Published in final edited form as:

Behav Brain Res. 2006 July 15; 171(1): 94–108.

Feeding, exploratory, anxiety- and depression-related behaviors are not altered in interleukin-6-deficient male mice

Artur H. Swiergiel^{a,b,*} and Adrian J. Dunn^a

^a*Department of Pharmacology, Toxicology and Neuroscience, Louisiana State University Health Sciences Center, P.O. Box 33932, Shreveport, LA 71130-3932, USA* ^b*Institute of Genetics and Animal Breeding, Polish Academy of Sciences, Jastrzebiec, Poland*

Abstract

Interleukin-6 (IL-6) has been implicated in behavioral responses associated with inflammation, sickness behavior and various nervous system disorders. We studied a range of different behaviors in IL-6-knockout (IL-6ko) and wild-type (WT) male mice. No significant differences were observed in ambulatory, exploratory, and stereotypic activities in home or novel cages, in an open field (OF), in the multicompartiment chamber (MCC), or in the elevated plus-maze (EPM). IL-6ko mice shed fewer fecal boli than WT mice in the OF, in novel cages and in the MCC although this effect was not statistically significant in the OF. In novel cages, intraperitoneal (i.p.) injection of IL-6 (1 µg) depressed ambulatory activity slightly more in IL-6ko than in WT mice. Restraint and interleukin-1β (IL-1β, 100 ng i.p.) decreased exploration of mice in the MCC and EPM, but there was no indication of altered sensitivity in IL-6ko mice. No significant differences were detected in the tail suspension and the Porsolt forced swim tests. IL-1β and lipopolysaccharide (LPS 1 µg i.p.) injection depressed sweetened milk and solid food intake similarly in IL-6ko and WT mice, but IL-6 had no effect, suggesting that IL-6 is not involved in these effects of IL-1 or LPS. However, IL-1β and LPS depressed body weight more in WT than in IL-6ko mice. Plasma corticosterone and basal concentrations of catecholamines, indoleamines and their metabolites in several brain regions were similar. The responses in these measures to IL-1β and LPS were also similar, except that there were no significant changes in tryptophan and serotonin metabolism in IL-6ko mice. This may reflect a role for IL-6 in the tryptophan and serotonin responses to IL-1 and LPS. It is concluded that the lack of IL-6 is not associated with substantial alterations in several different mouse behaviors, and in the responses to restraint, IL-1β, IL-6 and LPS.

Keywords

Interleukin-6-knockout; Behavior; Interleukin-1; Lipopolysaccharide; Catecholamines; Serotonin; Tryptophan; Corticosterone

1. Introduction

Infections and administration to mice of bacterial endotoxin (lipopolysaccharide, LPS) induce sickness behavior which includes hypophagia and decreases in locomotor and exploratory behavior [42,77,78]. The mechanisms underlying sickness behavior have not been fully elucidated, but it has been suggested that the cytokines, interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor-α (TNFα) are involved [17]. All three cytokines may be secreted in response to infections and LPS elevating their concentrations in plasma [18,29,

* Corresponding author. Tel.: +1 318 675 7869; fax: +1 318 675 7857. E-mail address: aswier@lsuhsc.edu (A.H. Swiergiel).

53,66]. Administration of IL-1 β has been well documented to induce many aspects of sickness behavior, such as depressed locomotor and exploratory activities, decreased feeding, and disturbed social interactions (see reviews [17,42]). However, IL-1 administration to animals induces secretion of IL-6 [45,66]. Thus, IL-6 could be responsible for some or all of these behavioral effects of IL-1. LPS administration initiates a cascade resulting in the secretion and consequent increase in plasma concentrations of TNF α , IL-1 β and IL-6 [29]. Because, IL-1 and TNF α can both induce IL-6 production [66], complex interactions among these three cytokines may occur in vivo following LPS administration, or infection with pathogens. Increases in brain concentrations of IL-6 have also been associated with certain stressful treatments and other pathologies [31,50,53,79].

Thus, it has been suggested that IL-6 mediates some aspects of sickness behavior [9,35], and that it may play a role in some disorders of mental health, including depression and anorexia nervosa [46,57]. Support for these hypotheses is circumstantial. IL-6 and receptors for IL-6 are thought to be present in neurons in various areas of the central nervous system (CNS), such as the hippocampus, the neocortex and the cerebellum [28,60]. Astrocytes have also been reported to express mRNA for both IL-6 and its receptors [64]. Peripheral administration of IL-6 was reported to produce a modest activation of the hypothalamo-pituitary-adrenal (HPA) axis [54,82], see also [19,68]. Peripheral administration of IL-6 also increases brain tryptophan and activates serotonin metabolism in mice [82,86,88]. These endocrine and neurochemical responses could affect behavior.

However, there are but few reports concerned with the effects of exogenous IL-6 on behavior and the observed responses have been few, rather subtle and often conflicting. Zalcman et al. [87] found that peripheral injections of IL-6 increased ambulatory exploration, digging, and rearing, with modest increases in locomotion and grooming in mice. Systemically administered IL-6 did not affect responding for rewarding hypothalamic stimulation that was disrupted by interleukin-2 [4], and did not affect feeding [76]. Intracerebroventricular (i.c.v.) IL-6 decreased locomotion and lever-pressing for food [65]. The latter findings are supported by the observations that autoimmune MRL-lpr mice with chronic upregulation of IL-6 displayed reduced sucrose consumption [63]. Also, mice infected with Ad5mIL6 adenovirus (known to induce excessive IL-6 concentrations over several days) decreased food, water and sucrose intake, novel object exploration, and swimming in the forced swim test [62]. On the other hand, Lenczowski et al. [43] reported that i.c.v. IL-6 did not result in changes in social investigatory and locomotor behavior; it reduced the behaviors only when it was administered along with IL-1.

Indirect evidence for a functional significance of IL-6 in mediating behavior has been more convincing. Alleva et al. [3] reported increased aggressive behavior in mice lacking functional genes for IL-6 (IL-6-knockout, IL-6ko mice) and facilitated affiliative-type social interactions in mice overexpressing IL-6. IL-6ko mice were also found to exhibit attenuated effects of LPS and IL-1 on social exploration and changes in body weight [9]. They also exhibited a facilitation of radial maze learning [11]. Furthermore, IL-6-deficient mice were found to differ from wild-type (WT) mice in both undisturbed and LPS-induced sleep and body temperature patterns [51,52].

In the present study, we examined the potential role of IL-6 in behavior, and in the behavioral responses to immune challenges and during stress. The effects of LPS, IL-1 β , IL-6, restraint and novel environments on a number of different behaviors were studied in male IL-6ko mice. We also studied the brain biogenic amines and plasma ACTH and corticosterone and their responses to IL-1 β and LPS.

2. Materials and methods

2.1. Animals and chemicals

Two separate groups of congenic IL-6 knockout (IL-6ko) male mice (BALB.B/Ai-[KO] IL6N9) and matched wild type (WT) controls (BALB.B/Ai-IL6N9) were obtained from Taconic Farms. The genotypes were verified by the supplier, and all mice were homozygous. The animals derived from mice rendered deficient in IL-6-production by targeted disruption of gene that encodes IL-6 and generated by Kopf et al. [38]. The original mice (C57BL/6 × 129 Sv) heterozygous for the mutation (IL-6^{+/-}) were interbred to obtain mice homozygous for the mutation. The line was sent to Dr. H.C. Morse at NIH/NIAID, who began backcrossing it onto BALB.B background in 1994. The line was placed with Taconic at N3 in 1995. Backcrossing at Taconic continued to N9 at which point the line was maintained through intracrossing to make it homozygous for the gene targeted allele (the first batch was F13, and the second batch was F17 of intracrossing). Upon arrival in our facilities, the animals were housed in individual plastic cages with wood shaving bedding, at a room temperature of 22–24 °C and under a 12 h light–dark cycle with lights on at 7 a.m. Mice were given free access to water and pelleted Purina rodent chow. At the beginning of the experiments the animals were six weeks old and weighed 23.4 ± 0.5 g (first batch, 12 IL-6ko and 12 IL-6WT mice) and 24.7 ± 2.1 g (second batch, 18 IL-6ko and 18 IL-6WT mice). The procedures were conducted in accordance with the NIH guide on the care and use of animals for research, and an in-house protocol approved by the LSUHSC-S Animal Care and Use Committee.

Recombinant mouse IL-6 (R&D Systems), recombinant mouse IL-1 β (R&D Systems), or lipopolysaccharide (LPS, Sigma Cat. No.: L-3755; extracted from *E. coli* 026:B6) dissolved in sterile saline were injected intraperitoneally (i.p.) 30, 90 or 120 min before the behavioral tests and 120 min before the biochemical assays. The time points chosen were those at which LPS, IL-1 or IL-6 had produced the most reliable or the largest behavioral and neurochemical effects in our previous experiments [19,21,22,25,76–78,81,82]. Because previous research has indicated the development of tolerance to endotoxin [84], each animal was treated with LPS only once. If an animal was treated more than once with IL-1, injections of IL-1 were at least two weeks apart. Many previous experiments have not indicated any tolerance to IL-1 when injections were separated by several days.

2.2. Behavioral paradigms and experimental design

Mice were tested in seven different behavioral paradigms in the following order: activity in home and novel home cages (clean and without bedding), activity in an open field (OF), the multicompartament chamber (MCC), the elevated plus-maze (EPM), the tail suspension test (TST), and the Porsolt forced swim test (FST). The OF, MCC, EPM, TST and FST observations were conducted in a dimly lit room adjacent but separate from the animal colony room, between 10 a.m. and 2 p.m. The animals had not been habituated to the observation room before the tests. Each behavioral test was performed on a different day, at least 2 days apart. Intake of solid chow and sweetened milk was assessed in home cages in the colony room after having completed the previously listed tests. Unless indicated otherwise, a 2 × 2 experimental design was used with six animals in each of four groups: WT-untreated; WT-treated; IL-6ko-untreated; IL-6ko-treated. In some experiments, mice were restrained by placing them for 25 min in 50 ml centrifuge tubes as previously described, and tested immediately after removal from the tubes [8]. Observers who scored behavior in the OF, MCC, EPM, TST, and FST were unaware of the specific experimental treatments or genotype of the mice. Following each trial, the behavioral apparatus were cleaned with 1% aqueous acetic acid.

2.3. Activity in home and novel cages

Behavioral activity of six mice of each genotype was scored for 10 min in the home cages. The remaining mice (six WT and six IL-6ko) were removed from their home cages and placed in identical clean cages without litter (“novel” cages) and activity assessed 2 min later for 10 min. The cages were inserted in activity frames (Omnitech Electronics, Inc., Columbus, OH). Infrared (IR) light diodes and photocells making 16 IR beams were spaced 2.5 cm apart along the long axis of the frame. The number and temporal pattern of IR beam interruptions were recorded in a computer and parameters determined by the software were scored. Briefly, the values generated by the system were designed to reflect: (1) total activity as the sum of ambulatory activity and stereotypic events, that is the total number of beam interruptions that occurred during the sample period; (2) ambulatory events—horizontal shifting of an animal interrupting the consecutive IR beams, (3) stereotypic events—body shift interrupting the same beam (or set of beams) repeatedly – detects head bobbing, grooming, etc., (4) discrete horizontal ambulatory episodes – continuous crossing through several consecutive beams – individual episodes are separated from each other by a rest period of at least 1 s, (5) stereotypic episodes – repeated breaking of the same beam(s) – a break in stereotypy of 1 s or more is required to separate one stereotypic episode from the next. The system generated both the number and the duration of events and episodes. Fecal boli in clean cages were counted after completing activity monitoring.

2.4. Activity in an open field (OF)

Spontaneous locomotor activity was quantified in an open field, a white plastic box 59 cm × 59 cm with its floor divided into 16 squares and illuminated by white light (20 W). Line crossings, rears and climbing were scored for 5 min. A line crossing was counted when all four paws were removed from one square and entered another. Line crossings were separated into central and peripheral based on the nature of the square entered. Rears were scored when a mouse raised both front paws from the floor, and climbs when an animal leaned its front paws against a wall. Time spent in the central and peripheral squares was also measured. Fecal boli were counted.

2.5. Multicompartment chamber (MCC)

The MCC apparatus and testing procedures have been described previously [8]. In brief, the apparatus consisted of a box divided into nine interconnecting compartments, each with a small hole in the floor. Recessed below each hole, a wire ball was rigidly attached to serve as a stimulus. A stimulus contact was defined when a mouse made any contact with that wire ball. The test was initiated by placing the mouse in the central compartment of the apparatus and the frequency and duration of contacts, the number of compartment entries as well as grooming, rears, and the number of fecal boli were scored for 25 min.

2.6. Elevated plus-maze (EPM)

The elevated plus-maze was made of a black Plexiglas cross (arms 30 cm long × 5 cm wide) elevated 40 cm above the floor [30]. Two opposite arms were enclosed by the transparent walls (30 cm long × 15 cm high) and two arms were open. The mouse was placed in the center of the apparatus facing an enclosed arm and observed for 5 min in red light. The time spent in the enclosed and open arms and the total number of entries into the enclosed and open arms was recorded.

2.7. Tail suspension test (TST)

The behavior of mice suspended by their tails was studied as described by Steru et al. [72]. A short piece of paper adhesive tape (about 6 cm) was attached along half the length of the tail (about 3 cm). The free end of the adhesive tape was attached to a 30 cm long rigid tape (made

from the paper tape folded several times), which was attached to a seesaw lever linked to a spring strain gauge that activated the hand of a spring balance. The animal was surrounded by a wooden enclosure (*H*: 45 cm, *W*: 40 cm, *D*: 40 cm) painted white such that the mouse's head was about 20 cm above the floor. Mice were observed for 6 min. Wriggling of the animal to avoid aversive situation produced movements of the hand on the balance that were recorded by an observer who could not see the animal directly. As recently pointed out, one of the confounding factors in the tail suspension test is tail-climbing behavior [48]. The tail-climbing periods tend to be scored as immobility by a mechanical device even though they clearly constitute avoidance behavior. Very few mice displayed tail climbing, but when this was observed, the data were omitted.

2.8. Forced swim test (FST)

The test was performed according to the method developed by Porsolt et al. for mice [58]. Mice were placed in a vertical glass cylinder (26 cm high, 12 cm in diameter) filled with 25 °C water to a depth of 16 cm. The water depth was chosen so that the animals must swim or float without their hind limbs or tail touching the bottom. For testing, each mouse was placed in the cylinder for 6 min, and the latency to float, and the duration of floating (i.e. the time during which mice made only the small movements necessary to keep their heads above water) was scored. As suggested by Porsolt et al. [58], only the data scored during the last 4 min were analyzed and presented.

2.9. Feeding behavior

Intake of sweetened condensed milk diluted with three parts of water was assessed as described previously [77]. Mice were habituated for at least 3 days to drink milk from 20 ml glass bottles fitted with metal spouts. The weighed bottles were placed in the cages at around 10 a.m. for 30 min, then removed and reweighed. All animals consumed the criterion of 1.5 g or more of milk during the session on the final day of habituation. Overnight intake of solid food was measured by placing two fresh and firm food pellets in a cage wire cover-feeder at 11 a.m. The remains of the pellets from the previous day were removed and weighed each morning at 8 a.m. Changes in body weight were followed by weighing the animals at the beginning of the experiment and before and after an experimental day. In all experiments overnight food intake and changes in body weight were recorded.

2.10. Brain biogenic amines and plasma corticosterone

Mice were killed by decapitation 120 min after injection of IL-1 β , LPS or saline. Trunk blood was collected in Eppendorf tubes containing 20 μ l of 0.1 M EDTA (disodium salt), centrifuged and plasma was stored at -30 °C. The brains were quickly removed from the skull and prefrontal cortex, hypothalamus, hippocampus and brain stem excised. They were weighed in the tared Eppendorf vials and frozen on dry ice as previously described [20]. The frozen samples were ultrasonicated in 0.1 M HClO₄ containing 1 mM EDTA and *N*-methyl dopamine (NMDA) as an internal standard, centrifuged and the supernatants analyzed using HPLC with electrochemical detection (for details see Dunn [23]). Plasma corticosterone concentrations were assayed using radioimmunoassay kits from ICN (Irvine, CA) as described by the manufacturer.

2.11. Statistical analyses

Multifactorial analysis of variance (ANOVA) was performed using SuperA-nova (Abacus Concepts, Inc.), followed by Fisher's least significant difference *t*-test for a priori designed pairwise comparisons. The main experimental factors were the genotype (WT or IL-6ko) and experimental treatments (IL-1 β , IL-6, LPS, novel environment or restraint). Covariance analysis (ANCOVA) was used when body weight could be a factor (feeding experiments).

Data are reported for the most representative experiment; there were no discrepancies between the two batches of animals or experiments. All results are presented as the mean $\pm$ S.E.M.

3. Results

All results are presented in the order in which the behavioral tests were performed.

3.1. Activity in home and novel cages

The basal activity of WT and IL-6ko mice was first observed in home and novel cages (six WT and six IL-6ko animals per environment) starting about 9 a.m. (2 h into the light phase) for 10 min. The total activity (ambulatory plus stereotypic events), the number of ambulatory events, the number of ambulatory episodes and their duration, the number of stereotypic events, the number and duration of stereotypic episodes, and, in novel cages only, the number of fecal boli were recorded (see Section 2 for the definitions). Fig. 1A-C shows the numbers of ambulatory and stereotypic events and the number and duration of ambulatory and stereotypic episodes in the home and novel cages. No statistically significant differences in any aspect of activity were observed between the genotypes, there were no effect of the cage environment, and no interaction between genotype and the cage environment (home or novel). Fig. 1A shows that ambulatory events (beam breaks) did not differ significantly between the genotypes ($F_{1,20} = 1.42$), nor were they affected by environment ($F_{1,20} = 2.94$), and there was no genotype $\times$ environment interaction ($F_{1,20} = 0.02$). Stereotypic events were not affected by genotype ($F_{1,20} = 0.03$) or environment ($F_{1,20} = 0.57$), and there was no genotype $\times$ environment interaction ($F_{1,20} = 0.23$). Fig. 1B shows that ambulatory episodes were not affected by the genotype ($F_{1,20} = 1.88$) or the environment ($F_{1,20} = 1.37$), and there was no interaction between the factors ($F_{1,20} = 0.01$). Neither the genotype ($F_{1,20} = 0.73$) nor the cage environment ($F_{1,20} = 0.29$) affected stereotypic episodes, and there was no significant interaction ($F_{1,20} = 0.37$). Fig. 1C indicates that the duration of ambulatory events did not differ significantly between the genotypes ($F_{1,20} = 0.90$), the cage environment ($F_{1,20} = 2.08$), nor was there a significant interaction ($F_{1,20} = 0.43$). Likewise, the duration of stereotypic events was not affected by the genotype ($F_{1,20} = 0.01$), or the environment ($F_{1,20} = 0.20$), and there was no interaction ($F_{1,20} = 0.02$). Fecal boli were counted in the novel cages without litter. IL-6ko mice defecated slightly less than WT animals in the novel cages ($F_{1,10} = 4.61$, $p = 0.057$), just short of statistical significance (Fig. 2B).

3.2. Activity after IL-6

The effects of administration of saline or IL-6 (1 μ g/mouse, i.p., 30 min before testing) on activity were assessed in the novel, clean cages (6 WT and 6 IL-6ko animals per treatment; Fig. 1D-F). A lower number of ambulatory events (Fig. 1D: beam breaks) in IL-6ko mice in comparison with WT animals did not quite reach statistical significance ($F_{1,20} = 4.08$, $p = 0.057$). Also, administration of IL-6 had no significant effect on ambulatory events (Fig. 1D: $F_{1,20} = 1.34$), and there was no significant genotype $\times$ treatment interaction ($F_{1,20} = 0.34$). The number of ambulatory episodes was not affected by genotype (Fig. 1E: $F_{1,20} = 0.99$) or IL-6 injections ($F_{1,20} = 2.49$) and there was no significant interaction between the genotype and IL-6 ($F_{1,20} = 1.94$). The duration of ambulation was significantly different between the genotypes (Fig. 1F: $F_{1,20} = 4.57$, $p < 0.05$), and was shortened by IL-6 in IL-6ko mice ($F_{1,20} = 4.45$, $p < 0.05$), but there was no significant interaction ($F_{1,20} = 2.28$).

Stereotypic events were not affected by genotype (Fig. 1D: $F_{1,20} = 3.38$, $p = 0.081$) or IL-6 injection ($F_{1,20} = 0.01$), and there was no significant genotype $\times$ treatment interaction ($F_{1,20} = 0.04$). The number of stereotypic episodes was higher in IL-6ko than in WT mice (Fig. 1E: $F_{1,20} = 4.51$, $p < 0.05$), but IL-6 administration had no effect ($F_{1,20} = 0.05$), and there was no significant interaction ($F_{1,20} = 0.27$). The duration of stereotypic behavior was not significantly

affected by genotype (Fig. 1F: $F_{1,20} = 2.41$), IL-6 injections ($F_{1,20} = 0.03$), and there was no significant interaction ($F_{1,20} = 0.01$). During exposure to the clean cages without litter, IL-6ko mice defecated significantly less ($F_{1,20} = 6.147$, $p < 0.05$) than WT animals, but there was no significant genotype $\times$ treatment interaction ($F_{1,20} = 0.683$; Fig. 2D).

3.3. Open field test

Mice were taken from their home cages, immediately placed in the open field and observed for 5 min. There were no statistically significant differences between the genotypes in the locomotor activity (Fig. 3). The animals displayed similar numbers of line crossings and the duration of stay in the central (aversive) part of the open field ($F_{1,22} = 0.19$ and 0.98 , respectively), and were equally active in the peripheral area ($F_{1,22} = 1.08$ and 0.143 , line crossings and duration of stay, respectively). IL-6ko mice defecated slightly less in the open field than WT animals, but this effect was not statistically significant (Fig. 2A).

3.4. Effect of IL-1 β on behavior in the multicompartiment chamber (MCC)

Animals were observed for 25 min in the MCC, 90 min after injection of IL-1 β (100 ng/mouse, i.p.) or saline. The behavior of WT and IL-6ko mice was similar in all behavioral measures (Fig. 4A-D). ANOVA indicated no effect of genotype on the mean stimulus-contact duration (Fig. 4A: $F_{1,20} = 0.001$), the number of stimulus contacts (Fig. 4B: $F_{1,20} = 0.82$), the number of compartment entries (Fig. 4C: $F_{1,20} = 0.98$), or the number of rears and climbs (Fig. 4D: $F_{1,20} = 3.90$, $p = 0.063$). Administration of IL-1 β strongly depressed all these parameters in both WT and IL-6ko mice ($F_{1,20} = 26$, 28 , 26 and 274 , respectively, all $p < 0.001$). However, there was no interaction between the genotype and IL-1 β for any of the parameters ($F_{1,20} = 0.35$, 0.34 , 1.03 and 2.66 , respectively). In the MCC, IL-1 β stimulated defecation ($F_{1,20} = 6.46$, $p < 0.05$) and, as in the OF and novel cage tests, IL-6ko mice defecated less than WT animals ($F_{1,20} = 6.46$, $p < 0.05$), but there was no significant interaction (Fig. 2C: $F_{1,20} = 0.10$).

3.5. Effect of restraint on behavior in the MCC

Mice were tested for 25 min in the MCC immediately after a 25 min period of restraint. Fig. 4E-H shows that as in the previous experiment, baseline parameters were similar in WT and IL-6ko mice. Genotype did not affect the mean contact duration (Fig. 4E: $F_{1,20} = 0.537$), the number of stimulus contacts (Fig. 4F: $F_{1,20} = 0.257$), or rears and climbs (Fig. 4H: $F_{1,20} = 0.91$). The number of compartment entries was lower in IL-6ko mice (Fig. 4G: $F_{1,20} = 4.47$, $p < 0.05$). Restraint decreased the mean contact duration ($F_{1,20} = 69$, $p < 0.001$) and increased the frequency of rears and climbs ($F_{1,20} = 8.76$, $p < 0.01$), but did not affect the number of stimulus contacts nor the number of compartment entries ($F_{1,20} = 0.80$ and 1.86). There were no significant interactions between the genotype and restraint for any of these measures ($F_{1,20} = 0.98$, 1.93 , 0.20 , and 0.002 , respectively).

3.6. Effect of IL-1 β on behavior in the EPM

Animals were observed for 5 min in the EPM, 90 min after IL-1 β or saline injections (100 ng/mouse, i.p.). As shown in Fig. 5, genotype did not affect the number of open arm entries (Fig. 5A: $F_{1,20} = 0.002$) nor the time spent on the open arms (Fig. 5B: $F_{1,20} = 0.005$), nor the total number of entries into the open and closed arms (Fig. 5C: $F_{1,20} = 2.49$). Administration of IL-1 β decreased the number of open arm entries, the time spent on the open arms and the total number of arm entries ($F_{1,20} = 6.02$, 4.67 , $p < 0.05$, and 14.0 , $p < 0.01$, respectively). However, there was no interaction between the genotype and IL-1 β with respect to the number of open arm entries ($F_{1,20} = 2.33$), the time spent on the open arms ($F_{1,20} = 0.13$), or the total number of arm entries ($F_{1,20} = 3.16$).

3.7. Effect of restraint on behavior in EPM

Mice were exposed for a second time to the EPM 5 days after the first test. They were placed for 5 min in the EPM immediately after a 25 min period of restraint. As in the experiment described above, genotype did not affect the number of open arm entries, the time spent on the open arms or the total entries (Fig. 5D-F: $F_{1,20} = 0.05, 0.02, 0.02$, respectively). ANOVA did not indicate statistically significant overall effects of restraint on the number of open arm entries, the time spent on the open arms or the total entries ($F_{1,20} = 1.13, 2.11, 0.416$, respectively). Also, there was no significant genotype $\times$ restraint interaction for the number of open arm entries, the time spent on the open arms, nor the total number of entries ($F_{1,20} = 1.41, 2.35, 0.41$, respectively).

3.8. Activity in the forced swim and tail suspension tests

Mice were observed for 6 min in the tail suspension test and, on a subsequent day, for 6 min in the forced swim test. The immobility times were remarkably similar in both WT and IL-6ko mice, and in neither test did the genotype affect the latency to immobility or the duration of immobility (Fig. 6).

3.9. Effect of mL-1 β on milk and food intake and body weight

In the three feeding experiments (IL-1, LPS and IL-6) six mice were used per genotype and treatment (four groups of six animals). Saline or IL-1 β (100 ng/mouse) was injected 90 min before presentation of the milk bottles (Fig. 7A–C). IL-1 β treatment significantly depressed the milk drinking and the subsequent overnight food intake ($F_{1,20} = 39$ and 18, respectively, $p < 0.001$), and body weight on the next day ($F_{1,20} = 7.76$, $p < 0.05$). The effect of genotype on milk or food intake and body weight was not statistically significant ($F_{1,20} = 1.28, 0.27$ and 0.35, respectively). There were no statistically significant genotype $\times$ IL-1 β interactions for milk or food intake, or body weight ($F_{1,20} = 0.38, 0.20$ and 0.55, respectively).

3.10. Effect of LPS on milk and food intake and body weight

LPS (1 μ g/mouse) significantly depressed milk intake observed 120 min after injection as (Fig. 7D: $F_{1,20} = 43$, $p < 0.001$), as well as overnight food intake (Fig. 7E: $F_{1,20} = 16$, $p < 0.001$), and body weight (Fig. 7F: $F_{1,20} = 5.74$, $p < 0.05$). There were no statistically significant differences between the genotypes in milk intake and body weight responses to LPS ($F_{1,20} = 1.25$, and 0.850). A significantly lower overnight consumption of food was observed in IL-6ko mice ($F_{1,20} = 5.45$, $p < 0.05$). However, after including body weight as a covariate in the ANOVA (the IL-6ko were slightly smaller than the WT mice), food intake was similar. Furthermore, ANOVA did not reveal significant genotype $\times$ LPS interactions for milk or food intake or body weight ($F_{1,20} = 0.009, 0.23$, and 1.02, respectively).

3.11. Effect of IL-6 on milk and food intake and body weight

IL-6 (1 μ g/mouse) or saline was injected 30 min before allowing access to milk. Administration of IL-6 did not alter milk intake, food intake or body weight (Fig. 7G-I: $F_{1,20} = 1.62, 0.07$, or 0.01, respectively). There was no effect of genotype on milk intake ($F_{1,20} = 0.21$) and no significant genotype $\times$ IL-6 interaction ($F_{1,20} = 0.21$). As in the preceding experiment, ANOVA indicated smaller overnight food intake and body weight changes in IL-6ko than in WT mice ($F_{1,20} = 7.09, 9.56$, $p < 0.01$). There was also a statistically significant genotype $\times$ IL-6 interaction for body weight changes ($F_{1,20} = 4.63$, $p < 0.05$). However, after including body weight as a covariate in the ANOVA, the effects of genotype and genotype $\times$ IL-6 interaction were no longer statistically significant.

3.12. Neurochemical measures

The responses of the noradrenergic, dopaminergic, and serotonergic systems in the prefrontal cortex, hypothalamus, hippocampus and brain stem were studied 120 min after i.p. injections IL-1 β and LPS. Ratios of catabolites to parent amines (MHPG:NE, DOPAC:DA and 5-HIAA:5-HT; measures of neurotransmitter turnover) are presented in Fig. 8. IL-1 β increased MHPG:NE ratios (Fig. 8A) in all four brain regions ($p < 0.001$). However, in none of the regions was there a significant effect of genotype or a genotype $\times$ IL-1 interaction. DOPAC:DA ratios (Fig. 8C) were increased by IL-1 β in hypothalamus and brain stem ($p < 0.001$), but not in the prefrontal cortex. An effect of genotype occurred only in brain stem ($F_{1,19} = 29.8$, $p < 0.001$). In none of the regions was there a significant genotype $\times$ IL-1 interaction. IL-1 β increased 5-HIAA:5-HT ratios (Fig. 8B) in prefrontal cortex ($F_{1,19} = 14.8$, $p < 0.001$) and hypothalamus ($F_{1,18} = 5.73$, $p < 0.05$), but this effect was only statistically significant in wild type mice. In none of the regions was there a significant effect of genotype, but a significant interaction was observed in the prefrontal cortex ($F_{1,19} = 12.9$, $p < 0.01$). IL-1 β increased tryptophan concentrations in all four brain regions (Fig. 8D). The effect of IL-1 β was statistically significant in prefrontal cortex ($F_{1,19} = 11.3$, $p < 0.01$), and almost significant in hypothalamus ($F_{1,18} = 4.33$, $p = 0.06$), hippocampus ($F_{1,16} = 3.25$, $p = 0.10$) and brain stem ($F_{1,19} = 4.21$, $p = 0.06$). A significant genotype $\times$ IL-1 interaction was observed in the pre-frontal cortex ($F_{1,19} = 5.37$, $p < 0.05$). Interestingly, these measures were only statistically significant in wild type mice.

Like IL-1 β , LPS elevated MHPG:NE ratios in all four brain regions, with significant increases in prefrontal cortex ($F_{1,22} = 4.35$, $p < 0.05$), hypothalamus ($F_{1,16} = 24.1$, $p < 0.001$), and brain stem ($F_{1,22} = 13.1$, $p < 0.01$), though not in hippocampus ($F_{1,15} = 2.29$). In none of the regions was there a significant effect of genotype or genotype $\times$ IL-1 interaction. DOPAC:DA ratios were not affected by LPS and there was no significant effect of genotype or a significant genotype $\times$ IL-1 interaction. LPS and genotype had no effect on 5-HIAA:5-HT ratios or tryptophan in any of the regions and there was no significant interaction.

3.13. Plasma corticosterone concentrations

Injection of IL-1 β (100 ng/mouse) and LPS (1 μ g/mouse) increased plasma concentrations of corticosterone at 120 min in both WT and IL-6ko mice (Fig. 9). IL-1 β induced significant elevations of concentration of corticosterone ($F_{1,18} = 13.4$, $p < 0.01$). However, ANOVA did not indicate any statistically significant effect of genotype ($F_{1,18} = 2.20$), and no treatment $\times$ genotype interaction ($F_{1,18} = 0.03$). Likewise, LPS induced significant increases in corticosterone ($F_{1,20} = 8.51$, $p < 0.01$), but there was no significant effect of genotype ($F_{1,20} = 0.26$), and no treatment $\times$ genotype interaction ($F_{1,20} = 0.02$). There were no significant differences between the genotypes in plasma ACTH concentrations (data not shown).

4. Discussion

Interleukin-6 plays an important role in immune system function [1,37,38,75,80]. Its primary established role is as the initiator of the acute phase response, an innate body defense mechanism observed during acute illness, which involves the production by the liver of acute-phase proteins (serum amyloid A, C-reactive protein (CRP), fibrinogen, alpha 1-antitrypsin, alpha 1-antichymotrypsin and haptoglobin [7,15,31]). IL-6 can be produced by a variety of cell types, including lymphocytes, fibroblasts, endothelial cells, macrophages and microglia [1]. IL-6 plays a critical role during immune responses, for example, stimulating the proliferation of B cells and immunoglobulin production, and the proliferation and differentiation of T cells [6,80]. Its basal blood plasma concentration can be increased more than 100-fold by infections, and peripheral administration of LPS or IL-1 [31,67]. In the brain, IL-6 appears to play a critical role in fever. I.c.v. administration, but not peripheral

administration of IL-6 elevates body temperature [40,81]. Moreover, IL-6ko mice failed to generate a fever following a turpentine-induced abscess, although they exhibited fever after influenza virus infection [40]. Abnormalities in IL-6 have also been implicated in a number of physiological and behavioral responses in stress [53,79] and in various diseases, such as depression [44,89], amyotrophic lateral sclerosis [41], systemic lupus erythematosus [34], Alzheimer's disease [32], post-traumatic stress disorder [5], and various CNS injuries [39] and infections [47].

Despite the many functions suggested for IL-6, the present experiments revealed very few differences in the behavior of IL-6-deficient and wild-type mice. Transgenic mice lacking a functional gene for IL-6 behaved in ways remarkably similar to those observed in the wild type animals. Moreover, the responses to various stimuli, be they psychogenic stressors (open field, novel environment, or restraint), or treatments such as IL-1 β , IL-6, or LPS were very similar. These treatments reliably affect locomotion and exploration, disturb feeding, and can produce anxiety- and depression-like behaviors, as well as stimulating the HPA axis, and cerebral noradrenergic and serotonergic activity in the brains of mice and rats. Thus, whatever roles IL-6 plays in these responses in wild type mice has probably been subserved by other factors in IL-6ko mice.

4.1. Locomotor and exploratory activity

The behaviors of the mice were assessed in several different tests: ambulatory and stereotypic activity in their home and novel cages, line crossings in the open field, the number of compartment entries, stimulus contacts and rears and climbs in the multicompartiment chamber (MCC), and the number of arm entries in the elevated plus maze (EPM). There were no significant differences between the genotypes in activity and exploratory patterns in any of the tests in untreated or treated mice (novelty, restraint, IL-1 β or IL-6). The results indicate that the absence of IL-6 does not alter baseline behavior nor IL-1 β - or restraint-induced changes in behavior in the MCC or the EPM. These results are consistent with most published studies which found no significant differences in activity between the WT and IL-6ko mice [11,40]. On the other hand, Butterweck et al. [12] found that IL-6ko animals showed higher horizontal, ambulatory activity (line crossings) in an OF and shorter exploration times of the open arms of the EPM than control animals, as well as lower vertical, exploratory activity (rears) in the OF. In an experiment in which the effects of IL-6 injections on activity in novel clean cages were studied, the IL-6ko mice displayed an increase in stereotypic activity (Fig. 1). However, the difference, although statistically significant ($p < 0.05$), was small and the trend was not observed in a separate study of activity in the home and novel cages. We conclude that IL-6 deficiency has little if any effect on stereotypic behavior, and does not significantly alter motor and exploratory activity.

4.2. Anxiety-related behaviors

In the EPM, the behavior of IL-6ko mice was very similar to that of wild-type mice, including the responses to restraint or administration of IL-1 β . IL-6ko mice exhibited a slightly enhanced sensitivity to IL-1 β in the EPM that manifested itself in a larger decrease in the total arm entries than in WT mice. However, the time spent on the open arms was almost identical in both groups of mice. There have been reports that IL-1 β decreased open arm entries in the EPM suggesting increased anxiety [70,71], but the changes reported were not distinguished from the overall decrease in activity induced by IL-1 β . In the present experiments, there were no significant differences between the genotypes in basal locomotion and exploration. The substantial decrease induced by IL-1 β in the number of total entries in IL-6ko mice suggests that the decrease in open arm entries and duration does not reflect anxiety, but rather a general decrease in locomotor activity. Together with the lack of a significant interaction between genotype and IL-1, the results do not suggest any increased anxiogenic effects of IL-1 β in IL-6ko mice or a

major role for IL-6 in anxiety-like behavior in the EPM. On the contrary, the lack of an effect of restraint on the total arm entries (the overall locomotor activity) in both genotypes, coupled with a lower number of open entries in restrained WT mice could be taken to suggest a specific anxiogenic effect of restraint in WT mice, but not in IL-6ko animals. Butterweck et al. [12] concluded that their data from the OF and the EPM could be interpreted as either increased or decreased anxiety in IL-6ko mice (see above), and suggest that IL-6 plays a role in the control of emotionality. Taken together, the present results suggest that the IL-6ko mice may be slightly less anxious than the WT mice, but these effects were small and not statistically significant.

4.3. Defecation

The frequency of defecations was assessed in novel cages, the OF and the MCC. In each of these tests, the IL-6ko mice consistently shed fewer fecal boli than the WT animals (Fig. 2). Statistically significant decreases were observed in the MCC and novel, clean cages, though not in the OF. Exposure to the OF is considered anxiogenic, and a slightly decreased defecation by IL-6ko mice in the OF suggests that those animals were less stress responsive than the wild type mice. Likewise, the smaller number of fecal boli observed in the MCC and novel cages suggests that mice devoid of IL-6 may be slightly less anxious than wild type mice. Increased defecation and urination is considered to reflect stimulation of autonomic responses in stress [10]. Mice selected for displaying high anxiety (low activity) in the OF were reported to produce high defecation/urination scores in this test [85]. Defecation is independent of locomotor activity that could be a significant confound in the EPM and OF [69]. However, emotionality in mice can be defined by the co-occurrence of defecation and activity in a novel environment and emergence into the open arms of the EPM [33]. In the present experiments, the low defecation activity in the IL-6ko mice did not appear to be due to impaired bowel function, because IL-1 administration stimulated defecation in both genotypes. Therefore, based on defecation scores, it can be tentatively concluded, consistent with the EPM data, that IL-6ko mice display slightly less anxiety than the WT animals.

4.4. Depression-related behaviors

Major depression has been reported to be associated with IL-6 dysregulation and significant diurnal elevations in blood plasma IL-6 concentrations were observed [2,46,89]. However, the importance of IL-6 in depression has been questioned [26]. For example, one study reported lower cerebrospinal fluid concentrations of IL-6 in depressed patients compared to nondepressed controls [44], while another found an increase in depressed geriatric patients [74], and yet another study found no differences [14]. To the authors' knowledge, there have been no reports of IL-6ko mice studied in paradigms reflecting behavioral despair or behaviors relevant to problems of depression. We observed no differences between the IL-6ko and WT mice in the tail suspension test and in the forced swim test. The time spent immobile that is considered a measure of behavioral despair [16,58,72] was remarkably similar in both WT and IL-6ko mice. Sakic et al. [62] reported that a spontaneous or Ad5mIL6 adenovirus-induced elevation in serum IL-6 concentrations coincided with a small increase in floating in lupus-prone mice (not statistically significant). However, lupus-prone mice display a constellation of behavioral deficits so such a finding does not necessarily suggest a specific role for IL-6 in this test [63]. It is relevant that a recent report indicated rather nonspecific effects of IL-1 and LPS in the tail suspension and forced swim tests [25]. It is concluded that IL-6 deficiency does not affect behavior in the tail suspension and forced swim tests.

4.5. Sickness and feeding behavior

A role of IL-6 in sickness behavior induced by infections or LPS has been suggested [9,43], but the evidence has not been consistent. Kozak et al. [40] studied sickness behavior in IL-6ko mice with a turpentine abscess or influenza infection. The lack of IL-6 completely prevented

fever, anorexia and cachexia induced by turpentine abscess. However, the symptoms of sickness after influenza virus infection were only slightly modified in IL-6ko mice. In one report, injection of 1 μ g of IL-6 reduced food intake and gastric emptying in rats [49].

In the present experiments, administration of LPS and IL-1 β , but not of IL-6, robustly decreased food intake and body weight in wild type mice. IL-6ko animals responded very much like WT mice. The results from these feeding tests clearly indicate that the absence of IL-6 does not alter the hypophagic responses to IL-1 β and LPS. It may be concluded that IL-6 is not critical for the IL-1- and LPS-induced depression of feeding behavior. On the other hand, changes in body weight in response to IL-1 β , IL-6 and LPS did differ between WT and IL-6ko mice (Fig. 6). This is consistent with a previous report that the effects of LPS and IL-1 on changes in body weight were attenuated in IL-6ko mice, and that there was a statistically significant interaction between genotype and IL-1 or LPS [9]. Other reports suggest that IL-6 plays a role in anorexia and metabolism. For example, in IL-10-deficient mice subjected to chronic mild stress, an increased expression of brain IL-6 was correlated with body weight loss in stressed mice [50].

IL-6 has been proposed as one of the major circulating cytokines in catabolic states. It was observed that patients infused with IL-6 showed many endocrine and metabolic changes found in catabolic states and increased resting energy expenditure [73]. Remick et al. [59] reported that WT mice displayed a significantly greater weight loss than the IL-6ko animals in the later stages of sepsis. Also, mice injected with OVCAR3 ovarian carcinoma cells and then treated with a monoclonal antibody (mAb) directed against the murine IL-6 receptor showed a significant increase in survival time with a partial and temporary attenuation of cachexia symptoms [55]. These results suggest that IL-6 in the OVCAR3 model may be an important cachectogenic factor when centrally released by tumor cells. Furthermore, cachexia induced by MCG 101 tumors was attenuated in IL-6ko mice and in WT mice treated with mAb directed against IL-6 [13]. A role of IL-6 in whole body metabolism is further supported by the finding that IL-6-deficient mice displayed reduced endurance and oxygen consumption during exercise, suggesting that IL-6 is necessary for normal exercise capacity [27]. In a recent review, Pedersen and Fabbraio [56] suggested that IL-6 released from skeletal muscle during exercise plays a role in whole body metabolism. Consistent differences in body weight changes between WT and IL-6ko mice in response to LPS, IL-1 and IL-6, even though they were small, suggest that further studies on the potential role of IL-6 in cachexia, anorexia or whole body metabolism in stress may be warranted.

4.6. Neurochemical and endocrine responses

There were no significant differences between WT and IL-6ko mice in any of the neurochemical parameters measured. Moreover, consistent with the literature [21,24,36], both IL-1 β and LPS administration caused the normal increases in MHPG and MHPG:NE ratios, reflecting an activation of brain noradrenergic systems, although the responses to LPS in this experiment were somewhat weaker than we usually observe. IL-1 β also increased DOPAC and DOPAC:DA ratios reflecting a modest activation of dopaminergic systems. There were no differences between WT and IL-6ko mice consistent with our earlier observations that treatment with a monoclonal antibody (mAb) to IL-6 failed to prevent the noradrenergic responses to IL-1 β and LPS [83]. 5-HIAA and 5-HIAA:5-HT ratios, reflecting an activation of serotonergic systems, were also increased by IL-1 β in the prefrontal cortex and hippocampus, but this effect was statistically significant only in wild type mice. IL-1 β also increased brain concentrations of tryptophan in the prefrontal cortex and hippocampus. There were no statistically significant differences between the responses in the WT and IL-6ko mice, but once again significant increases occurred only in wild type mice. Because IL-6

administration increases brain tryptophan and serotonin metabolism [82,86,88], this may reflect the contribution of IL-6 to these indoleaminergic responses to IL-1 β and LPS [83].

The increases in plasma corticosterone induced by IL-1 β and LPS administration did not differ between WT and IL-6ko mice. This is also consistent with our studies using mAb to IL-6 which indicated that the IL-6 mAb treatment attenuated the plasma ACTH and corticosterone responses to IL-1 and LPS, but only at 3 h and longer time points after their administration [83]. Perhaps the variability we observed in the response to LPS obscured the small reduction in the corticosterone responses in IL-6ko mice reported by Ruzek et al. [61].

In summary, the behavioral, neurochemical and endocrine results indicate no major differences between the wild type and IL-6ko mice, except perhaps for diminished responses in tryptophan and serotonin metabolism. Also, IL-6 does not appear to be critically involved in mediating the behavioral responses to IL-1 or LPS, nor to stressful stimuli. Thus, either IL-6 plays little or no important role in the behaviors and systems we studied, or whatever role IL-6 plays in the normal behavior of the organism has been subserved in the IL-6 knockout mouse. Overall, these results are consistent with those reported earlier by others. Small differences in behavior have occasionally been observed, generally those associated with behavioral measures thought to reflect anxiety. However, our results tended to suggest a lower susceptibility to anxiety in IL-6ko mice (Figs. 2 and 5), in contrast to earlier literature reports that suggested a tendency to increased anxiety in IL-6ko mice [12]. Thus, it is possible that these subtle effects reflect differences between the WT and IL-6ko mice we and others have studied, that are not directly attributable to the absence of the gene for IL-6. Likewise, there are no major differences in norepinephrine, dopamine and the indoleamines in the IL-6ko mice. However, whereas the responses in the catecholamines to IL-1 β and LPS were very similar, our results suggest a deficit in the responses in tryptophan and serotonin metabolism, supporting a role for IL-6 in these responses to IL-1 β and LPS.

Acknowledgements

We thank Charles Dempsey for technical assistance with the neurochemical assays. This work was supported by a grant from the National Institutes of Health (NS35370). AHS research is also supported by the EU Framework 6 NEWMOOD Integrated Project and the Polish Committee for Scientific Research (KBN) Grant No. 3P04C05825.

References

1. Akira S, Taga T, Kishimoto T. Interleukin-6 in biology and medicine. *Adv Immunol* 1993;54:1–78. [PubMed: 8379461]
2. Alesci S, Martinez PE, Kelkar S, Ilias I, Ronsaville DS, Listwak SJ, et al. Major depression is associated with significant diurnal elevations in plasma interleukin-6 levels, a shift of its circadian rhythm, and loss of physiological complexity in its secretion: clinical implications. *J Clin Endocrinol Metab* 2005;90:2522–30. [PubMed: 15705924]
3. Alleva E, Cirulli F, Bianchi M, Bondiolotti FP, Chiarotti F, De Acetis L, et al. Behavioural characterization of interleukin-6 overexpressing or deficient mice during agonistic encounters. *Eur J Neurosci* 1998;10:3664–72. [PubMed: 9875345]
4. Anisman H, Kokkinidis L, Borowski T, Merali Z. Differential effects of interleukin (IL)-1 β , IL-2 and IL-6 on responding for rewarding lateral hypothalamic stimulation. *Brain Res* 1998;779:177–87. [PubMed: 9473665]
5. Baker DG, Ekhtor NN, Kasckow JW, Hill KK, Zoumakis E, Dashevsky BA, et al. Plasma and cerebrospinal fluid interleukin-6 concentrations in posttraumatic stress disorder. *Neuroimmunomodulation* 2001;9:209–17. [PubMed: 11847483]
6. Barton BE. The biological effects of interleukin-6. *Med Res Rev* 1996;16:87–109. [PubMed: 8788215]
7. Baumann H, Gauldie J. The acute phase response. *Immunol Today* 1994;15:74–80. [PubMed: 7512342]

8. Berridge CW, Dunn AJ. Corticotropin-releasing factor elicits naloxonesensitive stress-like alterations in exploratory behavior in mice. *Regul Peptides* 1986;16:83–93.
9. Bluthé R-M, Michaud B, Poli V, Dantzer R. Role of IL-6 in cytokine-induced sickness behavior: a study with IL-6 deficient mice. *Physiol Behav* 2000;70:367–73. [PubMed: 11006436]
10. Bouvier M. Physiology of fecal continence and defecation. *Arch Int Physiol Biochim Biophys* 1991;99:A53–63. [PubMed: 1720692]
11. Braidà D, Sacerdote P, Panerai AE, Bianchi M, Aloisi AM, Iosue S, et al. Cognitive function in young and adult IL (interleukin)-6 deficient mice. *Behav Brain Res* 2004;153:423–9. [PubMed: 15265638]
12. Butterweck V, Prinz S, Schwaninger M. The role of interleukin-6 in stress-induced hyperthermia and emotional behaviour in mice. *Behav Brain Res* 2003;144:49–56. [PubMed: 12946594]
13. Cahlin C, Körner A, Axelsson H, Wang W, Lundholm K, Svanberg E. Experimental cancer cachexia: the role of host-derived cytokines interleukin (IL)-6, IL-12, interferon- γ , and tumor necrosis factor α evaluated in gene knockout, tumor-bearing mice on C57 Bl background and eicosanoid-dependent cachexia. *Cancer Res* 2000;60:5488–93. [PubMed: 11034092]
14. Carpenter LL, Heninger GR, Malison RT, Tyrka AR, Price LH. Cerebrospinal fluid interleukin (IL)-6 in unipolar major depression. *J Affect Disord* 2004;79:285–9. [PubMed: 15023509]
15. Castell JV, Gómez-Lechón MJ, David M, Andus T, Geiger T, Trullenque R, et al. Interleukin-6 is the major regulator of acute phase protein synthesis in adult human hepatocytes. *FEBS Lett* 1989;242:237–9. [PubMed: 2464504]
16. Cryan JF, Mombereau C. In search of a depressed mouse: utility of models for studying depression-related behavior in genetically modified mice. *Mol Psychiatry* 2004;19:1050–62.
17. Dantzer, R.; Bluthé, R-M.; Castanon, N.; Chauvet, N.; Capuron, L.; Goodall, G., et al. Cytokine effects on behavior. In: Ader, R.; Felten, D.; Cohen, N., editors. *Psychoneuroimmunology*. San Diego, CA: Academic Press; 2001. p. 703–27.
18. Dinarello CA. Biology of interleukin 1. *FASEB J* 1988;2:108–15. [PubMed: 3277884]
19. Dunn, A. Cytokine activation of the hypothalamo-pituitary-adrenal axis. In: Steckler, T.; Kalin, N.; Reul, JMHM., editors. *Handbook of stress and the brain Part 2: stress: integrative and clinical aspects*. 15. Amsterdam: Elsevier; 2005. p. 157–74.
20. Dunn AJ. Stress-related changes in cerebral catecholamine and indoleamine metabolism: lack of effect of adrenalectomy and corticosterone. *J Neurochem* 1988;51:406–12. [PubMed: 2455773]
21. Dunn AJ. Systemic interleukin-1 administration stimulates hypothalamic norepinephrine metabolism paralleling the increased plasma corticosterone. *Life Sci* 1988;43:429–35. [PubMed: 3260987]
22. Dunn AJ. Endotoxin-induced activation of cerebral catecholamine and serotonin metabolism: comparison with interleukin-1. *J Pharmacol Exp Therap* 1992;261:964–9. [PubMed: 1602402]
23. Dunn, AJ. Neurochemical methods for evaluating cerebral biogenic amine responses to cytokines and their involvement in the central actions of interleukin-1. In: De Souza, EB., editor. *Neurobiology of cytokines*. 17. San Diego: Academic Press, Inc; 1993. p. 209–22.
24. Dunn, AJ. Effects of cytokines and infections on brain neurochemistry. In: Ader, R.; Felten, DL.; Cohen, N., editors. *Psychoneuroimmunology*. New York: Academic Press; 2001. p. 649–66.
25. Dunn AJ, Swiergiel AH. Effects of interleukin-1 and endotoxin in the forced swim and tail suspension tests in mice. *Pharmacol Biochem Behav* 2005;81:688–93. [PubMed: 15982728]
26. Dunn AJ, Swiergiel AH, de Beaupaire R. Cytokines as mediators of depression: what we can learn from animal studies? *Neurosci Biobehav Rev* 2005;29:891–909. [PubMed: 15885777]
27. Faldt J, Wernstedt I, Fitzgerald SM, Wallenius K, Bergstrom G, Jansson JO. Reduced exercise endurance in interleukin-6-deficient mice. *Endocrinology* 2004;145:2680–6. [PubMed: 14988384]
28. Gadiant RA, Otten UH. Interleukin-6 (IL-6)—a molecule with both beneficial and destructive potentials. *Prog Neurobiol* 1997;52:379–90. [PubMed: 9304698]
29. Givalois L, Dornand J, Mekaouche M, Solier MD, Bristow AF, Ixart G, Siaud P, et al. Temporal cascade of plasma level surges in ACTH, and corticosterone, and cytokines in endotoxin-challenged rats. 1994;267:R164–70.
30. Gorman AL, Dunn AJ. β -Adrenergic receptors are involved in stress-related behavioral changes. *Pharmacol Biochem Behav* 1993;45:1–7. [PubMed: 8100069]

31. Gruol DL, Nelson TE. Physiological and pathological roles of interleukin-6 in the central nervous system. *Mol Neurobiol* 1997;15:307–39. [PubMed: 9457704]
32. Hampel H, Teipel SJ, Padberg F, Haslinger A, Riemenschneider M, Schwarz MJ, et al. Discriminant power of combined cerebrospinal fluid τ protein and of the soluble interleukin-6 receptor complex in the diagnosis of Alzheimer's disease. *Brain Res* 1999;823:104–12. [PubMed: 10095017]
33. Henderson ND, Turri MG, DeFries JC, Flint J. QTL analysis of multiple behavioral measures of anxiety in mice. *Behav Genet* 2004;34:267–93. [PubMed: 14990867]
34. Hirohata S, Miyamoto T. Elevated levels of interleukin-6 in cerebrospinal fluid from patients with systemic lupus erythematosus and central nervous system involvement. *Arthritis Rheum* 1990;33:644–9. [PubMed: 2346520]
35. Johnson RW. The concept of sickness behavior: a brief chronological account of four key discoveries. *Vet Immunol Immunopathol* 2002;87:443–50. [PubMed: 12072271]
36. Kabiersch A, del Rey A, Honegger CG, Besedovsky HO. Interleukin-1 induces changes in norepinephrine metabolism in the rat brain. *Brain Behav Immun* 1988;2:267–74. [PubMed: 3266560]
37. Kishimoto T. Interleukin-6: from basic science to medicine—40 years in immunology. *Ann Rev Immunol* 2005;23:1–21. [PubMed: 15771564]
38. Kopf M, Baumann H, Freer G, Freudenberg M, Lamers M, Kishimoto T, et al. Impaired immune and acute-phase responses in interleukin-6-deficient mice. *Nature* 1994;368:339–42. [PubMed: 8127368]
39. Kossmann T, Hans V, Imhof H-G, Trentz O, Morganti-Kossmann MC. Interleukin-6 released in human cerebrospinal fluid following traumatic brain injury may trigger nerve growth factor production in astrocytes. *Brain Res* 1996;713:143–52. [PubMed: 8724985]
40. Kozak W, Poli V, Soszynski D, Conn CA, Leon LR, Kluger M. Sickness behavior in mice deficient in interleukin-6 during turpentine abscess and influenza pneumonitis. *Am J Physiol* 1997;272:R621–30. [PubMed: 9124487]
41. Krieger C, Perry TL, Ziltener HJ. Amyotrophic lateral sclerosis—interleukin-6 levels in cerebrospinal fluid. *Can J Neurol Sci* 1992;19:357–9. [PubMed: 1393846]
42. Larson SJ, Dunn AJ. Behavioral effects of cytokines. *Brain Behav Immun* 2001;15:371–87. [PubMed: 11782104]
43. Lenczowski MJP, Bluthé R-M, Roth J, Rees GS, Rushforth DA, Van Dam A-M, et al. Central administration of rat interleukin-6 induces hypothalamus-pituitary-adrenal activation and fever but not sickness behavior in rats. *Am J Physiol* 1999;276:R652–8. [PubMed: 10070124]
44. Levine J, Barak Y, Chengappa KN, Rapoport A, Rebey M, Barak V. Cerebrospinal cytokine levels in patients with acute depression. *Neuropsychobiol* 1999;40:171–6.
45. Libert C, Brouckaert P, Shaw A, Fiers W. Induction of interleukin-6 by human and murine recombinant interleukin-1 in mice. *Eur J Immunol* 1990;20:691–4. [PubMed: 2318254]
46. Maes M, Bosmans E, Calabrese J, Smith R, Meltzer HY. Interleukin-2 and interleukin-6 in schizophrenia and mania: effects of neuroleptics and mood stabilizers. *J Psychiatric Res* 1995;29:141–52.
47. Matsuzono Y, Narita M, Akutsu Y, Togashi T. Interleukin-6 in cerebrospinal fluid of patients with central nervous system infections. *Acta Paediatr* 1995;84:879–83. [PubMed: 7488810]
48. Mayorga AJ, Lucki I. Limitations on the use of the C57BL/6 mouse in the tail suspension test. *Psychopharmacology* 2001;155:110–2. [PubMed: 11374330]
49. McCarthy DO. Tumor necrosis factor alpha and interleukin-6 have differential effects on food intake and gastric emptying in fasted rats. *Res Nurs Health* 2000;23:222–8. [PubMed: 10871537]
50. Mormedè C, Castanon N, Medina C, Moze E, Lestage J, Neveu PJ, et al. Chronic mild stress in mice decreases peripheral cytokine and increases central cytokine expression independently of IL-10 regulation of the cytokine network. *Neuroimmunomodulation* 2003;10:359–66.
51. Morrow JD, Opp MR. Diurnal variation of lipopolysaccharide-induced alterations in sleep and body temperature of interleukin-6-deficient mice. *Brain Behav Immun* 2005;19:40–51. [PubMed: 15581737]
52. Morrow JD, Opp MR. Sleep-wake behavior and responses of interleukin-6-deficient mice to sleep deprivation. *Brain Behav Immun* 2005;19:28–39. [PubMed: 15581736]

53. Morrow LE, McClellan JL, Conn CA, Kluger MJ. Glucocorticoids alter fever and IL-6 responses to psychological stress and to lipopolysaccharide. *Am J Physiol* 1993;264:R1010–6. [PubMed: 8498588]
54. Naitoh Y, Fukata J, Tominaga T, Nakai Y, Tamai S, Mori K, et al. Interleukin-6 stimulates the secretion of adrenocorticotrophic hormone in conscious, freely-moving rats. *Biochem Biophys Res Commun* 1988;155:1459–63. [PubMed: 2845968]
55. Negri DR, Mezzananza D, Sacco S, Gadina M, Benigni F, Cajola L, et al. Role of cytokines in cancer cachexia in a murine model of intracerebral injection of human tumours. *Cytokine* 2001;15:27–38. [PubMed: 11509006]
56. Pedersen BK, Febbraio M. Muscle-derived interleukin-6—a possible link between skeletal muscle, adipose tissue, liver, and brain. *Brain Behav Immun* 2005;19:371–6. [PubMed: 15935612]
57. Pomeroy C, Eckert E, Hu S, Eiken B, Mentink M, Crosby RD, et al. Role of interleukin-6 and transforming growth factor- β in anorexia nervosa. *Biol Psychiatry* 1994;36:836–9. [PubMed: 7893847]
58. Porsolt RD, Bertin A, Jalfre M. Behavioural despair in mice: a primary screening test for antidepressants. *Arch Int Pharmacodyn* 1977;229:327–36. [PubMed: 596982]
59. Remick DG, Bolgos G, Copeland S, Siddiqui J. Role of interleukin-6 in mortality from and physiologic response to sepsis. *Infect Immun* 2005;73:2751–7. [PubMed: 15845478]
60. Rothwell, NJ. The role of cytokines in neurodegeneration. In: Rothwell, NJ., editor. *Cytokines in the Nervous System*. Austin, TX: R.G Landes Co; 1996. p. 145-62.
61. Ruzek M, Miller AH, Opal SM, Pearce BD, Biron CA. Characterization of early cytokine responses and an interleukin (IL)-6-dependent pathway of endogenous glucocorticoid induction during murine cytomegalovirus infection. *J Exp Med* 1997;185:1185–92. [PubMed: 9104805]
62. Sakic B, Gauldie J, Denburg JA, Szechtman H. Behavioral effects of infection with IL-6 adenovector. *Brain Behav Immun* 2001;15:25–42. [PubMed: 11259078]
63. Sakic B, Szechtman H, Braciak T, Richards C, Gauldie J, Denburg JA. Reduced preference for sucrose in autoimmune mice: a possible role of interleukin-6. *Brain Res Bull* 1997;44:155–65. [PubMed: 9292205]
64. Schöbitz B, de Kloet ER, Sutanto W, Holsboer F. Cellular localization of interleukin 6 mRNA and interleukin 6 receptor mRNA in rat brain. *Eur J Neurosci* 1993;5:1426–35. [PubMed: 8287192]
65. Schöbitz B, Pezeshki G, Pohl T, Hemmann U, Heinrich PC, Holsboer F, et al. Soluble interleukin-6 (IL-6) receptor augments central effects of IL-6 in vivo. *FASEB J* 1995;9:659–64. [PubMed: 7768358]
66. Shalaby MR, Waage A, Aarden L, Espevik T. Endotoxin, tumor necrosis factor- α and interleukin 1 induce interleukin 6 production in vivo. *Clin Immunol Immunopathol* 1989;53:488–98. [PubMed: 2805453]
67. Silverman MN, Pearce BD, Biron CA, Miller AH. Immune modulation of the hypothalamic-pituitary-adrenal (HPA) axis during viral infection. *Viral Immunol* 2005;18:41–78. [PubMed: 15802953]
68. Silverman, MN.; Pearce, BD.; Miller, AH. Cytokines. HPA axis regulation. In: Kronfol, Z., editor. *Cytokines and mental health*. Norwell, MA: Kluwer Academic Publishers; 2003. p. 85-122.
69. Singer JB, Hill AE, Nadeau JH, Lander ES. Mapping quantitative trait loci for anxiety in chromosome substitution strains of mice. *Genetics* 2005;169:855–62. [PubMed: 15371360]
70. Song C, Leonard BE, Horrobin DF. Dietary ethyleicosapentaenoic acid but not soybean oil reverses central interleukin-1-induced changes in behavior, corticosterone and immune response in rats. *Stress* 2004;7:43–54. [PubMed: 15204032]
71. Song C, Li X, Leonard BE, Horrobin DF. Effects of dietary n-3 or n-6 fatty acids on interleukin-1 β -induced anxiety, stress, and inflammatory responses in rats. *J Lipid Res* 2003;44:1984–91. [PubMed: 12837849]
72. Steru L, Chermat R, Thierry B, Simon P. The tail suspension test: a new method for screening antidepressants in mice. *Psychopharmacology (Berlin)* 1985;85:367–70. [PubMed: 3923523]
73. Stouthard JML, Romijn JA, Van der Poll T, Endert E, Klein S, Bakker PJM, et al. Endocrinologic and metabolic effects of interleukin-6 in humans. *Am J Physiol* 1995;268:E813–9. [PubMed: 7762632]

74. Stübner S, Schön T, Padberg F, Teipel SJ, Schwarz MJ, Haslinger A, et al. Interleukin-6 and the soluble IL-6 receptor are decreased in cerebrospinal fluid of geriatric patients with major depression: no alteration of soluble gp130. *Neurosci Lett* 1999;259:145–8. [PubMed: 10025579]
75. Suzuki Y, Rani S, Liesenfeld O, Kojima T, Lim S, Nguyen TA, et al. Impaired resistance to the development of toxoplasmic encephalitis in interleukin-6-deficient mice. *Infect Immun* 1997;65:2339–45. [PubMed: 9169772]
76. Swiergiel AH, Dunn AJ. The roles of IL-1, IL-6 and TNF α in the feeding responses to endotoxin and influenza virus infection in mice. *Brain Behav Immun* 1999;13:252–65. [PubMed: 10469526]
77. Swiergiel AH, Smagin GN, Dunn AJ. Influenza virus infection of mice induces anorexia: comparison with endotoxin and interleukin-1 and the effects of indomethacin. *Pharmacol Biochem Behav* 1997;57:389–96. [PubMed: 9164599]
78. Swiergiel AH, Smagin GN, Johnson LJ, Dunn AJ. The role of cytokines in the behavioral responses to endotoxin and influenza virus infection in mice: effects of acute and chronic administration of the interleukin-1-receptor antagonist (IL-1ra). *Brain Res* 1997;776:96–104. [PubMed: 9439800]
79. Takaki A, Huang Q-H, Somogyvári-Vigh A, Arimura A. Immobilization stress may increase plasma interleukin-6 via central and peripheral catecholamines. *Neuroimmunomodulation* 1994;1:335–42. [PubMed: 7671121]
80. Van Snick J. Interleukin-6: an overview. *Ann Rev Immunol* 1990;8:253–78. [PubMed: 2188664]
81. Wang JP, Ando T, Dunn AJ. Effect of homologous interleukin-1, interleukin-6 and tumor necrosis factor- α on the core body temperature of mice. *Neuroimmunomodulation* 1997;4:230–6. [PubMed: 9650815]
82. Wang JP, Dunn AJ. Mouse interleukin-6 stimulates the HPA axis and increases brain tryptophan and serotonin metabolism. *Neurochem Int* 1998;33:143–54. [PubMed: 9761458]
83. Wang JP, Dunn AJ. The role of interleukin-6 in the activation of the hypothalamo-pituitary-adrenocortical axis induced by endotoxin and interleukin-1 β . *Brain Res* 1999;815:337–48. [PubMed: 9878816]
84. West MA, Heagy W. Endotoxin tolerance: a review. *Crit Care Med* 2002;30:S64–73.
85. Wirth-Dzieciolowska E, Lipska A, Wesierska M. Selection for body weight induces differences in exploratory behavior and learning in mice. *Acta Neurobiol Exp (Wars)* 2005;65:243–53. [PubMed: 16130798]
86. Zalcman S, Green-Johnson JM, Murray L, Nance DM, Dyck D, Anisman H, et al. Cytokine-specific central monoamine alterations induced by interleukin-1, -2 and -6. *Brain Res* 1994;643:40–9. [PubMed: 7518332]
87. Zalcman S, Murray L, Dyck DG, Greenberg AH, Nance DM. Interleukin-2 and -6 induce behavioral-activating effects in mice. *Brain Res* 1998;811:111–21. [PubMed: 9804916]
88. Zhang J-J, Terreni L, De Simoni M-G, Dunn AJ. Peripheral interleukin-6 administration increases extracellular concentrations of serotonin and the evoked release of serotonin in the rat striatum. *Neurochem Int* 2001;38:303–8. [PubMed: 11137624]
89. Zorrilla EP, Luborsky L, McKay JR, Rosenthal R, Houldin A, Tax A, et al. The relationship of depression and stressors to immunological assays: a meta-analytic review. *Brain Behav Immun* 2001;15:199–226. [PubMed: 11566046]

Fig 1.

Locomotor activity in home and novel cages in the untreated WT and IL-6ko mice (panels A–C) or in novel cages in WT and IL-6ko mice injected with saline or mIL-6 (1 μ g/mouse, i.p.) 30 min before scoring activity (panels D–F). Activity was recorded for 10 min. Panels A and D: number of short-lasting ambulatory or stereotypic infrared beam breaks; panels B and E: number of long-lasting ambulatory or stereotypic episodes; panels C and F: cumulative duration of activities shown in panels B and E. * $p < 0.05$ compared with saline-treated mice ($N = 6$).

Fig 2. Defecation scores in four independent behavioral tests

(A) Untreated mice in the open field (the same experiment as presented in Fig. 3). (B) Untreated mice in novel, clean cages (the same experiment as in Fig. 1A–C). (C) Effect of IL-1 β (100 ng/mouse, i.p.) in the multicompartiment chamber (MCC—the same experiment as in Fig. 4A–D). (D) effect of IL-6 (1 μ g/mouse, i.p.) in novel, clean cages (the same experiment as in Fig. 1D–F). * p < 0.05 compared with saline-treated IL-6ko mice; # p < 0.05 compared with saline-treated WT mice.

Fig 3.

Activity of WT and IL-6ko mice in the open field (5 min). The time spent in the center of the field and line crossings in the central and peripheral areas of the field are depicted ($N = 12$).

Fig 4. Effect of IL-1 β (100 ng/mouse, i.p., panels A–D) or 25 min restraint (panels E–H) on behavior in the multicompartiment chamber (MCC) in WT and IL-6ko mice. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ compared with saline-treated or quiet mice, respectively ($N = 6$).

Fig 5. Effect of IL-1 β (100 ng/mouse, i.p., panels A–C) or 25 min restraint (panels D–F) on behavior in the elevated plus-maze (EPM) in WT and IL-6ko mice. * $p < 0.05$; ** $p < 0.01$ compared with saline-treated mice, respectively ($N = 6$).

Fig 6. Latency and duration of immobility in the tail suspension test (TST, top) and latency and time spent floating in the forced swim test (FST, bottom) in untreated WT and IL-6ko mice ($N = 12$).

Fig 7. Effect of IL-1 β (100 ng/mouse, i.p.; left-hand panels A–C) or LPS (1 μ g/mouse, i.p.; vertical, middle panels D–F) or IL-6 (1 μ g/mouse, i.p.; right-hand panels G–I) on milk intake (top: A, D, G), food intake (middle: B, E, H) and changes in body weight (bottom: C, F, I). * p < 0.05; ** p < 0.01; *** p < 0.001 compared to saline-treated mice (N = 6).

Fig 8. Effect of saline (100 µl, i.p.), IL-1β (100 ng/mouse, i.p.) or LPS (1 µg/mouse, i.p.) on brain MHPG:NE (panel A), DOPAC:DA (panel B), 5-HIAA/5-HT ratios (panel C) and tryptophan concentrations (ng/mg wet tissue; panel D) in WT and IL-6ko mice. Mice were decapitated 120 min after injection. **p* < 0.05; ***p* < 0.01; ****p* < 0.001 compared with saline-treated mice (*N* = 6).

Fig 9. Effect of IL-1 β (100 ng/mouse, i.p.) or LPS (1 μ g/mouse, i.p.) on plasma corticosterone (ng/ml of plasma) of WT and IL-6ko mice. Trunk blood was collected 120 min after the injections in the same experiment as in Fig. 8. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ compared with saline-treated mice ($N = 6$)