tion of the contribution of contextual stimuli to tolerance (*10, 15*).

Opiate-inexperienced male rats (Wistar-derived, 90 to 110 days old) with permanent jugular cannulas (*16*) were intravenously injected with diacetylmorphine hydrochloride (heroin) 15 times, one injection every other day. The dose was increased according to the following schedule: first injection, 1 mg/kg; second and third injections, 2 mg/kg; fourth through seventh injections, 4 mg/kg; and eighth through fifteenth injections, 8 mg/kg. Each rat also received one volumetrically equated injection of the vehicle (5 percent dextrose solution) on days when it was not injected with heroin.

The injections were given in two different environments. One was the colony, where the rats were individually housed. The animal was removed from its cage, injected, and returned to its cage. The other environment was a different room with constant white noise (60 dB SPL). Rats were injected 15 minutes after being transferred to this room and were kept there for an additional 2 hours. One group of rats received heroin in the distinctive room and dextrose in the colony; a second group received heroin in the colony and dextrose in the distinctive room. Finally, the subjects in each group were placed in one of the two environments and injected with 15 mg of heroin per kilogram. This procedure permitted evaluation of the effects of a high dose of heroin in the context of cues that had previously signaled lower doses of the drug [similarly tested (ST) rats] and in the context of cues not previously associated with the drug [differently tested (DT) rats]. It should be emphasized that, throughout the study, both experimental groups were injected an equal number of times with the same doses of heroin at the same intervals between injections [results obtained from the two counterbalanced conditions were not significantly different (*17*)]. A third group received 30 daily injections of dextrose in each of the two environments on an alternating schedule and then an injection of heroin (15 mg/kg) in one of the two environments. Thus the control rats had no experience with the opiate before the final session.

Chi-square analysis indicates that mortality differed significantly among groups ($P < .001$) (*18*). Both groups with pretest experience with sublethal doses of heroin were more likely to survive the highest dose than control animals ($P < .002$), suggesting that tolerance resulted from the sublethal heroin injections independent of the environment associated with those injections. However, mortality was significantly higher in DT than in ST rats ($P < .001$), indicating that identical pretest pharmacological histories do not necessarily result in the display of equivalent tolerance to the lethal effect of heroin. The experiment was conducted in six replications (three involving testing in each of the two environments), and in every replication a greater proportion of DT than ST rats died ($P < .02$, binomial test). The combined results for all replications are summarized in Table 1.

In conclusion, groups of rats with the same pharmacological history of heroin administration can differ in mortality following administration of a high dose of the drug: rats that received the potentially lethal dose in the context of cues previously associated with sublethal doses were more likely to survive than animals that received the dose in the context of cues not previously associated with the drug.

SHEPARD SIEGEL
Department of Psychology, McMaster University, Hamilton, Ontario L8S 4K1, Canada
RILEY E. HINSON
Department of Psychology, University of Western Ontario, London, Ontario N6A 5C2
MARVIN D. KRANK
JANE MCCULLY
Department of Psychology, McMaster University

References and Notes

1. C. C. Hug, in *Chemical and Biological Aspects of Drug Dependence*, S. J. Mulé and H. Brill, Eds. (CRC Press, Cleveland, 1972), pp. 307–358.
2. D. B. Louria, T. Hensle, J. Rose, *Ann. Intern. Med.* **67**, 1 (1967); P. Mason, *Mt. Sinai Hosp. J. N.Y.* **34**, 4 (1967); D. W. Maurer and V. H. Vogel, *Narcotics and Narcotic Addiction* (Thomas, Springfield, Ill., 1973), p. 101.
3. P. H. Abelson, *Science* **168**, 1289 (1970); M. Helpern, *Hum. Pathol.* **3**, 13 (1972).
4. J. H. Jaffe and W. R. Martin, in *The Pharmacological Basis of Therapeutics*, A. G. Gilman, L. S. Goodman, A. Gilman, Eds. (Macmillan, New York, ed. 6, 1980), pp. 494–534.
5. J. L. Duberstein and D. M. Kaufman, *Am. J. Med.* **51**, 704 (1971).
6. D. W. Fraser, *J. Am. Med. Assoc.* **217**, 1387 (1971); J. C. Garriott and W. Q. Sturner, *N. Engl. J. Med.* **289**, 1276 (1973); D. H. Huber, *J. Am. Med. Assoc.* **229**, 689 (1974).
7. E. M. Brecher, *Licit and Illicit Drugs* (Little, Brown, Boston, 1972), pp. 101–114; T. Reed, *Int. J. Addict.* **15**, 359 (1980).
8. *Final Report of the Commission of Inquiry into the Non-Medical Use of Drugs* (Information Canada, Ottawa, 1973), pp. 310–315.
9. A. B. Light and E. B. Torrance, *Arch. Intern. Med.* **44**, 1 (1929).
10. S. Siegel, in *Psychopathology in Animals: Research and Clinical Applications*, J. D. Keehn, Ed. (Academic Press, New York, 1979), pp. 143–168; *Fed. Proc. Fed Am. Soc. Exp. Biol.*, in press; in *Research Advances in Alcohol and Drug Problems*, Y. Israel *et al.*, Eds. (Plenum, New York, in press).
11. I. P. Pavlov, *Conditioned Reflexes* (Oxford Univ. Press, London, 1927), pp. 35–37.
12. F. Obál, *Acta Physiol. Acad. Sci. Hung.* **30**, 15 (1966); A. Wikler, *Cond. Reflex* **8**, 193 (1973).
13. C. Advokat, *Behav. Neural Biol.* **29**, 385 (1980); R. K. Ferguson and C. L. Mitchell, *Clin. Pharmacol. Ther.* **10**, 372 (1969); G. J. LaHoste, R. D. Olson, G. A. Olson, A. J. Kastin, *Pharmacol. Biochem. Behav.* **13**, 799 (1980); R. F. Mucha, C. Volkovskis, H. Kalant, *J. Comp. Physiol. Psychol.* **95**, 351 (1981); S. Siegel, *Science* **193**, 323 (1976); *J. Comp. Physiol. Psychol.* **92**, 1137 (1978); ———, R. E. Hinson, M. D. Krank, *J. Exp. Psychol. Anim. Behav. Processes* **4**, 188 (1978); S. T. Tiffany and T. B. Baker, *J. Comp. Physiol. Psychol.* **95**, 747 (1981).
14. S. Siegel, R. E. Hinson, M. D. Krank, *Behav. Neural Biol.* **25**, 257 (1979).
15. R. L. Hayes and D. J. Mayer, *Science* **200**, 343 (1978).
16. The cannula was a modified version of that described by R. J. Brown and C. B. Breckenridge [*Biochem. Med.* **13**, 280 (1975)]. Subjects were cannulated 1 week before the experiment. The rate of injection was controlled by infusing the injected substance at a rate of 0.005 ml/sec through the intravenous cannula with a Harvard model 902 infusion pump. The concentration of heroin (in sterile 5 percent dextrose) was 3.125, 6.250, 12.500, 25.000, and 50.000 mg/ml for the doses of 1, 2, 4, 8, and 15 mg/kg, respectively. Thus the duration of the infusion for all rats at all dose levels was equivalent (about 1 min/kg).
17. Of the 37 ST rats, 17 were injected with heroin in the distinctive room and 20 were injected with heroin in the colony. Half of the 42 DT rats were injected with heroin in each of the environments.
18. For subjects that died as a result of the injection of heroin at 15 mg/kg, the median times for the start of the injection until death (as determined by lack of heartbeat) in ST, DT, and control groups were 187, 174, and 164 seconds, respectively. Thus these animals died soon after the start of the lethal injection (differences between groups were insignificant), as is frequently the case with human victims of drug overdose (*7, 8*).
19. Supported by grants from the Natural Sciences and Engineering Research Council of Canada and the National Institute on Drug Abuse. The assistance of D. Mitchell and W. Stephaniv is gratefully acknowledged.

30 November 1981

Tumor Rejection in Rats After Inescapable or Escapable Shock

Abstract. *Rats experienced inescapable, escapable, or no electric shock 1 day after being implanted with a Walker 256 tumor preparation. Only 27 percent of the rats receiving inescapable shock rejected the tumor, whereas 63 percent of the rats receiving escapable shock and 54 percent of the rats receiving no shock rejected the tumor. These results imply that lack of control over stressors reduces tumor rejection and decreases survival.*

Psychological states involving the loss of control, such as helplessness, bereavement, and depression, are associated with an increased incidence of cancer (*1*). The influence that psychological variables may have on the development and maintenance of malignancies is difficult to determine from correlational studies of humans: the psychological states may have preceded cancer onset, resulted from it, or occurred at the same time. Therefore, animal studies in which psy-

0036-8075/82/0423-0437$01.00/0

chological factors are manipulated independently of the onset of cancer are required.

One means for assessing the contribution of the psychological factor of stressor controllability to tumor susceptibility is the "yoked" testing procedure. In this procedure, two groups of subjects receive identical exposure to an aversive event and differ only in the extent to which their responses influence the event. One group controls the event and can terminate it, as by pressing a bar. The second group helplessly receives the stimulus as governed by the first group. A third group receives no aversive stimulation, and therefore provides a control for the effects of the tumor itself.

Experiencing an uncontrollable aversive event produces behavioral and physiological effects that do not occur when the event is controllable. For example, animals that have received inescapable shock show severe deficits in learning to escape from other aversive stimuli (*2*), become submissive in competition for food (*3*), fail to escape from frustrating situations (*4*), and show less aggression when subjected to additional shock (*5*). Physiologically, inescapable shock leads to catecholamine depletion in the central nervous system (*6, 7*), activation of the hypothalamic-pituitary-adrenal axis (*8*), weight loss (*9*), and gastric ulceration (*9, 10*). Finally, uncontrollable aversive events increase psychological distress in humans (*11*).

Tumor growth is also increased by the same amounts of uncontrollable shock which produce the behavioral deficits and physiological changes in animals. Sklar and Anisman (*12*) found that tumors grow more quickly in mice given inescapable shock than in mice given escapable shock or no shock. However, since the dose of tumor cells was large enough to ensure tumor development in every animal, the effect of inescapable shock on the process of tumor rejection could not be assessed. The physiological mechanisms that promote the growth of an established tumor are different from those that influence the rejection or initial development of a tumor and subsequent metastasis (*13, 14*). As Sklar and Anisman pointed out, in order to determine whether uncontrollable stressful events increase the risk of cancer, we need to investigate the influence of this psychological variable on tumor rejection.

In our experiment, we measured tumor rejection as a function of the controllability of shock by using a dose of tumor cells designed to induce tumors in only 50 percent of unshocked rats. If the shock, as a physical stressor, was sufficient to influence tumor development, then the escapable- and inescapable-shock groups would show the same rate of tumor rejection. However, if the psychological state induced by uncontrollable shock inhibited tumor rejection, then only rats exposed to inescapable shock would show a reduced ability to resist the tumor challenge.

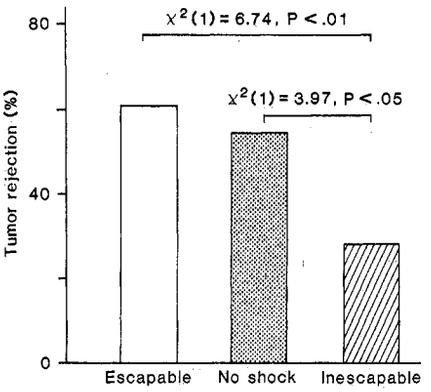

Fig. 1. Tumor rejection in rats subjected to escapable shock, no shock, or inescapable shock. The statistical data are based on chi-square analysis with Yates' correction for continuity.

The subjects, 93 Sprague-Dawley adult male rats (Holtzman), were housed individually in the University of Pennsylvania's general colony environment (*15*). They were placed on a photoperiodic cycle of 14 hours of light and 10 hours of darkness and given unlimited access to food and water. All experimental procedures were run during the light phase.

For the shock manipulation, two identical sound-attenuating chambers (background noise, 76 dB) were used, each containing one shock box (*16*). A 600-V a-c transformer with a limiting resistor supplied constant current through a Lafayette scrambler (model 82500). The shock (0.7 mA) was delivered to the grid floor and the metallic sides of each box. Depression of a bar in the escapable-shock box terminated the shock in both boxes. A bar press in the inescapable-shock box had no effect.

Syngeneic Sprague-Dawley rats with growing Walker 256 sarcoma tumors were killed after a tumor mass was palpable for 14 days. The tumor was dissected into pea-sized pieces, which were forced through a 40-mesh surgical sieve and mixed with equal parts (by weight) of Gey's solution. For implantation, 0.2 cm^2 of the tumor preparation (*17*) was injected subcutaneously into the left lower anterior flank of each subject. Twenty-four hours later the rats were divided randomly into three groups: escapable shock ($N = 30$), inescapable shock ($N = 30$), and no shock ($N = 33$). The rats were exposed to 60 shock trials at random intervals. The rats in the escapable shock group could terminate the shock at any time by pressing the bar. If they did not press the bar, the shock ended after 60 seconds. As described earlier, the rats in the yoked condition simultaneously received the same amount of shock as their partners, but were helpless to control it. After the trials, the rats were returned to their home cages and were maintained on the same feeding and light-dark schedule as before.

Tumor rejection was defined as the absence of a tumor 30 days after implantation. The 30-day cutoff was chosen as a result of extensive pilot work showing that all rats that had this tumor 30 days after cell injection died within another 60 days and that no rats that were tumor-free at 30 days developed the tumor or died in the next 60 days. Although all tumors were palpable, tumor absence or presence was confirmed by dissection.

Rats receiving inescapable shock were only half as likely to reject the tumor and twice as likely to die as rats receiving escapable shock or no shock (Fig. 1). Only 27 percent (8 of 30) of the rats given inescapable shock rejected the tumor, compared to 63 percent (19 of 30) of the rats given escapable shock and 54 percent (18 of 33) of the rats given no shock. Thus, inescapable shock increased the probability that an animal would die by decreasing the rate of tumor rejection.

Immunological activity is probably an important line of defense against cancer metastasis, and there is evidence that the immune system is suppressed after uncontrollable aversive events. For example, immunosuppression (defined by decreased spleen cell reactivity) was found in animals exposed to uncontrollable acceleration stress (*18*). Monjan and Collector (*19*) found that uncontrollable noise stress produced T and B cell suppression in mice. Suppression of lymphocyte function is related to the intensity of aversive stimulation (*20*). In humans, immunosuppression has been reported during bereavement (*21*).

One possible connection between the psychological experience of uncontrollable stress and immunosuppression is suggested by Selye's general adaptation syndrome (*22*). When an animal is exposed to a stressor, the arousal releases adrenocorticotropic hormone and adrenocortical activation occurs. This in turn causes immunosuppression. The more

aroused the animal, the greater the immunosuppression. Subjects receiving inescapable stress may experience greater arousal, as indicated by emotional responses and physiological changes, than subjects receiving escapable stress (*6–11*).

Recent evidence suggests that two immunological mechanisms are involved in tumor defense. After the primary tumor is established, the nonsensitized macrophages and lymphocytes destroy the developing tumor mass and inhibit regrowth (*13*). The second mechanism involves defense against metastasis, whereby sensitized T cells destroy cells that dislodge from the primary mass (*14*).

In summary, inescapable shock decreased tumor rejection. The low rate of tumor rejection was not a function of shock per se, but resulted from the animals' lack of control over shock. The psychological experience somehow interfered with the ability of the organism to resist tumor development. These results are consistent with those of Sklar and Anisman (*12*), and demonstrate that a psychological variable can decrease an animal's ability to reject a tumor.

MADELON A. VISINTAINER
JOSEPH R. VOLPICELLI
MARTIN E. P. SELIGMAN
Department of Psychology, University of Pennsylvania, Philadelphia 19104

References and Notes

1. B. H. Fox, *J. Behav. Med.* **1**, 45 (1978); A. H. Schmale, *Adv. Psychosom. Med.* **8**, 20 (1972).
2. S. F. Maier and M. E. P. Seligman, *J. Exp. Psychol. Gen.* **105**, 3 (1976).
3. P. M. Rappaport and S. F. Maier, *Anim. Learn. Behav.* **6**, 160 (1978).
4. R. Rosellini and M. E. P. Seligman, *J. Exp. Psychol. Anim. Behav. Processes* **1**, 149 (1975).
5. S. F. Maier, C. Anderson, D. A. Lieberman, *J. Comp. Physiol. Psychol.* **81**, 94 (1972).
6. J. M. Weiss, E. A. Stone, M. Harrell, *ibid.* **72**, 160 (1970); ———, H. J. Glazer, L. A. Hohorecky, in *Animal Models in Human Psychobiology*, G. Serban and A. Kling, Eds. (Plenum, New York, 1976), pp. 141–173.
7. H. Anisman, in *Psychopharmacology of Aversively Motivated Behavior*, H. Anisman and G. Bignami, Eds. (Plenum, New York, 1976), pp. 119–172.
8. S. F. Maier, S. Davies, J. W. Grau, R. L. Jackson, D. H. Morrison, J. Moye, J. Madden IV, J. D. Barchas, *J. Comp. Physiol. Psychol.* **94**, 1172 (1980); J. Rossier, E. D. French, C. Rivier, N. Ling, R. Guillemin, F. E. Bloom, *Nature (London)* **270**, 618 (1977).
9. J. M. Weiss, *J. Comp. Physiol. Psychol.* **77**, 14 (1971).
10. ———, *ibid.*, p. 1; *ibid.*, p. 21.
11. W. R. Miller and M. E. P. Seligman, *J. Abnorm. Psychol.* **84**, 228 (1975).
12. L. S. Sklar and H. Anisman, *Science* **205**, 513 (1979).
13. P. Alexander, in *Cancer Invasion and Metastasis: Biologic Mechanisms and Therapy*, S. B. Day, W. P. L. Meyers, P. Stansly, S. Grattini, M. G. Lewis, Eds. (Raven, New York, 1977), pp. 5–10; R. B. Herberman and H. T. Holden, *Adv. Cancer Res.* **27**, 6 (1977).
14. R. J. Oehler and R. B. Herberman, *Int. J. Cancer* **21**, 221 (1978); H. F. Pross and M. G. Baines, *Immunol. Immunother.* **3**, 78 (1977).
15. V. Riley, *Science* **189**, 465 (1975).
16. Each shock box was 30.5 cm long, 20.4 cm wide, and 19.7 cm high. The sides were clear plexiglass and the end walls were stainless steel. The floor was a stainless steel grid with bars 0.65 cm in diameter and 1.90 cm apart. On the left-hand wall was a lever 6.80 cm wide protruding 1.5 cm into the box and 6.0 cm above the grid floor.
17. Ten 0.2-cm^2 portions of the preparation were randomly sampled and tested for cell viability by trypan blue exclusion. Portions yielding 6×10^3 viable cells were used for implantation.
18. H. Folch and B. H. Waksman, *J. Immunol.* **112**, 127 (1974).
19. A. A. Monjan and M. J. Collector, *Science* **196**, 307 (1977).
20. S. E. Keller, J. M. Weiss, S. J. Schleifer, N. E. Miller, M. Stein, *Science* **213**, 1397 (1981).
21. R. W. Bartrop, L. Lazarus, C. Luckhurst, L. G. Kiloh, R. Penny, *Lancet* **1977-I**, 834 (1977).
22. H. Selye, *The Stress of Life* (McGraw-Hill, New York, 1956), pp. 56–73.
23. We thank J. Radcliffe for his advice and for supplying the tumor. Supported by National Institutes of Health medical scientist training grant GM 07170 to J.R.V. and by National Institute of Mental Health grant 19604 and University of Pennsylvania biomedical research grant 524 341 to M.E.P.S.

30 December 1981

Behavioral Sequences During Dominance Hierarchy Formation in Chickens

Abstract. *Dominance hierarchies near linearity (containing mostly transitive and few intransitive triads) are common in many species. Analysis of the possible sequences for forming dominance relationships shows that two ensure transitivity, and two others produce either transitive or intransitive triads. Experiments with chickens show that in groups of three and four they most often use the two sequences that ensure transitivity and thus linear hierarchies. Examination of such sequences may help explain the formation of near linear hierarchies in other species.*

Although research in social ethology has provided information about the impact of social relationships and roles on behavior and fitness in animals (*1*), much less is known about the processes or mechanisms through which social relationships are formed and roles occupied (*2*). This study describes behavioral processes used by chickens in forming one of the classic social structures in ethology: linear and near linear dominance hierarchies. Such hierarchies are found across a broad range of species including, for example, wasps, bumble bees, chickens, cows, buffaloes, rhesus monkeys, and young humans (*3, 3a*).

Earlier research has attempted to explain the structural form of dominance hierarchies by differences in individual attributes like aggressiveness, size, hormone levels, and past social performance or differences in pairwise competitive ability (*1*). However, analytical work by Landau and Chase (*4*), and experimental results of Bernstein and Gordon and King (*5*) indicate that although individual difference and pairwise ability models provide useful information about dominance relationships, they do not explain hierarchy structures themselves.

In order to discover the behavioral processes used in hierarchy formation I observed the establishment of dominance relationships in groups of chickens. Chickens are a good choice because they readily form linear hierarchies in small groups, and their dominance behavior is well defined. In the first experiment I used 24 groups, each with three white Leghorn hens (triads), and in the second experiment I used 14 groups of four (tetrads). The hens in each group were either unacquainted, or if acquainted, had been separated for several months—enough time to forget previous relationships (*6*). Hens were housed individually before triad and tetrad grouping and were observed in a neutral cage in a separate room.

When put together, all occurrences of three aggressive contact behaviors were recorded: peck (including feather pull), scratch (with the claws), and jump on. An SSR keyboard (*7*) was used to record data for the triads and an Apple microcomputer for the tetrads. The triads were observed for 4 hours each and the tetrads for 12 hours (*8*) each; a combined total of 2801 aggressive acts were recorded for the triads and 7402 acts for the tetrads (*9*).

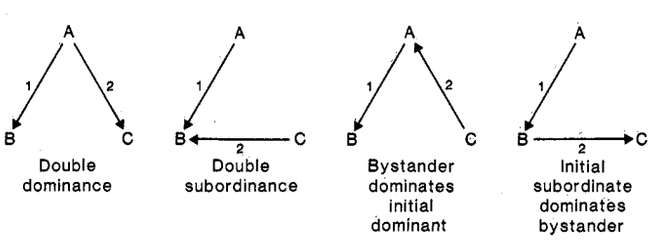

Fig. 1. The four possible sequences in the formation of the first two dominance relationships in triads. Relationships are numbered in order of formation.

0036-8075/82/0423-0439$01.00/0