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Neonatal dexamethasone and chronic tianeptine treatment inhibit ligature-induced periodontitis in adult rats

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Objective: The responsiveness of the hypothalamic–pituitary–adrenal (HPA) axis has been found to play a significant role for susceptibility and resistance to periodontal disease. In the present study we have investigated the effects of two different treatment strategies, which have been found to down-regulate the HPA axis, on ligature-induced periodontitis.

Methods: In experiment 1, newborn rats were treated with the synthetic glucocorticoid hormone dexamethasone-21-phosphate, which permanently down-regulates HPA axis responsiveness. In experiment 2, adult rats were treated with the novel antidepressant drug tianeptine, which opposes the action of stress. Periodontitis was inflicted upon all rats. Just before decapitation the animals received gram-negative bacterial lipopolysaccharide (LPS) to induce a robust immune and HPA axis response.

Results: Compared to the saline-treated control rats, dexamethasone-treated rats had significantly less periodontal bone loss ($p < 0.01$), reduced expression of glucocorticoid receptors in the hippocampus ($p < 0.001$), lower corticosterone ($p = 0.01$) and higher plasma levels of the cytokine tumor necrosis factor (TNF)-α ($p < 0.05$) after LPS challenge. Also the tianeptine-treated rats showed significantly reduced periodontal bone loss ($p = 0.01$), enhanced plasma levels of TNF-α ($p < 0.05$), and transforming growth factor-1β ($p < 0.01$), whereas no significant difference was found in corticosterone levels.

Conclusion: An individual's responsiveness to danger signals, whether they are of immunological, chemical, or psychological origin, may be an important factor for explaining variability in susceptibility to periodontal disease. The results may provide new insight into the mechanisms of periodontal disease development, and open new vistas for disease prevention.

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Recent research indicates that periodontitis may be a result of a dysregulated or misguided specific immune response, where the pathology may be a consequence of too weak T helper 1 (Th1) and T regulatory (Treg) immune responses (1–5). Misguided immune regulation may also explain the overgrowth of pathogenic dental plaque microorganisms (periodontopathogens) in subgingival dental plaque (4, 5). The subsequent immune-induced

destruction of the tooth supporting tissues (periodontium) seems to be caused by components, such as reactive oxygen metabolites and matrix metalloproteinases, belonging to the innate immune system (6–8). The tissue destructive components are most likely released when the immune cells fight and destroy periodontopathogens, and thereby protect the gingival tissues as well as the entire organism from getting infected (4, 5).

Both genetics (9), ageing (10, 11), and environmental factors, such as smoking (12), poorly regulated diabetes mellitus (13), traumatic life experiences, such as the loss of a spouse by death (14), poorly developed coping strategies to manage such traumatic experiences (14–16), and depressive mood and anxiety triggered off by negative life events, such as death in the family or financial problems (14, 16), have been found to be associated with severe and/or rapidly progressive periodontitis (17). The mechanisms by which these multifarious risk factors may increase the colonization of periodontopathogens and subsequently induce tissue destructive immune responses have, however, been poorly understood.

The hypothalamic–pituitary–adrenal (HPA) axis is one major biological system that is highly involved in immune regulation. Inappropriate responses may be genetically determined, but all the known periodontal disease risk factors are capable of inducing temporal or permanent changes or dysregulation (17–19). Being one of the major stress response pathways, the HPA axis is activated by danger signals interpreted by the brain as threatening to the organism, including emotional load (stress) and peripheral immune signals, such as those induced by cytokines released from lipopolysaccharide (LPS)-stimulated immune cells (18–20). The HPA axis may also be activated by LPS in gingival pockets (21). Upon activation, the HPA axis regulates the release of endogenous glucocorticoid hormones, predominantly cortisol in humans and corticosterone in rodents (20). Recent discoveries have revealed that the immune system interacts with the brain to regulate immune responses and control inflammation via the stress response system, including the HPA axis (17–20). In line with this, it has become clear that HPA axis over-responding individuals are susceptible to periodontitis, whereas under-responders are relatively more resistant to the disease (21–24).

Rats with genetically determined differences in their HPA axis responsiveness to danger signals significantly differ in their susceptibility to periodontitis (21, 22). As pointed out, the HPA axis responsiveness can be temporarily or permanently changed or 'reprogrammed' by environmental load, especially so when encountered in early life (25–27). Moreover, the release of glucocorticoid hormones from the adrenal glands during HPA axis activation (in response to danger signals) has been found to play a significant role for the ability of the early environment to reprogram the HPA axis (25–27). In this respect, the synthetic glucocorticoid 'stress hormone', dexamethasone, has turned out to be a useful tool with which to investigate the impact of early environmental stress on the HPA axis responsiveness in later adult life (27). Because dexamethasone is frequently used to prevent lung disease in preterm infants, neonatal dexamethasone experiments are also used to study the consequences of neonatal dexamethasone treatment on adult HPA axis responsiveness, health and disease (28).

Early life reprogramming of the brain–neuroendocrine regulatory response system profoundly influences a number of body functions, particularly behavioural responses to different types of environmental stress, cognition, body weight, immune system responses, and resistance and susceptibility to diseases in later life (26–30). Thus, long lasting effects upon immune system responses and the susceptibility and resistance to inflammatory diseases, including the development and progression of periodontitis, are anticipated. This assumption is supported by recent animal studies showing that postnatal experiences, such as manipulation in the maternal environment and exposure to LPS, can modulate the susceptibility to experimental periodontitis in the animals as adults (31, 32).

The aim of the present study was to further investigate the effects of stressful experiences on the development on ligature-induced periodontitis. The synthetic glucocorticoid hormone dexamethasone is often used to mimic a stressful situation and is known to inhibit the HPA axis responsiveness to danger signals in later life (25–30). Accordingly, we investigated whether postnatal treatment with dexamethasone would predict the susceptibility to periodontitis in adulthood. To reveal underlying mechanisms we also measured the effect of the neonatal dexamethasone treatment on the expression of glucocorticoid receptors in the hippocampus. These brain receptors are highly involved in the negative feedback regulation of HPA axis, and their expression plays an important role for the responsiveness of the HPA axis, and for the ability to cope with stress (33). In addition, we studied whether tianeptine, a novel drug that protects the hippocampus against deleterious consequences of stress and attenuates the HPA axis response to cytokine inducers such as LPS (34, 35), would influence immune responses and the susceptibility to periodontitis.

Material and methods

Animals

For experiment 1, 10-days pregnant Wistar rats were obtained from Möllegaard Breeding Center (Ejby, Denmark). They were housed individually, had free access to standard rat pellets and tap water, and were maintained under a 12–24 h light/dark cycle (light on 07.00 to 19.00 h) with temperature and humidity at 22°C and 40–60%, respectively. Pups were born on days 21–23 of gestation and weaned at day 21 of age. Eighteen females (nine dexamethasone treated and nine controls) were housed four to five per cage and used for the study.

For experiment 2, 20 male Fischer 344 rats weighing about 300 g at the time of tianeptine induction were obtained from Möllegaard Breeding Center (Ejby, Denmark). They were

included into the study after 2 weeks of acclimatisation to their housing condition. The rats were kept in groups of five, and 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 to 19.00 h) 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 investigate whether neonatal dexamethasone treatment, which has been used as a model to mimic stressful experience in early life as well as synthetic glucocorticoid treatment to newborn babies (27, 28), would influence immune and HPA axis responses to an LPS challenge, as well as the susceptibility to ligature-induced periodontitis.

The rat pups were injected intraperiotonally (i.p.) with dexamethasone on life day 1 (0.5 μg/g body weight), day 2 (0.3 μg/g) and day 3 (0.1 μg/g). Controls had equal volumes (10 μg/g) of sterile pyogen-free saline according to the experimental design by Kamphuis *et al.* (27). All pups in a nest received either dexamethasone or saline.

Experiment 2 — This experiment was designed to investigate whether adult rats chronically treated with tianeptine, which has been found to oppose and reverse the action of stress and successfully treats depression, would influence immune and HPA axis responses to an LPS challenge, as well as the susceptibility to ligature-induced periodontitis.

The rats were randomly assigned to two groups, each consisting of 10 rats. The animals in group 1 were injected i.p. with 10 mg/(kg day) of tianeptine sodium salt (a generous gift from Stablon, Servier, France) dissolved in saline. Fresh solutions were made every day, and the dose was selected on the basis of available literature. This dose has been shown to be effective in numerous reports (36, 37), including those dealing with the effect of tianeptine on stress, LPS, and cytokine-induced HPA axis activation (35, 38–41). Group 2 had a similar amount of saline and served as controls.

Experimental periodontal disease

At 3 months of age, all the animals in experiment 1 were anaesthetized with a subcutaneous injection in the neck with Hypnorm-Dormicum (fentanyl/fluanozon, 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 2nd molar teeth. All the animals in experiment 2 were treated in a similar way. The ligatures were left in the same position during the entire experiment and served as a retention device for oral microorganisms. Thirty-four days after application of the ligatures in experiment 1, and 35 days after application of the ligature and chronic tianeptine treatment in experiment 2, all animals were killed by decapitation. The maxillae were excised and fixed in 4% formaldehyde.

Radiographic examination of periodontal bone loss

The rat maxillary jaws were placed and stabilized with dental wax on a Trophy digital X-ray sensor, oriented with the axis of the teeth parallel to the sensor surface. The distance between the cemento-enamel junction and the most coronal portion of alveolar bone on mesial and distal surfaces of the 2nd molars was displayed digitally. The examination was done blinded. The reliability of the method has been tested earlier (5) and shown to have a standard error of the mean difference between two readings of 0.16 mm.

Lipopolysaccharide challenge

To assess whether the treatment regimen influenced cytokine and corticosterone responses, the animals were injected i.p. with LPS (*Escherichia coli* Serotype 0111:B4, Sigma; 100 μg/ml) 2 h before ending the experiments. In experiment 2, LPS (150 μg/kg) was, in addition, injected 24 h before decapitation to assess specific cytokine responses. 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 clot 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 prior to analysis of cytokines and corticosterone.

Assay of plasma cytokines

The levels of the cytokines tumor necrosis factor (TNF)-α, transforming growth factor (TGF)-1β, interleukin (IL)-6 and IL-10 in the plasma samples were measured by means of enzyme-linked immunosorbent assays (ELISA) kits from R & D Systems, Inc., Minneapolis, MN, USA, with catalogue numbers RAT00 for TNF-α, MB100 for TGF-1β, R6000 for IL-6, 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.

Assay of corticosterone

Plasma corticosterone was measured with a radioimmunoassay (RIA) Coat-A-Count kit from Diagnostic Products Corporation, Los Angeles, CA, USA, catalogue Number TKRC1. Limit of detection was 5.7 ng/ml. The antibody was highly specific, with the highest crossreactivity with 11-deoxycorticosterone of less than 2%. Plasma corticosterone values are expressed as ng/ml of plasma.

Isolation of RNA from hippocampus and reverse transcription–polymerase chain reaction assay of mRNA for the glucocorticoid receptor

The hippocampus from the rats in experiment 1 was isolated 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.) prior to use in reverse transcription–polymerase chain reaction (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 (version 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. NM022514) was preferred to those from rat β-actin (accession no. V01217 J00691) signal. Primers for L27 and β-actin were also designed using Oligo version 6.

RT–PCR was carried out using the Qiagen One-Step RT–PCR kit (Qiagen GmbH, Hilden, Germany). RT–PCR products were analyzed by agarose-electrophoresis using Gelstar fluorescence dye (BMA Inc., Cambrex Corp., East Rutherford, 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

Results are expressed as mean ± SD. The effects of saline, neonatal dexamethasone and tianeptine treatment on plasma corticosterone and cytokine, as well as tissue destruction were assessed with two-factorial repeated measures analysis of variance (ANOVA) test. The animal is used as the analytic unit, the data are expressed as means ± SD, and α level set at $p < 0.05$.

Results

In experiment 1, neonatal dexamethasone treatment reduced the body weight of the animals, which supports other studies using the same neonatal dexamethasone treatment (29). Neonatal dexamethasone-treated and saline-treated animals weighed 203.0 ± 10.4 g and 215.8 ± 12.2 g, respectively, at ligature induction, and 238.8 ± 13.2 g and 262.9 ± 30.9 g, respectively ($p < 0.05$ between groups), at the termination of the experiment 34 days after ligature induction (Table 1).

The tianeptine treatment (experiment 2) had no effect on the weight of the animals. The tianeptine-treated and control animals weighed 299.2 ± 8.2 g and 289.1 ± 4.8 g, respectively, at tianeptine induction, and 357.0 ± 12.7 g and 357.2 ± 13.2 g, respectively, at the termination of the experiment, 59 days later, and 35 days after ligature induction (Table 2).

Effect of neonatal dexamethasone and tianeptine treatment on periodontal tissue destruction

The neonatal dexamethasone-treated animals (experiment 1) were killed 34 days after application of the ligature. The mean bone loss was 0.70 ± 0.10 mm in the dexamethasone-treated rats, vs. 0.86 ± 0.10 mm in the control rats (Table 1 and Fig. 1) ($p < 0.01$ between groups). As the neonatal dexamethasone-treated rats showed reduced weight, the length of their teeth could be shorter. We therefore compared the root-length of the right 2nd molar teeth in the two groups by measuring the distance between the cemento-enamel junction and apex on mesial root surfaces. There was no difference between the root-length in the dexamethasone-treated and saline-treated control rats (1.91 ± 0.28 mm in dexamethasone-treated rats vs. 1.93 ± 0.09 mm in the controls; $p = 0.87$).

The tianeptine animals (experiment 2) were killed 35 days after application of the ligature. The bone loss was 0.88 ± 0.11 mm, compared with 0.99 ± 0.07 mm in the control rats (Table 2). The bone loss in the treatment group was significantly reduced compared to that seen in the untreated controls ($p = 0.03$).

Effects of neonatal dexamethasone and tianeptine treatment on corticosterone plasma levels

In experiment 1, the neonatal dexamethasone-treated rats showed a significant lower corticosterone response 2 h after LPS injection (dexamethasone-treated rats 1265.0 ± 345.7 nm/l; controls 1650.0 ± 213.0 nm/L; $p = 0.001$; Table 1 and Fig. 2).

Table 1. Effects of neonatal dexamethasone treatment in Wistar rats as adults

	Treatment		
	Neonatal DEX ($n = 9$)	Saline ($n = 9$)	*p*-values
Weight at ligature induction (g)	203.0 ± 10.4	215.8 ± 12.2	**0.03**
Weight at death (g)	238.8 ± 13.2	262.9 ± 30.9	**0.05**
Bone loss, X-ray (mm)	0.70 ± 0.10	0.86 ± 0.10	**< 0.001**
Corticosterone (nm/l, 2 h after i.p. LPS, day of death)	1265.0 ± 345.7	1650.0 ± 213.00	**0.01**
Expression of hippocampal glucocorticoid receptors (relative levels of mRNA 2 h after i.p. LPS, day of death)	0.86 ± 0.18	1.18 ± 0.13	**< 0.001**
TNF-α (pg/ml, 2 h after i.p. LPS, day of death)	3555.8 ± 3370.1	815 ± 528.8	**0.03**
IL-6 (pg/ml, 2 h after i.p. LPS, day of death)	1734.8 ± 1536.7	2323.0 ± 1780.7	0.43
IL-10 (pg/ml, 2 h after i.p. LPS, day of death)	52.9 ± 31.9	65.2 ± 59.7	0.53
TGF-1β (ng/ml, 2 h after i.p. LPS, day of death)	41.2 ± 10.2	41.4 ± 7.1	0.96

All data are shown as means ± standard deviation.
DEX; dexamethasone; TNF-α: tumor necrosis factor-α; IL-6 and 10: interleukin-6 and 10; TGF-$^{1\beta}$: transforming growth factor-$^{1\beta}$; i.p.: intraperitonally.

Table 2. Effects of chronic tianeptine treatment on male Fischer 344 rats

	Treatment		
	Tianeptine ($n = 10$)	Saline ($n = 10$)	p-value
Weight at tianeptine induction (g)	299.2 ± 8.2	289.1 ± 4.8	0.68
Weight at death (g)	357.0 ± 12.7	357.2 ± 13.2	0.71
Bone loss, X-ray (mm)	0.88 ± 0.11	0.99 ± 0.07	**0.01**
TNF-α (pg/ml, 2 and 24 h after i.p. LPS, day of death)	153.0 ± 115.6	66,8 ± 38.3	**0.04**
TGF-1β (pg/ml, 2 and 24 h after i.p. LPS, day of death)	16.3 ± 6.1	8.5 ± 2.6	**< 0.01**
IL-6 (ng/ml, 2 and 24 h after i.p. LPS, day of death)	900.7 ± 1034.5	303.3 ± 106.0	0.10
IL-10 (pg/ml, 2 and 24 h after i.p. LPS, day of death)	150.7 ± 98.7	90.1 ± 53.0	0.10
Corticosterone (nm/l, 2 and 24 h after i.p. LPS, day of death)	1382.2 ± 210.5	1335.6 ± 229.9	0.65

All data are shown as mean ± standard deviation.
TGF-1β: transforming growth factor-1β; IL-6 and 10: interleukin-6 and 10; TNF-α: tumor necrosis factor-α; i.p.: intraperitoneally.

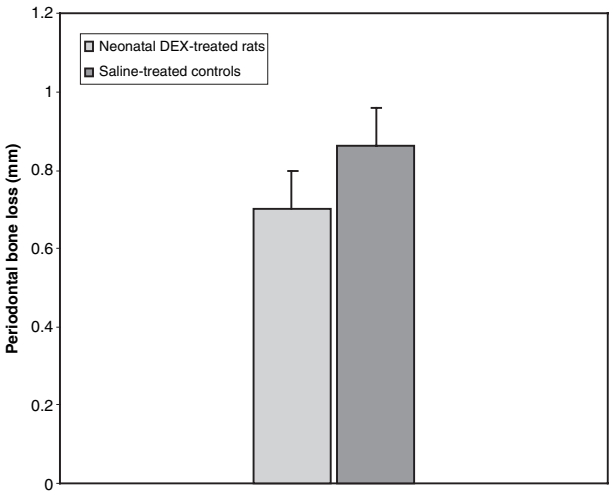

Fig. 1. The mean distance from the cemento-enamel junction to the alveolar bone crest in mm in neonatal dexamethasone (DEX)-treated rats and saline-treated controls as measured on digital radiographs. $p < 0.01$.

In experiment 2 with tianeptine, there was no difference in corticosterone response after LPS injection (tianeptine-treated rats 1382.2 ± 210.5 nm/L; controls 1335.6 ± 229.9 nm/L; $p = 0.65$; Table 2).

Effect of neonatal dexamethasone treatment and tianeptine on cytokine plasma levels

The neonatal dexamethasone-treated rats showed significantly higher TNF-α plasma level (3555.8 ± 3370.1 pg/ml) compared to controls (815.2 ± 528.8 pg/ml; $p < 0.05$; Table 1 and Fig. 3). The plasma levels of IL-6 as lower (1734.8 ± 1536.7 pg/ml) in the neonatal dexamethasone-treated rats compared to controls (2323.0 ± 1780.7 pg/ml), but this difference was not statistically significant ($p > 0.05$; Table 1). Also the plasma levels of IL-10 (52.9 ± 31.9 pg/ml) were lower in the neonatal dexamethasone-treated rats compared to controls (65.2 ± 59.7 pg/ml), but this difference was not significant (Table 1). TGF-1β plasma levels did not differ between groups (41.2 ± 10.2 ng/ml vs. 41.4 ± 7.1 ng/ml; $p = 0.96$; Table 1).

The tianeptine-treated rats showed significantly higher plasma levels of TNF-α (153.3 ± 115.6 pg/ml) compared to controls (66.8 ± 38.3 pg/ml; $p = 0.04$). The plasma levels of TGF-1β were also significantly higher in the tianeptine-treated rats (16.3 ± 6.1 ng/ml vs. 8.5 ± 2.6 ng/ml; $p < 0.01$). In addition we found a tendency to higher plasma levels of IL-6 (900.7 ± 1034.5 pg/ml vs. 303.3 ± 106.0 pg/ml; $p = 0.10$), and IL-10 (150.7 ± 98.7 pg/ml vs. 90.1 ± 53.3 pg/ml; $p = 0.10$; Table 2).

Effect of neonatal dexamethasone treatment on hippocampal expression of mRNA for the glucocorticoid receptor

The expression of mRNA for the glucocorticoid receptor in the hippocampus was significantly lower in the neonatal dexamethasone-treated rats compared to saline-treated controls. 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 (house-keeping gene, used as internal control). The mean ratio obtained for the glucocorticoid receptor was 0.86 ± 0.18 in the dexamethasone-treated rats and 1.18 ± 0.13 in saline-treated controls ($p < 0.001$ between groups; Table 1 and Fig. 4).

Discussion

Experiment 1 demonstrates that early postnatal treatment of rats with the synthetic glucocorticoid 'stress hormone' dexamethasone increases the resistance to experimental ligature-induced periodontal disease in adult rats. The data also show that neonatal dexamethasone-treated rats had significant reduced glucocorticoid receptor expression in the hippocampus as adults. In addition, we measured significantly lower plasma levels

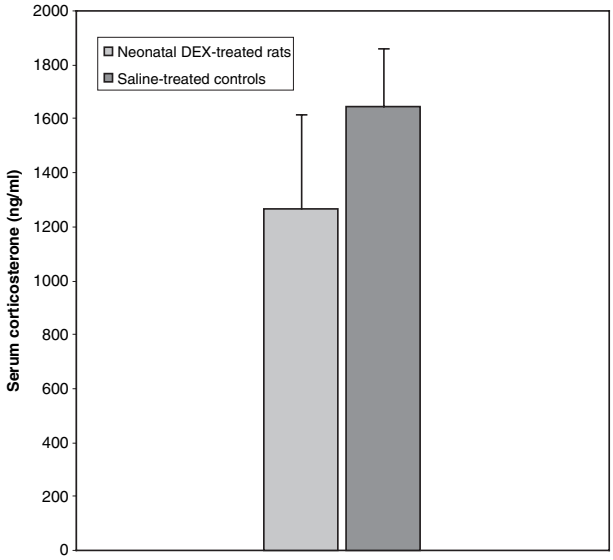

Fig. 2. Levels of corticosterone in serum 2 h after intraperitoneal injection of lipopolysaccharide (250 μg/kg) in neonatal dexamethasone (DEX)-treated rats and saline-treated. $p = 0.01$.

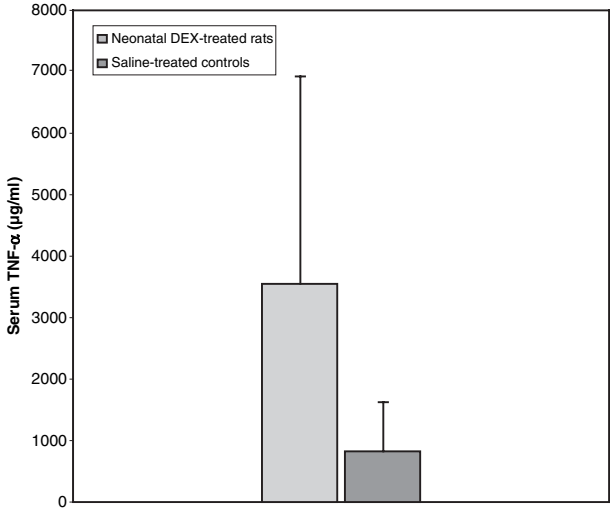

Fig. 3. Levels of tumor-necrosis factor-α (TNF-α) in serum 2 h after intraperitoneal injection of LPS (250 μg/kg) in neonatal dexamethasone (DEX)-treated rats and saline-treated controls. $p < 0.05$.

of corticosterone after a robust LPS challenge in the adult rats treated neonatally with dexamethasone, which demonstrates that the HPA responsiveness to LPS was decreased. Finally, the neonatal dexamethasone-treated rats showed an altered immune response in adulthood to gram-negative bacterial antigens, as demonstrated with significantly higher blood levels of the pro-inflammatory cytokine TNF-α in response to a robust LPS challenge.

Experiment 2 demonstrates that chronic treatment with the antidepressant drug tianeptine significantly inhibits tissue breakdown in ligature-induced periodontitis in periodontal disease susceptible Fischer 344 rats. In addition, we found that the tianeptine treatment influenced the immune system by significantly enhancing the plasma concentrations of the pro-inflammatory cytokine TNF-α and the T regulatory cytokine TGF-1β in response to the gram-negative bacterial antigen LPS. Tianeptine-treated rats also tended to produce more IL-6 and IL-10 in response to LPS.

Together, the two experiments demonstrate that treatments that change the responsiveness of the stress response system, whether in early childhood or in adult life, can change immune responses to gram-negative bacterial LPS antigens and the susceptibility/resistance to periodontitis. This is, to our knowledge, the first report demonstrating that neonatal dexamethasone treatment and treatment with an antidepressant drug inhibit the development of periodontitis.

Decreased reactivity of the HPA axis to stressors in adult life after neonatal glucocorticoid exposure, including dexamethasone, is supported by other studies (27, 29, 42–46). The finding that the neonatal dexamethasone-treated rats are responding with a decreased HPA axis response to an antigenic (LPS) challenge from gram-negative bacteria, is also in accordance with recent studies (29). Actually, adult rats treated with dexamethasone shortly after birth have been found to respond with significantly weaker HPA axis and glucocorticoid responses to differential environmental loads (stressors), such as novelty stress, conditioned fear, restraint stress, the pro-inflammatory cytokine IL-1β, and LPS (27, 29, 42–46). This shows that the effect of neonatal dexamethasone treatment is not specific for LPS, but involves several danger signals or environmental stressors that are well known to activate the HPA axis. Thus, neonatal synthetic glucocorticoid hormones exposure (or environmental stressors in early life that lead to HPA axis activation and subsequent increased release of endogenous glucocorticoids) may permanently change an individual's genetically determined HPA axis responsiveness to stressful stimuli, including the pathogenic bacterial antigen LPS.

The decreased glucocorticoid receptor expression in the hippocampus is also confirmed by other investigators

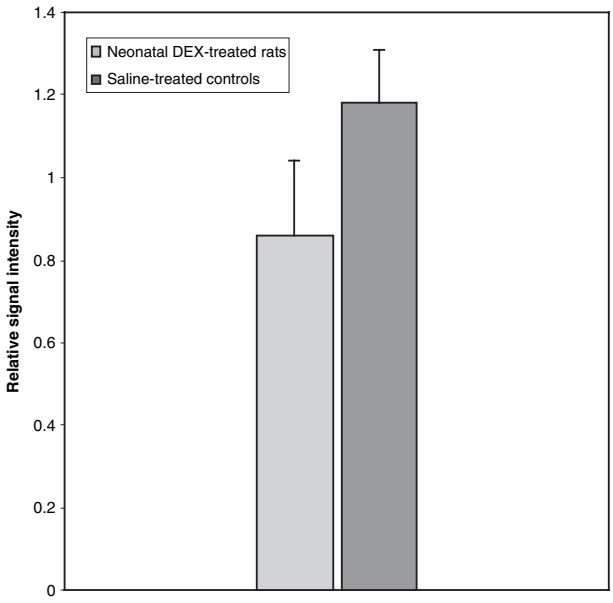

Fig. 4. Relative levels of mRNA for the glucocorticoid receptor in hippocampus expressed relative to the level of ribosomal protein L27 (house-keeping gene used as internal control) in neonatal dexamethasone (DEX)-treated rats and saline-treated controls. $p < 0.001$).

(42, 44). This finding was unexpected because down-regulation of hippocampal glucocorticoid receptors is usually followed by weaker negative feedback inhibition and thus stronger HPA axis responses to danger signals (33). However, Kamphuis and colleagues have recently shown that this discrepancy is most likely due to hypersensitivity of the HPA axis to negative feedback by glucocorticoids at the hypothalamic level induced by the neonatal dexamethasone treatment, and it may represent a compensatory adaptive response (27). Glucocorticoid receptors are conceivably down-regulated in the hippocampus to compensate for the hypersensitivity to glucocorticoids. Thus, the neonatal dexamethasone treatment may have changed the neurotransmission in the developing brain and induced a very strong hyper-sensitivity to glucocorticoid negative feedback inhibition, and this effect may be responsible for the weaker HPA axis response in adult life.

Interestingly, stressful experiences in early life can both down-regulate the HPA axis (as in the present experiment) and make the animals less susceptible to periodontitis (32) but more susceptible to Th1-mediated autoimmune diseases (19, 29), or up-regulate the HPA axis and make the animals more susceptible to periodontitis (31, 32) but less susceptible to Th-1 mediated autoimmune diseases, such as experimental autoimmune encephalomyelitis (19, 29). This disease mimics multiple sclerosis in humans. Thus, strong pro-inflammatory Th1 responses, which are unfavourable for Th1-mediated autoimmune diseases such as multiple sclerosis, may be favourable for periodontitis.

The results in experiment 2 in the present study, which showed that tianeptine altered TNF-α and TGF-1β responses to LPS, support other studies showing that chronic treatment with tianeptine, as well as with other antidepressants, can alter immune responses to pathogenic antigens (35, 38, 39, 47). Tianeptine is a modified novel tricyclic antidepressant (TCA), which is not associated with anti-cholinergic side-effects or with cordiotoxicity in overdose. Like TCAs, monoamine oxydase inhibitors, and selective serotonin reuptake inhibitors, it opposes and reverses the action of stress and successfully treats depression (48, 49). This drug is also devoid of the side-effects characterizing many antidepressants, such as sedation, sleeping trouble, and weight gain (50).

Recent studies have shown that negative stressful life experiences may down-regulate the production of new brain cells or neurons. The accompanying shrinkage of brain structures is especially evident in the hippocampus, which is highly involved in learning, memory and coping, and the negative feedback regulation of the HPA axis and sympathetic nervous system (51). Structural changes in the morphology of brain cells and their dendrites, as well as the cognitive impairment often associated with severe depressive illness, are opposed or reversed by antidepressant treatment (52). Tianeptine, in particular, has been found to stimulate the production of new neurons (neurogenesis) (52). This may also serve to normalize the neurotransmission of glutamate, which is cardinal in this process (53). The ability of tianeptine to reverse the structural changes in the hippocampus may partly account for its effect on the reactivity of the neuroendocrine systems and immune regulation (53, 54).

Interestingly, we have recently found that surgically inflicted lesions in the hippocampus up-regulate the HPA axis reactivity and dramatically increase periodontal disease susceptibility (55). Other procedures with similar effects on the HPA axis, e.g. chronic treatment with the glutamate receptor antagonist MK-801, and postnatal glutamate-induced central nervous system lesions, likewise worsen periodontitis (28, 56).

Glutamate plays a significant role in immune-to-brain signalling and HPA axis regulation. For example, in response to peripheral gram-negative LPS or the pro-inflammatory cytokine IL-1β there is an increased release of glutamate within the nucleus tractus solitarious (57). This is a brain cell nucleus receiving direct input from afferent sensory nerves during peripheral immune and inflammatory processes. Glutamate also augments retrovirus-induced immunodeficiency through stimulation of the HPA axis (58, 59). Alteration in glutamate neurotransmission may in part also explain the powerful inhibiting effect of dietary glycine treatment on ligature-induced periodontal disease (60).

Glutamate therefore probably plays an important role in tianeptine-induced alterations in neurotransmission as well as periodontal disease receptiveness and progression.

Glutamate is the principal excitatory neurotransmitter in the brain. It mediates central elements of the cellular effects of stressful stimuli by binding to the *N*-methyl-D-aspartate (NMDA) receptor subtypes. Tianeptine counteracts the increase in NMDA-receptor expression during stress (53). Different serotonin reuptake inhibitors and TCAs, the mood stabilizer lithium, and electroconvulsive shock therapy, up-regulate glutamate in the same brain regions (53, 61). Thus, a common cellular mechanism may explain the actions of antidepressants on immune responses and its effect on the outcome of ligature-induced periodontitis as found in this study, and glutamate is likely to play an important role.

Recent studies have shown that chronic treatment with tianeptine decreases the HPA axis response to danger signals, including those induced by psychological stress or coming from the immune system during LPS stimulation (35, 38, 39). It was therefore expected that chronic tianeptine treatment would reduce the serum levels of corticosterone in response to the LPS challenge, and thereby influence on the cytokine response and the outcome of periodontal disease. However, in contrast to the above-mentioned studies, we did not find reduction in HPA axis responsiveness (as measured by plasma corticosterone) induced by LPS stimulation in the chronic tianeptine-treated rats compared to vehicle-treated controls. This discrepancy may be caused by difference in chronic tianetine administration. For example, in the studies by Castanon and colleagues (38, 39) tianeptine was given twice daily, compared to once daily (with the same dose) in our experiment. In these studies, tianeptine was also administrated immediately after the LPS challenge, whereas LPS was given 2 h after the last tianeptine injection in the present experiment. Our finding is, however, in line with some other studies suggesting that chronic tianeptine treatment does not reduce the HPA axis response to stressors (34).

It is also possible that tianeptine may have altered the cytokine response and inhibited periodontal disease progression via other mechanisms. Antidepressant drugs are known to down-regulate the enhanced sympathetic nervous system as well as enhanced HPA axis responses during severe depression and stress (61). Thus, tianeptine may have influenced immune cell responsiveness and periodontal disease susceptibility by down-regulating the sympathetic nervous system or other brain-to-immune regulatory pathways. For example, we have recently found that peripheral chemical sympathetic nervous system denervation induced by the neurotoxic drug 6-hydroxydopamine, which destroys peripheral noradrenalin releasing sympathetic nervous system terminals, inhibits experimental periodontal disease in Fischer 344 rats (62), suggesting that over-responsiveness of the sympathetic nervous system may also play an important role in the pathogenesis of periodontal disease. Tianeptine may also have altered immune cell responsiveness directly. For example, *in vitro* studies have revealed that human monocytes, stimulated with LPS, show altered cytokine responses when treated with antidepressant drugs (63).

In conclusion, the current data demonstrate that exposure to glucocorticoid hormones in early postnatal life reprograms the responsiveness of the immunoregulatory HPA axis. The change in HPA axis responsiveness is associated with a change in brain glucocorticoid receptor expression, immune system responses, as well as altered susceptibility to periodontitis in adult life. These data suggest that exposure of endogenous glucocorticoids (which is typical during environmental stressful experiences) just after birth and subsequent alteration in the HPA axis responsiveness, may play a significant role for the susceptibility to periodontitis throughout life. The present study also suggests that dexamethasone therapy in newborn individuals, which is routinely used to prevent respiratory distress in preterm infants and increases the susceptibility to Th1-mediated autoimmune diseases (29, 30), may reduce genetically determined susceptibility to periodontitis.

The data also demonstrates that chronic treatment with the antidepressant drug tianeptine significantly inhibits ligature-induced periodontal breakdown. In addition, tianeptine significantly enhanced the pro-inflammatory cytokine TNF-α and the T regulatory cytokine TGF-β in response to a robust LPS challenge in stress over-responding and periodontal disease susceptible Fischer 344 rats. Several biological mechanisms may be involved, including its effect on glutamate NMDA receptors, the HPA axis, and the sympathetic nervous system.

Thus, an individual's responsiveness to stressors (psychological, chemical, or immunological), whether it is induced in early childhood or adult life, may importantly determine the variable susceptibility to periodontitis. These findings may also help us to understand the association found between periodontitis and adult stressful experiences, such as those experienced by soldiers at war (17), the loss of a loved one by death (14), and financial strain (16). Our data may also help us to understand the association found between periodontitis and an individual's ability to cope with stressful experiences (14–16). The results may provide new insight into the mechanisms of periodontal disease development, and open new vistas for disease prevention. Apart from dental plaque control, treatment approaches capable of altering brain–neuroendocrine–immune regulatory pathways may modulate the predisposition to periodontitis. Epidemiological studies in humans are needed to study the consequences of early postnatal life experiences on susceptibility/resistance to periodontitis in adulthood.

References

1. Tokoro Y, Matsuki Y, Yamamoto T, Suzuki T, Hara K. Relevance of local Th2-type cytokine mRNA expression in immunocompetent infiltrates in inflamed gingival tissue to periodontal diseases. *Clin Exp Immunol* 1997;**107:**166–174.

2. Chapple CC, Srivastava M, Hunter N. Failure of macrophage activation in destructive periodontal disease. *J Pathol* 1998;**186:**281–286.
3. Fokkema SJ, Loos BG, Slegte C, van der Velden U. A type 2 response in lipopolysaccharide (LPS)-stimulated whole blood cell cultures from periodontitis patients. *Clin Exp Immunol* 2002;**127:**374–378.
4. Breivik T, Rook GA. Oral treatment with SR299 (killed Mycobacterium vaccae) inhibits experimental periodontal disease in Wistar rats. *J Clin Periodontol* 2003;**30:**931–936.
5. Breivik T, Opstad PK, Engstad R, Gundersen G, Gjermo P, Preus H. Soluble β-1,3/1,6-glucan from yeast inhibits experimental periodontal disease in Wistar rats. *J Clin Periodontol* 2005: **32:** 347–352.
6. Birkedal-Hansen H. Role of cytokines and inflammatory mediators in tissue destruction. *J Periodont Res* 1993;**28:**500–510.
7. Ding Y, Haapasalo M, Kerosuo E, Lounatmaa K, Kotiranta A, Sorsa T. Release and activation of human neutrophil matrix metallo- and serine proteinases during phagocytosis of Fusobacterium nucleatum, Porphyromonas gingivalis and Treponema denticola. *J Clin Periodont* 1997;**24:**237–248.
8. Lohinai Z, Benedek P, Fehér E *et al.* Protective effects of mercaptoethylguanidine, a selective inhibitor of inducible nitric oxide synthase, in ligature-induced periodontitis in the rat. *Br J Pharmacol* 1998;**123:**353–360.
9. Michalowicz B. Genetic and heritable risk factors in periodontal disease. *J Periodontol* 1994;**65:**479–488.
10. Hugoson A, Jordan T. Frequency distribution of individuals aged 20–70 years according to severity of periodontal disease. *Comm Dent Oral Epidemiol* 1982;**10:**187–192.
11. Burt BA. Periodontitis and ageing: reviewing recent evidence. *J Am Dent Assoc* 1994;**125:**273–279.
12. Martinez-Canut P, Lorca A, Magan R. Smoking and periodontal disease severity. *J Clin Periodontol* 1996;**22:**743–749.
13. Thorstensson H, Hugoson A. Periodontal disease experience in adult long-duration insulin-dependent diabetics. *J Clin Periodontol* 1993;**20:**352–358.
14. Hugoson A, Ljungquist B, Breivik T. The relationship of some negative events and psychological factors to periodontal disease in an adult Swedish population 50–80 years of age. *J Clin Periodontol* 2002;**29:**247–253.
15. Wimmer G, Janda M, Wieselmann-Penkner K, Jakse N, Polansky R, Pertl C. Coping with stress: Its influence on periodontal disease. *J Periodontol* 2002;**73:**1343–1351.
16. Genco RJ, Ho AW, Grossi SG, Dunford RG, Tedesco LA. Relationship of stress, distress, and inadequate coping behaviors to periodontal disease. *J Periodontol* 1999;**70:**711723.
17. Breivik T, Thrane PS. Psychoneuroimmune interactions in periodontal disease. In: Ader R, Felten DL, Cohen N, eds. *Psychoneuroimmunlogy*, 3rd edn, Chapter 63. San Diego, CA: Academic Press, 2001: 627–644.
18. Chrousos GP. The hypothalamic–pituitary–adrenal axis and immune-mediated inflammation. *N Engl J Med* 1995;**332:**1351–1361.
19. Elenkov IJ, Chrousos GP. Stress hormones, Th1/Th2 patterns, pro/anti-inflammatory cytokines and susceptibility to disease. *Trends Endocrinol Metab* 1999;**10:**359–368.
20. Lightman SL, Windle RJ, Ma XM *et al.* Hypothalamic–pituitary–adrenal function. *Arch Physiol Biochem* 2002;**110:**90–93.
21. Breivik T, Thrane PS, Gjermo P, Opstad PK, Pabst R, von Horsten S. Hypothalamic–pituitary–adrenal axis activation by experimental periodontal disease in rats. *J Periodont Res* 2001;**36:**295–300.
22. Breivik T, Opstad PK, Gjermo P, Thrane PS. Effects of hypothalamic–pituitary–adrenal axis reactivity on periodontal tissue destruction in rats. *Eur J Oral Sci* 2000;**108:**115–122.
23. Breivik T, Thrane PS, Gjermo P, Opstad PK. Glucocorticoid receptor antagonist RU-486 treatment reduces periodontitis in Fischer 344 rats. *J Periodont Res* 2000;**35:**285–290.
24. Breivik T, Gundersen Y, Osmundsen H, Opstad PK, Fonnum F. Chronic treatment with the glutamate receptor antagonist MK-801 alters periodontal disease susceptibility. *J Periodont Res* 2005;**40:**28–35.
25. Welberg LAM, Seckel JR. Prenatal stress, glucocorticoids and the programming of the brain. *J Neuroendocrinol* 2001;**13:**113–128.
26. Matthews SG. Early programming of the hypothamo–pituitary–adrenal axis. *Trends Endocrinol Metabol* 2002;**13:**373–380.
27. Kamphuis PJGH, Bakker JM, Broekhoven MH *et al.* Enhanced glucocorticoid feedback inhibition of hypothalamo–pituitary–adrenal responses to stress in adult rats neonatally treated with dexamethasone. *Neuroendocrinol* 2002;**76:**158–169.
28. Mammel MC, Johnson DE, Green TP, Thomson TR. Controlled trial of dexamathasone in infants with bronchopulmonary dysplasia. *Lancet* 1983;**1:**1356.
29. Bakker JM, Kavelaars A, Kamphuis PJGH *et al.* Neonatal dexamethasone treatment increases susceptibility to experimental autoimmune disease in adult rats. *J Immunol* 2000;**165:**5932–5937.
30. Shanks N, Larocque S, Meany M. Neonatal endotoxin exposure alters the development of the hypothalamic–pituitary–adrenal axis: Early illness and later responsivity to stress. *J Neurosci* 1995;**15:**376–384.
31. Sluyter F, Breivik T, Cools A. Manipulation in the maternal environment reverse genetically predisposed periodontitis in rats. *Clin Diagn Lab Immunol* 2002;**4:**931–932.
32. Breivik T, Stephan M, Brabant GE, Straub RH, Pabst R, von Hörsten S. Postnatal lipopolysaccharide-induced illness predisposes to periodontal disease in adulthood. *Brain Behav Immunol* 2002;**16:**421–438.
33. De Kloet ER, Vreugdenhil E, Oitzl MS, Joëls M. Brain corticosteroid receptor balance in health and disease. *Endocr Rev* 1998;**19:**269–301.
34. Czeh B, Michaelis T, Watanabe T *et al.* Stress-induced changes in cerebral metabolites, hippocampal volume, and cell proliferation are prevented by treatment with tianeptine. *Proc Natl Acad Sci USA* 2001;**98:**12796–12801.
35. Castanon N, Mediana C, Mormende C, Danzer R. Chronic administration of tianeptine balances lipopolysaccharide-induced expression of cytokines in the spleen and hypothalamus of rats. *Psychoneuroendocrinology* 2004;**29:**778–779.
36. Conrad DC, Galea LAM, Kuroda Y, McEwen BS. Chronic stress impairs rat spatial memory on the Y maze and this effect is blocked by tianeptine pretreatment. *Behav Neurosci* 1996;**110:**1321–1334.
37. Malagie J, Deslandes A, Gardier AM. Effects of acute and chronic tianeptine administration on serotonin outflow in rats: comparition with paroxetine by using *in vivo* microdialysis. *Eur J Pharmacol* 2000;**403:**55–65.
38. Castanon N, Bluthe RM, Danzer R. Chronic treatment with the atypical antidepressant tianeptine attenuates sickness behaviour induced by peripheral but not central lipopolysaccharide and interleukin-1 beta in the rat. *Psychopharmacology* 2001;**154:**50–60.
39. Castanon N, Konsman J-P, Mediana C, Chauvet N, Danzer R. Chronic treatment with the antidepressant tianeptine attenuates lipopolysaccharide-induced Fos expression in the rat paraventricular nucleus and HPA axis activation. *Psychoneuroendocrinology* 2003;**28:**9–34.
40. Fontantages R, Mimouni J, de Grieve X, Picard J, Pugeat M, Bange C. Effect of tianeptine on neuroendocrine, enzyme and behavioural responses to restraint stress in male rats. *Eur J Psychiat* 1993;**8:**67–73.

41. Delbende C, Tranchand Bunel D, Tarozzo G *et al*. Effect of chronic treatment with the antidepressant tianeptine on hypothalamic–pituitary–adrenal axis. *Eur J Pharmacol* 1994;**251:**245–251.
42. Felszeghy K, Gaspar E, Nyakas C. Long-term selective down-regulation of brain glucocorticoid receptors after neonatal dexamethasone treatment in rats. *J Endocrinol* 1996;**8:**483–499.
43. Zoli M, Agnati LF, Fuxe K *et al*. Long-lasting reduction of glucocorticoid receptor immunoreactvity in the hippocampus field CA1 but not in the dentate gyrus after neonatal treatment with corticosterone in the rat. *Acta Physiol Scand* 1990;**138:**577–579.
44. Plageman A, Straut A, Gotz F *et al*. Long-term effects of early postnatally administrated interleukin-1β on the hypothalic-piutary-adrenal (HPA) axis in rats. *Endocrine Regul* 1998;**32:**77–85.
45. Plotsky PM, Meany MJ. Early, postnatal experiences alters hypothalamic corticotrophin-releasing factor (CRF) mRNA, median eminence CRF content and stress-induced release in adult rats. *Mol Brain Res* 1993;**18:**195–200.
46. Liu D, Diorio J, Tannenbaum B *et al*. Maternal care, hippocampal glucocorticoid receptors, and hypothalamic–pituitary–adrenal responses to stress. *Science* 1997;**277:**1659–1662.
47. Kubera M, Maes M, Holand V, Basta-Kaim A, Roman A, Shani J. Prolonged desipramine treatment increases the production of interleukin-10, an anti-inflammatory cytokine, in C57BL/6 mice subjected to the chronic mild stress model of depression. *J Affect Disord* 2001;**63:**171–178.
48. Cassano GB, Heinze G, Loo H, Mendlewicz J., Paes de Sousa M. A double-blind comparison of tianeptine, imipramine and placebo in the treatment of major depressive episodes. *Eur Psychiatry* 1996;**11:**254–259.
49. Costa de Silva JA, Ruschel SI, Caetano D *et al*. Placebo-controlled study of tianeptine in major depressive episodes. *Neuropsychobiol* 1997;**35:**24–29.
50. Wagstaff AJ, Ormrod D, Spencer CM. Tianeptine. A review of its use in depressive disorders. *CNS Drugs* 2001;**15:**231–259.
51. McEwen BS. Stress and hippocampal plasticity. *Ann Rev Neurosci* 1999;**20:**9104–9110.
52. Malberg JE, Eisch AJ, Nesler EJ, Duman RS. Chronic antidepressant treatment increases neurogenesis in adult rat hippocampus. *J Neurosci* 2000;**20:**9104–9110.
53. Kole MHP, Swan L, Fuchs E. The antidepressant tianeptine persistently modulates glutamate receptor currents of the hippocampal CA3 commissural associational synapse in chronically stressed rats. *Eur J Neurosci* 2002;**16:**807–816.
54. Leonard BE. The immune system, depression and the action of antidepressants. *Prog Neuro-Psychopharmacol Biol Psychiat* 2001;**25:**767–780.
55. Breivik T., Thrane PS, Gjermo P, Cools A, Myhrer T. Effects of hippocampal lesioning on experimental periodontal disease in Wistar rats. *J Periodont Res* 2002;**37:**360–365.
56. Breivik T, Thrane PS, Gjermo P, Fonnum F. Postnatal glutamate-induced central nervous system lesions alter periodontal disease susceptibility in adult Wistar rats. *J Clin Periodontol* 2001;**28:**904–909.
57. Mascarucci P, Perego C, Tarrazzino S, Simoni MG. Glutamate release in the nucleus tractus solitarius induced by peripheral lipopolysaccharide and interleukin-1β. *Neuroscience* 1998;**86:**1285–1290.
58. Ziegler AR, Basil AS. Glutamate augments retrovirus-induced immunodeficiency through stimulation of the hypothalamic–pituitary–adrenal axis. *J Immunol* 1999;**162:**4998–5002.
59. Ziegler DR, Herman JP. Local integration of glutamate signalling in the hypothalamic paraventricular region: regulation of glucocorticoid stress responses. *Endocrinology* 2000;**141:**4801–4804.
60. Breivik T, Gundersen Y, Osmundsen H, Opstad P-K, Fonnum F. Chronic glycine treatment inhibits ligature-induced periodontal disease in Wistar rats. *J Periodont Res* 2005;**40:**43–47.
61. Gold PM, Licinio J, Wong ML. Corticotropin-releasing hormone in the pathophysiology of melancholic and atypical depression and in the mechanism of action of antidepressant drugs. *Ann N Y Acad Sci* 1995;**771:**716–729.
62. Breivik T, Gundersen Y, Opstad PK, Fonnum F. Chemical sympathectomy inhibits periodontal disease in Fischer 344 rats. *J Periodont Res* 2005;**40:**325–330.
63. Xia Z, De Poere JW, Nassberger L. TCA's inhibit IL-1, IL-6 and TNF release in human blood monocytes and IL-2 and interferon in T-cells. *Immunopharmacology* 1991;**34:**27–37.