adulthood to late middle age. The mean life-span on the Fischer 344 strain of male rats is 29 months with a maximal survival of 35 months under barrier conditions (*8*). This increase in number of adipocytes need not imply that the cells are derived from mitotic activity of stem cells, but rather they could originate from preadipocytes becoming lipid laden (*9*).

The perirenal depot is of great importance in this strain since it accounts for a large fraction of the total adult adipose mass. A study of the effect of age on lean body mass and adipose tissue mass suggests that the epididymal and perirenal depots each account for about 20 percent of the total adipose tissue mass at 6 months of age (data not shown). However, between 6 and 18 months, the perirenal depot mass increased 2.3-fold compared to a 1.35-fold increase in total adipose mass and a 1.56-fold increase in epididymal depot mass.

The studies on which constant adipocyte theories are based in rats were usually terminated at about 6 months of age (*3*) since an asymptote in adipocyte number seemed to have been reached by that time. However, only a small part of the adult life-span had been studied. The experimental investigations in humans involved a time span of only about 6 months (*1*). Although most data on clinical obesity support the concept that adipocyte number is fixed in adults, there have been some reports of adipocyte hyperplasia in what appears to be adult-onset obesity (*10*).

Studies indicating that adipocyte number may not be constant in the adult have been largely ignored either because a marked dietary perturbation was involved (*7*), or the data could be otherwise interpreted, or the changes noted were not significant. For example, DiGirolamo and Mendlinger (*4*) found that the perirenal depots from 1-year-old guinea pigs have a much larger number of adipocytes than did those from 6-week-old animals; however, it was not clear that this increase occurred during the adult stage of life. A similar problem exists in interpreting the work of Enesco and Leblond (*11*) and Zingg *et al.* (*5*). Although this difficulty is not involved in interpreting the work of Stiles *et al.* (*6*), the age-related increase in the number of epididymal adipocytes was not great.

The data in our report demonstrate that the sole mode of increasing the mass in the perirenal depot of the adult Fischer 344 male rat, a strain not prone to obesity, involves increasing the number of adipocytes either by the genesis of new adipocytes or by the maturation of preadipocytes. In contrast in the same rats the sole mode of increasing the epididymal depot mass is one of adipocyte hypertrophy. However, even in this depot, hyperplasia can occur in adult life; in an earlier study a 30 percent increase in adipocyte number was seen in this depot between 24 and 30 months of age, during senescence. To what extent our findings apply to depots other than the epididymal and perirenal and to other rat strains remains to be explored.

H. A. BERTRAND, E. J. MASORO
Department of Physiology, University of Texas Health Science Center, San Antonio 78284

B. P. YU
Research Service, Veterans Administration Hospital, San Antonio, Texas 78284

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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 forearm.

In each trial, the arm was either loaded or unloaded; 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

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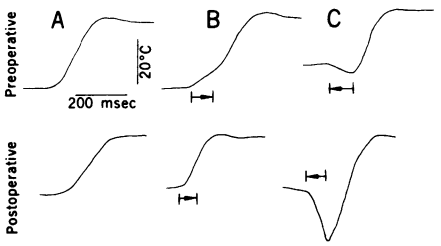

Fig. 1. (Preoperative) Visually elicited arm movements made by an intact monkey during pointing task. (A) Normal, unloaded movement. (B) Arm movement with which the torque motor moved the limb toward the target zone. (C) An instance in which the torque motor had displaced the forearm away from the target. In both (B) and (C), the forearm reached the correct final position. Timing and duration of the load application are indicated by arrows. (Postoperative) Visually evoked arm movement made by the same monkey after bilateral dorsal rhizotomy from the second cervical to the third thoracic vertebrae in response to visual targets but without sight of its arm. In (B) a transient torque displaced the arm toward the target zone, whereas in (C) the load displaced it away from the target zone. The target light was on during actual pointing. In both preoperative and postoperative conditions, the initial forearm position was different from trial to trial.

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 afferent 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 retested 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 the pointing response (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 (*3*), as well as the few myelinated fibers described in some species (*4*), 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 undershoot 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.

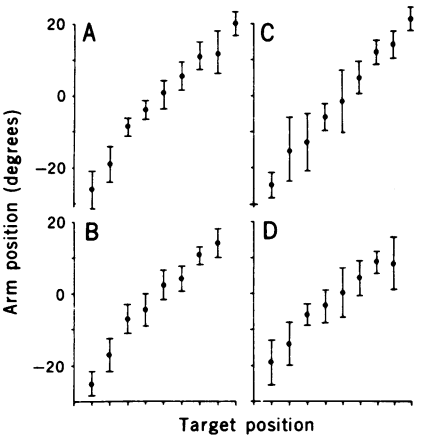

Fig. 2. Means and standard deviations of final arm positions as a function of target position. Each point represents the average of 10 to 40 movements. Final arm positions recorded from (A) intact and (B) deafferented monkeys. (C and D) Final position reached by the arm in which initial position was displaced in (C) intact and (D) deafferented animals. (Both movements toward and away from final position were computed.) The numbers on the ordinates represent arm position in degrees. The negative numbers represent flexion and the positive, extension with respect to a reference position (zero degree) that corresponds to an elbow angle of 90°. Target positions are shown on the abscissa. Intertarget distance equals 5°.

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-

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*).

ANDRES POLIT
EMILIO BIZZI

Department of Psychology, Massachusetts Institute of Technology, Cambridge 02139

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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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