carrier in the ER that transports the substrate across the membrane to the phosphatase (*12, 13*). Karnovsky *et al.* (*14*) found that this carrier is absent in the brain and that G6P gains access to the phosphatase only by slow diffusion across the ER membrane. This would further slow the phosphohydrolytic activity of whatever G6Pase is present in the brain.

The report of Huang and Veech (*6*) has led to debate on the role of G6Pase in the brain and to speculations about futile cycles in cerebral tissue (*15*). It has also been used to argue against the validity of the deoxyglucose method for measuring local utilization of glucose in the brain in animals and humans (*6, 14*), a method that in its earliest form assumed negligible loss of deoxyglucose-6-phosphate in the brain during the experimental period (*16*). It now seems that such speculations and extrapolations are without foundation.

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Regional Brain Dopamine Metabolism: A Marker for the Speed, Direction, and Posture of Moving Animals

Abstract. *Brain dopamine is necessary for normal movement. To determine whether there is a precise relation between the intensity of movement and changes in brain dopamine metabolism, the investigators ran rats on straight and circular treadmills at different speeds and with different body postures. Concentrations of dopamine and its metabolite 3,4-dihydroxyphenylacetic acid increased in the caudate and accumbens nuclei in direct relation to the speed and angular posture of the animals. Dopamine metabolism in the nucleus accumbens was more strongly linked to the speed and direction of movement, while in the caudate nucleus dopamine and 3,4-dihydroxyphenylacetic acid were affected most by posture and direction.*

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The neurotransmiter dopamine has an important role in the control of motor behavior. In patients with Parkinson's disease, loss of dopaminergic neurons leads to dopamine depletion in the brain and drastically reduced voluntary movement (*1*). Treatment with the dopamine precursor L-dopa can increase the dopamine concentration and restore motor function (*2*). Animal studies have provided more detailed evidence about the neurophysiological action of dopamine. Electrical stimulation of the dopaminergic cells in the substantia nigra on one side of the brain causes animals to run in circles in a direction away from the stimulated side (*3*). The asymmetric movement is presumably the result of asymmetric release of dopamine in the caudate and putamen, nuclei innervated by the substantia nigra. There is also functional specificity in the motor nuclei receiving dopamine input. The caudate and putamen appear to regulate posture (*4*), while another dopaminergic structure, the nucleus accumbens, is more involved in the expression of general locomotor activity (*5*).

We previously reported a new approach to the study of the role of dopamine in movement (*6*). Rather than using electrical stimulation, drugs, or lesions to change brain physiology and behavior, we trained rats to turn in circles. We then looked for specific changes in dopamine metabolism produced by the act of running in circles. The results showed that the turning animals had increased release, synthesis, and catabolism of dopamine in the caudate and accumbens nuclei on the side of brain contralateral to the circling direction. Because there were increases in both dopamine and its metabolite 3,4-dihydroxyphenylacetic acid (DOPAC), the increase in dopamine synthesis was even greater than the increase in the rate of dopamine release and metabolism. These results were evidence that a neurotransmitter could be selectively activated by voluntary motor behavior.

We have now developed a series of motor tasks to determine whether there is a precise relation between the intensity of motor behavior and the degree of activation of dopamine metabolism in the caudate and accumbens nuclei. We ran rats on powered straight, and circular treadmills, with which we could regulate the animals' speed and posture. To see whether movement of the head in space could change dopamine production, we also spun the animals in tube rotometers.

Male Sprague-Dawley rats (300 to 350 g) were run in three different treadmills and a tube rotometer. The animals had been deprived of water, and a water dropper was provided in front of each apparatus to keep them oriented in a forward-moving direction. They were run for one practice session on the day before the experiment to familiarize them with the apparatus. Motor behavior was allowed to proceed for 20 minutes and then the animals were killed. The caudate was removed from 0.0 to 1.5 mm anterior to bregma and dorsal to the anterior commissure (*7*). The nucleus accumbens was cut from a section 1.5 to 3.0 mm anterior to bregma dorsal to the olfactory tubercle and ventral to the anterior commissure. Tissues were assayed for dopamine and DOPAC by high-performance liquid chromatography with electrochemical detection (*8*). All data were analyzed by two-way analysis of variance followed by post hoc Dunnett's and Tukey's tests.

In the first series of experiments, animals were placed on a straight treadmill having a powered belt of emory cloth 10 cm wide and 30 cm long. Groups of six

rats were run for 20 minutes at speeds ranging from 144 to 720 cm/min. Figure 1 shows the relation between speed on the straight treadmill and dopamine and DOPAC concentrations in the two nuclei. Results are reported for the left and right sides of the brain. Dopamine in the nucleus accumbens increased progressively with speed ($r^2 = 0.71, P < 0.001$); DOPAC concentrations changed in parallel with dopamine. Caudate dopamine and DOPAC showed less correlation with speed. Dopamine in the caudate showed a smaller speed-related increase than in the nucleus accumbens and was significantly increased only at a treadmill speed of 720 cm/min. The DOPAC concentration exceeded control levels at all rates tested ($P < 0.05$), although the correlation with speed was low ($r^2 = 0.47$). Therefore, dopamine and DOPAC concentrations in the nucleus accumbens but not the caudate were closely related to the speed of running on the straight treadmill.

To study the effect of changes in posture as well as speed, other rats were placed in cylindrical drums with a rotating turntable floor. One cylinder was 24 cm in diameter; animals walking in circles in this device had a body arc that subtended about 120°. A smaller (12 cm in diameter) drum was used to increase the postural asymmetry; animals in this cylinder had a head-to-tail arc subtending about 300°. A partition extending from the center to the curved wall of each drum kept animals from walking around the full circumference. Cylinders were suspended a few millimeters above the rotating turntable so that the animals were forced to walk in place.

Figure 2 shows dopamine and DOPAC concentration data for animals moving in the 24-cm treadmill. The angular velocities at 2, 5, and 10 rev/min were the same as the linear speeds of the straight treadmill. Dopamine and DOPAC increased more on the side contralateral to the circling direction in both nuclei, suggesting that both are sensitive to the direction of movement. As with straight running, dopamine and DOPAC in the nucleus accumbens increased in direct linear relation to speed, although the increase was primarily on the side contralateral to the circling direction. The

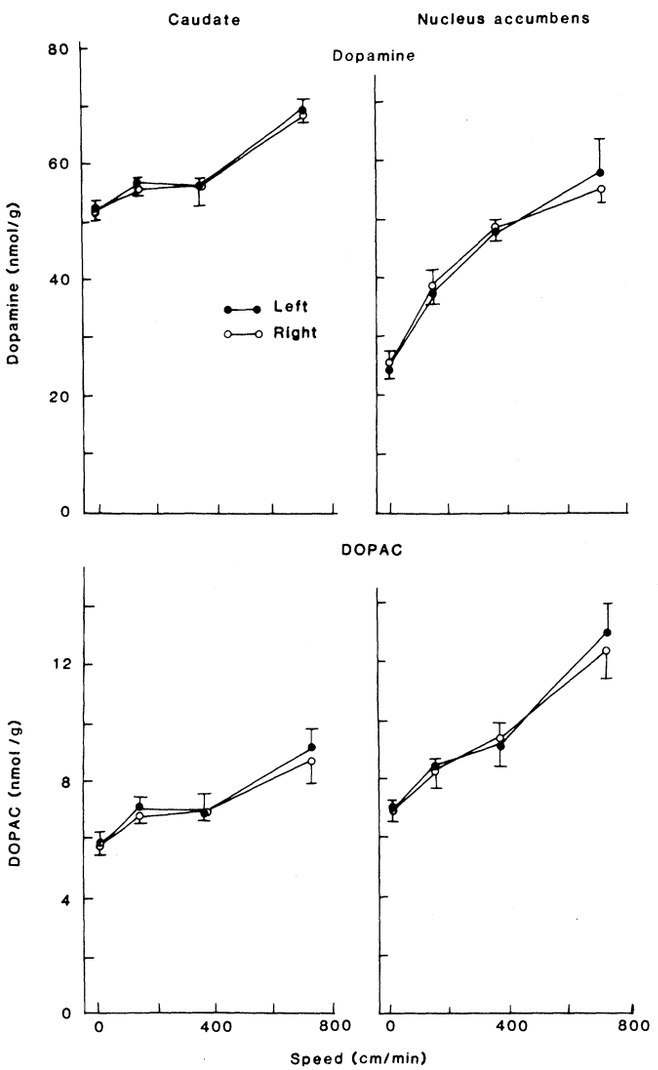

Fig. 1 (left). Dopamine and DOPAC concentrations in the caudate and accumbens nuclei after 20 minutes of running at different speeds on the straight treadmill. There is a rate-related increase in dopamine and DOPAC concentrations in both nuclei, especially the nucleus accumbens.

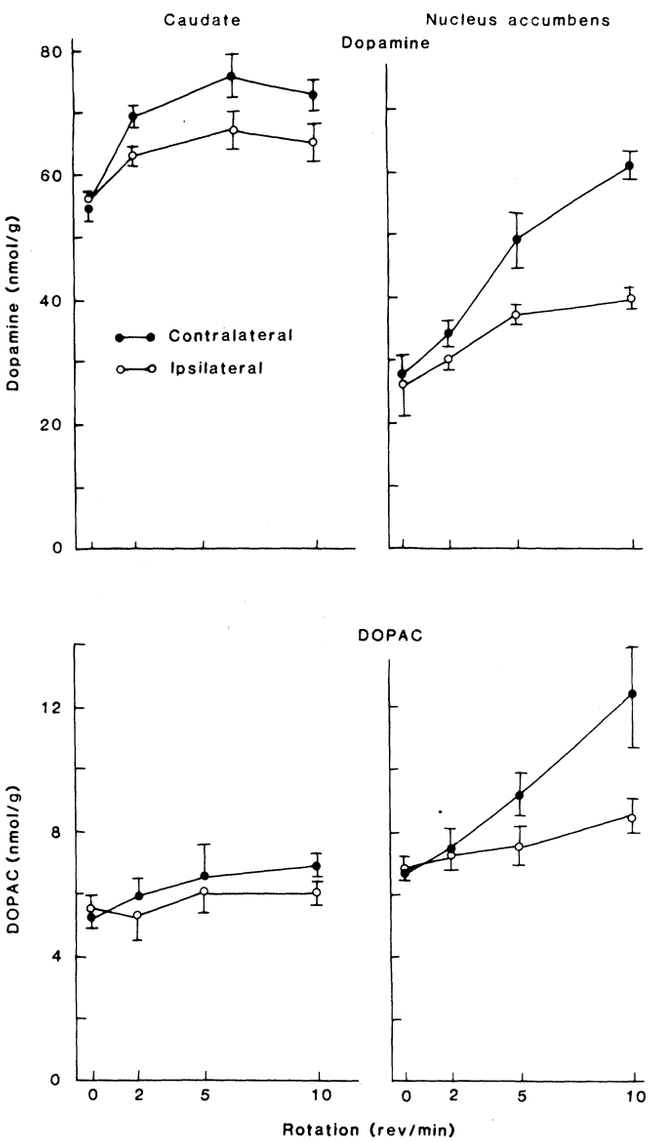

Fig. 2 (right). Dopamine and DOPAC concentrations in the caudate and accumbens nuclei after 20 minutes of running on the 24-cm-diameter rotating treadmill. Contralateral and ipsilateral refer to the sides of the brain in relation to the circling direction. Both nuclei show larger increases in dopamine and DOPAC on the contralateral side. The increases in the nucleus accumbens are linearly related to the speed of rotation while the changes in the caudate plateau at 5 and 10 rev/min.

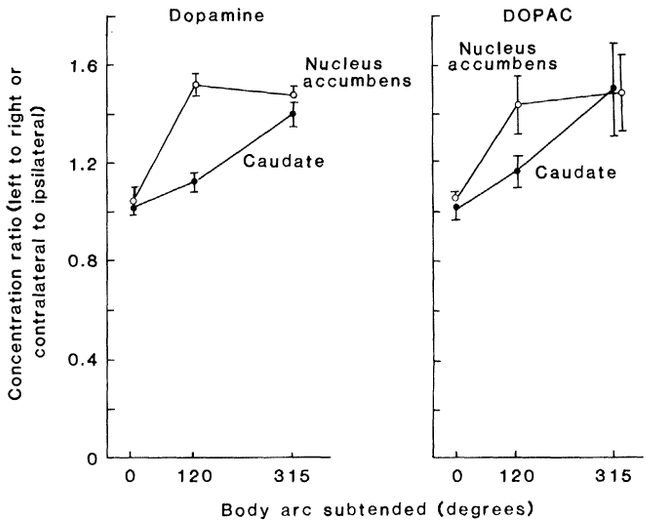

Fig. 3. Lateralization of dopamine and DOPAC in the caudate and accumbens nuclei with changes in body posture. All animals ran at the same speed. Animals with 0° of body arc were running on the straight treadmill at 720 cm/min. Animals with 120° and 315° of body arc were running at 10 rev/min in the 24- and 12-cm cylinders, respectively. The nucleus accumbens shows maximal lateralization at a body arc of 120° while the caudate shows a linear increase in laterality with more extreme posture.

correlation between dopamine concentration in the contralateral nucleus accumbens and speed was $r^2 = 0.75$ ($P < 0.01$); that between DOPAC and speed was $r^2 = 0.61$ ($P < 0.01$). Dopamine and DOPAC concentrations in the contralateral caudate were also significantly higher than control levels at all speeds for dopamine and at 10 rev/min for DOPAC ($P < 0.05$), but no significant differences were seen between the 5 and 10 rev/min groups. These results show that dopamine and DOPAC concentrations in the caudate and accumbens nuclei are closely related to the direction of circling and that dopamine in the nucleus accumbens is also linked to the speed of circling.

The effect of changes in body posture was studied by comparing results for the 24-cm drum with those for the 12-cm drum. Rotational speed was held constant at 10 rev/min, but body curvature was altered by the diameter of the running path. Dopamine and DOPAC concentration ratios (Fig. 3) show that the increased body curvature led to increased dopamine and DOPAC concentrations in the contralateral caudate; the nucleus accumbens showed a maximum increase in dopamine and DOPAC at a body arc of 120°. These differences are reflected in the correlation coefficients for the relation between dopamine and posture in the caudate ($r^2 = 0.88$, $P < 0.01$) and the nucleus accumbens ($r^2 = 0.66$, $P < 0.01$). Dopamine metabolism in the caudate therefore appears to be a good marker for the posture of animals.

To see whether movement of the head in space without locomotor activity caused lateralization of brain dopamine metabolism, we used the 24-cm drum. However, in this experiment the cylinder was placed directly on the turntable so that the cylinder and the rat rotated passively. The animals rode in circles but did not walk. Because this motion did not provide changing visual information, additional experiments were done to study head movement by spinning animals in the horizontal plane in a 5-cm-diameter tube rotometer at 10 and 30 rev/min. The animals' heads projected from the tubes, so both visual and vestibular stimuli were available. Results from both of these experiments showed no changes in dopamine metabolism in the caudate or nucleus accumbens at any speed. Therefore, passive movement of the head in space does not change brain dopamine metabolism.

These experiments indicate a close relation between movement and dopamine production in the caudate and accumbens nuclei. Speed of running on both circular and straight treadmills was strongly correlated with dopamine turnover in the nucleus accumbens (Figs. 1 and 2). Changes in speed on either treadmill led to greater changes in dopamine concentration in the nucleus accumbens than in the caudate nucleus. By contrast, a small change in posture led to maximum changes in lateralization of dopamine in the nucleus accumbens, while dopamine concentration in the caudate rose progressively with increasing postural asymmetry. Thus dopamine concentration in the nucleus accumbens appears to be a marker for the speed of animals while dopamine concentration in the caudate is more related to posture. Since both nuclei are sensitive to lateralization, dopamine in both nuclei is a marker for the direction of movement.

Others have shown with selective drug injections, electrical stimulation, and lesions that the caudate nucleus plays a role in controlling posture while the nucleus accumbens is more involved in regulating overall locomotor activity (*4, 5, 9*). Electrical stimulation of the nucleus accumbens or direct injection of dopamine agonists into this area increases locomotor activity, while lesions in this region cause hypoactivity (*5*). The same electrical or drug stimulation of the caudate nucleus causes animals to orient themselves away from the side of stimulation (*4, 9*). In our previous study we found that voluntary movement leads to changes in dopamine metabolism in these nuclei (*6*). The present results demonstrate that the changes in dopamine production in the caudate and accumbens nuclei are related to the function of the nuclei and, more importantly, are proportional to the speed, direction, and posture of the animals. They also show that a neurochemical measurement can be quantitatively related to all measures of locomotion. It is possible, although certainly not established, that the observed changes in dopamine turnover are uniquely related to the motor activity that preceded death.

Our results do not show whether changes in dopamine synthesis are necessary for the expression of motor behavior. Injection of dopamine agonists into the caudate or nucleus accumbens can change posture and initiate locomotor activity (*4, 5, 9*). On the other hand, animals with significant lesions of dopaminergic systems have apparently normal motor behavior, and fetal substantia nigra transplants can restore motor behavior in such rats even though the transplants restore only 20 percent of the normal dopamine concentration (*10*). Therefore the increase in dopamine production seen during motor behavior may not be essential for motor function. However, activation of dopaminergic systems does appear to be a reliable marker for the function of brain nuclei and may offer a neurochemical correlate of global motor activity.

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A Role for Glycosylation of the α Subunit in Transduction of Biological Signal in Glycoprotein Hormones

Abstract. *The biological properties of recombinants of glycoprotein hormones in which the α and β subunits were differentially deglycosylated have been investigated. Specific deglycosylation of the α subunit generated a recombinant that had more receptor-binding activity but did not produce hormone response in the target cells. The deglycosylated α + β recombinant was also an antagonist of the action of the native hormone. Thus, the carbohydrates in the α subunit play a dominant role in the transduction of the hormone signal into the cell.*

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Glycoproteins are ubiquitous in nature, performing important functions as enzymes, immunoglobulins, hormones, and receptors. One or more carbohydrate moieties may be involved in stabilization of protein conformation, regulation of metabolic half-life, uptake by cells, recognition, differentiation, growth, and metabolism (*1*). The oligomeric glycoprotein hormones of the pituitary and placenta are unusual because of the high carbohydrate content in the noncovalently linked α and β subunits (*2*). The removal of terminal sialic acid residues in human choriogonadotropin (hCG), follicle-stimulating hormone (FSH), and human luteinizing hormone (hLH) destroys the in vivo biological activity of the hormones (*3*) by rapid elimination from circulation but in vitro biological response is retained (*4*). However, removal of additional sugars by exoglycosidases or a mixture of exo- and endoglycosidases as in hCG (*5*) or chemical treatment of all the glycoprotein hormones (*6, 7*) has shown that the carbohydrate residues are not essential for binding to the receptors (*7*). We have observed that the carbohydrate moiety in the α subunit (*8*) of the pituitary glycoprotein hormone plays a critical role in coupling the hormone-receptor complex to the adenylate cyclase and thus the transduction of the biological signal.

Appropriate recombination of the isolated α and β subunits of the glycoprotein hormones generates a product that is virtually identical to the native hormone in all respects including biological activity (receptor binding, adenylate cyclase activity, and cellular response) (*2*). The complete integrity of the carbohydrate units in ovine LH is not required for subunit interaction or receptor binding (*7, 9*). Specific chemical deglycosylation of the ovine LH-α (DG-LH-α) enhanced its ability to combine with the β subunit, as reflected by the increase in receptor-binding activity of the DG-LH-α + LH-β recombinant (Fig. 1). In less than 10 minutes after recombination of the subunits, full receptor-binding activity was regenerated in this incubation mixture; by comparison, about 24 hours were required to attain maximal activity in LH-α + LH-β (in which both subunits are fully glycosylated). Specific deglycosylation of the ovine LH-β did not significantly affect its ability to regener-

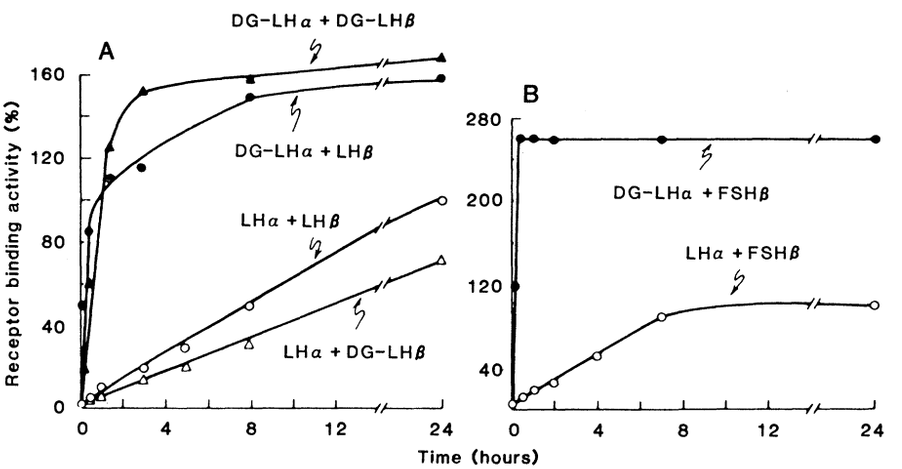

Fig. 1. Effect of deglycosylation of the α subunit on regeneration of receptor-binding activity. Native and deglycosylated subunits (1 mg/ml) were dissolved in 0.05*M* phosphate buffer, *p*H 7.5, and recombination was initiated by mixing equal volumes of these solutions. The solutions were incubated at 37°C, and at specified intervals aliquots were withdrawn and frozen at −20°C for receptor-binding assay. LH and FSH receptor-binding activities were determined with rat testicular homogenate (*7*) which contains different population of cells with separate and specific receptors for LH and FSH. In each receptor assay, about 75,000 count/min of ^{125}I-labeled ovine LH or ovine FSH was incubated with the homogenate for 2 hours at 37°C in a total incubation volume of 500 μl. The specific radioactivity bound to the membrane pellet after centrifugation was determined. Nonspecific binding was determined in the presence of 1 μg of unlabeled ovine LH or ovine FSH. The activity of the native hormone in each case was set as 100 percent for the calculation of relative receptor-binding activity. (A) LH receptor assay; (B) FSH receptor assay. Neither the isolated subunits nor their deglycosylated counterparts had receptor-binding activity. Similar data were obtained with pig ovarian preparations which also have both LH and FSH receptors. Deglycosylation of the α subunit did not affect the receptor-binding specificity of the complex. The same preparation of LH-α and DG-LH-α subunits was used for recombination in both assays as had been used in our previous work (*9*). The LH-β preparation was prepared by the salt precipitation method (*9*) but was also further purified by reversed-phase high-performance liquid chromatography. The major component, which had no intrinsic receptor-binding or biological activity, was selected for the recombination.