ELSEVIER

Neurobiology of Disease

www.elsevier.com/locate/ynbdi
Neurobiology of Disease 30 (2008) 439–450

Aged wild-type and APP, PS1, and APP+PS1 mice present similar deficits in associative learning and synaptic plasticity independent of amyloid load

A. Gruart,[a] J.C. López-Ramos,[a] M.D. Muñoz,[b] and J.M. Delgado-García[a,*]

[a]*División de Neurociencias, Universidad Pablo de Olavide, 41013 Sevilla, Spain*
[b]*Departamento de Investigación, Hospital Ramón y Cajal, 28034 Madrid, Spain*

Received 4 June 2007; revised 29 February 2008; accepted 10 March 2008
Available online 21 March 2008

Wild-type and single-transgenic (APP, PS1) and double-transgenic (APP+PS1) mice were studied at three different (3-, 12-, and 18-month-old) age periods. Transgenic mice had reflex eyelid responses like those of controls, but only 3-month-old mice were able to fully acquire conditioned eyeblinks, using a trace paradigm, whilst 12-month-old wild-type and transgenic mice presented intermediate values, and 18-month-old wild-type and transgenic mice were unable to acquire this type of associative learning. 18-month-old wild-type and transgenic mice presented a normal synaptic activation of CA1 pyramidal cells by the stimulation of Schaffer collaterals, but they did not show any activity-dependent potentiation of the CA3–CA1 synapse across conditioning sessions, as was shown by 3-month-old wild-type mice. Moreover, 18-month-old wild-type and transgenic mice presented a noticeable deficit in long-term potentiation evoked *in vivo* at the hippocampal CA3–CA1 synapse. The 18-month-old wild-type and transgenic mice also presented a significant deficit in prepulse inhibition as compared with 3-month-old controls. Except for results collected by prepulse inhibition, the above-mentioned deficits were not related with the presence of amyloid β deposits. Thus, learning and memory deficits observed in aged wild-type and transgenic mice are not directly related to the genetic manipulations or to the presence of amyloid plaques.

Keywords: CA3–CA1 synapse; Eyeblink; Hippocampus; Old mice; Long-term potentiation; Prepulse inhibition; Synaptic potentiation; Transgenic mice; APP; PS1; Classical conditioning

Introduction

A still unanswered question regarding animal models of Alzheimer's disease is whether learning and memory deficits are a consequence of amyloid β deposits in hippocampal and neocortical structures (Kelly et al., 2003; Schwab et al., 2004; Ewers et al., 2006). Furthermore, the putative relationships between functional deficits observed in genetically manipulated animals and those produced by the normal aging process of the same species are still a matter of debate (Woodruff-Pak, 2001). It has been proposed that some functional changes precede amyloid β plaque deposits (Moechars et al., 1999), and that early phenomena such as synaptic depression, evoked by excessive production of amyloid β peptides, can underlie the initial cognitive deficits reported in both experimental animals and Alzheimer's patients (Kamenetz et al., 2003). The availability of transgenic mice that mimic human Alzheimer's disease (Selkoe, 2002; Kamenetz et al., 2003) has made it of interest to develop learning tasks applicable in these small mammals. Classical conditioning of nictitating membrane/eyelid responses is a suitable associative learning procedure for mice (Takatsuki et al., 2003; Domínguez-del-Toro et al., 2004) and it can be compared with similar studies carried out in Alzheimer's patients (Woodruff-Pak, 2001; Weiss et al., 2002).

The aim of this study was to determine the learning capabilities of 3-, 12-, and 18-month-old wild-type and single-transgenic (APP751SL, PS1M146L) and double-transgenic (APP751SL/PS1M146L) mice using the classical conditioning of eyelid responses with a trace paradigm, which is a known hippocampally dependent associative learning task (Thompson, 1988; Moyer et al., 1990; McEchron and Disterhoft, 1997; Clark and Squire, 1998; Múnera et al., 2001). Field excitatory postsynaptic potentials (fEPSPs) evoked in the hippocampal CA1 pyramidal cell layer by the electrical stimulation of the Schaffer collateral-commissural pathway in behaving mice were also recorded (Gruart et al., 2006b). Long-term potentiation (LTP) of the pyramidal CA1 layer was experimentally evoked in behaving 3-month-old wild-type mice and in 18-month-old wild-type and transgenic mice. As a further control of the functional state of sensory-motor cortical centers (Borrell et al., 2002) in both young and old wild-type and transgenic mice, the startle response/

* Corresponding author. División de Neurociencias, Universidad Pablo de Olavide, Ctra. de Utrera, Km. 1, 41013 Sevilla, Spain. Fax: +34 954 349375.
E-mail address: jmdelgar@upo.es (J.M. Delgado-García).
Available online on ScienceDirect (www.sciencedirect.com).

0969-9961/$ - see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.nbd.2008.03.001

prepulse inhibition test was also carried out. Indeed, the presence of amyloid β deposits in extrahippocampal sites, including prefrontal areas, in single-transgenic APP and in double-transgenic APP+PS1 mice has been shown recently (Blanchard et al., 2003).

The current results support a similar derangement of conditioned eyelid responses and of activity-dependent hippocampal synaptic plasticity in aged wild-type and transgenic models of Alzheimer's disease, not directly related to the appearance of neuritic amyloid plaques. Part of this work has been presented in abstract form (Gruart et al., 2006a).

Materials and methods

Subjects

Male single-transgenic (APP751SL, PS1M146L) and double-transgenic (APP751SL/PS1M146L) mice bred in a C57Bl/6 background for at least six generations at Charles River (Lyon, France) were used in the present study, along with wild-type littermate controls (Blanchard et al., 2003; Schmitz et al., 2004). For behavioral studies, animals were divided as follows: i) classical conditioning: 3-, 12-, and 18-month-old wild-type, APP, PS1, and APP+PS1 mice ($n=6$ each) for the experiment illustrated in Figs. 2 and 3; ii) classical conditioning and hippocampal recordings: 3-month-old wild-type mice ($n=8$) and 18-month-old wild-type, APP, PS1, and APP+PS1 mice ($n=8$ each) for the experiments illustrated in Fig. 4; and iii) LTP of behaving mice: 3-month-old wild-type mice ($n=8$) and 18-month-old wild-type, APP, PS1, and APP+PS1 mice ($n=8$ each) for the experiments illustrated in Fig. 5. Some 12-month-old wild-type, APP, PS1, and APP+PS1 mice ($n\leq5$ each) were also included in the LTP study. The experiment illustrated in Fig. 6 (prepulse inhibition) was carried out with animals selected at random from the above-mentioned (i and ii) groups 2 weeks after their classical conditioning. All studies were carried out following the guidelines of the European Union Council (86/609/EU) and Spanish regulations (BOE RD 1201/2005) for the use of laboratory animals in acute and chronic experiments.

Surgical procedures

Animals for experiments (i, classical conditioning) and (ii, classical conditioning and hippocampal recordings) were anesthetized with a mixture of Ketolar (ketamine, 35 mg/kg) and Rompum (xylazine, 2 mg/kg), i.p., and implanted with bipolar stimulating electrodes on the left supraorbitary branch of the trigeminal nerve and with bipolar recording electrodes in the ipsilateral orbicularis oculi muscle (Fig. 1A). Electrodes were made of 50 μm, Teflon-insulated, annealed stainless steel wire (A–M Systems, Carlsborg, WA-98324, USA), with their tips cleaned of the isolating cover for ≈0.5 mm. The electrode tips were bent as a hook to facilitate a stable insertion in the upper eyelid. Animals for experiment (ii) were also implanted with bipolar stimulating electrodes aimed at the right (contralateral) Schaffer collateral-commissural pathway of the dorsal hippocampus (2 mm lateral and 1.5 mm posterior to Bregma; depth from brain surface, 1.0–1.5 mm) and with a recording electrode aimed at the ipsilateral stratum radiatum underneath the CA1 area (1.2 mm lateral and 2.2 mm posterior to Bregma; depth from brain surface, 1.0–1.5 mm; Paxinos and Franklin, 2001). These electrodes were made of 50 μm, Teflon-coated tungsten wire (Advent Research Materials Ltd., Eynsham,

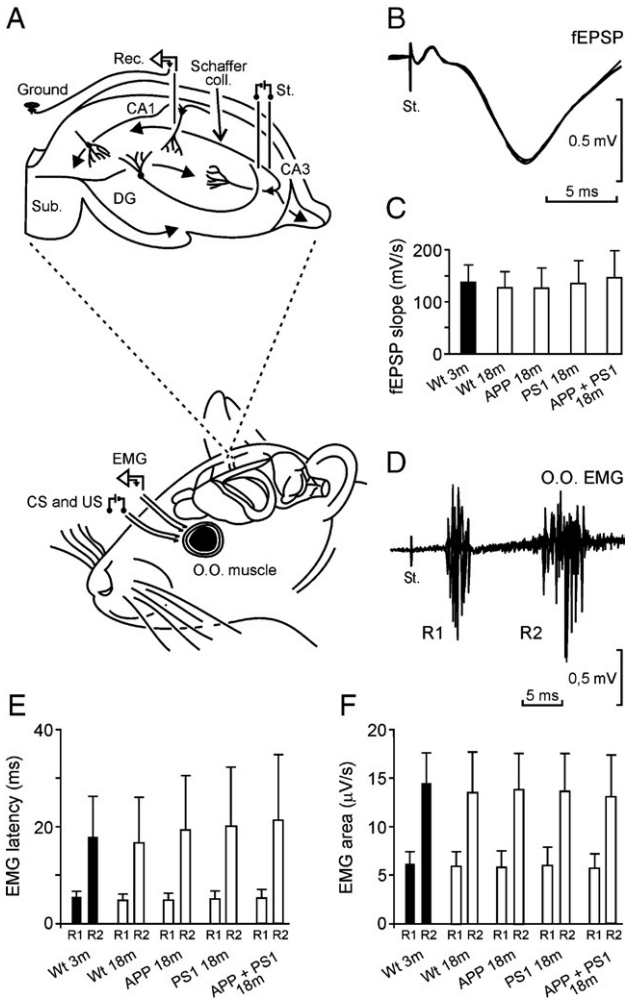

Fig. 1. Experimental design. (A) Electrodes for electromyographic (EMG) recordings were implanted in the orbicularis oculi (O.O.) muscle of the upper eyelid. Bipolar stimulating electrodes were implanted on the supraorbitary nerve for presentation of conditioned (CS) and unconditioned (US) stimuli. Classical conditioning was achieved using a trace paradigm. For this, a short (50 μs), weak (1.5× threshold) pulse was presented as a CS. The US consisted of a long (500 μs), strong (3× threshold) pulse. The US started 500 ms after the CS. The upper inset illustrates that animals were also implanted with recording (Rec.) electrodes in the hippocampal CA1 area and with stimulating (St.) electrodes at the ipsilateral Schaffer collaterals. (B) The three superimposed records illustrate the extracellular synaptic field potential recorded in the stratum radiatum of the CA1 area following electrical stimulation of Schaffer collaterals in an 18-month-old PS1 mouse. (C) Mean (±S.D.; $n=50$) fEPSP slopes from controls (Wt 3 m, black bar) and from the four 18-month-old experimental groups (white bars). No significant difference was observed between control and experimental groups ($P=0.13$, one-way ANOVA). (D) The three superimposed records correspond to the blink reflex evoked in the O.O. muscle by the electrical stimulation of the trigeminal nerve in 18-month-old APP mice. Note the two short (R1) and long (R2) latency components characterizing the blink reflex in mammals. (E, F) Mean (±S.D.; $n=50$) values collected for EMG latency (E) and amplitude (F) of both R1 and R2 components of electrically evoked blinks in controls (Wt 3 m; black bars) and in the four 18-month-old experimental groups (white bars). No significant difference ($P\geq0.23$, one-way ANOVA) was observed between groups for any of the four parameters.