aptic transmission and implies a single transmitter.

To further examine the possibility that one transmitter could produce opposite actions on a single follower cell, we applied acetylcholine (ACh) iontophoretically to the cell body of L7 with an external microelectrode. Iontophoretic application has the advantage of limiting the action of ACh to an area probably less than 50 μ in diameter (*5*) and provides the best experimental approximation of a single synaptic knob. Figure 4A shows the responses of L7 to pulses of ACh of increasing duration. A brief pulse gives a pure depolarizing response (Fig. 4, A1) while (at constant current) longer ones give a diphasic (depolarizing-hyperpolarizing) response (Fig. 4, A2 and A3), The diphasic ACh response is similar to the summated diphasic, synaptic response produced in L7 by a high-frequency discharge of L10 (Fig. 2, B2); the depolarizing phase of the ACh response causes L7 to fire, whereas its hyperpolarizing phase inhibits it. This response to ACh is quite distinct from the monophasic responses shown by other identified cells receiving single actions from L10. For example, L3, which receives only IPSP's from L10, responds to ACh by pure hyperpolarization; whereas R15, which receives only EPSP's from L10, responds with a pure depolarization. Indeed, diphasic responses have not been previously reported in surveys of the cells in this ganglion which categorized the response to ACh as being either purely depolarizing (D) or hyperpolarizing (H) (*6*). Our finding implies the existence of a new pharmacological cell type (D-H) with dual receptor properties (*7*). The ability to produce the dual response with highly localized applications of ACh also suggests that the two receptor types are spatially closely related.

With iontophoretic application of ACh we were also able to explore two alternative mechanisms—one presynaptic, the other postsynaptic—which could account for inversion of the postsynaptic potential: (i) The EPSP and IPSP might be mediated by independent branches of L10 ending separately on the two receptor patches, and the decrement of the EPSP could be caused by a presynaptic failure of the excitatory branch due to blocking at high rates of stimulation. Alternatively (ii) the EPSP and IPSP might be mediated by a single branch of L10 which ends on both receptors, and the decrement could represent a rapid desensitization of the excitatory receptor. With repeated iontophoretic application of ACh, the depolarizing component invariably decreased rapidly, providing support for the desensitization hypothesis. In addition, the hyperpolarizing component of the ACh response often increased with repeated applications. In the extreme case, we could demonstrate that the response properties of the two receptors were sufficiently different that the first of two closely spaced ACh pulses produced a pure depolarizing response, whereas the second pulse produced a pure hyperpolarizing response (Fig. 4, B2).

The simplest model to account for the diphasic response of L7 is that of a single branch ending on two separate (perhaps overlapping) postsynaptic receptors for ACh, one giving rise to excitation and the other to inhibition. The excitatory receptor has a low threshold and a rapid risetime and is quickly desensitized; the inhibitory receptor has a higher threshold and a slower risetime and is not readily desensitized. The existence of two different receptors to ACh is consistent with the recent finding of Chiarandini, Stefani, and Gerschenfeld (*8*) that the depolarization and hyperpolarization produced by ACh in different molluscan cells (H and DILDA) involve different receptor mechanisms producing permeability changes to Na^+ and Cl^-, respectively. It is likely that L7 has both the Na^+ and Cl^- permeability mechanisms to ACh and that these generate the separate components of the dual response.

Cell L7 therefore appears to combine, in a single cell, the receptor properties of cells receiving either purely excitatory or purely inhibitory branches from the same interneuron (L10). These findings provide additional support for the hypothesis (*4*, *6*, *9*) that in this ganglion the sign of the synaptic action is determined, not by the chemical nature of the transmitter substance released by the presynaptic neuron, but by type of receptor and the number of receptor types of the follower cell. The presence of L7 in the only quarter ganglion to contain both excitatory and inhibitory follower cells of L10 also suggests that the distribution of the two types of receptors among different follower cells and the permissible combinations of receptors on a given cell are specified regionally within each quarter ganglion.

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2 August 1967

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Mice: Individual Recognition by Olfactory Cues

Abstract. *Mice discriminated between two male mice of the same inbred strain on the basis of olfactory cues. Mice could also discriminate by olfactory cues between two different species, C3H* Mus musculus *and* Peromyscus maniculatus, *and between males and females.*

Recent investigations with mice have uncovered several important olfactory phenomena. Odors from mice have proved to act as pheromones (*1*) by modifying estrous cycles (Whitten effect), increasing the number of spontaneous pseudopregnancies (Lee-Boot effect), and blocking pregnancy (Bruce effect). There is also evidence of species and sex discrimination by mice on the basis of odor cues alone (*2*).

The well-known work of Bruce *et al.* (*3*) on blockage of pregnancy indicates that individual recognition within strains may occur: they report that females may distinguish between familiar and unfamiliar males of the same strain. Other investigators have argued, however, primarily from the lack of evidence of stable dominance hierarchies, that individual recognition is not found in mice (*4*).

Whether individual recognition occurs is a fundamental issue in the understanding of social behavior in any species. Since odor stimuli reportedly have several profound effects on the social behavior of mice, olfaction seemed the logical modality for an investigation of the presence or absence of intrastrain individual discrimination. In five separate experiments, mice were presented with progressively more difficult odor discriminations, beginning with two chemical odors and ending with a discrimination test between the odors of two genetically similar mice from the same inbred strain.

The subjects were three male and three female C57B1 *Mus musculus*, 90 days old at the beginning of the experiment; they were tested in a Y-maze designed to function as an olfactometer (Fig. 1). A slow, continuous stream of fresh air was blown through two odor-source boxes from which hoses directed the airflow into the arms of the maze. A simple valve system permitted alternation of the odors in each arm between trials. Clear plexiglass was mounted tightly against foam rubber over the entire top of the maze.

The six subjects were preadapted to the apparatus by allowing them to live in all sections of the maze with the blower on and with no odor in the boxes for 2 days. They were then removed and placed on a 23-hour water-deprivation schedule, and the experiment was begun. During each trial, after the subject had made a choice and entered a goal arm, the alley door was lowered to prevent retracing. A selective reinforcement procedure was used for all experiments: a single drop of water (*5*) in a small dish served as a reward whenever the correct odor was chosen. The animal was given 15 seconds in the arm and then replaced in the starting box for 1 minute before the next trial was initiated. By alternation of the correct arm of the maze according to a standardized series (*6*), auditory, visual, and spatial characteristics of the maze arms, as well as odors that lingered between trials, were eliminated as relevant cues.

After each subject had completed its trials for the day, the maze was cleaned with trisodium phosphate and dried. The order of testing of the subjects was varied randomly, and each subject was run through 20 massed trials daily. In all experiments the mice were run to a criterion of 18 correct responses in 20 consecutive trials. If learning did not occur within 140 trials, testing was terminated.

In experiment I, 30 cm^3 of oil of cinnamon, contained in a small flask, was placed in odor-box 2 and was the reinforced odor. A similar quantity of oil of juniper was placed in odor-box 1 and was the unreinforced odor. All subjects reached criterion within 130 trials; the mean number of trials to criterion was 83, standard deviation being 32.5. Individual scores ranged from 30 to 130 trials. Group performance curves for this and the other four experiments appear in Fig. 2; scores of individual subjects, in Table 1.

In experiment II, three male C3H *Mus* were placed in odor-box 1 and served as the source of reinforced odor; three male *Peromyscus maniculatus* in odor-box 2 gave the unrein-

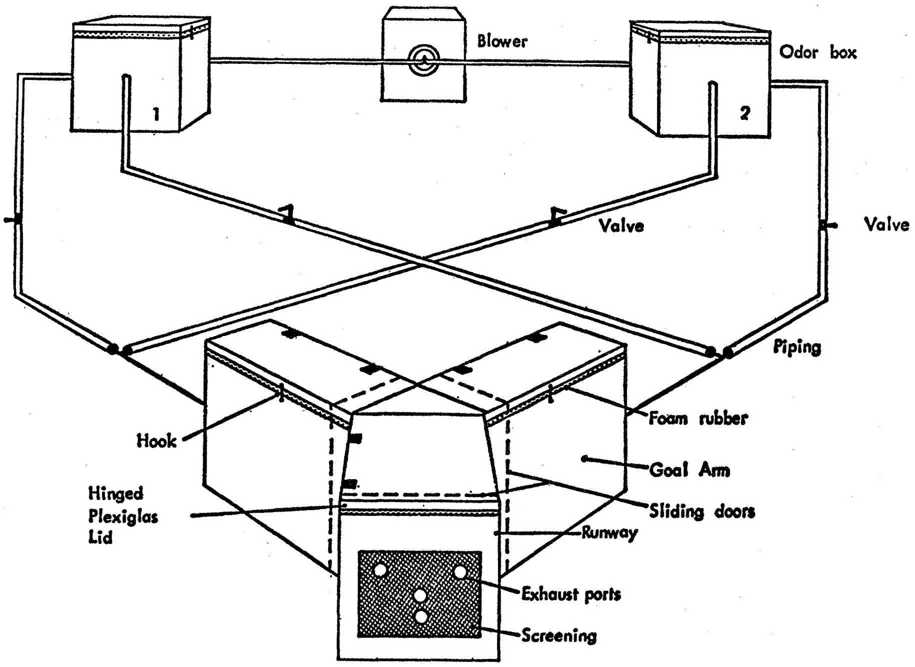

Fig. 1. Olfactory-discrimination apparatus.

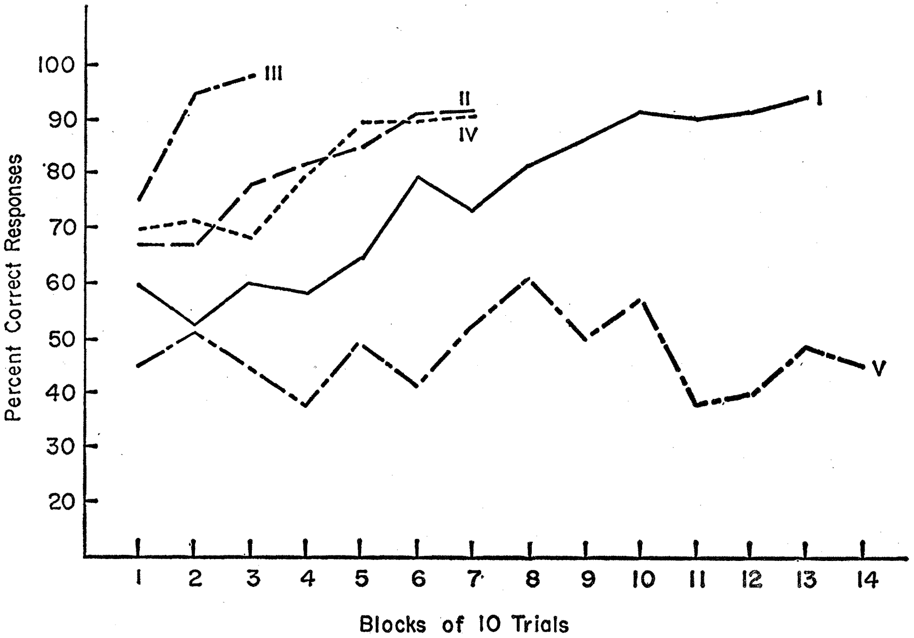

Fig. 2. Acquisition curves in chemical-odor discrimination (I), species-odor discrimination (II), sex-odor discrimination (III), individual-odor discrimination (IV), and control discrimination (V).

Table 1. Trials to criterion for six subjects in five experiments.

Experiment				
I	II	III	IV	V
110	50	30	70	140
30	40	20	40	140
80	50	30	60	140
90	60	20	30	140
130	70	20	50	140
60	50	20	50	140
		Means		
83	53	23	50	140
		S.D.		
32.5	9.4	4.7	12.9	0

forced odor. In this experiment all subjects reached criterion within 70 trials; the mean number of trials to criterion was 53, with a standard deviation of 9.4.

Males and females provided the odor stimuli to be discriminated in experiment III: two male C57B1 *Mus musculus* in odor-box 2 supplied the reinforced odor; two such females were the source of unreinforced odor. All subjects reached criterion within 30 trials; the mean number of trials to criterion was 23, with a standard deviation of 4.7. Each of two subjects required 30 trials; the remaining four required 20 trials each.

For experiment IV the discrimination to be made was between the odors of two male C57B1 *Mus musculus*; the two were neither litter mates nor litter mates of the test animals. All subjects reached criterion within 70 trials; the mean number of trials to criterion was 50, with a standard deviation of 12.9. Individual scores ranged from 30 to 70 trials.

The data from experiments II, III, and IV suggest that the mice may have had an initial preference for the rewarded odor stimuli; the question of whether or not such preferences existed is irrelevant to the purpose of this study. Ability in odor discrimination is demonstrated equally well whether performance results from reinforcement of natural preferences or from development of new ones.

In experiment V the six subjects followed the same procedure as for the previous four experiments, with the blower on, except that no odor stimuli were placed in the odor boxes. This procedure was designed to control for extraneous cues emanating from the odor boxes: for example, auditory or residual odor characteristics peculiar to one of the odor boxes. If such cues were significant the mice should have demonstrated discrimination in this experiment. The results show that after 140 trials none of the subjects reached criterion or showed any consistent improvement. The possibility that discrimination in experiments II–IV was based on sounds produced by the mice in the different boxes can be excluded on the basis of two considerations: First, since adult mice produce noise only sporadically, the probability was extremely small that these cues sufficed to enable the subjects to reach the 90-percent criterion of performance. Second, the noise produced by the blower was very loud and covered a wide range of frequency, probably masking any animal sounds.

These results indicate that, under appropriate experimental conditions, mice can readily discriminate closely related species (experiment II) and sexes (experiment III) on the basis of odor cues alone. This ability in discrimination can be interpreted in terms of its functional and adaptive value for the social behavior of mice. Ability to discriminate the odors of conspecific from odors of extraspecific animals probably serves as part of the isolating mechanism, ensuring the survival and integrity of a species by keeping it separated from sympatric and adjacent species. Similarly, sexual recognition facilitates the distant location of sex partners, making reproduction in this semisolitary animal a more efficient process.

Functional interpretation of the individual-discrimination ability found in mice (experiment IV) is more difficult. Individual recognition may be important to ambulatory pups, aiding them in locating the proper nest. However, there is no real support for the implication of individual discrimination in this and similar behaviors, since other environmental cues may guide them equally well. An alternative hypothesis for the existence of this highly developed individual-discrimination capacity is that it evolved only as a by-product of the necessity to discriminate between their own and closely related species. Interspecies discrimination requires the development of an extremely acute olfactory sense, because an animal often shares part of his range with genetically similar species probably having very similar odors.

One should not assume from the results of this study that individual recognition occurs in nature, or that olfaction is necessarily the primary modality employed for individual recognition; however, the knowledge that odor cues, and the capacity to use them, do exist should prove useful in future investigations of the behavior of wild mice.

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20 October 1967

Osmotic Mechanism and Negative Pressure

Scholander's conclusion that a solvent attains a negative hydrostatic pressure in solution (*1*) is a logical consequence of his assumption that the interaction between solute and solvent is localized to the near vicinity of the solute molecule. It does not depend upon the solute bombardment mechanism for osmotic pressure which he presents. He makes this assumption explicitly in his earlier paper (*2*) by the statement " . . . the water fabric, as it were, although *interrupted* by the solute molecules, evidently is not otherwise altered." This assumption essentially restricts any changes in the activity of the water in a solution to changes in hydrostatic pressure. If the only way the activity of water in a solution can change is through its hydrostatic pressure, at equilibrium the hydrostatic pressure of water must be the same in all parts of a system. Since it is an experimentally proved fact that the hydrostatic pressure of pure water in equilibrium with a solution through a semipermeable membrane is negative with respect to that of the solution, it follows from the assumption stated above that the hydrostatic pressure of the water within the