Life Sciences 138 (2015) 57–63

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Age differences in the role of the cannabinoid type 1 receptor on glutamatergic neurons in habituation and spatial memory acquisition

Anne Legler, Krisztina Monory, Beat Lutz *

Institute of Physiological Chemistry, University Medical Center of the Johannes Gutenberg University, Duesbergweg 6, 55128 Mainz, Germany

ARTICLE INFO

Article history:
Received 23 October 2014
Accepted 30 January 2015
Available online 2 March 2015

Keywords:
CB1 receptor
Glu-CB1-KO
Spatial learning
Habituation
Aging
Morris Water Maze
Water Cross Maze

ABSTRACT

Aims: Aging is typically linked with a decline in memory performance and alterations in neural integrity. In pathological aging such as Alzheimer's disease, these effects are aggravated. Studies using cannabinoid CB1 receptor-deficient mice have shown a role of the endocannabinoid system in memory processing and neuroprotection. As the CB1 receptor is expressed in various neuronal populations, in this study, we aimed at investigating the consequences of CB1 receptor gene inactivation in cortical glutamatergic neurons in mice (Glu-CB1-KO) in regard to age-related alterations in spatial memory performance.
Main methods: Juvenile (5.5–7.5 weeks), adult (5.5–7 months), and old (11.5–14 months) Glu-CB1-KO and Glu-CB1-WT mice were compared in two spatial learning tasks, the Morris Water Maze (MWM) using both visible and hidden platforms, and the Water Cross Maze (WCM).
Key findings: In the MWM with a visible platform, adult and old Glu-CB1-KO mice showed a delayed acquisition of the task, suggesting an age-dependent function of the endocannabinoid system in habituation. Juvenile and adult Glu-CB1-KO mice exhibited increased time and path length to the hidden platform in the MWM. However, these characteristics were accompanied by increased thigmotaxis in both age groups, suggesting anxiety-like behavior as a confounding factor. To exclude this possible bias, the animals were tested in a simplified spatial learning assay, the WCM, revealing a decreased accuracy of juvenile but not of adult Glu-CB1-KO to find the platform, therefore strongly suggesting spatial memory impairment in juvenile mice.
Significance: Our results suggest an age-dependent role of the CB1 receptor on cortical glutamatergic neurons in both habituation and spatial learning.

1. Introduction

With life expectancy steadily increasing in industrialized countries, the aging process and the resulting pathologies are a primary challenge to health and social care systems today. Declines in learning and memory and in motor performance are prominent age-related changes in brain functionality [11,30]. The broadly distributed endocannabinoid system, constituted by the cannabinoid receptors, their ligands, the endocannabinoids, and the endocannabinoid synthesizing and degrading enzymes [17], has been implicated in physiological and pathological aging (reviewed in [5,6]). Particularly, studies using mutant mice lacking CB1 receptor in all tissues reported an accelerated age-dependent cognitive decline [2,3,7] and aggravations in pathological aging [33], suggesting protective roles for the CB1 receptor in age-dependent memory decline.

The CB1 receptor is expressed in different neuronal populations in the mammalian brain. Particularly in cortical areas which are vital for memory processes, the CB1 receptor is present in glutamatergic as well as in GABAergic neurons [20]. To dissect the roles of CB1 receptors in these two neuronal populations, we have previously generated two mutant mouse lines lacking CB1 receptor specifically in forebrain GABAergic (GABA-CB1-KO mice; [22]) or cortical glutamatergic neurons (Glu-CB1-KO mice; [22]). Measurement of CB1 receptor protein and mRNA levels revealed that glutamatergic neurons contain only a minor amount of CB1 receptor; however, this receptor population is responsible for the majority of CB1 receptor-mediated G protein activation [31]. Accordingly, behavioral analysis of Glu-CB1-KO mice revealed essential roles of this receptor on glutamatergic neurons in several functions, such as protection against excitotoxic insults, stress coping, social behavior, fear and anxiety [12–14,16,19,21,22,32].

In this study, we investigated the effect of aging on spatial learning abilities in Glu-CB1-KO mice using the Morris Water Maze. Spatial learning in juvenile and adult Glu-CB1-KO and Glu-CB1-WT mice was further investigated by testing the mice in the Water Cross Maze, a two-choice paradigm with considerably fewer possible confounding variables.

2. Materials and methods

2.1. Animals

Mutant mice, lacking CB1 receptor specifically in cortical glutamatergic neurons (Glu-CB1-KO; [22]) and their wild-type littermates

* Corresponding author. Tel.: +49 6131 3925912; fax: +49 6131 3923536.
E-mail address: blutz@uni-mainz.de (B. Lutz).

http://dx.doi.org/10.1016/j.lfs.2015.01.034
0024-3205/

(Glu-CB1-WT) in a predominant C57BL/6N background (>10 backcrosses into C57BL/6N) were used. In this study, 3 age groups of male mice were used, termed juvenile (5.5–7.5 weeks old), adult (5.5–7 months old) and old (11.5–14 months old). Animals were housed in a temperature- and humidity-controlled room (22 ± 1 °C; 50 ± 1%) with a 12 h light–dark cycle (lights on at 7 am) and had ad libitum access to food and water. All the experimental protocols were carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and approved by the Ethical Committee on Animal Care and Use of Rhineland-Palatinate, Germany.

2.2. General procedure for behavioral experiments

Mice were separated and single-housed one week before the behavioral experiments started. Mice were in the behavioral room for at least 45 min before the experiment started. All behavioral experiments were performed during the light phase of the animals. Cages were not cleaned for the duration of an experiment. Scientists performing experiments, or scoring and analyzing behavioral data were always blind to the genotype of the animals. The Morris Water Maze and Rotarod experiments were performed on the same animals; with the Rotarod experiment starting on the last day of the MWM reversal task.

2.3. Morris Water Maze

A pool with a diameter of 1.20 m was used with four distant visual cues installed on the surrounding cubicle at four different directions (North, N; East, E; South, S; West, W). The target platform was centrally arranged in one quadrant at a point 25 cm distant from the pool wall and was submerged 1 cm beneath the surface of the water. The water (22–24 °C) was made opaque with white tempera. Additionally, 4 distant visual cues were added to the inside of the pool wall. The experimenter entered the cubicle through a door in the SW corner. Light intensity was 5 lx. The animals were tracked with the Video Tracking System EthoVision XT. Latency to platform, total path length, average swim speed, percentage of time spent not moving (floating) and thigmotaxis were measured.

The mice were tested in groups of four, allowing each mouse to rest for about 10 min between trials. Each starting point was used once per day for each mouse, with the order of the starting points being randomized over the days. The animals were released into the water facing the pool wall. A trial ended when the mouse reached the platform and remained there for at least 5 s. If the mouse did not find the platform within 70 s, it was gently guided to the platform. The mouse had to stay on the platform for 30 s before it was removed, dried and put back into its home cage. A heat lamp was used to warm the animals.

During the pretraining phase, a visible platform was marked with a flag and all visual cues were removed from the walls. The acquisition phase started after a two-day break. For 7 consecutive days, the platform was hidden and remained at a fixed position in the SE quadrant. In the reversal phase, the platform position was changed to the opposite quadrant (NW). The first trial of the reversal phase served as a probe trial for spatial retention. The starting point during the probe trial was randomized within the groups. The platform was removed for the probe trial. After 70 s, it was placed in the new position (NW); the animals were guided to the platform and had to stay there for 30 s.

2.4. Water Cross Maze

The protocol used in this study was adapted from Kleinknecht et al. [18]. The maze, custom made, was made of 0.5 cm thick clear Plexiglas, had four arms forming a cross, with each arm 10 cm wide, 50 cm long and 30 cm high. For spatial orientation, four extra-maze visual cues were installed on the surrounding cubicle. The platform was located E; the starting point was either N or S. The arm opposite to the respective starting position was always blocked with a removable Plexiglas panel. The maze was filled with water at 22–23 °C, until the platform was submerged 1 cm beneath the water surface. The light intensity was 5 lx. The animals were tracked with the Video Tracking System EthoVision XT.

The mice were tested for four days with eight trials per day. The eight trials were performed in two sessions with four trials, allowing each mouse to rest for about 2 h between sessions. Each starting point was used twice per session, with the order of the starting points being randomized over the days. A trial ended when the mouse reached the platform and remained there for at least 5 s, or when the mouse did not find the platform within 30 s. In this case, the mouse was gently guided to the platform. The mouse had to stay on the platform for 20 s before it was removed, dried and put back into its home cage. A heat lamp was used to warm the animals. The mice were trained in groups of six, resulting in an inter-trial interval (ITI) of about 10 min within a session.

Arm entries were manually scored. A trial was scored as accurate (i.e., value 1), if the animal swam from the starting arm directly into the goal arm and climbed onto the platform. A trial was counted as non-accurate (i.e., value 0), if a mouse entered the arm without platform or if it visited an arm several times. Accuracy was defined as the percentage of accurate trials.

2.5. Rotarod

A Rotarod apparatus from Ugo Basile (Comerio, Italy) was used. On day 1, mice were first pretrained for 300 s with a constant speed of 4 rpm (revolutions per minute). Mice which fell down during the 300 s pretraining sessions were immediately placed back on the rod. In the following two days, the animals were tested with speed accelerating from 4 to 70 rpm over 300 s. Rotarod tests were performed directly after the MWM test, starting with the pretraining after the last session of the reversal phase of the MWM. Mice in the Rotarod test were the same as in the MWM test.

2.6. Data analysis

Data were analyzed using the IBM SPSS Statistics Software (IBM Corporation, Armonk, NY, USA). Differences were considered significant at $p < 0.05$. All data are expressed as mean ± SEM. A 2-way mixed ANOVA with time or quadrant as a repeated factor and genotype as a between-subjects factor was used for repeated measures followed by simple-effects analysis if appropriate. The effect of quadrant in the probe trial of the MWM was specified in a planned contrast test by comparing the goal quadrant with the mean of the non-goal quadrants.

3. Results

3.1. Glu-CB1-KO mice have an age-dependent decline in habituation

The Morris Water Maze (MWM) is a complex learning paradigm, as during the acquisition of a spatial memory many different processes can interfere with the learning of the platform location. One of these processes is habituation to the stress resulting from the forced swim, in order to learn to swim efficiently and to learn that the only way to escape the pool is to find the platform [34]. Therefore, we began our MWM protocol with a pretraining phase with a visible platform before the animals were tested with a hidden platform.

Over two days, mice were included in eight pretraining trials (four trials each day) with a visible platform. Juvenile Glu-CB1-KO and Glu-CB1-WT mice both showed an improvement over the trials during the pretraining with no genotype difference present ($p = 0.1098$; Fig. 1A). Analysis of the adult group of Glu-CB1-KO and Glu-CB1-WT animals (Fig. 1B) revealed a significant effect of genotype during the pretraining, with the mutants performing worse than the wild-types ($p = 0.0058$). Simple-effects analysis for the first and last trials of the pretraining showed no differences between groups, suggesting equal