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Enhanced susceptibility to periodontitis in an animal model of depression: reversed by chronic treatment with the anti-depressant tianeptine

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Abstract

Objective: To test the hypothesis that the olfactory bulbectomy model of depression in rats could influence susceptibility to ligature-induced periodontitis, and that chronic treatment with the anti-depressant drug tianeptine could attenuate this effect.

Material and Methods: Tianeptine was given twice daily (10 mg/kg, i.p.) during the entire experiment, starting 29 days before induction of olfactory bulbectomy and periodontitis. Olfactory bulbectomized (OB) rats and sham-operated rats were given saline in a similar manner. Periodontal disease was assessed when the ligatures had been in place for 21 days. Two hours before decapitation, rats were injected with lipopolysaccharide (LPS;100 μg/kg, i.p.) to induce a robust immune and stress response.

Results: Compared with sham-operated controls, OB rats developed significantly more periodontal bone loss, exhibited characteristic behavioural responses in a novel open field test, and showed a decreased expression of glucocorticoid receptors (GRs) in the hippocampus. LPS provoked a significantly larger increase in circulating levels of the stress hormone corticosterone and the cytokine transformation growth factor (TGF)-1β but smaller tumour necrosis factor (TNF)-α levels. Tianeptine treatment of OB rats significantly inhibited peridodontal bone loss, normalized behavioural responses, enhanced TGF-1β levels, and abolished TNF-α decrease, but did not attenuate the increased corticosterone response and the decreased hippocampal GR expression.

Conclusions: These experimental results are consistent with an emerging literature showing that life stress, anxiety, depression, pathological grief, and poor coping behaviour may dysregulate regulatory mechanisms within the brain involved in immune regulation, and thereby alter immune responses and influence the susceptibility/resistance to inflammatory disorders.

Key words: Brain–neuroendocrine–immune interactions; cytokines; depression; LPS; periodontitis; rats

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Immune system dysregulation and concomitant increased susceptibility to periodontitis among people who have experienced negative life events and show poor coping behaviour is a significant but poorly understood problem (Breivik et al. 1996, 2005e, Breivik & Thrane 2001, Hugoson et al. 2002). Recently, it has become clear that the immune system is controlled and regulated by bi-directional brain-to-immune communicating mechanisms, whose responsiveness may be dysregulated by emotional load (stress) as well as other internal and external danger signals (Ader et al. 1995, Chrousos 1995, Breivik et al. 1996, O'Connor et al. 2000, Pavlov & Tracey 2004). The sympa-

thetic nervous system (SNS) and the hypothalamic–pituitary–adrenal (HPA) axis, which together comprise the stress response system, as well as the parasympathetic nervous system (PSNS) and the peptidergic/sensory nervous system, are among the central pathways of the brain-to-immune regulatory mechanisms (Hori et al. 1995, Turnbull & Rivier 1999, Elenkov et al. 2000, 2005, Habib et al. 2001). The stress response system is activated by all kinds of danger signals or stressors that are interpreted by the brain as threatening for the organism, including pathogenic microorganisms and emotional stress (Song 2000, Elenkov et al. 2005).

The immune-to-brain-to-immune regulatory pathways strive to skew pro-inflammatory responses towards the anti-inflammatory side, and these brain reflexes play an important role in fine tuning immune system responses to bacterial and other immunological challenges. Results from our own and other laboratories show that these overarching immunoregulatory pathways control immune system responses to antigenic challenges in a manner that plays a significant role in the development and progression of infections and inflammatory diseases, including periodontitis (Breivik et al. 2000a, b, c, 2001a, b, 2002a, b, 2005a, b, c, d, e, Eskandary & Sternberg 2002, Stephan et al. 2002, Elenkov et al. 2005). Thus, dysregulation of the stress response system may alter immune responses to microbial challenges, and may help to explain the now well-documented association between altered immune system responses, chronic infections, and stress, depression, and anxiety (Cohen 1991, Song 2000, Yang & Glaser 2000, Zorrilla et al. 2001). It also aids in understanding how environmental factors may influence susceptibility to periodontitis (Breivik et al. 1996, Breivik & Thrane 2001, Hugoson et al. 2002).

The present study was carried out to further investigate how the bi-directional communication between the brain and the immune system controls the susceptibility and progression of periodontitis. For this purpose, we combined a rat model of experimental periodontitis with one of major depression. The olfactory bulbectomized (OB) rat model is considered to be one of the best animal models of depression in terms of construct validity (Harkin et al. 2003). The OB rat model has also been found to be particularly suitable for studying the complex relationship between the brain, behaviour, the nervous and neuro-endocrine systems, and the immune system, as well as for testing the efficacy of anti-depressant and anxiolytic drugs like tianeptine, a novel drug that formerly has been shown to inhibit the development of periodontitis in high stress-responding Fischer 344 rats (Breivik et al. 2005e).

Specifically, we wanted to test whether olfactory bulbectomy influences stress and immune response to lipopolysaccharide (LPS) and, moreover, whether it modifies the susceptibility to ligature-induced periodontitis. At the same time, we wanted to measure how concomitant chronic treatment with an anti-depressant drug (tianeptine) influences the OB-induced alterations in stress and immune responses to LPS, and, consequently, susceptibility/resistance to periodontitis.

Material and Methods

Animals

Subjects were male Wistar rats weighing about 300 g at the time of ligature and olfactory bulbectomy. The rats were obtained from Möllegaard Breeding Center (Ejby, Denmark). They were included in the study after 2 weeks of acclimatization. The rats were kept in groups of three and four, and they had free access to standard rat pellets and tap water. They were maintained under a 12–24 h light/dark cycle (light on 07:00 –19:00 hours) with temperature and humidity at 22°C and 40–60%, respectively. The experiments were registered and approved by the Norwegian Experimental Animal Board.

Experimental Design

Experiment 1

This experiment was designed to test whether surgical bilateral removal of the olfactory bulbs would affect ligature-induced periodontal breakdown. The rats were randomly assigned to two groups, each consisting of 10 rats. Periodontitis was induced in all rats. Thereafter, bilateral olfactory bulbectomy was immediately performed in one group, while the others were sham-operated only. All animals were killed by decapitation 62 days after application of the ligatures and the induction of the bulbectomy/sham-operation.

Experiment 2

This experiment was a follow-up study to elucidate putative mechanisms for the results in Experiment 1. The rats in experiment 2 were randomly assigned to three groups, viz.: tianeptine-treated OB rats, saline-treated OB rats, and saline-treated control rats, each group consisting of 10 rats.

The tianeptine-treated OB rats received two daily intra-peritoneal (i.p.) injections (10 mg/kg between 08.00–09.00 and 14.00–15.00 hours), starting 29 days before the induction of periodontitis and the bulbectomy. Tianeptine sodium salt (a generous gift from Stablon; Servier, France) was dissolved in saline. Fresh solutions were made every day. The dosing was selected on the basis of the available literature. After induction of the disease and the bulbectomy, the daily injections were continued until decapitation. The last dose of tianaptine was given immediately before the LPS injection on the day of killing. The OB rats were handled in the same way, but instead of tianaptine they received the same volume of saline. The controls were treated as the OB rats, except that the olfactory bulbs were not removed. All animals were killed by decapitation 21 days after application of the ligatures and 2 h after the LPS injection.

Experimental periodontitis

All animals (experiments 1 and 2) were anaesthetized with a subcutaneous injection in the neck with Hypnorm-Dormicum (fentanyl/fluanizone, midazolam), 0.2 ml/100 g body weight. A sterile silk ligature (Ethicon Perma-hand® seide, Norderstedt, Germany) was tied around the neck of the maxillary right second molar tooth. The ligatures were left in the same position during the entire experiment and served as a retention device for oral microorganisms. At the end of the experiment, the maxillae were excised and fixed in 4% formaldehyde.

Olfactory bulbectomy

Immediately following placement of the ligatures, bilateral olfactory bulbectomy was performed as described by O'Connor and Leonard (1986). In short, the heads were shaved and a midline sagittal incision was made extending at least 1 cm rostral to bregma. The underlying

skull was penetrated, and two holes with a diameter of 2 mm were drilled 8 mm anterior to bregma and 2 mm from either side of the midline. Bilateral olfactory bulbectomy was performed by gentle aspiration into a syringe with a cannule (21G, diameter 0.8 mm) that was connected to a vacuum pump. A hole in the syringe (with the piston removed) made it possible to control suction power. Care was taken to avoid damage to the frontal cortex. The incisions were closed with a sterile polyamide ligature (Supramid, size 3/0, B. Braun Aesculap AG & CO. KG, Tuttlingen, Germany). Sham-operated control rats were treated similarly, but only their scalps were deflected and bone defects were prepared.

Behavioural testing

The open field test (experiment 2) was used to evaluate behavioural changes typically found in OB rats, including locomotor activity, explorative behaviour, and anxiety. The rats were tested on day 14 after induction of experimental periodontitis and removal of the olfactory bulbs, and seven days before decapitation. The apparatus consisted of a 100 cm × 100 cm square-shaped box, with the floor divided into 10 cm^2 squares. The walls surrounding the base were black and 40 cm high. The only illumination was provided by a 15 W bulb, positioned centrally and 50 cm above the floor. Each animal was gently placed in the centre of the apparatus and allowed to explore the arena over a 5 min period. An observer manually recorded movements and types of behaviour (grooming, rearing, and defaecation) between 9:00 and 12:00 hours Each animal was tested once, and after each testing, the apparatus was cleaned with water and bleach solution and dried.

The following parameters were evaluated:

1. Movements across square boundaries. The number of squares crossed for each animal was summarized for each animal and used as an indicator of the locomotor activity in a novel environment. This is a classical and sensitive behavioural parameter affected by bulbectomy (Zueger et al. 2005). This activity has been found to be significantly attenuated by chronic treatment with anti-depressants, but not by psychotropic drugs lacking anti-depressant activity (van der Stelt et al. 2005). Therefore, the locomotor activity was used as an indicator of major depression and anti-depressant activity.
2. Distance moved in the centre of the illuminated open field apparatus, defined as the inner middle of the arena (60 cm × 60 cm; 360 cm^2). This test is commonly used to measure anxiety, and OB rats usually spend less time in the illuminated centre of the open field apparatus than their sham-operated controls. Some anti-depressants attenuate this effect (Hallam et al. 2004).
3. Vertical activity (rearing). The extent of rearing in a new environment reflects exploratory behaviour. This activity has been found to be typically increased in OB animals, and reversed by treatment with some anti-depressants (Connor et al. 2000).
4. The time spent with self-grooming behaviour was recorded in seconds, and the number of defaecations was counted. Both measurements are used as an indicator of apprehension (Breivik et al. 2002a).

LPS challenge

The peripheral blood monocytes of humans with periodontal disease release an altered profile of immune mediators when stimulated by LPS (Fokkema et al. 2002). LPS is a potent activator of the immune system and the HPA axis, which in turn regulates or modulates various immune cell activities (Turnbull & Rivier 1999). Therefore, the animals were injected with LPS (*E.coli* serotype 0111:B4, Sigma) i.p. (100 μg/kg) 2 h before ending the experiments to assess whether the treatment regimen influenced corticosterone or cytokine responses to this inflammatory agent. After decapitation of the rats, the blood samples were collected (6–10 ml from each animal) in vacutainer tubes (10 ml without additives) and allowed to coagulate on ice for 1 h. Thereafter, the samples were centrifuged for 20 min. at 2000 × g, and the serum samples were removed, aliquoted, and stored at −20°C before analysis of corticosterone and cytokines.

Radiographic examination of periodontal bone loss

The rat maxillary jaws were placed and stabilized with dental wax on a Sidexis digital X-ray sensor, oriented with the axis of the teeth parallel to the sensor surface. The distance between the cemento-enamel junction (CEJ) and the alveolar bone (B) on mesial surfaces of the 2nd molars was displayed digitally. The examination was performed blinded. The reliability of the method has been tested earlier (Breivik et al. 2000a), and has been shown to have a standard error of the mean difference between two readings of 0.16 mm.

Serum corticosterone, tumour necrosis factor (TNF)-α, interleukin (IL)-10, and TGF-β analysis

Corticosterone, TNF-α, transforming growth factor (TGF)-1β, and interleukin (IL) − 10 are related to immune system regulation and depression (Kelly et al. 1997, Song 2000, Leonard & Song 2002). Serum corticosterone was measured with a radioimmunoassay (RIA) Coat-A-Count kit from Diagnostic Products Corporation, Los Angeles, CA, USA, catalogue Number TKRC1. The limit of detection was 5.7 ng/ml. The antibody is highly specific, with the highest cross-reactivity with 11-deoxycorticosterone of less than 2%. The serum levels of TNF-α, IL-10, and TGF-β were measured by means of enzyme-linked immunosorbant assay (ELISA) kits from R&D Systems Inc., Minneapolis, MN, USA, with catalogue numbers RAT00 for TNF-α, MB100 for TGF-1β, and R1000 for IL-10, respectively. The minimum detectable level/concentration for TNF-α is less than 12.5 pg/ml, less than 31.2 pg/ml for IL-10 and TGF-1β, and less than 62.5 pg/ml for IL-6.

Isolation of RNA from hippocampus and reverse transcriptase-polymerase chain reaction (RT-PCR) assay of mRNA for the glucocorticoid receptor

The left hippocampus from the rats in experiment 2 was surgically removed immediately after decapitation and transferred into 1 ml of RNA-later (Ambion Inc., Austin, TX, USA) for later processing. RNA was extracted using RNA-Whiz (Ambion Inc.), yielding RNA preparations of A_{260}/A_{280} of 1.7–1.8. These were DNAase treated (DNA-Free, Ambion Inc.) before use in RT-PCR analysis.

RT-PCR primers were designed from the published base-sequence of mRNA for the rat glucocorticoid-receptor

(accession no. Y12264) using the Oligo program (v.6) (Molecular Biology Insights Inc., Cadcade, CO, USA). For internal standard, the signal from primers designed from the mRNA sequence of rat ribosomal protein L27 (accession no. NM 022514) was preferred to those from rat β-actin (accession no. V01217 J00691) signal. Primers for L27 and β-actin were also designed using Oligo v.6.

RT-PCR was carried out using the Qiagen One-Step RT-PCR kit (Qiagen GmbH, Hilden, Germany). RT-PCR products were analysed by agarose-electrophoresis using Gelstar fluorescence dye (BMA Inc. Cambrex Corp., New Jersey, NJ, USA) for detection. Fluorescence-signals were quantified by using the ProExpress imager (Perkin Elmer Life and Analytic Sciences Inc., Boston, MA, USA). The RT-PCR analysis was carried out so as to ensure linearity between amount of RT-PCR product and integrated fluorescence signal.

Statistical methods

The open field data were analysed using a two-way ANOVA and the biochemical data were subjected to a three-way ANOVA, followed by Fisher's LSD post hoc multiple comparisons. The animal is used as the analytic unit, the data are expressed as mean ± SD, and α level is set at $p<0.05$.

Results

Effect of olfactory bulbectomy and tianeptine treatment on the weight of the animals

In experiment 1, there was no significant weight difference between the two groups, neither at the start of the experiment nor at the time of decapitation 62 days after ligature placement and removal of the olfactory bulbs. OB rats and sham-operated animals weighed 309.8 ± 17.7 and 320.1 ± 14.6 g, respectively, at the induction of the disease and olfactory bulbectomy, and 413.0 ± 31.4 and 406.8 ± 34.2 g, respectively, at the end of the experiments ($p>0.05$ between groups).

In experiment 2, there was no weight difference at the start of the experiment when tianaptine was administrated, i.e.: 29 days before ligature placement and bulbectomy. The rats weighed 310.8 ± 9.4 g in the tianaptine-treated OB group, 308.2 ± 11.0 g in the OB group, and 309.7 ± 8.1 g in the controls. At the time of the ligature placement and the bulbectomy, there was a tendency towards reduced weights in the tianeptine-treated animals (347.3 ± 19.5 g), while the mean weights in the OB rats (359.6 ± 24.1 g) and the controls (354.5 ± 24.1 g) were quite similar ($p>0.05$ between groups). The rats were decapitated 21 days after induction of the disease and the bulbectomy. In contrast to experiment 1 (where the animals were decapitated 62 days after the disease induction and bulbectomy), there was a significant decrease in the weights of the OB animals, and this reduction was significantly inhibited by the tianeptine treatment. At the time of killing, the tianeptine-treated OB rats weighed 374.0 ± 25.6 g, the OB animals weighed 352.7 ± 26.7 g, and the controls weighed 382.5 ± 28.3 g ($p<0.05$ between tianaptine-treated OB rats and OB rats, $p<0.01$ between OB rats and controls; $p>0.05$ between tianeptine-treated OB rats and controls).

Effect of olfactory bulbectomy and tianeptine treatment on periodontal tissue destruction

The OB animals had significantly more alveolar bone loss compared with controls in both experiment 1 and experiment 2. In experiment 1, where periodontitis was induced for 62 days, the mean bone loss was 1.06 ± 0.25 mm in the OB rats, *versus* 0.90 ± 0.13 mm in the sham-operated control rats ($p<0.01$ between groups). In experiment 2 (periodontitis for 21 days), the mean bone loss was 0.96 ± 0.09 mm in the OB rats, *versus* 0.81 ± 0.05 mm in the controls ($p<0.001$ between groups, Fig. 1). As the OB rats showed lower weight gain in experiment 2, the length of their teeth could be shorter. We therefore compared the root length of the right second molar teeth in the groups by measuring the distance between the CEJ and apex on mesial root surfaces. There was no difference between the root length in the OB rats and control rats (2.25 ± 0.07 mm in OB rats *versus* 2.26 ± 0.15 mm in the controls; $p>0.05$).

The tianeptine treatment completely abolished the enhanced periodontal

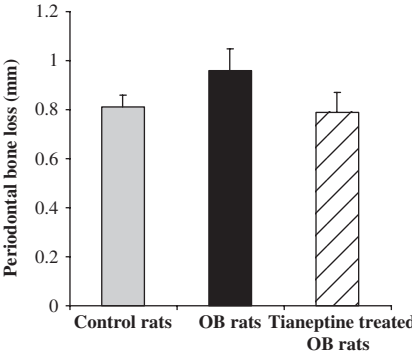

Fig. 1. The mean distance from the cemento-enamel junction to the alveolar bone crest in control rats, olfactory bulbectomized (OB) rats, and tianeptine-treated OB rats as measured on digital radiographs. $p<0.001$ between controls and OB rats, and between OB rats and tianeptine-treated OB rats.

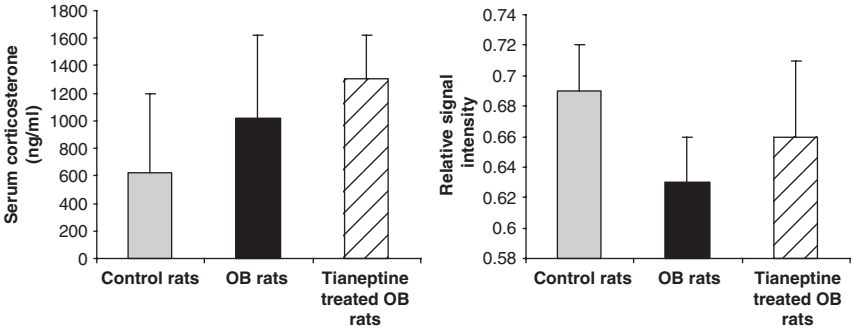

Fig. 2. Analysis of levels of corticosterone in the serum (left diagram), and relative levels of mRNA for the glucocorticoid receptor in the hippocampus (right diagram) expressed relative to the level of ribosomal protein L27 (housekeeping gene used as internal control), 2 h after intra-peritoneal injection of lipopolysaccharide (100 μg/kg) in control rats, olfactory-bulbectomized (OB) rats, and tianeptine-treated OB rats. OB rats showed significantly higher corticosterone levels compared with sham-operated controls ($p<0.05$). Tianeptine tended to increase this effect ($p>0.05$). The levels of mRNA for the glucocorticoid receptor in the hippocampus were significantly lower in OB rats compared with controls ($p=0.05$), and tianaptine tended to reduce this effect ($p>0.05$).

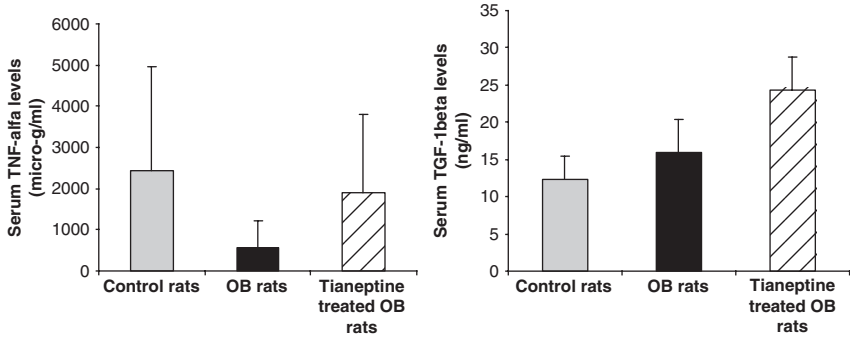

Fig. 3. Analysis of serum levels of the pro-inflammatory cytokine tumour-necrosis factor (TNF)-α (left diagram), and the anti-inflammatory cytokine transforming growth factor (TGF)-1β (right diagram) 2 h after intra-peritoneal injection of lipopolysaccharide (100 μg/kg) in control rats, olfactory-bulbectomized (OB) rats, and tianeptine-treated OB rats. OB rats demonstrated significantly lower serum levels of TNF-α ($p<0.05$), and tianaptine almost completely abolished the OB-induced TNF-α reduction ($p<0.05$). The serum levels of (TGF)-1β were, on the other hand, significantly higher in the OB rats ($p<0.05$), and tianeptine further increased this effect ($p<0.01$)

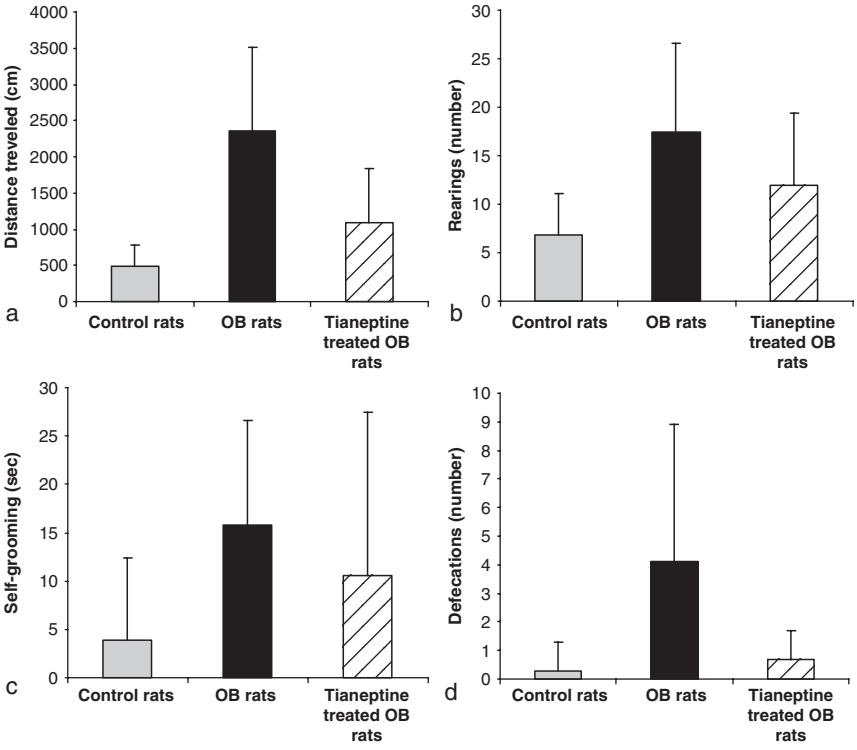

Fig. 4. Analysis of emotionality- and anxiety-related behaviour in the open field test of control rats, olfactory-bulbectomized (OB) rats, and tianeptine-treated OB rats. (a) OB rats demonstrated significantly increased locomotor activity compared with control rats ($p<0.001$) in the novel environment, and chronic tianeptine treatment significantly attenuated the OB-related hyperactivity ($p<0.01$). (b) OB rats reared significantly more than controls ($p<0.001$), and tianeptine significantly attenuated these effects ($p<0.05$). (c) Compared with controls, OB rats spent significantly more time with self-grooming ($p<0.01$). The self-grooming effect was reduced by tianeptine, but this effect was not significant ($p>0.05$). (d) OB rats showed significantly more numbers of defaecations than controls ($p<0.001$), and tianeptine significantly reduced this effect ($p<0.001$).

breakdown induced by the bulbectomy. Compared with the bone loss in the OB rats (0.96 ± 0.09 mm), the bone loss was significantly lower in OB rats treated with tianeptine (0.79 ± 0.08 mm; $p<0.001$). The bone loss was even lower than in the control rats without bulbectomy (0.81 ± 0.05 mm), but this difference was not significant ($p>0.05$; Fig. 1).

Effects of olfactory bulbectomy and tianeptine treatment on corticosterone plasma levels after LPS challenge

In experiment 1, the OB rats had significantly higher serum corticosterone levels compared with sham-operated controls (1140.4 ± 387.5 and 756.0 ± 422.7 ng/ml; $p=0.05$), which demonstrates that the basal HPA axis reactivity is enhanced in OB rats.

In experiment 2, where the animals in all three groups were exposed to an LPS challenge 2 h before killing, LPS tended to induce a strong immune and subsequent HPA axis response (Turnbull & Rivier 1999). Compared with controls (627 ± 569.4 ng/ml), we measured significantly higher serum corticosterone levels in the OB rats (1017.3 ± 606.4 nm/L; $p<0.05$; Fig. 2), demonstrating that the bulbectomy induced a stronger HPA axis responsiveness to the inflammatory LPS. The serum corticosterone levels in the tianeptine-treated OB rats (1310.7 ± 310.2 ng/ml) tended to be higher than in the OB rats (1017.3 ± 606.4 ng/ml; $p>0.05$; Fig. 2), which demonstrates that tianeptine does not inhibit the OB-induced hyper-responsiveness of the HPA axis.

Effect of olfactory bulbectomy and tianeptine treatment on expression of mRNA for the glucocorticoid receptor

The integrated fluorescence signals of the PCR products obtained with primers for the glucocorticoid-receptor were expressed as a fraction of the signals obtained using primers from ribosomal protein L27 (housekeeping gene, used as internal control). The mean ratio obtained for the glucocorticoid receptor was 0.63 ± 0.03 in the OB rats, 0.66 ± 0.05 in tianeptine-treated OB rats, and 0.69 ± 0.03 in controls (Fig. 2). The level of mRNA for the glucocorticoid-receptor in the hippocampus was significantly lower in the OB rats compared with controls ($p=0.05$), and there was a tendency towards reduced effect of the bulbectomy in the tianeptine-treated OB rats (Fig. 2).

Effect of olfactory bulbectomy and tianeptine treatment on cytokine serum levels to LPS challenge

Serum cytokine levels were only measured in rats from experiment 2. Compared with control rats (2449.9 ± 2506.3 pg/ml), OB rats exhibited significantly lower TNF-α serum levels

(561.6 ± 643.6 pg/ml) in response to the LPS challenge ($p < 0.05$; Fig. 3). Tianeptine almost completely abolished the reduction in TNF-α serum levels to the LPS challenge in the OB rats. The serum level of TNF-α was 1896.4 ± 1908.3 pg/ml in the tianeptine-treated OB rats ($p < 0.05$ between saline-treated OB rats and tianeptine-treated OB rats; Fig. 3).

On the other hand, the serum levels of TGF-1β were significantly higher in the saline-treated OB rats (15.9 ± 4.4 ng/ml) compared with saline-treated controls (12.3 ± 3.2 ng/ml; $p < 0.05$), and the treatment significantly enhanced this effect. The mean serum levels of TGF-1β in the treated animals were 24.3 ± 4.4 ng/ml ($p < 0.05$ between tianaptine-treated OB rats and saline-treated OB rats; $p < 0.01$ between tianaptine-treated OB rats and controls; Fig. 3). Also, the serum levels of IL-10 were significantly higher in OB rats compared with controls (46.9 ± 17.4 versus 33.8 ± 9.6 pg/ml; $p < 0.05$), and even higher after tianeptine (60.2 ± 36.2 pg/ml). The differences between the IL-10 serum levels in the tianaptine-treated OB rats and the saline-treated OB rats were, however, not significant ($p > 0.05$).

Effect of olfactory bulbectomy and tianeptine treatment on emotionality- and anxiety-related behaviour

In keeping with the findings from other studies (Kelly et al. 1997), OB rats were significantly more active in a new environment (the open field apparatus) than the controls. Tianeptine treatment significantly attenuated the OB-related hyperactivity. The mean distance travelled was 2355.8 ± 1150.2 cm for OB rats, 485.0 ± 291.1 cm for controls, and 1091.7 ± 739.9 cm for OB rats chronically treated with tianaptine ($p < 0.001$ between OB rats and controls, and $p < 0.01$ between OB rats and tianaptine-treated OB rats; Fig. 4a).

Despite their locomotor hyperactivity, OB rats moved less than controls in the illuminated anxiety-related centre field of the open field apparatus, and tianeptine significantly attenuated this effect (2.1% of the total distance travelled in OB rats, *versus* 15.1% in controls, and 10.2% in OB rats treated with tianeptine; $p < 0.001$ between groups). Also, the reported general increase in explorative behaviour, as measured by their rearing activity (Kelly et al. 1997, Zueger et al. 2005), was supported in this experiment. OB rats showed a significantly increased rearing activity (17.4 ± 9.2 *versus* 6.8 ± 4.3 in controls; $p < 0.001$). Chronic tianeptine treatment tended to attenuate this effect (12.0 ± 97.4; $p > 0.05$; Fig. 4b). The mean time spent in self-grooming behaviour was significantly higher in OB rats (15.8 ± 10.4 s) compared with controls (3.9 ± 8.5 s; $p < 0.01$), and there was a tendency towards reduced self-grooming in tianaptine-treated OB rats (10.6 ± 16.9 s; $p > 0.05$; Fig. 4c). The incidence of defaecation in the open field test was significantly increased in the OB rats compared with controls (4.1 ± 4.8 *versus* 0.3 ± 1.0 in controls; $p < 0.001$), and tianeptine significantly reduced this effect (0.7 ± 1.0; $p = 0.001$; Fig. 4d).

Discussion

Previous studies have demonstrated that rats responding to danger signals with strong stress responses are significantly more susceptible to experimental ligature-induced as well as spontaneously occurring periodontitis than lower stress-responding individuals (Breivik et al. 2000a, b, 2001a, b, 2002a, b, 2005a, Takada et al. 2004). Earlier, we have also found that chronic treatment with the glucocorticoid receptor antagonist and anti-depressant mifepristone (RU 486), as well as the anti-depressant tianeptine inhibits ligature-induced periodontitis in stress high-responding Fischer 344 rats (Breivik et al. 2000c, 2006).

In the present study, we have elaborated the above findings and shown that depressive OB rats develop significantly more periodontitis than sham-operated controls. Furthermore, chronic treatment with the anti-depressant drug tianeptine, which is known to reverse many of the OB-induced changes (Leonard 2001), significantly attenuated the OB-induced increased susceptibility to the disease. Compared with control rats, the OB-operated study group responded to an *in vivo* LPS challenge with reduced blood levels of TNF-α, which correlated with an increase in OB-induced serum corticosterone levels. These results are corroborated by other investigators (Connor et al. 2000), and support recent studies in humans showing that cytokine responses are abnormal in humans having major depression (O'Brian et al. 2004). Thus, as glucocorticoid hormones like corticosterone down-regulate the production and release of pro-inflammatory cytokines (Aupan et al. 1995), the OB-induced depressive mood and concomitant up-regulation of HPA axis may in part be responsible for the reduced TNF-α response to LPS. Up-regulation of the stress response system by changes in mood may also explain the increased blood levels of the anti-inflammatory cytokines TGF-1β and IL-10. This suggestion is substantiated by results from a recent study showing that exposure to a psychophysiological stressor (swim stress) increases the production of IL-10, while at the same time suppressing TNF-α after challenge with LPS (Connor et al. 2005). In that study, it was also demonstrated that the stress-induced elevation of IL-10 was mediated by activation of the SNS via β-adrenergic receptors. Thus, emotional stress seems to induce differential effects on the production and release of pro- and anti-inflammatory cytokines, resulting in an immunosuppressive cytokine phenotype.

The demonstration that chronic treatment with tianeptine abolishes depression-induced reduction in the pro-inflammatory cytokine TNF-α and increases the production of anti-inflammatory cytokines like TGF-1β and IL-10 in response to an antigenic challenge is in accordance with several recently published studies (Connor et al. 2000, Kubera et al. 2001, Yirmiya et al. 2001, Plaisant et al. 2003, Connor et al. 2005). Our results also coincide with studies describing reduced weight gain during the first weeks of OB-treatment, a characteristic hyperactivity, and increased exploratory, fear, and anxiety-related behaviour to a novel stressful environment (Hallam et al. 2004, Zueger et al. 2005). Finally, the OB rats showed reduced expression of glucocorticoid receptors (GRs) in the hippocampus. The reduced weight gain and most of the OB-induced effects on emotions were inhibited by tianeptine. Tianeptine did, however, not reverse the OB-induced stronger corticosterone response, and the reduction in hippocampal GR expression. These findings confirm other recent studies showing that anti-depressants like tianeptine, in contrast to the selective serotonin reuptake inhibitors (SSRIs) like fluoxetine, do not increase GRs in the hippocampus. Neither do they down-regulate the HPA axis responsiveness to danger signals (Kelly & Leonard 1994, Yau et al. 2004).

The hyperactivity and increased explorative and anxiety-related behaviour provoked by bulbectomy have been interpreted as a failure to habituate or cope with a novel situation or environment (Harkin et al. 2003, Zueger et al. 2005). The inability to adjust to novel situations is suggested to reflect an increased stress and/or anxiety-related response (McNish & Davis 1997, Zueger et al. 2005). Other behavioural deviations relevant to depression, and typically found in OB rats, include reduced sexual behaviour and impaired learning and memory. All of them may be reversed partially by chronic treatment with anti-depressants (Song 2000, Leonard & Song 2002).

Our previous animal studies have revealed that HPA axis and SNS over-responsiveness is associated with increased susceptibility to periodontitis (Breivik et al. 2000a, b, c, 2001a, b, 2005a, d, 2006). Human studies confirm that stressful negative events inducing trait anxiety and severe depression are associated with severe periodontitis (Moss et al. 1996, Genco et al. 1998, 1999, Hugoson et al. 2002, Vettore et al. 2003). It has also been substantiated that tianeptine may normalize emotional stress-induced dysregulation of numerous neurotransmitter systems (McEwen & Olie 2005), including the glutamateric, serotonergic, and adrenergic systems (Kole et al. 2002, Reagan et al. 2004). This quality may in part explain its efficacy in depression and immune regulation (Song 2000), as well as the ability of chronic tianeptine treatment to ameliorate ligature-induced periodontitis in rats with strong stress responses (Breivik et al. 2006).

Our finding that tianeptine does not inhibit OB-induced hypersecretion of corticosterone fits in with results obtained by some investigators (Kelly & Leonard 1994, Czeh et al. 2001), while others report that tianeptine down-regulates the HPA axis response to stressors like LPS (Delbende et al. 1994, Nickel et al. 2003). The lack of effect of tianeptine on the OB-induced HPA axis over-activity, and also on the expression of GRs in the hippocampus (which play significant roles in HPA axis feedback regulation) may indicate that other mechanisms are responsible for abrogating the OB-induced behavioural responses to novelty, the cytokine responses to LPS, or the subsequent increased susceptibility to periodontitis. For example, both tianeptine and other anti-depressant drugs restrain glucocorticoid receptor-mediated gene transcription (Budziszewska et al. 2002). Thereby, tianeptine may also have an impact on immune responses regulated by glucocorticoid hormones, and consequently also affect the changes in cytokine responses to LPS and the reduced susceptibility to ligature-induced periodontitis in the glucocorticoid overresponding OB rats. It is also possible that tianeptine may have normalized other mechanisms within the central nervous system involved in immune regulation, such as the SNS or PSNS.

Danger signals like LPS increase the release of catecholamines (noradrenalin and adrenalin) from the SNS, acetylcholine from the PSNS, and glucocorticoids from the HPA axis. The net result is suppression of cellular immunity and boosting of humoral immunity (Breivik et al. 1996, Elenkov & Chrousos 1999; Palvlov & Tracey 2004). Activation of the PSNS and increased release of acetylcholine, for instance, down-regulates the production and release of pro-inflammatory cytokines like TNF-α (Palvlov & Tracey 2004). It is, however, not likely that the PSNS is responsible for the reduced TNF-α response to LPS found in the OB rats. Recent studies have shown that olfactory bulbectomy causes hypo-function of the central cholinergic system (Hozumi et al. 2003, Hallam et al. 2004).

Taken together, the present study indicates that OB-induced dysregulation of immunoregulatory mechanisms controlled by the brain may be responsible for the altered outcome of cytokine responses and the increased susceptibility to ligature-induced periodontitis in the OB rats. Prevention of this dysregulation by chronic treatment with tianeptine may have inhibited these effects by inhibiting glucocortiocoid gene transcription or by restoring other immunoregulatory mechanisms dysregulated by the bulbectomy. The results imply that major depression and poorly developed psychological coping strategies, which are associated with enhanced susceptibility to periodontitis (Breivik et al. 1996, Moss et al. 1996, Genco et al. 1999, Hugoson et al. 2002, Wimmer et al. 2002), are mediated in part by dysregulation of the stress response and immune systems. Changes in the stress response have profound effects on the immune system (Elenkov & Chrousos 1999, Eskandary & Sternberg 2002, Song 2000). Over-responsiveness of the stress response system may down-regulate immune system responses controlling the growth of pathogenic micoorganisms in subgingival dental plaque (periodontopathogens), and this may represent an important mechanism responsible for alteration in the susceptibility to periodontitis in emotionally stressed individuals (Breivik et al. 1996, Breivik & Thrane 2001). The present results may help to explain our previous report showing that widows, widowers, and people with poorly developed psychological coping strategies are at a significant risk of developing severe peridontitis (Hugoson at al. 2002). As all the periodontal disease risk factors have been found to be associated with over-responsiveness of the stress response system (Breivik & Thrane 2001), these effects may help us to understand the aetiology and pathophysiology of periodontitis. An individual's responsiveness to danger signals may thus significantly determine the variable susceptibility to periodontitis. The discovery that peripheral immune system responses are regulated by the brain via stress response systems strongly indicates that emotional stress and other environmental factors may modify a patient's risk of developing periodontitis. In addition to dental plaque control, treatment approaches normalizing a misguided or dysregulated brain-neuro-endocrine-immune balance may therefore be important to diminish the predisposition to periodontitis.

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Clinical Relevance

The rationale for the animal experiment described herein was clinical observations and epidemiological studies showing that patients with severe periodontitis often have experienced negative life events, have poorly developed coping behaviour to manage negative life experiences, and have anxiety and depression. The data from the present study support our previous work in the inter-disciplinary field of brain-neuro-endocrine-immune-periodontology by demonstrating that alteration in behaviour and stress responsiveness may change immune function to bacterial antigens, and thereby alter the susceptibility to periodontitis. Treatment approaches (psychological, physical, and/or pharmacological) normalizing a dysregulated/misguided brain-neuro-endocrine-immune balance may modulate the predisposition to severe periodontitis.