number of adequate shells increases. *Pagurus hirsutiusculus* lives in an environment where adequate shells are scarce (*11, 28*). Sensitivity to calcium may enable this species to locate partially buried shells, thus increasing the number of shells available for habitation. Living in an area with a siliceous-based substrate and little coral, *P. hirsutiusculus* may be able to discriminate empty shells from those housing living gastropods (*31*) as well as from pebbles or unsuitable homes.

Calcium is one of the most important control and regulatory substances in physiological systems (*32*), but reports of the elicitation of behavior by metal ions such as calcium are rare (*33*). Studies of the effects of calcium on whole animal systems may reveal a role for calcium sensitivity in other species.

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8. Replicas of several different compositions that varied in weight and internal configuration were also examined (K. A. Mesce, in preparation).
9. Coated shells (shells without a calcium cue) were only explored if the crab's chelae accidently entered the shells' aperture and chelae probing was released or the crab was presented with one in a no-choice situation after being without a shell for hours. Shells were coated with Au-Pd film, paraplast wax, varathane, silicone rubber, or cyanoacrylate glue. At least ten crabs were tested with each type of coating.
10. This contrasts with the findings of E. S. Reese (*2*) who reported that the hermit crab species *P. samuelis* will quickly explore shells that are coated and hence lack a calcium cue.
11. R. R. Vance, *Ecology* **53**, 1062 (1972).
12. Outside molds of natural shells were made from silicone rubber. Inside molds were made from Blend Impression Material (Westone Labs., Inc.). The two molds were well aligned with one another to ensure accurate wall thickness. Core molds were carefully twisted out from both the original and plaster shells and could be used over again many times.
13. Hermit crabs were not found to prefer boiled shells over those that were well scrubbed and air or oven dried. Gloves were polyvinyl chloride (VWR Scientific).
14. Full exploratory behavior consisted of grasping and extensive scratching of the object with the chelae and repeatedly turning it over between the appendages. Because the objects did not have an aperture, chelae probing could not be fully expressed.
15. The same hermit crabs were also observed to select (100 percent) natural and replica shells over ones that had been coated and lacked a calcium cue in the same $CaSO_4$ saturated seawater. Shell selection behavior also appeared normal under these conditions. It was concluded that the crab's detection of calcium or sulfate was not impaired by raising the levels of $CaSO_4$ in the seawater and that animals could still detect a greater amount of calcium at the shell surface.
16. Possible effects of SO_4 are not completely ruled out because the solubility of celestite is less than that of gypsum. However, the calcium carbonates are even less soluble than $SrSO_4$ and they were effective in promoting exploratory behavior.
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19. *Pagurus samuelis* was tested under the same conditions as *P. hirsutiusculus*. Crabs were given a choice between natural shells (*Tegula funebralis*) or their plaster replicas and natural or replica shells coated with paraplast wax or silicone rubber. Because *P. samuelis* is visually attracted to particular shells (*2*) animals were tested in complete darkness or with black paint covering their eyes [K. A. Mesce, *Am. Zool.* **20**, 727 (1980)].
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27. N. Fotheringham, *ibid.* **57**, 570 (1976).
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31. Many living gastropods, especially young ones, secrete a periostracum around their shells which presumably reduces the amount of calcium lost from the surface.
32. A. Scarpa and E. Carafoli, Eds., *Calcium Transport and Cell Function* (New York Academy of Sciences, New York, 1978).
33. A significant influence of calcium was demonstrated in the homing of sock-eye salmon [D. Bodznick, *J. Comp. Physiol.* **127**, 157 (1978)], which are sensitive to calcium ion concentrations in the range of 10^{-6} to 10^{-3} *M*.
34. I thank G. Hoyle, S. Zill, R. Franklin, J. Mittenthal, K. Thompson, L. Muske, C. Cypher, and the reviewers for valuable discussions and comments on the manuscript. I also thank H. Howard for photographic assistance and B. Orr for providing the minerals. Supported in part by NSF grant BNS-79 04513 to D. Hoyle.

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Monoclonal Antibody to Acetylcholine Receptor: Cell Line Established from Thymus of Patient with Myasthenia Gravis

Abstract. *A human B cell line producing a monoclonal antibody to an antigenic determinant of acetylcholine receptors was established by cloning B cells that had been transformed in vitro by Epstein-Barr virus. The B cells were obtained from the thymus of a patient with myasthenia gravis. The antibody produced by the cell line precipitated acetylcholine receptors from denervated and innervated rat muscle and from human muscle, but did not show detectable response to the acetylcholine receptors from the electric organs of* Narke japonica. *The monoclonal antibody showed identical binding patterns in innervated and denervated rat muscles. Passive transfer of the monoclonal antibody into rats induced moderate muscle weakness and electromyographic changes characteristic of myasthenia gravis.*

Myasthenia gravis (MG) is an autoimmune disease in which neuromuscular transmission is impaired by autoantibodies to acetylcholine receptors (AChR). Monoclonal antibodies against *Torpedo* AChR produced by hybridoma cells have been used to identify individual determinants of AChR (*1, 2*). Only some of the antigenic determinants of *Torpedo* AChR proved to be shared with mammalian AChR and to induce MG (*2*).

Using B cells from the thymus of a patient with MG, we have now established a human B cell line producing a monoclonal antibody against AChR by cloning the cells after their transformation in vitro by Epstein-Barr virus. We chose the thymus as a source of B cells sensitized with AChR because antibodies to AChR have been found in 70 percent of the tissue extracts and culture fluids of the thymus and in germinal centers in the thymus of patients with MG (*3–5*).

Thymus lymphocytes from a patient with MG were suspended in RPMI-1640 medium with 20 percent fetal calf serum (*6*). Eight parts of thymus lymphocyte suspension (2×10^7 cells per milliliter) were mixed with two parts of the culture fluid of B95-8 cells (used as a source of Epstein-Barr virus) (*7*). After incubation at 37°C for 3 hours, enough culture medium was added to adjust the cell concentration to 2×10^6 per milliliter. The lymphocytes were cultured further for 1 month in Linbro plates (76-033-05) in a humidified atmosphere of 5 percent CO_2 in air at 37°C. Half the volume of medium was changed once or twice a week. One month after the culture was started, the amount of antibody to AChR in the supernatant of each well of the Linbro plates was assessed, and the cell clusters producing relatively high titers of the antibody were dispersed to form new clusters. This procedure was repeated two times, until a cluster producing a reasonably high titer of the antibody was obtained. This cluster was used as a source for cloning the antibody-producing cells. Cells producing antibody to AChR were cloned twice by limiting dilution (0.4 cell in 0.2 ml per well) in Microtest II plates (Falcon 3040) in the presence of feeder cells (10^4 cells per well) prepared by x-ray irradiation (2000 rads) of autologous lymphocytes that did not secrete antibody to AChR or by treating the cells with mitomycin C at a concentration of 50 μg/ml for 30 minutes at 37°C. Feeder cells were not proliferative under these conditions. Four weeks after the cells were cloned, supernatants

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from each well were assayed for antibody to AChR by an immunoprecipitation radioimmunoassay. For the assays, solubilized AChR was obtained from innervated rat muscle, denervated rat muscle, IT45R92 (a myogenic cell line from Wistar rat thymus) cell homogenates (*8*), amputated human thigh muscle, and *Narke japonica* complexed with an excess of α-[^{125}I]bungarotoxin (*6*). Muscle action potentials were recorded by the method of Satyamurti *et al.* (*9*).

The cell line producing monoclonal antibody to AChR has been passaged continuously for more than 8 months without loss of specific antibody production (Fig. 1A). When 3.5×10^4 cells were cultured in a conical tube containing 2 ml of culture medium, the doubling time of the cells during the logarithmic growth phase was 3 to 4 days and the production rate of antibodies to AChR was 7.7 fmole per 10^4 cells per day. These values varied with culture conditions such as cell density.

The specificity of the antibody produced by this cell line was examined by measuring its binding to AChR from different sources. The antibody precipitated AChR extracted from denervated and innervated rat muscles, from IT45R92, and from amputated human muscle, but did not precipitate detectable amounts of AChR from the electric organ of *Narke japonica* (Fig. 1B). However, the antibody from the serum of the lymphocyte donor precipitated the *Narke* AChR moderately (data not shown). According to Weinberg and Hall (*10*), the antibody in the serum of MG patients recognizes two classes of determinants on AChR: one that occurs only on extrajunctional AChR, and the other common to junctional and extrajunctional AChR. To test which determinants the monoclonal antibody responds to, we incubated various amounts of the antibody with equal amounts of AChR from denervated rat muscle and from innervated rat muscle complexed with toxin (Fig. 1C). Binding patterns of AChR from denervated and innervated rat muscles were similar over a wide range of antibody concentrations. These results in vitro indicate that the monoclonal antibody might be directed to a determinant common to the AChR from denervated and innervated rat muscles and that it binds to antigen determinants exposed on AChR in innervated rat muscle in vivo. To confirm this assumption, rats (160 g) were given, for 5 days, daily intravenous injections of 2 ml of monoclonal antibody to AChR produced in culture (45 to 50 pmole/ml). Clinical weakness was moderate at the end of the experiment. To ensure the biological activity of the antibody, the rats were anesthetized with 75 mg of chloral hydrate, and muscle action potentials evoked by stimulation of sciatic nerve were recorded. Repetitive nerve stimulation at a rate of 10 sec^{-1} decreased the response. The response was restored from a 32 percent decrement to a 9 percent decrement after an injection of edrophonium chloride (20 μg) (Fig. 2). Tests for nerve stimulation showed that the passively transferred antibody to AChR in rat serum was 6.5 pmole/ml.

The muscle weakness observed in rats treated with antibody to AChR seems to be induced by the monoclonal antibody bound to a determinant apart—but not far—from the binding site for acetylcholine on the α subunit of AChR. The capacity of the monoclonal antibody to interfere with the binding of α-bungarotoxin, which binds to the site for acetylcholine on AChR, appears to be as small as 10 percent when tested with human AChR and denervated rat muscle AChR (data not shown). Lennon and Lambert found that monoclonal antibodies to AChR produced by rat hybridoma cells impair neuromuscular transmission without affecting the binding of neurotransmitter to its binding site (*2*). Thus, antibodies to AChR bound to some determinants—not necessarily the binding site

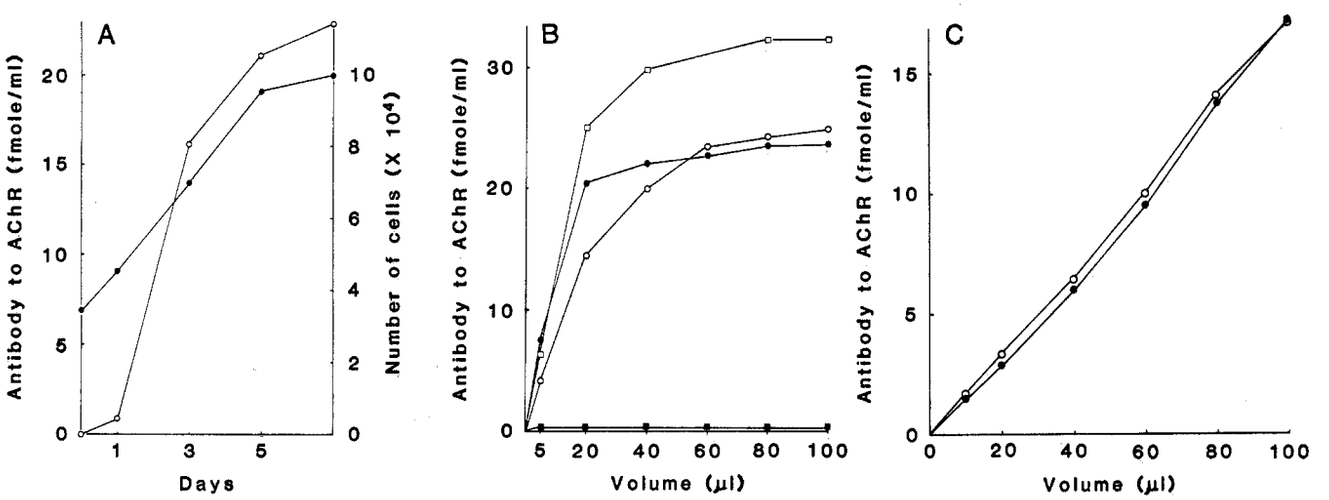

Fig. 1 (above). (A) Growth curve of cells and levels of antibodies released by cells producing monoclonal antibody to AChR. Samples of culture supernatant were incubated with an excess of denervated rat muscle AChR for 12 hours at 4°C, and 225 fmole of α-bungarotoxin was added to the reaction mixture. Culture medium was not changed during the experiments. Each point represents the average of duplicate assays; (●) number of cells and (○) amount of antibody to AChR. (B) Precipitation of AChR from various sources by the monoclonal antibody. The abscissa indicates the volume of culture medium containing monoclonal antibody added to the reaction mixture. All assays were carried out in duplicate with a single batch of culture fluid and the same amount of AChR (53 fmole) from (□) denervated rat muscle, (■) *Narke*, (○) IT45R92, and (●) human. (C) Binding patterns of AChR from denervated and innervated rat muscles to the monoclonal antibody. The abscissa indicates the amounts of culture medium containing the monoclonal antibody. Each point was determined by duplicate assay. The batches of denervated rat AChR and culture medium were different from those used in (B). Both receptors were adjusted to 79 fmole for assay; AChR from (○) innervated rat muscle and (●) denervated rat muscle.

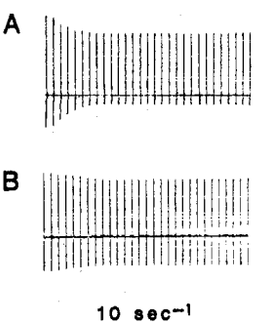

Fig. 2 (bottom right). Evoked potentials recorded over plantar muscles with sciatic nerve stimulation at 10 sec^{-1}. (A) Rat given monoclonal antibody to AChR. (B) Same rat 10 minutes after intravenous injection of edrophonium chloride. Electromyographic responses were recorded by a spark writing recorder (DISA 1500, Denmark).

for cholinergic ligands—are likely to induce the degeneration of AChR (*11*) or affect transmembrane ion exchange (*12*), resulting in muscle weakness. Failure to precipitate *Narke* AChR suggests that our monoclonal antibody is directed to a particular determinant present on mammalian AChR.

The fact that antibodies to human AChR share some idiotype specificities suggests that the number of idiotypic determinants is limited (*13*). Thus, it might be possible to prepare specific immunosuppressants by raising antibodies against different types of idiotypic determinants on molecules of human antibody to AChR.

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Eye Movements of Preschool Children

Abstract. *Accurate recordings of eye movements of children 4 and 5 years old show that their eye movements differed from those of adults. During maintained fixation, saccades were large (1° to 2°) and smooth eye movement speeds were high (45 minutes of arc per second). Saccade latencies were highly variable during target step tracking. Smooth pursuit latencies were longer than those of adults. These hitherto unknown characteristics limit a child's ability to use eye movements to acquire visual information.*

Eye movements are essential for effective visual processing. For example, saccades bring selected retinal images to the central fovea where visual acuity is best. Once the image is foveal, saccades and smooth eye movements must maintain fixation so that visual details can be discerned. Despite the importance of eye movements, little is known about their development (*1*).

We undertook to study oculomotor development by making accurate recordings of the two-dimensional eye movements of two preschool children, Philip (4 years 7 months) and Jennifer (5 years 3 months) (*2*). These children were asked to perform simple oculomotor tasks, which were chosen because they require little instruction and have been studied carefully in adults (*3*). We found that the children did not fixate as well as adults. This result has implications for understanding the development of visual processing.

The children were asked to fixate a small bright stationary point. This target, displayed on a cathode-ray tube located at optical infinity, was visible in an otherwise darkened room. The children were asked to "look at the star" and reminded to do so throughout the recording sessions (*4*).

Fixation by preschool children is not like adult fixation. The children's line of sight was unstable. This finding is best summarized by the two-dimensional scatter of the line of sight, specifically, the mean bivariate contour ellipse area—

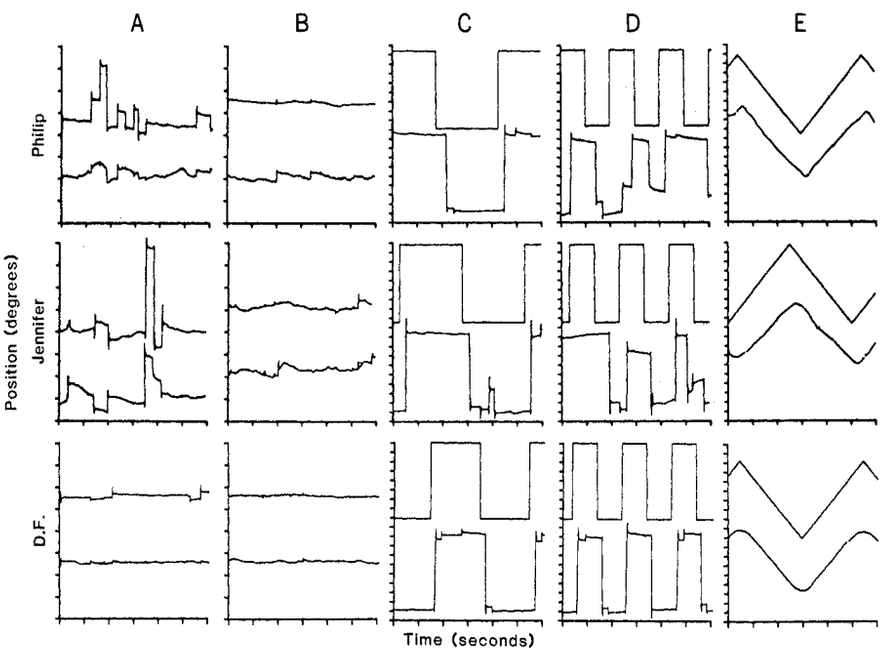

Fig. 1. Representative eye movement records. The time scale shows 1-second intervals and the position scale, 1° rotations. (A) Fixation of a stationary target. Top traces show horizontal and bottom traces vertical eye movements. Saccades (high-velocity rotations of the eye) 1° or larger are frequent in the children's records. The large overshoots at the end of saccades are caused in part by slippage of the crystalline lens in its capsule. (B) Examples of saccade-free intervals during fixation. (C) Saccadic tracking of low-frequency periodic horizontal target steps (0.4 step per second). Top traces show the stimulus and bottom traces horizontal eye movements. (D) Saccadic tracking of higher frequency target steps (1 step per second). (E) Smooth pursuit of periodic horizontal constant velocity (2.4° per second) target motion. Top traces show the stimulus and bottom traces, horizontal eye movements. Saccades have been removed from these records. Eye traces were corrected for the changes in position introduced by saccades by assuming that smooth eye movements continued during the saccade at the velocity present just before saccade onset.

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