a Dry Ice and methanol bath to keep the sample well frozen. The cold finger in the vacuum system contained liquid nitrogen. After the sample had been lyophilized to dryness the vacuum was replaced with dry nitrogen gas and the samples were stored for a moment over $P_2O_5$ until diffraction patterns had been run under a dry nitrogen atmosphere.

Although the lyophilized samples gave no visual evidence of shrinkage relative to the volume of wet gel, the diffraction patterns showed that the crystal 001 spacings decreased to 12 A. This is an even greater collapse than that caused by the freezing process, but it indicates that there is still a small amount of interlayer water since the crystals would collapse to a 10 A spacing upon oven drying.

Thus, x-ray diffraction studies indicate that either freezing or lyophilizing bentonite gels greatly alters their structure. Visual evidence of this was also obtained, by microscopic observation of the lyophilized samples (Fig. 1). Note particularly the fibrous nature of the gel in the edge view; subsequent studies indicate that this is due to the developmental pattern of the ice lenses. The dark areas in the photographs are voids left by the sublimed ice uptake.

Quick-freezing of thin (1 mm) samples of thixotropic bentonite gels on aluminum foil resulted in the freezing front's entering the sample from both surfaces at equal rates. The lyophilized specimens showed a parallel fibrous structure, similar to that shown in Fig. 1*b* except that the ice lenses entering from the two surfaces did not form all the way to the center of the sample. The center layer was without the fibrous structure. Thin gel samples froze more rapidly and developed a finer structure.

Word just received from Australia indicates that Norrish and Rausell-Colm (*5*) have found results similar to ours (*6*).

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#### References and Notes

1. I. T. Rosenqvist, *Proc. Am. Soc. Civil Engrs.* **85**, 31 (1959).
2. J. D. Lopez-Gonzalez and C. Del Pino Vazquez, *Ann. Spanish Roy. Soc. Phys. Chem.* **55**, 711 (1959).
3. K. Norrish, *Discussions Faraday Soc.* **15**, 120 (1954).
4. L. A. G. Aylmore and J. P. Quirk, *Nature* **183**, 1752 (1959).
5. K. Norrish and J. A. Rausell-Colm, abstracts of papers presented at the 1st Australian Conference on Clay Minerals and Related Topics, University of Melbourne, Melbourne, Australia, 12–13 Feb. 1962.
6. This report is journal paper No. 1906 of the Purdue University Agricultural Experiment Station, Department of Agronomy, Lafayette, Ind.

27 March 1962

## Classical Conditioning in Newborn Rats

*Abstract.* Newborn albino rats were trained according to classical conditioning procedure wih one of four intervals between conditioned and unconditioned stimuli. A vibrotactile stimulus (conditioned stimulus) paired with an electric shock (unconditioned stimulus) was presented to the forelimb 80 times. The results demonstrate that conditioning takes place in newborn rats. However, levels of performance as a function of time intervals between stimuli did not resemble the levels traditionally reported for older animals.

There is a paucity of experimental evidence demonstrating learning in newborn animals. In the experiment reported here we undertook to determine the ability of newborn rats to learn a simple conditioned response. The time intervals between presentation of conditioned and unconditioned stimuli were varied to determine whether the performance of the newborn rat is similar to that of older animals.

Newborn albino rats of the Sprague-Dawley strain (*1*) were tested on an apparatus for measuring delayed conditioned leg flexion. The animal was suspended in a harness to immobilize it in a nontraumatic manner. The conditioned stimulus was a vibrotactile stimulus delivered to the animal's chest by a glass rod attached to a speaker cone. The vibration of 128 cy/sec was produced by an audio oscillator set at an amplitude just sufficient to cause the rod to vibrate. No sound was emitted at these values. The unconditioned stimulus was delivered to the right foreleg by means of saline-saturated felt electrodes. This stimulus, of 50-msec duration, was a direct-current electric shock of 1.0 ma delivered through a current-regulating device which insured a constant current value regardless of the change in the animal's body resistance. All time intervals were controlled by electronic timers. Leg flexions were recorded by means of a microtorque motion-displacement transducer connected to the animal's leg by a fine thread. Spring tension on the transducer arm made it possible to record the leg movement. The output of this transducer, fed into an Offner (model A) electroencephalograph, was recorded, together with the time of presentation of the conditioned stimulus.

All animals received initial training with the conditioned stimulus only, within a period of from 1 to 8 hours after parturition, to insure that the stimulus would not elicit a leg-flexion response. The criterion for completion of this phase of training was ten consecutive presentations (conditioned stimulus only) without leg movement. Immediately after this, eight blocks of ten trials were presented, with random intertrial intervals of either 10, 15, 30, 45, or 50 seconds and an interblock interval of 3 minutes. Four groups of animals ($N = 9$ per group, randomly divided between males and females) were given paired conditioned and unconditioned stimuli on four different schedules, with intervals between the stimuli of 300, 600, 1200, and 2400 msec, respectively. For each experimental group a control group ($N = 4$ per group) was tested in a pseudoconditioning situation, with the same conditioned- and unconditioned-stimulus parameters, but with the order of the stimuli randomly varied.

The data presented in Fig. 1 demonstrate that newborn rats are capable of learning a simple conditioned response. Data for males and females within each of the experimental and

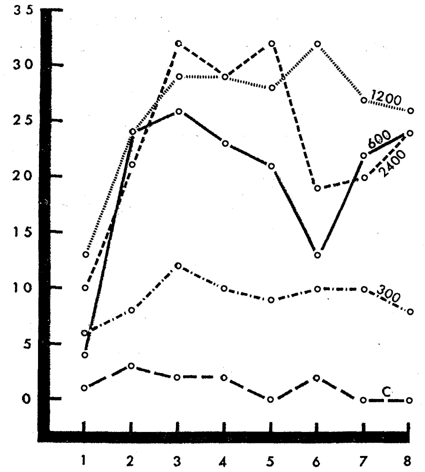

Fig. 1. Mean percentages of conditioned responses for newborn rats trained according to classical conditioning procedure with intervals between conditioned and unconditioned stimuli of 300, 600, 1200, and 2400 msec, respectively.

control groups were combined when analyses for sex differences in conditioning proved nonsignificant. Furthermore, data for the four control groups were combined, since no statistical differences were found for these groups.

The performance of the four experimental groups was significantly superior to that of the control groups ($p < .002$ in a two-tailed Mann-Whitney $U$ test for all comparisons). There were no significant differences in levels of performance of the 600-, 1200-, and 2400-msec groups. However, the performance of these groups did differ significantly from that of the 300-msec group, as indicated by an analysis of variance ($p < .01$, two-tailed test). The highest level of performance attained was 32 percent for the 1200- and 2400-msec groups. This level of performance is below that traditionally reported in the literature on classical conditioning of adult rats (*2*). However, in another investigation (*3*) a maximum performance of 39 percent was reported for classical conditioning; this is similar to the performance of the 2400-msec group. In the study reported here, the optimum interstimulus intervals for learning were found to be between 600 and 2400 msec, with the 1200-msec group manifesting the highest mean percentage of conditioning. This is in contrast to the finding, reported in the experimental literature (*2*), that the best interstimulus interval for learning in adult animals is between 300 and 600 msec. The higher percentage of learning found with longer intervals in the newborn rat may be due, in part, to the lack of maturation in neural development at this age, as compared to development in the older animal, and is probably related to the low degree of myelinization and the low speed of neural conduction concomitant with this stage of neural development. On the basis of this interpretation, a linear relationship between total mean percentage of conditioning and length of interstimulus interval would be expected—the longer the interval the larger the mean percentage of conditioning. It should be noted, however, that the experimental data show a curvilinear relationship for the four stimulus conditions, with the 1200-msec interval resulting in the greatest amount of conditioning. Such a curvilinear relation is similar to that reported in the literature for classical conditioning in the adult, but it differs markedly in that the peak is shifted to a longer interstimulus interval. The performance decrement found for the 300-msec group during the fifth trial block and for the 600- and 2400-msec groups during the sixth trial block may represent a fatigue reaction resulting from the massed presentation of stimuli. The need for additional research on the learning capacities of the newborn organism is clearly indicated (*4*).

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### References and Notes

1. The test animals were the offspring of first-conception gravid animals purchased from Rawley Farms, Plymouth, Michigan, and shipped a distance of 30 miles at 16 to 20 days of gestation.
2. E. R. Hilgard and D. G. Marquis, *Conditioning and Learning* (Appleton-Century-Crofts, New York, ed. 2, 1961).
3. W. C. Biel and D. D. Wickens, *J. Comp. Psychol.* **32**, 329 (1941).
4. This research was supported in part by the U.S. Atomic Energy Commission under contract AT-(11-1)-821.

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## Estimates of Energy Budgets for a Typha (Cattail) Marsh

*Abstract.* Yearly utilization of total solar radiation by a *Typha* marsh shows approximately equal allotment to reflection (albedo), evapotranspiration, and conduction-convection. Reflection during the growing season is proportionally lower because of greater light absorption by the vegetation. Photosynthesis is a negligible quantity, although in relation to visible radiation during the growing season it nearly equals reflection.

Measurements of production of above-ground organic matter, reflection of visible light, and evapotranspiration have been made for a *Typha* (cattail) marsh at Cedar Creek Natural History Laboratory, Bethel, Minnesota (*1*). These measurements, together with solar radiation data from the nearby weather station at St. Cloud, Minnesota, provide material for estimating the total energy budget of the marsh.

Mean total solar radiation measured by an Eppley pyrheliometer at the St. Cloud station from 1950–1959 is given in Table 1 for the growing season, May through September, and for the entire year (*2*). Visible radiation (390 to 760 m$\mu$) was calculated as 50.4 percent of total radiation for the growing season on the basis of data in Tables 137, 148, and 170 of List's report (*3*) by the method outlined by Bray (*4*).

The mean net above-ground production of oven-dry organic matter of *Typha* over a 3-year period was 1360 g/m$^2$ per year. The energy content of this matter was 4340 g cal per gram, as measured by a Parr oxygen bomb calorimeter, for a net production of 590 g cal/cm$^2$ per year (*5*). Underground production could not be measured. The weight of underground material in 1957 was 2960 g/m$^2$ (*6*). Underground production of *Zizania aquatica*, a marsh annual, was 10 percent of above-ground matter. Underground production for *Typha*, which has a large and thick root and rhizome system, is estimated to be at least 20 percent of above-ground matter. Respiration is estimated to be about 15 percent of gross production, a value which represents the median respiration estimate from a wide survey of the literature. Consumption by animals is estimated to be about 1.0 percent of gross production, a value similar to that measured for tree leaves of an angiosperm forest by Bray. (*7*). When these estimates of underground production, respiration and consumption are used in the calculation, the gross photosynthetic production is approximately 842 g cal/cm$^2$ per year.

The reflection of visible radiation toward the zenith (visible albedo) in 1960 was 3.0 percent under a clear sky, with an incoming visible radiation of 110,870 lux. Total albedo for *Typha* was estimated by including measurements of reflection of infrared (760 to $5000 + \mathrm{m}\mu$) from leaf and plant surfaces (*8–11*). These estimates were averaged for each interval of 200 m$\mu$ and weighted by the mean energy content of each interval as listed in Table 130 of List (*3*). The mean of these weighted values was 42 percent. This value, when averaged on a total energy basis with the measured reflection of 3 percent of the visible radiation, gave an estimate of total albedo for the *Typha* marsh of about 22 percent. No measurements of total albedo for a *Typha* marsh are available in the literature, but estimates of 26 percent for high fresh grass and 22 percent for wet grass by List (*3*) indicate that the estimate of 22 percent is within a reasonable magnitude.

Total albedo for the year was calculated to be 34 percent, an integration of the above value of 22 percent on a