ecution of the learned motor performance in the deafferented animals was contingent upon their bodies' being in a fixed relation to the arm apparatus. Whenever we changed the center of rotation of the elbow 1 or 2 inches forward from a monkey's body, the animal's pointing response to the target was inaccurate. All of our intact monkeys, in contrast, were able to compensate quickly for any variations in their accustomed position with respect to the arm apparatus. The inability of the deafferented monkeys to point accurately in an unusual postural setting underscores the importance of afferent feedback. These findings suggest that, in the performance of visually evoked, learned movements, one of the major functions of afferent feedback is in the adaptive modification of learned motor programs.

With respect to our major observation, we stress that although we have detected one of the processes underlying arm movement, there are obviously other processes that occur concurrently. It is clear, for instance, that the arm movements monkeys use to reach a given position can vary in velocity. Consequently, the mechanism elucidated here by which intended posture is achieved must coexist with a mechanism specifying intended arm velocity.

The results of this study, as well as our previous investigations on the termination of head movements in both intact and deafferented monkeys (*1*), provide experimental evidence that visually evoked movements may result in part from commands that shift the equilibrium point between agonist and antagonist muscles. Similar theories have been proposed in the past. For instance, in order to account for speech production MacNeilage suggested that speech may be controlled by "commands" that specify final vocal tract configuration rather than a particular movement pattern (*5*). On the basis of a different line of investigations—the analysis of involuntary changes in the posture of the human arm following changes in load—arm movements have been hypothesized to result from shifts in the equilibrium point of the muscle-load system (*6*). Finally, certain motor disorders that depend upon damage to the left hemisphere have been interpreted to result from pathological disruption of the process controlling sequential attainment of postures (*7*).

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Vaccination of Experimental Monkeys Against *Plasmodium falciparum*: A Possible Safe Adjuvant

Abstract. *Owl monkeys (*Aotus trivirgatus griseimembra*) were effectively immunized against a human malaria parasite,* Plasmodium falciparum. *Two injections of antigen, primarily mature segmenters with fully developed merozoites, mixed with adjuvant (6-*O*-stearoyl-*N*-acetylmuramyl-*L*-alanyl-*D*-isoglutamine and liposomes) were administered intramuscularly at a 4-week interval. Approximately 2 weeks after the second vaccination, the monkeys were challenged with the homologous strain of* P. falciparum. *All immunized monkeys survived the challenge. The substitution of Freund's complete adjuvant is an encouraging step toward the development of an effective and safe vaccine for human malaria.*

We previously reported that the owl monkeys (*Aotus trivirgatus griseimembra*) could be immunized against a human malaria parasite (*Plasmodium falciparum*) infection (*1*), and this result was confirmed (*2*). In those studies Freund's complete adjuvant was essential for effective immunization. The use of Freund's adjuvant in humans is not considered safe because of data that relate its use to potentiation of plasma cell tumors in mice, induction of autoimmune reactions, formation of disseminated focal granulomata, and long-term persistence of mineral oil in animals (*3*). Muramyl dipeptide (MDP), an agent that has been substituted for whole tubercle bacilli in Freund's complete adjuvant, has been shown to enhance immune responses (*4*). However, this compound could be used in human immunization only after elimination of the mineral oil in the adjuvant mixture which is partly responsible for undesirable side reactions. We reported that the replacement of the primary hydroxyl group at the C-6 position of *N*-acetylmuramyl-L-alanyl-D-isoglutamine MDP by a lauroyl, stearoyl, or docosanoyl group produced an MDP derivative with adjuvant activities (*5*). We now report that 6-*O*-stearoyl-*N*-acetylmuramyl-L-alanyl-D-isoglutamine can replace Freund's complete antigen in the immunization of owl monkeys against infection with *P. falciparum*.

The Uganda–Palo Alto strain (FUP) of *P. falciparum* has been maintained in our laboratory by serial passages of blood-induced infections of owl monkeys (*6*). The immunizing antigen was prepared by short-term in vitro cultivation of this parasite in RPMI 1640 medium supplemented with fetal calf serum (FCS) and fatty acid–free bovine albumin (FAF albumin) (*7*). Parasitized blood from owl monkeys was cultured in sterile 500-ml side-arm flasks fitted with stoppers, with entry ports for a gas mixture of 90 percent N_2, 8 percent CO_2, and 2 percent O_2. Whole heparinized parasitized blood (7 ml) was introduced into each culture flask containing 63 ml of RPMI 1640, 8.8 ml of FCS, and 6 ml of FAF albumin (12.5 mg/ml). The medium was changed after approximately 12 and 24 hours of incubation. At the end of 35 to 40 hours of incubation, most of the parasites had developed to mature segmenters with fully developed individual merozoites. These mature segmenters were concentrated and harvested, relatively free of other cellular elements (*8*). The final antigenic material consisted of 50 to 60 percent segmenters with individual merozoites; the remainder consisted of other developmental stages of the parasites. Antigen was stored at −20°C.

The adjuvant 6-*O*-stearoyl-*N*-acetylmuramyl-L-alanyl-D-isoglutamine was used with carrier liposomes (*5*). Adjuvant-incorporated liposomes were prepared by the method of Inoue (*9*) except that 10 μmole of cholesterol (grade 99+ percent; Sigma) and 10 μmole of lecithin (dipalmitoyl-DL-α-phosphatidyl choline, grade I approximately 99 percent; Sigma) were dissolved in 5 ml of chloroform in a 10-ml round-bottomed flask. The

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chloroform was removed (at < 30°C) by a rotary vacuum evaporator. The adjuvant (0.025, 0.5, or 1.0 mg) in 0.5 ml of phosphate-buffered saline was added to the lipid-coated flask. Liposomes were produced by sonication at 0°C in an argon atmosphere. The vaccine was prepared by thoroughly mixing antigen and the liposomes incorporated with adjuvant in a double-hubbed needle and two syringes.

The seven owl monkeys in our study weighed approximately 900 g. On the basis of physical characteristics and coloration, the monkeys were judged to belong to phenotype group B, which Ma *et al.* described (*10*). The standard chromosome preparation method of Moorehead *et al.* (*11*) indicated that the monkeys belonged to karyotype II (*10*). Three monkeys (A287, A291, and A303) were controls; the other four monkeys (A283, A284, A286, and A294) were immunized with *P. falciparum* (FUP strain) merozoite-enriched antigen mixed with the adjuvant-incorporated liposomes. Two intramuscular injections were given at a 4-week interval. A total of 2.86 mg of parasite protein (1.0 mg on day 0 and 1.86 mg on day 28) was administered to three monkeys (A286, A284, and A283), and animal A294 received a total of 2.80 mg of parasite protein (1.4 mg each on day 0 and day 28). Immunization never produced a detectable infection. On day 45, all seven monkeys were challenged with an intravenous injection of 7.5×10^5 parasites of the FUP strain of *P. falciparum* derived from an ongoing infection in an owl monkey.

Thick or thin blood films from all the animals were made daily after the challenge injection (Fig. 1). Of the control monkeys, A303 died on day 8 after challenge with 60.0 percent of its red cells being infected, and A287 died on day 15 with 54.4 percent of its red cells parasitized. Although A291 eventually survived the infection, more than 25 percent of its erythrocytes contained parasites at the peak of infection. In contrast, all four immunized monkeys survived. Animals A286 and A294 developed low-grade infections that lasted for 1 week; animals A284 and A283 had between 5.0 and 14.0 percent of their red cells infected for 1 week. By day 24, A284, A283, and A294 had become negative for parasites, and none were detected in A286 by day 32. Three months after the challenge, all these monkeys remained negative. Although the number of animals in this experiment was small, a difference was evident between the course of infection in immunized and nonimmunized monkeys. Spontaneous recovery of a control monkey (A291) in this sort of experiment is a very rare occurrence. Within the last 4 months, blood-induced infections (FUP strain of *P. falciparum*) were initiated in the same manner in three other *A. trivirgatus griseimembra* monkeys, and all showed a typical course of infection and died. These three monkeys were also of karyotype II and phenotype group B.

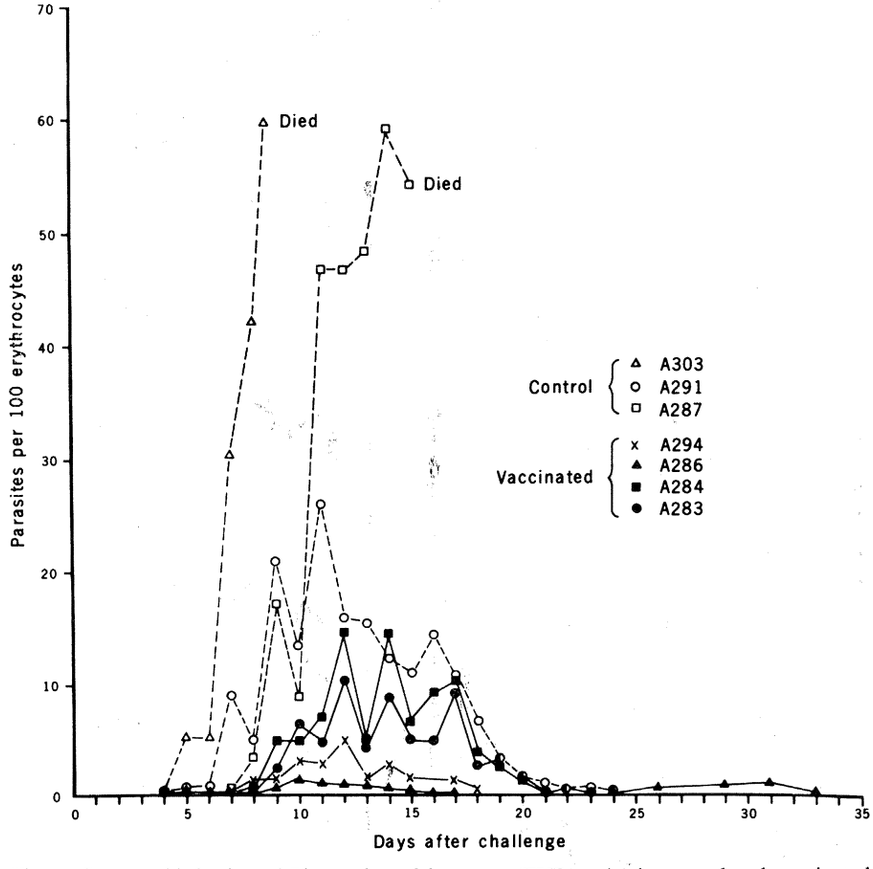

Fig. 1. Course of infection of *Plasmodium falciparum* (FUP strain) in control and vaccinated monkeys (*Aotus trivirgatus griseimembra*).

Serums obtained from monkeys during the course of vaccination significantly inhibited parasite multiplication (*12*). Indirect fluorescent antibody studies demonstrated the presence of antibodies to *P. falciparum* in all vaccinated animals after the second vaccination. In addition, lymphocytes that were sensitized to *P. falciparum* antigens could be detected as early as 14 days after first vaccination in some animals by antigen-induced blast transformation.

We conclude that *P. falciparum* merozoite vaccination, when 6-*O*-stearoyl-*N*-acetylmuramyl-L-alanyl-D-isoglutamine was used with liposomes as an adjuvant, protects against homologous infection with intraerythrocytic stages of the normally lethal *P. falciparum* parasites. The administration of *P. falciparum* antigen with adjuvant and liposomes did not produce any reaction at the site of injection. The only side effect associated with the adjuvant was anorexia for a few days immediately after vaccination, which resulted in some loss of weight. However, all vaccinated and adjuvant control monkeys regained weight within 2 weeks after vaccination. The loss of weight was considerably less in A294, which received only one-third the concentration of the adjuvant given to the other monkeys, an indication of some correlation between the concentration of adjuvant and the degree of side effect observed.

Our results are significant with regard to the substitution of Freund's adjuvant with a possible safe agent that leads to effective immunization of experimental monkeys against *P. falciparum*, suggesting that it may also be effective and safe in a vaccine for human malaria.

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Retention of an Associative Behavioral Change in *Hermissenda*

Abstract. *The nudibranch mollusk* Hermissenda crassicornis *is normally attracted to a test light. Three days of training consisting of 50 trials per day of light paired with a rotational stimulus led to a significant increase, lasting for days, in the animal's response latency to enter a test light. The group that received light associated with rotation was significantly different from groups subjected to nonassociative control procedures. Modifications of well-known sensory networks may be related to a behavioral change that shares several operational features with associative learning.*

Invertebrates have been selected for study in investigations of the physiology of learned behavior because their simpler nervous systems are more amenable to a cellular analysis (*1–4*). Some of the characteristics of learned behavior have been examined at the behavioral and cellular levels for nonassociative behavioral modifications such as habituation and sensitization (*2*). For gastropod mollusks, behavioral changes that may be dependent on the temporal association of two sensory stimuli have only recently been explored (*3*, *4*). Methodological questions have been raised concerning behavioral changes reported to be examples of aversive conditioning for the mollusk *Pleurobranchaea californica* (*3*). In addition, for this preparation an analysis has not been made of the neuronal interactions within the relevant sensory pathways.

Stimulation of two sensory systems in the nudibranch mollusk *Hermissenda crassicornis* with natural stimuli has resulted in a short-term change in both the intact preparation and the isolated nervous system (*4*, *5*). We now report a long-term behavioral change that shares several operational features with associative learning. This behavioral change lasts for several days, persists during repeated testing, is reversible, and is dependent on the temporal association of two sensory stimuli. An examination of the cellular mechanisms underlying this behavioral change in *Hermissenda* may be useful, therefore, in understanding cellular mechanisms of associative learning.

The visual and statocyst pathways of *Hermissenda* consist of a relatively small number of cells whose synaptic relations and cellular organization have been examined in detail with intracellular recording and histological techniques (*6*). Stimulation of these pathways with light and rotation in subjects trained and tested en masse has resulted in a short-term change in the animals' normal attraction to a light stimulus (*4*) and in correlated neural changes (*5*).

To determine whether this behavioral change is long-term and dependent on the association of light and rotation we examined changes in response latencies of individual animals to enter a test light following paired, unpaired, and random presentations of light and rotation.

Animals (N = 115) weighing 0.2 to 1.2 g were maintained separately in plastic mesh containers in a fresh flow-through seawater system (1 liter/min at 15°C). The animals (*7*) were fed small pieces of squid daily and placed on a cycle of $6^1/_2$ hours of light in 24 hours. Training and testing were carried out during the daily light cycles. After at least 3 days of this light schedule, the animals' baseline response to enter a light spot was measured. The data collection system and training procedures were completely automated. The animals were transferred from the plastic containers to glass tubes 228 mm long filled with seawater (Fig. 1). A plug inserted through an opening in the tubes confined the animal to one end of the tube. The tubes were then inserted into ten holders on a modified turntable (Fig. 1) enclosed in an incubator at 15°C. A light spot (*8*) was projected onto the center of the turntable, illuminating a circular area 10.16 cm in diameter with ten photocells on its circumference. Response latencies were recorded when a *Hermissenda*, entering the light spot, interrupted the light between the source and a photocell, and thus triggered an event marker on a ten-channel recorder.

After the baseline measurements, the animals were randomly assigned to treatment and control groups. Training during the acquisition phase consisted of a total of 150 trials (50 trials per day). A trial for the treatment group (N = 22) consisted of 30 seconds of light (*8*) paired with 30 seconds of rotation (*9*) with a variable intertrial interval (range, 1 to 3 minutes). Five groups were run to control for ef-

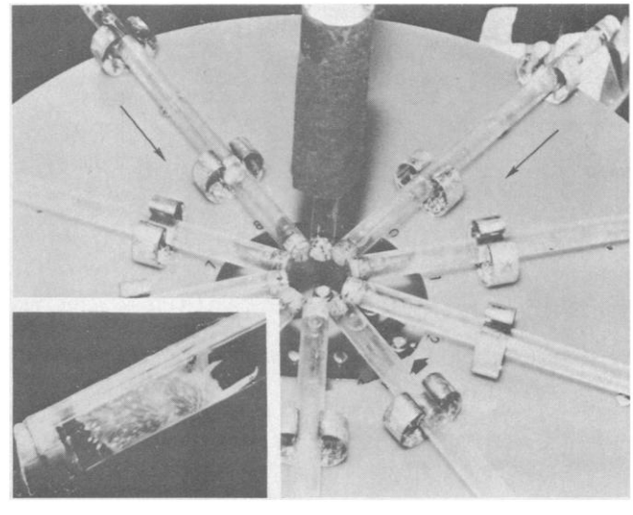

Fig. 1. Training and testing apparatus. The response latencies to enter a light spot projected onto the center of the turntable by an overhead illuminator were recorded automatically when the *Hermissenda* moved toward the light source (direction of arrows) and interrupted the light between illuminator and photocells (arrowhead). (Inset) *Hermissenda* were subjected to different behavioral treatments consisting of light and rotation while confined to the end of glass tubes filled with seawater.

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