

classes respond differently to tests involving repeated stimulation and the manipulation of the rat's level of arousal.

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#### References and Notes

1. This technique was used extensively in neurophysiological studies from Helmholtz to the early 1950's. K. Lucas [*J. Physiol.* **51**, 1 (1917)] used it to show that there were two populations of neurons innervating the adductor muscle of the claw of *Astacus*. For references verifying Lucas' conclusions, by single unit recording, see E. J. Furshpan, in *Handbook of Physiology: Neurophysiology*, J. Field and H. W. Magoun, Eds. (American Physiological Society, Washington, D.C., 1959), vol. 1, pp. 240-242.
2. C. R. Gallistel, *J. Comp. Physiol. Psychol.*, in press.
3. J. A. Deutsch, *ibid.* **58**, 1 (1964).
4. There is both internal and external evidence consistent with our interpretation of these data. The refractory period value for the reward effect is characteristic of mammalian A fibers with diameters of 2 to 4  $\mu\text{m}$  [H. Grundfest, *Annu. Rev. Physiol.* **2**, 213 (1940)]. The period of latent addition in such fibers is 0.2 msec. The hump near the abscissa, which we ascribe to latent addition in the fibers receiving subthreshold excitation from the first pulse, has a width of 0.2 msec. The refractory period value for the priming effect is characteristic of mammalian A fibers with diameters of 1 to 2  $\mu\text{m}$ . The period of latent addition in these fibers is also 0.2 msec—again consistent with the width of the hump near the abscissa. Finally, the refractory period estimates are consistent with the following histological and pharmacological data. The self-stimulation system probably utilizes norepinephrine as a transmitter substance [L. Stein and C. D. Wise, *J. Comp. Physiol. Psychol.* **67**, 189 (1969); C. D. Wise and L. Stein, *Science* **163**, 299 (1969)]. The noradrenergic fibers in the medial fore-brain bundle area have diameters ranging from 1 to 4  $\mu\text{m}$  [K. Fuxe, *Acta Physiol. Scand.* **64**, suppl. No. 247, 47 (1965)].
5. The fixed parameters of stimulation during the self-stimulation test were: train duration, 0.3 second; pulse width, 0.1 msec; 100 pulse/sec. The pulses throughout this study were negative-going, but fed through a large capacitance, to prevent polarization of the electrode.
6. Since the first synapse probably acts as a nearly perfect temporal integrator over the relevant range (0.1 to 0.2 msec), refractory period effects probably arise before the first synapse and remain independent of the characteristics of subsequent units in the chain. The Helmholtz nerve-muscle preparation yields the neural refractory period, even when the muscle refractory period is twice as long [H. C. Bazett, *J. Physiol.* **36**, 414 (1908)].
7. The refractory period was estimated by eye from the plotted data on burst size. In doubtful cases (including Fig. 2C) we applied a statistical decision rule based on the  $\chi^2$  test.
8. The neurons shown in Fig. 1B with refractory period values of 0.7 msec and 1.2 to 1.4 msec do not agree with the behaviorally determined refractory period values of the neurons mediating the rewarding and priming effects, respectively. But both sets of neurons were in the same brain sites and responded to further tests in the same manner as the neurons with refractory periods agreeing with the behavioral determinations. Since urethane prolongs the refractory period of nerve bundles [I. Tasaki, *Nervous Transmission* (Thomas, Springfield, Ill., 1953), p. 104], one might include these units in the appropriate populations. However, their inclusion must remain tentative.
9. This histogram was compiled from units recorded in rats tested for self-stimulation and in rats with stimulating electrodes lowered into highly reliable self-stimulation sites at the start of the acute experiments. Results from tested and untested preparations were alike.
10. Supported by NIMH grant 2 R01 MH-13628.
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## Laughing Gull Chicks: Recognition of Their Parents' Voices

**Abstract.** *Laughing gull chicks between 6 and 13 days old responded to the calls of their own parents with orientation toward the sound, approach, increased locomotion, and vocalization. In response to the same kinds of calls from other adults they tended to orient away from the sound, withdraw, and sit or crouch. Chicks as young as 6 days can identify their parents from individual characteristics in the calls of adult gulls.*

Laughing gulls *Larus atricilla* breed colonially (1). Consequently, when the young become mobile, they frequently encounter adults other than their parents. Because the young are usually cared for only by their parents and are treated with hostility by other adults, it must be assumed that there is some means by which parents and young are directed to one another in a gullery. Laughing gull chicks behave differently toward their parents than the way they do toward other adults, even at the same place of encounter (2), which implies that the chick can recognize its parents. I now report an experiment to test the possibility that laughing gull chicks can

distinguish their parents on the basis of individual characteristics of voice. This possibility was suggested by the fact that individual characteristics are discernible in some of the types of calls given by adults (2), and by the fact that the vegetation height and density in the gullery after hatching are such that parents and young are often cut off visually from one another. Recognition by chicks of the voices of their parents has been conclusively demonstrated by experiment with the guillemot *Uria aalge aalge*, a colonially breeding bird in the same order (3). Less-direct evidence has been obtained for it in black-billed gulls *Larus bulleri* (4), sandwich terns *Sterna sandwicensis* (5),

and king penguins *Aptenodytes patagonica* (6).

Laughing gull chicks were taken from their nest areas (7) and tested indoors, one at a time, with recordings of calls of their parents and other adults. The testing situation was a wooden box (120 by 30 by 30 cm) open at the top and with the two end walls consisting of cheesecloth screens. The interior of the box was flat gray. The floor was marked off transversely into 24 strips (5 cm wide) and longitudinally into 2 strips (15 cm wide) to give a reference grid for noting the position of a chick in the box. A portable speaker-amplifier (Nagra DH) connected to a portable tape-recorder (Nagra III) was used to broadcast calls through the cheesecloth screen at one end or the other of the box during a test. The volume of the broadcast sound approximated that of natural calls and was the same in each test, but no measurements of the sound intensity in the box were made. An overhead light source gave even illumination over the floor of the box. The observer sat behind a screen and was not directly visible to a chick in the box. A mirror enabled him to observe the position and behavior of the chick being tested.

The chicks tested came from nests at which recordings of the calls of the parents had been obtained (on the Nagra, with a Sennheiser MKH404 microphone). These chicks were captured in the field and tested at ages which ranged from 6 to 13 days. This range was selected because within it chicks show the first clear signs of being able to recognize their parents. Twelve chicks were tested. For each test session two chicks were selected which were from parts of the gullery remote from one another, and whose ages were as close as possible. Each chick was tested with two tapes—one was a recording of calls of its parents (its "parental" tape); the other was a recording of calls of the other chick's parents (the "foreign" tape). Thus each test tape was used both as a "parental" tape and as a "foreign" tape. This strategy was used to balance out the possible effects of differences between tapes incidental to the identities of the adults recorded.

The tapes were played for 5 minutes, and each contained instances of all the types of call that, as field observation had suggested, might influence filial responses of a chick. Before each test with a tape (sound test) a chick was

Table 1. Responses of laughing gull chicks to recordings of calls of their parents and of other adults. Median scores were derived from the numbers of 15-second intervals (out of 40) in which the item was registered, except for total calls and the position scores (see text). Probabilities are derived from two-tailed Wilcoxon matched pairs tests (matching by chicks). Probabilities involve the comparisons of tests indicated.  $N = 12$ .

Item	Median scores on tests				Probabilities			
	Parental tests		Foreign tests		Silence and sound		Parental and foreign	
	Silence	Sound	Silence	Sound	Parental	Foreign	Sound	Silence
Orientation to speaker	0	23.0	0	1.5	< .01	*	< .01	*
Orientation to other end	2.5	1.0	0	19.0	*	< .01	.01	*
Position score, speaker side	0	118.5	0	28.0	< .01	*	< .01	*
Position score, other side	22.0	31.0	0	48.0	*	.05	*	*
Position change	0.5	18.0	1.0	3.5	< .02	*	< .01	*
Calling	0	20.0	0	0.5	< .02	*	< .01	*
Total calls	0	80.0	0	1.0	< .01	*	< .01	*
Sitting	2.5	0.5	1.0	16.0	*	*	< .02	*

\* Not significant.

observed for 5 minutes in the box with no sound played to it (silence test). The chick was tested with each tape twice, with the speaker placed first at one end and then at the other end of the box. The entire testing session for a chick therefore consisted of four silence-sound sequences. About 12 minutes intervened between each sequence; during these intervals the chick was kept in a room away from the test room although not completely out of earshot of it. The order of presentation of tapes was parental-foreign-parental-foreign for five of the chicks; foreign-parental-foreign-parental for the other seven. The order of speaker location was similarly varied between chicks but independently of the order of tapes.

Every 15 seconds a chick's orientation was scored as either toward the speaker end, or toward the other end, or nil, according to whether it was pointed in a direction within  $20^\circ$  to either side of the direct line from it to one end or the other. The chick's position, determined by the cell on the floor grid in which its feet were placed, was also noted and scored according to whether it was on the speaker or nonspeaker side of the central transverse division, and values were assigned to each transverse strip of the floor grid—the central transverse line was assigned a value of 0 and the strips were assigned values which increased from 1 to 12 reading from the central transverse line toward one end or the other. The chick was also scored as to whether it changed position or not during the preceding 15 seconds. The chick was scored as to whether it called in that interval or not; and the total number of calls uttered by the chick in the whole 5-minute period was tallied. Finally it was noted, at the end of each interval, whether the chick was sitting or on its feet.

The results were subjected to com-

parison of the two 5-minute observation periods in each category (for example, the two foreign silence tests, the two foreign sound tests); comparison of silence tests and sound tests; and comparison of parental tests with foreign tests. To examine whether behavior was regularly different between first and second sound tests or silence tests because of the order in which they occurred, Wilcoxon matched-pairs comparisons (8) were made of first and second sound tests and of first and second silence tests in both the parental and foreign categories. In the same way the possibility was examined that position of the speaker affected behavior. There were no significant differences associated with order, as far as 5-minute observation periods in the same category were concerned, or the position of the speaker. To simplify the other two types of comparison the results of the two 5-minute observation periods, in each category, for each chick, were summed. The subsequent analyses were therefore carried out as though sound tests and silence tests were 10 minutes in duration and hence consisted of 40 intervals of 15-seconds each. Forty was therefore the maximum possible score that a chick could obtain for orientation (to one end or the other or both ends taken together), for position change, for calling, and for sitting. The maximum possible position score, for one side or the other or for both sides taken together, was 480 ( $12 \times 40$ ). The maximum score for total calls was unrestricted by this scoring system (Table 1).

The chicks moved about more, called more, positioned themselves closer to the speaker, and oriented themselves more often toward the speaker, in response to parental calls than they did in response to silence or to the calls of other gulls. Also they spent less time sitting during the parental tests

than during the foreign tests. Behavior in the silence tests consisted, for the most part, of inactivity—a chick stood or sat in silence at or near where it had been placed in the box, occasionally preening, stretching, or nibbling at the wall or floor. On hearing its parents' calls the chick, in most cases, immediately raised its head and started calling (9); it stood up if it had been sitting, oriented itself toward the speaker and walked or ran to the speaker end. There some of the chicks pushed against, scrabbled at, or jumped against the end wall as though attempting to get to the source of the sound. In general, the behavior in the parental sound tests consisted of locomotion and calling by the chick.

The chicks in the foreign sound tests tended to orient away from the speaker and to spend more time in the nonspeaker half of the box than in the speaker half. But there was more variability in the results of the foreign sound tests than in the results of the parental sound tests—some chicks showed more locomotion and calling when the foreign calls were played than in the preceding silence test, although less, as a rule, than in the parental sound tests (two gave more calls than the median number for the parental sound tests); other chicks showed no change in behavior whatever between the silence test and the foreign sound test.

Since each test tape was used both as a parental tape and as a foreign tape, tapes instead of chicks could be matched to give an alternative comparison of scores in the parental and foreign sound tests. Wilcoxon comparisons of the scores matched by tapes gave results essentially in agreement with those obtained from matching by chicks: for orientation to the speaker, position score for the speaker side, position change, calling and total

calls, the scores were significantly higher ( $P < .05$ , two-tailed) when a tape was parental than when it was foreign; for orientation to the nonspeaker end the scores were higher when a tape was foreign than when it was parental ( $P < .05$ , two-tailed); position scores for the nonspeaker side and scores for sitting were not significantly different between a tape that was parental and one that was foreign.

A laughing gull chick's immediate response, in the field, to arrival of one of its parents is usually orientation toward the parent, calling, and approach. In the proximity of an adult other than the parent the chick, particularly if it is outside the family territory, usually orients away from the adult, crouches silently, or flees. Such selective responsiveness by the chick is evident in the field as early as 6 days after hatching. Whatever the total range of characteristics on which the chick can base this selective responsiveness, it is clear from my experiment that individual characteristics in the calls of the adults are sufficient for it, at least after a certain age or degree of experience. What these individual characteristics are, when and how a chick's discrimination of them develops, and what the consequences are of the development of such discriminations for the later behavioral development and social relationships of a chick, are not known.

Recognition by chicks of individual characteristics of the voices of parents is not unique to the guillemot, although the ability in laughing gull chicks may not be established prior to hatching, as Tschanz (3) has shown is the case in the guillemot. The laughing gull chick, unlike the guillemot chick, does not hatch where it is immediately confronted with proximity of numerous adults other than its parents to which it must react selectively. Only after several days when the parents begin leaving them unattended for periods and they start wandering away from the nest, do laughing gull chicks come into frequent close contact with adults other than their parents; and there is reason to believe that ability to recognize the parents develops during the early days after hatching as a consequence of positive conditioning in which association of the parental calls with feeding plays a prominent part (2). In the black-billed gull it seems that chick recognition of the parents' voice is not manifest until after at least 1 day after hatching (4).

Although the details of its develop-

ment and adaptive significance may vary from species to species, individual recognition by young of voices of their parents may be widespread in colonially breeding species of birds (4-6). Recognition of their young by parents (10) and of one adult by another (11) on the basis of individual characteristics of voice may also be more common than is presently realized.

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#### References and Notes

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6. B. Stonehouse, *Falklands Island Dependencies Survey Report* 23 (1960).

7. The chicks were taken from the gullery in the Brigantine National Wildlife Refuge, New Jersey, and the experiment was carried out in the Field Station of the Institute of Animal Behavior, Rutgers University, which is situated in the Refuge.
8. S. Siegel, *Nonparametric Statistics* (McGraw-Hill, New York, 1956).
9. The calls given by the chicks in the experimental situation were harsh in quality. Sonagrams show more or less unstructured bands extending from 1 to 7 khz. The calls were either disyllabic ["chiz-ik" according to M. Nice, *Trans. Linnaean Soc. N.Y.* 8, 1 (1962) and J. P. Hailman, *Behaviour*, Suppl. 15 (1967)] or polysyllabic ("chirirah" according to C. G. Beer, in preparation).
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## Elution of Glomerular Bound Antibodies in Experimental Streptococcal Glomerulonephritis

**Abstract.** *Immunoglobulin G, eluted from glomeruli of rats with streptococcal glomerulonephritis, reacts with type 12 M protein of the streptococcus but not with other streptococcal or renal antigens. Therefore, this disease may be mediated by fixation of antigen-antibody complexes consisting of streptococcal M protein and type-specific antibody.*

Clinical and immunopathologic findings associated with poststreptococcal glomerulonephritis in man suggest that this disease is mediated by the fixation of antigen-antibody complexes in the region of the basement membrane of the glomerulus (1). Which, if any, streptococcal antigen or antigens is involved in the antigen-antibody complex is not certain. The elution and characterization of the bound  $\gamma$ -globulins would provide a direct assessment of their reactivity. However, such studies have not been performed and are

limited by the necessity for more tissue than can be obtained by the usual biopsy techniques.

The availability of an experimental model which fulfills many of the epidemiological, clinical, and laboratory features of the disease, as seen in man, offers an accessible alternative opportunity to study this aspect of the pathogenesis of the disease (2). In this model of experimental streptococcal glomerulonephritis in rats, the disease is restricted to animals exposed to a nephritogenic strain of group A, type 12 streptococcus; it is characterized by proteinuria and by tissue-bound  $\gamma$ -globulin and streptococcal M protein in the region of the glomerular basement membrane. Our characterization of the fixed  $\gamma$ -globulins provides direct support for the hypothesis that streptococcal M protein and type-specific antibody are the immunologic reactants in the experimentally induced disease.

Twenty-six Sprague Dawley rats were exposed to the nephritogenic (A12N) strain of type 12 streptococcus and 15 to the nonnephritogenic (A12) strain (2). All animals were killed at 65 days. There was no difference in the amounts of type 12 specific hemagglutinins in

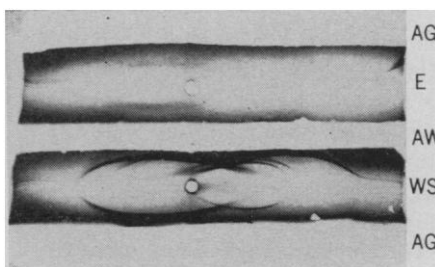


Fig. 1. A comparison by immunoelectrophoresis of serum proteins with those present in the acid eluate of the kidney. E, eluate; WS, whole rat serum; AG, goat antibody to rat globulins (Hyland Labs., lot No. S228C1); and AW, goat antibody to whole rat serum (Hyland Labs., lot No. 822DOO1A1).