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Interleukin-18 deficiency reduces neuropeptide gene expressions in the mouse amygdala related with behavioral change

Yuta Yamamoto [a], Toshihito Tanahashi [a,*], Sakurako Katsuura [a], Ken Kurokawa [a], Kensei Nishida [a], Yuki Kuwano [a], Tomoko Kawai [a], Shigetada Teshima-Kondo [a], Sachiko Chikahisa [b], Yoshihiro Tsuruo [c], Hiroyoshi Sei [b], Kazuhito Rokutan [a]

[a] Departments of Stress Science, Institute of Health Biosciences, The University of Tokushima Graduate School, Tokushima 770-8503, Japan
[b] Departments of Integrative Physiology, Institute of Health Biosciences, The University of Tokushima Graduate School, Tokushima 770-8503, Japan
[c] Department of Anatomy and Cell Biology, Wakayama Medical University School of Medicine, Wakayama 641-8509, Japan

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ABSTRACT

In this study, we examined the effects of *IL-18* deficiency on behaviors and gene expression profiles in 6 brain regions. *IL-18*$^{-/-}$ mice reduced depressive-like behavior and changed gene expressions predominantly in the amygdala compared with wild-type mice. Pathway analysis of the differentially expressed genes ranked behavior as the top-scored biological function. Of note, the absence of *IL-18* decreased *Avp*, *Hcrt*, *Oxt*, and *Pmch* mRNA levels and the number of arginine vasopressin- and oxytocin-positive cells in the amygdala, but not in the hypothalamus. Our results suggest that *IL-18*-dependent vasopressinergic and oxytocinergic circuitry in the amygdala may regulate depressive-like behaviors in mice.

1. Introduction

Pro-inflammatory cytokines play a major role in inflammatory and immune responses, and also have an important role of physiological and behavioral responses in the central nervous system (CNS) (Kronfol and Remick, 2000; Rothwell and Luheshi, 2000). Pro-inflammatory cytokines regulate locomotion, emotion, social interaction, and sleep (Dantzer, 2001), and are suggested to be involved in the pathogenesis of several mood disorders (Kronfol and Remick, 2000). For example, an increased release of interleukin (IL)-1, IL-2, IL-6, and interferon (IFN)-γ has been reported in major depression patients (Kronfol and Remick, 2000; Schiepers et al., 2005). In addition, IL-6 deficient mice displayed a reduction in depressive-like behavior (Chourbaji et al., 2006). Several lines of evidence have suggested an important crosstalk between pro-inflammatory cytokines and CNS signaling, while little is known about specific roles of pro-inflammatory cytokines in the regulation of distinct neuronal networks.

IL-18, originally identified as an IFN-γ inducing factor (Okamura et al., 1995), is a member of the IL-1-superfamily and is now recognized as a multifunctional cytokine facilitating an up-regulation of Fas (CD95/APO-1) ligand expression, increase in cytolytic activity of T cells, and production of T helper 2 cytokines (Boraschi and Dinarello, 2006; Nakanishi et al., 2001a; Nakanishi et al., 2001b). Several recent studies have suggested a potential role for IL-18 in psychological stress responses (Nishida et al., 2009; Sekiyama et al., 2005; Yang et al., 2005). Our recent report showed that acute psychological stress stimulated IL-18 secretion from the adrenal gland, which was necessary for the subsequent production of cytokines, including IL-6 (Sekiyama et al., 2005). Isolation stress increased the expression and production of IL-18 in the mouse rectum (Nishida et al., 2009). In the CNS, IL-18 was especially expressed in both astrocytes and microglia cells (Conti et al., 1999), and its expression was stimulated by corticotropin-releasing hormone (CRH) (Yang et al., 2005). Moreover, it was reported that the expression of IL-18 was increased in the posterior cortex in a mice model of depression (Kroes et al., 2006). These studies led us to speculate that IL-18 might be involved in physiological and behavioral responses.

Behavioral responses are likely influenced by many genes (Seong et al., 2002). However, reflecting the anatomical and functional differences of individual brain regions, gene expression shows the region-specific pattern (Letwin et al., 2006; Nadler et al., 2006; Pavlidis and Noble, 2001). Moreover, considering the complex connection to the other various brain regions, it is complicated to determine which particular genes in any given region are responsible

* Corresponding author. Department of Stress Science, Institute of Health Biosciences, The University of Tokushima Graduate School, 3-18-15 Kuramoto-cho, Tokushima 770-8503 Japan. Tel.: +81 88 633 9004; fax: +81 88 633 9008.
E-mail address: tana@basic.med.tokushima-u.ac.jp (T. Tanahashi).

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for behavioral changes (Nestler and Carlezon, 2006). For example, the prefrontal cortex intelligently regulates the behaviors, thought, and emotion through connections with the amygdala, striatum, and brain stem under the normal conditions (Arnsten, 2009). The olfactory bulb, along with the amygdala and hippocampus, forms a part of the limbic system that contribute to the emotional components of behavior (Song and Leonard, 2005). Under the stressful conditions, the amygdala activates the hypothalamus, striatum, and brain stem, which induce high release of catecholamines (Arnsten, 2009).

In this study, we evaluated behavioral changes in *IL-18*$^{-/-}$ mice with a test battery consisting of 4 evaluations (open field, light-dark transition, elevated plus maze, and forced swimming tests) to examine the effects of IL-18 deficiency on behavioral changes. Using whole mouse genome microarrays, gene expression signatures were analyzed across 6 different brain regions (olfactory bulb, prefrontal cortex, striatum, amygdala, hypothalamus, and hippocampus) involved in the regulation of physiological and behavioral responses. Finally, a pathway analysis was conducted to reveal specific gene related to behavioral changes in *IL-18*$^{-/-}$ mice.

2. Materials and methods

2.1. Mice and breeding plan for *IL-18*$^{-/-}$ mice

We purchased *IL-18*$^{-/-}$ mice and C57BL/6 mice from Charles River Japan (Kanagawa, Japan). *IL-18*$^{-/-}$ mice were backcrossed to C57BL/6 for at least 8 generations at the Jackson Laboratory (Bar Harbor, ME). To reduce differences in genetic backgrounds between *IL-18*$^{-/-}$ and C57BL/6 mice, we conducted an additional breeding plan (Fig. S1). To produce F1 generation mice, 3 male *IL-18*$^{-/-}$ mice were mated with 6 female C57BL/6 J mice. We mated F1 mice and created their F2 littermates. From this breeding strategy, we prepared 50 F2 male mice.

2.2. Housing and genotyping

Male mice littermates at six weeks of age were weaned. They were housed in plastic mouse cages (282 mm × 451 mm × 157 mm), and allowed free access to tap water and a laboratory animal chow (MF; Oriental Yeast Co., Tokyo, Japan). All mice were maintained on a 12 h light/dark cycle (lights on at 9:00) at 24 ± 2 °C and 50–60% humidity. They were treated in accordance with the APS Guiding Principles in the Care and Use of Animals. All procedures were approved by the Animal Care Committee of the University of Tokushima. Mice were subjected to behavioral tests and sacrificed under general anesthesia with diethyl ether. Fifty male mice were genotyped with PCR according to the protocol of the Jackson Laboratory from the liver after finishing the behavioral tests. As a result, wild-type ($n = 15$), *IL-18*$^{+/-}$ ($n = 17$), and *IL-18*$^{-/-}$ mice ($n = 18$) were prepared for this study. We measured body weights at 20:00 h once a week. At 12 weeks, body weights of wild-type, *IL-18*$^{+/-}$, and *IL-18*$^{-/-}$ mice were 27.9 ± 0.6, 27.0 ± 0.3, and 27.6 ± 0.8 g (means ± SEM).

2.3. Behavioral test procedures

Behavioral tests were conducted as previously described (Yamamoto et al., 2009). Briefly, these tests were performed in the order from the least invasive to the most invasive test (open field, light–dark transition, elevated plus maze, and then forced swimming tests) (McIlwain et al., 2001; Paylor et al., 2006). At 12 weeks, wild-type ($n = 10$), *IL-18*$^{+/-}$ ($n = 12$), and *IL-18*$^{-/-}$ mice ($n = 13$) were sequentially subjected to one behavioral test per day over 5 days (Fig. S2). Behavioral tests were performed between 13:00 and 15:00 h. Before each test, mice were habituated to the experimental room for 2 h prior to the behavioral test. Mouse behaviors were videotaped and measured using an automated image analysis system (Image J OP, LD, EP, and FZ, O'hara, Tokyo, Japan), which was modified with the public domain Image J software (http://www.rsb.info.nih.gov/ij/). All experiments were done in a blinded and randomized fashion, because the genotype of all mice was verified after a set of behavioral tests.

2.4. Open field test

Locomotor activity and anxiety-like behavior were assessed by the open field test (Prut and Belzung, 2003). In this test, a white acrylic chamber (45 cm long, 45 cm wide, and 30 cm high) was placed in the experimental room and illuminated by four indirect and homogenous lamps (150–200 lx). Each mouse was individually placed at a corner of the open field arena. Locomotor activity, distance traveled, total time in the central area, and movement speed were measured for 10 min. Increased time spent in the central area has been shown to be a valid index of reduced anxiety-like behavior (Prut and Belzung, 2003).

2.5. Light–dark transition test

Anxiety-like behavior was assessed using the light–dark transition test (Crawley and Goodwin, 1980). A chamber was divided into two equal-sized compartments (22 cm long, 24 cm wide, and 30 cm high). The light compartment (450–500 lx) was composed of white flooring, transparent walls, and lid. The dark compartment (0–5 lx) was composed of black flooring, walls, and lid. This compartment was completely enclosed except for a small opening (5 cm wide and 3.5 cm high) to allow movement between the dark and light compartments. Mice were placed in the dark compartment, and the latency to move to the light compartment for the first time was recorded. The time spent in each compartment was also recorded for 5 min. Increased time spent in the light compartment generally indicates a reduction in anxiety-like behavior (Bourin and Hascoet, 2003).

2.6. Elevated plus maze test

Anxiety-like behavior was also examined using the elevated plus maze test (Lister, 1987). The plus maze consisted of two connected runways. One runway had closed arms (30 cm long and 7 cm wide) protected by 15 cm high walls. Another runway had open arms (30 cm long and 7 cm wide) without walls. The four maze arms originated from a central platform (7 cm square). The maze was situated 70 cm above the floor. Each mouse was individually placed at the center of the maze facing the closed arm, and behavior in the maze was recorded for 5 min. The number of entries and time spent in the open and closed arms were measured automatically.

2.7. Forced swimming test

Depressive-like behavior was evaluated by the forced swimming test (Cryan et al., 2005). Mice were individually placed in a plastic cylinder (25 cm high and 20 cm diameter) containing water 15 cm deep at 25 °C. Behavior was recorded for 5 min. When a mouse was observed floating in the water in an upright position without moving, it was considered to be immobile.

2.8. Tissue collection

Wild-type ($n = 5$), *IL-18*$^{+/-}$ ($n = 5$), and *IL-18*$^{-/-}$ mice ($n = 5$) were sacrificed under general anesthesia with diethyl ether. After systemic perfusion with cold phosphate-buffered saline through the heart using a syringe attached to a 21-G needle, the whole mouse brain was removed. Coronal brain sections (1 mm thick) were prepared on ice using a brain slicer (Muromachi Kikai, Tokyo, Japan). The olfactory bulb section was sliced between 3.5 and