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IL-6 deficiency alters spatial memory in 4- and 24-month-old mice

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ABSTRACT

Significance of interleukin 6 (IL-6) deficiency in cognitive processes was evaluated in 4- and 24-month-old C57BL/6J IL-6-deficient (IL-6 KO) and control (WT) mice in Morris water maze (MWM), holeboard test (HB) and elevated plus maze (EPM). During 3-day learning escape latency time (ELT) was longer in IL-6 KO than in WT mice, however their swimming was slower, floating longer, and path length did not differ. The comparison of ELT and the distance traveled between the first and the third learning day within each group revealed significant decrease of ELT in all groups with the highest difference in 4-month-old WT mice, and significant decrease of distance traveled only in both groups of WT mice. In a single probe trial, performed 24 h after the last learning session, there were no major differences in the absolute values of ELT, but ELT turned out to be significantly shorter in both IL-6 KO groups, when it was compared to the ELT on the last learning day, indicating on better memory retrieval. In HB test only significant increase in number of rearings in aged WT mice, and in EPM significant prolongation of open arm time and higher number of open arm entries in 4-month-old IL-6 KO mice were observed. Results of HB and EPM tests showed that alterations of learning and reference memory observed in MWM were specific to cognition.

Attenuation of learning ability in young adult IL-6-deficient mice assessed in MWM suggests that physiological level of IL-6 is involved in mechanisms engaged in proper memory formation, and it may also indicate on the importance of IL-6 signaling in brain development. Maintained on similar level in both 4- and 24-month-old IL-6 KO mice learning ability and its attenuation in 24-month-old vs 4-month-old WT mice indicates on slower age-related memory decline in mice not expressing IL-6. Better performance of IL-6 KO mice in the probe trial points to their reference memory improvement and may also indicate that IL-6 plays a role in mechanism responsible for cognitive flexibility.

1. Introduction

Interleukin 6 (IL-6), a mediator of inflammation and immune responses (Yirmiya and Goshen, 2011), turned out to be an important signaling molecule within the central nervous system (CNS), where it is able to exert an effect on neuronal and synaptic functions independently of its immunoregulatory properties (Gruol, 2015; McAfoose and Baune, 2009; Spooren et al., 2011). Up-to-date observations on humans have indicated that the expression of IL-6 increases with age both in the periphery and in the CNS (Cohen, Pieper, Harris, Rao, & Currie, 1997; Hager et al., 1994; Maggio, Guralnik, Longo, & Ferrucci, 2006). In the aged humans the increased IL-6 plasma level was correlated with cognitive decline (Maggio et al., 2006; Weaver et al., 2002), suggesting a putative role of this pleiotropic cytokine in age-related cognitive dysfunctions. It has been also shown that IL-6 is overexpressed in neurodegenerative diseases, such as Alzheimer's, Parkinson's and Huntington's disease, as well as in multiple sclerosis (Maggio et al., 2006; Spooren et al., 2011), and in disorders such as major depression and schizophrenia (Spooren et al., 2011). Therefore, it has been postulated that IL-6 contributes to cognitive dysfunctions present in the pathological conditions mentioned.

Studies performed on animals have also demonstrated that IL-6 concentration is increased in the CNS of aging rodents in comparison to young ones. Elevated level of IL-6 was detected especially in cerebral cortex, hippocampus and cerebellum (Prechel, Halbur, Devata, Vaidya, & Young, 1996; Ye and Johnson, 2001). Furthermore, in the brains of 10-month-old senescence accelerated mice a significant increase in the IL-6 protein level has been reported (Tha et al., 2000). These observations are in agreement with cognitive impairment in animals with an elevated level of IL-6 in the brain, caused by peripheral LPS injection (Burton and Johnson, 2012; Chen et al., 2008), implying that IL-6 could be involved in the impairment of learning and memory processes also in normal aging (Godbout and Johnson, 2004; Weaver et al., 2002; Wright et al., 2006).

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The different role of physiological and elevated levels of IL-6 in cognitive processes was supported in experiments with induction and maintenance of long term potentiation (LTP), an established model of mechanism underlining memory storage. In hippocampal slices prepared from transgenic mice with cerebral overexpression of IL-6, LTP in the dentate gyrus was markedly reduced as compared to control animals (Bellinger, Madamba, Campbell, & Siggins, 1995). Similarly, the exposure of hippocampal slices to IL-6 attenuated LTP in the CA1 region (Li, Katafuchi, Oda, Hori, & Oomura, 1997). This effect was mediated by IL-6 receptor, as it was dose-dependently blocked by antibodies against this receptor. The authors of the latter study have suggested that at low doses IL-6 seems to be involved in the process of transformation between short and long-term plasticity, but at higher concentrations it can interrupt the LTP process itself. In experiments performed on moving rats, Balschun et al. (2004) reported that endogenous IL-6, induced in the hippocampus by neuronal activity, plays a role in LTP as a negative regulator of its maintenance, by limiting long-term storage of certain types of memory information, but it is not necessary for LTP induction. The finding that LTP increases IL-6 gene expression, together with the finding of longer LTP preservation after IL-6 neutralization, suggests that IL-6 may have a physiological role in LTP termination (Balschun et al., 2004). Therefore, this physiological regulation of memory mechanisms may be essential for fine-tuning memory consolidation and resetting memory mechanisms, so that new information can be encoded. This hypothesis was supported by the impairment of reversal learning in IL-6-deficient mice evaluated in Morris water maze (Baier, May, Scheller, Rose-John, & Schiffelholz, 2009; Erta, Giralt, Esposito, Fernandez-Gayol, & Hidalgo, 2015), as well as after neutralization of IL-6 by antibody directed against this cytokine, in stressed and non-stressed rats in the attentional set-shifting test (Donegan, Girotti, Weinberg, & Morilak, 2014). Also reported in our recently published study the improvement of long-term memory in Morris water maze in IL-6 KO mice, supported the role of this cytokine in the process of memory consolidation (Bialuk and Winnicka, 2018).

Although the involvement of IL-6 in the impairment of cognitive functions in neurodegenerative disorders was supported by experiments with IL-6 overexpression (Bellinger et al., 1995; Heyser, Masliah, Samimi, Campbell, & Gold, 1997), a little is known about its role in normal aging. Given that the elevated levels of IL-6 in the brain of aging mice was described (Ye and Johnson, 2001) and that the elevated levels in the blood have been found to correlate with poorer cognitive performance in normal subjects (Wright et al., 2006), as well as in subjects showing age-related cognitive decline (Economos et al., 2013; Lekander, et al., 2011) the aim of the present study was to evaluate the effect of total IL-6 deficiency on learning and memory processes and on age-related memory decline. The study was performed on 4- and 24-month-old IL-6-deficient mice and on age-matched controls under physiological conditions in Morris water maze, a task being strongly correlated with hippocampal synaptic plasticity. Evaluated in this test spatial memory provides a common ground between species, where age-related deficits are described consistently for humans, non-human primates, dogs and rodents (Lister and Barnes, 2009).

2. Materials and methods

Naive 4-month-old (young adult) and 24-month-old (aged) male IL-6-deficient mice C57BL/6J$^{IL\text{-}6\text{-}/\text{-}TMKopf}$ and reference wild type (WT) animals (C57BL/6), obtained from the Centre for Experimental Medicine of the Medical University of Białystok, were originally purchased from Jackson Laboratory (Bar Harbor, ME, USA). The experiments were performed after at least 14 days of acclimatization to the laboratory conditions. The mice were maintained in a temperature-controlled environment (22 ± 1 °C), humidity (45–55%), with a 12 h light-dark cycles beginning at 7 a.m. and were housed in polycarbonate cages, five animals per cage, with water and commercial food available ad libitum. Behavioral tests were performed on 20 young adult animals of both genotypes, on 16 WT aged animals and on 20 IL-6 KO aged mice with comparable body weight in both genotypes within age groups (Fig. 1). Experiments were performed between 9.00 a.m. and 1.00 p.m. in an air-conditioned, sound-isolated room with regulated light intensity. Each animal was subjected to three behavioral tests: on day 0 a holeboard followed by an elevated plus maze test; on day 1, 2, 3 and 4 – Morris water maze. All tests were carried out by an investigator, who was blinded to the animal's genotype and age. The recorded material from experiments was later evaluated by an independent researcher who was given an evaluation form. During one experimental week only five or six animals of both genotypes were subjected to behavioral tests. Genotype of animals has been confirmed. All experiments were approved by the Local Animal Ethics Committee in Białystok, Poland and were performed in compliance with the European Communities Council Directive 2010/63/EU.

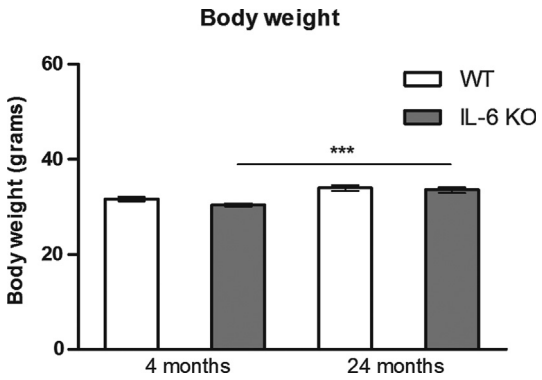

Fig. 1. Body weight of 4- and 24-month-old IL-6-deficient and WT mice measured on day 0. Values represent means ± SEM. *** $p < 0.005$, Kruskal-Wallis test followed by Dunn's test.

2.1. Behavioral tests

2.1.1. Holeboard test (HB)

The test was performed according to the modified method described by File and Wardill (1975). The apparatus was a grey wooden box with a square floor of 53.5 cm × 53.5 cm divided into 25 equal parts and surrounded by a 42-cm high wall. Four holes in the floor (2.5 cm in diameter) were designed as objects of possible interest to the animals. The apparatus was placed on the floor and lit with the intensity of 30 lx. The animal was placed in the center of the holeboard box and its behavior was observed for 5 min. The apparatus was cleaned after each animal. Locomotor activity (ambulation) was measured as the number of squares crossed with all four limbs. Exploratory activity was measured as the number of rearing events (rises of an animal on its rear limbs, either with forelegs leaning against the wall or away from the wall) and the number of head-dips (dips of the head into the hole). Moreover, during assessment of ambulation, peripheral and central locomotor activities were recorded. The number of crossed squares adjacent and not adjacent to the apparatus walls was used to measure peripheral and central activity, respectively. Moreover, latency time to leave the central area was also recorded to measure anxiety level.

2.1.2. Elevated plus maze (EPM)

The procedure was performed according to Pellow, Chopin, File, and Briley (1985) immediately after the holeboard test. The apparatus, made of the same material as the holeboard box, was raised 80 cm above the floor with constant illumination of 75 lx at its level. The EPM apparatus consisted of four arms: two open, 30 cm × 7 cm, and two closed arms 31 cm × 7 cm × 35 cm, all having an open roof. The arms were arranged such that the two open arms were opposite to each other and connected with the central area 7 cm × 7 cm. Mice were placed in the central area of the maze, facing one of the open arms. The apparatus