The underlined bases code for J_H3, which is found in the A4, E109, and A47N protein sequences; J_H3 might also code for X44 and J539 protein sequences. Our sequence is in substantial agreement with the sequence of this area as determined by H. Sakano and S. Tonegawa (personal communication).
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25. J_H1 codes for M104E, Hdex 2, Hdex 3, Hdex 6, Hdex 7, Hdex 8, Hdex 9, Hdex 10, T15, S63, S107, Y5236, H8, M603, and M511. It is also possible that J_H1 might code for proteins W3207, M167, and T601, which each differ from J_H1 by one amino acid residue. Similarly, J_H2 might also code for X24, which differs from J_H2 by only one amino acid residue.
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* Present address: Biology Department, Reed College, Portland, Ore. 97202.
† Present address: Department of Microbiology, University of Texas, Southwestern Medical School, Dallas 75235.

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Electrical Stimulation of the Midbrain Mediates Metastatic Tumor Growth

Abstract. *Pulmonary metastases were counted 10 days after female rats received tail-vein injections of Walker-256 carcinosarcoma cells. Previous observations that halothane anesthesia plus hind-limb amputation increases the number of metastases were confirmed. Amputation under the analgesia of electrical stimulation of the midbrain was found to increase metastatic activity. However, the stimulus-produced analgesia alone also increased the number of metastases. Systemically administered naloxone blocked the analgesic effect of midbrain stimulation but did not block the increase in the number of pulmonary metastases.*

Recent evidence suggests that certain anesthetic agents, used alone or followed by surgery, are immunosuppressive and associated with an increased number of pulmonary metastases in syngeneic mice inoculated with fibrosarcoma (*1*, *2*). Barbiturates alone increase the induction rate and the metastatic spread of several types of tumors in rodents (*3*). A recent report (*4*) documented accelerated growth of testicular cancer after cytoreductive surgery (there was no obvious explanation for this exacerbation of the disease). Halothane, however, does not increase tumor growth even with prolonged, repeated exposure (*5*). Our intent in this study was to isolate the role of surgery from that of inhalation anesthesia in the development of induced pulmonary metastases. Therefore, we amputated the hind limbs of rats anesthetized by electrical stimulation of the mesencephalic periaqueductal gray (PAG) region.

Stimulus-produced analgesia (SPA) has been elicited by delivering electric current to various brain locations, especially those around the mesencephalic cerebral aqueduct (*6*). Stimulation of the PAG region and nearby sites abolishes pain responses to noxious stimuli mediated by spinal cord reflex, yet does not affect motor activity. Morphine also blocks these reflexes (*7*). Both morphine analgesia and SPA can be impeded by the specific opiate antagonist naloxone (*8*). The analgesia elicited by stimulating the PAG region was more than sufficient for easy amputation of the hind limbs of our rats.

Although much of our data has been obtained from mouse studies, our neurosurgical experience in stereotaxic techniques is well developed with rats. We therefore chose the female Sprague-Dawley rat as the experimental animal. Before proceeding to the SPA experiments, then, it was necessary to recapitulate in the rat model the preliminary halothane and surgery studies done in the mouse.

Walker-256 carcinosarcoma cells, maintained in ascites form in Fischer rats, were harvested by sterile peritoneal tap and washed three times in phosphate-buffered saline (PBS). Cell viability, as determined by trypan blue dye exclusion, was greater than 95 percent in each experiment. Next, a dose-response curve for production of pulmonary metastases was established. On day 0, we injected 10^2 to 10^6 tumor cells in PBS (0.2 ml) into the tail veins of the rats after exposing them to ether for 30 seconds. Ten days later, the animals were killed with an overdose of ether, and the number of pulmonary metastases was counted by the India ink method of Wexler (*9*).

We chose a dose of 1×10^3 cells for further experiments because it produced a consistently low number of metastases. Anesthesia was induced with 4 percent halothane in oxygen for 1 minute and maintained with 1 percent halothane for 6 minutes (the length of time necessary to perform the amputations) (*10*). We found that the mean number of metastases in group 2 (tumor plus halothane) was similar to that in the controls (tumor alone). However, in group 3 (tumor plus halothane plus surgery), the number was markedly increased. The effect of surgery alone could not be deduced by following this protocol.

In order to evaluate the effects of surgery under SPA, we anesthetized the rats with ketamine HCl (150 to 250 mg/kg) and positioned them in a stereotaxic frame. A concentric 30-gauge electrode (*11*) was then implanted into the substantia grisea centralis at the junction of

Table 1. Number of pulmonary metastases in the different treatment groups (mean ± standard error).

Group	Treatment	Rats (No.)	Metastases	
			Raw data	Transformed data
1	Tumor	10	0.7 ± 0.2	0.64 ± 0.29
2	Tumor and halothane	10	1.9 ± 0.3	1.26 ± 0.25
3	Tumor, halothane, and surgery	9	17.1 ± 2.6	4.05 ± 0.70
4	Tumor and electrodes	7	1.1 ± 0.3	0.89 ± 0.34
5	Tumor and ESM (SPA)	9	15.9 ± 2.3	3.92 ± 0.63
6	Tumor, ESM, and surgery (SPA)	9	16.9 ± 1.8	4.05 ± 0.49
7	Tumor and naloxone	8	4.1 ± 1.0	1.91 ± 0.37
8	Tumor, naloxone, and ESM (no SPA)	8	12.6 ± 2.7	3.36 ± 1.21

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the pars lateralis and the pars medialis (*12*). The electrode wires were attached to an integrated-circuit connector. The entire array was fixed with self-curing plastic to two anchor screws in the skull. This permitted deferred stimulation. (The accuracy of the implant was determined by delivering a coagulating radiofrequency current to the electrode to mark the target immediately before the animal was killed on experimental day 10.)

After implanting the electrodes, we waited 14 days before performing any further manipulations. This was deemed a sufficient period to avoid sequelae of the implantation procedure. The animals receiving electrical stimulation of the midbrain (ESM) were stimulated for 7 minutes; 0.2-msec pulses at 1 μA, given at the rate of 100 pulses per second for 1 minute, were sufficient to abolish all aversive responses to pain, although motor activity was unimpaired (Table 1). There were no significant differences in the number of metastases between the implanted controls (group 4) and the unimplanted controls (group 1). When stimulation was added, there was a dramatic increase in the number of metastases. In this case, surgery did not extend that effect; there were no significant differences in the number of metastases between the SPA and SPA plus surgery groups (*13*).

It is clear that the release of endogenous opiates is increased during midbrain stimulation and that this mediates the resultant analgesia (*14*). Thus naloxone was administered to animals before stimulation to determine whether opiate receptor binding was necessary to achieve the increase in the number of metastases induced by ESM. Naloxone HCl (1.0 mg/kg) was given intravenously 15 minutes before the manipulations on day 0. This dose was found to be maximally effective in inhibiting SPA in the rats (*15*). When naloxone was given to unstimulated rats (group 7), the number of metastases they developed did not differ significantly from that of the controls (*13*). When analgesia was blocked by naloxone, stimulation sufficient to cause analgesia even in its absence resulted in an increase in the number of metastases (group 8).

Our data suggest that the analgesia associated with ESM may not play a major role in mediating the observed increase in metastastic activity, since naloxone administration was not associated with a decrease in metastasis. However, naloxone may not block all opioid peptide receptors. Receptors for opioid peptides are present on lymphocytes, cells that can be responsible for the immune destruction of tumor cells (*16*). In addition, Kosterlitz *et al.* (*17*) demonstrated low effects of naloxone against delta receptors for natural opioid peptides in the mouse. Beta endorphin has a high affinity for both mu and delta receptors.

There has been much controversy regarding the effects of stress (the release of hormones or neurotransmitters) on tumor growth. Riley (*18*) subjected C3H/He mice to environmental conditions producing various degrees of chronic stress, and found an increased incidence of mammary carcinoma in the most highly stressed groups. Spackman *et al.* (*19*) found that stress in the murine host rapidly elevates plasma corticosterone levels, produces involution of the thymus, and reduces the number of circulating T cells. However, Peters and Kelly (*20*) showed that total adrenalectomy followed by laparotomy and manipulation of the mesentery did not prevent an increase in tumor growth in mice. This suggests that in the mouse, released endogenous steroids do not necessarily play a primary role in promoting tumor growth.

Sklar and Anisman (*21*) demonstrated that a single session of inescapable shock resulted in earlier tumor appearance, increased tumor size, and decreased survival in DBA/2J mice bearing a syngeneic P815 mastocytoma. However, they did not propose a mechanism for this phenomenon. Invoking an immune-mediated effect is tempting on the basis of a study by Monjan and Collector (*22*). They found that acute stress is associated with decreased T and B cell mitogenesis and higher cortisol levels, whereas long-term stress results in normal cortisol levels and enhanced immune responses. This observation is supported by Sklar and Anisman's data, which show that multiple shock (that is, extended stress) is not associated with increased tumor growth. Elevated levels of somatotropic hormone were noted, and hypophysectomized animals were unable to regain immune responsiveness.

The mechanism responsible for this phenomenon cannot be explained in the face of the conflicting data. In previous studies with mice, we found that the increased metastatic activity associated with thiopental or halothane and surgery was clearly related to immunosuppression (*2*). We cannot yet define the mechanism, immunologic or otherwise, by which ESM alters metastatic activity. Understanding this phenomenon necessitates definition of the role of the immune system in response to ESM. It will also be necessary to separate the endorphin system from the stress mechanism, if indeed they are different.

RICHARD H. SIMON
EDMUND J. LOVETT III
DAVID TOMASZEK, JOEL LUNDY
Department of Surgery, University of Connecticut Health Center, Farmington 06032

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10. The anesthetic was generated by a Frazer-Sweatman VMS machine with a Fluotec adapter. A modified port was used to fit snugly over the muzzles of the rats.
11. Steel wire (0.04-inch in diameter) was connected in continuity to a 30-gauge needle. Copper wire (40-gauge) coated with nylon was passed through a slot in the side and out the tip. The entire array was doped with insulating epoxy. The tip was ground to expose the steel of the needle and the copper core. No more than 2 kilohms of impedance was allowed between the ground and the copper, and a minimum impedance of 250 kilohms was tolerated between the conductors.
12. The inclination of the head was set such that the bregma was 2.25 ± 0.5 mm above the lambda. The electrode was passed through a small hole drilled in the skull. The coordinates were 7.10 mm beneath the skull surface, 5.00 mm posterior, and 0.5 mm left-lateral.
13. After the data were subjected to a square root transform, a one-way analysis of variance was performed. An F score of 22.5 indicates significance at $P < .001$. Duncan's new multiple range test was applied to the raw data for intergroup comparisons. The control groups (1, 4, and 7) and the group receiving halothane alone (2) were alike (no significant difference at $P = .05$). Groups 1, 2, 4, and 7 were significantly different from the stimulated groups 3, 5, and 8 ($P < .01$ in all groups we considered significantly different).
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