Supplementary online material

The impact of social isolation on immunological parameters in rats

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Materials and Methods

Animals and housing conditions

Adult male outbred Wistar rats (RjHan:WI, own breed; 7 weeks, n=32) were housed in standard laboratory cages in groups of four before the housing protocol. The animals had free access to food and water under a 12-hours light-dark schedule (lights on 7:00 a.m., 60 lux within the cages), room temperature of 22±2°C, humidity of 50% and weekly changed bedding. The experiments were approved by the relevant Animal Welfare Office according to the guidelines for the use of animals in biomedical research of the European Communities Council Directive (86/609/EEC).

Beginning from the ninth week of age up to the following four weeks, sixteen rats were housed individually in separate cages and further sixteen animals were housed in groups of four familiar animals in the common stock. The body weight of all animals was registered at start of the housing protocol and again at day 28. Otherwise, the animals were left undisturbed.

Behavioral testing

The behavioral outcome of individually and group-housed animals was measured in eight rats per group by forcing them to swim in an inescapable situation to detect symptoms of “behavioral despair” (Porsolt et al., 1977; Regenthal et al., 2009). In a preceding trial at day 27, rats were placed in a cylindrical basin (0.5 m height x 0.25 m diameter) filled with water (25±1°C) up to 0.35 m height for 15 minutes. In the experimental trial 24 hours later, the immobile time (floating and movements to keep the head out of the water only) and the time of escape attempts (climbing at the basin wall) were registered for 7 minutes.

Cytokine measurement

Blood samples of the remaining eight animals per group were taken under isoflurane anesthesia between 9 and 11 a.m. by heart puncture at day 28. Plasma concentrations of IL-2, IL-4, IL-10, IL-22, TNF-α, IFN-γ and ACTH were measured in duplicates using a Cytometric Bead Array (CBA; Becton, Dickinson and Company [BD] Biosciences, San Jose, CA), IL-22 was detected using rat IL-22 Quantikine ELISA Kit (R&D Systems, Minneapolis, MN).
Data analysis

The Mann-Whitney-U test for unpaired data was performed using SPSS (IBM SPSS Statistics 20). A level of asymptotic 2-tailed P-value of <0.05 was considered to indicate significant difference.

Results

Figure 1: Effects of housing conditions of Wistar rats on plasma cytokine concentrations of IL-2 and IL-4 (left panel) and IL-10, IL-22, IFN-γ and TNF-α (right panel). Adult rats were housed in groups or social isolation for 28 days (n=8 each). Data are shown as means ± standard errors of the means (SEM); * indicates a significant difference for the values of single housed compared to group housed animals.

Table 1: Influence of group and single housing of Wistar rats on body weight, behaviour in the forced swim test and plasma ACTH concentration.

<table>
<thead>
<tr>
<th></th>
<th>Group housing (mean ± SEM)</th>
<th>Single housing (mean ± SEM)</th>
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<tbody>
<tr>
<td>Body weight (at start)</td>
<td>(n=16 each) 318 ± 8 g</td>
<td>316 ± 9 g</td>
</tr>
<tr>
<td>Body weight (day 28)</td>
<td>(n=16 each) 392 ± 9 g</td>
<td>358 ± 9 g *</td>
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<tr>
<td>Forced swim test</td>
<td></td>
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<tr>
<td>Immobility</td>
<td>120 ± 28 sec</td>
<td>262 ± 11 sec *</td>
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<tr>
<td>Escape attempts</td>
<td>57 ± 7 sec</td>
<td>24 ± 8 sec *</td>
</tr>
<tr>
<td>Plasma ACTH (pg/ml)</td>
<td>(n=8 each) 436 ± 93</td>
<td>816 ± 117 *</td>
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</table>

* indicates a significant difference for the values of single housed compared to group housed animals.
References
