the lung gas by a single passive expiration and inspiration.

We interpret a constant esophageal pressure during expiration to mean that the lungs and surrounding chest walls are moving in at the same rate with respect to each other. With active expiration, muscles "push" the lung and pressures on surfaces of the lungs increase. With passive expiration, recoiling lungs may "pull" the chest wall in and cause pressures on surfaces of the lungs to decrease. When pressure on a surface of the deflating lungs remains constant, muscles are not "pushing" lungs nor are lungs "pulling" the chest walls.

Pressures on different surfaces of the lungs probably vary, and esophageal pressures reflect changes in pressure more accurately than absolute pressures on the lungs. When a whale surfaces, the esophagus and lungs lie dorsally near the water level. Laterally the water pressure may be absorbed partly by ribs. Ventrally some of the buoyant force on the abdominal surface may be transmitted through the diaphragm to the lungs.

There appears to be little advantage to active blowing since the lungs nearly empty themselves in 0.5 second. This whale passively expires even when hyperventilating in response to carbon dioxide. She increases ventilation, as does man, by inspiring more deeply and thereby creating greater recoil pressures to drive the next expiration. Morever, she could hyperventilate to 30 times the resting levels through increased frequency of breathing alone. By contrast, man lacks this reserve in frequency and must use his muscles to augment expiration when hyperventilating beyond six or eight times the resting levels (*4*).

Immersion in water should favor passive deflation of the lungs (*5*). Anatomical features that may facilitate emptying of as much as 88 percent of lung gas are cartilaginous supports of bronchi that maintain patency of small branches (*6*) and a diaphragm which, lacking a central tendon and being positioned horizontally (*7*), probably moves easily toward the spine between the buoyed-up abdomen below (*8*) and the recoiling lungs above.

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13 January 1968

Operant Conditioning of Cortical Unit Activity

Abstract. *The activity of single neurons in precentral cortex of unanesthetized monkeys* (Macaca mulatta) *was conditioned by reinforcing high rates of neuronal discharge with delivery of a food pellet. Auditory or visual feedback of unit firing rates was usually provided in addition to food reinforcement. After several training sessions, monkeys could increase the activity of newly isolated cells by 50 to 500 percent above rates before reinforcement.*

Neural mechanisms of motor activity can be investigated by recording the activity of single neurons in unanesthetized animals performing a specific behavioral response. In such experiments, subjects are trained to a behavior pattern designed to test hypotheses concerning the function of the cells investigated (*1*), or to provide a well-timed motor response to which cell activity can be related (*2, 3*). When the response was overtrained until it recurred in a repeatable, stereotyped manner, its variability was reduced, and correlations between cell and muscle activity were enhanced. However, such correlations are not sufficient to establish functional relations when many elements of the motor system are activated in synchronized patterns. Indeed, the relations revealed in such repetitive situations sometimes disappeared during more random behavior (*1*).

To test the functional relations between neurons and muscles, it seemed desirable to study a more flexible situation in which the animal could be trained to activate specific cells or muscles directly. Reports that individual motor units can come under voluntary control (*4*) and Olds' original work on operant conditioning of neuronal activity (*5*) encouraged this approach. This report describes a technique for conditioning the activity of individual cortical cells in awake mulatta monkeys by direct operant reinforcement of high rates of unit activity.

Unit recording techniques described by Luschei *et al.* (*3*) were used to record from single neurons in precentral "motor" cortex of unanesthetized *Macaca mulatta.* A stainless steel bone plug, permanently implanted over the precentral cortex and sealed with a thin sheet of Silastic rubber, held a removable Trent Wells hydraulic microdrive, which advanced tungsten microelectrodes (*6*) through the Silastic and intact dura into the cortex. Signals recorded by the microelectrode were relayed by a field-effect transistor source follower on the microdrive to a Grass preamplifier (at 0.5 to 30 khz bandpass) and were displayed on a Tektronix 565 oscilloscope. When a single-unit spike was well isolated from background activity, the oscilloscope sweep was triggered from the rising phase of the action potential and was set sufficiently fast to display the action potential over the entire screen (usually 0.1 msec per division). Such continuous observation of the expanded action potential provided assurance that the same single unit was monitored throughout the session. The electrode penetrated the cortex at different points each day, which made repeated observation of the same cell unlikely; all cells were located within a circle 5 mm in diameter over the precentral hand area.

High rates of cortical unit activity

were reinforced by delivery of a banana-flavored pellet to a food cup in front of the monkey's mouth. Cell firing rates were continuously monitored and were appropriately reinforced by an electronic "activity integrator," consisting of a simple resistor-capacitor voltage integrator with a variable exponential decay and a threshold level for triggering the feeder. This mechanism continuously integrated rectangular voltage pulses triggered from the cell's action potentials, so that the integrator voltage underwent a step increment for each cell spike and decreased exponentially toward zero in the absence of activity. The running voltage was consequently roughly proportional to the cell's firing rate. Sufficiently high rates brought the voltage to triggering level for the feeder and reset the integrator voltage to zero. The two parameters of the activity integrator—the decay constant and the size of threshold relative to the step increment—could be varied according to the firing pattern of the cell. As cell activity increased, the feeder-triggering level could be raised or the decay constant could be decreased to maintain an approximately constant reinforcement frequency of about 5 to 12 pellets per minute.

During training sessions the monkey sat in a primate restraint chair inside a dimly illuminated sound-attenuating chamber (IAC 400). The monkey could move his limbs and head freely and could lick the pellets from the food cup. During reinforcement periods, the monkey usually received either auditory or visual feedback related to the unit's firing rate, as a possible discriminative stimulus to aid in training. Two monkeys were exposed to a moderately intense click for each firing of the unit; thus, after several sessions, high rates of clicking could become discriminative for reinforcement. Three subjects faced an illuminated meter showing deflections proportional to the integrator voltage; for these subjects, extreme deflections of the meter arm to the right could become discriminative for reinforcement.

In a typical daily training session only one cortical cell was reinforced. The cell's spontaneous activity was first monitored in the absence of any feedback or reinforcement, to determine its unconditioned rate or operant level. Average firing rates of units during this initial control period were generally steady. In records of operant rates lasting 15 or more minutes, successive 3-minute averages varied from the mean by an average deviation of 5 to 50 percent. After this control period there was a reinforcement period during which the monkey received continuous feedback signaling the unit firing rate and obtained a food pellet for achieving sufficiently high rates. There was no clear evidence of an increase in unit firing rates during the first three to eight training sessions. In several subsequent sessions the rates typically increased gradually, often

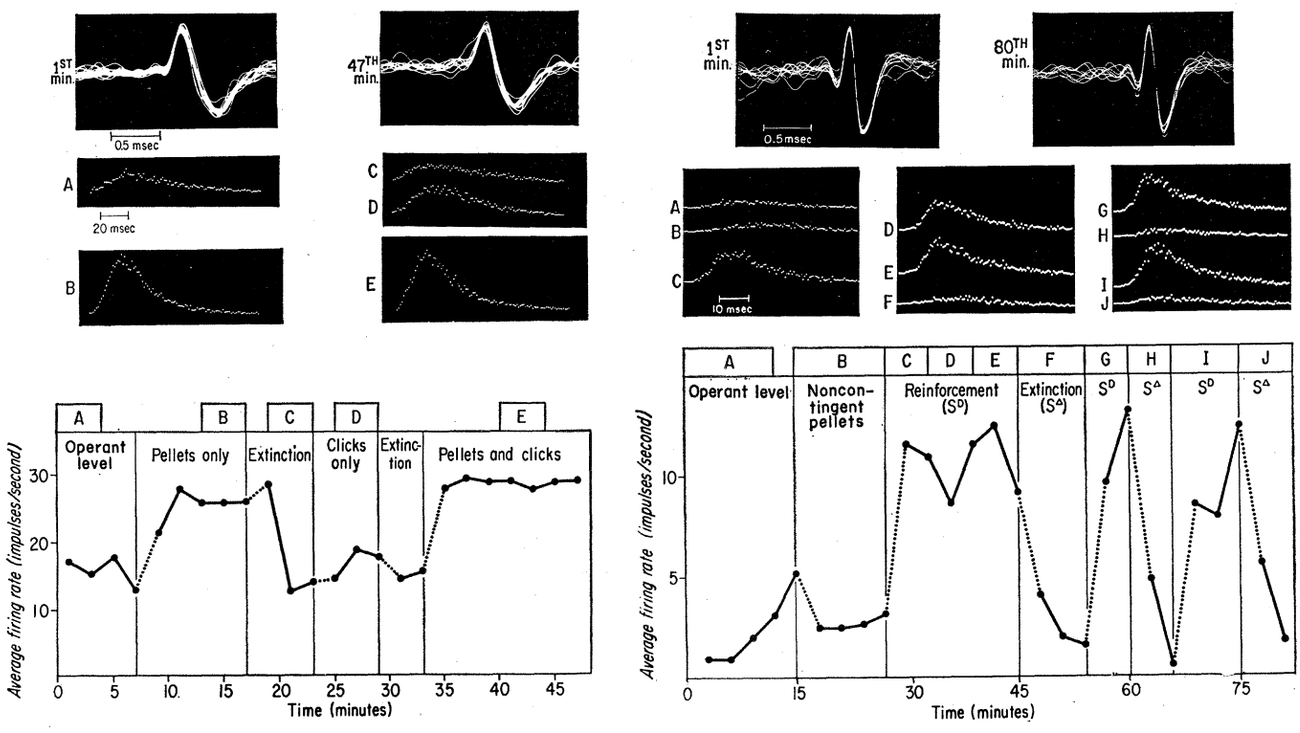

Fig. 1 (left). Firing rate of precentral cortex cell as a function of reinforcement schedule. During operant level and extinction periods neither food nor click feedback was presented. During pellets only period the highest firing rates were reinforced with delivery of a food pellet, without click feedback. During clicks only period a click was presented for each firing of the cell; finally, both pellets and clicks were provided. Interspike interval histograms above the graph show the relative number of intervals from 0 to 125 msec occurring during the specified 4-minute segments of the session. Several superimposed examples of the cell's action potential from the first and last minute of the session are illustrated at top (*9*). Fig. 2 (right). Firing rate of a precentral cell in a session with visual feedback and noncontingent reinforcement. Each point represents the average firing rate for the preceding 3-minute interval. During operant level and extinction (S^{Δ}) periods, no food or feedback was provided. During the noncontingent pellets period, the meter was illuminated and pellet delivery and meter deflection were determined by a tape recording of a previous session. The only change from noncontingent pellets to reinforcement (S^{D}) period was the correlation of pellet delivery and meter deflection with the activity of the monitored cell. In succeeding periods reinforcement (S^{D}) alternated with extinction (S^{Δ}). Interspike interval histograms taken during the specified time segments show the number of intervals between 0 and 62.5 msec, all at the same vertical scale. Several superimposed action potentials from the first and last minute of the session are shown at top (*9*).

over reinforcement periods lasting 10 to 30 minutes. After sufficient training sessions, monkeys consistently and rapidly increased the activity of newly isolated cells. During reinforcement periods of these later sessions, firing rates of units generally increased to a plateau level 50 to 500 percent above their operant levels (Figs. 1 and 2). Typically, firing patterns changed to bursts of activity of 100 to 800 msec in duration, which were sometimes accompanied by specific, coordinated movements such as flection of the elbow or rotation of the wrist. After activity had reached a plateau, an extinction period was introduced during which food reinforcement and feedback were withdrawn. Under these conditions, cell activity decreased and usually returned to operant levels.

Several explanations for the increased firing rates must be considered. Microelectrode damage to the cell is one possibility. Cell injury discharges usually appear as a high, sustained firing rate, often of sudden onset; records with such a pattern were discarded. More subtle cell injury is an unlikely explanation of the remaining cases because the reinforced activity usually changed to a pattern of prolonged bursts separated by periods of normal firing rates. Furthermore, since rates returned to operant levels during extinction, they were obviously correlated with the reinforcement schedule.

Another explanation is that the visual and especially the auditory feedback provided during reinforcement periods may have stimulated cell activity directly. If a cell responded to a click produced by its own firing, the resulting positive-feedback loop would drive the activity up. The effect of sensory feedbacks alone was tested by introducing them independently of food reinforcement. Either auditory or visual feedback alone could temporarily increase cell activity slightly in trained animals, but this effect would more likely be due to the acquired reinforcing properties of the feedback than to its driving properties as a physiological stimulus. Feedback as a behavioral conditioned reinforcer is a function of conditioning, as distinguished from a physiological driving stimulus which produces responses closely locked to the stimulus. Feedback alone increased firing rates only after the feedback had been repeatedly correlated with food reinforcement, and not in naive animals. Furthermore, when food was withheld while feedback was continued, the cell activity returned to operant levels. (In the case of click feedback, a direct physiological response can be detected from histograms of the interspike interval. If any cell had fired in a direct response to a click triggered by a previous firing, a peak should appear on its histogram at the cell's response latency. In these experiments no such peak occurred when click feedback was introduced.) As a further control, feedback was sometimes withheld while only pellet delivery was provided for high firing rates; under these conditions experienced monkeys had no difficulty in increasing the rates of newly isolated cells. Figure 1 illustrates a session in which a monkey previously trained with auditory feedback increased cell activity without such feedback.

A third explanation for increased firing rates is that they were a direct consequence of food presentation. If cells became more active when monkeys oriented to the food cup and consumed the pellet, the increases in average firing rates would be a trivial consequence of "reinforcement." Such a possibility seems unlikely since pellet delivery to naive animals did not raise firing rates. Furthermore, in most cases, cell firing rates actually decreased within 70 to 100 msec after discharge of the feeder. Generally this suppression of unit activity lasted several hundred milliseconds, after which the rates returned to operant levels, before a new burst of activity triggered the feeder again (*7*). Very few cells exhibited increased activity immediately after the feeder discharge. These patterns of suppression and activation after pellet delivery are most simply explained as part of the orienting response to the feeder.

Another consequence of food delivery and feedback may have been an increased general motor activity resulting in indiscriminate activation of many precentral cells. To test for such a generalized excitation, food and feedback were presented randomly. In such "yoked control" sessions the pellet delivery and sensory feedback were determined by a tape recording of cell activity during a reinforcement period from a previous session and were therefore uncorrelated with the unit being monitored. In all trials with random reinforcement, the average firing rates remained at or below operant levels. Only when reinforcement was contingent on high rates of firing of the monitored cell did these rates increase. Figure 2 illustrates such a session with a monkey previously trained with visual feedback; pellets and feedback did not increase cell activity until they became correlated with firing rates.

Whether these precentral cortical cells are activated predominantly by central neural systems, independent of sensory feedback, or mainly in response to stimulation of peripheral receptors remains untested. Since changes in activity of "motor" cortex cells sometimes precede the first recorded electromyogram in a reaction-time situation (*2*, *3*), many of these cells may be activated prior to any correlated movement. Evidence that monkeys can emit and learn new movements with deafferented limbs and without auditory or visual feedback (*8*) suggests that some cells in the motor system may be driven in the absence of sensory feedback. However, since many precentral cells respond to peripheral stimulation, they may also be activated consequent to movements which stimulate receptors in joints, muscles, or skin. When we reinforced activity of postcentral "somatosensory" cortex cells, monkeys quickly learned to stimulate the appropriate receptive fields.

The technique of conditioning the activity of specific central structures by direct operant reinforcement will be useful for investigating neural mechanisms. A generalized version of the activity integrator could be used to monitor and reinforce more general patterns of neural or muscular activity. By integrating and reinforcing rectified electromyograms, we were able to train animals to contract specific muscles. With multiple inputs, providing either positive or negative contributions to the integrator voltage, one may differentially reinforce one activity with simultaneous suppression of another. The degree to which one neural or muscular activity can be behaviorally dissociated from another could be a relevant test for causal connections between the underlying structures.

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10. Supported by NIH grant FR 00166 and PHS 5T1 NB 5082-13. I thank Dr. E. Luschei for instruction in chronic unit recording techniques and Mrs. S. Barrow for assistance with the behavioral techniques.

18 November 1968, revised 13 January 1969

Ice Crystals

Odencrantz *et al.* (*1*) report that replicas of ice crystals prepared with the vapors of methyl-2-cyanoacrylate monomer exhibit thin whiskers (about 0.5 μ in diameter) over the surfaces of the replicas. They assume that these whiskers represent real ice whiskers present on the original ice crystals grown in their laboratory chamber, which led them to suggest that the breakup of these mechanically fragile whiskers could be a mechanism for the multiplication of ice crystals in the atmosphere.

Having had considerable experience in replicating crystals by this resin-vapor replication technique (*2*), I believe that the whiskers observed by Odencrantz *et al.* are artifacts produced during replication. For some time I had been mystified by the appearance of these whiskers on replication until I discovered that they could be entirely eliminated by (i) carefully removing excess moisture and other foreign materials from the surface on which the ice crystals were to be replicated, (ii) not overexposing the ice particles to the replicating vapor, (iii) making certain the cold-chamber atmosphere contained no residue of resin vapors from earlier replications before forming the ice fog, and (iv) adding a resin-polymerization catalyst (NH_3) to the chamber air before replication.

The reasons for these precautionary measures follow. To accomplish replication of an ice particle by the resin-vapor technique, the particle is exposed to the monomer vapor, which condenses and polymerizes over the particle surface to form a thin plastic shell or replica. The resin vapor, however, can quite readily react with moisture to produce globular or snake-like artifacts (often observed as "background" material deposited over substrate) (Fig. 1) as well as the whisker-like artifacts typified in Odencrantz's pictures. The substrate (glass slides) should be rinsed in ethanol and chloroform to remove surface water and foreign materials, especially acidic substances. The polymerization of the cyanoacrylate monomer is very sensitive to bases. Since water can serve as a weak base, the polymerization is initiated by the contact with the ice, but the addition of ammonia promotes more complete polymerization and therefore stronger, more artifact-free replicas.

For best results when replicating small ice particles, the following is suggested. About 5 cm^3 of ammonia gas should be introduced into the experimental chamber for every 10 liters of air, usually just prior to replication. The slide coated with the liquid monomer should be held 1 ml over the desired particles for about 10 seconds. This slide, initially at room temperature, should be backed with a thin slab of insulating plastic foam so that the temperature of liquid monomer does not decrease too rapidly during replication. (A critical amount of resin is needed to produce a complete replication, and the major force driving the resin vapor diffusion is the temperature gradient between the resin liquid and the ice.) All ice should be sublimed away from within the replicas before they are brought to room temperature.

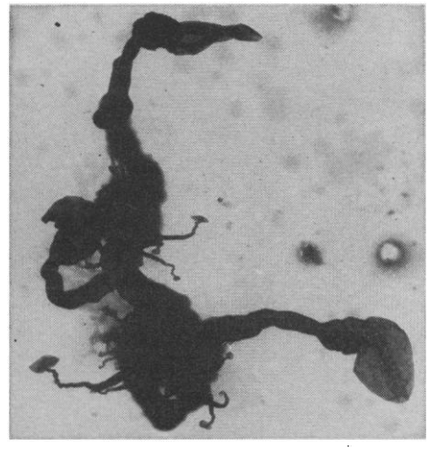

Fig. 1. Ice crystals and artifacts.

A more sophisticated procedure for replicating ice crystals with the cyanoacrylate monomer has been reported by Odencrantz and Humiston (*3*). However, the occurrence of artifacts was not considered, and the above remarks should be kept in mind while reading their paper.

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1 July 1968

Too Much Noise in the Autoradiogram?

Reports regarding autoradiographic localization of noncovalently bound substances are very conflicting, and it appears that frequently such pictorial data are accepted without sufficient concern for their validity.

For instance, there are six reports of ^{3}H-estradiol localization in the uterus; not one of these agrees with another. Radioactivity was found to be concentrated in the lumen of glandular tubes in contact with the apical poles of cells (*1*); in the cytoplasm of the luminal epithelium (*2*); in the nuclei of endometrial and glandular cells (*3*); at the apex and base of luminal cells (*4*); in the cytoplasm preferentially at the cellular membrane of uterine eosinophilic cells in the connective tissue, while no nuclear labeling was detected (*5*); and in nuclei of luminal and glandular epithelium, the substantia propria, and muscularis as well (*6*).

Studies of the pituitary have yielded similarly conflicting results. ^{3}H-Estradiol was found to be concentrated over the nucleoli and at nuclear membranes of eosinophiles (*4*); in the cytoplasm of basophiles (*7*); and in nuclei of eosinophiles, basophiles, and chromophobes as well—however, not over nucleoli and at nuclear membranes (*8*).

In the brain, ^{3}H-estradiol was described as being localized in neurons of the nucleus supraopticus and nucleus paraventricularis (*7*); in neurons and glial cells throughout the brain as well as in the spinal cord, without "exclusive uptake by or absence of uptake from any particular type of nerve cell or