Sci. **104**, 190 (1963); K. L. Melmon, M. E. Webster, S. Goldfinger, J. E. Seegmiller, in preparation.
4. M. J. Cline and K. L. Melmon, *J. Clin. Invest.* **68**, 33 (1966).
5. L. M. Greenbaum, R. Freer, K. S. Kim, *Federation Proc.* **25**, 287 (1966).
6. M. J. Cline, *Nature*, in press; *J. Lab. Clin. Med.*, in press.
7. H. Eagle, *Science* **130**, 432 (1959).
8. C. R. Diniz and I. F. Carvalho, *Ann. N.Y. Acad. Sci.* **104**, 77 (1963).
9. M. E. Webster and J. P. Gilmore, *Biochem. Pharm.* **14**, 1161 (1965).
10. M. E. Webster and J. V. Pierce, *Ann. N.Y. Acad. Sci.* **104**, 91 (1963).
11. H. Moriya, J. V. Pierce, M. E. Webster, *ibid.*, p. 172.
12. O. D. Ratnoff and J. E. Colopy, *J. Clin. Invest.* **34**, 602 (1955); J. Margolis, *Ann. N.Y. Acad. Sci.* **104**, 133 (1963).
13. G. Weissmann and L. Thomas, *J. Clin. Invest.* **42**, 661 (1963); G. Weissmann, B. Becher, V. Bevans, *Federation Proc.* **23**, 332 (1964); G. Weissmann, *Blood* **24**, 594 (1964).
14. We thank Mrs. B. Bartuska, Miss Laura Matheson, and Miss Doris Handrock for unusually skillful technical assistance. Supported by USPHS grants CA-07723, HE-09964, and HE-06285.

16 May 1966

Odor Discrimination in Pigeons

Abstract. *An operant procedure was employed to investigate odor discrimination in the pigeon. Amyl acetate concentrations of 6-percent saturation in air, and lower, when paired with electric shock markedly reduced key-pecking during the odor stimulus period. Sectioning the olfactory nerves eliminated this selective suppression behavior. After the operation, the suppression was again conditioned when the concentration of amyl acetate was increased to 15-percent saturation in air. This is theoretically possible through mediation by the trigeminal system.*

Although it has long been known that all birds possess an olfactory epithelium and an olfactory bulb (*1*), it is only recently that Tucker (*2*) has studied the functional status of the receptors. Electrical activity was recorded from the primary olfactory nerves in pigeons, quail, turkey vultures, and eleven other species of birds during olfactory stimulation with amyl acetate and other odorants. Tucker concluded that the olfactory receptors of birds are functional.

Behavioral studies have produced controversial results. By such methods as studying change in heart rate and respiration, some evidence has been obtained that birds can perceive olfactory stimulations (*3*). However, most experiments designed to study the learning of olfactory discriminations have yielded negative results (*4*). Other investigators (*5*) have shown some behavioral change in learning to discriminate olfactory stimuli. Michelsen, using an operant conditioning technique, reported successful odor discrimination in the pigeon. Fink (*6*), using the gray goose as a subject, was unable to repeat the work of Michelsen.

Calvin (*4*), using a classical conditioning technique developed by Orgel and Smith (*7*), was unable to show discrimination of olfactory stimuli in pigeons. Later work by Smith and others in our laboratory has shown that the classical conditioning methods with birds are quite insensitive, and has led to the development of a more nearly adequate technique for the study of sensory phenomena in these animals (*8*). This technique is a variety of the conditioned suppression method and employs dual contingencies of positive reinforcement and aversive stimulation. Base lines for steady, on-going behavior are generated, and can be discretely disrupted by repeatedly pairing the warning stimulus with electric shock. The elimination of responding during the warning stimulus, when compared with base-line responding, gives high signal-to-noise ratios.

The purpose of the present study was to use olfactory stimulation as the warning signal in utilization of the conditioned suppression technique to study olfactory discriminations in the pigeon. The apparatus used for delivery of the olfactory stimulus, and the bird test chamber, are illustrated in Fig. 1. The air was cleaned with silica gel and activated charcoal and was saturated with either distilled water or amyl acetate. The breathing chamber was equipped with a pigeon key and an opening allowing access to a grain hopper. Perforated discs at the intake and exhaust ports of the breathing chamber allowed for more nearly uniform flow of air. A "white" masking noise was present throughout the sessions.

The subjects for the experiment were two Silver King pigeons, 6 and 7 years old, and one 2-year-old white Carneaux. The three subjects were run at approximately 80 percent of normal body weight. Stainless-steel wires were implanted around the pubis bones and terminated in a small plug which was attached to the back of a light canvas jacket worn by the bird (*9*). In the presence of air flow in the chamber (94 cm^3/sec) each of the birds was trained to peck the key for grain reinforcement. Training on a variable interval (V.I.) schedule of reinforcement (average inter-reinforcement period equaled 1 minute) ensued for 1 hour a day for approximately 14 days, at which time the rate of responding was stable. After stabilization of the response rate, suppression training was initiated. During the 1-hour session while the bird was pecking the key (average, approximately 100 pecks per minute), ten suppression trials, three control trials, and two base-line control trials were given. A suppression trial consisted of a 30-second period of stimulation with amyl acetate (6 cm^3/sec) added to the air flow, followed by an 85-msec electric shock administered to the pubis bones. Recordings of the key pecks during the olfactory stimulation and during the 30-second period prior to stimulation were made on two electrical impulse counters. To insure that the animal was responding to the presentation of amyl acetate rather than to an increase in air flow, control trials were run in exactly the same manner except that a volume of air equivalent to that of the amyl acetate

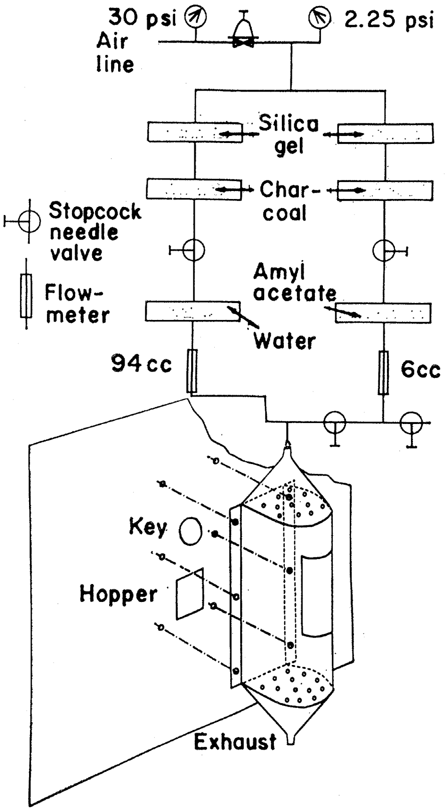

Fig. 1. Schematic diagram of the odorant system. The gas-washing bottles containing the charcoal, distilled water, and amyl acetate were continuously bathed in water at 20°C. Stopcocks and needle valves permitted control and mixture of the air in the two channels, as measured by the flow meters. The resultant concentration (6 percent shown in the diagram) was passed through the pigeon's breathing chamber and exhausted with a vacuum pump.

stimulation was introduced and the shock was omitted. In order to measure the stability of the on-going responding of the birds, base-line controls were introduced, consisting of merely recording the number of key pecks in two consecutive 30-second intervals. The degree of suppression on all trials was measured by the Fleshler-Hoffman suppression ratio (*10*) as given by the following formula:

$$\frac{\text{Pre-stimulus responses}-\text{Stimulus responses}}{\text{Pre-stimulus responses}}$$

By this formula no suppression of responding (as should be seen in base-line controls) yields a ratio of 0.0, and complete suppression during the olfactory stimulation would yield a suppression ratio of 1.0.

Later in the sessions it was found that a stimulus period of 18 seconds was as satisfactory as 30 seconds to show the suppression, and bird No. 3 (the Carneaux) was trained entirely with this schedule. In addition it was found that a V.I.–2-minute schedule yielded more sensitive base lines than the V.I.–1-minute originally used.

Bird No. 1 was trained to suppress its key-pecking response when presented with amyl acetate in a 6-percent vapor saturation. The mean suppression ratios for the odor stimulations were 0.20 on session 5, 0.58 on session 15, 0.67 on session 25, and 0.97 on session 33. Bird No. 3 was trained in a similar manner with a 5-percent saturation of amyl acetate. This bird reached a mean suppression ratio of 0.90 after only 23 training sessions. At the rate of approximately 100 responses per minute, a mean suppression ratio of 0.90 and above indicated the birds made only three or less responses during the warning stimulus. Typically these responses occurred within the first 2 seconds, followed by complete suppression for the remainder of the warning stimulus.

Bird No. 2 failed to suppress key-pecking when presented with the low concentration of amyl acetate, but showed the characteristic suppression when the concentration was increased to 15 percent. No additional data are available on this bird because it did not survive the surgery following this training.

Throughout the experiment, the mean suppression ratios of the air control and base-line trials did not differ significantly from zero. This indicated that the response was due to the odorant and not to an increase in total air flow, and that the base line of responding remained stable throughout the experiment.

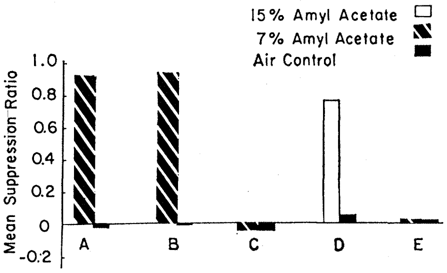

Fig. 2. Mean suppression ratios for bird No. 3: (*A*) last 20 trials prior to sham operation; (*B*) 30 trials after sham operation; (*C*) 20 trials after sectioning the olfactory nerve; (*D*) last 30 trials of retraining at 15-percent concentration; (*E*) 20 trials of retest at 7-percent concentration.

After suppression in response to the olfactory stimulus was established, attempts were made to determine the intensity threshold. (The procedure and results of the threshold measurements are in preparation.) The Carneaux (bird No. 3) suppressed reliably in response to concentrations as low as 0.2 percent of saturation, which is close to the threshold observed by Tucker (*2*) in electrophysiological preparations.

Following the threshold measurements, birds Nos. 1 and 3 were given several sessions with a stimulus concentration of 7 percent. After stabilization of the suppression on this schedule, the birds were anesthetized with sodium pentobarbital and a sham operation was performed. In this operation, the olfactory nerves were exposed, but not cut. Two days after the operation the first of three additional suppression-training sessions was given. After these sessions the birds were anesthetized and the olfactory nerves were severed. On the second day after this operation, the first of two additional training sessions was initiated. Figure 2 shows the results of these sessions for bird No. 3. The results for bird No. 1 were comparable. Prior to the sham operation the mean suppression ratio was 0.90. It can be seen that the sham operation had no deleterious effect on the suppression in response to olfactory stimulation. Cutting the olfactory nerves, however, completely destroyed the olfactory discrimination.

Both birds were retrained with amyl acetate at 15-percent saturation, and stable suppression was established in six to eight sessions. When the concentration was again reduced to 7 percent, both birds failed to discriminate, in spite of the recent history of training at the higher level. Postmortem examination indicated that the olfactory nerves were indeed severed in both birds.

Electrophysiological data have indicated that the threshold concentration for the trigeminal nerve response to amyl acetate is approximately 10 percent in the rabbit and in the gopher tortoise (*11*). It is proposed here that the conditioned suppression in response to the 15-percent saturation, obtained after the olfactory nerves were cut, was possible because of the presence of the trigeminal nerves.

The difference in the maximum suppression obtained with the olfactory nerves intact (at 7 percent amyl acetate) and severed (at 15 percent amyl acetate) does not reflect an absolute difference in discrimination, but is due to an approximately 2-second longer latency in the cessation of responding with the olfactory nerves severed. With latency effects equated, the degree of suppression under both conditions is equal.

These experiments agree with the work of Michelsen in showing that a pigeon can learn an olfactory discrimination. In addition, a procedure for threshold measurement has been developed. These behavioral data relate quite closely to Tucker's electrophysiological threshold measurements for both the olfactory and the trigeminal nerves.

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References and Notes

1. B. G. Bang, *Nature* **205**, 513 (1965); **188**, 547 (1960); S. Cobb, *Perspectives Biol. Med.* **3**, 383 (1960); A. J. Marshall, *The Biology and Physiology of Birds* (Academic Press, N.Y., 1960).
2. D. Tucker, *Nature* **207**, 34 (1965).
3. W. Neuhaus, *Olfaction and Taste*, Y. Zotterman, Ed. (Pergamon, Oxford, England, 1963), p. 111; B. M. Wenzel, *2nd. International Symposium on Olfaction and Taste*, T. Hayashi, Ed. (Pergamon, Oxford, in press).
4. A. D. Calvin *et al.*, *Am. J. Physiol.* **188**, 255 (1957); W. G. Walter, *Arch. Neerl. Physiol.* **27**, 1 (1943).
H. Frings and W. A. Boyd, *Am. Midland Naturalist* **48**, 181 (1952); H. Frings and J. Jumber, *Science* **119**, 318 (19 C. L. Hamrum, *Am. Midland Naturalist* **49**, 872 (1953); W. J. Michelsen, *Science* **130**, 630 (1959).
6. E. Fink, *Zool. Jahrb. Abt. Allgem. Zool. Physiol. Tiere* **71**, 429 (1965).
7. A. R. Orgel and J. C. Smith, *Science* **120**, 891 (1954).
8. I. Dinc and J. C. Smith, *Radiation Res.* **25**, 185 (1965); J. C. Smith *et al.*, *ibid.* **22**, 237 (1964); J. Hendricks, *J. Exp. Anal. Behav.*, in press; D. D. Morris, *ibid.* **9**, 29 (1966).
9. N. Azrin, *ibid.* **2**, 161 (1959).
10. H. S. Hoffman *et al.*, *ibid.* **4**, 575 (1963).
11. D. Tucker, *J. Gen. Physiol.* **46**, 453 (1963).
12. Supported by AEC contracts AT-(40-1)-2903 and AT-(40-1)-2690, and PHS grant MH 11955-01.

18 April 1966