The Journal of Neuroscience, November 1989, 9(11): 4033–4041

Regional Cerebral Glucose Utilization Reveals Widespread Abnormalities in the Motor System of the Rat Mutant *dystonic*

Lucy L. Brown and Joan F. Lorden

Department of Neurology and Department of Neuroscience, Albert Einstein College of Medicine, Bronx, New York 10461, and Department of Psychology and Neurobiology Research Center, University of Alabama at Birmingham, Birmingham, Alabama 35294

Rats with an inherited movement disorder (*dystonic, dt*), their phenotypically normal littermates, and normal unrelated controls were studied using a metabolic mapping technique, 2-deoxyglucose autoradiography. This approach was used to identify potential sites of abnormality underlying the movement disorder, as no morphological abnormalities using light and electron microscopic techniques have been identified in this mutation. There was a significant overall glucose utilization (GU) reduction in the *dt* rats and their littermate controls when they were at rest and not displaying abnormal movements. Conversion of GU values to standard scores showed abnormalities in *dt* compared with both control groups in the following areas: deep cerebellar nuclei, locus coeruleus, pontine gray, ventrolateral–ventromedial thalamic complex, nucleus of the third nerve, lateral habenula, and basolateral amygdala. Littermates were different from nonlittermates in several regions, including the dentate and red nuclei. A study of relative GU performed in animals displaying dystonic movements also showed abnormalities in the deep cerebellar nuclei and locus coeruleus, and in the red nucleus, external cuneate, and medial septum. Correlations computed for GU in pairs of regions with known anatomical connections suggested that cerebellar, substantia nigra, and basal ganglia efferents may be abnormal. These studies complement existing biochemical and neuropharmacological data which show abnormalities in the cerebellum of the *dt* rat. Additionally, the function of brain stem and even basal ganglia nuclei is affected in this mutant, perhaps as a consequence of abnormal cerebellar activity. The partial effects in the littermates suggest that abnormalities in only a few regions are not sufficient to produce the movement disorder, and a gene dose effect may exist. Thus, although there are no identifiable morphological abnormalities in *dt*, there are major functional deficits in many brain regions, and a global deficit in energy metabolism may accompany the syndrome.

The rat mutant *dystonic* (*dt*) is a model of an inherited movement disorder discovered in the Sprague-Dawley strain (Lorden et al., 1984). This mutant shows twisting movements of the limbs and axial musculature in the absence of any light and electron microscopic morphological signs of lesions or degeneration in either the central or peripheral nervous system. Breeding studies have indicated that the movement disorder is the result of an autosomal recessive mutation; however, the nature of the primary gene defect is unknown.

Behavioral and biochemical studies have indicated that the cerebellum may be the primary focus of abnormalities in the mutant. For example, the *dt* rat is insensitive to the tremorogenic effects of the drug harmaline (Lorden et al., 1985), which is known to cause a tremor in normal rats by the rhythmic activation of an olivocerebellobulbar pathway. The finding that *dt* rats did indeed show a tremor when treated with oxotremorine, however, suggested that this mutant did not lack the spinal mechanism but that a defect in the supraspinal pathways including the cerebellum might exist. Thus it is of special interest that the Purkinje cells of the mutant cerebellum show an abnormal electrophysiological response pattern elicited by harmaline (Stratton et al., 1988). Moreover, the *dt* rat shows low levels of cerebellar cyclic nucleotide 3′,5′-guanine monophosphate (cGMP), a compound that is particularly abundant in the cerebellum and rapidly altered by changes in excitatory input. cGMP levels are low in the *dt* even in response to harmaline which activates excitatory input (Lorden et al., 1985). In addition, activity of the synthetic enzyme for GABA, glutamic acid decarboxylase (GAD), is increased in the deep cerebellar nuclei within days of the appearance of clinical symptoms (Oltmans et al., 1986) but not in the caudate-putamen or globus pallidus (Oltmans et al., 1984). The selective change in GAD activity in the cerebellar nuclei may reflect a specific abnormality in the function of the cerebellum and Purkinje cells, and not a general defect in GABAergic neurons. Because the Purkinje cells are the major output neurons of the cerebellar cortex, it is possible that a change in their activity would affect their efferent targets and other motor nuclei, and that such a change would be detectable with a functional metabolic assay. However, it is also possible that abnormal GAD synthesis in the deep nuclei actually reflects little functional change and another part of the motor system would show major metabolic abnormalities.

To identify regions and systems of abnormal neural activity in the *dt*, we used the 2-deoxy-D-[1-^{14}C]glucose (DG) technique of Sokoloff et al. (1977). The use of the DG technique by others has suggested a close relationship between glucose utilization (GU) in a particular brain region and local functional activity at that site (Sokoloff, 1981). This technique has been applied successfully in the mouse mutant *rol*, or Rolling, for the same

Received Dec. 27, 1988; revised May 15, 1989; accepted May 23, 1989.

This work was supported by NIH Grants NS 18062 and RR02499. We wish to thank Diane Smith and Sandra Salinas for their excellent technical assistance.

Correspondence should be addressed to Dr. Lucy L. Brown, Albert Einstein College of Medicine, 1300 Morris Park Ave., Bldg. K, Rm. 601, Bronx, NY 10461.

purpose (Kato et al., 1982). The Rolling mouse shows an ataxia thought to be of cerebellar origins (Ohno, 1979); however, the DG studies suggested changes in GU at several sites in the basal ganglia, in addition to changes in the cerebellum (Kato et al., 1982).

In the present study, a total of 62 sites from throughout the neuraxis were selected for investigation. These included both motor and nonmotor regions. The study compared GU in 3 groups: *dt* rats, their phenotypically normal littermates, and unrelated normal control rats. The nonlittermate controls were used to assess whether rats from the dystonia colony showed any general abnormalities in glucose utilization, regardless of genotype. Because quantitative analyses of the data indicated that the mutant rats and a subset of the littermates had a global reduction in glucose metabolism in comparison with normal controls, standardized scores were used to identify sites of abnormal glucose utilization. In addition, a correlational analysis was used to explore alterations in functional relationships between sites with direct anatomical connections. The study revealed abnormalities in cerebellum and efferent targets, in locus coeruleus, in functional relationships between the substantia nigra and its projection sites, and in functional relationships among basal ganglia nuclei.

Materials and Methods

Groups with previous anesthesia, GU quantified. Studies which used the methods of Sokoloff et al. (1977) to estimate GU (μmols/100 gm/min) were carried out on 19 rat pups. The groups consisted of 19 to 24-d-old dystonics ($n = 6$), littermates ($n = 8$), or nonlittermates ($n = 5$) 19–24 d of age. Femoral arterial and venous catheters (PE 50 tubing) were implanted under halothane anesthesia and sewn coiled under the skin (length, 8–10 cm, 20 μl dead space) so that the animals could be freely moving after the surgery without having access to the catheters. Two to three hours after recovery from anesthesia the catheters were uncoiled and animals were injected with 10–15 μCi DG (55 mCi/mM; American Radiolabeled Chemicals Inc., St. Louis, MO) in 0.1 cc saline. Over the 45 min between DG injection and sacrifice, 6 arterial samples were drawn for glucose assay and ^{14}C determination. The number (8) and volume (40 μl) of samples were less than are typically used in adult animals to minimize blood loss in the pups; sample times were chosen to capture the peak and describe the curve: 1 sec, 1, 2, 10, 20, 30, 45 min. During the experiment, animals were allowed to move in a restricted area (15 × 15 cm) and restrained by hand if necessary. At 45 min post-DG injection, animals were killed by decapitation and brains were frozen in isopentane cooled to −35°C. Cryostat sections, 20 μm thick, were incubated against Kodak SB-5 film for 7 d.

Arterial blood gases were determined for separate groups of dystonics ($n = 5$), littermates ($n = 5$), and nonlittermates ($n = 5$) that underwent halothane anesthesia to implant femoral arterial catheters. Samples were taken 10–15 min after animals showed normal movements following cessation of anesthesia.

Groups without previous anesthesia, relative GU measured with ratios. To avoid the possible effects of anesthesia and trauma of surgery on brain GU and to confirm our major findings in the groups with anesthesia, we used a method to estimate GU which does not require anesthesia. An intraperitoneal (i.p.) injection of DG (Meibach et al., 1980) was given to 24-d-old dystonics ($n = 10$) and littermates ($n = 8$). For these animals 10–15 μCi DG and a 45 min experimental period was used. Samples were taken from blood produced by decapitation and plasma was analyzed for ^{14}C and glucose. Autoradiographic analysis of data for this technique uses a ratio of optical densities. The validity of comparisons between groups using this approach depends upon the between-group similarity of GU rate of the denominator of the ratio. We knew that our assumption for the denominator might not be entirely valid in this instance, but large differences would be detectable and could confirm or question major findings.

Data analysis. Densitometric readings for 62 brain regions were made using an image-analysis system (Quantimet 970, Cambridge Instruments, Buffalo, NY). A digital tablet and a mouse were used to outline the region of interest on a monitor; left and right measurements were taken for each region and averaged; for the cerebellar hemispheres and vermis the entire area on the section was included in the measurement; for the neocortex, all layers were included in the measurement. GU was estimated according to the formula of Sokoloff et al. (1977) for the groups with previous anesthesia and surgery for which data from plasma variables were available. As mentioned above, a ratio of optical densities was used for the groups with i.p. injections. The denominator for the optical density ratio was the value for the interpeduncular nucleus because it was found to have a similar GU rate in dystonics and the majority of the littermates (Table 2) in the fully quantitative study.

A standard score (z) analysis was used to compare data among groups for which GU was fully quantified because GU rate was globally lower in dystonics than in nonlittermates. The z-score produced a relative value for a given brain region GU for each animal by calculating

$$z\text{-score} = \frac{\text{Brain region GU} - \text{Mean GU of 62 regions}}{\text{Standard deviation of 62 regions}}.$$

The z-score made comparisons possible despite overall differences in GU. Mean z-scores were calculated for each region in each group, and an analysis of variance and *posthoc* t tests were calculated for the z-scores. A significant difference in brain region z-score between groups demonstrates that the brain region has changed its position within the distribution of GU of all regions measured. Although this is a relative measure, it still demonstrates abnormalities in GU.

Product–moment correlation coefficients between selected brain regions were determined for each group. Previous positron emission tomographic studies of GU in humans have used a correlation coefficient to describe the functional coupling between regions (Clark et al., 1984; Horwitz et al., 1986, 1987). This is a potentially revealing measure of functional relationships in brain systems, and studies of human focal dystonias have shown differences from normal in the basal ganglia/thalamus correlation (Stoessl et al., 1986). In the present study, regions with known anatomical connections within the motor system and regions which showed significantly different z-scores were selected for analysis. Each correlation was evaluated with a t test (Rosner, 1986).

Statistical analyses for most comparisons were an analysis of variance followed by 2-tailed t tests if the analysis of variance showed $p < 0.05$. This procedure is appropriate for a survey study searching for unsuspected, *post hoc* differences. On the other hand, the deep nuclei of the cerebellum were evaluated with a t test without the analysis of variance, as a *planned* comparison (Keppel, 1973) because previous studies had shown differences between dystonics and littermates in those nuclei and tests of their differences addressed a specific hypothesis.

Results

Groups with previous anesthesia, GU quantified

Physiological variables. Plasma glucose did not significantly differ among groups. The values (mean ± SEM) for nonlittermates, littermates, and dystonics, respectively, were 1.54 ± 0.05, 1.46 ± 0.07, and 1.44 ± 0.11 mg/ml. Body weight was significantly different between dystonics and nonlittermates ($p < 0.01$). The values (mean ± SEM) for nonlittermates, littermates, and dystonics, respectively, were 59 ± 5, 50 ± 8, and 34 ± 2 gm. Arterial pCO_2 in dystonics was slightly higher than both control groups ($p < 0.05$) and pH was slightly lower ($p < 0.05$ compared with nonlittermates), but the values were within normal range (Table 1). Arterial O_2 was normal in all groups.

Behavior. All animals were alert and capable of moving but showed few or sporadic movements during the experiments. The dystonics lay quietly with some visible increase in muscle tone or occasional limb extension but no consistent movements.

Glucose utilization rates. GU rates in dystonics were significantly lower than nonlittermate controls. All 62 brain regions measured were lower in dystonics than nonlittermates with the average whole-brain difference between groups being −37% and the range being −16 to −49% (Table 2). The only regions which were not significantly lower than nonlittermates were the deep nuclei of the cerebellum, red nucleus, third nerve nucleus, and

medial geniculate. In the littermate control group, 5 out of 8 animals also showed a low average GU rate similar to *dt* rats (mean of 62 regions: 48 ± 5 μmol/100 gm/min; dystonics, 46 ± 6), while 3 littermates showed average GU rates similar to nonlittermates (80 ± 5 μmol/100 gm/min compared with 74 ± 9 for nonlittermates). Thus, the low littermates, animals with a mean brain GU of 38–56 μmol/100 gm/min, were compared with dystonic group, whose mean brain GU ranged from 41–50 μmol/100 gm/min. The regions in the *dt* rats which showed a significant difference in GU rate when compared with the matched low littermates were the deep nuclei of the cerebellum (Table 2). All of the deep nuclei showed increased GU over littermates. In the dentate nucleus there was an increase of 37% ($p = 0.02$), in the fastigial nucleus the increase was 30% ($p = 0.03$), and in the interpositus the increase was 41% ($p = 0.01$).

z-Scores. The *z*-score analysis confirmed that the deep nuclei of the dystonics were different from littermates ($p < 0.01$), and also showed that the fastigial nucleus and interpositus nucleus were different from nonlittermates ($p < 0.05$, Table 2). Other regions in *dt* rats which were different from both control groups, using the *z*-score analysis, were the locus coeruleus, the pontine gray, the third nerve nucleus, the ventrolateral and ventromedial nuclei of the thalamus, the lateral habenula, and the basolateral nucleus of the amygdala. There were other miscellaneous effects of the *z*-score analysis: the red nucleus of both dystonics and littermates was different from the nonlittermates; this suggests abnormalities in the littermates as well as in *dt*. The littermates also showed differences from nonlittermates in the dentate nucleus, cerebellar hemispheres, inferior cerebellar peduncle, cuneate, and lateral geniculate. None of the other brain regions throughout basal ganglia, cortex, thalamus, or hypothalamus showed significant differences among groups in either GU or *z*-score analyses.

Correlation analysis

The product–moment correlations of GU rates were calculated for motor and nonmotor areas with known anatomical connections. The regions in which correlations were not similar among groups were the deep cerebellar nuclei, cerebellum, inferior olive, locus coeruleus, and basal ganglia nuclei (Table 3). Significant positive correlations were obtained for both control groups, but not dystonic rats, between the medial accessory inferior olive and the cerebellar vermis; the interpositus and the red nuclei; and the locus coeruleus and cingulate cortex (Table 3). On the other hand, for correlations between the fastigial nucleus and vermis, only the dystonic group showed a high correlation (0.95, $p < 0.05$) while the 2 control groups showed lower correlations (0.84, 0.72, n.s.). For both the dentate nucleus to cerebellar hemisphere and the dentate nucleus to ventrolateral thalamus correlations, the *dt* rats and their littermates showed higher, significant correlations than the nonlittermate group (Table 3). The locus coeruleus/cerebellar hemisphere correlation was not significant for any of the groups, but it was highest in the nonlittermate group (0.82 nonlittermate, 0.56 littermate, 0.49 *dt*). The locus coeruleus correlations with hippocampus and forelimb sensorimotor cortex were not significant for either the dystonics or the littermates, but they were significant for the nonlittermates.

In the basal ganglia and their thalamic efferent targets, the dystonics were different from both control groups for each correlation computed. For the striatum/substantia nigra and substantia nigra/ventromedial thalamus, the *dt* rats showed a significant negative correlation (−0.81, $p < 0.05$), while the 2 control groups showed positive correlations (+0.93, +0.91, $p < 0.05$). For the other basal ganglia correlations (Table 3), the 2 control groups also showed positive correlations but the dystonics showed no significant correlation. For example, the striatum/globus pallidus correlation was 0.87 ($p < 0.05$) and 0.93 ($p < 0.05$) for the nonlittermates and littermates, but −0.31 (n.s.) for the dystonics.

Normal, significant correlations for the dystonics were found in the auditory system and for interhemispheric measurements (0.92, $p < 0.05$). However, for the cuneate/VPL thalamus correlation, dystonics were again abnormal (0.15, n.s., Table 3).

Table 1. Comparisons of mean ± SEM arterial blood variables in dystonics and the 2 control groups

Variable	Nonlittermates (5)	Littermates (5)	Dystonics (5)
pH	7.48 ± 0.01	7.44 ± 0.02	7.40 ± 0.02[b]
pO_2	97 ± 8.0	91 ± 6.0	95 ± 5.0
pCO_2	22 ± 1.0	25 ± 1.0	28 ± 1.0[a,b]

(*n* in parentheses).
[a] $p < 0.05$ different from littermates, *t* test.
[b] $p < 0.01$ different from nonlittermates, *t* test.

Groups with no previous anesthesia, relative GU measured with ratios

Physiological variables. Plasma glucose levels were not reliably different between the *dt* and littermate control groups (*dt* = 1.17 ± 0.06, littermates = 1.29 ± 0.06, $p = 0.13$). However, the *dt* rats were significantly lower than littermates in body weight (means = 26 ± 2 vs 58 ± 6 gm, respectively, $p < 0.01$), and higher than littermates in plasma ^{14}C (2316 ± 1194, 1178 ± 461, respectively). The lower plasma glucose and higher plasma ^{14}C in dystonics than in littermates suggests an overall lower GU rate for dystonics, but differences in μCi/gm body weight injected made it impossible to draw firm conclusions from the data.

Behavior. During the experiment all mutant pups were constantly engaged in the movements that are characteristic of the model. These included head and limb extension, limb-crossing, and forepaw clasping. The littermates were alert and moved occasionally. This is in contrast to the previous groups, which had received anesthesia 2–3 hr prior to the experiment and were generally quiet and without dystonic movements. Further discussion of these groups will refer to them as quiet and active.

Optical density ratios. The data obtained from these studies were autoradiographic ratios, or relative GU. With this analysis, the deep nuclei of the cerebellum ($p < 0.001$), and the locus coeruleus ($p < 0.05$) were again found to differ from control values in the *dt* rats (Table 4). Differences were also found in the red nucleus ($p = 0.001$), external cuneate ($p < 0.001$), and medial septum ($p = 0.01$, Table 4). All these regions showed ratios which suggested higher glucose utilization rates in *dt* rats than in littermates. These results are very similar to the previous groups' results and suggest that the cause of the cerebellar nuclei changes and other changes in the red nucleus and locus coeruleus are not related to the anesthesia and surgery.

Correlation analysis. Correlations for the active, no-anesthesia groups were different from the quiet anesthesia groups but showed abnormalities in the same regions. The quiet/active

Table 2. Mean ± SEM glucose utilization (μmol/100 gm/min) and *z*-score comparisons in the 62 brain regions analyzed

Brain region	Glucose utilization rate comparisons			*z*-Score comparisons	
	Nonlittermate (5)	Littermate (5)	Dystonic (6)	Dystonic	Littermate
Cerebellum, pons, medulla					
Dentate n.	86 ± 13	45 ± 3[b]	62 ± 5[d]	*f*	*a*
Fastigial n.	82 ± 11	50 ± 3[b]	65 ± 5[d]	*e,a*	
Interpositus n.	86 ± 13	51 ± 3[b]	72 ± 6[e]	*f,b*	
Cerebellar hemisphere	44 ± 6	24 ± 2[b]	25 ± 2[b]	*d*	*a*
Inferior cerebellar peduncle	48 ± 7	29 ± 2[b]	28 ± 4[b]	*d*	*a*
Cerebellar vermis	68 ± 9	39 ± 3[b]	42 ± 2[b]		
Locus coeruleus	58 ± 8	36 ± 4[b]	43 ± 2[a]	*f,a*	
Pontine gray	59 ± 9	37 ± 3[b]	41 ± 1[b]	*e,a*	
Cuneate	72 ± 8	39 ± 2[c]	45 ± 2[b]	*e*	*b*
External cuneate	70 ± 7	44 ± 6[b]	39 ± 2[c]		
Inferior olive, m.	73 ± 10	46 ± 2[b]	49 ± 5[b]		
Inferior olive, l.	77 ± 9	49 ± 3[b]	50 ± 3[b]		
Vestibular n.	100 ± 12	62 ± 3[b]	64 ± 6[b]		
Median raphe	90 ± 13	53 ± 3[b]	53 ± 2[b]		
Cochlear n.	113 ± 14	77 ± 7[a]	77 ± 4[a]		
Superior olive	107 ± 10	75 ± 6[a]	78 ± 7[a]		
Midbrain					
Red n.	64 ± 8	50 ± 4	53 ± 2	*c*	*a*
Third nerve n.	77 ± 9	54 ± 4[b]	64 ± 1	*b,d*	
Midbrain retic.	61 ± 9	43 ± 3[b]	41 ± 2[a]		
SN compacta	68 ± 10	48 ± 4[a]	46 ± 1[a]		
SN reticulata	56 ± 7	41 ± 3[a]	38 ± 2[b]		
Superior colliculus	60 ± 9	42 ± 3[a]	41 ± 2[a]		
Inferior colliculus	102 ± 15	67 ± 5[a]	75 ± 4[a]		
Medial genic	71 ± 15	54 ± 4	47 ± 2		
Interpeduncular n.	95 ± 13	61 ± 3[b]	60 ± 2[b]		
Thalamus					
Thalamus, VL	78 ± 13	49 ± 3[a]	41 ± 3[b]	*a,d*	
Thalamus, VM	107 ± 14	63 ± 4[b]	56 ± 4[b]	*b,d*	
Thalamus, VA	92 ± 13	54 ± 6[b]	47 ± 4[b]		
Thalamus, VPL	75 ± 12	45 ± 3[b]	45 ± 3[b]		
Lateral geniculate	75 ± 12	43 ± 2[b]	40 ± 2[b]	*a*	*a*
Basal ganglia					
Subthalamic n.	81 ± 11	53 ± 5[b]	53 ± 2[b]		
Entopeduncular n.	57 ± 5	42 ± 3[b]	35 ± 2[c]		
Globus pallidus	56 ± 8	37 ± 3[b]	34 ± 2[b]		
Striatum anterior D	78 ± 10	53 ± 3[a]	51 ± 4[b]		
Striatum anterior V	70 ± 9	46 ± 3[b]	45 ± 2[b]		
Striatum mid1 D	79 ± 8	55 ± 5[b]	51 ± 3[b]		
Striatum mid1 V	67 ± 7	47 ± 5[b]	46 ± 3[b]		
Striatum mid2 D	81 ± 12	47 ± 2[b]	45 ± 2[b]		
Striatum mid2 V	65 ± 8	47 ± 3[a]	46 ± 2[b]		
Cortex and splenium					
Visual	72 ± 12	41 ± 3[b]	45 ± 3[a]		
Auditory	87 ± 13	56 ± 3[a]	53 ± 3[b]		
Hindlimb	86 ± 12	55 ± 9[a]	52 ± 2[b]		
Forelimb S-M	84 ± 9	58 ± 5[b]	54 ± 3[c]		
Forelimb motor	86 ± 7	59 ± 5[b]	53 ± 4[c]		
Forelimb sensory	82 ± 14	52 ± 7[a]	52 ± 3[a]		
Head sensory	85 ± 12	52 ± 6[b]	48 ± 3[b]		
S2 area	78 ± 11	47 ± 2[b]	48 ± 4[b]		
Cingulate, posterior	83 ± 12	49 ± 3[a]	45 ± 2[b]		
Cingulate, anterior	83 ± 12	49 ± 3[b]	45 ± 2[b]		
Parietal	78 ± 10	55 ± 4[a]	49 ± 4[b]		
Splenium	41 ± 7	30 ± 2	23 ± 1[b]		

Table 2. Continued

Brain region	Glucose utilization rate comparisons			z-Score comparisons	
	Nonlittermate (5)	Littermate (5)	Dystonic (6)	Dystonic	Littermate
Limbic system					
Amygdala, basolateral	81 ± 9	54 ± 5[b]	44 ± 3[b]	[b,d]	
Lateral habenula	108 ± 12	74 ± 5[c]	65 ± 3[b]	[b,d]	
Medial habenula	87 ± 10	57 ± 4[b]	51 ± 4[c]		
Hippocampus CA3	60 ± 8	42 ± 4[a]	36 ± 3[c]		
Hippocampus CA1	52 ± 5	37 ± 5[a]	30 ± 3[c]		
Septum medial	57 ± 4	40 ± 4[b]	36 ± 3[b]		
Septum lateral	50 ± 5	35 ± 4[a]	28 ± 2[b]		
Mamillary bodies	90 ± 15	60 ± 6[a]	53 ± 3[b]		
Hypothalamus, VMN	41 ± 5	30 ± 3	23 ± 3[b]		
Hypothalamus, PVN	46 ± 7	34 ± 3	27 ± 2[b]		
Hypothalamus, LH	52 ± 8	37 ± 3	30 ± 3[b]		

The littermate group is a subset of animals which showed a low overall glucose utilization rate similar to dystonics. *N* in parentheses. D, dorsal, V, ventral, M, medial, L, lateral, A, anterior, S-M, sensory-motor, SN, substantia nigra.

[a] $p \leq 0.05$, vs nonlittermate.

[b] $p \leq 0.01$ vs nonlittermate.

[c] $p \leq 0.001$ vs nonlittermate.

[d] $p \leq 0.05$ vs littermate.

[e] $p \leq 0.01$ vs littermate.

[f] $p \leq 0.001$ vs littermate.

comparisons may reveal particularly devastating, abnormal points in the system for carrying out movement in the dystonics. For example, in the quiet groups, dystonics showed a normal positive correlation for dentate nucleus/cerebellar hemisphere (0.94, $p < 0.05$) but a significant negative correlation while active ($-0.82, p < 0.05$), unlike littermates, which were positive in both situations (0.91, 0.80, $p < 0.05$; Table 3). Similarly, the locus coeruleus/cerebellar hemisphere correlation was positive in the quiet dystonics (0.49) but was negative in the active dystonics ($-0.84, p < 0.05$), unlike littermates (0.56 quiet, -0.11 active; Table 3). For these correlations, only the active state revealed differences between dystonics and littermates. The substantia nigra correlation with the deep layers of the superior colliculus was different from littermates in both the quiet and

Table 3. Product-moment correlations between GU rates of selected nuclei in nonlittermates, littermates, and dystonics

Brain regions	Nonlitter-mates (5)	Littermates		Dystonics	
		Quiet (5)	Active (8)	Quiet (6)	Active (10)
R cochlear n./R inferior colliculus	0.92[a]	0.94[a]	0.71[a]	0.92[a]	0.76[a]
L sensorimotor cortex/R sensorimotor cortex	0.99[a]	0.99[a]	0.90[a]	0.92[a]	0.88[a]
L cuneate/R VPL, thalamus	0.91[a]	0.86	0.42	0.15	0.04
L inferior olive/R cerebellar hemis.	0.93[a]	0.86	0.88[a]	0.78	−0.29
L inferior olive, m. accessory/Vermis	0.97[a]	0.88[a]	0.56[a]	0.46	−0.57
L fastigial n./Vermis	0.84	0.72	0.29	0.95[a]	−0.51
R dentate n./L cerebellar hemisphere	0.77	0.91[a]	0.80[a]	0.94[a]	−0.82[a]
R dentate/L VL	0.81	0.88[a]	0.49	0.83[a]	−0.50
R interpositus/L red n.	0.96[a]	0.89[a]	−0.80[a]	0.34	−0.12
L locus coeruleus/L cerebellar hemis.	0.82	0.56	−0.11	0.49	−0.84[a]
L locus coeruleus/L sensorimotor cortex	0.91[a]	0.84	−0.48	0.44	0.18
L locus coeruleus/L cingulate cortex	0.95[a]	0.95[a]	−0.20	0.01	0.40
L locus coeruleus/L hippocampus, CA1	0.95[a]	0.67	−0.10	−0.36	0.21
L dorsal striatum/L substantia nigra	0.86	0.73	0.06	−0.85[a]	0.23
L substantia nigra/L VM thalamus	0.93[a]	0.91[a]	0.03	−0.81[a]	0.21
L substantia nigra/L superior collic., v.	0.89[a]	0.98[a]	−0.55	−0.03	0.90[a]
L dorsal striatum/L globus pallidus	0.87[a]	0.93[a]	0.21	−0.31	0.35
L globus pallidus/L VL thalamus	0.86	0.93[a]	0.13	−0.02	0.10

Quiet refers to the group treated with anesthesia prior to the experiment for which GU was fully quantified and the animals were quiet during the experiment. Active refers to the group which was not treated with anesthesia prior to the experiment, ratio data were obtained, and the animals were active. L, left; R, right. Number of animals per group in parentheses.

[a] $p < 0.05$, *t* test.

Table 4. Active groups, no previous anesthesia

Brain region	Littermate (8)	Dystonic (10)
Dentate n.	0.99 ± 0.03	1.20 ± 0.04[c]
Fastigial n.	0.98 ± 0.02	1.17 ± 0.02[c]
Interpositus n.	1.01 ± 0.02	1.19 ± 0.03[c]
Locus coeruleus	0.66 ± 0.03	0.75 ± 0.03[a]
External cuneate	0.75 ± 0.01	0.87 ± 0.02[c]
Red nucleus	1.00 ± 0.03	0.87 ± 0.02[c]
Septum, medial	2.03 ± 1.01	0.79 ± 0.02[b]
Cerebellar vermis	0.76 ± 0.02	0.84 ± 0.03
Cerebellar hemisphere	0.59 ± 0.03	0.63 ± 0.02
Inferior olive, medial	0.82 ± 0.03	0.84 ± 0.02
Pontine gray	0.78 ± 0.04	0.84 ± 0.03
Vestibular n.	1.12 ± 0.02	1.19 ± 0.02
Cuneate	0.79 ± 0.04	0.86 ± 0.02
Midbrain retic.	0.81 ± 0.04	0.88 ± 0.04
Third n. n.	1.10 ± 0.04	1.03 ± 0.04
SN reticulata	0.68 ± 0.07	0.68 ± 0.01
Superior colliculus D.	0.72 ± 0.02	0.72 ± 0.03
Superior colliculus V.	0.82 ± 0.01	0.82 ± 0.04
Thalamus, VL	0.89 ± 0.03	0.93 ± 0.02
Thalamus, VM	1.16 ± 0.04	1.14 ± 0.05
Entopeduncular n.	0.59 ± 0.02	0.61 ± 0.02
Globus pallidus	0.65 ± 0.02	0.67 ± 0.02
Striatum anterior D.	0.93 ± 0.05	0.85 ± 0.03
Striatum anterior V.	0.84 ± 0.03	0.74 ± 0.03
Amygdala, basolateral	0.89 ± 0.03	0.84 ± 0.03
Cingulate cortex	0.93 ± 0.02	0.96 ± 0.03
Hypothalamus, PVN	0.58 ± 0.04	0.54 ± 0.02

Mean ± SEM optical density ratios for littermates and dystonics 24 d old. Data from all 61 regions are not shown. Regions shown are those which were significantly different between groups and a sample of other regions. N in parentheses. t tests were performed if the analysis of variance was $p < 0.05$.

[a] $p = < 0.05$.

[b] $p = < 0.01$.

[c] $p = < 0.001$.

active states: In littermates the correlation was positive in the quiet state (0.98, $p < 0.05$) and switched to negative in the active state (−0.55); in dystonics a lack of any correlation (−0.03) in the quiet state switched to positive in the active state (0.90, $p < 0.05$, Table 3). The cerebellum and substantia nigra outputs may be particularly affected in *dt*.

Discussion

We used cerebral GU as an index of neural activity to identify abnormal regions or systems in the *dt* rat. GU abnormalities in the deep nuclei of the cerebellum and locus coeruleus confirm that regions with biochemical abnormalities in *dt*, such as GAD and norepinephrine increases, do, indeed, reflect significant functional abnormalities. Suggestions from other studies of locus coeruleus abnormalities in dystonia in both rats and humans (Jacquet and Abrams, 1982; Wolfson et al., 1983; Hornykiewicz et al., 1986; Zweig et al., 1988) may be applicable to this mutant and suggest that norepinephrine plays a significant role in dystonia. However, in this model of dystonia, abnormalities were seen throughout the motor system, also, and overall cerebral GU was lower than normal. Abnormal z-scores in the cerebellum, pontine gray, red nucleus, ventrolateral and ventromedial thalamus suggest that the cerebellum and its efferents to brainstem regions and thalamus play an important role in the disorder. Studies of human dystonia have tried to localize pathology either to the brain stem or basal ganglia. The present results with the mutant rats show that cerebellum, brain-stem, and basal ganglia can be involved. Widespread pathology in the form of an enzymatic deficit or other unidentified deficit may be necessary for the syndrome or cerebellar pathology may have widespread effects on the motor system.

General considerations

GU increases measured with DG have been found to reflect changes in neuronal activity in several defined neural pathways, including the visual system, spinal cord, and hippocampus and may reflect either excitatory or inhibitory activity at axon terminals (Mata et al., 1980; Sokoloff, 1981; Ackerman et al., 1984; Kadekaro et al., 1985, 1987). However, the locus of metabolic events measured with DG is still unknown and may differ for brain areas with different densities of axons, terminals, and cell somata, as well as under different conditions. Thus, although we can identify regions of abnormal GU in the dystonic pup, we can only speculate about the abnormal neural events. Also, the technique may not be sensitive enough to reflect all abnormal neural activity, and the lack of change in a given region such as the basal ganglia must be interpreted with caution, as the correlation analysis suggests.

The global decrease in GU in the dystonics and some littermates compared with nonlittermates might be caused by genetic, developmental, or experimental factors. Experimental factors could be increased susceptibility of dystonics and littermates to the halothane anesthesia and to the trauma of the surgical procedure. The finding that the body weight of the dystonics was significantly lower than nonlittermates may reflect decreased feeding caused by the interfering movements or a primary metabolic and developmental defect. In any case, the low body weight could contribute to a heightened susceptibility to anesthesia and stress. The study which used i.p. injections of DG was carried out to minimize stress and eliminate the anesthesia. Such a study cannot detect global decreases or increases between groups but can show large relative changes in individual nuclei. The results of the i.p. study corroborate those of the quantitative experiment and showed that the increases in GU found in cerebellar nuclei and other regions could not have been caused by the indirect effects of anesthesia and stress. A possible explanation for the low overall GU in the dystonics and some littermates is that it reflects retarded development because normal younger animals than those used in this study show a low GU rate (McCann et al., 1986). Additional evidence which suggests that a global decrease might be an important feature of the dystonia comes from studies of adult rats with a mild dystonia syndrome caused by the drug iminidipropionitrile (IDPN) (Cadet et al., 1989). Of 49 regions measured following IDPN treatment, 47 showed decreases in GU, with 14 regions being significantly lower.

The abnormal movements of dystonia in man are often associated with abnormalities in the basal ganglia, especially in the putamen, although in idiopathic and inherited forms of dystonia there has been no identifiable site of pathology (Zeman, 1970; Hedreen et al., 1988). Similarly, in the *dt* rat, no macroscopic or microscopic pathology is evident in the basal ganglia, nor have any transmitter abnormalities been detected as yet in basal ganglia (McKeon et al., 1984; Oltmans et al., 1984; Lorden et al., 1988). Thus, it is of special interest that the mutant showed

GU rates similar to littermate controls but abnormal correlation coefficients in the basal ganglia and with their efferent targets. Although this indicates basal ganglia dysfunction, other data suggest that in this mutant a primary site of pathology may be in the cerebellum. Hedreen et al. (1988) raised the question of whether primary, inherited human dystonia could be caused by brain-stem pathology. For the *dt* rat, the brain stem is clearly involved. An interesting question is whether brain-stem defects could produce basal ganglia activity abnormalities that characterize the symptoms.

Littermates, though behaviorally normal, also showed abnormal *z*-scores in some nuclei. Littermates were significantly different from both nonlittermates and *dt* rats in the dentate nucleus, cerebellar hemispheres, inferior cerebellar peduncle, and cuneate nucleus. Both littermates and dystonics were different from normal pups in the red nucleus, suggesting that abnormalities at this site are not essential for the syndrome. The data clearly suggest that the genetic defect responsible for the dystonia partially affects the heterozygotes.

Correlation analyses

The correlation analysis, a measure of coupling between 2 brain regions in terms of general neural activity measured by glucose metabolism, has been used by others (Clark et al., 1984; Stoessl et al., 1986; Horwitz et al., 1987). Stoessl et al. (1986) used it in positron tomographic studies of glucose metabolism in patients with idiopathic torticollis. They found an abnormal correlation between caudate nucleus and thalamus associated with torticollis, although there was no significant difference in metabolic rate between controls and patients. The meaning of such correlations remains to be determined by future studies using other methods, but when regions with known anatomical connections are used or an external stimulus is used to drive 2 regions (Clark et al., 1984), an abnormal correlation indicates sites of abnormal neural activity.

We found several interesting abnormal correlations in the *dt* pup. Indeed, similar to the findings for patients with torticollis (Stoessl et al.), the striatal/pallidal/thalamic correlations were significant in controls but not in *dt* rats. This suggests abnormalities in the striatal efferents which eventually influence the cortex. However, the striatal to substantia nigra correlation and the substantia nigra correlation with ventromedial thalamus and deep layers of the superior colliculus were the most radically different from controls. The *dt* rats showed significant *negative* correlations whereas the controls showed positive correlations. The abnormal correlations imply altered function in the basal ganglia and its two major output systems. The change in the substantia nigra correlation with the deep layers of the superior colliculus from positive to negative in the active dystonics compared to the quiet dystonics suggests that the nigrotectal projection is especially different from normal in the dystonic brain. Abnormal GU has been found in the substantia nigra and superior colliculus of the IDPN model of dyskinesia, also (Cadet et al., 1989). This is probably related to the head and neck movements of both models, and strongly implicates abnormalities in the nigrotectal system. The abnormal correlations seen for the basal ganglia and their output systems were particularly surprising in light of earlier negative findings for dopaminergic, cholinergic, GABAergic, and serotonergic markers in the striatum of the *dt* rat (McKeon et al., 1984; Oltmans et al., 1986; Lorden et al., 1988). The correlational analysis indicates that motor systems in the brains of *dt* rats are more broadly affected than the previous biochemical investigations have suggested. It is still possible that a focal defect, such as an abnormality in cerebellar output, is capable of altering neural activity at sites of convergence between cerebellar and basal ganglia pathways. For example, in the ventromedial thalamus and superior colliculus, where there is overlap between substantia nigra and cerebellar output (Faull and Mehler, 1978; Haroian et al., 1981; Bentivoglio and Kuypers, 1982), abnormal correlations were found. These changes could then have an impact on many other regions.

The correlation of the inferior olive with the cerebellum was significant in the nonlittermates but not in the dystonic rats or littermates. The olive/vermis correlation was abnormal only in dystonic rats. These results are consistent with previous abnormal electrophysiological and pharmacological findings with harmaline (Lorden et al., 1985; Stratton et al., 1988) which affects olivocerebellar activity. Also, the correlation between red nucleus and interpositus nucleus was low in *dt* rats compared with controls. Thus, in the *dt* rats, there is evidence of unique abnormality throughout the circuit from the olive, through the cerebellum to the red nucleus. This may be significant for the motor syndrome of the mutant in light of the importance of the vertebrate rubrospinal tract in the orientation of limbs for terrestrial locomotion (ten Donkelaar, 1988).

Another finding was the high positive correlation in dystonics between the fastigial nucleus and vermis, and the lower, insignificant correlations in both control groups. This higher-than-normal correlation in the *dt* rats was also true for the dentate nucleus/cerebellar hemisphere relationship. Littermates showed high correlations compared with nonlittermates, too. Thus, regions normally not significantly correlated were highly correlated in *dt* rats. This high correlation may reflect uniformity of activity between nuclei which usually have a more complex pattern of interaction. The fact that littermates are similar to *dt* rats for some of the correlations again suggests that some abnormalities can exist without symptoms and that the littermates express part of the defect. It is important to note that not all correlations for the dystonics were abnormal. Interhemispheric correlations were normal, as were correlations in the auditory system.

An important nucleus which showed both altered *z*-score and altered correlations in the *dt* rats is the locus coeruleus. Several studies have suggested that norepinephrine could play a role in dystonia. Abnormalities in the noradrenergic system have recently been reported in the brains of dystonic patients (Hornykiewicz et al., 1986). In childhood-onset dystonia, Wolfson et al. (1983) found decreased norepinephrine metabolite in ventricular fluid. In the *dt* model an increase in cerebellar norepinephrine levels was found, although other terminal fields of the locus coeruleus appeared unaffected (McKeon et al., 1986). Pharmacologic evidence also suggests a role for norepinephrine in the *dt* model. The alpha 2 receptor agonist clonidine diminishes the dystonic movements in the *dt* pups (Lutes et al., 1985). In the present study, the locus coeruleus in dystonics was different from littermates and nonlittermates using the *z*-score analysis and showed slightly higher GU than littermates. Correlation coefficients between the locus coeruleus and cerebellum were not significant for any quiet group, but they were lowest for the dystonics and significantly negative for the dystonic active group. Whereas correlation coefficients for the cingulate cortex were significant for the 2 control groups, they were not significant for the dystonics. This is further evidence for an

abnormality in the *dt* rat in the locus coeruleus and its projections, but it does not clarify the nature of the abnormality.

Other regions which showed abnormal *z*-scores in *dt* include sensory and limbic nuclei. Sensory nuclei such as the cuneate and lateral geniculate were abnormal in littermates in addition to dystonics, ruling out feedback effects from grossly abnormal movements as the cause of the GU abnormality. The effect in the cuneate suggests that somatosensory information may be affected in dystonics at a very early stage in CNS processing. The effect in the third nerve nucleus could reflect any number of abnormalities there, or in its afferents, and emphasizes the widespread effects in the motor system. There is no obvious pattern of regions affected which suggests a specific transmitter or receptor system deficit.

Conclusions

The data suggest that if there is a primary site or system of pathology in *dt,* it can have a profound influence on the rest of the brain. Thus, it is possible that a primary defect in the cerebellum could affect all of its principal output regions and subsequent projections. Alternatively, there may be a global metabolic, transmitter, or enzyme deficit which might influence many systems with the cerebellum and Purkinje cell being more affected. Given the severity and characteristics of the movement disorder in *dt,* it is not surprising that brain-stem systems show abnormal GU or abnormal relationships to the cerebellum. The animals show head and neck extension, axial muscle extension, and limb extension similar to antigravity responses. Because many systems are involved, it seems unlikely that a single pharmacological treatment could abolish all symptoms; however, several different drugs might be able to ameliorate some symptoms of the motor syndrome. A major question is whether abnormalities in a single anatomical site or a single biochemical deficit can be found to cause the disorder. The Purkinje cell is a major focus for further investigation, along with the nigrotectal projections, noradrenergic, and basal ganglia systems.

References

Ackerman, R. F., D. M. Finch, D. L. Babb, and J. Engel (1984) Increased glucose metabolism during long-duration recurrent inhibition of hippocampal pyramidal cells. J. Neurosci. *4:* 251–264.

Bentivoglio, M., and H. G. J. M. Kuypers (1982) Divergent axon collaterals from rat cerebellar nuclei to diencephalon, mesencephalon, medulla oblongata, and cervical cord: A fluorescent double-labelling study. Exp. Brain Res. *46:* 339–356.

Cadet, J. L., A. Della Puppa, and E. London (1989) Involvement of nigrotectal-reticulospinal pathways in the iminodipropionitrile (IDPN) model of spasmodic dyskinesias: A 2-deoxy-d-[1-14C]glucose study in the rat. Brain Res. *484:* 57–64.

Clark, C. M., R. Kessler, M. S. Buchsbaum, R. A. Margolin, and H. H. Holcomb (1984) Correlational methods for determining regional coupling of cerebral glucose metabolism: A pilot study. Biol. Psychiatry *19:* 663–678.

Faull, R. L. M., and W. R. Mehler (1978) The cells of origin of the nigrotectal, nigrothalamic, and nigrostriatal projections in the rat. Neuroscience *3:* 989–1002.

Haroian, A. J., L. C. Massopust, and P. A. Young (1981) Cerebellothalamic projections in the rat: An autoradiographic and degeneration study. J. Comp. Neurol. *197:* 217–236.

Hedreen, J. C., R. M. Zweig, M. R. DeLong, P. J. Whitehouse, and D. L. Price (1988) Primary dystonias: A review of the pathology and suggestions for new directions of study. Adv. Neurol. *50:* 123–132.

Hornykiewicz, O., S. J. Kish, L. E. Becker, I. Farley, and K. Shannak (1986) Brain neurotransmitters in dystonia musculorum deformans. N. Engl. J. Med. *315:* 347–353.

Horwitz, B., R. Duara, and S. I. Rapoport (1986) Age differences in intercorrelations between regional cerebral metabolic rates for glucose. Ann. Neurol. *19:* 60–67.

Horwitz, B., C. L. Grady, N. L. Schlageter, R. Duara, and S. I. Rapoport (1987) Intercorrelations of regional cerebral glucose metabolic rates in alzheimer's disease. Brain Res. *407:* 294–306.

Jacquet, Y. F., and G. Abrams (1982) Postural asymmetry and movement disorder after unilateral microinjection of adrenocorticotropin 1–24 in rat brainstem. Science *218:* 175–176.

Kadekaro, M., A. M. Crane, and L. Sokoloff (1985) Differential effects of electrical stimulation of sciatic nerve on metabolic activity in spinal cord and dorsal root ganglion in the rat. Proc. Natl. Acad. Sci. USA *82:* 6010–6013.

Kadekaro, M., W. H. Vance, M. L. Jerrell, H. Gary, Jr., H. M. Eisenberg, and L. Sokoloff (1987) Effects of antidromic stimulation of the ventral root on glucose utilization in the ventral horn of the spinal cord in the rat. Proc. Natl. Acad. Sci. USA *84:* 5492–5495.

Kato, M., S. Hosokawa, S. Tobimatsu, and Y. Kuroiwa (1982) Increased local cerebral glucose utilization in the basal ganglia of the rolling mouse nagoya. J. Cereb. Blood Flow Metab. *2:* 385–393.

Keppel, G. (1973) *Design and Analysis: A Researchers Handbook,* Prentice-Hall, Englewood Cliffs, NJ.

Lorden, J. F., T. McKeon, B. Williams, H. Henry, N. Cox, and S. U. Walkley (1984) Characterization of the rat mutant dystonic (dt): A new animal model of dystonia musculorum deformans. J. Neurosci. *4:* 1925–1932.

Lorden, J. F., G. A. Oltmans, T. W. McKeon, J. Lutes, and M. Beales (1985) Decreased cerebellar 3',5'-cyclic guanosine monophosphate levels and sensitivity to harmaline in the genetically dystonic rat (dt). J. Neurosci. *5:* 2618–2625.

Lorden, J. F., G. A. Oltmans, S. Sutton, and L. E. Mays (1988) Neuropharmacological correlates of the motor syndrome of the genetically dystonic (dt) rat. Adv. Neurol. *50:* 277–297.

Lutes, J., J. F. Lorden, and G. A. Oltmans (1985) Effects of clonidine on the motor syndrome of the genetically dystonic rat. Soc. Neurosci. Abstr. *11:* 670.

Mata, M., D. J. Fink, H. Gainer, C. B. Smith, L. Davidsen, H. Savaki, W. J. Schwartz, and L. Sokoloff (1980) Activity-dependent energy metabolism in rat posterior pituitary primarily reflects sodium pump activity. J. Neurochem. *34:* 213.

McCann, E. L., H. W. Holloway, D. M. Larson, and S. I. Rappaport (1986) The developmental sequence of local cerebral glucose utilization (LCGU). Soc. Neurosci. Abstr. *12:* 1232.

McKeon, T. W., J. F. Lorden, G. A. Oltmans, M. Beales, and S. U. Walkley (1984) Decreased catalepsy response to haloperidol in the genetically dystonic (dt) rat. Brain Res. *308:* 89–96.

McKeon, T. W., J. F. Lorden, M. Beales, and G. A. Oltmans (1986) Alterations in the noradrenergic projection to the cerebellum of the dystonic (dt) rat. Brain Res. *366:* 89–97.

Meibach, R. C., S. D. Glick, D. A. Ross, R. D. Cox, and S. Maayani (1980) Intraperitoneal administration and other modifications of the 2-deoxy-D-glucose technique. Brain Res. *11:* 167–176.

Ohno, T. (1979) Animal models of ataxias (Rolling mouse Nagoya ataxia, falter). Adv. Neurol. Sci. *23:* 937–948.

Oltmans, G. A., M. Beales, J. F. Lorden, and J. H. Gordon (1984) Alterations in cerebellar glutamic acid decarboxylase (GAD) activity in a genetic model of torsion dystonia. Exp. Neurol. *85:* 216–222.

Oltmans, G., M. Beales, and J. F. Lorden (1986) Glutamic acid decarboxylase activity in micropunches of the deep cerebellar nuclei of the genetically dystonic (dt) rat. Brain Res. *385:* 148–151.

Rosner, B. (1986) *Fundamentals of Biostatistics,* 2nd ed., Duxbury Press, Boston.

Sokoloff, L. (1981) Localization of functional activity in the central nervous system by measurement of glucose utilization with radioactive deoxyglucose. J. Cereb. Blood Flow Metab. *1:* 7–36.

Sokoloff, L., M. Reivich, C. Kennedy, M. H. DesRosiers, C. S. Patlak, K. D. Pettigrew, O. Sakurada, and M. Shinohara (1977) The 14C deoxyglucose method for the measurement of local cerebral glucose utilization: Theory, procedure, and normal values in the conscious and anesthetized albino rat. J. Neurochem. *28:* 897–916.

Stoessl, A. J., W. R. W. Martin, C. Clark, M. J. Adam, W. Ammann, J. H. Beckman, M. Bergstrom, R. Harrop, J. G. Rogers, T. J. Ruth, C. I. Sayre, B. D. Pate, and D. B. Calne (1986) PET studies of cerebral glucose metabolism in idiopathic torticollis. Neurology *36:* 653–657.

Stratton, S. E., J. F. Lorden, L. E. Mays, and G. A. Oltmans (1988)

Spontaneous and harmaline-stimulated Purkinje cell activity in rats with a genetic movement disorder. J. Neurosci. *8:* 3327–3336.

ten Donkelaar, H. J. (1988) Evolution of the red nucleus and rubrospinal tract. Behav. Brain Res. *28:* 9–20.

Wolfson, L. I., N. S. Sharpless, L. J. Thal, J. M. Waltz, and L. Shapiro (1983) Decreased ventricular fluid norepinephrine metabolite in childhood-onset dystonia. Neurology *33:* 369–372.

Zeman, W. (1970) Pathology of the torsion dystonias (dystonia musculorum deformans). Neurology *20:* 79–88.

Zweig, R. M., J. C. Hedreen, W. R. Jankel, M. F. Casanova, P. J. Whitehouse, and D. L. Price (1988) Pathology in brainstem regions of individuals with primary dystonia. Neurology *38:* 702–706.