Introduction
Parkinson's disease (PD) is a neurodegenerative disorder characterized by the progressive degeneration of dopaminergic neurons in the substantia nigra (SN) and the presence of Lewy body inclusions in residual neurons. In recent years, increasing evidence has strongly suggested a role for inflammation in the brain in the pathogenesis of PD [1]. Bacterial endotoxin lipopolysaccharide (LPS) is one of common toxins produced by Gram (-) bacteria, including Escherichia coli. This agent can elicit a multitude of pathophysiological effects, including inflammation, macrophage activation, fever, and septic shock [2]. The blood-brain barrier can become leaky as a result of sepsis [3], allowing LPS to enter the cerebrospinal fluid. Previous studies have demonstrated that bacterial LPS exposure, that mimics Gram (-) bacterial infections, could cause a significant loss of dopamine (DA) neurons in the substantia nigra (SN) of rat. Along with DA neuron loss are the α-synuclein positive Lewy body-like inclusion formation and innate immunity dysfunction manifested by increase in number of reactive microglia, increase in pro-inflammatory cytokine levels, and blood-barrier leakage [4]. Numerous DA neurotoxins including 6-hydroxydopamine (6-OHDA) [5], rotenone [6], dieldrin [7] and paraquat [8] kill DA neurons in a variety of animal models. However, LPS exposure also displayed additive or synergetic effects with 6-OHDA (additive) or rotenone (synergistic) on DA toxicity [9]. So, these results suggested that LPS exposure might also be a risk factor of PD. Animals exposed to LPS exhibit numerous indices of neuroinflammation in the nigrostriatal pathway including life-long elevations in tumor necrosis factor alpha (TNF-α), increased numbers of activated microglia, increased levels of oxidized proteins, and reduced amounts of glutathione (GSH) [10].

The current study is designed to assess whether disordered immune responsiveness regulation via substantia nigra neuron loss is associated with LPS exposure, a risk factor for DA neuron loss.

Material and Methods
Animals
30 male Wistar rats weighing 180 ± 50 g at the start of the experiment were used. The animals were housed in a temperature- and light-controlled room (22°C, a 12-h cycle starting at 08:00 h) and were fed and allowed to drink water ad libitum. Rats were treated in accordance with the guidelines of Animal Bioethics from the Act on Animal Experimentation and Animal Health and Welfare Act from Romania and all procedures were in compliance with the European Communities Council Directive of 24 November 1986 (86/609/EEC).

Neurosurgery and drug administration
The rats were anesthetized with sodium pentobarbital (45 mg/kg b.w. i.p., Sigma). The substantia nigra was right unilateral lesioned by stereotaxic microinjections of 6-OHDA (Sigma, 8µg/4µl), administered through the Hamilton microsyringe (10µl) over 4.50 minutes. The syringe was left in place for 5 minutes after injection before being slowly removed. The rats were pretreated 30 minutes before the 6-OHDA infusion with 25 mg/kg i.p. desipramine (Sigma) to protect noradrenergic projections. Sham-operated rats received an injection of desipramine, followed by vehicle only in the substantia nigra. The following coordinates were used: 5.5 mm posterior to bregma; 2.0 mm lateral to the midline; 7.4 mm ventral to the surface of the cortex [11]. Lipopolysaccharide (LPS from Escherichia coli serotype 0111:B4, Sigma) was dissolved in pyrogen-free 0.9% NaCl to concentrations of 250 μg/kg and i.p. injected in 6-OHDA-lesioned rats for a period of 7 continuous days. In order to study the involvement of post lesion neuronal structures in these results, some immunological parameters (total serum protein, lymphocyte and antibody titer) were determined 1 week after right unilateral lesion of the substantia nigra (SN) by means of 6-hydroxydopamine and LPS exposure.

Blood sampling protocol
Blood samples were withdrawn via the Biotrol sampling catheter from 15 sham-operated and 15 LPS 6-OHDA-lesioned rats. Blood samples (0.5 ml approximately/sample) were collected in vials containing EDTA for hematological investigations. Hematological parameters were assayed by Hematology Analyzer MS9-5 VET Automatic Full Digital Cell Counter, Melet Schloesing Laboratoires - precision instruments for hematology research. To determine total serum protein and antibody level, we used the Weichselbaum method (biuret test for proteins and the technique for albumins).

Histological control
At the end of the experiment, all rats were killed with an overdose of sodium pentobarbital (100 mg/kg i.p., Sigma) followed by a transcardial infusion of 0.9% 30% sucrose/formalin solution. The brains were removed and placed in a 30% sucrose/formalin solution.

Results

The brains were frozen and cut into coronal sections (50 μm) using a freezing microtome and stained with cresyl violet for verification of the point of the syringing needle. Only experimental data from lesions correctly located in the substantia nigra were used for statistical analysis.

Statistical analysis
The results are expressed as means ± S.E.M. The results were analyzed using Student’s-t test. Values of p<0.05 were regarded as significant.

References
4. Wang S, Yan J, Lo Yuk, Carvey PM, Ling Z. Dexamethasone and serum levels of inflammatory cytokines in young adult rats prenatally exposed to the bacterial lipopolysaccharide. Brain Res. 2009; 1265: 196-204.