

IL-6 knockout mice exhibit resistance to stress-induced development of depression-like behaviors

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Cytokine-dependent mechanisms in the CNS have been implicated in the pathogenesis of depression. Interleukin-6 is upregulated in depressed patients and downregulated by antidepressants. It is, however, unknown whether IL-6 is involved in the pathogenesis of depression.

We subjected IL-6-deficient mice (IL-6^{-/-}) to depression-related tests (learned helplessness, forced swimming, tail suspension, sucrose preference). We also investigated IL-6 in the hippocampus of stressed wild-type mice.

IL-6^{-/-} mice showed reduced despair in the forced swim, and tail suspension test, and enhanced hedonic behavior. Moreover, IL-6^{-/-} mice exhibited resistance to helplessness. This resistance may be caused by the lack of IL-6, because stress increased IL-6 expression in wild-type hippocampi.

This suggests that IL-6 is a component in molecular mechanisms in the pathogenesis of depression. IL-6^{-/-} mice represent tools to study IL-6-dependent signaling pathways in the pathophysiology of depression in vivo. Moreover, these mice may support the screening of compounds for depression by altering cytokine-mediated signaling.

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Introduction

Cytokines whose primary function is to act as signaling molecules of the immune system have recently also been implicated in the pathogenesis of mood disorders (Anisman et al., 2005; Capuron and Dantzer, 2003; Hayley and Anisman, 2005). Thus, chronic cytokine elevations have been proposed to evoke neurotransmitter changes that are interpreted by the brain as stressors and contribute to the development of depression (Anis-

man et al., 2005; Hayley and Anisman, 2005). With respect to the proinflammatory cytokine IL-6, clinical studies have demonstrated an upregulation in depressive patients, which is alleviated following antidepressive treatment (Benedetti et al., 2002; Frommberger et al., 1997; Griffiths et al., 2000). Furthermore, elevated levels of cytokines including IL-6 may be associated with a variety of medical conditions which are known to favor the development of a depressive state (Pollak and Yirmiya, 2002). Moreover, therapeutic application of interferon- α in patients with specific forms of cancer or hepatitis C is also associated with increased levels of IL-6 and can give rise to depressive symptoms (Anisman and Merali, 2003; Musselman et al., 2001; Wichers et al., 2005). Plasma concentrations of IL-6 are even increased after “psychological stress”, a well-known risk factor for the development of depressive episodes (Cryan and Mombereau, 2004). However, there is still a controversy on whether single cytokines like IL-6 are directly involved in pathogenetic processes of depressive disorders, or whether their dysregulation rather represents an epiphenomenon, e.g., in form of a class of dysregulated “marker molecules” (Leonard, 2001). These questions can be answered using animal models, in particular mice with targeted mutagenesis (Urani et al., 2005).

Several strains of mice have been generated that over- or underexpress IL-6 (Heyser et al., 1997; Kopf et al., 1994; Raber et al., 1997). Studies in IL-6-deficient mice have implicated this cytokine in emotional behaviors, in particular exploratory- and anxiety-related behaviors (Armario et al., 1998; Butterweck et al., 2003). Mice with a transgenic overexpression of IL-6 in the CNS demonstrated increased glucocorticoid levels after stress (Raber et al., 1997), suggesting an increased stress sensitivity with a potential vulnerability for the development of depressive-like features. However, specific depressive-like behaviors have so far not been studied in any of these transgenic mouse strains. Therefore, the present study was designed to investigate whether IL-6 plays a crucial role for the development of stress-associated depression-like behaviors in mice. For this purpose, we subjected mice deficient for IL-6 (IL-6^{-/-}) to a standardized behavioral test

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battery, including detailed analyses of depressive-like behavior such as learned helplessness, despair (i.e., Porsolt forced swim test and tail suspension test) and (an)hedonic symptoms (i.e., sucrose preference test). Additionally, we investigated whether the helplessness procedure causes changes of IL-6 expression in the hippocampus, a key structure for murine depression-like behaviors (Campbell and Macqueen, 2004; Shirayama et al., 2002). Our results suggest that IL-6 expression is related to the stress-induced development of depression-like behaviors in mice.

Methods and materials

Animals

Mice with a conventional IL-6 knockout (Kopf et al., 1994) were backcrossed to a C57BL/6J genetic background (C57BL/6J, Harlan Winkelmann, Borcheln, Germany) for more than 10 generations. All experiments were performed with 3- to 6-month-old male animals. We investigated always groups of animals coming from different litters, containing 1 mutant and 1 wild-type littermate from an individual mother. Prior to the experiments, mice were individually housed for 2 weeks in a reversed dark–light cycle with lights on at 6 p.m. Animals were supplied with food and water ad libitum. All animal experiments were conducted in adherence to the European Directive of 24th November 1986 (86-609/EEC) and the decree of 20 October 1987 (87–848) and were approved by the German animal welfare authorities.

Behavioral experiments

All behavioral tests were conducted during the dark cycle, i.e., in the animals' active phase. Prior to each test, mice were acclimatized to the experimental room for at least 15 min. Test procedures were essentially performed as described earlier (Fleischmann et al., 2003; Ridder et al., 2005). The animals were subjected to several tests assessing locomotion, exploration, and anxiety, as well as to depression-relevant paradigms such as the learned helplessness model, the Porsolt forced swim test and the tail suspension test. One cohort of animals (IL-6^{-/-} $n = 15$, wild type $n = 15$) was studied in the novel cage test, open field and learned helplessness model, another cohort (IL-6^{-/-} $n = 8$, wild type $n = 8$) was analyzed in sucrose consumption, dark–light box, forced swim and tail suspension tests. The use of several test on the same group of animals and the order of the tests followed earlier recommendations ranking the tests from least stressful to more stressful (McIlwain et al., 2001; van Gaalen and Steckler, 2000). Between individual tests, there was a break of at least 24 h. In all experiments, the investigator was blind to the genotype of the mice during behavioral testing.

Novel cage

The number of rearings in a novel home cage was analyzed for 5 min as an indicator for vertical exploratory activity. Observation was conducted with red light illumination of the test room (IL-6^{-/-} $n = 15$, wild type $n = 15$).

Open field

Activity monitoring was conducted in a square shaped, white open field, measuring 50 × 50 cm² and illuminated from

above by 25 Lux. Mice were placed individually into the arena and monitored for 15 min by a video camera (Sony CCD IRIS). The resulting data were analyzed using the image processing system EthoVision 2.3 (Noldus Information Technology, Wageningen, The Netherlands). For each sample, the system recorded position, object area and the status of defined events. Parameters assessed for the present study were total distance moved, velocity, and time in center, which was defined as the area 10 cm distant from the walls (IL-6^{-/-} $n = 15$, wild type $n = 15$).

Dark–light box

The dark–light box, which exposes the animals to an approach–avoidance conflict, thereby revealing anxiety-related behaviors, consisted of two plastic chambers, connected by a small tunnel. The dark chamber measured 20 × 15 cm² and was covered by a lid. The other chamber, measuring 30 × 15 cm², was white and illuminated from above with an intensity of 600 Lux. Mice were placed into the dark compartment and latency to first exit, number of exits, and total time in the light compartment were recorded for 5 min (IL-6^{-/-} $n = 8$, wild type $n = 8$).

Learned helplessness

In the learned helplessness paradigm as described earlier (Chourbaji et al., 2005b), the animals were exposed to a transparent plexiglas shock chamber (18 × 18 × 30 cm), equipped with a stainless steel grid floor (Coulbourn precision regulated animal shocker, Coulbourn Equipment Bilaney Consultants, Düsseldorf, Germany), through which they received 360 scrambled footshocks (0.150 mA) on 2 consecutive days, respectively. The footshocks applied were unpredictable with varying shock duration (1–3 s) and interval episodes (1–15 s), amounting to a total session duration of approximately 52 min. Twenty-four hours after the second shock procedure, learned helplessness was assessed by testing shuttle box performance (Graphic State Notation, Coulbourn Equipment Bilaney Consultants, Düsseldorf, Germany). The shuttle box consisted of two equal-sized compartments (18 × 18 × 30 cm) that were separated by a small gate (6 cm wide and 7 cm high). The shuttle box also contained a grid floor, through which current could be applied, and a signaling light at the top of both compartments. Spontaneous initial shuttles from one compartment to the other were counted during the first 2 min by infrared-light beams at the bottom of each of the two divisions. Performance was analyzed according to the behavior during 30 shuttle escape trials. Each trial started with a light stimulus of 5 s, announcing a subsequent footshock of maximum 10-s duration. The intertrial interval was 30 s. The following behavioral reactions were defined: avoidance as adequate reaction to the light stimulus by changing to the other compartment immediately, escapes as shuttling to the other section in reaction to the electric shock, and failures, when no attempt to escape was made. Escape latency was assessed and defined as the time it took the animal to escape. For determination of the activity during the intervals, shuttles in between the trials were recorded. Total time of testing for helplessness was about 20 min, the exact time period depending on the animal's ability to learn the paradigm. To exclude altered pain sensitivity as a confounding factor, all mice were tested on the hotplate (ATLab, Vendargues, France) at a temperature of 52°C for 45 s. Latency to first reaction, licking hind paws or jumping was assessed (IL-6^{-/-} $n = 15$, wild type $n = 15$).

Porsolt forced swim test

To assess despair behavior, mice were placed into a glass cylinder (23 cm height, 13 cm diameter), which was filled with water (22°C) up to a height of 8 cm, as described earlier (Zörner et al., 2003). A testing period of 6 min was used to determine the onset and the percentage of time spent immobile. Immobility was defined as motionless floating in the water, only allowing movements necessary for the animal to keep its head above the water. In contrast, swimming was defined as time spent with active escape or struggling movements (Porsolt et al., 1977a) (IL-6^{-/-} $n = 8$, wild type $n = 8$).

Tail suspension

In the tail suspension test, which precipitates another type of despair behavior than the forced swim test, mice were suspended by their tails with adhesive tape attached to a hook, which was coupled to a computer-assisted energy measurement (Bioseb, Chaville, France). A test session lasted 6 min, during which the immobility scores of each mouse were determined (IL-6^{-/-} $n = 8$, wild type $n = 8$).

Sucrose preference

As correlate of possible hedonic alterations, the reinforcing properties of sucrose were assessed by using a procedure recently developed in our laboratory (Sanchis-Segura et al., 2004, 2005). This method is based on a free choice two-bottle sucrose consumption paradigm using the principles of the matching law (Hernstein, 1970), and it provides a quantitative index of the steady (i.e., molar) internal evaluation of a particular reward (Sugrue et al., 2005). The protocol was composed of two main phases. First, for 5 weeks, 5 days a week, 2 h per day (10:00–12:00 a.m.), the tap water bottles were replaced by two bottles containing sucrose solutions in different concentrations (0, 1, 2.5 or 5%, w/v) to habituate the mice to the restricted availability of sucrose. During the following 3 weeks, a two-bottle choice test was used, again 5 days a week, 2 h per day. During these 3 weeks 15 choice conditions (detailed in the supplementary table) between two solutions of different sucrose concentrations were concurrently available, and the consumption from each bottle was calculated by weighing the bottles after each session using an electronic scale ($n = 8$ per group).

IL-6 expression analyses

C57BL/6N mice were individually exposed to the learned helplessness procedure described above. An unshocked cohort served as control. The animals were sacrificed 24 h after the stress session: brains were removed, and the hippocampus was rapidly dissected and immediately frozen on dry ice. All samples were stored at -80°C until ready for assay. The RT-PCR for IL-6 using real-time technology has been described previously (Sallmann et al., 2000). In brief, RNA from brain tissues was extracted with peqGOLD RNAPure (PEQLAB, Erlangen, Germany), according to the manufacturer's instructions. 5 µg RNA was transcribed with Moloney murine leukemia virus reverse transcriptase and random hexamers. The following primers were used for PCR amplification: mIL-6 5' AAAGAGTTGTGCAATGGCAATTCT; mIL-6 3' AAGTGCATCATCGTTGTTTCATACA (mouse IL-6, PCR product (51 bp); mcycm-1, AGGTCCTGGCATCTTGTCAT; mcycm-2, GAACCGTTTGTGTTTGGTCCA (cyclophilin, PCR product 51 bp). PCR was performed according to the following protocol: 10 min at 95°C, 15 s at 95°C, and 1 min at 60°C (40 cycles). Amplification

was quantified with the Gene Amp 5700 sequence detector and the SYBR Green kit (PE Diagnostik, Weiterstadt, Germany). Linear concentration–amplification curves were established by diluting pooled samples. Using these curves, results for individual cDNAs were quantified and normalized to cyclophilin. The purity of the amplified products was checked by the dissociation curve and gel electrophoresis. Furthermore, the identity of the product was verified by sequencing after subcloning into the vector pCR II-TOPO (Invitrogen, Groningen, The Netherlands).

Statistical analyses

All analyses, except otherwise noted, were performed using the STATISTICA 4.1 software (Statsoft Inc. 1991–1994). Correlation studies were performed by using Pearson's index, and regression lines were calculated by using the least squares method. Mean differences were analyzed by means of Student's *t* test or ANOVAs. When a significant interaction between factors was found, post hoc analyses were conducted using the Newman–Keuls test. The data on sucrose consumption in the two bottle free choice experimental protocol were analyzed by using the “generalized matching law equation”. This equation states that $\log B_1/B_2 = a(\log r_1/r_2) + \log c$, where *B* represents the allocation of the behavioural responses to alternatives 1 and 2, and *r* represents the rate or relative reinforcing magnitude of the two alternatives, and *a* and *c* are empirically obtained parameters which illustrate the individual sensitivity to the ratio reward and bias for one or the other alternatives respectively. When applying this equation to a two bottles free choice procedure, the consumed volume (V_X) of each bottle provides the index of the relative behavioural allocation, whereas the concentration of the available solution (C_X) represents the magnitude of the reinforcer. Thus, for each rat the ratio of the consumed volume from the bottle located in the left over that located in the right (V_L/V_R) as well as the concentration of the respective solutions (C_L/C_R) were calculated. When any of the terms was zero, 0.1 substituted this value. This value represents the accuracy limit of our measurement method, and by this substitution division by 0 (and posterior undefined logarithm) is avoided with minimal distortion of the subsequent calculations. In a second step, the logarithms (base *e*) of these ratios were plotted on arithmetic co-ordinates and, by using the method of the least squares, the best-fit of regression line was estimated. Thus, for each subject, the bias (intercept) and sensitivity to the reward ratio (slope) indexes were calculated and compared by means of one-way ANOVA.

Results

IL-6^{-/-} mice demonstrate regular open field activity

In the novel cage test the vertical activity of mice – as assessed by the number of rearings – was not different between both genotypes (Fig. 1A). The locomotor and exploratory behavior of the mice in the open field was evaluated under dim light conditions to reduce the emotional component of the test. Under these conditions, IL-6-deficient mice, when compared to their wild-type littermates, demonstrated normal horizontal locomotor activity, indicated by an unchanged total distance moved (Fig. 1B), an unaltered velocity (Fig. 1C) and an unaltered number of visits in the center (data not shown).

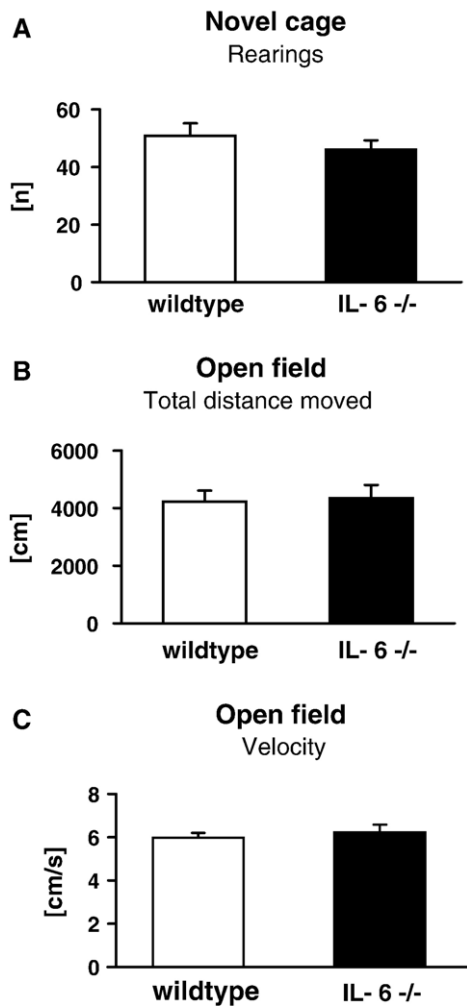


Fig. 1. Basal activity. IL-6^{-/-} mice, when compared to wild-type littermates, demonstrate regular vertical activity in the novel cage test (A), as well as unaltered locomotion in the open field, indicated by the parameters total distance moved (B) and velocity (C). Columns represent means + SEM.

IL-6^{-/-} mice display increased anxiety-like behavior in the dark–light box

The dark–light box test is based on the fact that mice prefer dark sheltered places over brightly illuminated areas. In this test, the IL-6-deficient mice spent significantly less time in the anxiety-related lit compartment ($P = 0.026$, Fig. 2).

IL-6^{-/-} mice exhibit decreased depression-like behavior in the learned helplessness paradigm

The learned helplessness paradigm evaluates the coping capabilities of mice in an aversive test situation after two days of intense stress, evoked by exposure to a series of unpredictable and uncontrollable footshocks. When tested for helpless behavior, IL-6^{-/-} mice showed significantly reduced numbers of escape failures ($P = 0.005$, Fig. 3A) and significantly decreased escape latencies ($P = 0.0003$, Fig. 3B). When mice were scored individually (Fig. 3C), none of the helpless individuals were IL-6^{-/-} mice (helplessness defined as more than 6 escape failures occurring together with an escape latency of more than 4.75 s). These results were most likely not caused by general changes in locomotor activity (Fig. 1) or

by altered pain sensitivity, as investigated by a hot plate test (29.5 ± 2.8 s vs. 26.5 ± 2.9 s to react to the heat, $P = 0.47$; data not shown, however, not the same pain circuits are affected).

IL-6^{-/-} mice show reduced despair behavior and altered sucrose consumption

The forced swim test and the tail suspension test represent two standardized paradigms for the assessment of despair behavior (i.e., giving up) by analyzing immobility scores in inescapable aversive situations. In the Porsolt test, IL-6^{-/-} mice start significantly later to float ($P = 0.05$, Fig. 4A) and demonstrate significantly lower total floating times ($P = 0.044$, Fig. 4A) than wild-type littermates. The reduced floating times were mirrored in the tail suspension test, in which IL-6-deficient mice also demonstrated less immobility than wild-type controls ($P = 0.04$, Fig. 4B).

The reinforcing properties of sucrose were used as an index of hedonic alterations. Such alterations could be related to differences between genotypes and/or a correlate of helplessness. Because not a single IL-6^{-/-} mouse displayed helplessness (see above), the initially planned bi-factorial design was reduced to comparisons of the reward indices of our procedure (slope and intercepts, respectively) in three groups of mice: IL-6^{-/-}, wild-type-non-helpless and wild-type-helpless (Fig. 5). Two independent one-way ANOVAs indicated significant differences between these groups [$F(2,18) = 9.20$, $P < 0.002$] in the slope but not in the intercept values of the regression line of their preference pattern. Post hoc comparisons revealed that IL-6^{-/-} mice were most sensitive to the rewarding effects of sucrose, indicated by significant steeper slopes than both groups of wild-type mice ($P < 0.05$ and $P < 0.01$, respectively, Fig. 5), which is indicative of a higher sensitivity to the rewarding effects of sucrose. Furthermore, wild-type-non-helpless mice had a higher sensitivity to the rewarding properties of sucrose than wild-type-helpless mice ($P < 0.05$, Fig. 5).

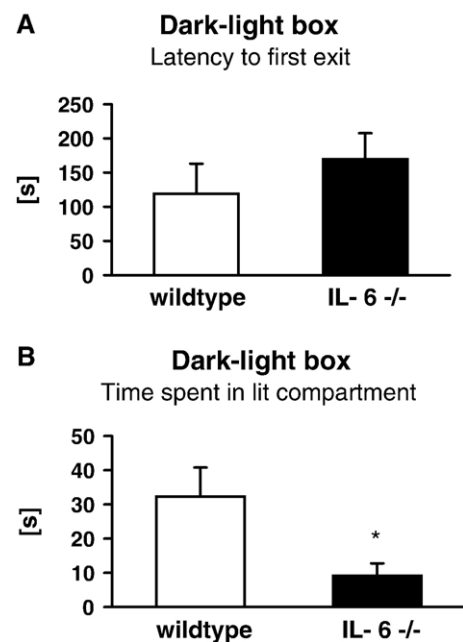


Fig. 2. Anxiety-like behavior in the dark–light box test. In the dark–light box test IL-6^{-/-} display unaltered latency to enter the aversive lit compartment (A), but spend significantly less time therein (B, $P = 0.026$). Columns represent means + SEM.

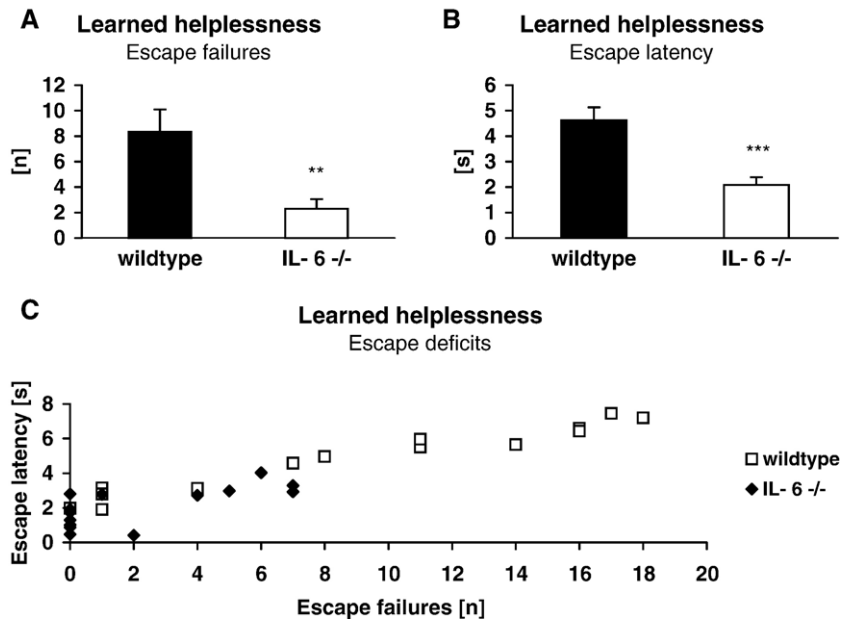


Fig. 3. Escape deficits in the learned helplessness paradigm. In the learned helplessness paradigm IL-6^{-/-} mice display significantly less escape deficits, indicated by a reduced number of escape failures (A, $P = 0.005$) and a decreased escape latency (B, $P = 0.0003$). When scored individually for both parameters, only wild-type mice reach the criterion of helplessness, defined by more than 6 escape failures and an escape latency higher than 4.75, while IL-6^{-/-} mice failed to meet both criteria (C). Columns represent means + SEM.

The learned helplessness procedure induces an increase of IL-6 in the hippocampus

To investigate, whether uncontrollable and unpredictable stress leads to alterations of IL-6 levels in brain regions critically involved in the pathogenesis of depression, IL-6 expression was analyzed in C57Bl/6N mice 24 h after exposure to the learned

helplessness procedure. Compared to sham-treated mice, animals subjected to the helplessness paradigm had a significant increase of IL-6 RNA levels in the hippocampus (Fig. 6).

Discussion

The present study examined the behavioral consequences of a deletion of the IL-6 gene in male mice under basal and stressful conditions, as well as the learned helplessness-induced expression of IL-6 in wild-type mice. Both experimental approaches revealed a significant association of IL-6 with behavioral features related to depression. IL-6 knockout animals demonstrated reduced despair behavior in the forced swim test and in the tail suspension test, as well as enhanced hedonic behavior indicated by increased sensitivity to the reinforcing properties of sucrose. Furthermore, IL-6^{-/-} mice exhibited a strong resistance to develop learned helplessness following intense stress by footshocks. Further evidence that this resistance is related to the lack of IL-6 is indicated by the fact that in wild-type mice the stress preceding the helplessness procedure significantly increases IL-6 levels in the hippocampus, a key structure of the limbic system thought to be involved in murine depression-like behaviors (Campbell and Macqueen, 2004; Shirayama et al., 2002).

IL-6^{-/-} mice show reduced depression-like behavior and are more resistant to behavioral stress

The learned helplessness paradigm represents a depression model for rodents and other species with good face and construct validity (Maier, 1984; Maier and Watkins, 2005; Nestler et al., 2002; Vollmayr and Henn, 2001). It is based on the concept that exposure to stress, in particular to aversive unavoidable environmental conditions, can lead to an impairment of coping behavior

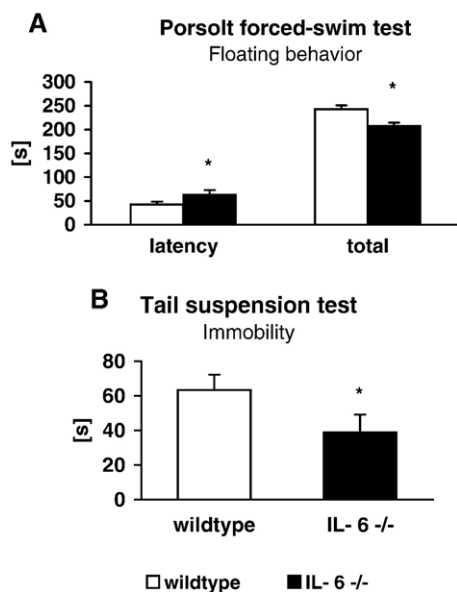


Fig. 4. Despair behavior in the forced swim and tail suspension tests. In the Porsolt test, IL-6^{-/-} mice started later to float ($P = 0.05$) and floated significantly less than wild-type littermates during the 6 min of testing ($P = 0.044$). In the tail suspension test IL-6-deficient mice demonstrated also significantly less immobility than wild-type controls ($P = 0.04$).

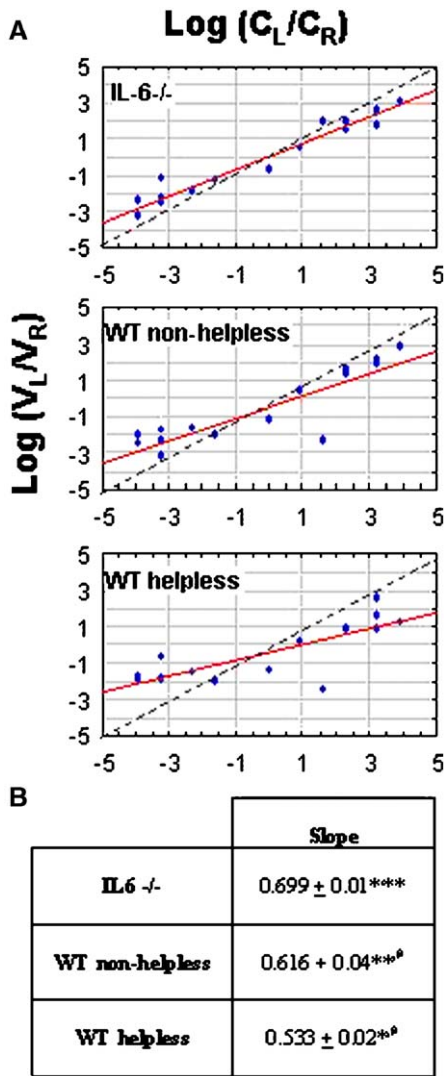


Fig. 5. Rewarding properties of sucrose. (A) Representative scattergrams of individuals belonging to the 3 groups of mice that were analyzed for sucrose consumption: IL-6^{-/-}, WT-non-helpless and WT-helpless. For each mouse, the ratio of the consumed volume (V_L/V_R) as well as the concentration of the respective solutions (C_L/C_R) was calculated (see Supplementary table for details about the testing conditions). The logarithms of these ratios were plotted on arithmetic co-ordinates and by using the method of the least squares the best-fit of regression line was calculated. The steepness of the slope of this regression line correlates with the sensitivity to sucrose rewarding. For a better comparison of the different slopes, the theoretical perfect matching behavior is shown as dashed line. (B) The table shows the means + SEM of the slope values of the 3 groups of mice. One-way ANOVAs demonstrate significant differences between the three groups (IL-6^{-/-} vs. WT-non-helpless, *P < 0.05, and WT-helpless, ***P < 0.01, WT-non-helpless vs. WT-helpless, [#]P < 0.05).

(Shumake and Gonzalez-Lima, 2003). This model has recently been used successfully to study the sensitivity/resistance of mutant mice to develop stress-induced depressive symptoms. In the present study, IL-6-deficient mice, when subjected to the helplessness procedure consisting of two consecutive days of inescapable and unpredictable footshocks, exhibited significantly reduced helplessness, i.e., improved coping behavior. This indicates a resistance to develop depression-like behavior in IL-6^{-/-} mice. These data

are in accordance with a recent study demonstrating reduced coping behavior in a similar task in transgenic mice with IL-6 overexpression in the brain (Heyser et al., 1997), also suggesting a critical role of IL-6 in the pathophysiology of depression.

Besides the determination of depressive-like behavior in the learned helplessness paradigm, the present study also assessed other depression-linked behavioral features in IL-6-deficient mice. The Porsolt forced swim and the tail suspension test represent well established standardized paradigms analyzing the extent of “despair” in animals, i.e., taking an immobile and passive posture without making attempt to escape from an unpleasant situation (Porsolt et al., 1977a,b; Willner, 1991). Indeed, a positive correlation of learned helplessness performance and despair behavior in the forced swim test was described in WKY rats with congenital depression-like behavior (Pare, 1994). In the present study, IL-6^{-/-} mice revealed decreased despair behavior in both, the Porsolt and the tail suspension test, supporting the finding of a non-depressive and stress-resistant like phenotype of this strain obtained in the learned helplessness paradigm. This interpretation is further strengthened by the increased hedonia of IL-6-deficient mice when tested for sucrose consumption, which represents a critical and representative measure for an alteration in emotional behavior (Willner, 1991). The alteration of several behavioral parameters related to depressive-like features in IL-6^{-/-} mice strongly indicates that this strain has reduced depression-like behavior and is resistant to stress.

In our behavioral analysis of IL-6^{-/-} mice, we included also an assessment of general behaviors under basal, i.e., non-stressful conditions. Thus, the animals were exposed to a standardized test battery, including the analyses of locomotion, exploration and anxiety. While locomotion and exploration were not changed by the mutation, IL-6^{-/-} mice differed with respect to anxiety-related parameters. The dark–light box paradigm exposes the animals to a conflict-avoidance situation, i.e., novel environment and light (Bourin and Hascoet, 2003). IL-6^{-/-} mice demonstrated increased anxiety as indicated by a slightly elongated latency to first exit and significantly decreased duration of stay inside the aversive lit compartment. These changes in emotionality confirm the results of previous behavioral studies in which increased anxiety-like behavior in the elevated plus maze and in a holeboard test were described in these mice (Armario et al., 1998; Butterweck et al., 2003). The increased anxiety scores are not necessarily in contradiction to the non-depressive phenotype of IL-6-deficient mice. As recently described by our group and others, anxiety-like features do not always positively correlate with depression-like

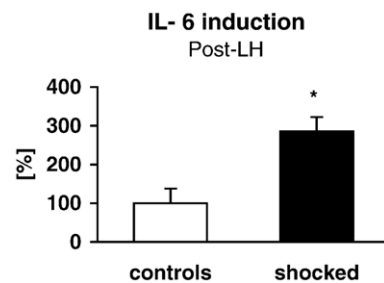


Fig. 6. IL-6 induction in helpless animals. Analysis of IL-6 expression in separate groups of helpless (shocked) and control (sham-shocked) wild-type animals revealed a significant increase of IL-6 mRNA in the hippocampus of helpless mice (P = 0.018). Columns represent means + SEM.

changes in learned helplessness or despair behavior (Andreatini and Bacellar, 1999; Chourbaji et al., 2005a; Strelakova et al., 2004). Since IL-6^{-/-} mutant mice only have a single mutation but depression represents a multigenic disease, it seems plausible that these mice do not have alterations in all features ascribed to depression. With regard to the recently developed concept of endophenotypes (Cryan and Mombereau, 2004; Urani et al., 2005), that tries to develop genetic models for cohorts of patients with a limited and defined cluster of symptoms, we describe here a targeted mutation in mice causing resistance to develop helplessness, despair and anhedonia.

IL-6 induction is modulated during the learned helplessness procedure

To search for additional evidence for an involvement of IL-6 in the development of a depressive-like phenotype, wild-type mice of similar age and gender as the IL-6^{-/-} animals were exposed to the same learned helplessness procedure and their hippocampus was analyzed subsequently for IL-6 expression. The significant induction of IL-6 mRNA in footshocked compared to sham-treated animals further indicates a role of this cytokine in the pathogenesis of depression. This interpretation is also supported by the finding that psychological stress—a strong risk factor for the development of depressive episodes—induces cytokine expression. Plasma levels of IL-6 are increased upon psychological stress in laboratory animals and humans (Takaki et al., 1994; Zobel et al., 2001). Moreover, elevated IL-6 levels are found in the brain, cerebrospinal fluid and plasma of depressed patients (Maes, 1992; Maes et al., 1995). In addition, repeated cytokine induction as a result of infection is considered as a potential trigger for major depression (Maes et al., 1995).

IL-6^{-/-} mice may represent a tool to study molecular mechanisms of cytokine-dependent depression-like behaviors

The chronic absence of IL-6 in our knockout mice in combination with the stress-induced elevation of IL-6 in wild-type mice during the learned helplessness procedure represents a promising constellation for the investigation of cytokine-dependent mechanisms with respect to resistance and vulnerability to depression. There are several mechanisms by which IL-6 may be involved in the pathogenesis of depression (Anisman et al., 2005; Capuron and Dantzer, 2003; Miller and O'Callaghan, 2005). One possibility is that IL-6 modulates the activity of the HPA axis (Raber et al., 1997), potentially by influencing the glucocorticoid negative feedback on the release of corticotrophin-releasing hormone (Miller et al., 1999). Furthermore, perinatal exposition to endotoxins could sensitize CNS activity in response to subsequent endotoxin or stressor exposure during adulthood, which raise the possibility, that early life infectious events may modulate psychological responses to stressors by affecting the activity of neuronal processes (Shanks et al., 2000). Another potential target is the serotonergic system, where cytokines induce indoleamine 2,3-dioxygenase, an enzyme that compromises the synthesis of serotonin (Lestage et al., 2002). IL-6 deficient as well as IL-6 overexpressing mice (Raber et al., 1997) represent valuable tools to study the role of IL-6 in these pathophysiological mechanisms in vivo, both under baseline conditions as well as following stress exposure. Moreover, using depression models like the learned helplessness paradigm in combination with antidepressant

therapeutic may elucidate how to influence the levels of cytokines and their downstream molecular signaling pathways. The combined use of transgenic techniques and behavioral models could help in the preclinical selection of compounds which influence depression by altering cytokine-mediated signaling (Cryan et al., 2002).

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.nbd.2006.05.001.

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