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Effects of LPS and serotonergic drugs on hygienic behavior in mice

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ABSTRACT

Hygienic self-grooming is a behavioral adaptation for removing litter particles and pathogenic agents from animal fur and skin. We studied contribution of brain serotonin system into mechanisms regulating hygienic behavior in intact mice and mice with LPS(lipopolysaccharide)-induced sickness. A spot of fluorescent dye was applied on the back of a mouse, and the decrease in its fluorescence served as an index of fur cleaning efficiency estimated using original classifier algorithm. Agonist of 5-HT_{1A} receptor (8-OH-DPAT) or 5-$HT_{2A/2C}$ receptor (DOI) attenuated fur cleaning at a dose of 1 mg/kg but not of 0.2 mg/kg. MAO-A inhibitor clorgyline decreased hygienic self-grooming at a dose of 10 but not of 5 mg/kg. SSRI paroxetine had no effect while fluoxetine diminished hygienic behavior at the higher dose used (20 mg/kg). Inhibitory effect of LPS treatment (50 μg/kg) on fur cleaning was not altered by administration of p-MPPI (5-HT_{1A} receptor antagonist, 1 mg/kg) or DOI (1 mg/kg) while 8-OH-DPAT (1 mg/kg) produced additive effect. The results suggest the involvement of 5-HT_{1A} and 5-$HT_{2A/2C}$ brain serotonin receptors and MAO-A in the inhibition of hygienic behavior in mice. However, LPS-induced depression of fur cleaning appeared to be mediated via different mechanisms and enhanced by 5-HT_{1A} receptor activation.

1. Introduction

Body care (hygienic) behavior in most animal species is a natural adaptation aimed at removing litter particles, pathogenic microbes, and parasites from animal fur and skin (Hart, 1990). It also serves other functions: attracting mates, improving predation or avoidance of predators (via removal of odors), thermoregulation, stimulation of pheromone release, self-stimulation, increasing or decreasing arousal, and decreasing irritation. Many of these functions overlap within the same behavioral action (Fentress, 1988; File et al., 1988; Sachs, 1988; Spruijt et al., 1992). Body cleaning is a sequence of body movements (self-grooming), i.e. licking the paws, washing movements over the head, fur licking, and genital cleaning (Berridge, 1990; Hart and Pryor, 2004). Hygienic behavior also serves as an indicator of animal health. A healthy rat spends about 30–50% of its waking time for the body care, and its fur is usually neat and tidy. On the contrary, the pelage of a sick animal is dirty and oily due to the lack of grooming (Hart, 1988).

Although hygienic self-grooming is very important for animal welfare and survival, mechanisms regulating this type of behavior remain poorly investigated due to the absence of direct and objective methods estimating fur cleaning efficiency. Nowadays, hygienic behavior is assessed by indirect indices only, usually by number and duration of grooming bouts or by numerical score of the state of fur coat in some studies (Yalcin et al., 2005; Piato et al., 2008; Vancassel et al., 2008).

Recently we have developed a new technique which allows measuring hygienic component of self-grooming, i.e. ability to clean fur in response to its "dirtiness", directly (Kulikov et al., 2010a). According to this method, a spot of fluorescent dye is applied on animal's caudal part of a back, and a decrease in the spot fluorescence serves as an index of fur cleaning efficiency. Fluorescence level is detected using original classifier algorithm implemented in the ColorScan software. In the previous study, we found that the efficiency of fur cleaning in mice was suppressed substantially by administration of lipopolysaccharide (LPS) (Kulikov et al., 2010a). In laboratory, LPS is widely used to induce sickness, i.e. complex of alterations in behavior and physiological functions (fever, reduced food intake, body weight, social investigation, locomotion, sucrose preference, etc.) (Dantzer, 2001). However, detailed mechanisms causing sickness and its certain components remain unclear. Prospective direction in this respect is investigation of mechanisms mediating effects of LPS on fur cleaning efficiency in mice.

It is well-known that LPS causes myeloid cells to synthesize cytokines such as interleukin-1, interleukin-6, and tumor necrosis factor-α, which then stimulate the various systems responsible for the acute phase response (Hart, 1988). However, the CNS mechanisms that control hygienic self-grooming and are modulated by LPS-induced cytokine response are not clear. An extensive literature on grooming behavior proposes the involvement of multiple neurotransmitters and

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brain regions in control of this type of behavior (Bressers et al., 1995, 1998; Cromwell and Berridge, 1996; Drago et al., 1999; Lumley et al., 2001). Serotonin attracts particular attention in respect of mediating the suppression of hygienic behavior during acute phase response since the agonists of serotonin receptors are known to inhibit spontaneous, prandial, and waterspray-induced self-grooming in rats (Frambes et al., 1990; Halford et al., 1997; Kennett et al., 1997; Carey et al., 2008; Hartley and Montgomery, 2008). On the other hand, LPS activates central serotonin turnover (Dunn, 1992; Linthorst et al., 1996; Lacosta et al., 1999; Pitychoutis et al., 2009) and increases expression of 5-HT_{2A} serotonin receptor in midbrain and decreases expression of 5-HT_{1A} serotonin receptor in cortex in mice (Kulikov et al., 2010b). Moreover, a significant role of brain serotonin system was shown for anorexia that is another behavioral phenomenon observed in a few hours after LPS administration (Hrupka and Langhans, 2001; von Meyenburg et al., 2003; Langhans, 2007; Asarian and Langhans, 2010).

The present study was aimed to estimate the effects of various doses of LPS and some widely used serotonergic drugs (SSRIs, MAO-AI, 5-HT_{1A} and 5-$HT_{2A/2C}$ receptor agonists and 5-HT_{1A} receptor antagonist) on hygienic self-grooming using novel assay for efficiency of fur cleaning in mice.

2. Materials and methods

2.1. Experimental animals

In the study we used mice of C57BL6/J inbred strain since they had demonstrated high rate of fluorescent spot removing in previous studies (Kulikov et al., 2010a). All experiments were performed on adult mouse males weighing 25–30 g. C57BL6/J strain was maintained by brother–sister inbreeding for at least 50 generations at the Institute of Cytology and Genetics SB RAS (Novosibirsk, Russia). After weaning mice were separated by sex and kept by 10 per cage (40×25×15 cm) until the age of 3–4 months under standard conditions (temperature: 18–22 °C, relative humidity: 50–60%, standard food and water ad libitum). Two days before the experiment, animals were isolated in cages of the same size to eliminate group effects. Experimental sessions were conducted between 12:00 and 15:00 h.

All experimental procedures were in compliance with the European Communities Council Directive of 24 November 1986 (86/609/EEC). All efforts were made to minimize the number of animals used and their suffering.

2.2. Drugs and drug administration

All drugs were purchased from Sigma-Aldrich Co., they were diluted in saline and injected intraperitoneally. *LPS* (Lipopolysaccharides from *Escherichia coli* 055:B5; 2, 10, 50 or 500 μg/kg) was injected 2 h prior painting (Kulikov et al., 2010a). Serotonergic drugs were administered 30 min prior applying of dye spot. The doses of serotonergic drugs that were used in the present study are within the range of those typically employed for behavioral studies: 5-HT_{1A} receptor agonist *8-OH-DPAT* — 0.2 and 1 mg/kg (Blanchard et al., 1997; Grewal et al., 1997; Popova and Amstislavskaya, 2002); a selective 5-HT_{1A} receptor antagonist 4-iodo-N-[2-[4-(methoxyphenyl)-1-piperazinyl]ethyl]-N-2-pyridinyl- benzamide (*p-MMPI*) — 1 mg/kg (Parsons et al., 1998; Griebel et al., 2000; Wang et al., 2008); 5-$HT_{2A/2C}$ receptor agonist (+/−)-1-(2,5-dimethoxy-4-iodophenyl)-2 aminopropane (*DOI*) — 0.2 and 1 mg/kg (Eison and Wright, 1992; Kouhata et al., 2001; Bishop et al., 2004); SSRI *fluoxetine* — 10 and 20 mg/kg (Dhir and Kulkarni, 2008; Enginar et al., 2008; Gomes et al., 2009; Taksande et al., 2009); SSRI *paroxetine* — 5 and 10 mg/kg (Guzzetti et al., 2008; Taksande et al., 2009; Umathe et al., 2009; Weber et al., 2009); irreversible MAO-A inhibitor *clorgyline* — 5 and 10 mg/kg (Cohen et al., 1999; Popova et al., 2000). Saline was injected as a control. In the series with combined treatment with LPS and serotonergic drugs, LPS at a dose of 50 μg/kg (for LPS-treated groups) or saline (for the rest groups) was injected 2 h prior painting while serotonergic drugs or saline were injected 30 min prior painting (1.5 h after the first injection).

2.3. Fluorescent spot application and scanning

To compare differences in the fur cleaning dynamics, a spot of green fluorescent dye (about 3 cm^2 in size) was applied on the animal's caudal part of a back using a standard marker (Text Plus, Centropen a.s., Dačice, Czech Republic). A mouse was taken from its home cage, held still by the experimenter (holding it by the scruff of the neck and tip of the tail) under the objective of a digital camera (Olympus C-770, Olympus Corporation, Tokyo, Japan) under blue light (450 nm) which was harmless to the eyes, and scanned. Then the mouse was placed back in the home cage. The spot was rescanned 1 h later.

2.4. Measurement of spot fluorescence intensity

We have developed the ColorScan software (www.ethostudio.com/colorscan/) to measure spot intensity using original classifier algorithm based on proximity to green color (Kulikov et al., 2010a). The efficiency of fur cleaning was calculated as the ratio (%) of the difference between the intensity of spot fluorescence at the selected time point (1 h) and the initial value to the initial value.

$$E_t = (x_o - x_t) * 100 / x_o$$

where E_t represents the efficiency of cleaning for t period of time, and x_o and x_t represent the spot intensities at their initial application and at the t time point, respectively. In other words, this index corresponds to the amount of dye cleaned by a mouse (%) of the total amount of dye (assumed as 100%) applied on its fur.

2.5. The open field test

The open field test was held in order to assess correlation between inhibitory effects of LPS on hygienic self-grooming and general locomotion. The test was carried out in 1 h after the test for fur cleaning efficiency (mice rested for 1 h in their home cages). The animals were tested in a round (40 cm in diameter) arena. The plastic walls were 25 cm high. The floor of the arena was made of mat and semitransparent plastic. The arena was placed on the mount at 40 cm above two halogen lamps of 12 W each. The light from the lamps diffused by the semitransparent floor was transmitted through the arena to the objective of digital camera (Panasonic) placed at 80 cm above the arena. A mouse was placed at a wall of the arena, and its position and movements were tracked for 5 min. The arena was carefully cleaned after each test. The video stream from the camera was analyzed frame-by-frame using the original EthoStudio software (Kulikov et al., 2008). Distance travelled (cm) of a mouse was detected automatically while number of rearings was measured by an observer blind to experimental design.

2.6. Statistics

The data were presented as mean ± SEM and compared using one-way ANOVA followed by Newman–Keuls post-hoc comparison. To evaluate the contribution of the 5-HT_{1A} or 5-HT_{2A} serotonin receptor activity to the effect of LPS on hygienic behavior two-way ANOVA followed by Newman–Keuls post-hoc comparison was used. The independent variables were LPS treatment (controls, LPS) and administration of serotonergic drug (controls, DOI or 8-OH-DPAT or p-MPPI). Correlation between inhibitory effects of LPS on hygienic self-grooming and general locomotion was assessed using simple linear correlation for means of the groups.

3. Results

LPS administration affected fur cleaning dramatically ($F(4,47) = 24.7$, $p<0.001$). All doses used, except for the dose of 2 μg/kg, were effective. Moreover, a dose of 50 or 500 μg/kg attenuated fur cleaning for greater extent than a dose of 10 μg/kg did ($p<0.001$). At the same time, the effects of LPS administration at doses of 50 and 500 μg/kg did not differ significantly (Fig. 1). For the further study of interaction between LPS and serotonergic drugs we used the dose of 50 μg/kg. It exerted substantial decrease in fur cleaning and appeared to be maximally effective since a dose 10 times as much as this one had similar effect on fur cleaning.

LPS also inhibited general locomotion. A significant reduction in horizontal ($F(4,44) = 26.6$, $p<0.001$) or vertical ($F(4,44) = 10.7$, $p<0.001$) locomotor activity was observed in LPS-treated mice (Table 1). There was a significant positive correlation between LPS-evoked decrease in hygienic self-grooming and horizontal ($r = 0.92$, $p<0.05$) or vertical ($r = 0.89$, $p<0.05$) locomotor activity.

A selective agonist of 5-HT_{1A} serotonin receptor 8-OH-DPAT suppressed fur cleaning in mice ($F(2,24) = 15.3$, $p<0.001$). However, the dose of 0.2 mg/kg was ineffective while the dose of 1 mg/kg reduced hygienic self-grooming dramatically (Fig. 2a). Similarly, an agonist of 5-$HT_{2A/2C}$ serotonin receptor DOI decreased hygienic cleaning significantly ($F(2,24) = 9.3$, $p<0.01$) with the dose of 1 mg/kg ($p<0.01$) but not 0.2 mg/kg (Fig. 2a).

The effect of acute administration of SSRI fluoxetine ($F(2,18) = 10.3$, $p<0.01$) but not paroxetine ($F(2,21)<1$) on the cleaning of dye spot applied on the fur of a mouse was revealed. Noteworthy, fluoxetine reduced efficiency of fur cleaning only when a dose of 20 mg/kg (68.9 ± 4.8% compared to 95.9 ± 2.3% in control group, $p<0.01$) but not of 10 mg/kg (89.3 ± 5.4%, $p>0.05$) was used. Paroxetine failed to affect hygienic self-grooming with all doses studied (93.0 ± 4.9% or 99.5 ± 0.2% in the groups treated with 5 or 10 mg/kg of paroxetine compared with 92.2 ± 5.2% in control group, $p>0.05$). Since fluoxetine treatment with high doses is known to inhibit MAO-A, we examined the effect of a selective MAO-A inhibitor clorgyline on cleaning behavior. Indeed, clorgyline was shown to diminish fur cleaning in mice ($F(2,18) = 9.3$, $p<0.01$). The drug attenuated efficiency of fur cleaning only when the dose of 10 mg/kg was used (63.5 ± 11.5% compared to 99.9 ± 0.1% in control group, $p<0.01$) while the dose of 5 mg/kg was ineffective (97.7 ± 1.4%, $p>0.05$).

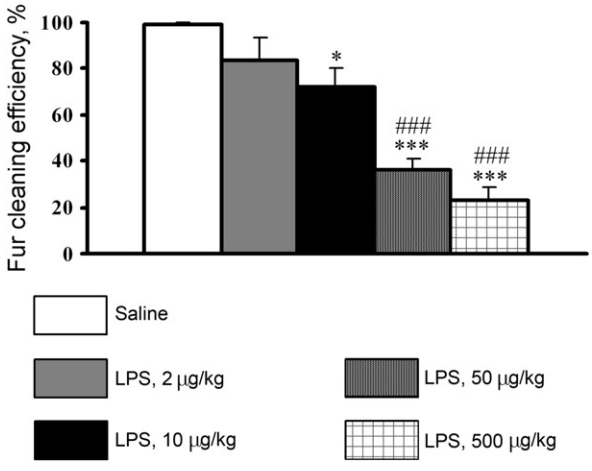

Fig. 1. Dosage effects of LPS on the efficiency of fur cleaning in C57BL6 male mice. Magnitude of the efficiency of fur cleaning was calculated as the ratio (%) of the difference between the intensity of spot fluorescence at selected time point (1 h) and the initial value to the initial value, this index corresponds to the amount of dye cleaned by a mouse (%) of the total amount of dye (assumed as 100%) applied on its fur. Each bar and vertical line represent the mean ± SEM of the values obtained in an independent group of animals ($n = 10$–12 per group). Statistically significant differences: *$p<0.05$, ***$p<0.001$ vs. control group treated with saline; ###$p<0.001$ vs. group treated with LPS at a dose of 10 μg/kg (one-way ANOVA followed by post-hoc Newman–Keuls test).

Table 1
Dosage effects of LPS administered intraperitoneally on the indices of locomotor activity in the open field test in C57BL6 mice.

Dose	Horizontal locomotor activity (distance travelled, cm)	Vertical locomotor activity (no. of rearings)
Control, 0 μg/kg (saline)	690.6 ± 54.9	16.3 ± 3.6
2 μg/kg	502.4 ± 47.0** ###	6.8 ± 1.9**
10 μg/kg	443.0 ± 17.6*** ###	5.0 ± 1.2**
50 μg/kg	392.7 ± 33.8*** ##	1.5 ± 0.5***
500 μg/kg	143.4 ± 13.1***	0.22 ± 0.15***

Data are presented as the mean ± SEM of the values obtained in an independent group of animals ($n = 9$–10 per group). Statistically significant differences: **$p<0.01$, ***$p<0.001$ vs. control group treated with saline; ##$p<0.01$, ###$p<0.001$ vs. group treated with LPS at a dose of 500 μg/kg (one-way ANOVA followed by post-hoc Newman–Keuls test).

Significant influence of LPS administration ($F(1,52) = 8.4$, $p<0.01$) and the interaction of the factors of LPS and DOI injection ($F(1,52) = 10.3$, $p<0.01$) on the efficiency of fur cleaning was demonstrated. Like in the first series, LPS injected at a dose of 50 μg/kg decreased fur cleaning significantly ($p<0.001$). The effect of LPS was very similar to that observed after the injection of DOI. However, combined treatment with LPS and DOI decreased efficiency of fur cleaning to the level observed after injection of each compound alone (Fig. 3).

Significant influence on the efficiency of fur cleaning of LPS ($F(1,23) = 12.2$, $p<0.01$) or 8-OH-DPAT ($F(1,23) = 16.0$, $p<0.001$) injection but not interaction of the factors ($F(1,23)<1$) was revealed. The effect of LPS was very similar to that observed after the injection of 8-OH-DPAT: LPS-treated mice removed 68.5 ± 10.0% of fluorescent dye and 8-OH-DPAT-given mice — 64.0 ± 7.6% ($p>0.05$). Nevertheless, combined treatment with LPS and 8-OH-DPAT was even more effective than the treatment with each compound alone ($p<0.05$) suggesting an additive effect of the drugs (Fig. 3).

To evaluate the contribution of 5-HT_{1A} serotonin receptor activity to the effect of LPS on hygienic behavior, the effects of combined treatment with LPS and p-MPPI (a selective 5-HT_{1A} receptor antagonist) were studied. Significant influence of LPS injection ($F(1,35) = 16.8$, $p<0.001$) on the efficiency of fur cleaning was found whereas effects of p-MPPI injection ($F(1,35)<1$) or interaction of the factors ($F(1,35)<1$) were insignificant. While LPS inhibited fur cleaning substantially ($p<0.05$), p-MPPI had no effect per se and failed to block LPS effect (Fig. 3).

4. Discussion

Recently, we have proposed a new technique which allows measuring efficiency of fur cleaning in mice (Kulikov et al., 2010a). Activation of immune system due to an acute LPS administration induces sickness behavior at 2–6 h (Frenois et al., 2007). We showed in the present study that acute LPS treatment produced a dose-dependent decrease in fur cleaning in C57BL6 mice. This finding is consistent with LPS-induced reduction of grooming bouts observed by other authors (Yirmiya et al., 1994; Hollis et al., 2006). The decrease of the cleaning efficiency can be proposed as a valuable index of sickness.

We studied the effects of some serotonergic drugs that regulate functioning of brain serotonin system on hygienic behavior and possible involvement of serotonergic mechanisms in the inhibiting effect of LPS on fur cleaning.

Our results did not evidence significant role of serotonin reuptake mechanism in the inhibition of fur cleaning efficiency in mice. Indeed, SSRI paroxetine failed to alter high level of fur cleaning efficiency in C57BL6 mice at all doses studied while another SSRI fluoxetine reduced fur cleaning significantly only when a dose of 20 mg/kg was used. However, the effect of fluoxetine seems to be mediated via another mechanism. Previously, treatment with high doses of

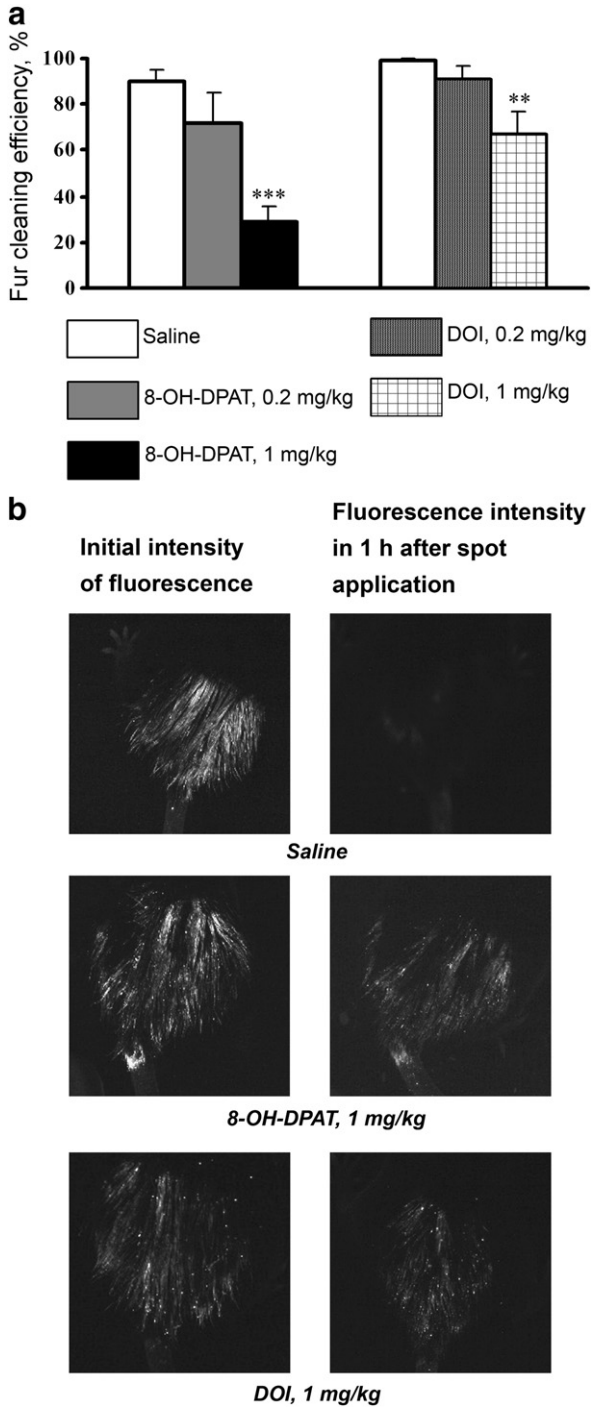

Fig. 2. Dosage effects of the agonist of 5-HT_{1A} receptors (8-OH-DPAT) or 5-$HT_{2A/2C}$ receptors (DOI) on the efficiency of fur cleaning in C57BL6 male mice. *a* — magnitude of the efficiency of fur cleaning which was calculated as the ratio (%) of the difference between the intensity of spot fluorescence at selected time point (1 h) and the initial value to the initial value, this index corresponds to the amount of dye cleaned by a mouse (%) of the total amount of dye (assumed as 100%) applied on its fur. Each bar and vertical line represent the mean ± SEM of the values obtained in an independent group of animals ($n = 8$–10 per group). Statistically significant differences with respect to the control group treated with saline: $^{**}p<0.01$, $^{***}p<0.001$ (one-way ANOVA followed by post-hoc Newman–Keuls test). *b* — photographs of the green fluorescent spot applied on an animal's back: before and in 1 h after administration of saline or 8-OH-DPAT (1 mg/kg) or DOI (1 mg/kg).

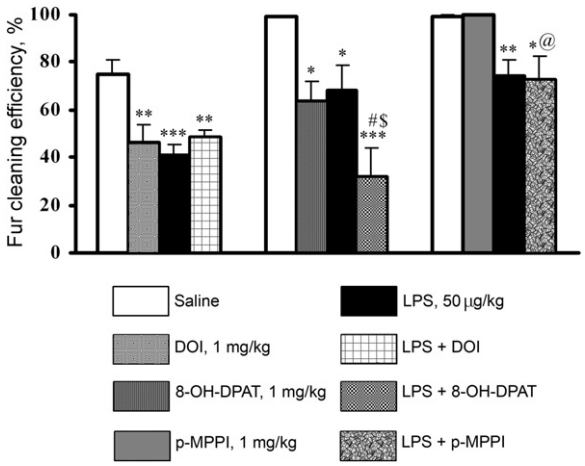

Fig. 3. Effects of acute treatment with LPS and co-administration of LPS with DOI (5-$HT_{2A/2C}$ receptor agonist) or 8-OH-DPAT (5-HT_{1A} receptor agonist) or p-MPPI (selective 5-HT_{1A} receptor antagonist) on the efficiency of fur cleaning in C57BL6 male mice. Magnitude of the efficiency of fur cleaning was calculated as the ratio (%) of the difference between the intensity of spot fluorescence at selected time point (1 h) and the initial value to the initial value, this index corresponds to the amount of dye cleaned by a mouse (%) of the total amount of dye (assumed as 100%) applied on its fur. Each bar and vertical line represent the mean ± SEM of the values obtained in an independent group of animals ($n = 6$–7 per group for LPS + 8-OH-DPAT series; $n = 8$–11 per group for LPS + p-MPPI series; $n = 14$ per group for LPS + DOI series). Statistically significant differences: $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ vs. saline-treated group; $\#p<0.05$ vs. LPS-treated group; $\$p<0.05$ vs. 8-OH-DPAT-treated group; $@p<0.05$ vs. p-MPPI-treated group (two-way ANOVA followed by post-hoc Newman–Keuls test).

fluoxetine was shown to inhibit MAO-A (Mukherjee and Yang, 1999). We suggested that fluoxetine might suppress hygienic self-grooming via blockade of MAO-A activity. Hence, we examined the effect of a selective MAO-A inhibitor clorgyline and confirmed that inhibition of MAO-A diminished fur cleaning in mice. Since MAO-A enzyme metabolizes other brain monoamines in addition to serotonin, the involvement of other monoaminergic systems of brain in control of hygienic self-grooming can be supposed.

Activation of presynaptic 5-HT_{1A} receptors by 5-HT_{1A} agonists is one of the most potent mechanisms regulating function of serotoninergic neuron. It causes inhibition of 5-HT cell firing, synthesis, and release in forebrain areas (Pineyro and Blier, 1999). This study showed that acute administration of a selective agonist of 5-HT_{1A} receptor (8-OH-DPAT) interrupted fur cleaning in mice. However, this effect was observed when the higher dose (1 mg/kg) of the drug was used. Activation of presynaptic 5-HT_{1A} receptor does not appear to play crucial role in depression of fur cleaning behavior, since much lower dose (10 μg/kg, i.v.) of 8-OH-DPAT maximally inhibits raphe neurone firing (Crespi et al., 1990). Moreover, serotonin reuptake blockade which did not exert profound effect on hygienic self-grooming leads to activation of presynaptic 5-HT_{1A} receptors in midbrain (Pineyro and Blier, 1999). Thus, inhibiting effect of 8-OH-DPAT on fur cleaning should be attributed to activation of postsynaptic 5-HT_{1A} receptors.

Earlier, it was demonstrated that 8-OH-DPAT injected both peripherally or directly into the median raphe nucleus attenuated LPS-induced anorexia in rats (Hrupka and Langhans, 2001; von Meyenburg et al., 2003). On the contrary, in the present study 8-OH-DPAT injection exerted similar inhibiting effect on fur cleaning as LPS did. Noteworthy, an additive effect was observed when the drugs were administered together. Moreover, blockade of 5-HT_{1A} receptors by p-MPPI had no effect on LPS-induced alterations in hygienic self-grooming. We can conclude that LPS effects on feeding and hygienic self-grooming are conducted via different mechanisms. Thus, 5-HT_{1A} receptor mechanisms do not seem to mediate the effects of LPS on hygienic behavior.

LPS and 5-$HT_{2A/2C}$ receptor agonist DOI had antagonistic effects in vivo and in vitro studies. Acute treatment with LPS inhibited 5-HT_{2A} receptor-mediated behavior (DOI-induced wet dog shakes) in rats (Kouhata et al., 2001) while DOI produced a dose-dependent suppression of the LPS-induced nitrite levels in glioma cells (Miller et al., 1997). Surprisingly, both drugs decreased efficiency of fur cleaning in mice. The

rate of inhibition of fur cleaning by DOI was very similar to LPS. Hence, it was hard to estimate whether LPS blocked DOI-induced inhibition of hygienic behavior, since it reduced efficiency of fur cleaning per se. Absence of the additive effect of co-administration of the drugs suggested that LPS and DOI might use the same mechanisms to decrease hygienic self-grooming in mice. Another possible explanation is the negative effect of LPS administration on pathways activated by DOI and mediating its effects on fur cleaning.

We also measured effects of LPS administration on locomotor activity in the mice. Locomotor inhibition is a typical feature of the sickness behavior induced by LPS (Dantzer, 2001). Here, we assessed correlation between LPS-induced inhibition of fur cleaning efficiency and locomotor activity. Significant positive correlation between LPS-evoked decrease in hygienic self-grooming and horizontal and vertical locomotor activity was found. These data suggest that reduction in fur cleaning and locomotion may be regulated with the same mechanisms. However, particular common mechanisms controlling these two components of the early phase of sickness condition and their interrelations remain to be determined. At the same time, effects of serotonergic drugs on hygienic self-grooming do not seem to be associated with locomotor decrease. It was shown that 5-$HT_{2A/2C}$ agonist DOI produced increase in locomotion in C57BL6/J mice with doses 0.625–5.0 mg/kg (Halberstadt et al., 2009). Neither fluoxetine (de Angelis, 1996; Rodrigues-Filho and Takahashi, 1999) nor clorgyline (Popova et al., 2000; Villegier et al., 2006) or 8-OH-DPAT (Matsushita et al., 2005) produced significant effects on locomotor activity in mice with the doses inhibiting efficiency of hygienic self-grooming.

It should be noted that the technique for estimation of hygienic self-grooming used in our study allows measuring efficiency of the behavioral response to application of foreign substance (a dye) on animal's fur exactly. It does not allow measuring a sequence of washing movements or their duration or the initial stages of self-grooming (cleaning the muzzle, neck area, and ears). These questions were out of scope of the study.

In conclusion, the results of the study suggested the involvement of 5-HT_{1A} and 5-$HT_{2A/2C}$ brain serotonin receptors and MAO-A in the inhibition of hygienic self-grooming in mice while serotonin reuptake mechanism was not shown to play key role in the process. However, LPS-induced depression of fur cleaning efficiency appeared to be mediated via different mechanisms and enhanced by combined treatment with 5-HT_{1A} receptor agonist 8-OH-DPAT.

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