30. J. I. Miyazaki, E. Appella, K. Ozato, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 9537 (1986).
31. The functional significance of a partial homology between the IRS region and the repressor region of the IRE (Fig. 1) remains to be evaluated, since dissociation of the repressor from the IFN β IRE is controlled by virus or double-stranded RNA, not by IFN itself.
32. Radioimmunoassays were performed as described (*10*) with the antigen-specific monoclonal antibodies 28-14-8, (αH-2D^b); 20-8-4, (αH-2K^b); or 11-4-1, (αH-2K^k).
33. J. LaLanne, C. Delabre, G. Grachelin, P. Kourilsky, *Nucleic Acids Res.* **11**, 1567 (1983).
34. Time course studies determined the appropriate time lengths for IFN incubations. Endogenous H-2 antigen expression on BL5 and BLK SV cells was fully induced in 48 hours by type I and 60 hours by type II IFN (determined by radioimmunoassay). CAT gene expression driven by the H-2 class I promoter was fully induced in 12 hours by type I IFN, and 36 hours by type II IFN. All inductions were stable for up to 5 days. Therefore 3-day IFN incubation periods were chosen for these experiments. IFN α + β (1000 unit/ml), and IFN γ (20 unit/ml), were fully saturating and used for all experiments described. Murine IFN α + β was purchased from LEE BioMolecular. CAT assays were performed as described (*10*).
35. MPC11 nuclear extracts and footprinting reactions were performed as described in B. Korber, L. Hood, and I. Stroynowski [in *Major Histocompatibility Genes and Their Role in Immune Function*, C. S. David, Ed. (Plenum, New York, in press)]. AP-1 purification and footprinting reactions were performed as described (*21*).
36. We thank K. Blackburn and M. Krempin for superb professional assistance with tissue culture, and D. Bohmann for advice during AP-1 purification. We thank M. Shepard of Genentech for providing the recombinant murine IFN γ, D. Vapnek of AMGen for human α consensus IFN, and T. Hansen for monoclonal antibodies. Supported by a National Research Service Award (T32GM7616) from the National Institute of General Medical Sciences and by an NIH grant AI 19624.

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Salivary Gland Lysates from the Sand Fly *Lutzomyia longipalpis* Enhance *Leishmania* Infectivity

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Leishmaniasis is a parasitic disease transmitted by phlebotomine sand flies. The role of sand fly saliva in transmission of the disease was investigated by injecting mice with *Leishmania major* parasites in the presence of homogenized salivary glands from *Lutzomyia longipalpis*. This procedure resulted in cutaneous lesions of *Leishmania major* that were routinely five to ten times as large and contained as much as 5000 times as many parasites as controls. With inocula consisting of low numbers of *Leishmania major*, parasites were detected at the site of injection only when the inoculum also contained salivary gland material. This enhancing effect of sand fly salivary glands on cutaneous leishmaniasis occurred with as little as 10 percent of the contents of one salivary gland of one fly. Material obtained from other bloodsucking arthropods could not mediate the phenomenon.

PROTOZOAN PARASITES OF THE GENUS *Leishmania* are transmitted through the bite of infected phlebotomine sand flies. The sand fly can transmit several different species of *Leishmania*, causing different forms of the disease in the infected mammalian host. Cutaneous forms are characterized by the development of lesions at sites in the skin where the infected sand fly has probed for a blood meal (*1*). In a manner analogous to other bloodsucking arthropods, sand flies salivate into the skin of the host in the course of obtaining a blood meal. The saliva of arthropods in general (*2*), and sand flies in particular (*3*), has several potent pharmacological activities, including apyrase activity and factors that inhibit platelet aggregation and induce erythema. We therefore investigated the possibility that these activities, or others not yet described, in the salivary glands of the sand fly might affect transmission of *Leishmania*.

We worked with the sand fly *Lutzomyia longipalpis*, a known vector for visceral leishmaniasis in South America and the only sand fly for which pharmacological data are available for its salivary secretion (*3*). The parasite we used was *Leishmania major*, the causative agent of cutaneous leishmaniasis in the Old World and a parasite for which there is an established murine model of infection. Promastigotes of *Le. major* were mixed with lysates of the salivary glands of *Lu. longipalpis*. An inoculum consisting of 10^5 parasites and 0.5 of one salivary gland was injected into the footpads of either CBA mice [animals genetically resistant to infection with *Le. major* (*4*)] or into BALB/c mice [animals genetically susceptible to the disease (*4*)]. Lesions on mice that received a mixture of *Le. major* promastigotes and lysates from the sand fly salivary gland were substantially more severe than lesions on control animals both in CBA and in BALB/c mice (Fig. 1).

We then examined the effect that lysates of the salivary glands of *Lu. longipalpis* would have on the course of lesions induced in mice injected with decreasing numbers of *Le. major* promastigotes. To this end, CBA mice were injected in the footpad with inocula consisting of 10^4, 10^3, 10^2, or 10^1 *Le. major* promastigotes admixed with the lysate of 0.5 of one salivary gland. We found (Fig. 2) that the effect on the course of cutaneous leishmaniasis was especially pronounced when the infecting inoculum contained low numbers of parasites. Lesions on mice receiving 10^2 or 10^1 *Le. major* promastigotes mixed with salivary gland lysates were often five to ten times as large as lesions on control mice receiving parasites alone.

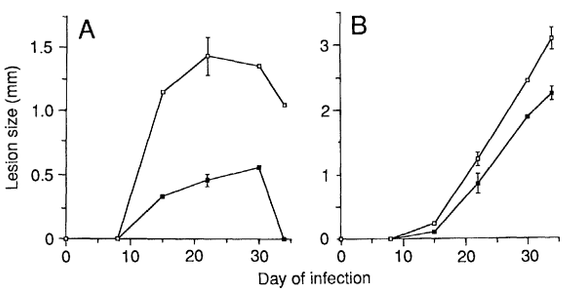

Fig. 1. Enhancement of the infectivity of *Le. major* by lysates of the salivary glands of *Lu. longipalpis*. Groups of five (**A**) CBA/T6 or (**B**) BALB/c mice (Jackson Laboratory) were injected subcutaneously in one hind footpad with either 10^5 *Le. major* promastigotes in 20 μl of 0.1% bovine serum albumin, *p*H 7.0 (BSA) (Sigma) in phosphate-buffered saline (PBS) (■) or 10^5 promastigotes in 20 μl of BSA/PBS containing 0.5 of one gland of *Lu. longipalpis* (□). *Leishmania major* promastigotes (LV 39) were maintained as described (*7*); parasites from stationary phase (*6*) cultures were used in all the experiments. *Lutzomyia longipalpis* were reared by the procedure of Modi and Tesh (*8*). Salivary glands from mated, non–blood-fed, 5- to 7-day-old adult female sand flies were dissected, placed in 0.1% BSA in water (*p*H 7.0), and frozen to achieve complete disruption (*3*). The glands were stored at −70°C until use, at which time they were brought to isotonicity by the addition of 10× PBS. We determined lesion size by measuring, with a vernier caliper, the thickness of the infected footpad compared to the thickness of the contralateral uninfected footpad. The values given are means ± SEM.

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Next we determined whether the increase in lesion size induced by lysates of the salivary gland of *Lu. longipalpis* was accompanied by a parallel increase in the number of parasites present in the lesion. This was important because lesion development in cutaneous leishmaniasis is characterized by a heavy mononuclear cell infiltrate, and thus an increase in lesion size might represent only an increase in the cell infiltrate and not an increase in parasite numbers. Limiting dilution analysis (*5*) to determine the number of viable parasites present in the lesions was performed on footpads from the groups of mice depicted in Fig. 2. The numbers of parasites in lesions induced with *Le. major* promastigotes and salivary gland lysates were profoundly elevated, and the greatest enhancement of parasite numbers occurred in mice injected with low numbers of *Le. major* promastigotes mixed with salivary gland lysates. When 10^2 parasites mixed with salivary gland material were injected, parasite numbers in the lesions were as much as 5000 times the numbers in controls; when 10^1 parasites were injected, the presence of salivary gland lysate in the infecting inoculum determined whether or not parasites could be detected in the lesions (Table 1).

We also examined the possibility that the infectivity of *Le. major* would be enhanced by the salivary glands of bloodsucking arthropods that transmit other parasites. Salivary glands from mosquitoes (*Aedes aegypti*), triatomine bugs (*Rhodnius prolixus*), and ticks (*Ixodes dammini*) were prepared in the same fashion as sand fly salivary glands, and the four preparations were injected into CBA mice. None of the other glands added at equivalent protein concentrations had any effect in the system (Fig. 3). This result suggested that the enhancing effect of the salivary glands of *Lu. longipalpis* on cutaneous leishmaniasis was an attribute unique to the sand fly.

The mechanism by which the salivary gland constituents from the sand fly exacerbate cutaneous leishmaniasis is unknown. However, the potency of the effect is quite striking, and in four independent experiments the salivary gland lysates exacerbated the disease to an equivalent extent. As little

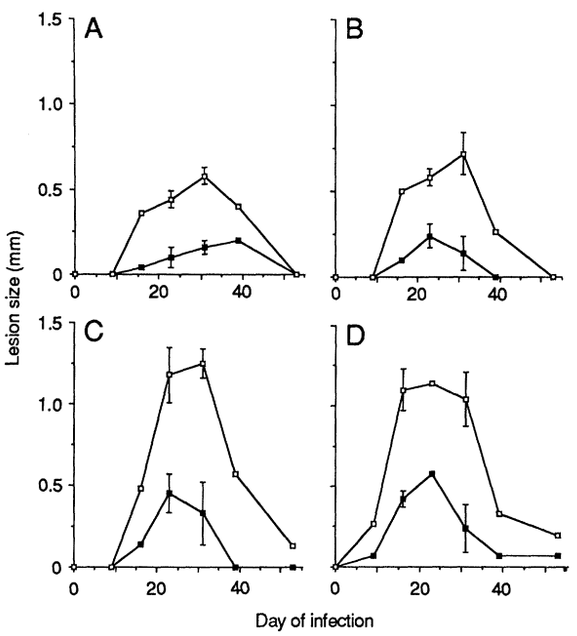

Fig. 2. Enhancement of the infectivity of *Le. major* by lysates of the salivary glands of *Lu. longipalpis*. Groups of five CBA/Ca mice (Jackson Laboratory) were injected with (**A**) 10^1, (**B**) 10^2, (**C**) 10^3, or (**D**) 10^4 *Le. major* promastigotes in the presence (□) or absence (■) of lysate of *Lu. longipalpis* salivary glands. For methods used, see Fig. 1. The values given are means ± SEM.

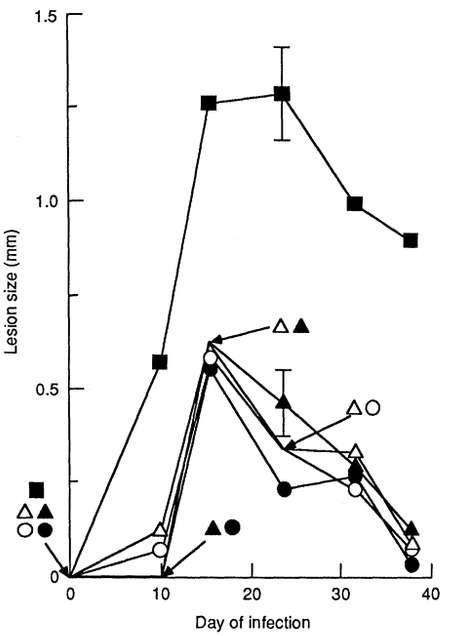

Fig. 3. The effect of salivary glands from other bloodsucking arthropods on the course of cutaneous leishmaniasis. Groups of five CBA/Ca mice were injected with 10^5 *Le. major* promastigotes in the presence of lysates of the salivary glands from *Lu. longipalpis* (■); *Aedes aegypti*, mosquito (△); *Rhodnius prolixus*, triatomine bug (●); *Ixodes dammini*, tick (○); or no salivary gland material (▲). *Lutzomyia longipalpis* salivary glands were prepared as described in Fig. 1. *Aedes aegypti* glands [5 μg of protein per pair of glands (*9*)] were prepared from female mosquitoes in a similar fashion (*10*), placed in 0.1% BSA in water, and stored at −70°C until use. *Rhodnius prolixus* glands were removed from adult bugs under saline, placed without medium in tubes, and stored at −70°C until use (*10*). *Ixodes dammini* salivary glands were obtained from adult female ticks fed on rabbit ears for 4 days. The salivary glands were dissected out under saline, placed without medium in tubes, freeze-dried, and stored at −70°C until use. When used, both the *R. prolixus* and *I. dammini* salivary glands were suspended in water, disrupted in a glass Ten Broeck tissue homogenizer, centrifuged at 10,000*g* for 2 minutes and adjusted to isotonicity with 10× PBS and a final concentration of 0.1% BSA. Protein concentrations were assayed (Bio-Rad) before addition of BSA for each salivary gland preparation, and 250 ng (the protein content of 0.5 of one sand fly salivary gland) of each material were injected. For other methods, see legend to Fig. 1. Values represent the mean ± SEM.

Table 1. The number of *Le. major* in lesions of mice injected with a mixture of parasites and salivary gland contents (0.5 of one gland). The number of viable parasites in lesions of CBA/Ca mice (legend to Fig. 2) were counted by means of a limiting dilution assay (*5*). Dilutions of infected footpad homogenates were placed in the wells of the microtiter plates that contained blood agar slants. The plates were incubated for 10 days at 26°C, and the wells containing viable parasites were identified with an inverted microscope. Estimation of the frequency of *Le. major* present in lesions was determined by minimum χ^2 analysis applied to a Poisson distribution. Duplicate animals were assayed to generate each value in the table. Duplicate assays agreed within ±52.7%.

Treatment	Number of *Le. major* per footpad ($\times 10^{-4}$)	
	Day 12 of infection	Day 33 of infection
10^1 *Le. major*	None detected	None detected
10^1 *Le. major* plus gland	0.02	0.3
10^2 *Le. major*	None detected	0.06
10^2 *Le. major* plus gland	0.1	334.8
10^3 *Le. major*	0.6	0.07
10^3 *Le. major* plus gland	19.5	43.1
10^4 *Le. major*	46.0	17.5
10^4 *Le. major* plus gland	2094.3	431.2

as 0.1 of one gland [approximately 50 ng of protein (*3*)] consistently exacerbated the course of cutaneous leishmaniasis to a degree equivalent to that obtained with 0.5 of one gland. Amounts less than 0.1 of one salivary gland had less effect in the system, but infection was enhanced later in the course of the disease. Sacks and Perkins (*6*) reported that developmental stages of *Leishmania* occur in the sand fly and result in the generation of highly infective parasites. Thus, vectorial capacity of a given phlebotomine species should be a reflection of the ability of the fly to generate infective forms of *Leishmania* and subsequently to enhance transmission of the parasite by means of the contents of the salivary gland.

REFERENCES AND NOTES

1. E. K. Markell and M. Voge, *Medical Parasitology* (Saunders, Philadelphia, 1971).
2. J. M. C. Ribeiro, *Annu. Rev. Entomol.* **32**, 463 (1987).
3. ———, P. A. Rossignol, A. Spielman, *Comp. Biochem. Physiol. A* **83**, 683 (1986).
4. R. Behin, J. Mauel, B. Sordat, *Exp. Parasitol.* **48**, 81 (1979); E. Handman, R. Ceredig, G. F. Mitchell, *Aust. J. Exp. Biol. Med. Sci.* **57**, 9 (1979).
5. R. G. Titus, M. Marchand, T. Boon, J. A. Louis, *Parasite Immunol.* **7**, 545 (1985).
6. D. L. Sacks and P. V. Perkins, *Science* **223**, 1417 (1984).
7. R. G. Titus, R. Ceredig, J.-C. Cerottini, J. A. Louis, *J. Immunol.* **135**, 2108 (1985).
8. G. B. Modi and R. B. Tesh, *J. Med. Entomol.* **20**, 568 (1983).
9. J. M. C. Ribeiro, J. J. F. Sarkis, P. A. Rossignol, A. Spielman, *Comp. Biochem. Physiol. B* **79**, 81 (1984).
10. J. M. C. Ribeiro and E. S. Garcia, *J. Insect Physiol.* **26**, 303 (1980).

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Neural Model of Adaptive Hand-Eye Coordination for Single Postures

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A neural network model has been developed that achieves adaptive visual-motor coordination of a multijoint arm, without a teacher. The model learns to position an arm so that it reaches a cylinder arbitrarily positioned in space. The model uses a new neural architecture and a new algorithm for modifying neural-connection strengths. Computer simulations show that the model performs with an average position error of 4% of the arm's length and with an average orientation error of 4°. The model is designed to be generalized for coordinating any number of topographic sensory inputs with limbs of any number of joints.

THE HUMAN BRAIN DEVELOPS accurate sensorimotor coordination despite many unforeseen changes in the dimensions of the body, strength of the muscles, and placements of the sensory organs. This is accomplished for the most part without a teacher. How is this done? I present some new hypotheses and computer simulations of distributive neural representations and computations that suggest how at least one type of adaptive sensorimotor coordination might be developed and maintained. The hypotheses rely on the self-consistency between sensory and motor signals to achieve unsupervised learning. They also rely on the topography of units in a network. (Topography is the ordered contiguous representation of inputs or outputs across a surface with possible overlap of neighboring representations.) Topographic mappings have been found in most sensory and motor brain structures (*1*), and their computational properties are just beginning to be studied (*2*).

This study combines the constraints of self-consistency and topography toward the problem of adaptively coordinating a multijoint arm to reach a cylinder arbitrarily positioned in space, as viewed by two eyes.

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The first hypothesis is that representations of postures emerge out of the correlation between posture sensation and target sensation. Such a correlation allows sensation and manipulation to become self-consistent. The self-consistency hypothesis is an extension of results from developmental studies in coordination behavior. Studies in the kitten (*3*) show that visually guided behavior develops only when changes in visual stimulation are systematically related to self-produced movement. The hypothesis is also consistent with the motor theory of speech perception (*4*).

The second hypothesis explores one of the ways a correlation between sensation and manipulation can be developed, called the circular reaction, which is an extension of one of Piaget's developmental stages (*5*). This reaction comes in two phases (Fig. 1). Self-produced motor signals are first generated to explore a large range of arm postures. During each posture, with object in hand, topographic sensory information about the object is projected to a target map through modifiable gating factors, called weights, which produce computed motor signals. Differences between the actual (self-produced) motor signals generated for each posture and the computed motor signals are used to change the weights so that these differences are minimized. These weight changes, for all possible motor postures, constitute the sensorimotor correlation and allow the system to become self-consistent. This is not simply feedback error correction. The weight changes must be structured in a way to allow global consistency for similar targets in all possible positions.

The second phase of the circular reaction takes effect after the correlation has been developed. In this phase, the self-consistency developed in the first phase is used to grasp objects found free in space. Sensory information about the object projects to a target map through the correlated weights and thereby evokes the appropriate motor signals to grasp that object.

The neural model of the circular reaction was implemented by means of discrete arithmetic and difference equations operating on matrices of numbers. The mechanical system that the model controls was rendered on a graphics workstation (Fig. 2).

Arm-muscle signals a_{pq} activate antagonistic muscle pairs ($p = 1,2$) in five degrees of freedom ($q = 1, \ldots, 5$) for the upper and lower limbs: shoulder roll ($q = 1$),

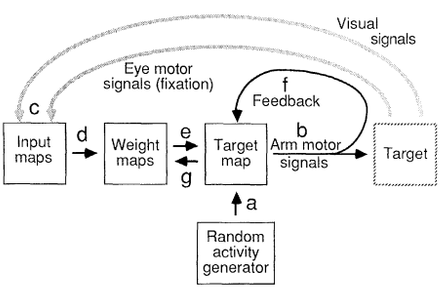

Fig. 1. The circular reaction. Self-produced motor signals that manipulate an object target are correlated with target sensation signals. The sequence for training is a, b, c, d, (e+f), g. Correlated learning is done in step g. After the correlation is achieved, target sensation signals alone can evoke the associated motor signals to accurately manipulate the target. The sequence for performance is c, d, e, b.