physiol. **38**, 90 (1975). This device allows the electrode to be raised or lowered within the brain after implantation and thus permits the experimenter to select the most suitable recording sites.

5. Platinum-iridium wires (60 μm) that were coated with Teflon except at the tip were inserted into the hypoglossal nucleus for recording of neural activity during licking and into other bulbar regions for control recordings. Placement of the tips of recording electrodes in the hypoglossal nucleus was achieved by recording antidromic responses to electrical stimulation of the tongue during the implantation procedure. A ground connection was provided by bare stainless steel wires that were wrapped around stainless steel screws implanted in the skull. The wires from the recording electrodes and ground wire were connected to a miniature plug (Amphenol 223-1205) that was cemented to the skull.
6. The steel enclosure was 45 cm high, 30 cm wide, and 46 cm long. The skull-mounted plug was connected to an Amphenol 223-1105 socket that had a field effect transistor (TI 2N5045) in line with each recording electrode lead. A flexible multiconductor cable ran from the field effect transistors to a mercury slip-ring commutator (Scientific Prototype Co.). The rat could reach all parts of the cage without losing slack in the cable. From the commutator, signals were conventionally amplified and recorded on FM magnetic tape. A stainless steel drinking tube with a 3.6-mm opening was attached through a silicone rubber stopper to a glass bottle filled with tap water. The end of the drinking tube was placed 4 mm outside a 1.3-cm hole that was centered opposite an elliptical opening in the back wall of the cage. This arrangement, which approximated the high-restriction licking environment described by L. Marowitz and B. P. Halpern [*Physiol. Behav.* **11**, 259 (1973)], required the rat to protrude its tongue to make contact with the liquid-filled drinking tube and thereby prevented contact with the lips. Tongue contact by the rat was detected by a solid-state drinkometer circuit (<1-μa short-circuit current) that emitted a brief pulse within 1 msec of the end of each lick contact. A single tongue contact with the drinking tube at the observed licking rates was expected to last 70 msec [B. P. Halpern, in *Olfaction and Taste*, D. A. Denton and J. P. Coghlan, Eds. (Academic Press, New York, 1975), vol. 5, pp. 47–52]. Each lick was reinforced with approximately 5 μl of tap water at room temperature. The lickometer pulses were tape-recorded on a second channel. The neural and lickometer responses were analyzed off-line with a digital computer (PDP 8-E) under a LAB-8/E PST program.
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Classical Nictitating Membrane Conditioning in the Awake, Normal, Restrained Cat

Abstract. *Present knowledge of its central nervous system makes the cat a desirable subject for studies of brain-behavior relationships. Response frequencies and latency characteristics in conditioning and control groups indicate that the response of the nictitating membrane can be classically conditioned in a new restraint system in which detailed brain and behavior measures can be easily obtained.*

The cat has long been a favored subject for studies of basic neural processes in both the brain and spinal cord and has perhaps the best understood of all mammalian nervous systems. Thus, it would be desirable to analyze simple learning processes in the cat nervous system. However, the cat's inherent dislike of restraint has precluded the development of an easily used and readily quantifiable behavioral paradigm in the cat such as was developed by Gormezano and his collaborators (*1*) for the rabbit and used for brain recording in more recent work (*2*). Behavioral studies of the cat have frequently been performed with free-ranging or minimally restrained subjects. Other experimenters have used long periods of adaptation, paralyzed preparations, or invasive measures such as skull bolts to achieve adequate restraint for behavioral measurements (*3*). The need for such procedures has precluded the use of large numbers of subjects in studies requiring recording of discrete responses, such as leg lift or eyelid closure. We now report our successful experimental efforts at showing unequivocal evidence of classical conditioning of the discrete, easily quantifiable, nictitating membrane (NM) response in cats restrained in a system requiring no adaptation.

Sixty adult, mongrel cats were randomly assigned to squads of four subjects with three squads in each of five experimental or control conditions. One subject was randomly eliminated from each condition after one subject died during the study. Each subject was prepared the day before training by shaving the region around the right eye and suturing a 6-0 monofilament nylon loop into the outer edge of the membrane. Stainless steel wound clips placed 0.5 cm above and below the eye allowed delivery of the 100-msec, 3-ma a-c shock unconditioned stimulus (UCS). The conditioned stimulus (CS), a 72-db sound pressure level (SPL), 1000-hertz, 500-msec tone was delivered through a speaker in the ventilated, dimly lit, deactivated refrigerator shells that served as experimental chambers. A 65-db SPL white-noise background was continuously present. The response of the NM was recorded by hooking a thread to the NM loop. The thread was coupled to the arm of a minitorque potentiometer mounted on the restraint box. Membrane extensions were recorded on a polygraph (Grass), and response latency was also measured by a digital recorder. A response was any NM movement of at least 0.5 mm starting within the appropriate scoring interval.

The details of the restraint system will be available elsewhere (*4*). Briefly, the animal is placed in a box patterned after the Gormezano rabbit restrainer (*1*). The box is of heavy Plexiglas with a lid and a movable back plate that allows the cat's body to be completely enclosed while the cat's head protrudes through a slanted front plate. A stock comes down over the top of the neck and is secured at a point allowing free neck movement but not allowing the head to be pulled back. The chin rests in a slot on a plate projecting from the front of the box below the neck stock. A bite bar attached to the chin plate is positioned through the mouth behind the lower canine teeth and is tightened sufficiently to preclude head turning but not to produce discomfort. Two nylon releasable cable ties, which fit through slots in the chin plate, are tightened around the head, in front of and behind the ears. The ties are the major head restraint and leave room for skull electrode implants or surgery that might be desired. The cat is thus held sufficiently securely to permit eyelid or nictitating membrane movements to be measured by a transducer mounted on the box. The cat accepts this restraint for as long as 2 hours with little struggling. No appreciable increase in resistance to being placed in the apparatus has developed for as long as 9 days in our studies. The animal's legs could also be made accessible if holes were made in the bottom of the restrainer.

All subjects received nine daily experimental sessions. The first session was an adaptation session, during which no stimuli were given, and which was used to assess the random response rate by recording as though stimuli had been delivered. Six acquisition (or control) ses-

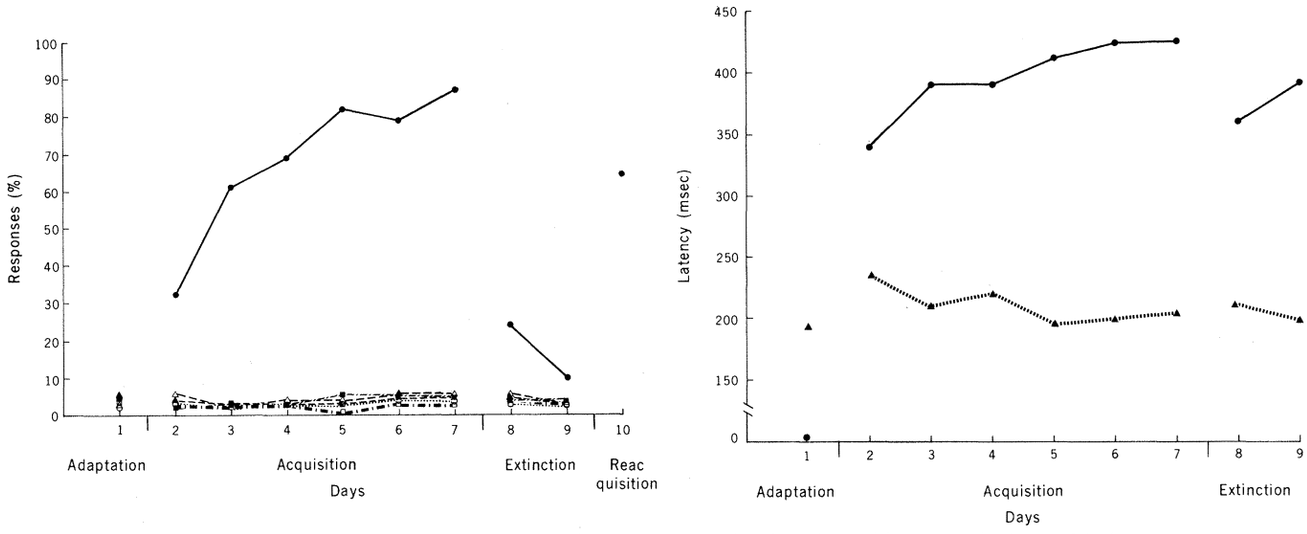

Fig. 1 (left). Responses (as percentages) for adaptation, acquisition, and extinction for all conditions [unpaired (△), CS alone (■), shock alone (□), nothing (▲), and preshock unpaired (○)] and reacquisition sessions for the paired condition (●). Fig. 2 (right). Response onset latency (..... and ▲) and regular trials and peak latency (—— and) on test trials for the paired group in adaptation, acquisition, and extinction. These measures are not shown for the control conditions because of the small number of responses.

sions and two extinction (or control) sessions followed. The experimental group received a total of 101 classical conditioning and test trials in each acquisition session followed by CS-alone trials in extinction. An extra reacquisition session was given this group after extinction. In acquisition, the CS preceded the UCS by 400 msec, and they terminated simultaneously; trials were 50, 60, or 70 seconds ($\bar{X}$ = 60 seconds) apart. The initial and each subsequent tenth trial was a CS-alone test trial in acquisition, for which the response scoring interval was 500 msec rather than the 400 msec of regular trials. Test trials were scored in extinction. The unpaired control group received 101 CS and 90 UCS presentations randomly sequenced with an interpresentation interval of 20, 30, or 40 seconds ($\bar{X}$ = 30 seconds) in acquisition. The appropriate 11 CS-alone presentations were scored as test trials. In addition, for the preshock unpaired condition, responses were scored during the 400 msec prior to UCS delivery in acquisition. Extinction was the same as in the paired group. The CS-alone and shock alone control groups received only CS presentations and UCS presentations, respectively, throughout acquisition and extinction and were scored as were those in the paired condition. The "nothing" control group was given no stimuli during the eight training sessions and was scored as though paired trials had occurred.

Responding to the CS increased over sessions only in the paired group (Fig. 1). In other groups, response rates remained at essentially adaptation level throughout. In extinction, the response level of the paired group dropped rapidly, and it rose again in reacquisition. Analysis of variance showed no between-group differences in adaptation, significant differences in acquisition between groups (d.f. = 5, 60; F = 133.08; $P < .01$), over sessions (d.f. = 5, 300; F = 12.94; $P < .01$), and as an interaction between groups and sessions (d.f. = 25, 300; F = 11.33; $P < .01$). The same factors were significant in extinction (group: d.f. = 5, 60; F = 16.98; $P < .01$; sessions: d.f. = 1, 60; F = 7.70; $P < .01$; and groups by sessions: d.f. = 5, 60; F = 5.39; $P < .01$). For the paired group, onset latency decreased steadily during acquisition, while peak latency increased (Fig. 2). No such trends were apparent during extinction. Between-session trends were tested with analysis of variance (latency: d.f. = 5, 50; F = 2.51; $P < .05$; peak latency: d.f. = 5, 30; F = 2.58; $P < .05$). In addition, peak response amplitude on test trials in the paired group showed a significant increase in acquisition (d.f. = 5, 30; F = 4.58; $P < .01$) from about 1.3 to 2.8 mm; it dropped to about 1.6 mm in extinction.

The results of the experimental paired group when compared with the control conditions are unequivocal evidence for classical conditioning of the cat NM response. The absence of a higher response rate during acquisition than during adaptation for animals in any control condition indicates that the response system is not contaminated with nonassociative responses to any significant degree. The random blink rate of about 5 percent is comparable to that normally expected in the well-documented rabbit NM preparation (*1*). In addition, the shifts in onset and peak latency parallel those seen in the rabbit NM response system under similar conditioning parameters (*1*, *5*). In extinction, response rates declined more rapidly than in many rabbit NM conditioning studies which show more prolonged responding or resistance to extinction under conditioning parameters similar to those we used (*1*, *5*). Parametric stimulus manipulations may increase resistance to extinction in the cat, but it is also possible that a basic difference in neural control of the cat and rabbit NM is being manifest. The NM's of both cat and rabbit are extended by abducens nerve activity either directly or through eyeball retraction; however, the cat NM is actively withdrawn through autonomic activity while the rabbit membrane withdrawal is primarily passive but also has a small active component due to striate muscles innervated by the oculomotor nerve (*6*). Therefore the rapid extinction of the cat NM response may reflect strong autonomic inhibitory activity brought on by the extinction procedure. The delineation of the conditioning of NM response in the cat and development of a simple restraint system thus allows direct comparisons between the cat and rabbit NM response systems and provides a simple and ideal preparation for the study of brain activity in the cat during conditioning and other physiological manipulations.

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Measuring Plutonium Concentrations in Respirable Dust

In their report on the plutonium hazard in respirable dust, Johnson *et al.* (*1*) state that "the respirable fraction of surface dust was separated by ultrasonic dispersion and a standard water-sedimentation procedure." It is apparent that their respirable fraction includes particulate that is too large to fall within the respirable size range, and that the analytical results obtained after the sample preparation techniques described will not show the concentration of plutonium associated with the in situ respirable surface dust. My criticism has as its basis the following reasons.

1) Wet digestion with hydrogen peroxide and particle dispersion by sonication reduces or eliminates the binding mechanisms that hold respirable-size plutonium particles to nonrespirable-size dust particles in the surface soil. After altering the real in situ particle associations, it is wrong to assign the final value for the soil concentration of plutonium to the original respirable size fraction of the surface soil.

2) In using the sedimentation technique to isolate the respirable size fraction, it is wrong to base "threshold parameters" on particles having an effective maximum diameter of 5 μm and a density of 11.45 g/cm^3 because (i) A 5-μm PuO_2 particle having a density of 11.45 g/cm^3 has an equivalent aerodynamic size of about 17 μm, which is well above the respirable size range. (ii) By using the above threshold parameters, one includes in the respirable fraction much of the ordinary dust present that is well beyond the respirable size. For example, by Stokes' law, dust particles with a density of 1.5 g/cm^3 and a size of 23 μm may be shown to have sedimentation characteristics similar to those of PuO_2 with a size of 5 μm and a density (ρ) of 11.45 g/cm^3. A 23-μm (ρ = 1.5 g/cm^3) dust particle has an aerodynamic diameter of about 28 μm. It is the aerodynamic diameter that determines respirability.

This selection of threshold parameters may or may not give conservative results in assessing the hazard of plutonium in soil. The plutonium attached to host dust particles that are well above the respirable size range is included as respirable particulate, while nonrespirable dust particles with no attached plutonium are also included in the respirable dust fraction.

In any event, these methods of sample preparation and data analysis will not yield valid results. The most conservative approach would be to call all of the plutonium respirable, since that which is outdoors is virtually all in the respirable size range (the mean size at Rocky Flats is on the order of 0.3 μm or less, depending on source) when considered as unassociated with host soil particles. If one wants to know the concentration of plutonium actually associated with respirable dust particles, then a valid technique must be used. One such technique would be to sample by vacuum and collect by impaction, using an impactor that classifies the dust according to its aerodynamic size.

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We believe that it is valid to disperse the in situ particle associations. The procedure is used in an effort to overcome the variables associated with microaggregate stability, in order to achieve reproducible results and provide data that are comparable from season to season or site to site. Our definition of the respirable size fraction is that fraction of soil that includes plutonium oxide particles of the given size. This fraction does include other mineral particles of lower density and larger diameter (to 12.6 μm, based on an average mineral particle density of 2.65 g/cm^3, according to Stokes' equation).

It is irrelevant whether these other mineral particles are ever retained within the lung, although there is evidence of some retention (*1*). However, that does not render it unacceptable to use the weight of the entire fraction as a basis for expressing the concentration of the plutonium. This fraction does comprise the orders of particle sizes of concern for health.

We agree that the selection of threshold parameters could be based on an appropriate equivalent aerodynamic size in place of the actual particle size. However, this is not necessarily a more conservative approach for the conditions of this study. It is probably true that nearly all of the plutonium on the soil is in the respirable size range (*2*), and we have probably measured nearly all of the plutonium on the surface of the soil. A minor adjustment in threshold parameters as proposed would result in a small change in the weight basis for expressing concentration. We believe that this concentration difference is trivial, particularly when compared with the difference between the weight of the respirable fraction (following our definition), which we used, and the weight of the whole soil collected to arbitrary depths, which it has been the practice to use in the past.

Employing a vacuum device for sample collection may be a useful modification of our method, if the device is equipped to avoid loss of submicrometer particles. The respirable fraction may be separated by any procedure capable of performing the separation effectively. However, the separation procedure that we utilized to isolate the respirable fraction will probably yield more reproducible results (*3*).

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