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Neuroanatomical Substrates for Paroxysmal Dyskinesia in Lethargic Mice

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Abstract

The paroxysmal dyskinesias are a group of neurological disorders described by intermittent attacks of involuntary abnormal movements superimposed on a relatively normal baseline. The neuroanatomical substrates for these attacks are not fully understood, though available evidence from studies of affected people and animal models points to dysfunction in the basal ganglia or cerebellum. In the current studies, the anatomical basis for paroxysmal dyskinesias in lethargic mice was determined via histochemical methods sensitive to changes in regional brain activity followed by surgical elimination of the suspected source. Cytochrome oxidase histochemistry revealed increased activity in the red nucleus. Surgical removal of the cerebellum worsened ataxia but eliminated paroxysmal dyskinesias. These studies support the hypothesis that abnormal cerebellar output contributes to paroxysmal dyskinesias.

Introduction

The paroxysmal dyskinesias are a group of clinical neurological disorders characterized by attacks of involuntary abnormal movements superimposed on a normal baseline (Fahn and Marsden, 1994, Demirkiran and Jankovic, 1995, Bhatia, 1999). There are several subtypes that are classified according to a number of factors including the duration of the attacks, the nature of the abnormal movements, and the influences that trigger attacks.

The neurobiological mechanisms underlying the paroxysmal dyskinesias are not well understood. Both genetic and non-genetic causes are known. Inherited causes among humans include mutations affecting the myofibrillogenesis regulator protein and ion channels (Giffin et al., 2002, Ohtsuka et al., 2003, Lee et al., 2004, Rainier et al., 2004, Du et al., 2005). At the neuroanatomical level, there is limited information on the source of the abnormal movements. The types of abnormal movements seen in the paroxysmal

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dyskinesias are commonly associated with dysfunction of the basal ganglia, implying that these motor pathways are involved (Fahn and Marsden, 1994, Demirkiran and Jankovic, 1995, Bhatia, 1999). In keeping with this suggestion, lesions of the basal ganglia are found in some people with paroxysmal dyskinesias (Blakeley and Jankovic, 2002). However, such lesions are uncommon, and most affected individuals have none. In individuals without apparent brain lesions, functional imaging has been used to identify areas of abnormal metabolic activity. One study found abnormal function of the basal ganglia (Lombroso and Fischman, 1999), while another found abnormal function of the cerebellum (Kluge et al., 1998). These studies implicate specific brain regions, but they do not provide a definitive answer, because the primary source of the movements cannot be distinguished from secondary compensatory changes. The neuroanatomical foundations for paroxysmal dyskinesias in humans therefore remain uncertain.

Animal models can provide valuable tools for more rigorous studies of the responsible brain regions. An inbred line of hamsters with paroxysmal dyskinesias has no apparent neuropathological defects, but biochemical and physiological abnormalities are found in many regions (Loscher et al., 1989, Wahnschaffe et al., 1990, Loscher and Horstermann, 1992, Loscher et al., 1994, Pratt et al., 1995, Gernert et al., 1997, Nobrega et al., 1997, Gernert et al., 1998, Nobrega et al., 1999, Rehders et al., 2000, Bennay et al., 2001, Gernert et al., 2002). Abnormal metabolic activity detected by functional histochemical stains such as cytochrome oxidase (CO) histochemistry or [^{14}C] 2-deoxyglucose (2DG) uptake is found in several areas, including the basal ganglia and cerebellum (Nobrega et al., 1998, Richter et al., 1998). These widespread abnormalities make it difficult to determine the precise anatomical pathways for paroxysmal dyskinesia in these hamsters.

More recently, paroxysmal dyskinesias have been described in association with mutations affecting calcium channels in mouse models (Barclay et al., 2001, Fureman et al., 2002, Khan and Jinnah, 2002, Du et al., 2005). Tottering mice exhibit paroxysmal dyskinesias triggered by stress, caffeine, or alcohol (Fureman et al., 2002). Neuropathological studies of Nissl-stained material reveal no abnormalities, but maps of regional activity derived from c-*fos* in situ hybridization demonstrate selective activation of the cerebellum and connected structures such as the red nucleus, thalamus, and cortex (Campbell and Hess, 1998). 2DG autoradiography also reveals the most prominent abnormalities to be in the red nucleus and thalamus (Noebels and Sidman, 1979). The cerebellum is more directly implicated as the source of paroxysmal dyskinesias in tottering mice by observations that eliminating cerebellar output after cross breeding the animals with *pcd* mutants to cause selective degeneration of cerebellar Purkinje cells results in mice with ataxia, but without paroxysmal dyskinesias (Campbell et al., 1999).

Lethargic mice also exhibit paroxysmal dyskinesias (Khan and Jinnah, 2002). Unlike tottering mice, dyskinesias are triggered by procedures that promote motor activity, such as placing them in a large open cage or providing motor stimulants. The goal of the current studies was to delineate the neuroanatomical basis for paroxysmal dyskinesias in lethargic mutant mice, via histochemical methods sensitive to changes in regional brain activity and surgical elimination of the suspected source.

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Methods

Animals.

Because homozygous male lethargics do not reproduce, homozygous females (*cachb4*$^{lh/lh}$) and normal F1 hybrid (C3H x C57BL/6J) males obtained from The Jackson Laboratories (Bar Harbor, ME) were bred to generate heterozygotes. The heterozygotes were then crossed to generate homozygous lethargics. Their normal and heterozygous littermates served as controls. Mice were weaned at 4 weeks of age and housed in groups of 2-8 with a 14:10 hour light:dark cycle and free access to food and water. Animals were 8-14 weeks of age at the time of testing. All animal procedures were conducted in accordance with guidelines established by the Johns Hopkins University Animal Care and Use Committee and National Institutes of Health.

Experimental design.

We used a two-step approach to delineate the neuroanatomical substrates for paroxysmal dyskinesias. The first step involved mapping regions of abnormal brain activity with two well-established and complementary functional histochemical methods. Cytochrome oxidase (CO) is a mitochondrial enzyme that is regulated in part by metabolic demands. Multiple studies have shown that its activity is increased or decreased in parallel with changes in regional brain activity (Wong-Riley, 1989). Alterations in CO activity generally require chronic changes in regional activity. Acute or transient changes often have little influence. In comparison, the immediate early genes are less sensitive to chronic changes but instead respond to acute sustained increases in neural activity associated with calcium influx (Sharp et al., 1993). These two functional histochemical methods are sensitive to different metabolic influences, so they often provide overlapping but complementary results.

Studies using these methods often compare more than 40 brain regions, but such broad surveys increase the chances of finding spurious differences unless stringent statistical corrections are applied. Because the goal of the study was to address the substrates for the motor disorder, we focused on 10 regions relevant to motor control rather than a broad survey of multiple regions not relevant to the question under consideration. Though multiple sites within each region were sampled, the results for each region were combined because of the lack of any significant subregional differences between experimental and control groups.

Both histological methods are useful for correlating changes in regional brain activity with a specific neurological disorder, but their correlational nature precludes using them for establishing a definitive causal link between identified brain regions and the disorder. To establish a causal link, the second step of our experimental design involved an attempt to eliminate the paroxysmal dyskinesias by surgical removal of the suspected source. The histochemical studies of lethargic mice revealed evidence for abnormal activity of the red nucleus, and prior studies of paroxysmal dyskinesias in tottering mice implicated abnormal cerebellar activity (Campbell and Hess, 1998, Campbell et al., 1999). Together these findings suggest the hypothesis that abnormal cerebellar output might be responsible for paroxysmal dyskinesias in lethargic mice. To assess the role of the cerebellum, paroxysmal dyskinesias were evaluated in lethargic mice before and after cerebellectomy.

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c-fos in situ hybridization.

The method for in-situ hybridization followed previous descriptions (Campbell and Hess, 1998). Briefly, brains were collected after decapitation, rapidly frozen in isopentane cooled on dry ice, and stored at −70°C. Twenty μm coronal sections were cut at 200 μm intervals using a cryostat through the entire brain, thaw-mounted onto Fisher SuperFrost Plus microscope slides (Fisher, Newark, DE), and stored at −70°C. In situ hybridization for c-*fos* mRNA was conducted with [^{35}S]-labeled riboprobes. Processed sections were apposed to X-ray film for 2-4 days, and labeled regions were identified and quantified from the films with an MCID image analysis system (Imaging Research, St. Catherines, Ontario). Digital images were obtained from the films at a 640x480 pixel resolution and optical density was determined for predefined regions.

Cytochrome oxidase histochemistry.

The CO histochemistry was modified from previous methods (Strazielle et al., 1998). Brains were rapidly removed, frozen in isopentane cooled on dry ice, and stored at −70°C. Brains were sectioned as described above. Prior to staining, sections were fixed with 0.5% glutaraldehyde and 10% sucrose in 0.1 M phosphate buffer at pH 7.6 for 5 minutes. They were then washed 4 times for 5 minutes with 10% sucrose in 0.1 M phosphate buffer. Sections were incubated for one hour in the dark with constant stirring at 37°C in a solution consisting of 0.05% diaminobenzadine, 0.02% reduced cytochrome c from horse heart, 4% sucrose, and 0.02% catalase in 0.1M phosphate buffer, pH 7.4. They were then washed for 5 minutes with 4% sucrose in 0.1 M phosphate buffer, followed by immersion in 10% buffered formalin with 10% sucrose for 30 minutes. The 4% sucrose wash was repeated 3x5 minutes, sections were dehydrated in graded ethanol and xylenes, and slides were coverslipped with Permount.

The CO histochemical reaction was quantified with sections of whole homogenized brains at varying thicknesses to construct a standard curve as previously described (Gonzalez-Lima and Jones, 1994). To make these standards, whole brains were homogenized using a tissue homogenizer with a Teflon-glass tube and pestle in Tris buffer to a final concentration of 50% w/v. The homogenate solution was aliquotted into 2ml centrifuge tubes. Tubes were centrifuged for 5 minutes at 5000g and the supernatant removed. The remaining tissue was frozen in isopentane on dry ice. Discs of the frozen homogenate were cut on a cryostat at thicknesses of 10, 20, 30, 40, and 60 μm. Discs of increasing thicknesses were mounted on a slide, and stained in parallel with brain sections.

The specific activity of the homogenate was related to the stain intensity in the tissue discs by spectrophotometric assay of the original homogenate (Hess and Pope, 1953). Briefly, one centrifuge tube containing 50% w/v brain homogenate was diluted to 0.2%. 180 μl of the 0.2% homogenate was incubated at 30°C for 5 minutes in a warm microplate. Then 20 μl of 0.1% reduced cytochrome c was added to the homogenate to begin the assay. Absorbance at 550 nm was read on a SpectraMax Plus spectrophotometer (Molecular Devices, Sunnyvale) every minute for 30 minutes at 30°C. CO activity was calculated using an extinction coefficient of 21.1 mM^{-1} cm^{-1}. The specific activity of the homogenate was

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then related to the staining intensity of sections of the tissue discs to construct a standard curve (Gonzalez-Lima and Jones, 1994).

The correlation between stain intensity and tissue disc thickness was r>0.95. Quantification of staining in different brain regions was performed by comparing staining intensity to the standard curve. The intensity of staining was measured using the MCID image analysis system. Digital images of each stained section were obtained at a 640x480 pixel resolution and optical density was determined for predefined regions.

Cerebellectomy.

Removal of the cerebellum was performed as previously described for rats (LeDoux et al., 1993) in 6 lethargic mutants and 10 controls. Mice were anesthetized with 20 ml/kg of 2% 2,2,2-tribromoethanol in saline. The head was shaved and cleaned with Betadine. A 1 cm midllne vertical incision was made through the posterior scalp and a 5 mm^2 area of bone overlying the posterior cerebellum was removed. The cerebellum was removed by subpial suction and the resulting cavity filled with a collagen-based hemostat (Avitene, Davol, Cranston RI). The scalp was closed with sutures and animals placed on a heating pad at 30°C. They received 50 mg/kg chloramphenicol, 0.02 mg/kg buprenorphine, and 10% glucose in 0.45% saline twice daily for 3 days.

Intraoperatively 3 animals did not survive (1 mutant and 2 controls). Postoperatively, 3 additional animals (1 mutant and 2 controls) exhibited lateralized behavior with rolling around the longitudinal axis. This behavior is indicative of injury to nearby brainstem structures (LeDoux, personal communication) and prevented recovery of meaningful motor function, so these animals were not studied further. The remaining animals (4 mutants and 6 controls) resumed normal ingestive behaviors in 1-2 days. They lost ~10% of their body weight in the immediate postoperative period but recovered their preoperative weights by 2 weeks after surgery. Behavioral testing started 3 weeks after surgery. Lesions were confirmed following behavior testing in all of these animals.

Behavior analysis.

The analysis of motor behavior was conducted using several standard tests for motor function as well as methods developed specifically for paroxysmal dyskinesias (Khan and Jinnah, 2002). Gross motor activity was quantified using photocell activity chambers (San Diego Instruments, San Diego CA). Mice were placed in 20 x 40 cm Plexiglas cages with 4 infrared beams across the width and 8 beams across the length 1 cm off the bottom of the cage. The numbers of beam breaks were measured in 10 min bins for 1 hr during the light cycle with no habituation period.

Strength and agility were measured by the cling test. A 20 x 20 cm square platform with 6 mm grid wire mesh and 5 cm frame was suspended 50 cm above a padded surface. Mice were placed on the platform and allowed to habituate for 1 min. The platform was rotated 90° and held vertical for 1 min, then rotated again 90° and held inverted for 1min. Each mouse was timed until falling on 3 different trials.

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A treadmill (Columbus Instruments, Columbus OH) was used to assess walking duration (Khan and Jinnah, 2002). Mice were habituated for 2 hrs to the apparatus when it was off and then trained on 5 separate occasions for 5 minutes each to walk at a moderate pace of 9.0 m/min. If the mice could not maintain speed or stopped walking, the moving treadmill carried them to a small vestibule where they experienced a 0.1 mA electric shock for up to 2 seconds. During this period, they could escape the vestibule and resume walking on the treadmill. The test involved measuring walking duration. Any animal that remained in the vestibule for >2 seconds was assumed to be unable to continue walking, and was removed from the experiment. The average walking duration for a maximum of 5 minutes was determined for each mouse after 3 trials separated by at least an hour.

Paroxysmal dyskinesias.

Paroxysmal dyskinesias in lethargic mice are triggered by several influences, especially those that stimulate locomotion (Khan and Jinnah, 2002). A mild and non-invasive trigger involves merely moving the animals into a large cage, where natural tendencies for exploration increase locomotor activity and thereby cause paroxysmal dyskinesias. Stronger triggers include psychomotor stimulants such as caffeine or amphetamine, or forced locomotion on a treadmill.

For most of the behavioral studies, caffeine (15 mg/kg, subcutaneous) or forced ambulation on a treadmill (maximum duration 5 minutes) were used to trigger paroxysmal dyskinesias. With caffeine paroxysmal dyskinesias emerge within 5 minutes and last for at ~60 minutes, ensuring a lengthy opportunity for observing paroxysmal dyskinesias. With the treadmill paroxysmal dyskinesias also emerge within 5 minutes, and prevent further ambulation, offering a clear and objective endpoint. For the histological studies, the non-invasive procedure of moving animals to a larger cage was preferred, because all pharmacological stimulants have complex effects on regional brain metabolism. For these studies, pairs of lethargic and control mice were placed into empty 2000 ml beakers. These beakers were large enough for the animals to move about freely, but small enough to retard locomotion. After 3 hours of habituation to the beakers, one mouse from each pair was transferred to a large (24 x 42 cm) clear Plexiglas cage. The increased locomotion stimulated by the larger cage size triggered paroxysmal dyskinesias within 5 minutes, and brains were collected for histology 60 minutes later.

There are currently no automated behavioral tasks capable of discriminating and quantifying the different movement disorders relevant for our studies (paroxysmal dyskinesias and ataxia). To provide a semi-quantitative measure of these motor syndromes, an observation-based scoring method analogous to the scales used for human movement disorders was developed (Jinnah and Hess, 2005). The method is similar to that described in prior studies of mutant mice with abnormal motor syndromes (Khan and Jinnah, 2002, Khan et al., 2004) or rats with drug-induced dyskinesias (Winkler et al., 2002). In brief, animals were removed from their home cages and placed singly in clear Plexiglas boxes. They were observed by an investigator blinded to experimental condition for exactly 60 seconds at 10 minute intervals for an hour. At each interval multiple abnormal behaviors were recorded. The behaviors were recorded according to body part and included tremor (face, neck, trunk, limbs); tonic

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flexion or extension (face, neck, trunk, limbs); twisting (neck, trunk); clonus (face, limbs); head bobbing (vertical displacement); and head wagging (horizontal displacement). We also recorded several other problems not readily ascribed to an isolated body part including falling (with all 4 limbs off the ground), listing (tilting to one side with 2 limbs off the ground), stumbling, widened stance, circling (360° turn with forward ambulation), spinning (360° turn without forward ambulation), and reverse locomotion.

A total dyskinesia score was determined by calculating the simple arithmetic sum of all abnormal behaviors. The maximum total score was 30. Since total scores obscure important differences in the types of abnormal behaviors, several subscores for clinically defined motor disorders were also calculated by combining similar behaviors across body parts. Because paroxysmal dyskinesias have a predominantly dystonic quality while cerebellectomy leads ataxic movements, we focused on subscores for dystonia versus ataxia. The dystonia score reflected the arithmetic sum of all twisting movements and sustained tonic postures, as these are most characteristic of dystonic motor behavior. An ataxia score was derived by summing stumbling, listing, falling, and splayed stance. Other behaviors were included in the total dyskinesia score but not separately analyzed because they were rare or absent (tremor, circling, spinning, and bobbing or wagging the head).

Results

Metabolic mapping.

Lethargic mice exhibited a slowed and hesitant gait with slight unsteadiness reflective of mild ataxia at baseline as previously described (Khan and Jinnah, 2002). Transferring lethargic mutants from a small to a large beaker induced the appearance of paroxysmal dyskinesias that were clearly distinguishable from baseline motor behavior. The dyskinesias were dominated by dystonic movements but included also clonic movements, and rare listing or circling in one direction or another. The emergence of paroxysmal dyskinesias was reflected by a significant increase in the total dyskinesia score in the animals transferred to large beakers (Table 1).

Surveys for *c-fos* labeling revealed no differences in the pattern or intensity of *c-fos* expression between normal (n=6) and lethargic mice (n=6) for any brain region, whether or not dyskinesias occurred (not shown).

CO histochemistry revealed a diffuse semi-granular appearance throughout the brain with variations in regional intensity that permitted discrimination of many brain regions without counterstaining as previously described (Strazielle et al., 1998). The results were analyzed by ANOVA with main independent factors of brain region, genotype, and occurrence of paroxysmal dyskinesias. There was a significant main effect for region ($F_{9,119}$=8.1, $p<0.001$), indicating regional differences in CO activity. There were no significant main effects for genotype ($F_{1,119}$= 2.2, p=0.14) or paroxysmal dyskinesias ($F_{1,119}$= 1.3, p=0.26). Animals of each genotype were therefore combined for comparisons across different regions (Table 2). Post-hoc Tukey t-tests revealed significant differences between normal and lethargic mice (+65%) only in the red nucleus ($p<0.02$).

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Consequences of cerebellectomy.

The functional histochemistry suggested abnormal activity of the red nucleus. However, prior studies of tottering mice have suggested paroxysmal dyskinesia results from abnormal cerebellar activity, which is a major source of projections to the red nucleus. Together these results suggest that abnormal cerebellar output may be a source for paroxysmal dyskinesias in lethargic mice. The consequences of cerebellectomy on paroxysmal dyskinesias of lethargic mice were therefore evaluated.

Lethargic mice subjected to cerebellectomy recovered remarkably well. Their mild baseline ataxia worsened, while paroxysmal dyskinesias disappeared completely. The elimination of paroxysmal dyskinesias was most evident on treadmill testing. Unoperated lethargics could walk on the treadmill for an average of only 134±27 seconds, because of the emergence of paroxysmal dyskinesias that prevent further walking. After removal of the cerebellum all lethargic mice could walk for the entire 5-minute duration of the test period without emergence of paroxysmal dyskinesias. Though they could walk on the treadmill for the entire duration, their gait was not normal. In comparison to normal animals that maintained a relatively steady speed and a forward position on the treadmill, lethargic mice exhibited ataxia with an irregular rate, swaying or staggering sideways, and occasional stumbling or falling (see online video supplement).

To quantitatively describe the visibly apparent differences in the motor behavior of lethargic mice before and after cerebellectomy, scores for ataxic and dystonic motor behavior were evaluated. The animals were evaluated after administration of saline which does not affect baseline behavior, and after administration of caffeine to induce paroxysmal dyskinesias. As expected, cerebellectomy resulted in a significant increase in ataxia scores of lethargic mice, even in saline injected mice (Figure 1). Cerebellectomy did not cause dystonia, but unexpectedly increased dystonia scores, even in saline-injected mice. Careful inspection of the raw data revealed the increase in dystonia scores to be a compensatory reaction to worsened ataxia. Specifically, operated animals developed a compensatory strategy to stabilize the ataxic trunk by gaining support from tonic extension of a limb. Because these tonic extensions contribute to the dystonia score, the dystonia scores increased.

Despite the lack of 100% specificity, the elimination of paroxysmal dyskinesias by cerebellectomy was still evident. In unoperated animals, caffeine triggered paroxysmal dyskinesias, as reflected by a significant increase in the dystonia score. In operated animals this increase was absent, because of the absence of paroxysmal dyskinesias. Caffeine increased ataxia scores in operated lethargics, because the overall stimulation of motor activity increased abnormal as well as normal movements (Figure 1).

Normal mice subject to cerebellectomy also recovered remarkably well, except for obvious ataxia. The ataxia was evident as a significant increase in the ataxia score following cerebellectomy (Figure 2). Like lethargic mice, despite the ataxia, the mice were able to walk on the treadmill for the full duration of the test period (not shown). Also like lethargic mice, dystonia scores increased following cerebellectomy. This increase occurred because of the same behavioral compensations seen in lethargic mice, and was similar in magnitude.

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It is feasible that the apparent elimination of attacks in lethargic mice could reflect an artifact if severe ataxia can mask detection of underlying dystonia. To address this possibility, a further experiment was conducted to verify that the increase in ataxic movements caused by cerebellectomy would not obscure the detection of dystonic movements. Normal mice were challenged before and after cerebellectomy with 4 mg/kg of the L-type calcium channel agonist ±BayK 8644, which is thought to trigger dystonia via corticostriatal circuits (Jinnah et al., 2000, Kasim et al., 2006). The ±BayK 8644 increased dystonia scores to a similar extent in operated and unoperated mice, without increasing ataxia scores. These results show that ataxic movements resulting from cerebellectomy do not preclude detection of superimposed dystonic movements, and the ataxia and dystonia subscores reflect the clinically observable motor syndrome. Lethargic mice were not tested in a similar manner because their genetic defect affecting calcium channels predisposes them to severe toxicity from ±BayK 8644.

To obtain additional information on the functional consequences of cerebellectomy, all mice were subject to additional motor tests. Cerebellectomy did not impair performance of normal or lethargic mice on the cling test (Figure 3). Cerebellectomy reduced spontaneous locomotor activity in normal mice, presumably because the ataxia led to a more labored and slightly slower gait. Cerebellectomy did not reduce the spontaneous locomotor activity in lethargics, which have a slow and ataxic gait at baseline (Figure 3).

Lesions in all mice were confirmed following behavior testing. In all cases, the majority of the cerebellum was absent (Figure 4). Residua of the cerebellar peduncles could be identified in most animals. The flocculonodular lobe was intact in many, because the bony cavity in which it normally resides protected it from removal during surgery.

Discussion

These studies serve to pinpoint the neuroanatomical source for paroxysmal dyskinesias in lethargic mutant mice. In situ hybridization for c-*fos* did not identify any abnormalities between normal and lethargic mice. The absence of any abnormalities suggests that this method is not sufficiently sensitive to detect the relevant brain regions, or that paroxysmal dyskinesias are insufficient triggers for c-*fos* gene transcription. CO histochemistry revealed abnormal metabolic activity of the red nucleus in lethargic mice. It is tempting to speculate that the red nucleus is involved in a pathway responsible for paroxysmal dyskinesias, but this method is correlative and cannot definitively establish a role for the red nucleus. The abnormal activity of the red nucleus may reflect a compensatory response, and other involved regions may fall below the sensitivity of the method for detection. The strongest evidence comes from the results of cerebellectomy, which worsened ataxia but eliminated paroxysmal dyskinesias. This result provides strong evidence that the cerebellum is the source of paroxysmal dyskinesias. These results are consistent with prior studies linking abnormal cerebellar function to the expression of paroxysmal dyskinesias in tottering mutant mice, where genetic ablation of cerebellar Purkinje cells led to disappearance of the attacks (Campbell et al., 1999).

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The role of the cerebellum in abnormal motor behavior.

Although the evidence suggests that paroxysmal dyskinesias arise from cerebellar dysfunction, the dystonic movements characteristic of paroxysmal dyskinesias are quite distinct from the ataxic movements typically associated with defects of the cerebellum. These different motor syndromes could arise from the same motor pathway because different defects alter functional output in different ways (Jinnah and Hess, 2006). Ataxia results from lesions or degeneration of the cerebellum, with loss of functional output. Other abnormal movements may result from “gain” of cerebellar function, due to aberrant functional output.

Tremor, for example, is thought to arise from dysfunction of circuits that involve the cerebellum (Wilms et al., 1999). Tremor may not result from loss of cerebellar function, but rather abnormal synchronizations that cause rhythmic oscillations in the motor system. Another motor disorder that may arise from cerebellar dysfunction is dystonia. Like tremor, emerging evidence suggests that dystonia results from distorted function rather than loss of function (Jinnah and Hess, 2006). The concept that multiple motor disorders may arise from a single brain region is not unique to the cerebellum. An analogous situation occurs in the motor cortex. Some motor cortex defects (such as stroke) lead to loss of function manifested as weakness or lack of movement. Other motor cortex defects (such as epilepsy) lead to aberrant function, characterized by tonic and clonic convulsions.

The exact manner in which cerebellar function is altered to cause dystonia versus ataxia is not yet known. However the concept of cerebellar origin of different motor syndromes is consistent with prior suggestions from studies of the dystonic rat and weaver mutant mice that abnormal cerebellar function may be more detrimental than loss of cerebellar function (LeDoux et al., 1993, Grusser and Grusser-Cornehls, 1998, Grusser-Cornehls et al., 1999). In the case of lethargic mice and the dystonic rat, the reason is that dystonic motor dysfunction is functionally more disabling than ataxia for a quadriped. For weaver mice, the ataxia that results from lesioning the cerebellum is less disruptive to the gait than the involuntary hindlimb movements that cause constant falling and tumbling.

Assessment of abnormal motor behavior.

The current studies also highlight a growing appreciation of methodological shortcomings in the study of motor disorders in animals. One shortcoming involves descriptive terminology. The term “dyskinesia” is used broadly in the clinical literature to refer to a wide variety of abnormal movements. The term is sometimes generally used to describe any abnormal motor behavior, and sometimes for more specific purposes. In Parkinson disease, for example, the term “dyskinesia” refers to a variety of different types of movements affecting any body part that result from dopamine replacement strategies (Winkler et al., 2002). On the other hand, the term “tardive dyskinesia” refers specifically to stereotypical orolingual movements following exposure to dopamine receptor antagonists (Gershanik, 1998). The term “paroxysmal dyskinesia” refers to a disorder described by transient attacks of extrapyramidal movements superimposed on a relatively normal baseline (Fahn and Marsden, 1994, Demirkiran and Jankovic, 1995, Bhatia, 1999).

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The use of the term “dyskinesia” is even more varied in the basic sciences. Many investigators use it broadly to describe any abnormal movement (Lees et al., 1979, Cadet, 1989), while others use it in a manner that parallels specific clinical disorders (Bergamo et al., 1997, Winkler et al., 2002). The varied uses of the term “dyskinesia” are often confusing to those unfamiliar with the overlapping nomenclature of some motor disorders. Here, we use the term “dyskinesia” to describe any abnormal movement, including ataxia, dystonia, and others. We reserve the term “paroxysmal dyskinesia” to describe the specific syndrome in which a genetic defect causes transient attacks of abnormal movement.

The second and more important shortcoming in assessment of motor disorders in animals is that the use of non-specific terminology such as “dyskinesia” obscures important differences in the quality of the abnormal movements, which have differing underlying pathophysiologies. In clinical movement disorders, separate rating instruments are used to assess different motor syndromes such as ataxia, dystonia, Parkinsonism, chorea, and others. Similar scales are available for animals but they are less widely used because they are viewed as less objective than standardized motor tasks such as activity meters, Rotarod, pole and beam tests, treadmill, and others (Jinnah and Hess, 2005). These standardized motor tasks provide more readily quantified measures of motor function. On the other hand, these tasks are incapable of describing the nature of the motor syndrome. None is capable of discriminating ataxia from dystonia or other abnormal motor syndromes (Jinnah and Hess, 2005). If animal models are to be useful tools for exploring human disorders, a good description of the motor syndrome being studied is essential.

In the current studies, we used a combination of a series of standardized tests to monitor functional consequences together with an observation-based rating system to describe the nature of the disorder. Lethargic mice with or without cerebellectomy were too impaired to perform Rotarod or beam tests. Performance was normal on the cling test. This result is not surprising because this test depends chiefly on strength, and lethargic mice do not have weakness, even after cerebellectomy. The activity meters demonstrated a difference between normal and lethargic mice, but provided no information regarding the cause of the difference. Treadmill duration provided a very simple and direct measure of functional disability, but again was incapable of characterizing the reason for disability.

The observational rating scale was useful for both measuring and describing the abnormal movements. The subclassification of raw observational data into clinically meaningful subgroups is used extensively for movement disorders of both humans and animals (Jinnah and Hess, 2005). It is generally acknowledged that these methods lack 100% sensitivity or specificity. Sensitivity is limited by the fact that some very rapid movements or those involving small obscured body parts such as the face may be overlooked. Specificity is limited by the fact that scores for a particular motor syndrome may include movements that are normal or due to an alternate motor syndrome. For example, normal reaching for an object that is just out of reach may result in a sustained tonic posture of the limb that would contribute to the dystonia score. Falling or stumbling could arise from either dystonic or ataxic motor behavior. The lack of complete specificity was evident in mice subject to cerebellectomy, because compensatory movements could not be distinguished

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from pathological ones. Despite the lack of complete specificity, the scoring method had value in discriminating dystonic versus ataxic movement.

Paroxysmal dyskinesias and epilepsy.

The paroxysmal dyskinesias are often misdiagnosed as epilepsy, both in animals and clinical practice. One reason for the frequent confusion is that both disorders are characterized by transient attacks of abnormal movements superimposed on a relatively normal baseline. Indeed, some authorities suggest that paroxysmal dyskinesias should be considered as a form of epilepsy (Guerrini, 2001, Vercueil and Hirsch, 2002). Other evidence suggests that paroxysmal dyskinesias and epilepsy are not the same (Khan and Jinnah, 2002). First, the motor attacks in paroxysmal dyskinesias typically last for minutes to days, while those of motor epilepsy typically last for less than a minute. Second, the movements of paroxysmal dyskinesias are dominated by dynamic and repetitive twisting or choreiform movements, while those of epilepsy are mostly tonic and/or clonic. Third, treatments for the two disorders differ, though with some overlapping agents. Fourth, EEG abnormalities are absent in the paroxysmal dyskinesias, yet essential for the diagnosis of epilepsy. Finally, there is growing evidence that the neuroanatomical substrates are different. The current studies and prior studies of tottering mice indicate that paroxysmal dyskinesias originate from the cerebellum. Epilepsy arises from cortical circuits involving the thalamus and hippocampus, not the cerebellum.

Though there are many differences between the paroxysmal dyskinesias and epilepsy, the distinctions are sometimes blurred by families where the two disorders coexist. Several human families have been described where paroxysmal dyskinesias are combined with epileptic seizures (Szepetowski et al., 1997, Guerri et al., 1999, Ohtsuka et al., 2003, Du et al., 2005). One explanation for this is that the two disorders reflect distinct pleitropic consequences of a single pathomechanism (Cannon, 2006). Some of the best evidence for this comes from animal models such as tottering and lethargic mice, which are models for calcium channelopathy. Defective calcium channels in cortical circuits underlie absence epilepsy, while defective channels in the cerebellum underlie ataxia and paroxysmal dyskinesias.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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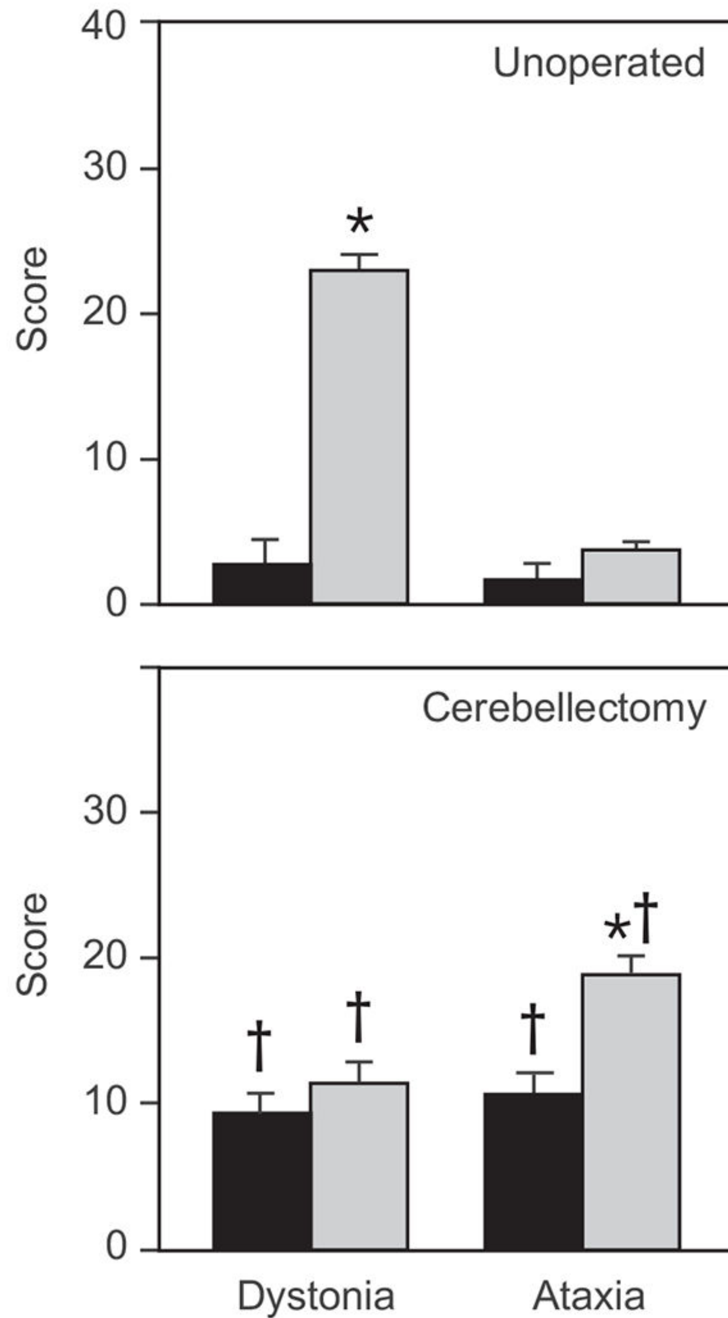

Figure 1.

Abnormal movement scores before and after cerebellectomy in lethargic mice. Results show average (±SEM) abnormal movement scores before (upper panel) and after (lower panel) cerebellectomy in 4 lethargic mice treated with saline (black bars) or caffeine (gray bars). Results for each type of abnormal movement were analyzed separately by 2-way ANOVA with cerebellectomy and drug treatment as the main factors. Asterisks show significant differences between saline and drug-treated animals, while daggers show

significant differences between unoperated and operated mice by post-hoc Tukey-tests ($p<0.05$).

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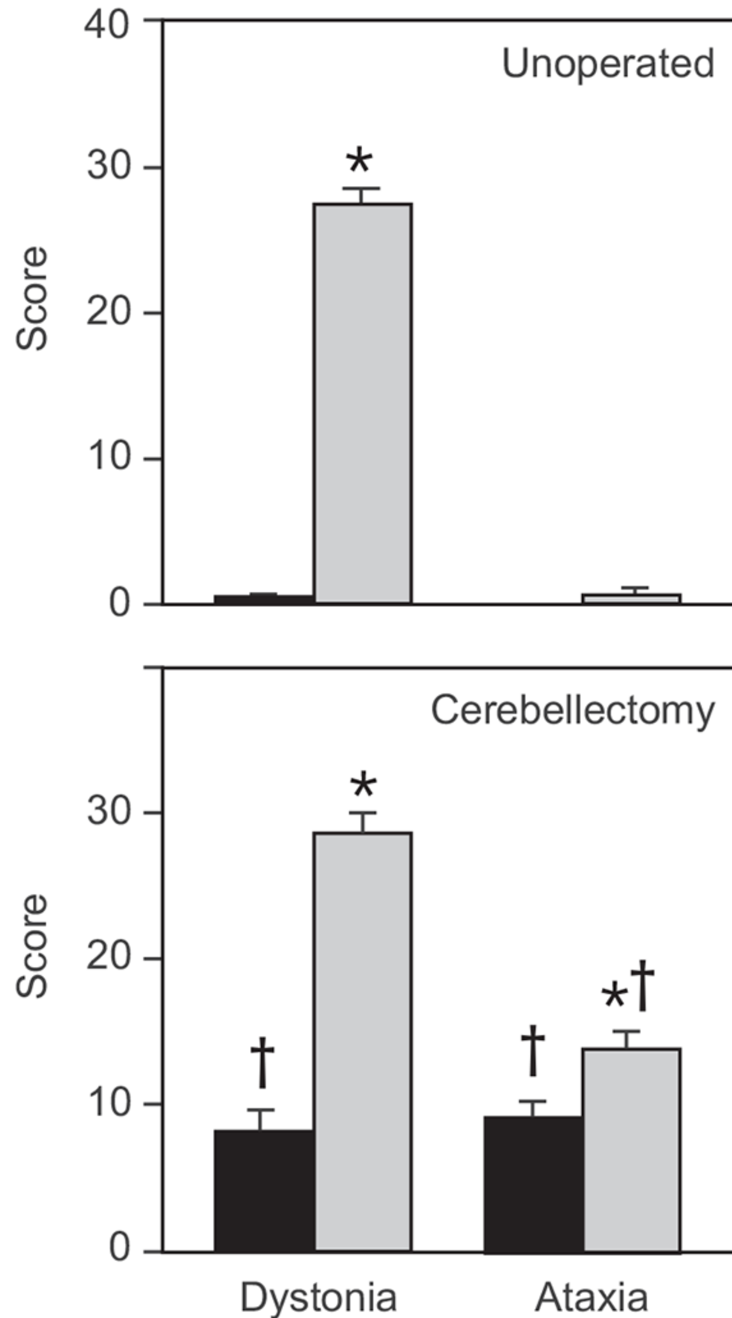

Figure 2.

Abnormal movement scores before and after cerebellectomy in normal mice. Results show average (±SEM) abnormal movement scores before (upper panel) and after (lower panel) cerebellectomy in 6 normal mice treated with saline (black bars) or ±BayK 8644 (gray bars). Results for each type of abnormal movement were analyzed separately by 2-way ANOVA with cerebellectomy and drug treatment as the main factors. Asterisks show significant differences between saline and drug-treated animals, while daggers show

significant differences between unoperated and operated mice by post-hoc Tukey-tests ($p<0.05$).

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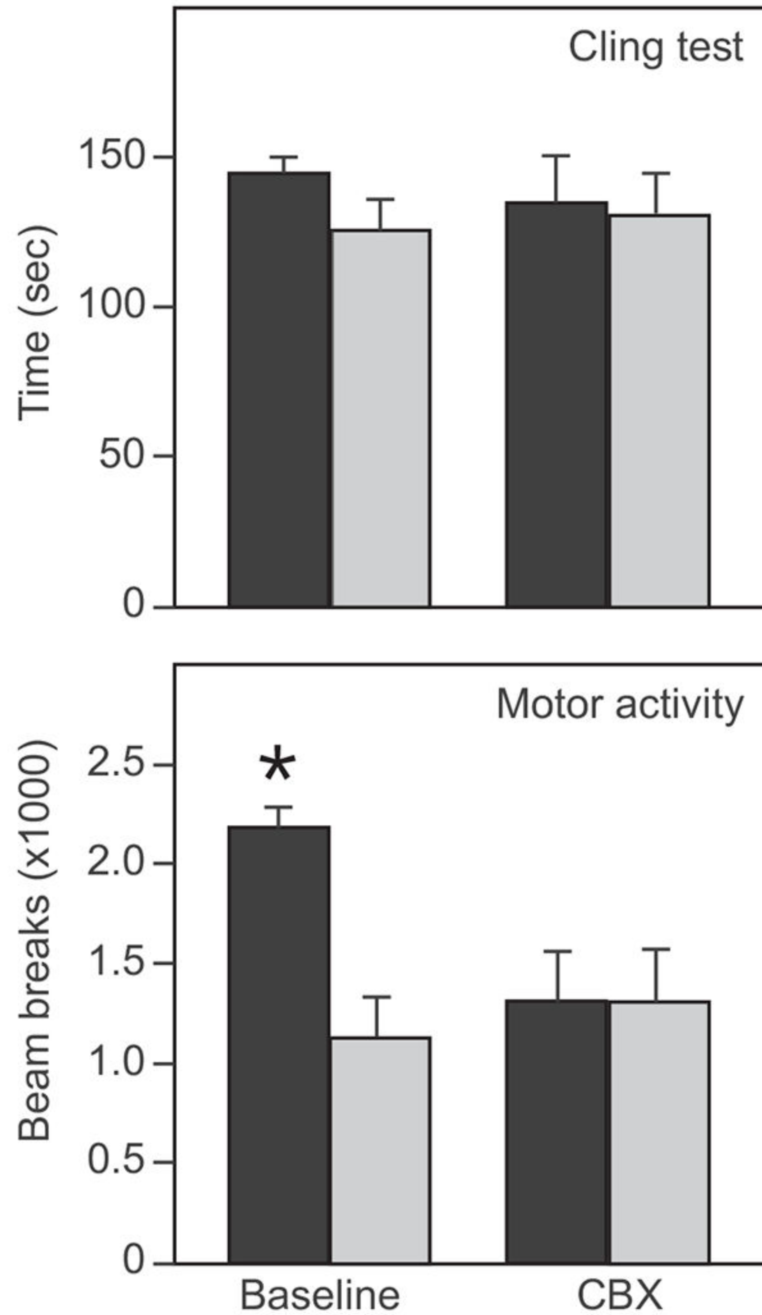

Figure 3.

Motor function tests. Results show average (±SEM) for the cling test (upper panel) and activity chambers (lower panel) for 6 normal (black bars) and 4 lethargic (open bars) mice. Results for each motor test were analyzed separately by 2-way ANOVA with genotype and cerebellectomy as the main factors. The asterisk shows a significant difference for one group compared with all other groups ($p<0.05$).

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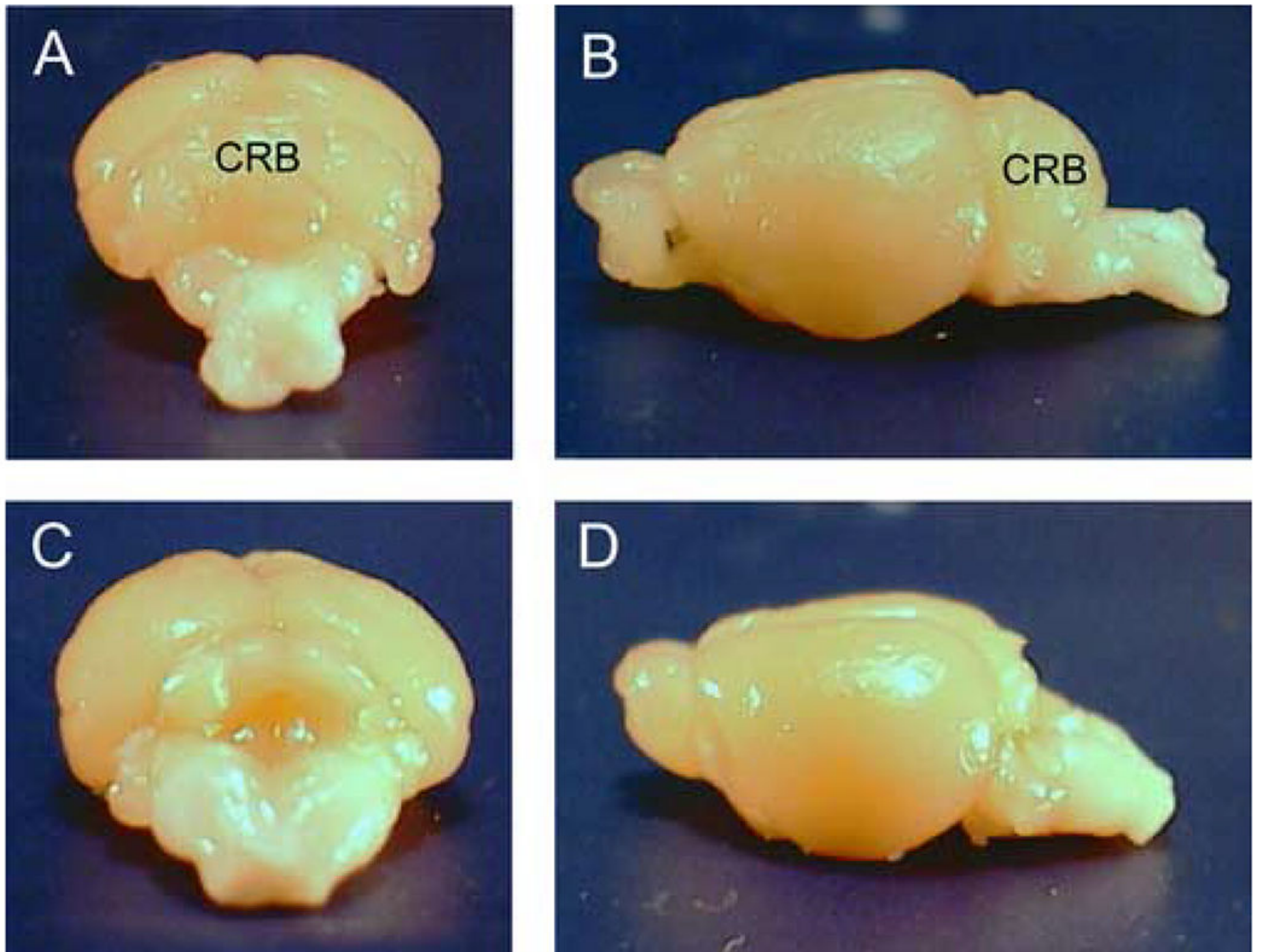

Figure 4.
Gross brain specimen after cerebellectomy. A normal mouse is shown in panels A (posterior) and B (lateral), while an operated mouse is shown in panels C (posterior) and D (lateral). The cerebellum has been completely removed, providing an unobstructed view of the tectum.

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Table 1.

Dyskinesias associated with changes in cage size

Condition	Normal	Lethargic
Small cage	0±0	0±0
Large cage	0±0	19.0±1.5

Results reflect average values (±SEM) for 4 mice per group.

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Table 2.

Cytochrome Oxidase Activity

Region	Normal	Lethargic
Cerebellum	46.0±7.5	47.3±4.5
Cortex	34.9±1.3	35.6±2.0
Dentate nucleus	53.0±8.5	60.0±5.5
Interpeduncular nucleus	32.7±5.7	44.7±6.8
Pontine nuclei	26.8±2.0	26.2±2.4
Red nucleus	22.2±4.2	36.6±5.9*
Striatum	34.9±1.7	39.6±1.5
Substantia nigra	35.9±2.8	33.7±2.8
Thalamus, ventral anterior	39.7±3.3	41.7±3.1
Thalamus, ventrolateral	48.2±3.1	41.4±3.5

Results show average (±SEM) specific activity of CO in μmol min^{-1} g^{-1} of tissue for 8 mice per group. The asterisk indicates $p<0.05$ by a Tukey t-test following ANOVA.