Processes Controlling Arm Movements in Monkeys

Abstract. The experiments identify some of the processes underlying arm movements in rhesus monkeys. Three monkeys were trained to point to a target with the hand and forearm and to hold that position for about 1 second to obtain a reward. Forearm movements were performed without sight of the arm before and after bilateral dorsal rhizotomy. In both intact and deafferented animals, we unexpectedly displaced the forearm prior to movement initiation and observed that the arm moved accurately to the target. These results are relevant to the question of what is being controlled by motor commands. The controlled variable appears to be an equilibrium point between agonist and antagonist muscles. The findings suggest that the feedback system plays a major role in updating and adjusting the central programs subserving the execution of learned motor patterns.

Certain limb movements elicited by visual stimuli are currently assumed to be controlled by "programs" that generate instructions appropriate for activating the spinal motoneurons. Nothing is known, however, about the organization and the characteristics of these programs; in particular, it is not yet clear which aspects of movement they may control. The experiments reported here are addressed to the latter question. Three adult rhesus monkeys were trained in a pointing task. The monkeys sat in a primate chair with the right forearm fastened to an apparatus that permitted flexion and extension of the forearm about the elbow in the horizontal plane. The pointing task required that the monkey position its limb in front of a small target light. Ten lights were spaced at 5° intervals along a small perimeter arc centered around the axis of rotation of the elbow. The monkeys were trained to point to whichever light was on and to hold the arm at that position for about 1 second. To obtain a reward, the monkey had to point to an electrically defined target zone centered on the target light. The zones were 12° to 15° wide. This width was found to make the task moderately difficult without requiring the monkey to hunt for the target zone with a zigzag approach. In the intertrial interval (3 to 5 seconds) the monkey was free to choose any arm position. The experiments were conducted in a dark room to restrict visual cues to the target; at no time during an experiment was the animal able to see its forelimb.

In each trial, the arm was either loaded or unloading; loads were applied on about 20 percent of the trials by way of a torque motor in series with the shaft of the apparatus. The load most often used was a constant torque load whose onset time, duration, amplitude, and direction were randomized. In most instances, the load was applied within the reaction time of the monkey. Hence, when the motor command specifying a given forearm movement occurred, the positional disturbance had altered the length of the agonist and antagonist muscles, and the proprioceptive stimulation resulting from this disturbance had altered their state of activation. In spite of these changes, the intended final arm position was always reached; this was true of...
whether the torque motor had displaced the forearm further away from, closer to, or even beyond, the intended final position (Fig. 1). For each target position, a t-test of the differences between the average final positions of undisturbed movements and of movements whose initial position had been changed by the torque motor was calculated. No significant differences were found.

Attaining the intended arm position could be explained by assuming that different proprioceptive information changed the original motor command, either through the action of known short and long proprioceptive loops or, given that the movements were as long as 600 msec, through reprogramming. However, previous results (1) suggested an alternative hypothesis, that the motor program underlying arm movement specifies, through the selection of a new set of length-tension curves, an equilibrium point between agonists and antagonists that correctly positions the arm in relation to the visual target. To a first approximation, this view can be represented by referring to a simple mechanical analog. If we assume that the muscles moving a body segment can be represented by springs whose resting length can be set at some value (for simplicity, a pair of springs acting across a hinge joint in the agonist-antagonist configuration) and by damping elements, whenever the tensions of the opposing springs are changed, movement will take place and a new posture will be attained.

To investigate the applicability of this hypothesis to arm movements, we tested the monkey’s performance after it had undergone a bilateral dorsal rhizotomy from the first cervical through the third thoracic root. We could soon elicit pointing responses (within 2 days in some of the animals) after surgery. Although the deafferented animals were able to perform adequately during the experimental trials, they did not otherwise engage in motor behavior for at least a week after deafferentation. A similar observation has been made by Taub et al. (2). The completeness of the dorsal root section was tested by making sure that there were no changes in the electromyogram indicative of a stretch reflex when the arm was unexpectedly displaced. It was also checked anatomically by examining serial sections of the spinal cord stained with modified Fink-Heimer. In addition, errors of predictable directions were made by our animals when constant forces of long duration opposing the intended movement were applied. This observation suggests that the deafferentation was functionally complete and implies that the slow-conducting unmyelinated fibers in the ventral roots (5), as well as the few myelinated fibers described in some species (6), do not provide the type of information about external disturbances that could have been used by the deafferented animals to adjust forearm movements. Fast motor relearning soon after deafferentation is also unlikely because our deafferented monkeys had considerable difficulty in adjusting their motor performance when bias loads were applied to the arm even while they could see the arm.

Postoperatively, when the forearm was displaced (in random trials) immediately after the appearance of the target light and released just before or during the activation of motor units, the arm moved accurately to the target. Figure 1 illustrates the cases in which the action of the torque motor brought the forearm toward (B) and away from (C) the final position approximately within the reaction time. Figure 2 displays the mean final arm position as a function of target position for intact (A) and deafferented monkeys (B) for undisturbed movement. Figure 2, C and D, shows the same result for all the movements in which the initial position was displaced either toward or away from the final position. For each target position, a t-test evaluated differences between the average final positions of undisturbed movements and of movements in which the initial arm position was changed. No significant differences were found in the three monkeys we have studied both before and after deafferentation. These results thus suggest that what is being programmed is an intended equilibrium point resulting from the interactions of agonist and antagonist muscles. Observations of the final arm position when a constant force opposing the intended movement was applied with the torque motor throughout the movement provided further support for this idea. The arm undershot observed in this case indicated that the intended equilibrium point of the arm muscles could be modified in a predictable way by the application of an external force.

If we assume that no radical change in motor programming occurred during the days immediately following the rhizotomy, we can conclude that in both intact and deafferented monkeys, visually evoked arm movements seem to depend, at least in part, upon a process that specifies final position. This conclusion needs an important qualification: despite the fact that intact and deafferented monkeys showed essentially similar motor behavior in a highly practiced task, dramatic differences were present under other circumstances. The successful ex-
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-acetyl muramyl-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-acetyl muramyl-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-acetyl muramyl-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 (FAB 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 N₂, 8 percent CO₂, and 2 percent O₂. 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 FAB 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-acetyl muramyl-L-alanyl-D-isoglutamine was used with carrier liposomes (5). Adjuvant–incorporated liposomes were prepared by the method of Iinoe (9) except that 10 μmole of cholesterol (grade 99.5 percent; Sigma) and 10 μmole of lecithin (dipalmitoyl-DL-α-phosphatidyl choline; grade 1 approximately 99 percent; Sigma) were dissolved in 5 ml of chloroform in a 10-ml round-bottomed flask. The