

NLP-66 New Son

Brackish water forms might be transported in bilge water or ballast tanks of ships. The American blue crab *Callinectes sapidus* was probably transported to Israel in ballast tanks⁹. Because ships passing through the Panama Canal must maintain a minimum draft, empty ships required to take on additional salt water ballast probably transport microscopic marine organisms from ocean to ocean¹⁰. Several ports in the Chesapeake Bay— for example, Baltimore, Norfolk and Newport News—are either large commercial ports, sites of naval bases or both. West Point, Virginia, adjacent to station P-30, is visited by ocean-going freighters loading paper products. The controlling depth of the river channel to this point is less than 6.5 m, so bilges and ballast tanks are pumped to enable more cargo to be loaded. This may be the way in which brackish water organisms from similar regions elsewhere are being introduced.

Three specimens of *M. inexpectata* from the Pamunkey River, Virginia, have been deposited in the National Museum of Natural Sciences, Ottawa, Canada. One of us (D. R. C.) thanks the National Research Council of Canada for support through a postdoctorate fellowship.

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Induced Bactericidal Response in a Sipunculid Worm

THERE is increasing evidence that inducible immunity may be demonstrated in several invertebrate species using appropriate methods. The bactericidal system^{1,2} seems to occupy an important position in the phylogenetic emergence of inducible immunity. Such responses and others have been reviewed recently^{2,3}.

Bang⁴ has reported an inducible lysis in the coelomic fluid of a sipunculid worm. This substance caused lysis of a parasitic marine ciliate and could be induced by the injection of foreign substances, including crab blood with or without the ciliate, and by injecting large numbers of bacteria.

The preliminary experiments described here demonstrate an inducible bactericidal system in another sipunculid species, *Dehidrostomum zosteriolum* Chamberlain, a worm used for other experiments on comparative immunology⁵.

Animals were collected, maintained and bled as previously described⁶. The bacterium used for immunization and assay was strain EMB-1, a Gram-negative bacillus isolated from the normal gut flora of a spiny lobster⁷. Antigen preparation and bactericidal assay procedures were identical to those used for lobsters⁸. Animals received a primary intracoelomic injection of 2×10^8 formalin-killed bacteria (0.1 ml.). Sixty days later the survivors received a secondary injection of 4×10^8 bacteria.

As Table 1 shows, coelomic fluids from fourteen non-immunized worms had no bactericidal activity. After a

Table 1. BACTERICIDAL TITRES OF CENTRIFUGED-CELOMIC FLUID

No. of antigen injections	Day	No. of animals in group	Individual titres	
None	0	11	0	
	One	1	9	0
			Pool*	10
		2	8	0
		4	Pool*	0
Two	7	4	0	
		1	10	
	60	5	0, 0, 40, 80, 160	
	62	5	0, 100, 160, 320, 320	
	67	6	10, 80, 640, 640, 640, 640	
		0		
		0		

* Pool of coelomic fluids from five worms.

primary injection of antigen, most animals were negative from days 1 to 7 although slight responses were observed in two cases. By the sixtieth day, three of five animals had developed significant titres without further immunization. On days 62 and 67, after an additional challenge with antigen on day 60, bactericidal activity was considerably enhanced. Another group of five non-immunized worms bled at this time were negative. This verified that the increase in coelomic fluid bactericidality was not a consequence of non-specific seasonal variation.

It is interesting that there was no significant response during the first week after primary immunization; thus the sipunculid seems to differ from other invertebrate systems¹⁻³ which often increase their activity significantly during the first day or two after immunization.

The accelerated secondary response of this sipunculid is somewhat reminiscent of the antibody response in mammals. Obviously studies of specificity will be required to assess the significance of this bactericidal system in the evolution of immunity. Such studies are in progress and will be reported elsewhere.

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NLP-66

Discrimination of Auditory Sequences by Monkeys

We have been successful in an attempt to train rhesus monkeys to discriminate among the four permutations of a two element auditory sequence in which each of the elements can be either a burst of tone or a burst of noise. This is an unusually complex task and as such should prove sensitive to manipulations of the central nervous system. Indeed, in experiments now under way, severe deficits have been found in the discrimination performance of monkeys following unilateral ablations of the cortex of the superior temporal gyrus (auditory "association" cortex).

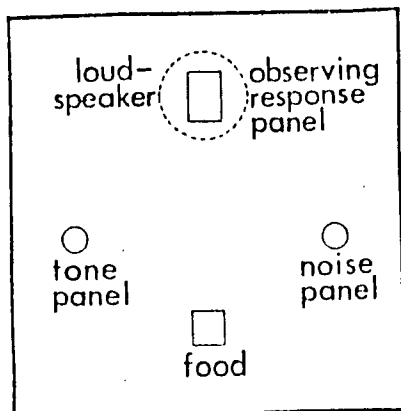


Fig. 1. Testing display.

Training the monkeys to make auditory discriminations is straightforward. From their 22 x 22 x 22 inch cage they can easily reach, through the bars, a 24 x 24 inch testing display (Fig. 1). Mounted behind this display is a loudspeaker through which the auditory stimuli are delivered. On the monkeys' side of the display, centred directly in front of the cone of the loudspeaker, is a 2 x 2 inch 'Perspex' panel which, if pressed at the appropriate time, causes the delivery of an auditory stimulus. Thus to get an auditory discriminative stimulus, the monkey must first give an "observing response".

Lower on the display, placed symmetrically at each side of the food-well, are two circular 'Perspex' response panels which, if pressed at the appropriate time, cause a burst of tone (left panel) or a burst of noise (right panel) to be delivered through the loudspeaker. In the initial stages of training, the animal is taught to press the left panel after a tone stimulus (1,000 Hz for 0.5 s) and the right panel after a noise stimulus (20,000 Hz low-pass "white" noise for 0.5 s). A trial begins therefore when the monkey presses the observing response panel: either a tone or a noise is delivered (at equal probability for each) on a random schedule. Immediately afterwards, the monkey must indicate the nature of the stimulus by pressing either the left or the right circular panel. A correct choice results not only in a tone or a noise (appropriate for the panel pressed) but also in food reward (GHA 190 mg banana-flake pellet). An incorrect choice results in a tone or a noise (appropriate for the panel pressed) and in a 3 s blackout during which a press on any panel is ineffective. After the blackout (or after the delivery of a food pellet when the choice is correct) a new trial may be initiated. Each of the three panels is illuminated from behind, so that at the beginning of a trial only the observing response panel is lit; when the stimulus has terminated, only the left and right panels are lit.

When this task has been learned (90 per cent correct responses in three consecutive daily 100 trial sessions), the monkey is further trained to discriminate between the stimuli when their durations range between 0.1 and 10.0 s.

Then training on sequence discrimination begins. Instead of a single tone or noise burst, a two element sequence (with a silent interval between the two elements) is delivered to the monkey when he presses the observing response panel. All four patterns of this two element sequence occur randomly, each at 0.25 probability of occurrence over a block of 100 trials. Two consecutive presses in the appropriate sequence on the circular panels are required before the next trial can be undertaken.

Five monkeys have so far learned this problem to the 90 per cent correct response criterion. This performance was achieved after 17, 26, 45, 80 and 125 daily sessions for the five animals. The average number of trials per session was 190, 300, 300, 180 and 185, so that the total

number of trials to criterion for each of the monkeys was, respectively, 3,230, 7,800, 13,500, 16,020 and 23,025. It is essential to point out that these figures are only for sessions during which no slumping of the animals' behaviour was undertaken; they therefore do not represent the total effort expended by either experimenters or monkeys but, rather, about a half.

We next investigated the effects on discrimination performance of altering the durational characteristics of portions of the sequence. This was done using a titration testing method² labelled "staircase" or "up-and-down" in which changes in the level of difficulty of discrimination are made within the session in the light of the immediately preceding performance by the subject^{3, 5}. This method allows the experimenter to force performance to a predetermined level for a wide range of stimulus conditions. It is also important that, through the principle of negative feedback, discrimination performance about the predetermined level of difficulty becomes progressively more stable.

This phase of the experiments required at least fifty daily sessions of 100-150 trials. At each session the duration of one portion of the sequence (either the elements or the silent interval between the elements) was manipulated according to a protocol drawn up before the first session, while the other portion was held constant. There were three basic types of sessions in which the duration of elements was increased, in which the interval between them was increased and in which the duration of elements was decreased. Increases and decreases were made from the point of origin of 0.5 s - the duration at which all monkeys were originally trained and for which their performance was best. Durations were from 0.03 s to 10.0 s, divided into fifteen steps - seven on either side of 0.5 s. The durations used as constants included 0.1, 0.3, 0.5, 0.75, 1.0, 1.5 and 2.0 s. During the course of a session, the duration of the critical portion of the sequence was changed one step in the direction of greater difficulty of discrimination after three correct responses in a row and one step in the direction of greater ease of discrimination after an incorrect response. Either an increase or decrease of element length from 0.5 s causes the animal's performance to deteriorate progressively from the stable 90 per cent level, as does an increase of the duration of the silent interval. (Decreasing the interval from 0.5 s to zero does not, in these experiments, adversely affect the monkeys' performance.) Throughout these determinations

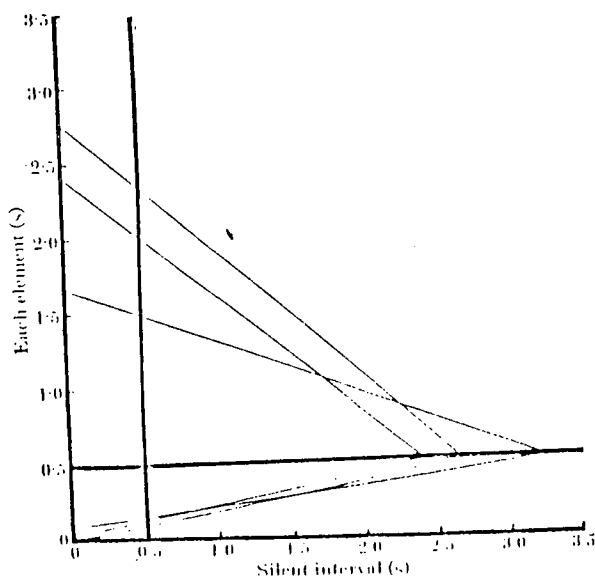


Fig. 2. Response areas for three monkeys. Combinations of sequence element and silent interval duration which fall outside the area yield discrimination performance below the 70 per cent correct response level.

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tions (at the end of every day's testing session) each monkey has been shown to be discriminating at the 90 per cent correct response level whenever elements and interval were set equal at 0.5 s. The first monkey of the present group of three has now been performing at a stable 96 per cent level for 10 months.

We have chosen to plot the data as response areas, within which the monkey can discriminate at the 79 per cent level or better, and outside which his performance falls below 79 per cent correct. This percentage level is a consequence of the testing rules, that is, requiring three-in-a-row correct before increasing the difficulty of the discrimination forces the animal to "home in" on the 79 per cent level⁹. Fig. 2 shows response areas for three monkeys.

A prime ingredient of auditory sequence discrimination which can be assessed from the data of Fig. 2 is short term or recent memory. The testing paradigm we have used resembles a technique developed by Konorski⁷ to study recent memory, and it is important to indicate the differences between the methods and explain why Konorski's technique is unsuitable for sequence discrimination problems. Konorski trained dogs to respond or to withhold response ("go-no go") according to whether two successively presented auditory stimuli were the same or different. This problem has also been mastered (though with considerable difficulty) by monkeys who learned to indicate whether two successive trains of acoustic clicks were made at the same or a different rate⁸. Clearly, an animal need not discriminate between trials where A is followed by B and where B is followed by A; both require the same response. Similarly, trials where A follows A and where B follows B are also equivalent in terms of response requirement. In our experiments, however, AA, AB, BA and BB trials each require different patterns of response, and only by remembering the sequence of presentation can the animal perform correctly. Stepien and Cordeau indicated that requiring their monkeys to withhold response as one of the two response alternatives was perhaps detrimental to the mastery of the problem. Instead, they felt that a "go left-go right" paradigm, using two manipulanda, might prove more successful (Cowey⁹ gave a detailed discussion). We have adopted this suggestion.

The fact that our animals must remember the order in which particular stimuli are heard has important implications. By introducing a delay between the end of the two-element sequence and the time when the animal can begin his responses, or by increasing the number of elements in the sequence, for example, to tone-tone-noise-tone, it should be possible to study short term memory and its decay or interference. It will then be feasible to perform with monkeys the type of digit span experiment that has proved so profitable in the study of short-term memory in man.

The ability to deal with auditory sequences is vital to verbal behaviour¹⁰. It is well known that, in man, severe deficits in verbal behaviour often follow damage to the dominant cerebral hemisphere. Cerebral dominance has never been demonstrated for any infra-human species. We feel that such a phenomenon might be uncovered by study of the effects of unilateral cortical lesions on the discrimination of auditory sequences by monkeys.

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GENERAL

Centroid Method of Integration

THE usefulness, for numerical integration in p dimensions, of the following "centroid method" seems to have been generally underestimated. The method is a generalization of the mid-point method for one dimension. The first approximation to the integral is $f(\bar{x})$ multiplied by the "volume" of the region of integration, where \bar{x} is the centroid of the region. This first approximation is itself often more accurate than might be expected. (It is exact if f is linear.) If f has a p -dimensional Taylor expansion about the centroid, valid in the region, then the integral is given exactly by the following equation

$$\int \dots \int f(x) dx = \sum_{v_1, v_2, \dots, v_p} \frac{m(v)}{v_1! v_2! \dots v_p!} \cdot \frac{\partial^p f(x)}{\partial x_1^{v_1} \dots \partial x_p^{v_p}} \Big|_{x = \bar{x}}$$

where $n = v_1 + v_2 + \dots + v_p$, where the terms for $n=1$ vanish, and where $m(v)$ is the generalized moment of inertia

$$\int \dots \int (x_1 - \bar{x}_1)^{v_1} \dots (x_p - \bar{x}_p)^{v_p} dx$$

Because any non-pathological domain can be "triangulated" into simplexes, it is convenient to have a formula for the generalized moments of inertia of a simplex the vertices of which are $x_k = (x_{k1}, \dots, x_{kp})$, ($k=0, 1, \dots, p$). It can be shown that $m(v)$ is equal to the coefficient of $t_1^{v_1} t_2^{v_2} \dots$ in

$$\frac{V p! v_1! v_2! \dots}{(p+n)!} \exp\left[\frac{1}{2} W_2 + \frac{1}{3} W_3 + \dots\right]$$

where V is the "volume" of the simplex, given by a familiar determinant, and where

$$W_n = \sum_{k=0}^p \left\{ \sum_{i=0}^p (x_{ki} - \bar{x}_i) t_i \right\}^n$$

If $1 \leq n \leq 3$ (but not if $n \geq 4$), it can be readily deduced that

$$m(v) = \frac{V p! (n-1)!}{(p+n)!} \sum_{k=0}^p (x_{k1} - \bar{x}_1)^{v_1} \dots (x_{kp} - \bar{x}_p)^{v_p}$$

and if $p=2$ and $1 \leq n \leq 5$ (but not if $n \geq 6$),

$$m(v) = V c_n \sum_{k=0}^2 (x_{k1} - \bar{x}_1)^{v_1} \dots (x_{kp} - \bar{x}_p)^{v_p}$$

where $c_2 = 1/12$, $c_3 = c_1 = 1/30$, $c_4 = 2/105$. An example of a fourth moment for arbitrary p is

$$m = (4, 0, 0, \dots, 0) = -\frac{3Vp!}{(p+4)!} \left\{ \left[\sum_{k=0}^p (x_{k1} - \bar{x}_1)^2 \right] + 2 \sum_{k=0}^p (x_{k1} - \bar{x}_1)^4 \right\}$$

As an example we take $f(x) = \exp(x_1 + \dots + x_p)$, and for the region of integration the simplex whose vertices