IBRO Reports 2 (2017) 81–86

Contents lists available at ScienceDirect

IBRO Reports

journal homepage: http://www.journals.elsevier.com/ibro-reports

Impaired retention of depression-like behavior in a mouse model of Alzheimer's disease

Xianwen Luo [a,b], Yuan Shui [a], Furong Wang [a,b], Ryo Yamamoto [a], Nobuo Kato [a,*]

[a] Department of Physiology, Kanazawa Medical University, Ishikawa 920-0293, Japan
[b] Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China

ARTICLE INFO

Article history:
Received 20 February 2017
Received in revised form 1 May 2017
Accepted 1 May 2017

Keywords:
Depression
Alzheimer's disease
Long-term potentiation
Excitability
Forced swimming
Homer1

ABSTRACT

By using a 5-day forced swimming test (FS) that we previously developed, swim immobility was induced in 3xTg Alzheimer's model mice and wild-type (WT) mice. After the initial 5-day FS, the next and last swimming session was performed at a 4-week interval, during which the immobility was reduced in 3xTg mice, but was maintained fully in WT mice. After FS, context-dependent fear learning was normally induced in WT mice, but was impaired in 3xTg mice, suggesting that FS may exaggerate cognitive deficits typical to 3xTg mice. Hippocampal long-term potentiation (LTP) at Schaffer collateral-CA1 synapses was suppressed by FS in WT mice, but not in 3xTg mice, indicating that FS modifies LTP in the WT mouse hippocampus, but not in 3xTg tissue. FS increased excitability of cingulate cortex pyramidal cells similarly in WT and 3xTg mice. Agreeing with our previous finding that expression of Homer1a protein is decreased in the cingulate cortex in harmony with FS-induced immobility, western blot showed that Homer1a expression is reduced by FS in the WT mice. In 3xTg mice, by contrast, FS failed to reduce Homer1a expression. The disrupted endurance of FS-induced immobility in 3xTg mice appears to be attributable to impaired cognition typical to this genotype. Failure of FS to alter LTP magnitude might be related to unaltered Homer1a expression after FS in 3xTg mice.

1. Introduction

The forced swimming (FS) test, a widely-used model for depression in rodents, was invented originally as a means by which to evaluate candidate compounds for antidepressant drugs in rats (Porsolt et al., 1977). In this test, a depression-like state represented by swim immobility or quiescent floating is induced on the first day, and its recovery by compounds is evaluated on the second day. In the later introduced mouse version of FS (Porsolt et al., 1978), both the induction and evaluation procedures are completed on one day only. More recently, however, it has frequently been questioned whether FS is a suitable model procedure for human depression (Holmes, 2003; Nestler and Hyman, 2010; Veenema et al., 2003). Changes in animal behavior induced by acute intensive insults in general may be less likely to simulate human depression than those induced by chronic succession of unpredictable mild stress (Holmes, 2003; Mineur et al., 2006; Nestler and Hyman, 2010; Willner, 1997, 2005). Such debate is related to the question on the physical characteristics of the stressor. More essentially, the swim immobility is not unanimously accepted as a manifestation of despair. Rather, the floating behavior is occasionally interpreted as active learning instead of depression-like behavior (De Pablo et al., 1989; West, 1990; Veenema et al., 2003). If mice save energy consumption by instinct, quiescent floating would be strategically advantageous in the inescapable environment rather than making useless efforts to desperately escape.

One way to approach such an argument would be to use mice defective in learning as the FS subjects, and check how much floating behavior they achieve. We previously used Homer1a-konckout (H1aKO) mice (Sun et al., 2015), which are defective at least in hippocampus-dependent contextual fear learning (Inoue et al., 2009). Homer1a is a member of the scaffold protein family Homer that link and regulate various receptors on the cell and endoplasmic reticulum membrane (Brakeman et al., 1997; Kato et al., 1997; Ango et al., 2001). In H1aKO mice, floating behavior was partially recovered in our original version of FS (Sun et al., 2011, 2015), in which the induction phase consisted of a 10-min-long daily swim for 5 consecutive days and the evaluation of floating was done 4 weeks later. Since no recovery is observed in wild-type (WT) mice, effects of genetic modification or antidepressant treatment during the interval could be examined. It was thus suggested that the partial recovery of the floating in H1aKO mice may be

* Corresponding author.
E-mail address: kato@kanazawa-med.ac.jp (N. Kato).

http://dx.doi.org/10.1016/j.ibror.2017.05.001

attributed to a defective learning ability, and that the floating may at least partly represent active learning. This interpretation is not necessarily straightforward, since Homer1 is implicated in major depression (Rietschel et al., 2010) and a possibly frail resilience to stress in H1aKO mice may preclude the full manifestation of cognitive ability. Therefore, the present study used 3xTg Alzheimer's model mice (Oddo et al., 2003), in which learning deficit has been well confirmed, to test if the floating behavior during FS is altered in cognitively impaired animals.

2. Experimental procedures

2.1. Animals

All the experiments were performed in accordance with the guiding principle of the Physiological Society of Japan and were approved by the Animal Care Committee of Kanazawa Medical University. Triple transgenic AD model mice (3xTg; Oddo et al., 2003) with 129/C57BL6 hybrid background, provided by Dr LaFerla (University of California, Irvine), were kept under day-night control (12:12 h), and allowed free access to food and water. Male 3xTg mice of 4–5 months of age were used. As the age-matched control, non-transgenic mice from the same hybrid background were used (wild-type).

2.2. Behavior

Forced swimming was performed as described (Sun et al., 2011). Briefly, transparent acrylate cylinder (24 cm diameter, 60 cm high) were filled with 25 °C water (25 cm deep). The mice swam for 10 min daily for 5 consecutive days and 4 weeks later on day 33. Swimming trajectory was analyzed by ANY-Maze (Stoelting Co., Wood Dale, IL, USA).

Fear conditioning was performed as described (Sun et al., 2015). Briefly, a dark-colored plastic chamber (25 W × 25 D × 27 cm H) located inside a sound-proof box (66W × 54 D × 54 cm H; Panlab s.l.u., Cornella, Spain) was positioned on the gravity sensor controlled by FREEZING software (Panlab). On day 1, after a 3-min habituation, the animals were exposed to white noise (80 dB) and illumination for 28 s, then to the same tone and illumination combined with electrical shock (0.4 mA) for 2 s, which was repeated twice at the interval of 30 s. On day 2, the animals were placed in the same chamber as on day 1, initially with no tone given. Freezing was measured for 5min. One hour later, the animals were placed in a white-colored chamber of the same size to assess the baseline activity for 3 min, and then the same tone as on day 1 was given for 3 min to assess tone-dependent fear conditioning.

2.3. Electrophysiology

Slice experiments were performed as described (Sun et al., 2011; Wang et al., 2015b; Yamamoto et al., 2011). After behavioral tests, mice were sacrificed by decapitation under ether anesthesia. The brain was soaked into a medium (pH 7.4; 25 °C) containing the following (in mM): 124 NaCl, 3.3 KCl, 1.3 KH_2PO_4, 26 $NaHCO_3$, 2.5 $CaCl_2$, 2.0 $MgSO_4$, and 10 glucose. Sections of the cingulate cortex or hippocampus were cut with a slicer at 200 μm (Zero-1, Dosaka). Slices were placed in a recording chamber on the stage of an upright microscope (Eclipse E600FN, Nikon) with a ×40 water-immersion objective (Fluor 40 × 0.80 W, Nikon). The chamber was continuously perfused with medium (25 °C) and bubbled with a mixture of 95% O_2 and 5% CO_2. For recording, we used patch pipettes (resistance, 4–10 MΩ) filled with a solution (pH 7.3) containing the following (in mM): 130 K-gluconate, 10 KCl, 2 $MgCl_2$, 2 Na-ATP, 0.4 Na-GTP, 0.2 EGTA, 10 HEPES, 5 K_2-Phosphocreatine, with pH adjusted to 7.2–7.3 using KOH.

Whole-cell recordings were made from layer II/III pyramidal cells that had sufficiently negative resting membrane potentials (< −55 mV) without spontaneous action potentials. Membrane potentials were recorded in the current-clamp mode (Axoclamp 200A and B, Molecular Devices) and digitized at 10 kHz (Digidata 1322 and pCLAMP10, Molecular Devices). To assess membrane excitability of recorded neurons, depolarizing currents (50–500 pA for 500 ms) were injected through the patch pipette.

For field potential recording at Schaffer collateral-CA1 synapses, recording electrodes (2–5 MΩ) filled with 2.5 M NaCl were placed in the stratum radiatum and a set of bipolar tungsten electrodes was inserted nearby. Long-term potentiation (LTP) was induced with theta-burst stimulation (TBS). The test pulse intensity was adjusted to be 50–75% of threshold for population spikes and two trains of TBS at the interval of 20 s were given to induce LTP.

2.4. Western blot

Western blot was done as described (Sun et al., 2011). Briefly, cingulate cortex tissue was homogenized in cold buffer (lithium lauryl sulfate, 2%; aprotinin, 1.7 mg/ml; phenylmethylsulfonyl fluoride, 10 mg/ml; sodium orthovanadate, 1 mM). The homogenate was centrifuged at 15,000 rpm for 15 min at 4 °C. Protein concentration was determined by the Bradford method. Protein samples (40 μg) were analyzed by SDS-PAGE with 5–15% or 10–20% Ready Gels (Bio-Rad). After blocking with a skim milk solution (5%), immunoblotting was done with goat anti-Homer1a (1:500; SC8922; Santa Cruz Biotechnology) and rabbit anti-β-actin antibodies (1:2000; IMG-5142A; Imgenex). After reaction with HRP-conjugated secondary antibodies (donkey anti-goat IgG, 1:1000. HAF109, R&D Systems; goat anti-rabbit IgG, 1:1000, catalog no. 32460, Thermo Scientific), bands were detected with a chemiluminescence substrate kit (Super-Signal West Femto kit, Thermo Scientific) and a detector (LAS-4000, FUJIFILM) and analyzed by using ImageJ software.

2.5. Data analysis

Data are expressed as averages ± SEM. For statistics, pairwise or unpaired *t*-test and repeated-measures or one-way ANOVA followed by Dunnett T3 test were used (SPSS, version 21; Japan IBM).

3. Results

3.1. Behavioral study

Depression-like behavior was induced by the 5-day forced swimming test that we have designed (Sun et al., 2011). In both WT and 3xTg mice, the immobility time developed during the initial 5-day phase of forced swimming (Fig. 1) and became longer on day 5 (WT, 333.9 ± 29.7 s, N = 15; 3xTg, 295.7 ± 37.2 s, N = 13) than on day 1 (WT, 191.4 ± 17.3 s, $P < 0.001$; 3xTg, 123.9 ± 28.0 s, $P < 0.001$, paired *t*-test). This pattern of immobility development was the same as we previously reported in WT mice (Sun et al., 2011) and in Homer1a knockout (H1aKO) mice (Sun et al., 2015). In the last swimming session on day 33, WT mice maintained the elongated immobility time (368.1 ± 19.2 s), whereas the immobility was significantly shortened in 3xTg mice (189.0 ± 39.6 s, $P = 0.017$ as compared to day 5 and $P = 0.18$ as compared to day 1). It is thus apparent that the depression-like behavior, as manifested by the immobility time, is recovered within a 4-week period in 3xTg mice, but is persistently maintained in WT mice.

The influence of forced swimming on cognitive performance was assessed by contextual fear conditioning (Fig. 2). After the induction of depression-like behavior by the 5-day forced