Linc. This allowed both control of the chambers from a PC, and recording of the activity measurements. The software used to control the chambers and process the data was written using Coulbourn Graphic State Notation.

For the tone test carried out on day 2, the contextual cues in the conditioning chamber were altered. The walls were replaced with a black and white striped alternating pattern using Perspex inserts. The floor grid was covered with a Perspex solid floor covered with a black and white checkerboard pattern. The floor was then covered with a thin layer of fresh sawdust. The odour cues were also changed. Whereas on day 1 the grid floor was wiped with 70% alcohol between each subject, during tone testing the Perspex floor was wiped with a damp cloth containing a diluted solution of Hibiscrub (AstraZeneca; 1 part Hibiscrub to 10 parts water) between each subject. Two test chambers were used in the present study and the assignment of the mice to each chamber was fully counterbalanced.