Cat odor exposure induces distinct changes in the exploratory behavior and Wfs1 gene expression in C57Bl/6 and 129Sv mice

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Abstract

129Sv and C57Bl/6 (Bl6) strains are two most widely used inbred mice strains for generation of transgenic animals. The present study confirms the existence of substantial differences in the behavior of these two mice strains. The exploratory behavior of Bl6 mice in a novel environment was significantly higher compared to 129Sv mice. The exposure of mice to cat odor-induced an anxiety-like state in Bl6, but not in 129Sv mice. The levels of Wfs1 gene expression did not differ in the prefrontal cortex, mesolimbic area and temporal lobe of experimentally naive Bl6 and 129Sv mice. However, after cat odor exposure the expression of Wfs1 gene was significantly lower in the mesolimbic area and temporal lobe of Bl6 mice compared to 129Sv strain. Dynamics of Wfs1 gene expression and exploratory behavior suggest that the down-regulation of Wfs1 gene in Bl6 mice might be related to the increased anxiety. Further studies are needed to test the robustness and possible causal relationship of this finding.

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129Sv and C57Bl/6 (Bl6) strains are two most widely used inbred mice strains for the generation of transgenic animals. Recent studies have consistently reported that 129Sv mouse lines display reduced locomotor activity and higher anxiety compared to Bl6 mice in the open field test and different anxiety models [12,16]. Predator odor or predators themselves can be highly effective stimuli for eliciting anxiety-like states in rodents [1]. Belzung et al. [4] have established that cat odor increases anxiety in Bl6 strain mice. There is indirect evidence suggesting that mice in 129Sv/C57Bl/6 mixed background do not exhibit increased anxiety in the elevated plus-maze test after exposure to cat odor [3]. This could indicate that 129Sv strain is less sensitive to cat odor-induced anxiety. To test this hypothesis, we compared the effect of cat odor exposure on the exploratory behavior of 129Sv and Bl6 mice.

Köks et al. [9] have demonstrated that the exposure of male Wistar rats to cat odor produces an up-regulation of Wfs1 (wolframin) mRNA in the amygdala. Mutations in WFS1 gene cause Wolfram syndrome (DIDMOAD), a rare multi-system neurodegenerative disorder of autosomal recessive inheritance [8]. Moreover, it has been suggested that mutations in WFS1 gene in humans are associated with increased susceptibility to mood disorders [13]. Using biochemical methods, Takeda et al. [14] have shown that the WFS1 protein is an integral, endoglycosidase H-sensitive membrane glycoprotein that localizes in the endoplasmic reticulum. WFS1 expression has been shown to confer cation channel activity and increased cytosolic Ca2+ levels in Xenopus oocytes [11]. Recent evidence suggests that WFS1 protein participates in the regulation of cellular Ca2+ homeostasis, at least partly, by modulating the filling state of the endoplasmatic reticulum Ca2+ store [15]. In the rat brain, at both protein and mRNA level, Wfs1 has been found to be highly expressed in the hippocampus CA1 region, amygdaloid area, olfactory tubercle and superficial layer of allocortex [14]. To investigate a possible role of Wfs1 gene in the regulation of emotional behavior, Wfs1 mRNA level was measured in three forebrain structures (pre-
frontal cortex, mesolimbic area, temporal lobe) of 129Sv and Bl6 mice after exposure to cat odor.

All studies were performed in female C57Bl/6 (Bl6, Scanbur BK) and 129Sv/SvEv/Tac (129Sv, Taconic) mice, 8–10 weeks old at the time of testing. There are many substrains of 129 mice, which can differ markedly in their behavioral profiles [5]. We have chosen 129Sv/SvEv/Tac substrain, because their embryonic stem cells are commonly used in targeted gene inactivation studies. For the present experiment, female mice were chosen as they tend to be more sensitive to predator odor stress than males [2]. Mice were housed in groups of 10–12 in the animal house at 20 ± 2 °C under a 12-h/12-light/dark cycle (lights on at 07:00 h). Tap water and food pellets were available ad libitum. The permission (No. 39, 7 October 2005) for the present study was given by the Estonian National Board of Animal Experiments in accordance with the European Communities Directive of 24 November 1986 (86/609/EEC).

Both 129Sv and Bl6 mice were divided into two groups. One group was exposed to a cloth impregnated with cat odor and the other to a clean cloth. The exposure was performed in two separate but similar rooms (one for cat odor and the other for clean cloth exposure with the same illumination intensity of 20 lx, humidity, ventilation, etc.). Animals were habituated to the experimental room and the experimenter for three consecutive days before the experiment. On the fourth day, after 30 min habituation in the testing room, mice were individually exposed to either a clean or a cat odor impregnated cloth. The exposure was performed in a cage (25 cm × 40 cm × 15 cm) similar to animals’ home cages. A mouse was placed in a cage and a clean or a cat odor impregnated cloth was placed in the corner of the cage on the bedding. The exposure to cat odor lasted for 30 min and the session was video-recorded. An observer, not aware about the manipulations performed with mice, analyzed the videotaped behavioral responses. To score horizontal activity, the cage was divided into two parts by a visible line and the number of transitions between these parts were counted. Also the following parameters were analyzed: number of contacts with the cloth, duration of cloth contact, number of rearings, number of digging events, duration of digging, number of burying events and duration of burying. Behavioral experiments were performed in randomized order, that is, all four groups of mice were studied in parallel.

In gene expression studies, animals from both strains were divided into three groups: experimentally naïve animals, animals exposed to a clean cloth and animals exposed to cat odor. Experimentally naïve mice were handled in the same way as animals from two other groups, except for performing the behavioral study. Mice were decapitated immediately after the cat odor or clean cloth exposure. Brains were quickly dissected into three parts: the prefrontal cortex, mesolimbic area (including the nucleus accumbens and olfactory tubercle) and temporal lobe (including the amygdala), and frozen in liquid nitrogen. These three regions were chosen according to high level of Wfs1 protein expression in the mouse brain (our unpublished data). Dissection was performed according to the coordinates obtained from the mouse brain atlas [6]. Total RNA was extracted individually from each brain structure of each mouse using Trizol®

Reagent (Invitrogen, USA) according to the manufacturer’s protocol. First strand cDNA was synthesized by using poly(T)_18 oligo-nucleotides and SuperScript™ III Reverse Transcriptase (Invitrogen, USA).

Measurements of Wfs1 mRNA expression level in cat odor exposed, control odor exposed and experimentally naïve animals were conducted in parallel. The relative amount of gene expression was estimated by TaqMan-based real-time quantitative-PCR (qPCR) assay. The qPCR was performed using ABI Prism 7900 Sequence Detection System (PE Applied Biosystems, USA) equipment and ABI Prism 7900 SDS Software. All reactions were performed in a final volume of 10 μl, using 50–100 ng of cDNA. Mouse hypoxanthine-guanine phosphoribosyltransferase (Hprt) was used as an endogenous reference gene as it is a constitutively expressed gene in the mammalian brain. Primers and probes for Hprt were designed with the Primer Express™ software (PE Applied Biosystems, USA). For Hprt the following primers were used: 5′-GACGTACAGCCCAAAATGG-3′ (forward) and 5′-AAACAAAGTCTGGCTGTATCCCAA-3′ (reverse). The probe for Hprt was 5′-VIC-AAGCTCTGCGTGTAGGAAA-AGGACCTTCGTAMRA-3′. Primers and probes specific for mouse Wfs1 gene exons 7 and 8 were ordered from PE Applied Biosystems as Assay-On-Demand ( assay no. Mm01220326-m1). Wfs1 expression level was determined relative to the housekeeper gene using the comparative ΔC<sub>T</sub> method [10]. Every reaction was performed in four parallel samples to minimize the effect of technical errors. Wfs1 mRNA level in experimentally naïve Bl6 animals was taken as a reference (defined as 1) and the mRNA level in other groups were expressed as fold changes. The results are expressed as mean values ± S.E.M. The significance of the effect of cat odor exposure on the behavior and Wfs1 gene expression was analyzed using two-way analysis of variance (strain and exposure as independent variables). The effect of cat odor exposure on gene expression inside the strain was analyzed by means of one-way ANOVA: Post hoc comparisons between individual groups were performed with Tukey HSD test in gene expression studies and Newman–Keuls test in the behavioral experiment using Statistica for Windows software (Statsoft, USA).

Behavioral recordings expectedly showed that in the presence of control cloth, Bl6 mice exhibited significantly higher exploratory activity than 129Sv mice (Table 1). Nearly all measured parameters of exploratory behavior were significantly higher in Bl6 mice (Newman–Keuls test, p < 0.05). Only time spent in contact with the control cloth did not differ between Bl6 and 129Sv mice. The exposure to cat odor suppressed exploratory behavior in Bl6 mice as evidenced by reduced horizontal and vertical exploratory activity and digging behavior. However, cat odor exposure did not change the number and duration of cloth contacts in Bl6 mice. By contrast, cat odor exposure had no effect on the exploratory activity of 129Sv strain mice and tended to increase the number and duration of cloth contacts. Two-way ANOVA revealed a significant strain effect for the number of contacts with cloth [F<sub>1,63</sub> = 65.7, p < 0.001 (strain); F<sub>1,63</sub> = 1.48, p = 0.23 (expo-
The effect of cat odor exposure on the behavior of 129Sv and C57Bl/6 mice

### Behavioral parameters

<table>
<thead>
<tr>
<th></th>
<th>129Sv clean cloth</th>
<th>129Sv cat odor</th>
<th>C57Bl/6 clean cloth</th>
<th>C57Bl/6 cat odor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of contacts with the cloth</td>
<td>18 ± 2.8</td>
<td>25 ± 2.5</td>
<td>48 ± 3.7†</td>
<td>49 ± 4.1</td>
</tr>
<tr>
<td>Duration of cloth contact (s)</td>
<td>462 ± 122</td>
<td>683 ± 96†</td>
<td>360 ± 33</td>
<td>364 ± 34</td>
</tr>
<tr>
<td>Number of transitions</td>
<td>20 ± 1.6</td>
<td>25 ± 1.4</td>
<td>43 ± 3.4*</td>
<td>30 ± 1.8†</td>
</tr>
<tr>
<td>Number of rearings</td>
<td>24 ± 5.2</td>
<td>26 ± 3.5</td>
<td>96 ± 5.2*</td>
<td>79 ± 3.3†</td>
</tr>
<tr>
<td>Number of digging</td>
<td>6.3 ± 1.1</td>
<td>5.8 ± 0.6</td>
<td>21 ± 2.1*</td>
<td>12 ± 1.1†</td>
</tr>
<tr>
<td>Duration of digging (s)</td>
<td>10 ± 2.3</td>
<td>11 ± 1.5</td>
<td>38 ± 4.5*</td>
<td>21 ± 3.1†</td>
</tr>
<tr>
<td>Number of burying</td>
<td>3.6 ± 1.0</td>
<td>2.2 ± 0.5</td>
<td>7.8 ± 0.9*</td>
<td>10.2 ± 0.9†</td>
</tr>
<tr>
<td>Duration of burying (s)</td>
<td>4 ± 1.2</td>
<td>3 ± 1.1</td>
<td>16 ± 2.6*</td>
<td>20 ± 2.2</td>
</tr>
</tbody>
</table>

Mean values ± S.E.M. are presented in the table. Number of mice in each group was 16–17.

† $p < 0.05$ (compared with 129Sv clean cloth group, Newman–Keuls test after the significant two-way ANOVA).

‡ $p < 0.05$ (compared with the respective clean cloth group).

§ $p < 0.05$ (compared with C57Bl/6 mice exposed to cat odor).

Contrary to our expectations, 129Sv mice spent significantly longer time in contact with the cat odor impregnated cloth than individuals from Bl6 strain (Table 1). Two-way ANOVA established significant strain as well as strain and exposure interaction effects for the following parameters of exploratory behavior: number of transitions [$F_{1,63} = 43.3, p < 0.001$ (strain); $F_{1,63} = 3.39, p = 0.07$ (exposure); $F_{1,63} = 16.5, p < 0.001$ (strain × exposure)], number of rearings [$F_{1,63} = 206.0, p < 0.001$ (strain); $F_{1,63} = 2.68, p = 0.11$ (exposure); $F_{1,63} = 4.29, p < 0.05$ (strain × exposure)], number of digging [$F_{1,63} = 56.9, p < 0.001$ (strain); $F_{1,63} = 11.9, p = 0.001$ (exposure); $F_{1,63} = 10.1, p < 0.01$ (strain × exposure)], duration of digging [$F_{1,63} = 37.3, p < 0.001$ (strain); $F_{1,63} = 6.41, p = 0.01$ (exposure); $F_{1,63} = 9.0, p < 0.01$ (strain × exposure)] and number of burying [$F_{1,63} = 55.0, p < 0.001$ (strain); $F_{1,63} = 0.48, p = 0.49$ (exposure); $F_{1,63} = 5.53, p < 0.05$ (strain × exposure)]. For time spent in burying, only strain effect was established [$F_{1,63} = 57.8, p < 0.001$ (strain); $F_{1,63} = 1.02, p = 0.32$ (exposure); $F_{1,63} = 1.64, p = 0.21$ (strain × exposure)]. Post hoc analysis demonstrated that cat odor exposure suppressed exploratory behavior in Bl6 mice, but not in 129Sv strain (Table 1).

The expression level of Wfs1 gene did not differ significantly in the corresponding brain structures of Bl6 and 129Sv mice not exposed to cat odor (Table 2). The ranking order of Wfs1 mRNA expression level in different brain structures was also rather similar in both strains. In Bl6 mice, the expression ratio between the mesolimbic area, temporal lobe and prefrontal cortex was 1:0.93:0.43. In 129Sv strain, the respective values were 1:0.95:0.57. After cat odor exposure, significant differences in Wfs1 gene expression were established between Bl6 and 129Sv strains in the mesolimbic area and temporal lobe (Table 2). Two-way ANOVA (strain and exposure as independent factors) revealed both strain and strain × exposure effects in the mesolimbic area [$F_{1,42} = 8.97, p < 0.01$ (strain); $F_{2,42} = 0.55, p = 0.58$ (exposure); $F_{2,42} = 3.68, p < 0.05$ (strain × exposure)] and strain × exposure effect in the temporal lobe [$F_{1,42} = 2.61, p = 0.11$ (strain); $F_{2,42} = 0.13, p = 0.88$ (exposure); $F_{2,42} = 4.97, p < 0.01$ (strain × exposure)]. Further analysis demonstrated that the established differences were mostly due to changes in the gene expression in Bl6 mice, but not in 129Sv strain. One-way ANOVA demonstrated a significant inhibitory effect of cat odor on the expression of Wfs1 gene in the temporal lobe ($F_{2,21} = 3.72, p < 0.05$) and mesolimbic area ($F_{2,21} = 3.53, p < 0.05$) of Bl6 animals. The subsequent use of Tukey HSD test demonstrated that the effect of cat odor exposure was significant ($p < 0.05$) if compared to naive and clean cloth exposed mice in the temporal lobe, and to naive animals in the mesolimbic area (Table 2). In the prefrontal cortex only strain effect was established ($F_{1,42} = 8.95, p < 0.01$ (strain); $F_{2,42} = 0.55, p = 0.58$ (exposure); $F_{2,42} = 0.53, p = 0.59$ (strain × exposure)]. However, the post hoc test did not establish any statistically significant differences between the groups (Table 2).

The present study confirms previously reported differences in the exploratory behavior between Bl6 and 129Sv strains [12,16] with Bl6 mice being significantly more active than 129Sv strain. Cat odor exposure induced an anxiety-like state in Bl6 mice by reducing horizontal and vertical exploratory activity and digging.
behavior. Additionally, Bl6 mice made more attempts to cover the cat odor impregnated cloth with bedding than the control cloth. The established changes in anxiety of Bl6 mice are in agreement with the findings of Belzung et al. [4] demonstrating that Bl6 belongs to the mouse strains that respond to cat odor exposure with increased anxiety. In contrast, cat odor exposure had no effect on the exploratory activity of 129Sv mice, but tended to increase time spent in contact with the cloth impregnated with predator odor. These results suggest that 129Sv mice do not respond to cat odor with increased anxiety-like behavior. It has been noted, and this is probably not a mere coincidence, that the outcome of genetic manipulations is also different in Bl6 and 129Sv mice. For example, genetic invalidation of serotonin transporter gene induced anxiety-like state in Bl6 mice, but had no observable effect on the behavior in 129Sv strain [7]. Thus, our results lend further support to the notion that Bl6 and 129Sv mouse strains display different anxiety-related behavioral profiles. Previously, a cat-odor-induced up-regulation of Wfs1 mRNA in the amygdaloid area of male Wistar rats has been reported [9]. In the present study, we identified significant differences in brain Wfs1 mRNA levels between Bl6 and 129Sv strains after cat odor exposure. Predator odor reduced Wfs1 gene expression level in the mesolimbic area and temporal lobe of Bl6 mice, whereas in 129Sv strain an opposite tendency was observed. Wfs1 expression level was 2.1-fold and 1.7-fold higher in the mesolimbic area and temporal lobe of cat odor-exposed 129Sv mice than corresponding Bl6 mice. Consequently, changes in the expression of Wfs1 gene in the mesolimbic area and temporal lobe, both of which are involved in the regulation of exploratory behavior, are apparently in relation to the behavioral changes induced by cat odor exposure in these two strains. Dynamics of Wfs1 gene expression and exploratory behavior observed in the present experiment suggest that the down-regulation of Wfs1 gene in Bl6 mice might be related to increased anxiety. Further studies are needed to test the robustness and possible causal relationship of this finding.

Acknowledgment

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