

Individual behavioral differences in recovery from abdominal sepsis in rats

A. Bauhofer¹, Y.-J. Ho², A. Schmitt¹, M. Köster¹, R. K.W. Schwarting³, C. R. Pawlak⁴

¹ Institute of Theoretical Surgery, Philipps-University of Marburg, 35043 Marburg, Germany, e-mail: a-bauhofer@web.de

² School of Psychology, Chung Shan Medical University, Tai-Chung 402, Taiwan, ROC

³ Experimental and Physiological Psychology, Philipps-University of Marburg, 35032 Marburg, Germany

⁴ Department of Psychopharmacology, Central Institute of Mental Health, 68159 Mannheim, Germany, Fax: +49 (0) 621 17036255, e-mail: cornelius.pawlak@zi-mannheim.de

Received 26 September 2008; returned for revision 25 October 2008; received from final revision 13 November 2008; accepted by M. J. Parnham 19 November 2008

Abstract. *Objective and Design:* In the present study we determined whether individual behavioral differences (high and low locomotor activity) differentially affected recovery from sepsis with high or low mortality.

Methods: Two trials were performed. Trial 1 with high mortality: Rats were randomly assigned to (1) control-A: anesthesia, (2) control-B: sham surgery, (3) sepsis: laparotomy and peritoneal contamination and infection (PCI) with human stool bacteria, (4) sepsis with antibiotic prophylaxis (cefuroxime/metronidazole), and (5) sepsis with antibiotic plus G-CSF prophylaxis. Trial 2 with low mortality: Comparison of groups 3 and 5. Endpoints were mortality, behavior (open field and social interaction tests), and proinflammatory cytokines (interleukin-6 = IL-6 and macrophage inflammatory protein-2 = MIP-2).

Results: The combination of antibiotics plus G-CSF was most effective in reducing mortality in both trials and modified sickness behavior. Behavioral deficits were not statistically significantly improved by G-CSF. However, high versus low responders were differentially affected in both behavioral tests. Furthermore, IL-6 and MIP-2 were increased 24 hours after inoculum only in high responders with untreated sepsis and high mortality.

Conclusion: Improvement of sickness behavior in sepsis with G-CSF/antibiotic prophylaxis is a promising approach. The course of recovery from sepsis may depend on pre-morbid individual differences.

Key words: Sickness behavior – Open field – Social interaction – Granulocyte-colony stimulating factor (G-CSF) – Individuality

Introduction

Sepsis represents an uncontrolled inflammatory response to an infection that results in significant morbidity and mortality. A recent review estimated the annual number of deaths resulting from sepsis in the United States to be more than 200,000 [1]. Survivors from critical illness, especially from sepsis, present long-term cognitive impairments, including alterations in memory, attention, concentration, and also altered pain perception and reduced emotional well-being [2–5]. Till today behavioral alterations of the individual sepsis patient were not registered adequately. Individual differences, not only in behavior, give a chance for a more patient oriented treatment compared to the current treatment which is patient group specific treatment. This conceptual change will give also a chance for more positive results in future sepsis trials, since the current approach failed in most trials. Furthermore, an often neglected issue in sepsis research concerns the improvement of quality of life. Several substances like antithrombin III [6] and granulocyte-colony stimulating factor (G-CSF) [7, 8] do not only support physical and immunological functions but also quality of life. The positive effect of G-CSF on inflammation and behavior was investigated in our current trials.

Recently, we showed in clinic modeling randomized trials (CMRTs) in rats that sepsis leads to reduced behavioral activity which can be monitored continuously in the home cage by radio-telemetry [9, 10] or in well-established behavioral tests like the open field, elevated plus-maze, and social interaction test [11]. Out of these, the open field test is widely used as a standard screening procedure to measure psychomotor activity and exploration [12, 13]. Furthermore, behavioral changes due to infections can be measured by the analysis of social interactions [14].

In previous years it became clear that an approach based on individual differences is not only necessary in humans,

but is also critical in animal models [15]. The individual approach helps to: (1) explain variability between behavioral, or physiological measures, (2) get a better prediction of pharmacological reactivity (e.g. different reactivity of “high” and “low responder” animals), (3) understand the mechanisms and susceptibilities to pathological conditions (e.g. influence of pharmacogenomics to diseases like infections [16], or cancer [17]).

Behavioral patterns in humans and animals in the course of infectious diseases are lethargy, anxiety, depression and anorexia [18]. These behavioral changes are defined in animals as sickness behavior [19], which is considered as an organized behavioral strategy to facilitate the role of fever in combating infections. The development of sickness behavior is triggered indirect by proinflammatory cytokines produced by peripheral phagocytic cells which are in contact with invading micro-organisms [20] or more direct by cytokines produced centrally. Systemic proinflammatory cytokine levels can be reduced with G-CSF, as demonstrated in infectious disease [21, 22]. Important cytokines in sepsis are IL-6 and macrophage inflammatory protein-2 (MIP-2). IL-6 is a proinflammatory cytokine and an independent marker of sepsis [23] and MIP-2 is a chemokine involved in the regulation of granulocyte trafficking during infection [24].

Here, the aim of these experiments was to analyze inter-individual behavioral differences during the course of untreated sepsis, standard treatment with antibiotics, or additional treatment with G-CSF in rats. The open field (activity and exploration) and social interaction (social exploration) tests were used to obtain more information from the psychological and social domain of sickness behavior during recovery from sepsis. Finally, in an additional sample of animals IL-6 and MIP-2 were measured and analyzed in relation to individual behavior and sepsis treatment.

Methods

Animals

The study population consisted of male adult outbred Wistar Unilever rats (Harlan Winkelmann, Borcheln, Germany) weighing between 220–270 g at the beginning of the experiment. For the social interaction test, additional young male Wistar rats were used as partners. These were obtained post weaning at an age of 22 days (Harlan Winkelmann, Borcheln, Germany). The housing room was maintained on a 12 h light:dark cycle (lights on: 7:00–19:00 h). Ambient temperature was 23 ± 1 °C. Rats were housed in groups of five per cage (length×width×height: 57×35×24 cm) under standard laboratory conditions and had free access to food and water, except the day before surgery. Upon arrival, rats were randomized to the treatment groups and were kept together in the same cages throughout the experiment. Permission for the experiments was given by the regional animal welfare committee Giessen, Hessa, Germany.

Trial design

Trial 1 – high mortality: (1) control A: anesthesia, (2) control B: sham surgery, (3) sepsis: laparotomy and peritoneal contamination and infection (PCI), (4) sepsis with antibiotic prophylaxis (cefuroxime/metronidazole), (5) sepsis with antibiotic plus G-CSF prophylaxis (n = 20 rats/group). PCI was performed with a standardized human stool inoculum of 1.5 ml/kg.

Trial 2 – low mortality: (1) sepsis: PCI only (identical with group 3 in trial 1), (2) sepsis with antibiotic plus G-CSF prophylaxis (identical with group 5 in trial 1; n = 35 rats/group). Furthermore, n = 10 rats/group were used for cytokine assays. PCI was performed with a standardized human stool inoculum of 0.8 ml/kg. The experiments were performed in five identical blocks with 5 or 10 rats per group.

Sepsis induction and treatment

Twelve hours before surgery and sepsis induction, rats were weighed and deprived of food. Rats with G-CSF prophylaxis were injected s.c. three times (12 h before, 12 h and 36 h after PCI) with 20 µg/kg body weight G-CSF (Filgrastim, Amgen, Munich, Germany). The rats in the other groups were treated with Ringer’s solution (Fresenius, Germany). Anesthesia was induced with fentanyl/droperidol 0.08/4 mg/kg i. p. one hour before surgery (Janssen-Cilag, Neuss, Germany). For PCI, a polymicrobial inoculum prepared from human stool was used [25], which had been stored at –70 °C until use. Laparotomy was performed through a midline incision of two-centimeter length and the inoculum was injected i.p. into the pelvic region of the rats [26]. The wound was closed in two layers using an interrupted absorbable Vicryl® 3–0 suture technique (Ethicon, Hamburg, Germany). For post-operative analgesia 20 mg/kg tramadol was injected s.c. (Mundipharma, Limburg, Germany). After surgery, the animals received food and water *ad libitum* again. Overall survival was recorded for 9 days. In trial 2 from additional rats 1 ml blood was sampled 1 hour before, 1 hour after, and 24 hours after PCI by puncture of the retro-bulbar plexus.

General behavior procedure

All adult animals underwent gentling and handling daily for four days (five minutes each) prior to behavioral testing. All behavioral tests were performed during the dark phase of the day. Preoperative data from an open field and social interaction test were recorded two nights before surgery. Horizontal locomotor activity from the open field test was used to perform a median split between high and low responder rats in each treatment group. PCI was performed during the daytime between 10:00–12:00 h. After surgery, the behavioral tests were repeated on day 2, 4, 6, 8 in trial 1 and on day 1, 2, 4, 6, 8 in trial 2.

All behavioral tests were started 2 h after the lights went off (21:00 hr). Animals were placed individually in a clean cage (41×25×19 cm) and transported to a dim observation room. Then they were placed immediately into the test apparatus. Before each test, the apparatus was cleaned with 0.1% acetic acid, followed by thorough drying. The behavioral parameters were analyzed by an automated computer program, or later scoring from videotapes.

Open field test

The open field consisted of an acrylic box (40×40×40 cm), which was monitored by an automated animal activity monitor system (Tru Scan™; Coulbourn Instruments; USA). One grid of infrared sensor beams was mounted horizontally 3 cm above the floor, and a second tier of beams was mounted 16.5 cm above the floor to measure vertical (rearing) activity. Additionally, a video camera was suspended 150 cm above the center. Open field activity was measured under red light with four bulbs (28 lux in the center).

The following measures were assessed over a time period of 10 minutes [27]: (1) locomotion, that is, the distance traveled in cm, and (2) the number of rearings.

Social interaction test

In trial 2, immediately after the open field, the social interaction test was performed similar to Castanon [14]. A grey plastic test box

(58×58×39 cm) including a grey base consisting of the same material was used. Video taping was performed under red light with four red bulbs emitting 28 lux in the center and with a camera mounted 150 cm above the test box. At the beginning of the test, the adult experimental animal and the young animal (25–32 days old) were diagonally separated by a white polypropylene square insert. The test (5 min) was started when the square was removed. The SI time of the adult animal was recorded as the time following the young, sniffing its anogenital tract, or grooming it. These young interaction partners were used only once with the same adult animal; however, they were introduced 4–6 times to different adult rats, depending on survival of the latter.

Cytokine analysis

In trial 2, in an extra series 5 high and 5 low responder animals were categorized for blood sampling 1 hour before, 1 hour after and 24 hours after PCI in the PCI and G-CSF group, respectively. Under light anesthesia with fentanyl/droperidol, 1 ml blood was withdrawn by puncture of the retro-orbital venous plexus with heparinized capillaries. For cytokine analysis, EDTA blood was immediately centrifuged and plasma was stored at -70°C until being assayed. Quantification of the cytokines interleukin (IL)-6 and macrophage inflammatory protein (MIP)-2 was performed with conventional ELISA kits (Biosource, Camarillo, CA, USA).

Data analysis

According to a standardized and widely used procedure [13], all animals were ranked using the distance traveled in cm in a novel open field. Those animals above the median were assigned to the high responder group and those below the median to the low responder group (high/low trial 1: control A – anesthesia $n = 7/6$ and $n = 10/10$ each in all other groups; trial 2: G-CSF $n = 20/15$, PCI $n = 15/20$). One block of 5 high responder animals was allocated to the G-CSF instead to the PCI group. In trial 2, an additional 5 high and 5 low responder animals from each treatment group ($n = 10$) were used for cytokine analyses. The animals for cytokine analyses were only screened for baseline behavior and not included in the behavioral analyses of trial 2. Finally, all groups were assigned in a balanced way, that is, the distance traveled was comparable in all subgroups between treatments.

Survival rates were analyzed with the Chi-square test between treatment groups. Animals which died during the course of the experiment were excluded from behavioural data analysis. All behavioral data was normally distributed, and were assessed with ANOVA for repeated measures over all time points for different treatments, and thereafter from each treatment group a high and low responder subgroup analysis was performed. All post hoc analyses between groups were performed with the Tukey HSD test. Multiple post hoc *t*-tests were corrected with the Bonferroni procedure. Cytokines were analyzed with the non-parametric Kruskal-Wallis ANOVA by ranks. Correlations between different parameters were determined with Pearson or Spearman correlations where appropriate. All *P*-values presented are two-tailed unless otherwise stated and regarded as significant when $P < 0.05$. Data are expressed as means \pm S.E.M.

Results

Survival analysis

Trial 1 (Figure 1A): In the control A and B groups (anesthesia only and sham-treated groups) all animals survived throughout the course of the experiment. In the PCI group the mortality rate was 55% (11/20, $P < 0.001$ versus sham, 6 high responder and 5 low responder rats). Antibiotic prophylaxis reduced mortality to 30% (6/20, $P = 0.20$ versus PCI,

2 high responder and 4 low responder rats). Antibiotics plus G-CSF prophylaxis reduced mortality further to 10% with two deaths in the low responder group (2/20, $P < 0.01$ versus PCI; $P = 0.24$ versus PCI plus antibiotic). Finally, the PCI ($P < 0.001$) and the PCI plus antibiotics group ($P < 0.05$) showed a significantly higher mortality rate compared to the sham animals, but the antibiotics plus G-CSF prophylaxis did not ($P = 0.49$). Analyses for individual differences showed no significant differences for different mortality rates between high and low responder in any group.

Trial 2 (Figure 1B): Using a reduced bacterial inoculum, 22% of the rats in the PCI group died (10/45, 7 high responder and 3 low responder rats). The mortality rate in the group with antibiotics plus G-CSF prophylaxis was reduced to 9% (4/45, 3 high responder and 1 low responder rat), but not significantly lower compared to PCI group ($P = 0.144$). Analyses for individual differences showed a trend for a higher mortality rate of the high compared to the low responder in the PCI group ($P = 0.083$).

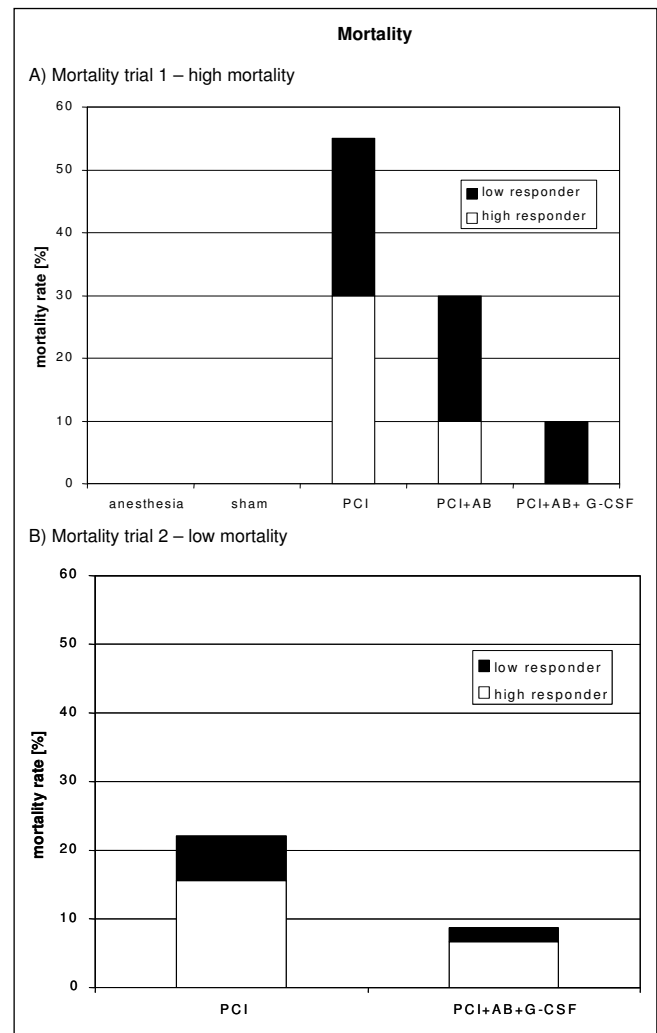


Fig. 1. Percentage of mortality rates for high (trial 1; A), and low mortality sepsis infection (trial 2; B) over 192 hours. Mortality rates are shown for different treatment groups and categorized preoperatively as high or low responder according to their horizontal locomotor activity in an open field.

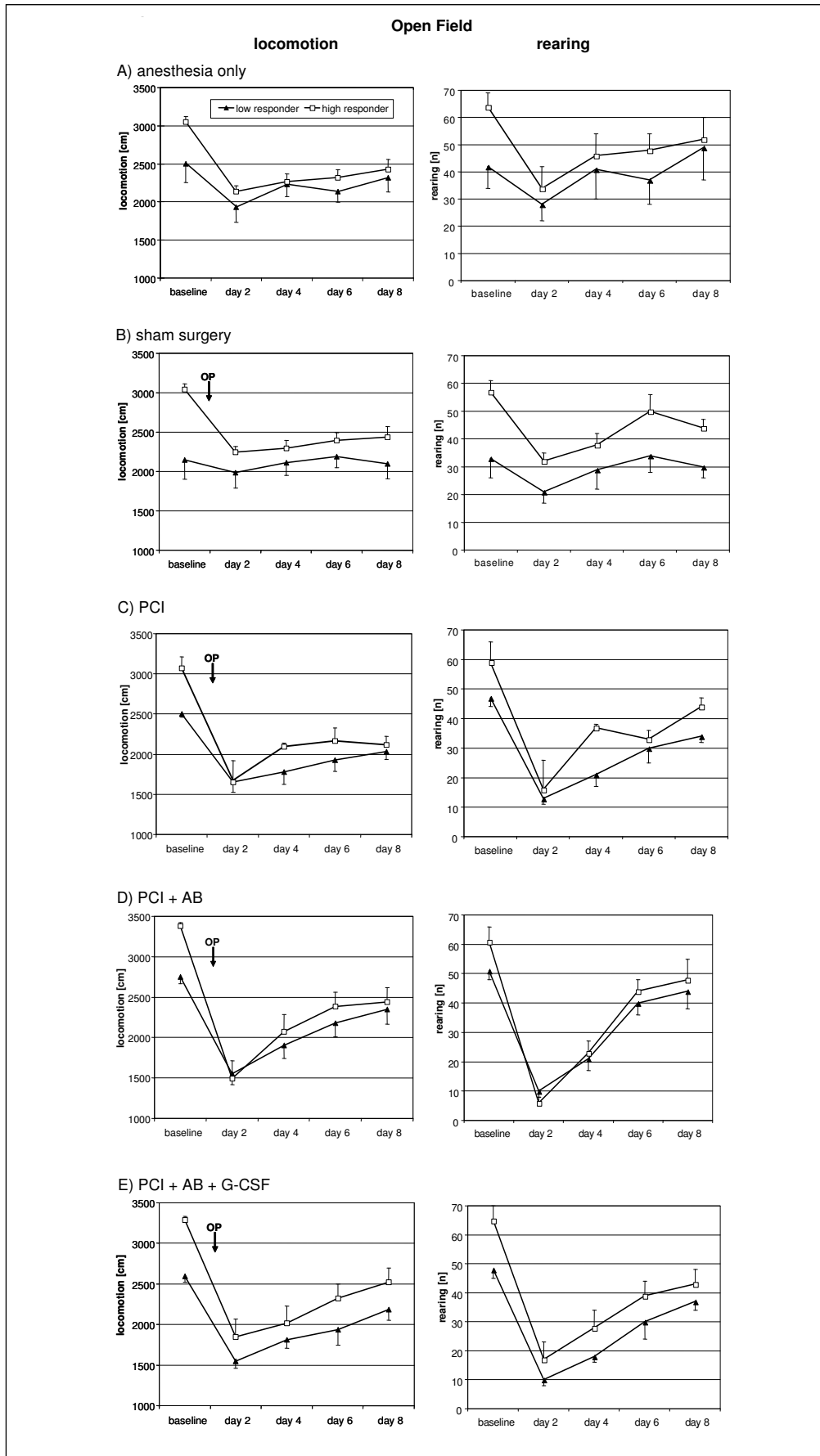


Fig. 2. Rat behavior in an open field was measured for horizontal locomotor (left column) and vertical rearing activity (right column) before and after inoculation (OP) with high mortality sepsis (trial 1). Rats were categorized preoperatively as high or low responder according to their horizontal locomotor activity. Measurements were taken before surgery and on day 2, 4, 6, and 8 thereafter. Data are presented as mean \pm S.E.M. See results for statistics.

Open field test

Trial 1 (Figure 2): ANOVAs for repeated measures were applied to detect treatment differences. For locomotion and rearings, significant effects were obtained for time, and time x group (P -values < 0.001), but not for groups (P -values < 0.10). No differences were obtained between any of the treatment groups with the Tukey post hoc test. Compared to preoperative baseline values, rearing activity and locomotion was significantly reduced in all groups on day 2 after surgery (P -values < 0.01).

Irrespective of treatment, ANOVA for repeated measures for both locomotion and rearings showed effects for time x group (P -values < 0.05), group (P -values < 0.005), and time (P -values < 0.001) comparing low and high responder groups during the course of the experiment. Further analyses showed that both locomotor activity (expected due to the median split after the preoperative open field), and the number of rearings were lower for low responder than for high responder at baseline before surgery (P -values < 0.001). These differences persisted until the end of the experiment, showing more locomotion for all high compared to all low responder on day 6 (P < 0.05, one-tailed), and day 8 (P = 0.055, one-tailed), but not for rearings (P -values < 0.10).

When comparing the subgroups for each treatment group for horizontal locomotion, high and low responder rats showed significant time x group and group effects for the

sham group (P -values < 0.05), and time effects for the sham and PCI group (P -values < 0.05). Analyses for vertical activity revealed also more rearings for the high compared to the low responder rats in the sham group and PCI animals (P -values < 0.05).

Trial 2 (Figure 3): The second trial showed similar results as trial 1. ANOVAs for repeated measures revealed significant differences in time, time x group, and between treatment groups of PCI and G-CSF plus antibiotic prophylaxis for locomotion and rearings (P -values < 0.01).

When comparing the subgroups for each treatment group, high and low responder rats showed significant time x group, group (P -values < 0.05), and time (P -values < 0.001) effects for locomotor activity in the PCI and the G-CSF plus antibiotic prophylaxis group, respectively. However, analyses of rearing activity showed that high and low responder rats produced only significant time effects in both groups (P -values < 0.001). Bonferroni corrected paired t-tests comparing day 0 (preoperative) versus day 8 (last day of testing) in the PCI group showed blunted locomotion (P -values < 0.001), and rearing activity (P -values < 0.05) for high and low responder rats, respectively. In parallel, the G-CSF plus antibiotics prophylaxis group revealed blunted locomotor (P < 0.001), and rearing activity (P < 0.05) for high responder. However, low responder displayed only reduced locomotor (P < 0.001) between day 0 versus day 8, suggesting a partial recovery for this G-CSF subgroup.

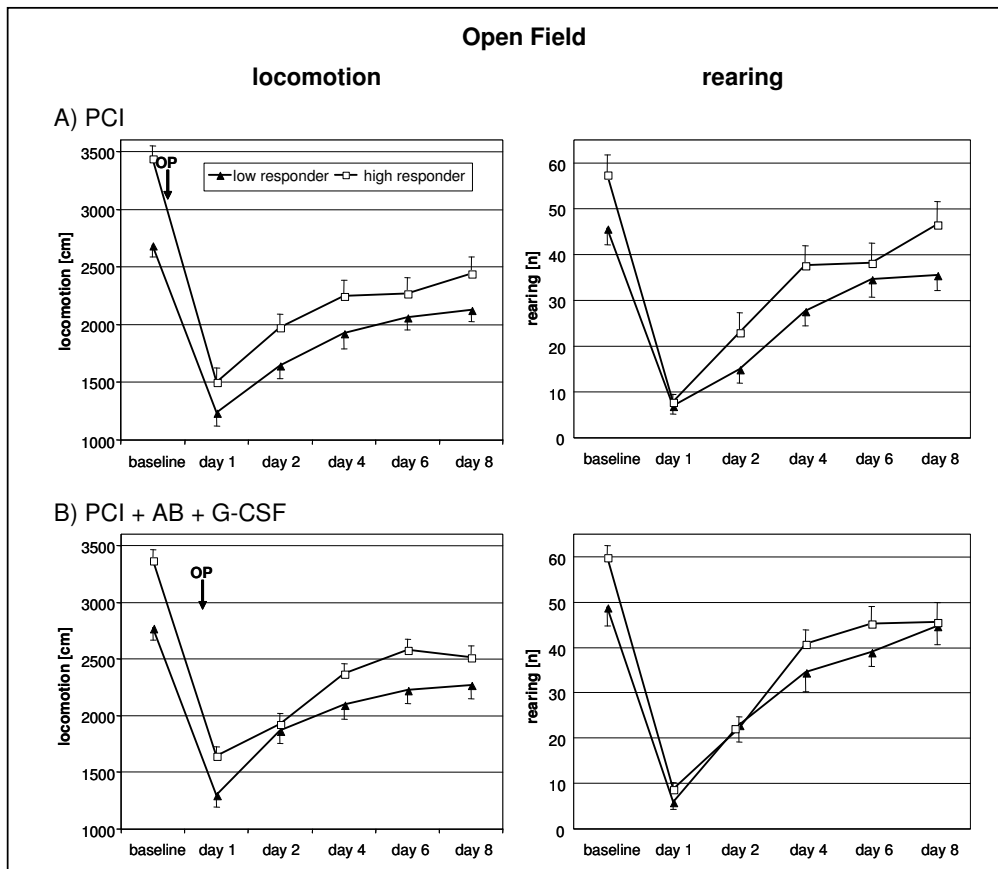


Fig. 3. Rat behavior in an open field was measured for horizontal locomotor (left column) and vertical rearing activity (right column) before and after inoculation (OP) with low mortality sepsis (trial 2). Rats were categorized preoperatively as high or low responder according to their horizontal locomotor activity. Measurements were taken before surgery and on days 1, 2, 4, 6, and 8 thereafter. Data are presented as mean \pm S.E.M. See results for statistics.

Social interaction test

Trial 2 (Figure 4): ANOVA for repeated measures of treatment groups showed a significant effect for time ($P < 0.001$), but no effects for group ($P = 0.72$), and time \times group ($P = 0.24$). When analyzing each treatment group separately, low versus high responder revealed no group and time \times group effects, but time effects (P -values < 0.001) for both groups, PCI and G-CSF plus antibiotic prophylaxis, respectively. Bonferroni corrected paired t -tests comparing day 0 (preoperative) versus day 8 (last day of testing) revealed that only high responder of the G-CSF plus antibiotics group showed a trend for blunted social activity ($P = 0.056$), but none of the other subgroups.

Relationship between behaviors

Before surgery, and for all rats, there was a significant positive correlation between locomotion and rearing (trial 1: $r =$

0.71, $P < 0.001$; trial 2: $r = 0.68$, $P < 0.001$) which remained stable during the course of the experiment (trial 1: r -values ≥ 0.64 ; trial 2: $r = 0.59$, P -values < 0.001).

In trial 2, the correlations between social activity and locomotor activity ($r = 0.15$, $P = 0.25$), and rearings, respectively ($r = 0.22$, $P = 0.08$), were relatively low before surgery. In contrast, social activity and locomotor activity ($r = 0.42$, $P < 0.001$) were significantly correlated on day 1. Similar positive relationships were observed between social activity and the number of rearings for day 1 ($r = 0.40$, $P < 0.01$), and day 2 ($r = 0.28$, $P < 0.05$). Thereafter, no significant correlations were observed until the end of the experiment (data not shown).

Cytokine analysis

Trial 2 (Figure 5): At baseline, the IL-6 levels were below the limit of quantification of the ELISA assay, and were excluded from the subsequent analysis. For the remaining two

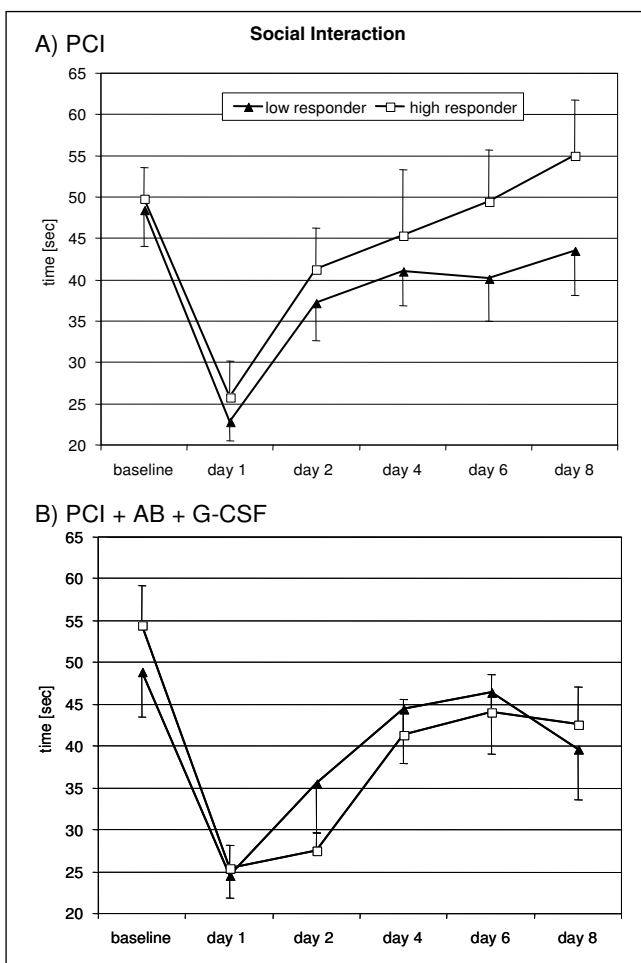


Fig. 4. Rat behavior in the social interaction test was measured as the time spent of the experimental adult animal in grooming, following, and sniffing a young male rat. Measurements were taken before surgery (OP) and on days 1, 2, 4, 6, and 8 thereafter. Data are presented as mean \pm S.E.M. See results for statistics.

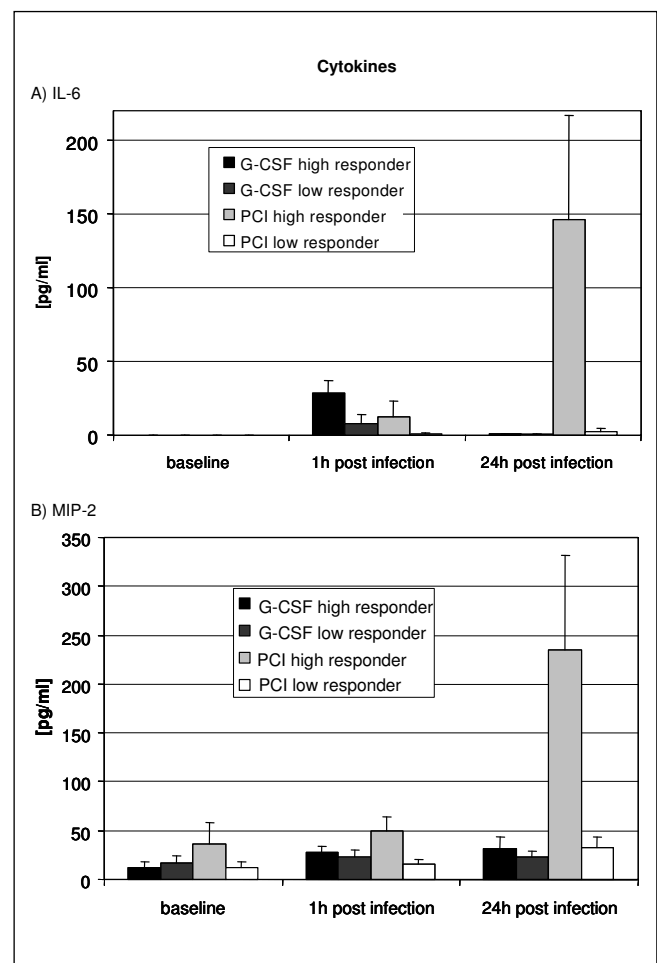


Fig. 5. In trial 2 ($n = 10$ rats/group) 1 ml blood was drawn 1 hour before, 1 hour after, and 24 hours after PCI by puncture of the retro bulbar plexus, and IL-6 and MIP-2 were analyzed. At baseline the IL-6 levels were below the limit of quantification of the ELISA assay. Data are presented as mean \pm S.E.M. Note: Scales of the figures are different. See results for statistics.

time points, i. e. 1 hour and 24 hours after surgery, Kruskal-Wallis ANOVA by ranks were calculated showing no difference between groups 1 hour after inoculation. However, a significant group difference revealed enormously high IL-6 levels for the PCI high responder group 24 hours after surgery ($P = 0.04$).

For MIP-2 levels, Kruskal-Wallis ANOVA showed no effects before and 1 hour after inoculation. However, highly increased MIP-2 levels for the PCI high responder group 24 hours following sepsis infection were observed ($P = 0.03$).

Discussion

Mortality

In both trials, as expected mortality rates were lowest for the antibiotic plus G-CSF prophylaxis following different concentrations of human stool inoculum. As demonstrated previously [25] and in trial 1, sepsis led to a high mortality rate which could be substantially reduced by prophylactic treatments, especially when using antibiotics plus G-CSF. Previously, with this combination, antimicrobial cellular functions (polymorphonuclear cells migration to bacteria, phagocytosis and oxygen radical formation) were improved and hyperinflammation (TNF- α , IL-6 and IL-8) was reduced [28]. Here, however, the lower number of animals dying in the G-CSF group was not significantly different from standard treatment with antibiotics (trial 1), or untreated sepsis (trial 2). Nonetheless, in trial 1 only the G-CSF rats showed a comparable low mortality rate to the sham animals, while the PCI and the PCI plus antibiotic groups had a significantly higher mortality rate compared to sham treatment. In trial 2, we suppose that a floor effect was yielded because the mortality rate was kept relatively low compared to trial 1. Interestingly, however, analyses of individual differences in trial 2 revealed a trend for a higher mortality rate of high compared to low responder rats in the PCI group. In any case, a lower mortality rate in G-CSF rats in both trials, and differential mortality rates in relation to premorbid behavior still merit further attention since dead individuals are important regardless of statistical effects.

Behavior

On the behavioral level, the present results demonstrate that immediately after infection and independent of the prophylaxis regimen, all behavioral activities were drastically reduced, such as locomotion and rearing in an open field and social interaction. Locomotor activity and rearing were decreased acutely (i. e., 1 or 2 days after surgery) in all groups, including sham and anesthesia controls. These acute decreases were more pronounced in the infected groups than in the controls as previously shown [29]. These data are in line with several previous results obtained in rats and mice which showed that various types of infections can reduce open field activity, including bacterial agents such as muramyl dipeptide, or LPS [30], or intact microbes such as schistosoma mansoni [31], staphylococcus aureus or pseudomonas aeruginosa [32].

Sepsis modified behavior in groups with prophylactic treatments; however, recovery to normal activity was not substantially improved in the open field and social interaction tests. After sepsis induction, preoperative high and low locomotion responders showed different activity in the open field, but not in social exploration. We provide first evidence that postoperative values for high versus low locomotor active rats showed differential behavioral results. All low responder animals showed less activity during the whole recovery period compared to all high responder rats in an open field. More importantly, the measures of high and low responder for locomotion and rearings (trial 1) showed interaction effects pointing at a differential role for baseline activity towards recovery from sepsis. More specific analyses revealed that all high compared to all low responder showed more locomotion on day 6 (significant), and day 8 (trend), but that these effects were not observed for rearings. When comparing the subgroups for each treatment group for horizontal locomotion, high and low responder rats showed interaction and group effects compared to the sham group. Finally, rearing activity was higher for the high compared to the low responder rats in the sham group and PCI animals. In trial 2, interaction effects of high and low responder rats were observed for locomotion, but not for the number of rearings (as opposed to trial 1). When looking for the time course, only low responder rats with antibiotics plus G-CSF prophylaxis showed no altered rearing activity between preoperative and end of the experiment, suggesting a partial recovery for the G-CSF subgroup in trial 2. Thus, the present data suggest that horizontal locomotion and vertical rearing activity in an open field appears to be affected more in active animals depending on their treatment. These results support previous evidence on individual differences to disease susceptibilities. For example, locomotor activity was used to analyze if individual differences of rats were related to cytokine production and other relevant parameters in an animal model of rheumatoid arthritis [33]. Based on the distance traveled in an open field low active and high active animals differed in their severity of bone destruction. Also, low active rats had less mitogen-induced splenic IL-10 and interferon- γ production during adjuvant-arthritis [33].

Alternatively, our rats may have shown impairments in memory function. Recently, Wistar rats tested ten days after sepsis induction, but without overt signs of sickness behaviour did not show differences in combined locomotor and rearing activity between novel exposure to an open field and a 24 hours retest [34]. In addition, the sepsis group showed a decreased performance in latency retention compared with the sham group in an inhibitory avoidance test after foot shock [34], suggesting reduced memory functions.

In trial 2, we also analyzed social interaction behaviour, a measure which presumably reflects exploratory activity and emotional variables such as anxiety [35]. Our analyses revealed that open field behaviour (locomotion, rearing) and social interaction were not correlated with each other, except for modest correlations in the two days after low mortality sepsis induction. The results showed that recovery of social exploration was neither different between PCI and G-CSF plus antibiotic prophylaxis groups nor between high and low responder rats. These non-significant results notwithstanding it is noteworthy that social exploration in PCI only treated

rats diverged between high and low responder beginning with postoperative day 1 and continued until the end of the experiment, where it was higher in high compared to low responder. In contrast, social exploration was differentially affected in the G-CSF prophylaxis group showing that low but not high responder returned to baseline social activity levels, although this difference was not quite significant.

Using the technique of radio-telemetry [9, 10], we had previously shown that antibiotic prophylaxis, especially when combined with G-CSF, positively altered sickness behavior measured in terms of behavioral activity and circadian rhythms of heart rate and blood pressure in septic rats. At first sight, the results from the present study appear not to support the data from the previous telemetric experiments [9, 10], when neither open field nor social interaction behavior yielded superior recovery in the G-CSF group. However, the results do not necessarily contradict our previous findings because telemetric data were yielded in the home cage while the present study used external environments. In addition, the observation time was much shorter in the present study than previously. Therefore, we suggest that longer observation times are needed to observe behavioral differences between groups underlying sepsis treatments.

Cytokines

Finally, analyses of blood cytokine levels (IL-6, MIP-2) were conducted in rats with low mortality sepsis infection in trial 2. While similar levels were observed for baseline and 1 hour post infection in both behavioral subgroups of untreated and G-CSF groups, 24 hours later an enormous increase of both cytokines was noted especially in high responder with untreated sepsis. High responders seem to be more at risk for an adverse outcome than low responders. Those rats with extremely increased cytokine levels died soon afterwards. High IL-6 levels are known to be an independent predictor of mortality in sepsis [23, 36]. Recently, also MIP-2 levels have been reported to be a feasible predictor of mortality in sepsis [23, 37]. In contrast to the mortality results we did not find a correlation between increased peripheral cytokine levels and other behavioural parameters such as rearing or time spent together in the social interaction test. There are first indices of individual behavioral differences to be related to central cytokine profiles. Higher (striatum) and lower (prefrontal cortex) endogenous IL-2 mRNA gene expression was shown in rats with high compared to low anxiety-like behavior [38]. Thus, the present results point to an unknown relationship between individual behavioral differences and sepsis mortality with increased cytokine levels.

It is unclear if the G-CSF effects on both cytokines in high responder were achieved directly by a reduction of the proinflammatory cytokine expression, or indirectly by an increase in the clearance of bacteria by phagocytic cells, or both. The induction of sickness behavior by a peripheral immune stimulation (e. g. PCI) requires the peripheral and/or central synthesis and release of cytokines [39]. The effect of G-CSF on centrally released cytokines has just begun to be studied. Recent work demonstrated that G-CSF administration reduced the recruitment of T cells to the CNS [40]. Further, G-CSF down-regulated the production of proinflam-

matory cytokines like TNF- α , IL-1 and IL-6 in the periphery [21, 25], and at the side of infection [26]. It is known that different components of sickness behavior may be mediated by different cytokine expression patterns of IL-1, IL-6 and TNF- α [41]. Finally, G-CSF prophylaxis improved survival and clearance of microbes and reduced MIP-2 in the lung [42].

Conclusion

A quick recovery after infection or trauma is intended by the addition of immune modulators, which do not only influence the immune system, but also modulate individual behavior and cytokine response. We are at the beginning of understanding the complex interaction of sickness behavior in animals and quality of life in humans. Conventional outcomes sepsis research does not provide prognostications to individual patients, but our data is suggested to be a first individualised approach to the treatment of sepsis in standardized animal models. Individual risk profiles including behavioral parameters and cytokines are an interesting tool for future sepsis research in animals and man. Individual behavior and cytokine levels may be linked together in a today nearly unknown way. The increasing interest and acceptance of quality of life as an important outcome in clinical research indicates further the necessity of complex animal models with respect to systematic individual behavioral differences to test new immune modulators in preclinical research.

Acknowledgements: This work was supported by the Deutsche Forschungsgemeinschaft (BA 1560-2/3 and PA 818/4-1), and by a Project Based Personnel Exchange Program from the NSC (0940042882) and from the German Academic Exchange Service (D/05/06869). We thank Amgen Inc. for providing us with filgrastim (G-CSF).

References

- [1] Angus DC, Linde-Zwirble WT, Lidicker J, Clermont G, Carcillo J, Pinsky MR. Epidemiology of severe sepsis in the United States: analysis of incidence, outcome, and associated costs of care. *Crit Care Med* 2001; 29: 1303–10.
- [2] Granja C, Dias C, Costa-Pereira A, Sarmento A. Quality of life of survivors from severe sepsis and septic shock may be similar to that of others who survive critical illness. *Crit Care* 2004; 8: 91–8.
- [3] Heyland DK, Hopman W, Coe H, Tranmer J, McColl MA. Long-term health-related quality of life in survivors of sepsis. Short Form 36: a valid and reliable measure of health-related quality of life. *Crit Care Med* 2000; 28: 3599–605.
- [4] Herridge MS, Cheung AM, Tansey CM, Matte-Martyn A, Diaz-Granados N, Al Saiti F et al. One-year outcomes in survivors of the acute respiratory distress syndrome. *N Engl J Med* 2003; 348: 683–93.
- [5] Davidson TA, Caldwell ES, Curtis JR, Hudson LD, Steinberg KP. Reduced quality of life in survivors of acute respiratory distress syndrome compared with critically ill control patients. *JAMA* 1999; 281: 354–60.
- [6] Rublee D, Opal SM, Schramm W, Keinecke HO, Knaub S. Quality of life effects of antithrombin III in sepsis survivors: results from the KyberSept trial. *Crit Care* 2002; 6: 349–56.
- [7] Lyman GH, Kuderer NM. Filgrastim in patients with neutropenia: potential effects on quality of life. *Drugs* 2002; 62 Suppl 1: 65–78.

- [8] Bauhofer A, Plaul U, Torossian A, Koller M, Stinner B, Celik I et al. Perioperative prophylaxis with granulocyte colony-stimulating factor (G-CSF) in high-risk colorectal cancer patients for an improved recovery: A randomized, controlled trial. *Surgery* 2007; 141: 501–10.
- [9] Bauhofer A, Witte K, Celik I, Pummer S, Lemmer B, Lorenz W. Sickness behavior, an animal equivalent to quality of life, is improved in septic rats by G-CSF and antibiotic prophylaxis. *Langenbeck's Arch Surg* 2001; 386: 132–40.
- [10] Bauhofer A, Witte K, Lemmer B, Middeke M, Lorenz W, Celik I. Quality of life in animals as a new outcome for surgical research: G-CSF as a quality of life improving factor. *Eur Surg Res* 2002; 34: 22–9.
- [11] Bauhofer A, Köster M, Schmitt A, Schwarting RKW, Lorenz W, Pawlak CR. Sickness behavior of rats with abdominal sepsis can be improved by antibiotic and G-CSF prophylaxis in clinic modeling randomized trials. *Inflamm Res* 2004; 53: 697–705.
- [12] Thiel CM, Muller CP, Huston JP, Schwarting RK. High versus low reactivity to a novel environment: behavioural, pharmacological and neurochemical assessments. *Neuroscience* 1999; 93: 243–51.
- [13] Pawlak CR, Schwarting RK. Object preference and nicotine consumption in rats with high vs. low rearing activity in a novel open field. *Pharmacol Biochem Behav* 2002; 73: 679–87.
- [14] Castanon N, Bluthé RM, Dantzer R. Chronic treatment with the atypical antidepressant tianeptine attenuates sickness behavior induced by peripheral but not central lipopolysaccharide and interleukin-1 β in the rat. *Psychopharmacology (Berl)* 2001; 154: 50–60.
- [15] Pawlak CR, Ho YJ, Schwarting RK. Animal models of human psychopathology based on individual differences in novelty-seeking and anxiety. *Neurosci Biobehav Rev* 2008; 32: 1544–68.
- [16] Hayney MS. Pharmacogenomics and infectious diseases: impact on drug response and applications to disease management. *Am J Health Syst Pharm* 2002; 59: 1626–31.
- [17] Robert J. Pharmacogenetics and pharmacogenomics as new tools to optimise cancer chemotherapy. *J Chemother* 2004; 16 Suppl 4: 22–4.
- [18] Gupta R, Rajani R, Primrose JN, Johnson CD. Body composition, physiological function and psychological changes in patients with predicted severe acute pancreatitis. *Pancreatol* 2001; 1: 58–62.
- [19] Hart BL. Biological basis of the behavior of sick animals. *Neurosci Biobehav Rev* 1988; 12: 123–37.
- [20] Konsman JP, Parnet P, Dantzer R. Cytokine-induced sickness behaviour: mechanisms and implications. *Trends Neurosci* 2002; 25: 154–9.
- [21] Hartung T, Volk HD, Wendel A. G-CSF an anti-inflammatory cytokine. *J Endotoxin Res* 1995; 2: 195–201.
- [22] Hareng L, Hartung T. Induction and regulation of endogenous granulocyte colony-stimulating factor formation. *Biol Chem* 2002; 383: 1501–17.
- [23] Remick DG, Bolgos GR, Siddiqui J, Shin J, Nemzek JA. Six at six: interleukin-6 measured 6 h after the initiation of sepsis predicts mortality over 3 days. *Shock* 2002; 17: 463–7.
- [24] Zhang P, Nelson S, Holmes MC, Summer WR, Bagby GJ. Compartmentalization of macrophage inflammatory protein-2, but not cytokine-induced neutrophil chemoattractant, in rats challenged with intratracheal endotoxin. *Shock* 2002; 17: 104–8.
- [25] Lorenz W, Reimund K-P, Weitzel F, Celik I, Kurnatowski M, Schneider C et al. Granulocyte colony-stimulating factor prophylaxis before operation protects against lethal consequences of postoperative peritonitis. *Surgery* 1994; 116: 925–34.
- [26] Bauhofer A, Stinner B, Kohlert F, Reckzeh B, Lorenz W, Celik I. Granulocyte colony-stimulating factor but not peritoneal lavage increases survival rate after experimental abdominal contamination and infection. *Br J Surg* 2002; 89: 1457–63.
- [27] Ho YJ, Eichendorff J, Schwarting RK. Individual response profiles of male Wistar rats in animal models for anxiety and depression. *Behav Brain Res* 2002; 136: 1–12.
- [28] Bauhofer A, Torossian A, Lorenz W, Middeke M, Plaul U, Schütz P et al. Dependence of positive effects of granulocyte colony-stimulating factor on the antibiotic regimen: evaluation in rats with polymicrobial peritonitis. *World J Surg* 2004; 28: 834–44.
- [29] Oarada M, Ito E, Terao K, Miyazawa T, Fujimoto K, Kaneda T. The effect of dietary lipid hydroperoxide on lymphoid tissues in mice. *Biochim Biophys Acta* 1988; 960: 229–35.
- [30] Engeland CG, Kavaliers M, Ossenkopp KP. Sex differences in the effects of muramyl dipeptide and lipopolysaccharide on locomotor activity and the development of behavioral tolerance in rats. *Pharmacol Biochem Behav* 2003; 74: 433–47.
- [31] Fiore M, Alleva E, Moroni R, Aloe L. Infection with *Schistosoma mansoni* in mice induces changes in nociception and exploratory behavior. *Physiol Behav* 1998; 65: 347–53.
- [32] Bradfield JF, Schachtman TR, McLaughlin RM, Steffen EK. Behavioral and physiologic effects of inapparent wound infection in rats. *Lab Animal Sci* 1992; 42: 572–8.
- [33] Sajti E, van MN, Kavelaars A, van der NJ, Gispen WH, Heijnen C. Individual differences in behavior of inbred Lewis rats are associated with severity of joint destruction in adjuvant-induced arthritis. *Brain Behav Immun* 2004; 18: 505–14.
- [34] Barichello T, Martins MR, Reinke A, Feier G, Ritter C, Quevedo J et al. Cognitive impairment in sepsis survivors from cecal ligation and perforation. *Crit Care Med* 2005; 33: 221–3.
- [35] Hooks MS, Juncos JL, Justice JB, Jr., Meiergerd SM, Povlock SL, Schenk JO et al. Individual locomotor response to novelty predicts selective alterations in D1 and D2 receptors and mRNAs. *J Neurosci* 1994; 14: 6144–52.
- [36] Osuchowski MF, Welch K, Siddiqui J, Remick DG. Circulating cytokine/inhibitor profiles reshape the understanding of the SIRS/CARS continuum in sepsis and predict mortality. *J Immunol* 2006; 177: 1967–74.
- [37] Walley KR, Lukacs NW, Standiford TJ, Strieter RM, Kunkel SL. Elevated levels of macrophage inflammatory protein 2 in severe murine peritonitis increase neutrophil recruitment and mortality. *Infect Immun* 1997; 65: 3847–51.
- [38] Pawlak CR, Schwarting RK, Bauhofer A. Cytokine mRNA levels in brain and peripheral tissues of the rat: relationships with plus-maze behaviour. *Brain Res* 2005; 137: 159–165.
- [39] Dantzer R. Cytokine-induced sickness behavior: where do we stand? *Brain Behav Immun* 2001; 15: 7–24.
- [40] Zavala F, Abad S, Ezine S, Taupin V, Masson A, Bach JF. G-CSF therapy of ongoing experimental allergic encephalomyelitis via chemokine- and cytokine-based immune deviation. *J Immunol* 2002; 168: 2011–19.
- [41] Besedovsky HO, del Rey A. Immune-neuro-endocrine interactions: facts and hypotheses. *Endocr Rev* 1996; 17: 64–102.
- [42] Bauhofer A, Lorenz W, Kohlert F, Torossian A. Granulocyte colony-stimulating factor prophylaxis improves survival and inflammation in a two-hit model of hemorrhage and sepsis. *Crit Care Med* 2006; 34: 778–84.