

have been obtained in another laboratory (11). A family of related type D retroviruses could conceivably cause a spectrum of immunodeficiency diseases in nonhuman primates.

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References and Notes

1. N. L. Letvin *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 2718 (1983).
2. R. V. Henrikson *et al.*, *Lancet* **1983-I**, 388 (1983).
3. R. D. Hunt *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 5085 (1983).
4. N. L. Letvin *et al.*, *Lancet* **1983-II**, 599 (1983).
5. Hematologic data for Mc 184-80 were as follows: hemoglobin, 7.4 g/dl with microcytic hypochromic indices; a leukocyte count of

$1.5 \times 10^3/\text{mm}^3$ with a differential count of 46 percent neutrophils, 6 percent band forms, 46 percent lymphocytes, and 2 percent monocytes. Hypoproteinemia was detected with total protein 3.3 g/dl, albumin 1.6 g/dl, and globulin 1.7 g/dl. The data for Mc 398-80 were: hemoglobin, 9.6 g/dl with microcytic hypochromic indices; a leukocyte count of $4.8 \times 10^3/\text{mm}^3$ with a differential count of 21 percent neutrophils, 5 percent band forms, 67 percent lymphocytes, 3 percent eosinophils, 2 percent monocytes, and 2 percent blast forms. Liver function tests were normal.

6. N. W. King, R. D. Hunt, N. L. Letvin, *Am. J. Pathol.* **113**, 382 (1983).
7. C. Barker, J. Wills, E. Hunter, in preparation.
8. I.-M. Chiu, P. R. Andersen, S. A. Aaronson, S. R. Tronick, *J. Virol.* **47**, 434 (1983).
9. D. L. Fine *et al.*, *J. Natl. Cancer Inst.* **54**, 651 (1975).
10. R. Desrosiers, in preparation.
11. E. Hunter, personal communication.
12. I.-M. Chiu and S. Aaronson, personal communication.
13. B. Hirt, *J. Mol. Biol.* **26**, 365 (1967).
14. E. M. Southern, *ibid.* **98**, 503 (1975).
15. R. C. Desrosiers, *J. Virol.* **39**, 497 (1981).
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Epinephrine Enables Pavlovian Fear Conditioning Under Anesthesia

Abstract. Rats underwent Pavlovian defensive conditioning (noise paired with shock) while under general anesthesia. Peripheral administration of epinephrine (0.01 to 1.0 milligram per kilogram of body weight) during training resulted in the acquisition of conditioned fear, as shown 10 days later by conditioned suppression of water drinking. Analysis of heart rate and measurement of reflexes during training indicated that epinephrine did not lighten the state of anesthesia. These results indicate that epinephrine enables the learning of conditioned fear in the anesthetized brain.

Sensory systems process stimuli during states of deep anesthesia. Most knowledge of sensory neurophysiology is based on recordings obtained from anesthetized animals. Yet the results of such sensory processing apparently are not remembered, or at least not retained in a form that is later expressed in behavior. For example, anecdotal reports and clinical studies of learning in humans under general anesthesia have failed to provide unequivocal evidence for learning. Apparent learning occurs only if the (usually auditory) stimuli have "high emotional content, coinciding with lightening of anesthesia" (1). Animal studies have yielded similar findings (2). Learning and memory can be facilitated by pharmacological agents and hormones (3). We asked whether a facilitatory treatment would enable learning to take place and be detectable behaviorally later, during a state of general anesthesia. We now report that epinephrine administered to deeply anesthetized rats enables Pavlovian fear conditioning. The effect is

not due to a lessening of the depth of anesthesia by epinephrine.

Male Sprague-Dawley rats ($N = 44$) 80 to 120 days of age at the time of testing were maintained in individual cages in a vivarium with a 12-hour light cycle. Some subjects had not been anesthetized previously, in which case the electroencephalogram (EEG) was recorded from needle electrodes (Grass) inserted bilaterally in the scalp after animals were anesthetized. Other subjects had screw electrodes implanted bilaterally over the cerebral cortex (7 mm posterior and 2 mm lateral to bregma) under sodium pentobarbital anesthesia (48 mg per kilogram of body weight, injected intraperitoneally) from 14 to 21 days before training (4).

On the day of training, animals were anesthetized with sodium pentobarbital (48 mg/kg, intraperitoneal) supplemented with chloral hydrate (30 to 60 mg/kg, intraperitoneal) (5). The animal was placed on a heating pad within an acoustic chamber and held lightly by the

mouth plate and snout bar of a rat stereotaxic apparatus. An earphone was placed within 1 cm of the right ear. The EEG leads were connected and bilateral needle electrodes were inserted subcutaneously into the thorax to record the electrocardiogram (EKG). Needles were inserted into the right posterior flank and attached to a constant current stimulator by way of a stimulus isolation unit (6). Reflexes were then tested; if present to any degree, the animal received a supplemental injection of chloral hydrate and was retested within 5 minutes. Training was not initiated until all reflexes were absent (7).

The conditioned stimulus (CS) was 15 seconds of white noise (90 dB); the unconditioned stimulus (US) was a 50-msec train of 50-Hz, 5.0-msec pulses (4 to 6 mA) delivered to the right hindlimb at the offset of the CS on paired trials (8). Responsiveness to the US was tested three times (average intertrial interval, 1.3 minutes) 4 minutes before and again 4 minutes after a subcutaneous injection of either saline or epinephrine (9). Ten paired trials of the CS and US were presented, with an average intertrial interval of 1 minute (range, 30 to 90 seconds on an irregular schedule). After trial 10, reflexes were tested, and the animal was allowed to recover from anesthesia in a warm environment.

To determine the effect of training, animals were tested for conditioned suppression in the presence of the white noise 10 days after training. Either 2 or 3 days after recovery from anesthesia, water bottles were removed, and the animals were given the opportunity to drink their entire supply during 5 minutes (at approximately 5:00 p.m.). Animals quickly learned to drink almost continuously while water was available. They lost not more than 15 percent of their weight (10). All animals were drinking continuously before the test for conditioned suppression, held on day 10. During testing, the animal's cage was placed near a loudspeaker. During the first minute of water availability, the speaker was off. During minutes 2 through 5, the speaker provided white noise (85 dB within the cage); during minutes 6 and 7, the noise was off. For each minute of testing, the experimenter recorded the cumulative number of seconds of drinking on electronic timers (11). The effects of training were assessed by determining a suppression ratio: duration of drinking during minute 2 divided by that during minute 1. Conditioned suppression to a CS after aversive training in waking animals is regarded as evidence of Pavlovian fear conditioning (12).

Four experimental groups were studied: epinephrine at doses of 0.01 (E0.01), 0.10 (E0.10), and 1.00 (E1.00) mg/kg and a saline group. Animals in one control group—unpaired epinephrine, 0.01 mg/kg—were treated as the trained E0.01 animals were, except that the CS and US were never paired but were presented ≥ 15 seconds apart. A second control group of untrained animals were neither anesthetized nor trained, but they did undergo the standard regimen of water deprivation, availability, and testing with noise.

During training under anesthesia, application of the US or the CS-US combination failed to elicit any consistent change in the EKG, and there was no observable change in the EEG (13).

The saline, unpaired epinephrine, and untrained groups exhibited minimal suppression (no significant differences in paired comparisons among these groups, Mann-Whitney *U* test) (Fig. 1). Thus, animals receiving saline exhibited no conditioned fear in the presence of the CS, and epinephrine itself did not alter drinking behavior in the presence of the CS. In contrast, all three trained epinephrine groups exhibited statistically significant suppression (Fig. 1).

Since the two groups given 0.01 mg/kg doses of epinephrine differed from each other (Fig. 1), the suppression of drinking was not due to some nonassociative effect of epinephrine, such as increased sensitivity to noise. And since the group receiving the lowest dose of epinephrine had the greatest suppression ratio (Fig. 1), epinephrine did not enable learning merely by lightening the level of anesthesia—it would indeed have had the least anti-anesthetic action for these animals. To assess this possibility more directly, we compared the reflex scores at the end of training with later performance. If the level of anesthesia at the end of training were related to conditioning, reflex scores and suppression ratios would be inversely correlated. There was virtually no relation ($r = 0.093$, $P < 0.28$, $N = 44$). Further, the average reflex scores did not differ significantly among groups; in fact, the group with the lowest ratio (greatest suppression), the E0.01 group, had the lowest mean reflex score (saline, 1.80; 0.01, 0.43; 0.10, 1.79; 1.00, 1.43).

As an additional check on anesthesia during training, we counted the number of heartbeats during the 5 seconds before and 5 seconds after the onset of the test shocks that preceded conditioning and before and during the presentations of white noise (14) for the saline and paired E0.01 groups. The stimuli elicited

Table 1. Average change in heart rate in beats per second as a result of stimulation with shock or white noise.

Stimulus	Group	
	Saline	E0.01
Shock	-0.017 \pm 0.007	-0.017 \pm 0.009
White noise	-0.007 \pm 0.004	-0.010 \pm 0.005

almost no change in heart rate (Table 1), and the groups did not differ (*t*-tests) (15). Therefore, the differential behavior during the test for conditioned suppression cannot be explained by an anti-anesthetic action.

Peripheral injection of epinephrine leads to a dose-dependent modulation of memory for avoidance tasks (3). Our findings indicate that epinephrine can enhance Pavlovian (classical) conditioning as well. Further, the enhancement lasts for at least 10 days, the period between training and testing. These results may help explain the difficulty in obtaining consistent findings of learning during surgical anesthesia in humans, despite anecdotal reports of such learning (1, 2). It is possible that some surgical episodes result in the release of epinephrine some minutes after anesthesia has been induced, and that in such cases patients can learn and remember events taking place within the operating room. Agents other than epinephrine may also be effective in enabling learning under anesthesia. Finally, our findings demonstrate that a severely depressed brain can quickly acquire Pavlovian defensive conditioning.

Our procedures permit the study of neural bases of learning in animals re-

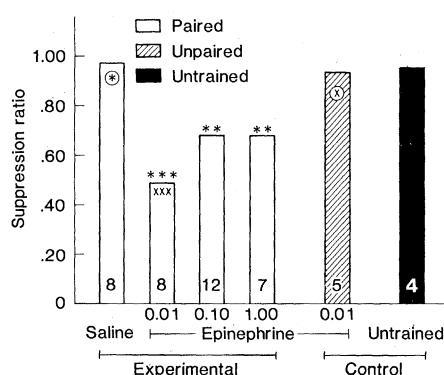


Fig. 1. Conditioned suppression of drinking: mean suppression ratios for each group. Bars marked with asterisks are compared with saline (**, $P < 0.01$; ***, $P < 0.001$), and E0.01 is compared with unpaired E0.01 (xxx, $P < 0.004$). Numerals in the bars indicate the number of animals in each group. Mann-Whitney *U* tests were used to assess differences.

ceiving identical stimulus presentations, but in which the stimulus processing either fails to find expression in behavior (saline) or supports behavioral learning (epinephrine). Learning in anesthetized animals can occur with epinephrine despite the depression of much brain function; the suppression of activity in areas of the brain that are not necessary for learning should make it easier to identify neural sites involved in learning.

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References and Notes

1. R. Trustman, S. Dubovsky, R. Titley, *Int. J. Clin. Exp. Hypn.* **25**, 557 (1977); A. Cherkov and P. Harroun, *Anesthesiology* **38**, 564 (1973).
2. J. Miller [*Am. J. Psychol.* **54**, 94 (1941)] reported eyelid conditioning in cats, and M. Lico, A. Hoffmann, and M. R. Covian [*Physiol. Behav.* **3**, 673 (1968)] obtained conditioned responses of blood pressure in rabbits only if the anesthesia was light enough to permit reflexes to be elicited. Conditioned taste aversion learning can take place if the subjects are anesthetized after presentation of the CS—that is, during the CS-US interval [B. Berger, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **29**, 749 (1970); O. Buresova and J. Bures, *Behav. Biol.* **20**, 41 (1977)], but not if anesthesia is induced before presentation of the CS (O. Buresova and J. Bures, *ibid.*).
3. J. L. McGaugh, *Annu. Rev. Psychol.* **34**, 297 (1983).
4. The numbers of animals prepared under these two methods did not differ among treatment groups, and there were no significant differences in learning within a group as a function of type of preparation for EEG recording. Therefore, data for the two types of preparation were pooled within each treatment group.
5. In pilot studies, we found that chloral hydrate deepened anesthesia without increasing the risk of death, in contrast to additional sodium pentobarbital, which had both effects.
6. The following reflexes were measured before and at the end of training under anesthesia: corneal reflex, by lightly brushing the cornea with a cotton-tipped applicator; pinna reflex, by repeatedly stroking the inside of the pinna with the wooden rod of the applicator; and tail reflex, by strong pinch of the tail by finger pressure. The strength of these reflexes was scored on a four-point scale from 0 (absent) to 3 (strong).
7. Grass S88 constant current stimulator and stimulus isolation unit. The EEG and EKG were recorded with a Grass 7 polygraph.
8. These parameters produced a train of two pulses. The standard current was 4 mA; in a few cases, 5 or 6 mA were used if the lower level did not visibly contract the musculature of the right hind limb. Subjects did not exhibit any behavior in response to shock other than a brief movement of the right hindquarter.
9. The purpose of these test shocks was to determine whether the shock caused a lightening of anesthesia. No such effect was found.
10. The restricted availability of water resulted in weight losses averaging about 10 percent of pretraining body weight. There were no significant differences in body weight between groups (analysis of variance).
11. On some occasions, testers were unaware of the treatment group of the animal, but there were no significant differences within or between groups on this basis. During testing and training, animals from various groups were run in an intermixed order to control for possible effects of time of day.

12. W. K. Estes and B. F. Skinner, *J. Exp. Psychol.* 29, 390 (1941).
13. In three animals of the E0.10 and E1.00 groups, epinephrine increased heart rate and EKG amplitude for 5 to 15 minutes. However, the shock and white noise elicited no EKG response.
14. During the CS, we counted the heartbeats for the 5-second period 1 to 6 seconds after onset of the white noise to allow time for the appearance of a change in heart rate to an acoustic stimulus.
15. Although there were no significant differences between groups, and although most stimuli elicited no change in heart rate, it was possible that the stimuli sometimes did elicit a slight change in heart rate. We thus analyzed heart rate of saline and E0.01 animals during "sham" trials—ran-

domly selected, adjacent 5-second periods in intertrial intervals between test shocks. The fluctuation in heart rate between the first and second 5-second periods during the sham trials did not differ significantly from that between pretrial and shock periods, either within or between saline and E0.01 groups.

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Prolongation of Rat Islet Allograft Survival by Direct Ultraviolet Irradiation of the Graft

Abstract. *Ultraviolet irradiation of rat dendritic cells completely abrogated their allostimulatory capacity in a mixed lymphocyte reaction. Rat islets of Langerhans similarly irradiated remained hormonally functional when transplanted into syngeneic diabetic rats. Allogeneic transplantation across a major histocompatibility barrier of islets initially treated in vitro with ultraviolet irradiation resulted in prolonged allograft survival without the use of any immunosuppressive agents.*

Although disparity between major histocompatibility complexes (MHC's) leads to rejection of grafted tissue, it is the recognition of this incompatibility by the host that appears to be critical in initiating the rejection process. Recognition of foreignness by the host seems to require the presence of class I and class II MHC antigens on the graft, while lymphoreticular cells accompanying the graft and bearing both classes of antigens are thought to be responsible for sensitizing the host toward a primary immune response (1). We report here that ultraviolet (UV) irradiation (sunbeam spectrum), at a dose that can abrogate a mixed-lymphocyte reaction (MLR) after irradiation of rat dendritic stimulator cells (2), can also attenuate the immunogenicity of pancreatic islets without altering their endocrine function and can prolong the survival of rat islet allografts in diabetic hosts without the use of immunosuppressive agents.

The exact nature of the "passenger leukocyte" implicated in causing graft rejection is not known. Rat dendritic cells (DC's) have been demonstrated to be extremely powerful as accessory cells in T-cell proliferation and in causing rapid rejection of rat kidneys otherwise depleted of passenger leukocytes (3). We first investigated the ability of UV irradiation of rat DC's derived from afferent lymph (3) to attenuate their stimulatory activity in an MLR. Abdominal lymph nodes were removed from rats 6 weeks before thoracic duct drainage. Lymph was collected over a 36-hour period and DC's among the resultant cells were enriched to approximately 70 percent by

high-density centrifugation (4, 5). These cells were gamma-irradiated (1600 rads) and then UV-irradiated in open petri dishes while suspended in Hanks balanced salt solution with constant stirring with a magnetic bar. The source of the UV irradiation was a bank of two FS20 lamps (Westinghouse), which have a flux of 1 mW/cm² at 310 nm (UVX-Radiometer, Ultra-Violet Products) measured 10 cm from the source. Using thoracic duct lymphocytes from rats of strain ACI (RT1^a) as responders and Lewis rat (RT1^b) DC's as stimulators, we obtained a high stimulation index (SI) (6) of > 400 with 10⁵ DC's (Fig. 1). When the number of DC stimulators was decreased to 0.125 × 10⁵ the SI remained markedly elevated at 162.

Dendritic cells subjected to UV irradiation (800 to 1000 J/m²) were completely ineffective as stimulators in the MLR (SI < 3). Although DC's are extremely powerful allogeneic stimulators in the MLR and cause graft rejection (2, 3),

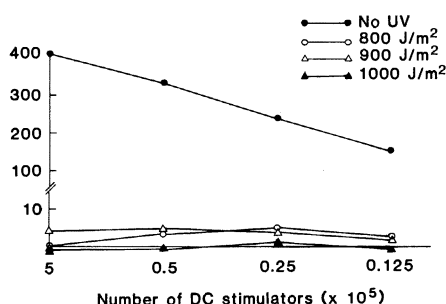


Fig. 1. Effect of UV irradiation on the stimulatory activity of Lewis rat DC's in the MLR. The proliferative response of ACI thoracic duct lymphocytes is expressed (SI) (6).

they appear to be inactivated as stimulators by UV but not gamma irradiation.

Once the dose range of UV irradiation necessary to attenuate the MLR was defined, we examined the ability of islets irradiated at the same dose range to reverse the diabetic state in syngeneic diabetic rats (Fig. 2). Rats were made diabetic with intravenously administered streptozotocin (60 mg/kg) and used as recipients if blood glucose was > 300 mg/dl on three weekly successive measurements. Lewis rat islets were isolated by collagenase digestion, Ficoll gradient separation (7), and subsequent handpicking under a stereomicroscope. Isolated islets were suspended in Hanks balanced salt solution in open petri dishes and irradiated while being constantly stirred with a magnetic bar. The UV source was the same as for the DC's. After irradiation the islets were incubated for 24 hours at 37°C and 5 percent CO₂ in CMRL medium 1066 with 10 percent fetal calf serum and transplanted through the portal vein. Islets irradiated with 1000 J/m² converted diabetic recipients to a normoglycemic state for less than 5 days; islets irradiated with 1200 J/m² failed to convert them. Irradiation of syngeneic islets with 600 or 900 J/m² resulted in permanent conversion of all diabetic recipients to normoglycemia. Thus the dose of UV irradiation that can abrogate the proliferative response in the MLR with 10⁵ DC stimulators has no deleterious effect on the in vivo endocrine function of syngeneic islet grafts irradiated with 900 J/m².

To determine whether the immunogenicity of allogeneic islets was reduced after such irradiation, Lewis islets were transplanted into ACI rats made diabetic with streptozotocin (Fig. 3 and Table 1). All the control ACI animals receiving Lewis islets cultured for 24 hours at 37°C rejected their grafts and became diabetic again after 6.8 ± 2.7 days (mean ± standard deviation). When Lewis islets were exposed to UV irradiation at 900 J/m², cultured for 24 hours, and transplanted into diabetic ACI recipients, the islets survived for more than 80 days in 8 of 11 animals (more than 110 days in four rats), and all eight remained normoglycemic. These results indicate that UV irradiation of allogeneic rat islets at a level that is not deleterious to their endocrine function reduces the islets' immunogenicity and permits prolonged allograft survival without immunosuppression.

The importance of passenger leukocytes in initiating allograft rejection has been a recurring theme in transplantation immunology (8). In islet transplantation various in vitro culture techniques (9) to