Effects of Stress on Susceptibility and Severity of Inflammation in Adjuvant-induced Arthritis[a]

ANTONIO J. CHOVER-GONZALEZ,[b] MICHAEL S. HARBUZ,[c] PURIFICACION TEJEDOR-REAL,[b] JUAN GIBERT-RAHOLA,[b] PHILIP J. LARSEN,[d] AND DAVID S. JESSOP[c,e]

[b]*Department of Neurosciences, University of Cadiz, Spain*
[c]*Department of Clinical Medicine, University of Bristol, United Kingdom*
[d]*Department of Medical Anatomy B, The Panum Institute, University of Copenhagen, Denmark*

ABSTRACT: We have utilized the open field and learned helplessness (LH) models of psychological stress to determine whether a differential response to stress can affect the severity of adjuvant-induced arthritis (AA) within a single rat strain. In response to open field stress, the corticosterone response of the low emotivity rats was significantly lower than that of the high emotivity rats. In spite of the differential corticosterone response to stress, no significant difference was found in paw volumes between the AA high and low emotivity groups. In another study, rats were subjected to a learned LH paradigm and separated into two groups based on failed (LH+) or successful (LH–) avoidance. Plasma corticosterone levels in response to avoidable foot shock in the LH– rats were significantly greater than in the LH+ group. Following injection with adjuvant, paw inflammation occurred earlier and was more severe in the LH– rats compared to the LH+ group. These data show that rats with a greater tendency to avoid foot shock have more severe inflammation, despite having a greater corticosterone response to stress. We conclude that an increased corticosterone response to stress does not affect susceptibility to or severity of inflammation in AA. Indeed, in the LH model a more robust response to stress is associated with increased inflammation and earlier onset of the disease.

There are many reports of an association between rheumatoid arthritis (RA) and a compromised hypothalamo-pituitary-adrenal (HPA) axis that is incapable of secreting adequate amounts of cortisol, thereby preventing a patient from mounting a robust anti-inflammatory response.[1–6] Although it has been proposed that the defect in HPA axis activity is located at the level of the hypothalamus,[3] other studies have demonstrated adrenal insensitivity,[4,7] and the precise location of any defect remains to be determined. In animal models, Lewis rats that have increased susceptibility to experimentally induced inflammatory diseases exhibit an impaired HPA axis re-

[a]This work was supported by The Wellcome Trust, The Oliver Bird Fund of the Nuffield Foundation, the European Neuroscience Programme, and the Spanish CICYT Grant SAF97-0225.

[e]Address correspondence to David S. Jessop, Department of Clinical Medicine, Division of Medicine, University of Bristol, Marlborough Street, Bristol BS2 8HW, UK. Tel: 00-44-117-9283402; fax: 00-44-117-9283315; e-mail: David.Jessop@bris.ac.uk

sponse to stress, whereas histocompatible Fischer rats that have a robust corticosterone response to acute stress are largely resistant,[8–10] although in one study the central neuroendocrine responses to stress were similar in Fischer and Lewis rats.[11] An association between HPA axis dysfunction and autoimmune disease has also been demonstrated in the MRL lupus-prone mouse and in the obese strain of chicken with autoimmune thyroiditis.[12] Consequently, it has been proposed that a blunted HPA axis response to stress may contribute to the onset and development of certain autoimmune diseases through inadequate production of anti-inflammatory glucocorticoids.[13] However, this hypothesis has largely evolved from observations of differences in the stress response between two separate rat strains and has never been directly tested in rats of the same strain. Susceptibility to autoimmune diseases in the Lewis rat may not be a consequence of an impaired HPA axis response, nor may resistance to inflammation in Fischer rats be due to the ability to mount a robust response to stress, because these strains could have quite separate central and peripheral mechanisms controlling inflammatory processes, to which HPA axis responses are secondary. When testing the hypothesis that an impaired HPA axis response is causally related to severity of autoimmune disease, it is important to use one strain only, to eliminate any genetic variability between strains, such as central neurotransmitter pathways which might alter HPA axis activity and susceptibility to inflammation. To conform with this rigorous objective, we subjected Wistar rats to two stress paradigms: open field and learned helplessness. On the basis of their corticosterone responses to these stressors, rats could be separated into high or low corticosterone secretors. The differential corticosterone responses of rats within a strain could then be related to the degree of inflammation in experimentally induced adjuvant arthritis (AA).

OPEN FIELD STRESS AND ADJUVANT-INDUCED ARTHRITIS

The open field experimental protocol has been fully described.[14] A pre-stress blood sample was taken from the tail vein of adult male Wistar rats and 24 h afterwards rats were subjected to open field stress. The number of fecal pellets excreted in a 5-min period was counted and rats were divided into two groups of high or low emotivity on the basis of pellet count. A second blood sample was collected from the tail vein 30 min after initiating the stress. Four hours following termination of the stress, groups of high or low emotivity rats were further divided into two groups and given either an intradermal injection of *Mycobacterium butyricum* in paraffin oil or vehicle for the induction of arthritis.[15] Fourteen days after injection rats were killed by decapitation, and trunk blood was collected. All plasma samples were assayed for total corticosterone by in-house radioimmunoassay using antiserum kindly donated by Dr. G. Makara (Institute of Experimental Medicine, Budapest, Hungary).

Pre-stress basal corticosterone concentrations between high and low emotivity groups were not significantly different (FIG. 1). High and low emotivity animals all responded to the open field stress with significantly elevated plasma corticosterone. However, the difference in the stress response between the high and low emotivity groups was highly significant ($p < 0.01$; FIG. 1), the low emotivity group being characterized by a 60% reduction in plasma corticosterone. Paw volumes in the AA high

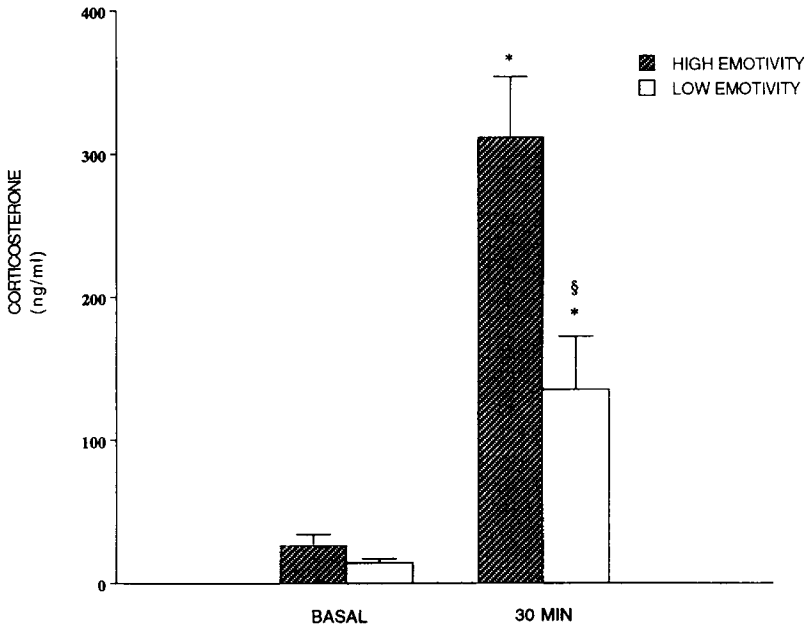

FIGURE 1. Plasma corticosterone concentrations in rats 24 h prior to (basal) and 30 min following a 5-min open field stress. Rats were divided into high ($n = 21$) and low ($n = 9$) emotivity groups on the basis of fecal pellet count during the stress. Values are means ± SEM. *$p <$ 0.05 compared to respective basal levels; §$p < 0.01$ compared to high emotivity group.

and low emotivity groups were significantly greater than their respective non-AA controls. However, in spite of the differential corticosterone response to open field stress, no significant difference in paw volumes was found between the AA high and low emotivity groups at day 14 (FIG. 2). Failure to detect differences in degree of inflammation between the high and low emotivity groups was not due to maximal hindpaw inflammation being attained, because we have observed significantly greater inflammation in other experimental paradigms at day 14.[16]

LEARNED HELPLESSNESS AND ADJUVANT-INDUCED ARTHRITIS

Fifty-two adult male Wistar rats received inescapable, unavoidable, and unpredictable foot shocks delivered in a 20 × 12 × 10 cm Plexiglas chamber. A constant current shocker delivered 60 shocks (duration 15 s; intensity 1 mA, intershock interval 60+/–20 s) to the grid floor (1.5 cm stainless steel mesh). In order to evaluate shock avoidance, avoidance training was initiated 48 h after the inescapable shock session in an automated two-way shuttlebox divided into two equal-sized chambers by a black Plexiglas partition with a gate providing access to the adjacent compartment. The animals were singly placed in the shuttlebox and subjected to 30 avoidable

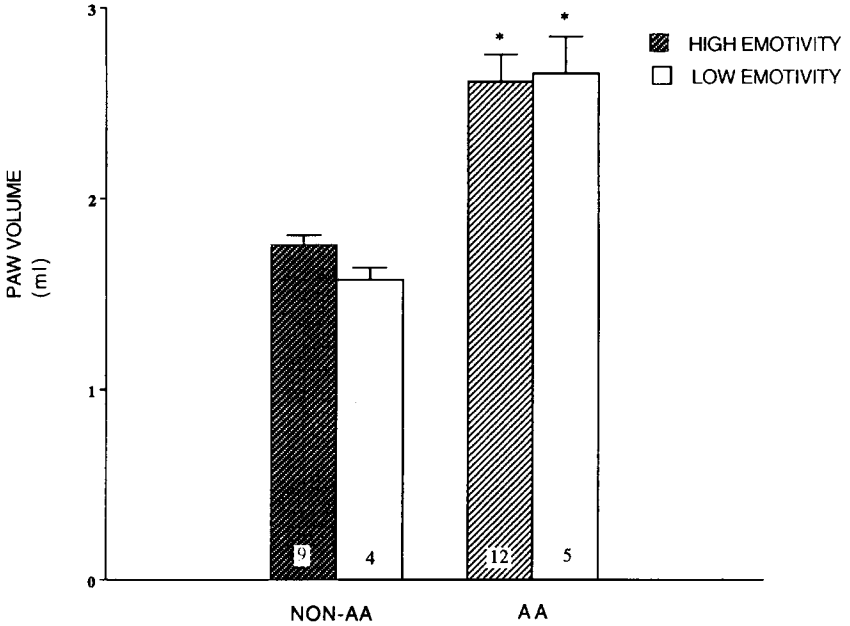

FIGURE 2. Paw volumes in high or low emotivity groups 14 days following injection with adjuvant (AA) or vehicle (non-AA). Values are means ± SEM. $*p < 0.05$ compared to respective controls (n = number in group).

shocks. During the first 3 s of each trial a light signal was presented. The animals were allowed to avoid shock during this period. If an avoidance did not occur, a 1 mA shock was delivered for 3 s. If no escape response occurred within 3 s, shock and light were terminated. The response required of the rat during each trial was to pass through the gate once into the other compartment of the shuttlebox. Intertrial interval was 30 s. Rats were divided into two groups according to the number of failures to pass into the adjacent compartment: LH+ = high number of failures and LH– = low number of failures. Tail-vein blood samples were collected 24 h prior to the unavoidable foot shock session and 30 min following the LH test. Four hours following the LH test, groups of LH+ and LH– rats were further divided into two groups and given either an intradermal injection of *Mycobacterium* or vehicle. Fourteen days after injection rats were killed by decapitation and brains, spleens, thymuses, and trunk blood were collected.

As in the open field study, all blood samples were assayed for corticosterone following termination of the LH experiment on day 14. Pre-stress basal corticosterone concentrations between LH+ and LH– groups were not significantly different (FIG. 3). Plasma corticosterone at the 30-min time point was significantly elevated in both the LH+ and LH– groups compared to controls, whereas levels in the LH– group were significantly higher than in the LH+ group (FIG. 3). Paw volumes in the AA LH+ group were significantly larger at day 14, but not at day 10, compared to vehi-

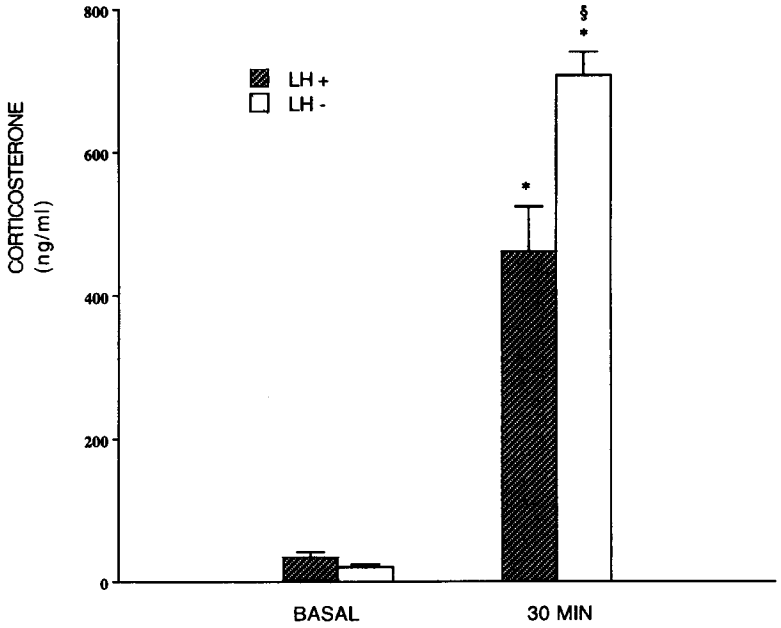

FIGURE 3. Plasma corticosterone concentrations in rats 72 h prior to (basal) and 30 min following the learned helplessness (LH) test. Values are means ± SEM. $*p < 0.01$ compared to respective basal levels; $\S p < 0.01$ compared to LH+ group ($n = 8–12$).

cle-injected LH+ rats, whereas paw volumes in the AA LH– group were significantly larger than their LH+ counterparts at both days 10 and 14 (FIG. 4). No significant correlation was found between plasma corticosterone at the 30-min time point and paw volumes 10 and 14 days after injection in individual rats. β-endorphin levels in splenic extracts of AA LH– rats were significantly greater than their LH+ counterparts (FIG. 5), but there were no significant differences in splenic contents of corticotropin-releasing hormone (CRH), adenocorticotropin (ACTH) or vasopressin (AVP). No differences were observed in thymic contents of any peptides measured (data not shown). All peptides were measured by specific in-house radioimmunoassays following acid extraction from tissues.

DISCUSSION

We have utilized the open field and learned helplessness paradigms to demonstrate that an attenuated corticosterone response to stress does not increase susceptibility to or severity of inflammation in AA. The hypothesis that an attenuated stress response increases susceptibility to inflammatory autoimmune disease is based on observations that immature female Lewis rats, a strain which is more susceptible to streptococcal cell wall (SCW) arthritis than immature females of the Fischer strain,[17]

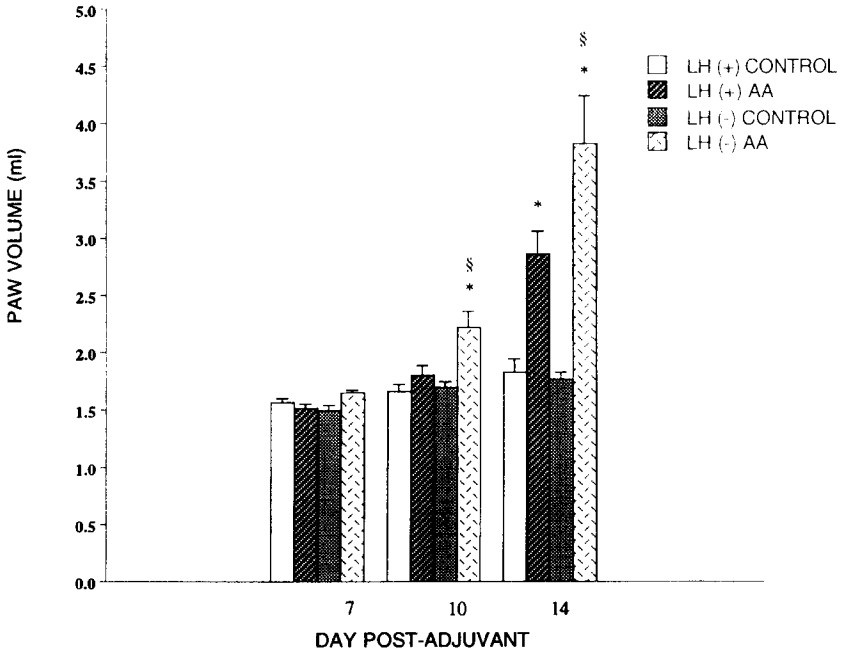

FIGURE 4. Paw volumes in LH+ and LH– rats 14 days following injection with adjuvant (AA) or vehicle (control). Values are means ± SEM. $*p < 0.05$ compared to respective controls ($n = 8$–12); $\S p < 0.05$ compared to LH+ AA group.

have an impaired HPA axis response to CRH or interleukin (IL)-2α[9] and to open field, restraint, ether or swim stressors.[10] However, the relationship between response to stress and susceptibility to arthritis is more complex in adult rats. Adult Lewis rats can perform poorly or similarly to the Fischer strain depending on the type of stress.[11,18,19] In a recent report, adult male and female Lewis and Fischer rats mounted similar responses to an immunological stress.[19] We have shown that, in spite of evidence that the CFY, Wistar, and Sprague-Dawley strains can all mount robust responses to various stressors, the CFY strain is relatively resistant to AA, whereas AA is readily induced in Wistar and Sprague-Dawley rats.[20]

Furthermore, the Piebald-Viral-Glaxo strain in which AA is readily induced has a very robust corticosterone response to stress.[16] This was also found in the Fischer strain, where both the conventional F344 and the germ-free (GF) F344 had an equally robust corticosterone response to IL-1α, yet the F344 rats were resistant to SCW arthritis while the GF F344 rats were susceptible.[21] Inflammation-susceptible and inflammation-resistant rat strains mounted equally strong corticosterone responses to lipopolysaccharide.[22] In a clinical study, patients with the parasitic disease trypanosomiasis exhibited an impaired HPA axis response to the CRH test during the course of the infection, but their ACTH and cortisol response to CRH following treatment was normal, as it presumably was prior to onset of the disease.[23]

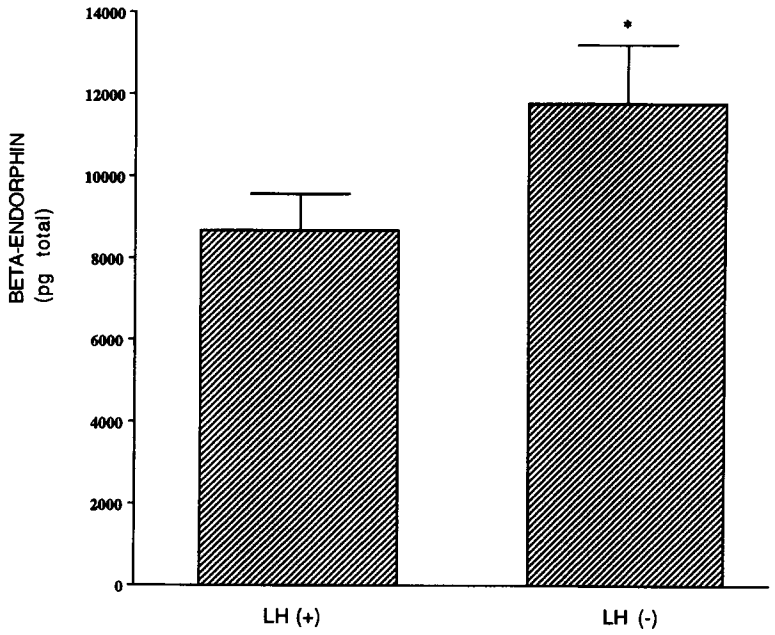

FIGURE 5. Total amounts of β-endorphin in extracts of spleens from arthritic LH– ($n = 9$) or LH+ ($n = 12$) rats. Values are means ± SEM. *$p < 0.05$ compared to LH+ group by unpaired Student's t test.

Therefore a robust response to stress does not necessarily protect humans or rodents from inflammatory disease. Inflammation was exacerbated in the LH– rats, in spite of a significantly increased corticosterone response to stress which might have been predicted to have a protective effect. In fact, not only was inflammation in the LH– group more severe following injection of adjuvant, onset was earlier because inflammation was significantly greater compared to the LH+ rats by day 10. This could not be accounted for by an underlying deficiency in corticosterone secretion in the LH– rats because basal levels were not different between the LH+ and LH– groups. This is consistent with the lack of correlation between basal corticosterone and susceptibility to inflammation in Lewis and Fischer strains, since basal corticosterone levels are similar in both strains.[10] In fact, basal levels of corticosterone are lower in male Lewis rats compared to females,[24] and yet the females are more susceptible to inflammation.

Differences in inflammation between the LH+ and LH– rats might be due to changes in central production of serotonin (5-HT). The LH model is considered to be a reliable animal model of depression,[25] which is characterized by a defect in central serotonergic function, as is depression in humans. Levels of 5-HT were decreased, and its metabolite 5-HIAA increased, in the cortex of LH+ rats.[26] Abnormalities in the 5-HT_2 receptor reported in depressed patients are also found in LH+ rats.[27] Treatment with antidepressant fluvoxamine, a serotonin selective reuptake inhibitor

(SSRI), which elevates central levels of 5-HT, prevented learned helplessness.[28] We have previously observed that depleting central 5-HT with a specific neurotoxin prevented the onset of AA, while increasing central 5-HT with an SSRI enhanced hindpaw inflammation.[29] Thus, increased central 5-HT exacerbates peripheral inflammation, possibly through stimulation of expression of a number of proinflammatory cytokines such as IL-6 and TNF-α,[30] although the mechanism is not yet clear. This may have important implications for arthritic patients who are prescribed SSRIs for depression. In the present study, if central levels of 5-HT are decreased in LH+ rats, this may explain why inflammation was less severe in this group compared to the LH– group. This hypothesis can be tested by treating LH+ rats with an SSRI and determining whether severity of inflammation increases during AA. However, the degree of paw inflammation in the LH+ rats was similar to that observed in normal animals, suggesting that it is the LH– rats which have abnormal inflammation. We intend to investigate whether this phenomenon in LH– rats may be associated with alterations in central 5-HT metabolism.

Paradoxically, increased inflammation in the LH– rats may also be a consequence of the enhanced response of this group to stress. It is now becoming clear that the indisputable anti-inflammatory effects of exogenously administered glucocorticoids in the clinic are not necessarily mirrored by increased secretion of endogenous glucocorticoids in response to stress. The stress response is extremely complex, involving the stimulated secretion of not only corticosterone but a myriad of other compounds, many of which—for example, cytokines, β-endorphin, AVP, and prolactin—are proinflammatory.[31] In the chronic inflammatory stress of AA, plasma corticosterone was elevated along with peripheral CRH, AVP, and interleukin-1β.[32,33] Thus, the net effects of stress may be to exacerbate an inflammatory condition, as has been reported for RA.[34,35] The degree to which a stressor may act in a pro- or anti-inflammatory way on a preexisting disease may be determined by the type of stressor, the specific neurochemical pathway activated, and the nature of the compounds secreted into the blood in response.

Finally, many HPA axis neuropeptides involved in the stress response are also synthesized within immune tissues and can modulate immune functions, thereby suggesting a potential mechanism whereby stress can influence the development of an inflammatory immune response mediated via neuropeptides in immune tissues.[36] A mechanism has been proposed whereby lymphocyte-derived β-endorphin can be targeted to specific sites of inflammation.[37] Elevated levels of splenic β-endorphin in the LH– group may be evidence that this opioid peptide is involved in the mechanisms that mediate increased inflammation in these animals.

CONCLUSIONS

Our data from the open field and learned helplessness studies show that increased severity of inflammation in AA is not a simple consequence of an attenuated response to stress. Thus the hypothesis of a causal relationship between these factors does not necessarily hold true. The power of our experimental design allows basal and stress-stimulated plasma corticosterone concentrations to be correlated with the

degree of inflammation within individual animals, thus permitting a direct comparison to be drawn between any variation in the response to stress and severity of arthritis in the individual rat. We conclude that, under our experimental conditions, an attenuated response to stress is not correlated with susceptibility to, or severity of, AA. This, however, does not preclude an association between ability to respond to stress and susceptibility to other forms of disease and infection. AA is an inflammatory response to an experimentally injected bacterial antigen, and it is possible that other types of disease such as those of true autoimmune etiology are more influenced by an attenuated response to stress. This experimental model should prove of considerable value in extending our observations on the relationship between the HPA axis response to stress and susceptibility to other diseases. Using this model, we could establish which diseases are associated with an attenuated response to stress (if any), and which are independent. We believe that this will provide important information about the involvement of the HPA axis in susceptibility to disease and infection.

REFERENCES

1. WEST, H. F. 1957. Corticosterone metabolism and rheumatoid arthritis. Ann. Rheum. Dis. **16:** 173–181.
2. NEECK, G. *et al.* 1990. Adrenal secretion of cortisol in patients with rheumatoid arthritis. J. Rheumatol. **17:** 24–29.
3. CHIKANZA, I. C. *et al.* 1992. Defective hypothalamic response to immune and inflammatory stimuli in patients with rheumatoid arthritis. Arthritis Rheum. **35:** 1281–1288.
4. GUDBJORNSSON, B. *et al.* 1996. Intact adrenocorticotropic hormone secretion but impaired cortisol response in patients with active rheumatoid arthritis. Effect of glucocorticoids. J. Rheumatol. **23:** 596–602.
5. MASI, A. T. & G. P. CHROUSOS. 1996. Hypothalamic-pituitary-adrenal-glucocorticoid axis function in rheumatoid arthritis. J. Rheumatol. **23:** 577–581.
6. HARBUZ, M. S. & D. S. JESSOP. 1999. Evidence for defective production of cortisol in RA. Rheumatol. In press.
7. HALL, J. *et al.* 1994. Abnormal hypothalamic-pituitary-adrenal axis function in rheumatoid arthritis. Arthritis Rheum. **37:** 1132–1137.
8. SPINEDI, E. *et al.* 1994. Sex differences in the hypothalamo-pituitary-adrenal axis response to the inflammatory and neuroendocrine stressors. Neuroendocrinology **60:** 609–617.
9. STERNBERG, E. M. *et al.* 1989. Inflammatory mediator-induced hypothalamic-pituitary-adrenal axis activation is defective in streptococcal cell wall arthritis-susceptible Lewis rats. Proc. Natl. Acad. Sci. USA **86:** 2374–2378.
10. STERNBERG, E. M. *et al.* 1992. Corticotropin releasing hormone related behavioural and neuroendocrine responses to stress in Lewis and Fischer rats. Brain Res. **570:** 54–60.
11. RIVEST, S. & C. RIVIER. 1994. Stress and interleukin-1β-induced activation of c-fos, NGF1-B and CRF gene expression in the hypothalamic PVN: Comparison between Sprague-Dawley, Fisher 344 and Lewis rats. J. Neuroendocrinol. **6:** 101–117.
12. WICK, G. *et al.* 1993. Immunoendocrine communication via the hypothalamo-pituitary-adrenal axis in autoimmune diseases. Endocr. Rev. **14:** 539–563.
13. STERNBERG, E. M. & J. LICINIO. 1995. Overview of neuroimmune stress interactions. Ann. N.Y. Acad. Sci. **771:** 364–371.
14. CHOVER-GONZALEZ, A. J. *et al.* 1998. A differential response to stress is not a prediction of severity in inflammatory disease. Stress **2:** 221–226.

15. HARBUZ, M. S. *et al.* 1992. Paradoxical responses of hypothalamic corticotropin-releasing factor (CRF) messenger ribonucleic acid (mRNA) and CRF-41 peptide and adenohypophysial proopiomelanocortin mRNA during chronic inflammatory stress. Endocrinology **130:** 1394–1400.
16. HARBUZ, M. S. *et al.* 1993. HPA axis responses to acute stress and adrenalectomy during adjuvant-induced arthritis in the rat. Am. J. Physiol. **264:** R179–R185.
17. WILDER, R. L. *et al.* 1987. Thymus-dependent and independent regulation of Ia antigen expression in situ by cells in synovium of rats with streptococcal cell wall-induced arthritis. J. Clin. Invest. **79:** 1160–1171.
18. DHABHAR, F. S. *et al.* 1993. Stress response, adrenal steroid receptor levels and corticosteroid-binding globulin levels—A comparison between Sprague-Dawley, Fischer 344 and Lewis rats. Brain Res. **616:** 89–98.
19. GROTA, L. J. *et al.* 1997. Corticosterone responses of adult Lewis and Fischer rats. J. Neuroimmunol. **74:** 95–101.
20. HARBUZ, M. S. *et al.* 1994. The effects of restraint or hypertonic saline stress on corticotrophin-releasing factor, arginine vasopressin, and proenkephalin A mRNAs in the CFY, Sprague-Dawley and Wistar strains of rat. Brain Res. **667:** 6–12.
21. VAN DE LANGERRIJT, A. G. M. *et al.* 1993. Regulation of resistance against arthritis in the Fisher rat. Clin. Exp. Immunol. **94:** 150–155.
22. ANDERSSON, I. *et al.* 1998. Analysis of adrenocortical secretory functions following acute and chronic immune challenges in inflammation-susceptible and resistant rat strains. Clin. Exp. Rheumatol. **16:** 633.
23. REINCKE, M. *et al.* 1994. Impairment of adrenocortical function associated with increased plasma tumor necrosis factor-alpha and interleukin-6 concentrations in African trypanosomiasis. Neuroimmunomodulation **1:** 14–22.
24. GRIFFIN, A. C. & C. C. WHITACRE. 1991. Sex and strain differences in the circadian rhythm fluctuation of endocrine and immune function in the rat: Implications for rodent models of autoimmune disease. J. Neuroimmunol. **35:** 53–64.
25. WILLNER, P. 1984. The validity of animal models of depression. Psychopharmacology **83:** 1–23.
26. HELLHAMMER, D. H. *et al.* 1984. Learned helplessness: Effects on brain monoamines and the pituitary-gonadal axis. Pharmacol. Biochem. Behav. **21:** 481–485.
27. NANKAI, M. *et al.* 1995. Increased 5-HT_2 receptor-mediated behaviour 11 days after shock in learned helplessness rats. Eur. J. Pharmacol. **281:** 123–130.
28. PETTY, F. *et al.* 1996. Serotonin dysfunction disorders: A behavioural neurochemistry perspective. J. Clin. Psychiatry **57 Suppl. 8:** 11–16.
29. HARBUZ, M. S. *et al.* 1998. Alteration of central serotonin onset and severity of inflammatory arthritis. Br. J. Rheumatol. **37:** 1077–1083.
30. POUSSET, F. *et al.* 1996. Effect of serotonin on cytokine mRNA expression in rat hippocampal astrocytes. Brain Res. **38:** 54–62.
31. CHIKANZA, I. C. & A. B. GROSSMAN. 1998. Hypothalamic-pituitary-mediated immunomodulation: Arginine vasopressin is a neuroendocrine immune mediator. Br. J. Rheumatol. **37:** 131–136.
32. JESSOP, D. S. *et al.* 1993. Interleukin-1 beta measured by radioimmunoassay in the rat spleen and thymus is increased during chronic inflammatory stress. Neuropeptides **24:** 367–371.
33. JESSOP, D. S. *et al.* 1995. Changes in ACTH and β-endorphin immunoreactivity in immune tissues during a chronic inflammatory stress are not correlated with changes in corticotropin-releasing hormone and arginine vasopressin. J. Neuroimmunol. **60:** 29–35.
34. ZAUTRA, A. J. *et al.* 1997. Examination of changes in interpersonal stress as a factor in dis-

ease exacerbation among women with rheumatoid arthritis. Ann. Behav. Med. **19:** 279–286.
35. AFFLECK, G. *et al.* 1997. A duel pathway model of daily stressor effects on rheumatoid arthritis. Ann. Behav. Med. **19:** 161.
36. JESSOP, D. S. 1998. Neuropeptides: Modulators of the immune system. Curr. Opin. Endocrinol. Diabetes **5:** 52–58.
37. JESSOP, D. S. 1998. Beta-endorphin in the immune system—Mediator of pain and stress? Lancet **351:** 1828–1829.