the latter condition demonstrates a dissociation of the P300 from a motor response [$F(1, 19) = 9.38$, $P < 0.01$].

The stimulus and behavioral requirements of this monkey study were similar to those used in human experiments measuring P300. Wave form morphology elicited in monkeys paralleled that of the human ERP. In the monkey the P300 was markedly attenuated if elicited by rare stimuli that were not relevant to the task; its amplitude varied in a systematic fashion with stimulus probability, and it was independent of motor responses. Thus the late positive peak recorded from monkeys in this study behaved essentially like the P300 component recorded from humans in similar experimental situations.

As far as we know, this is the first report of a nonhuman primate P300 obtained from animals engaged in a stimulus discrimination task (*14*). The component was robust and was reliably present in both animals tested. The demonstration in monkeys of a late positivity in the ERP that appears comparable to the human P300 component provides opportunities to further explore the neurophysiological and anatomical bases of its underlying neural processes.

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9. EEG (bandpass, 0.1 to 100 Hz; gain, 1000) was recorded on magnetic tape, digitized and averaged off-line, and stored on disks only when the behavior was at least 75 percent correct.
10. All three probability levels were not always tested in a given recording session.
11. The ERP's recorded from Maya were more than twice as large as those recorded from Bo at the same locations. For this reason, we normalized the data for each monkey by setting the base-to-peak amplitude of the P300 for the precentral region (Cz) at 100 percent. The amplitude of each of the other potentials was then recalculated as a percentage of P300's amplitude at Cz. The results are as follows: Cz, 100 percent (number of averages = 32); parieto-occipital region (Pz), 73 percent ($N = 37$); medial parietal region (C3), 80 percent ($N = 18$); medial parietal region (C4), 79 percent ($N = 24$); occipital region (P3), 70 percent ($N = 14$); and occipital region (P4), 61 percent ($N = 14$). The underlying cortical regions for these locations were derived from W. D. Winters, R. T. Kado, and W. R. Adey [*A Stereotaxic Brain Atlas for Macaca nemestrina* (Univ. of California Press, Berkeley, 1969)].
12. The data were amplitude-normalized for each monkey. The 0.10 and 0.50 conditions were significantly different (Duncan's multiple-range test).
13. The F ratio was 5.70 [$F(2, 19) = 5.70$, $P < 0.05$)]. The mean latencies of the 0.10 (286 msec) and the 0.30 (296 msec) conditions were significantly different from that of the 0.50 condition (323 msec) (Duncan's test).
14. E. Donchin, D. Otto, L. K. Gerbrandt, K. H. Pribram [*Electroencephalogr. Clin. Neurophysiol.* **31**, 115 (1971)] showed a P300-like wave recorded from the postcentral electrode in a nonhuman primate.
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Opioid Peptides Mediate the Suppressive Effect of Stress on Natural Killer Cell Cytotoxicity

Abstract. *The cytotoxic activity of natural killer cells was investigated in rats subjected to one of two inescapable footshock stress paradigms, both of which induce analgesia, but only one via activation of opioid mechanisms. Splenic natural killer cell activity was suppressed by the opioid, but not the nonopioid, form of stress. This suppression was blocked by the opioid antagonist naltrexone. Similar suppression of natural killer activity was induced by high doses of morphine. These results suggest that endogenous opioid peptides mediate the suppressive effect of certain forms of stress on natural killer cell cytotoxicity.*

Exposure to stress can suppress the immune system, and it is widely held that this process renders organisms more vulnerable to certain diseases, including neoplasia (*1*). For example, stress reduces the level of circulating antibodies (*2*), delays skin allograft rejection (*3*), and suppresses the reactivity of lymphocytes to mitogenic (*4*) and antigenic (*5*) stimulation. Natural killer (NK) cells are a subpopulation of lymphocytes that spontaneously recognize and selectively kill certain tumor cells and hence seem to be particularly involved in immune surveillance against neoplastic disease (*6*). Thus, it is especially noteworthy in this context that activity of NK cells is markedly reduced in animals by stressors such as surgery, starvation, and transportation (*7*). Moreover, NK activity is suppressed in college students who cope poorly with life-change stress (*8*).

Exposure to stress can also release opioid peptides from central and peripheral sites (*9*), and opioids have recently been implicated in immune regulation. For example, in vitro studies show that morphine and opioid peptides alter the percentage of T cells forming active rosettes (*10*), the reactivity of T cells to mitogenic stimulation (*11*), and the cytotoxic activity of NK cells (*12*). Additionally, opioid receptors have been identified on various components of the immune system, for example, granulocytes, monocytes, lymphocytes, and terminal complexes of complements (*10, 13*). Thus, it may be that opioid peptides released by stress mediate some of the effects of stress on the immune system.

To test this hypothesis, we investigated the effects on NK cell cytotoxicity of two types of inescapable footshock stress: (i) applied intermittently, causes analgesia that, by several criteria, appears to be mediated by opioid peptides ("opioid stress") and (ii) applied continuously, induces equally potent analgesia not involving opioids ("nonopioid stress") (*14*). We find that the opioid, but not the nonopioid, form of stress suppresses the cytotoxic activity of NK cells and that this suppression is blocked by the opioid antagonist, naltrexone. Furthermore, this suppression is mimicked by morphine administration (*15*).

Fischer 344 (F344) female rats, 50 to 60 days old, were maintained on a 12-hour light cycle with free access to food and water. Animals were subjected to one of two footshock paradigms identical in shock intensity and total "shock on" time but differing in the temporal parameters of their application. The intermittent footshock (2.0 mA, 60-Hz sine waves, on 1 of every 5 seconds for 10 minutes) caused an opioid-mediated analgesia; the continuous footshock (2.0 mA, 60-Hz sine waves, on continuously for 2 minutes) caused an equally potent

but nonopioid analgesia (*16*). Rats were given the opioid ($N = 28$) or nonopioid ($N = 15$) stress daily for four consecutive days. Another group ($N = 19$) was given daily injections of naltrexone (10 mg/kg, subcutaneously) 20 minutes before opioid footshock stress. Nonstressed control groups received either naltrexone (10 mg/kg, $N = 12$) or saline ($N = 5$) alone for 4 days or were untreated ($N = 27$).

Three hours after the last stress session or drug treatment, all animals were anesthetized with halothane and their spleens removed and dissociated into a single-cell suspension. Cells were then filtered through a nylon mesh, washed twice in phosphate-buffered saline and adjusted to a final concentration of 10^7 cells per milliliter in complete RPMI 1640 media (*17*). Natural killer activity was measured in a standard 4-hour chromium release assay using YAC-1 murine lymphoma cells labeled with sodium (chromium-51) chromate as target cells (*18*). Mixtures of 100 μl of spleen cell suspensions and 100 μl of labeled target cells were cocultured in microtiter plates in 100:1, 50:1, and 25:1 effector to target (E:T) ratios. Plates were centrifuged at 200*g* for 5 minutes and placed in an incubator. Four hours later, plates were centrifuged at 1000*g* for 10 minutes, and 100 μl of the supernatant was recovered from each well. The amount of radioactivity was determined in a gamma counter and was used to calculate percent specific cytotoxicity (*19*).

To correct for daily variations in percent specific cytotoxicity, scores for each animal were transformed to percent of the mean of untreated control values for that day. Data were analyzed using analysis of variance and post hoc Tukey tests for specific comparisons (*20*).

Exposure to the opioid, but not the nonopioid, form of footshock stress resulted in significant suppression of NK activity at all E:T ratios. Following opioid stress, percent specific cytotoxicity averaged over the three E:T ratios was suppressed to 74 percent of control values ($P < 0.01$) (Fig. 1A). This suppression was prevented by naltrexone administration. Natural killer activity in rats treated with naltrexone before stress was significantly higher than that of opioid-stressed rats not given this drug ($P < 0.05$) and did not significantly differ from any unstressed control group. Neither naltrexone nor saline injections alone significantly affected NK activity compared to untreated controls (Fig. 1A).

In another study, rats were given morphine to determine if this opiate drug would mimic the effect of endogenous opioids released by stress. F344 female rats were given daily injections of morphine sulfate for 4 days at doses of 10 mg/kg ($N = 5$), 30 mg/kg ($N = 7$), and 50 mg/kg ($N = 11$). Twelve rats served as untreated controls. Three hours after the last injection, spleens were removed and processed and NK activity was assessed as described above.

Morphine caused a dose-related suppression of NK activity (Fig. 1B). Whereas 10 mg/kg had no statistically significant effect, the two higher doses markedly suppressed NK activity ($P < 0.01$, compared to control values). This suppression was comparable to that caused by the opioid form of stress (compare A and B in Fig. 1).

The present results clearly show that stress can suppress the activity of NK cells. These findings are consistent with earlier results by others (*7*). More particularly, our results show that only the opioid form of stress suppresses NK activity, that this suppression is blocked by naltrexone, and that a suppression of similar magnitude is caused by morphine (*21*). These results support the hypothesis that opioid peptides mediate the suppressive effect of certain forms of stress on NK activity. They are also consistent with our earlier findings that opioid, but not nonopioid, stress suppresses the response of T lymphocytes to mitogenic stimulation, an effect that is blocked by naltrexone (*15, 22*).

That intermittent (opioid), but not continuous (nonopioid), footshock stress suppresses NK activity may in part be attributable to the perceived controllability of these stressors. Exposure to inescapable, but not escapable, shock causes "learned helplessness" (*23*), is associated with the release of opioids (*24*), and has been shown to enhance tumor development (*25*) and suppress immune function (*26*). Although both stress procedures used in this study are inescapable, only the intermittent footshock procedure causes learned helplessness (*27*). Controllability of stress influences NK activity in man (*8*), and psychological depression, thought to be modeled by learned helplessness in animals (*28*), has also been associated with immunosuppression (*29*).

Several physiological mechanisms could account for our findings. Morphine and opioid peptides released by stress might suppress immune function directly. As mentioned previously, in vitro studies show that opioids and morphine can alter immune function (*10–12, 30*), and opioid receptors have been identified on some immune system components (*13*). Alternatively, opioids and morphine might affect NK cells indirectly by causing or modulating the release of certain hormones. For example, adrenocorticotropic hormone (ACTH) and adrenocorticoids are known to suppress various aspects of immune function, including NK activity (*31*); and opioids have been implicated in mediating stress-induced release of these hormones (*32*). High doses of morphine similarly affect the pituitary-adrenal axis (*33*). Moreover, morphine suppresses interferon levels (*34*), and interferon powerfully induces and augments NK activity (*35*). Interesting relationships between interferon, ACTH, and opioids have recently been described (*36*); and, although the full functional significance of these findings remains to be determined, it has been suggested that ACTH and interferon compete for binding sites on NK cells (*37*). Finally, the opioid link in the stress-induced suppression of NK activity may

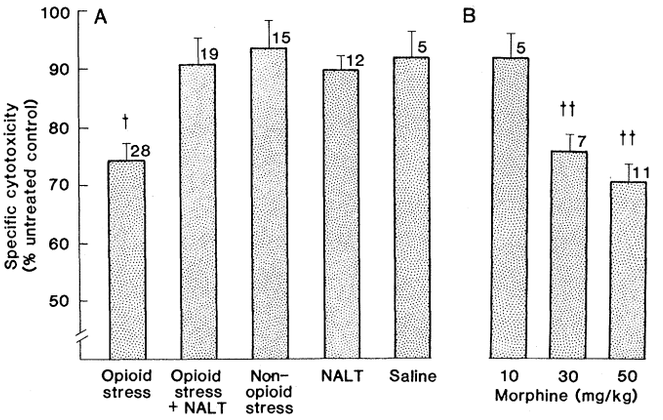

Fig. 1. Specific cytotoxic activity (expressed as percent of untreated controls) of splenic NK cells. Scores of individual untreated control animals ranged from 80 to 120 percent. (A) Effects of prior exposure (1 session per day for 4 days) to opioid or nonopioid footshock stress and administration of naltrexone (*NALT*) or saline. (B) Effects of prior administration of morphine (once daily injections for 4 days) at doses of 10, 30, or 50 mg/kg. (†) Significantly different from untreated controls and from the opioid stress + naltrexone group. (††) Significantly different from untreated controls and from the morphine 10 mg/kg group (analysis of variance and post hoc Tukey test). The number of rats in each group is indicated next to the standard error bars.

involve opioid modulation of sympathetic outflow (*38*), also thought to regulate immune function (*39*).

Although knowledge of the precise involvement of the immune system in surveillance against tumors is limited, there is evidence to suggest that NK cells play a pivotal role (*6*). In this regard, we found that the same footshock stress regimen which causes a naltrexone-sensitive suppression of NK activity also enhances development of an immunogenic, experimental mammary ascites tumor (MAT 13762B) in F344 rats (*40*). This effect also was prevented by naltrexone, and the nonopioid stress did not significantly affect tumor growth. Despite the apparent correlation between the effects of opioid stress on the immune system and on tumor growth, it is premature to conclude that NK suppression mediates the tumor potentiating effect of stress. More generally, our findings support the view that the nervous system, by significantly modulating immune function, exercises some measure of control over the inception and development of certain disease processes. These results also reinforce continuing efforts to dissect the underlying mechanisms of stress in order to account for some of the variance prevalent in studies of this kind.

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17. RPMI 1640 media was supplemented with 0.1 percent gentamicin, 1 percent l-glutamine, and 10 percent heat-inactivated fetal calf serum.
18. YAC-1 cells were propagated in tissue culture (in complete RPMI 1640 further supplemented with 1 percent sodium pyruvate and 1 percent nonessential amino acids) at 37°C, in a humidified atmosphere containing 5 percent CO_2. Cells were labeled with chromium-51 by incubation for 2 hours at 37°C. Labeled cells were washed and resuspended in complete RPMI 1640 at a concentration of 10^5 cells per milliliter.
19. Percent specific cytotoxicity was calculated using the formula $(w - s)/(m - s) \cdot 100$, where w is counts per minute in the test well, m is the maximum release counts per minute, and s is spontaneous release counts per minute. Test well counts per minute were determined in 100 μl supernatant from wells in which effector and target cells were cocultured. Portions of 100 μl of supernatant from wells in which only YAC-1 cells were incubated served to determine both spontaneous and maximum release counts per minute. In a typical assay, percent specific cytotoxicity for untreated control animals was approximately 30, 50, and 70 percent for E:T ratios 25:1, 50:1, and 100:1, respectively.
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Analysis of the Cretaceous-Tertiary Boundary Clay: Methodology Questioned

Rampino and Reynolds' study (*1*) of the clay mineralogy of Cretaceous-Tertiary (K/T) boundary clays from four localities concludes that these boundary-clay samples are neither mineralogically exotic nor distinct from clays above and below the boundary. Furthermore, Rampino and Reynolds could not detect the significant ejecta component in these boundary clays that is predicted by the asteroid-impact scenario, and they propose that volcanic material be consid-