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A Functional And Neurophysiological Examination Of Cerebellar Output In The Mutant Rat Dystonic (Dt).

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A FUNCTIONAL AND NEUROPHYSIOLOGICAL EXAMINATION OF CEREBELLAR OUTPUT IN THE MUTANT RAT DYSTONIC (dt)

by

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A DISSERTATION

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ABSTRACT

The genetically dystonic (dt) rat, an autosomal recessive mutant, exhibits a progressive motor syndrome that resembles the generalized idiopathic dystonia seen in humans. Even with supportive measures, dt rats die before reaching maturity. Previous work has identified the cerebellum of the dt rat as a site of biochemical, metabolic, and functional abnormality. To test the hypothesis that a cerebellar defect is critical to the expression of the motor syndrome, groups of dt rats and phenotypically normal littermates underwent cerebellectomy (CBX) at either 15 or 20 days of age. CBX that included the dorsal portions of the lateral vestibular nuclei (dLV) eliminated the dystonic motor syndrome of the dt rats, greatly improved motor function, and prevented early death.

Because CBX included all deep cerebellar nuclei (DCN) and the dLV, the selective elimination of cerebellar nuclei was used to determine the cerebellar components critical to the mutant's motor syndrome. Bilateral electrolytic and/or excitatory amino acid lesions of the medial cerebellar nucleus (MCN), nucleus interpositus (INT), lateral cerebellar nucleus (LCN), and dLV were created in separate groups of 15-day-old dt rats. Rats were observed for the

presence of abnormal motor signs (falls, twists, clasps, pivots) and tested on several measures of motor performance (activity, climbing, righting, homing, hanging) before surgery and again on Postnatal Day 20. All nuclear lesions produced significant improvements in motor function and decreases in the frequency of abnormal motor signs. Electrolytic lesions of the dLV were associated with the greatest improvement.

To determine the cellular correlates of the dt rat's abnormal cerebellar output, single-unit recordings were obtained from the DCN in awake normal and dt rats. Cells from the MCN, INT, and LCN demonstrated an abnormal firing pattern characterized by bursting. Bursting increased with age. Deep cerebellar nuclear cells from normal and dt rats did not differ in average spike frequency. These findings in the dt rat suggest that abnormal cerebellar output may play a critical role in the pathophysiology of human dystonia.

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TABLE OF CONTENTS

TABLE OF CONTENTS (Continued)

LIST OF TABLES

LIST OF FIGURES

LIST OF FIGURES (Continued)

LIST OF ABBREVIATIONS

3-AP, 3-acetylpyridine

3V, 3rd ventricle

4V, fourth ventricle

6, abducens nucleus

7, facial nucleus

7N, facial nerve

8N, vestibulocochlear nerve

10, dorsal motor nucleus vagus

12, hypoglossal nucleus

A, scaling parameter, gamma distribution

AH, anterior hypothalamic area

AM, anterior median nucleus

Amg, amygdaloid nuclear complex

ANCOVA, analysis of covariance

AP, area postrema

Arc, arcuate hypothalamic nucleus

ATP, adenosine triphosphate

BI1, bursting index 1

BI2, bursting index 2

C, shape parameter, single gamma distribution

C1, first shape parameter, double gamma distribution

C2, second shape parameter, double gamma distribution

CBX, cerebellectomy

cc, corpus callosum

CG, central gray

cMAO, caudal medial accessory olive

CN, cochlear nucleus

CPu, caudate-putamen

cu, cuneate fasciculus

Cu, cuneate nucleus

DC, dorsal cochlear nucleus

DCN, deep cerebellar nuclei

DG, dentate gyrus

dLV, dorsal lateral vestibular nucleus

DM, dorsomedial hypothalamic nucleus

DR, dorsal raphe nucleus

DV, dependent variable

ECu, external cuneate nucleus

Ento, entopeduncular nucleus

Fl, flocculus

G, gelatinosus thalamic nucleus

g7, genu 7th nerve

GP, globus pallidus

Gr, gracile nucleus

Hb, habenular nucleus

IBI, interburst interval

IC, inferior colliculus

icp, inferior cerebellar peduncle

INT, interpositus cerebellar nucleus

IO, inferior olivary nuclear complex

ISI, interspike interval

LC, locus coeruleus

LCN, lateral cerebellar nucleus

LH, lateral hypothalamic area

ll, lateral lemniscus

LPB, lateral parabrachial nucleus

LV, lateral vestibular nucleus

M, mode, single gamma distribution

M1, first mode, double gamma distribution

M2, second mode, double gamma distribution

MANOVA, multivariate analysis of variance

MCLH, magnocellular nucleus lateral hypothalamus

MCN, medial cerebellar nucleus

mcp, middle cerebellar peduncle

me5, mesencephalic trigeminal nucleus

ml, medial lemniscus

mlf, medial longitudinal fasciculus

Mo5, motor trigeminal nucleus

MV, medial vestibular nucleus

MVv, medial vestibular nucleus, ventral

opt, optic tract

p, proportionality parameter, double gamma distribution

Pa, paraventricular hypothalamic nucleus

PFl, paraflocculus

Po, posterior thalamic nuclear group

PO, superior olivary/paraolivary nuclear complex

Pr5, principal sensory trigeminal nucleus

PrH, prepositus hypoglossal nucleus

py, pyramidal tract

R2, late blink component

Re, reuniens thalamic nucleus

Rh, rhomboid thalamic nucleus

RPa, raphe pallidus

Rt, reticular thalamic nucleus

RtTg, reticulotegmental nucleus

scp, superior cerebellar peduncle

SD, sample standard deviation

SEM, standard error of the mean

Sol, nucleus solitary tract

SpV, spinal vestibular nucleus

st5, spinal trigeminal tract

STh, subthalamic nucleus

SuV, superior vestibular nucleus

t, time in milliseconds

Tz, nucleus trapezoid body

VC, ventral cochlear nucleus

VL, ventrolateral thalamic nucleus

VM, ventromedial thalamic nucleus

VMH, ventromedial hypothalamic nucleus

VP, ventroposterior thalamic nucleus

VTg, ventral tegmental nucleus

ZI, zona incerta

INTRODUCTION

Dystonia has been defined by an ad hoc committee of the Dystonia Medical Research Foundation as a syndrome of sustained muscle contractions, frequently causing twisting and repetitive movements, or abnormal postures (Fahn, 1988). This descriptive definition reflects the poor understanding of the pathophysiology of dystonia that exists at present. Dystonia can be classified by etiology into primary (or idiopathic) and secondary (or symptomatic.) Primary dystonia may be inherited or sporadic in occurrence. Secondary dystonia may be caused by a single brain lesion such as a putaminal infarct (Obeso & Giménez-Roldán, 1988), a metabolic derangement like that of Wilson's disease, the enzymatic defect seen in Lesch-Nyhan syndrome (Calne & Lang, 1988), or neural degeneration as in Joseph's disease (Rosenberg et al., 1978).

Human Dystonia

Human idiopathic dystonia may be generalized or focal. Family members of patients with primary generalized dystonia may have generalized, segmental, or focal dystonia suggesting that the primary dystonias have a common pathophysiology (Zeman et al., 1960; Marsden, 1976). The most common focal primary dystonia is torticollis. Generalized idiopathic dystonia, commonly called dystonia

musculorum deformans, typically begins in childhood and, in advanced stages, is characterized by fixed postures with marked distortion of the body and a profound impairment of motor function. Most patients with dystonia have a negative family history for the disorder. In the vast majority of inherited idiopathic dystonias, the mode of inheritance is autosomal dominant with incomplete penetrance and variable expressivity (Bressman et al., 1988). However, an autosomal recessive pattern may be present in several Spanish Gypsy families with idiopathic torsion dystonia (Giménez-Roldán et al., 1988) and an X-linked recessive form of dystonia is seen in Filipinos on the Island of Panay (Lee et al., 1976). In addition, a clinically distinct form of idiopathic dystonia, dopa-responsive dystonia, is a childhood-onset dystonia inherited in an autosomal dominant pattern and characterized by a dramatic therapeutic response to L-dopa (Nygaard et al., 1988). Therefore, based on this genetic and clinical data, it seems that different biochemical defects can produce very similar motor syndromes. However, these biochemical defects remain undetermined.

Because pathology in the majority of patients with secondary dystonia has been localized to the basal ganglia, it has been assumed that abnormalities in the basal ganglia are also responsible for primary torsion dystonia. However, magnetic resonance imaging (Rutledge et al., 1988), postmortem pathologic examination (Zeman et al., 1960; Zeman, 1970; Zweig et al., 1987) and positron emission

tomography (Chase et al., 1988; Gilman et al., 1988; Lang et al., 1988; Leenders et al., 1988; Martin et al., 1988; Perlmutter and Raichle, 1988; Eidelberg et al., 1992; Karbe et al., 1992; Magyar-Lehman et al., 1994; Schelosky et al., 1994) have not shown consistent basal ganglia abnormalities in idiopathic dystonia.

In contrast to studies that have focused on the basal ganglia, recent postmortem examinations have implicated other neural structures as potential sites of abnormality in dystonia. Examination of brains from four persons with idiopathic dystonia revealed histopathologic abnormalities in two cases. A 29-year-old man with generalized dystonia had abundant neurofibrillary tangles and mild neuronal loss within the locus coeruleus and sparse neurofibrillary tangles within the substantia nigra pars compacta, pedunculopontine nucleus, and dorsal raphe nucleus. A 68-year-old man with focal cranial dystonia had substantial cell loss in the locus coeruleus, raphe nuclei, pedunculopontine nuclei and substantia nigra pars compacta.

Neurochemical studies on brains from three patients with idiopathic dystonia have demonstrated abnormalities in serotonin and norepinephrine levels (Hornykiewicz et al., 1986; Jankovic et al., 1987). More specifically, Hornykiewicz et al. (1986) examined the brains of two patients with childhood-onset generalized dystonia. Norepinephrine concentrations were decreased in the lateral and posterior hypothalamus, mammillary body, subthalamic

nucleus and locus coeruleus and increased in the septum, thalamus, colliculi, red nucleus, and dorsal raphe nucleus. Serotonin levels were subnormal in the dorsal raphe nucleus and were elevated in the globus pallidus, subthalamic nucleus, and locus coeruleus.

The findings of Hornykiewicz et al. (1986) may be related, in part, to the multiple medications that their two patients were taking prior to death or to the abnormal sleep patterns typical of patients with idiopathic dystonia (Jankel et al., 1984). Also, the irregularities in norepinephrine and serotonin levels may merely be epiphenomena of another, more specific neural biochemical defect. The very limited availability of postmortem brains and the inability of imaging studies to demonstrate consistent abnormalities in idiopathic dystonia makes the study of animal models a potentially critical means of elucidating the pathophysiological mechanisms operative in dystonia.

Dystonic (dt) Rat

The dystonic (dt) rat is an autosomal recessive mutant discovered in the Sprague-Dawley strain (Lorden et al., 1984). The movement disorder of the dt rat closely resembles the idiopathic, generalized dystonia seen in humans and may provide a vehicle for understanding the biochemical and physiological defects in human idiopathic dystonia.

The dt rat exhibits a severe, generalized, progressive, dystonic motor syndrome after a period of normal development. Beginning at approximately Postnatal Day 12, locomotion is seriously impaired by twisting movements and postures that involve the muscles of the neck, trunk, and limbs (Lorden et al., 1984). The motor syndrome is characterized by twisting of the axial musculature, clasping of the paws, and frequent falls. The movements are reduced or absent when the animals are at rest. The gross morphology of the brain is normal, and there is no evidence of lesions or degeneration in the central or peripheral nervous systems in Golgi or Nissl-stained material (Lorden et al., 1984). Because anatomical examination of the brains from dt rats was unrevealing, biochemical, pharmacological, and neurophysiological studies were undertaken.

Twisting movements and postures similar to those of the dt rat are generally attributed to dysfunction of the basal ganglia (Calne & Lang, 1988). Neurochemical studies on the GABAergic, cholinergic, and dopaminergic systems of the basal ganglia of the dt rat have been uninformative (McKeon et al., 1984; Lorden et al., 1988; Beales et al., 1990). Both biochemical and physiological evidence, however, point to the olivo-cerebellar system as the site of a functional defect.

Behavioral Analysis

Behavior was analyzed before and after the systemic administration of pharmacologic agents from specific

functional groups known to be useful in dystonia and other movement disorders (Lorden et al., 1988). The benzodiazepine diazepam produced a dose-dependent decrease in dystonic movements. The cholinergic blocker scopolamine improved postural stability in the dystonic rats. The alpha-2 adrenergic agonist clonidine decreased dystonic movements and falls, even at low doses. Thus, the dt rat responds positively, but to a variable degree, to agents which have been shown to benefit patients with idiopathic dystonia. However, the pattern of response to these different agents does not clearly suggest a specific neural abnormality.

In addition to studies examining the effects of drugs that might have therapeutic benefit, other studies examined responses to drugs with specific motor effects. For example, in normal rats, harmaline causes a generalized tremor by inducing rhythmic activity in inferior olivary (IO) neurons which in turn evoke complex spike discharges in Purkinje cells in cerebellar cortex (Stratton et al., 1988). Dystonic rats do not exhibit a tremor in response to harmaline, although other tremorogenic agents with different mechanisms of action are effective in the mutants (Lorden et al., 1985).

Electrophysiology of dt Rat Cerebellum

Single-unit recording in dt rats demonstrates that IO neurons show a normal increase in the rate and rhythmicity of firing in response to harmaline, although Purkinje cells

do not (Stratton et al., 1988; Stratton & Lorden, 1991). Because harmaline is thought to influence the firing of Purkinje cells indirectly through action on IO neurons, this suggests a defect in the transmission of information from the IO to Purkinje cells in the dt rat, although it is not possible to specify whether the defect is pre- or postsynaptic.

Purkinje cells are the sole output neurons of cerebellar cortex. In normal rats given harmaline, cells in the MCN show a bursting pattern of response in which the average frequency of the bursts matches the average frequency of complex spikes in the Purkinje cells (Lorden et al., 1992). Extracellular single-unit recording from the DCN reveals increased spontaneous firing rates in dystonic rats in comparison with normal rats (Lorden et al., 1992). In addition, DCN cells in dystonic rats fire more rhythmically than DCN cells from normal rats and the firing pattern is not altered by harmaline administration. These results indicate a significant impairment in Purkinje cell regulatory control over the DCN.

If activation of the IO by harmaline fails to induce an alteration in the firing of DCN cells in dt rats, it is unlikely that natural stimuli that should drive IO neurons would have any effect on the output of the cerebellum. The abnormally rapid and regular firing of cells in the MCN could, furthermore, alter timing-sensitive relationships in many other regions, including the IO. Thus, abnormal

cerebellar output in the dt rat may have important implications for the motor syndrome of the mutant.

Inferior Olive

Recent studies demonstrate dense GABAergic innervation of the IO in rats and other species (de Zeeuw et al., 1989; 1990a; 1990b; 1990c; Nelson et al., 1989). IO afferents arise from multiple areas including the spinal cord, brainstem, DCN and frontal cortex (Swenson & Castro, 1983). GABAergic input from the DCN to the IO is thought to be important in the regulation of IO activity and particularly in governing electrotonic coupling between IO cells (Sotelo et al., 1986; de Zeeuw et al., 1989; 1990a; 1990b).

In addition to the somata and dendrites of IO neurons, the axons also receive GABAergic input (de Zeeuw et al., 1990c). The IO neuron axon hillock is unusually long and forms spiny appendages which are located within glomeruli. In the cat IO, 65 percent of afferent terminals on axonal spines, the shaft of the axon hillock, and the transition between the hillock and initial segment in the cat IO were shown to be GABAergic (de Zeeuw et al., 1990c). These synapses may serve a different function than those on the soma and dendrites. GABAergic projections to cell bodies and dendrites of IO neurons may arise from a variety of cerebellar and noncerebellar sites. The projection to the axon is, however, thought to be exclusively cerebellar in origin (de Zeeuw et al., 1989; 1990c). Therefore, GABAergic IO afferents may regulate both the electronic coupling of

cells and the subsequent transmission of impulses. The abnormally high firing rate of MCN cells in the DCN of the dt rat may significantly alter the relationship between excitatory and inhibitory influences on IO neurons. This may contribute to the relative ineffectiveness of harmaline in dt rats, despite the fact that IO cells are activated by the drug.

Climbing Fiber Projections

A critical issue in understanding the pathophysiology of the dt rat olivo-cerebellar loop has been the mechanisms underlying the insensitivity of the dt rat to the tremorogenic effects of harmaline (Stratton, 1991; Stratton & Lorden, 1991). Harmaline induces a fine generalized tremor by activation of IO cells with subsequent transmission of this information from the IO to cerebellar cortex and from there to the DCN and then to premotor and motor nuclei. Because harmaline activates IO neurons but does not produce a tremor in the dt rat, a defect in the transmission of information from the IO to the cerebellum must be present in the dt rat (Stratton & Lorden, 1991). Based on this finding, the pathway from the IO to cerebellar cortex was studied anatomically and physiologically in order to localize the defect. Two questions were asked: (1) do IO cells in the dt rat display anatomically normal projection patterns to cerebellar cortex; and (2) does cerebellar cortex in the dt rat respond appropriately to direct electrical stimulation of the IO?

The IO projection to cerebellar cortex was studied with both antero- and retrogradely transported horseradish peroxidase. The connectivity pattern was consistent with studies in normal rats, and cerebellar cortical terminations were normal at the level of light microscopy (Stratton, 1991).

In the stimulation experiment, the caudal medial accessory olive (cMAO) was stimulated and single-unit activity was recorded from vermal Purkinje cells in both normal and dystonic rats. The rate of elicited complex spikes for 2 Hz, 4 Hz and 8 Hz stimulation was compared for normal and dt rats in a repeated measures analysis of variance. There was no significant difference between the dt and normal rats (Stratton, 1991). Purkinje cells followed stimulation rates from 2-8 Hz, and stimulation decreased the frequency of simple spike activity. The stimulation data supports the anatomical observations suggesting that the pathway is intact and has a normal topography. There are two possible causes for the failure of transmission of action potentials from IO neurons to Purkinje cells: (1) a defect specific to the initial segment and/or axon hillock region of IO neurons; or (2) increased IO afferent GABAergic activity in the region of the axon hillock and initial segment.

Deep Cerebellar Nuclei

The DCN are a site of major abnormalities in the dt rat. In comparison to normal rats, glucose utilization is

significantly elevated in the DCN of the dt rat suggesting increased activity at this site (Brown & Lorden, 1989). The DCN are normally inhibited by Purkinje cells. The inhibitory transmitter of Purkinje cells is GABA. The synthetic enzyme for GABA, glutamic acid decarboxylase (GAD), is located primarily in the terminals of Purkinje cells. Neurochemical indices of abnormality, including increases in GAD activity and decreases in ^{3}H-muscimol binding, are specific to the DCN and have not been identified elsewhere in motor or non-motor regions (Beales et al., 1990). GAD activity is increased in the DCN of dystonic rats in comparison with normal controls, suggesting increased inhibition of the DCN (Oltmans et al., 1984). A similar increase in DCN GAD activity can be obtained by destroying the IO with 3-acetylpyridine (3-AP)(Oltmans et al., 1985).

Because of the apparently contradictory findings of increased metabolic activity (Brown & Lorden, 1989) in the presence of increased GAD activity (Oltmans et al., 1985) in the DCN, electrophysiological recordings were obtained from the DCN. Cells in the MCN had significantly increased firing rates and a more synchronous pattern of activity in comparison to normal rats (Lorden et al., 1992). These findings suggest impaired afferent control of DCN cells and, therefore, of cerebellar output. The abnormal signal from the DCN could have significant consequences at several sites within the motor system. Furthermore, since there is a

projection from the DCN to the IO, these results identify this projection as a potential source of abnormal input to the IO.

Rationale for Proposed Study

The primary defect underlying the motor syndrome inherited by the dt rat has not been identified, although the output of the cerebellum is clearly abnormal as shown in glucose utilization studies and electrophysiological recordings from the DCN. Studies completed to date do not, however, demonstrate that increased activity in the DCN of the mutant rats is causally related to the movement disorder. The first goal of the studies presented here was to establish that abnormal cerebellar output can generate a dystonic syndrome by showing that the syndrome can be improved by elimination of cerebellar output. The second goal was to identify the electrophysiological features of cerebellar output in the awake dt rat.

The specific aims of this research were to:

1. Determine the importance of cerebellar outflow in the generation of the dt rat motor syndrome. CBX was performed to test the hypothesis that the dt rat motor syndrome can be improved by interrupting abnormal cerebellar output.

2. Determine the role of individual cerebellar output structures in the generation of the dt rat motor syndrome. If CBX improves the dt rat motor syndrome, then it is important to establish the structures critical for the

improvement. Selective elimination of cerebellar output structures was used to test the hypothesis that the MCN, INT, LCN, and dLV each contribute to the dt rat motor syndrome.

3. Determine the cellular correlates of abnormal cerebellar output in the awake dt rat. Three critical questions were addressed by single-unit recordings from the DCN in awake dt rats. First, do normal and dt rats differ in terms of DCN neuronal firing rates and firing patterns in the awake preparation? Second, do the MCN, INT, and LCN demonstrate differences in neuronal activity in the dt rat? Third, are there any differences in DCN neuronal activity between the awake and anesthetized dt rat?

EXPERIMENT #1

Is Cerebellar Output Critical to the Expression of the dt Rat Motor Syndrome?

Numerous correlates of the movement disorder have been observed in the cerebellum of the dt rat. These correlates do not constitute evidence that cerebellar dysfunction is causally related to the movement disorder. The goal of Experiment #1 was to address this issue.

Damage to the cerebellum in adult or developing animals is known to produce severe postural and locomotor deficits (Ito, 1984). Experimental evidence shows, however, that the cerebellum is not essential to the generation of locomotor rhythms. Animals lacking a cerebellum instead provide evidence that the cerebellum plays a role in the coordination and calibration of movements and in the adaptive plasticity of motor systems (Ito, 1984; Thach et al., 1992). For example, Optican and Robinson (1980) reported that ablations of the cerebellum altered only the metrics of saccadic eye movements and the ability of animals to adaptively repair saccades made hypometric by weakening an eye muscle. Similarly, CBX has been shown to prevent adaptation of the vestibulo-ocular reflex, but not to block the reflex itself (Robinson, 1976). The presence of an

erroneous signal from an adaptive or error-correcting mechanism could mask the functional integrity of other mechanisms essential to posture and locomotion.

In order to test the hypothesis that cerebellar output is essential to the expression of the rat disease, normal and dt rats were subjected to CBX. Although ablation studies are generally used to disrupt normal behavior, there is evidence that the removal of an erroneous signal may in some cases restore function. One example is the Sprague effect in which transection of the commissure of the superior colliculus or lesions of the substantia nigra reinstate visual orienting behavior in hemianopic cats (Wallace et al., 1990).

To assess the effects of CBX on the mutant rats, a series of age-appropriate behavioral tests was selected (Altman & Sudarshan, 1975). These included righting on a surface, climbing a wire-mesh incline, and homing (traversing a passageway to return to a home cage). In normal rat pups, the ability to perform these tasks is acquired early in development, prior to the behavioral deterioration of the dt rats (Altman & Sudarshan, 1975; Lorden et al., 1984). The mutant rats were observed to determine how the progression of the disease affected previously established motor function. The effects of CBX were then evaluated by observing changes in the frequency of occurrence of motor signs of the rat disease as well as changes in task performance.

Method

Normal and dt rats (Jfl:SD-dt) bred in the laboratory were tested prior to surgery on either Postnatal Day 15 or 20. In dt rats, the severity of the motor syndrome was assessed by counting the occurrence of twisting movements of the neck and trunk, paw clasps, falls, and pivoting over a 5-min observation period (Lorden et al., 1984). Paw clasps were defined as movements in which an animal extended any two limbs and brought them into apposition, flexing the digits of one paw around those of the other. Falls were counted when movement resulted in an animal's lying on its flank with its limbs rigidly extended. Pivoting occurred when a animal rotated about an adducted hindlimb. Both normal and dt rats were examined for overall levels of activity and tested on righting reflex time, climbing, and homing (Altman & Sudarshan, 1975). Activity scores were obtained by counting interruption of infrared light beams spaced 3 cm apart in an 18- X 28.5-cm chamber. Counts were summed for a 15-min period, following 2 min of acclimation. To obtain righting reflex times, rats were placed in a supine position and released. Time required for all 4 limbs to contact the floor was measured on 5 trials and a median was obtained. In the climbing task, rats received scores of 1, 2, or 3 points, respectively, for climbing a 1-cm wire mesh incline set at 30, 45, or 70 degrees from horizontal. Three 1-min trials were run at each angle. Therefore, the maximum number of points possible on the climbing test was

18. A zero was recorded if a rat failed to move both hindlimbs at least 2 cm. Scores on all trials were added and the sum recorded. Locomotor ability was quantified in the homing task in which rats were required to traverse a 58-cm long, corrugated plastic tube, 5 cm in diameter, to reach a home cage from a start box placed over crushed ice. The longest distance covered in three trials was recorded. Time to complete the homing task was also recorded. Trials were terminated after 120 sec.

Rats were anesthetized for surgery with intraperitoneal injections of ketamine hydrochloride (45 mg/kg) and xylazine (5 mg/kg). Separate groups of 5 normal and 5 dt rats underwent CBX by subpial suction at either 15 or 20 days of age. An additional group of eight 15-day-old dt rats received bilateral kainic acid injections (0.25 µl of 9.4 nM kainic acid, Sigma Chemical Co.) into the entopeduncular nuclei (ENTO) as a control for non-specific lesion effects. Injections were made 5.9 mm deep to the cortical surface at a point 1.8 mm posterior and 2.3 mm lateral to bregma. This nucleus was chosen because, along with the substantia nigra pars reticulata, it serves as a main output nucleus of the basal ganglia (Heimer et al., 1985). There were no postoperative deaths in the normal or dt CBX groups or in the ENTO group. The rats were ambulatory upon recovery from anesthesia, at which point they were returned to their home cages. Behavioral testing was repeated 3 days after

surgery. Groups of 18- or 23-day-old dt and normal rats were also tested as age-matched unoperated controls.

After postoperative testing, rats were deeply anesthetized with sodium pentobarbital and transcardially perfused with normal saline followed by 10% buffered formalin. All CBX and ENTO brains were sectioned in the coronal plane and stained with cresyl violet.

A separate group of 6 dt rats underwent CBX on Day 15 and was allowed to survive indefinitely to assess the permanence of the lesion effects. In untreated dt rats, growth and body weight are maintained during the preweaning period (Lorden et al., 1984). Motor function, however, progressively deteriorates. Even with supportive measures such as hand feeding, postweaning dt rats deteriorate, usually dying by Day 40. Thus, survival of CBX rats serves as a good index of overall functional improvement. Although the rats in the long-term survival group were monitored, they received no care after surgery beyond standard animal husbandry.

Results

Histology

In most CBX cases, portions of the paraflocculi and flocculi were spared (Figures 1 and 2). However, the vast majority of vestibulo-cerebellar efferent fibers were included in the CBXs. Therefore, it is unlikely that the small portions of remaining cerebellar cortex could have exerted a significant anterograde influence on motor

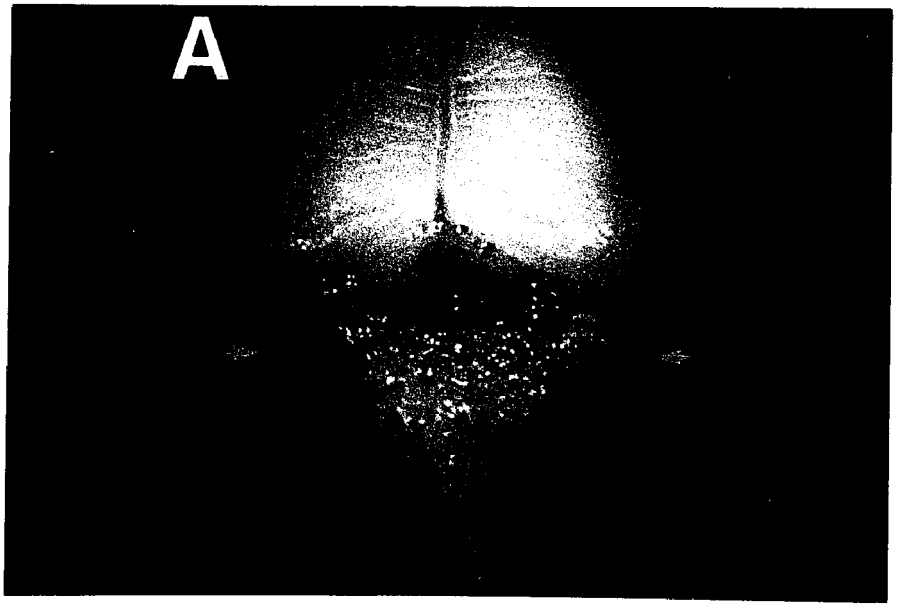
systems. Extra-cerebellar damage was limited to the dorsal-most portion of the lateral vestibular nucleus (Figure 2). In some cases small portions of the lateral cerebellar nuclei remained after CBX (Figure 2). However, the white matter rostral, caudal, and medial to this nucleus was removed.

The effectiveness of the ENTO lesions was verified by the presence of pyknotic nuclei, gliosis, and the absence of neuronal perikarya. All ENTO lesions extended into small portions of adjacent nuclei (Figure 3). A few spared cells were occasionally seen in the entopeduncular nucleus.

Untreated Rats

To provide a background against which the behavioral effects of CBX can be judged, the performance of groups of untreated normal and dt rats on behavioral tasks on Postnatal Days 15, 18, 20, and 23 (N = 5/group) is presented in Figure 4. The data for the Day 15 and 20 groups is the pre-operative performance of the CBX rats. Over the period from 15 to 23 days of age, normal rats showed little variation on the behavioral measures (Figure 4). In dt rats, the frequency of occurrence of clinical signs increased significantly ($p < .001$) (Figure 4A). Overall levels of activity did not change reliably in either normal or dt rats (Figure 4B). On three measures of motor performance (Figure 4C-F), the dt rats showed consistent and significant deterioration. Righting time was slow at Day 15 and increased monotonically. By Day 23, the dt rats were

Figure 1. (A, B) Examples of fixed brains after CBX. These brains show the typical extent of the lesions. The similarity between these two cases is also typical of the sample. Portions of the flocculi and paraflocculi are present bilaterally (arrows).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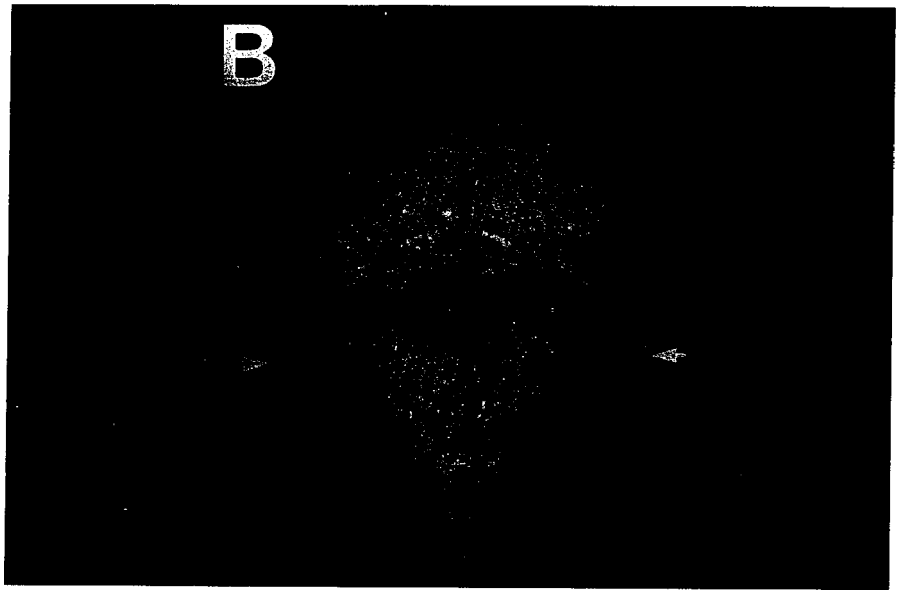

B

Figure 2. Serial reconstruction of the brainstem from a representative case illustrates the extent of CBX in cross section. Sections are 480 µm apart.

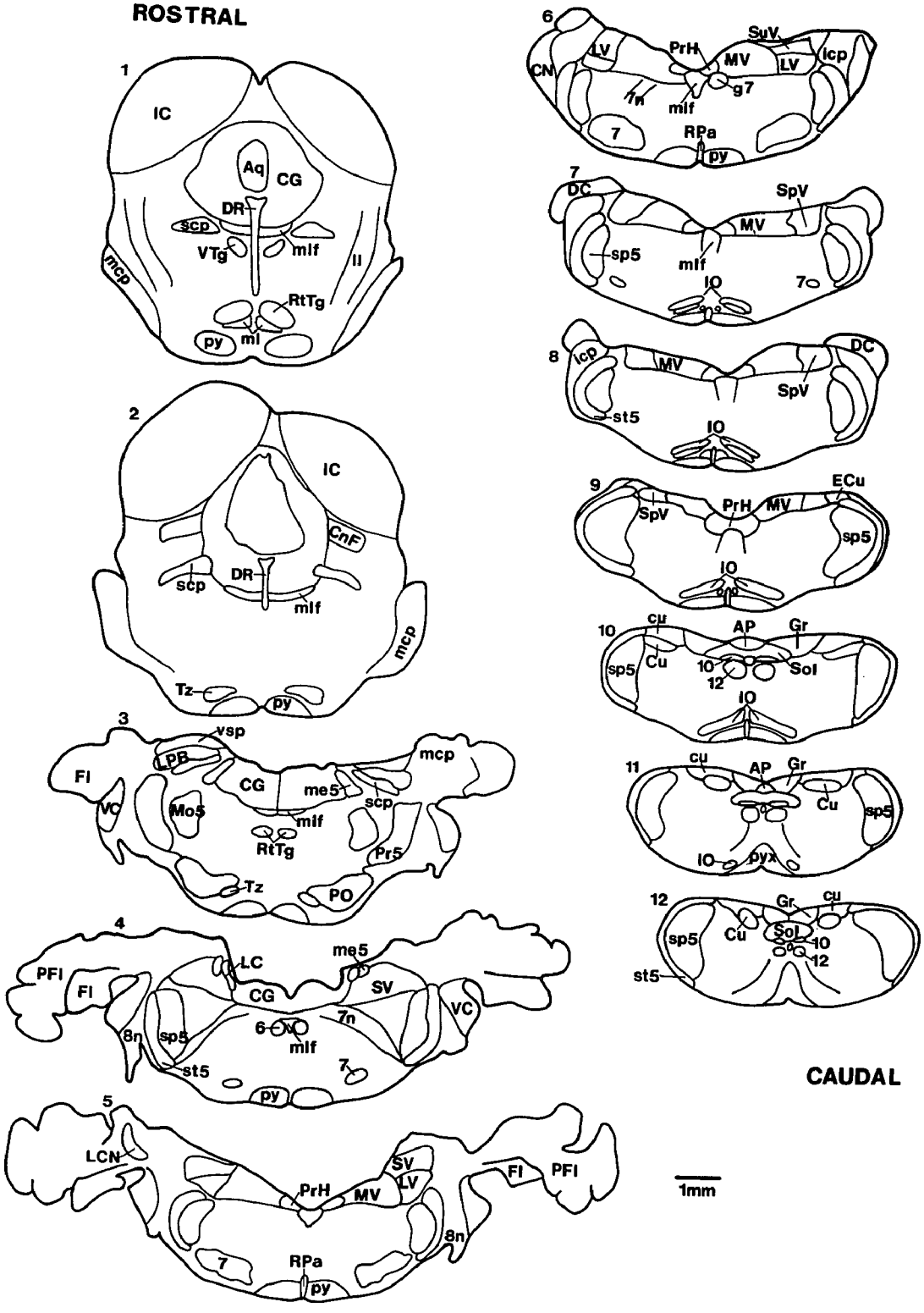

ROSTRAL
1
IC
Aq
CG
DR
scp
VTg
mlf
ll
mcp
RtTg
py
ml
2
IC
CnF
DR
scp
mlf
mcp
Tz
py
3
vsp
LPB
mcp
Fl
CG
me5
scp
VC
Mo5
mlf
RtTg
Pr5
Tz
PO
4
me5
PFl
LC
Fl
CG
SV
VC
7n
8n
sp5
6
mlf
st5
7
py
5
LCN
SV
PrH
MV
LV
Fl
PFl
8n
7
RPa
py
6
SuV
LV
PrH
MV
LV
lcp
CN
7n
mlf
g7
7
RPa
py
7
DC
SpV
MV
sp5
mlf
IO
7
8
lcp
MV
DC
SpV
st5
IO
9
ECu
SpV
PrH
MV
sp5
IO
10
cu
AP
Gr
sp5
Cu
10
12
Sol
IO
11
cu
AP
Gr
Cu
sp5
IO
pyx
12
Gr
cu
sp5
Cu
Sol
10
12
st5
CAUDAL
1mm

Figure 3. Serial reconstruction through the entire extent of the entopeduncular nuclei from a representative case. Extent of the kainic acid lesion is indicated by cross-hatching. Sections are 120 µm apart.

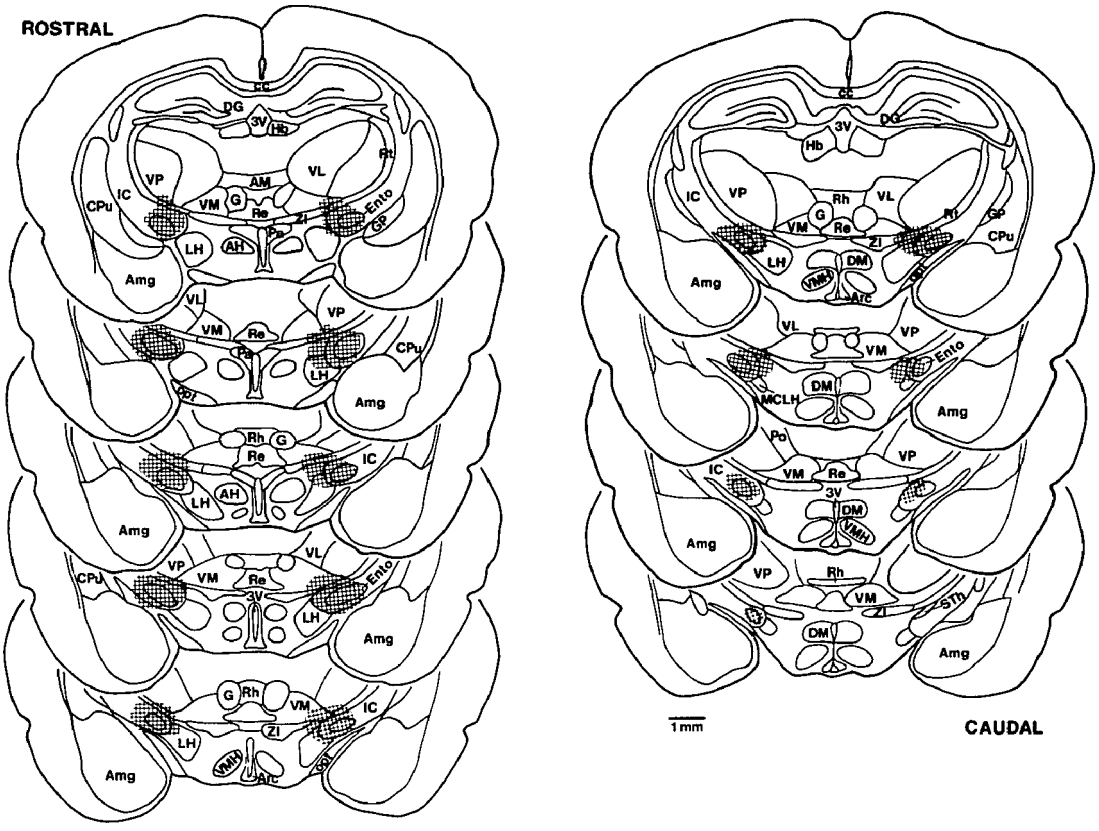
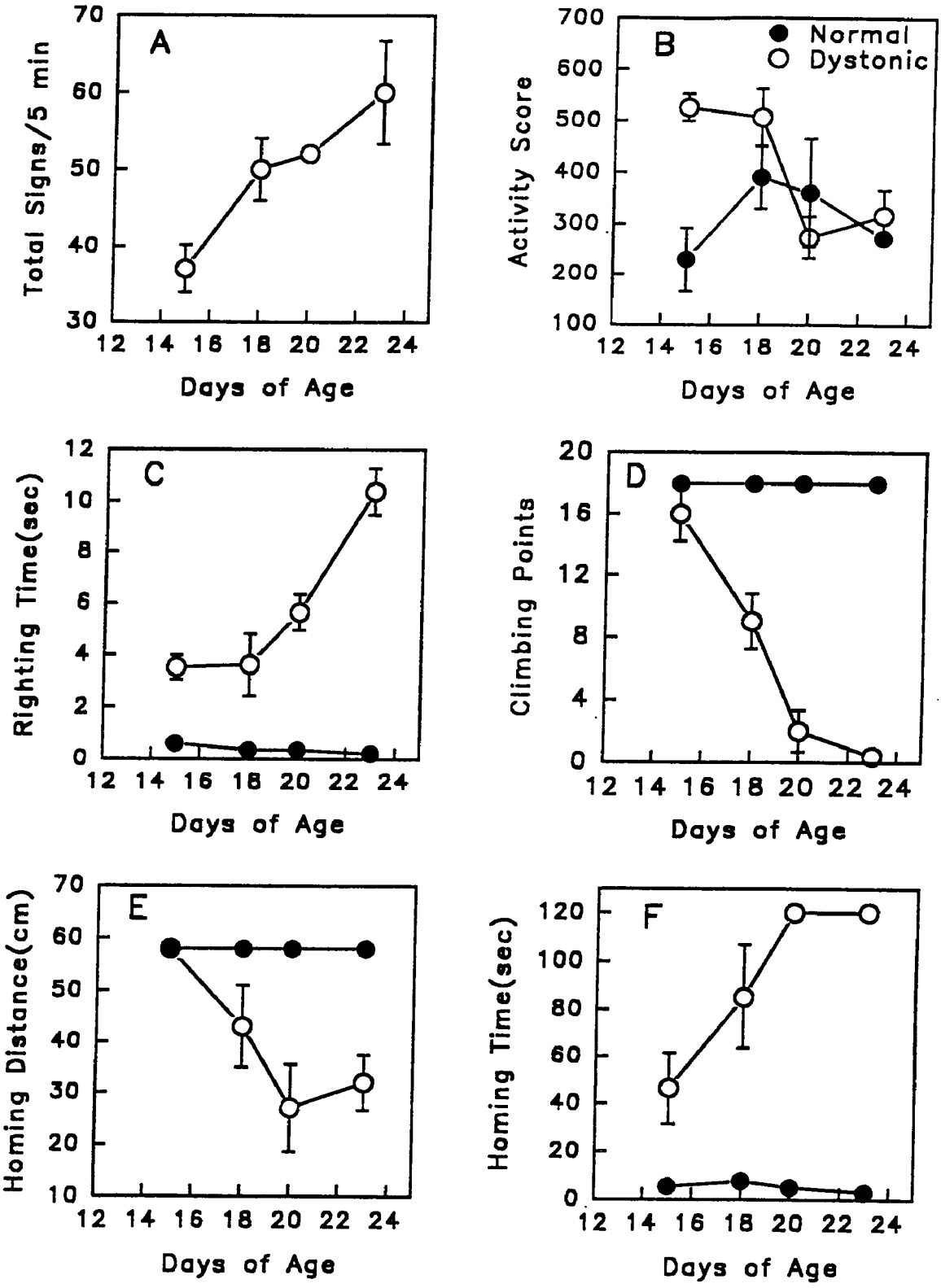

ROSTRAL
CC
DG
3V
Hb
Rt
VP
IC
AM
VL
CPu
VM
G
Re
ZI
Ento
GP
LH
AH
Po
Amg
Rh
MCLH
DM
Arc
CAUDAL
1 mm

Figure 4. The natural history of the rat disease was followed from Postnatal Day 15 to 23. All values are means ± SEM. (A) The total number of clinical signs over a 5-min period. (B) Activity scores for a 15-min period, following 2 min of acclimation. Although normal and dt rats differed on Day 15 ($p < .001$), the difference did not persist. (C) Righting reflex times; each point is an average of 5 trials. (D) Scores for climbing a wire mesh incline set at 30, 45, or 70 degrees from horizontal. Scores are total points earned in three trials at each angle. (E) Homing task; the longest distance covered in three trials was recorded. (F) Time to complete the homing task. The change in performance with age in A and C-F was statistically significant ($p < .004$) in the dt rats.


A
Total Signs/5 min
70
60
50
40
30
12 14 16 18 20 22 24
Days of Age
B
Normal
Dystonic
Activity Score
700
600
500
400
300
200
100
12 14 16 18 20 22 24
Days of Age
C
Righting Time(sec)
12
10
8
6
4
2
0
12 14 16 18 20 22 24
Days of Age
D
Climbing Points
20
16
12
8
4
0
12 14 16 18 20 22 24
Days of Age
E
Homing Distance(cm)
70
60
50
40
30
20
10
12 14 16 18 20 22 24
Days of Age
F
Homing Time(sec)
120
100
80
60
40
20
0
12 14 16 18 20 22 24
Days of Age

unable to complete either the climbing task (Figure 4D) or the homing task (Figure 4E-F). Linear trend analyses on all three tasks illustrated in Figure 4C-F indicated that the performance of the dt rats deteriorated with age ($p < .004$, for all).

Lesion Effects

In both the 15-day-old and 20-day-old dt groups, CBX produced a significant reduction in all the characteristic movements of the dt rats (Figures 5 and 6). Repeated measures analysis of variance indicated significant postoperative reductions in motor signs in both CBX dt lesion groups in comparison with preoperative values ($p < .001$). Comparisons between CBX and ENTO lesion groups and the age-matched unoperated controls (18C) at Day 18 indicated that the only reliable differences were between the CBX group and both the ENTO and 18C groups ($p < .0001$, for both). At Day 23, the CBX group also showed significantly fewer motor signs ($p < .0001$) than the unoperated group (23C).

In the CBX rats, pivoting movements were still evident. Pivoting appears during normal rat development and reaches a peak at about Postnatal Day 9 (Altman & Sudarshan, 1975; Petrosini et al., 1990). In normal rats, but not dt rats, pivoting is virtually absent by Day 15 (Lorden et al., 1984). Although pivoting declines in the dt rat with age, its disappearance is delayed by neonatal cerebellar lesions (Petrosini et al., 1990).

In contrast to the effects of CBX, ENTO lesions increased the frequency of clinical signs in the mutants (Figure 5). This effect was significant both in comparison with preoperative levels and in comparison with age-matched (18C) unoperated dt rats ($p < .025$, for both). Thus, the effects of the cerebellar lesions on the movement disorder cannot be attributed to a nonspecific lesion effect.

The results of behavioral evaluations of CBX and ENTO rats are presented in Figure 6. Data on both preoperative (Day 15 or 20) and postoperative (Day 18 or 23) performance are presented. The effects of CBX and ENTO lesions on the clinical signs of dt rats are summarized in Figure 6A. The reductions in motor signs that followed CBX were not secondary to a general decrease in activity levels. Figure 6B shows that postoperative activity levels were similar to preoperative levels. As seen in Figure 6C, an improvement in righting time ($p < .02$) was seen after surgery. Other comparisons between pre- and postoperative performance for this group were not statistically significant (Figure 6D-F).

In the young dt CBX group that underwent surgery on Day 15, the lesion generally prevented deterioration in motor performance. This was evident in analyses of the Day 18 performance of CBX, ENTO, and age-match unoperated dt rats and normal CBX rats. The young dt CBX group did not differ from the normal CBX rats on any measure and scored significantly better than the unoperated 18C dt group, shown in Figure 4, on all measures, except homing distance

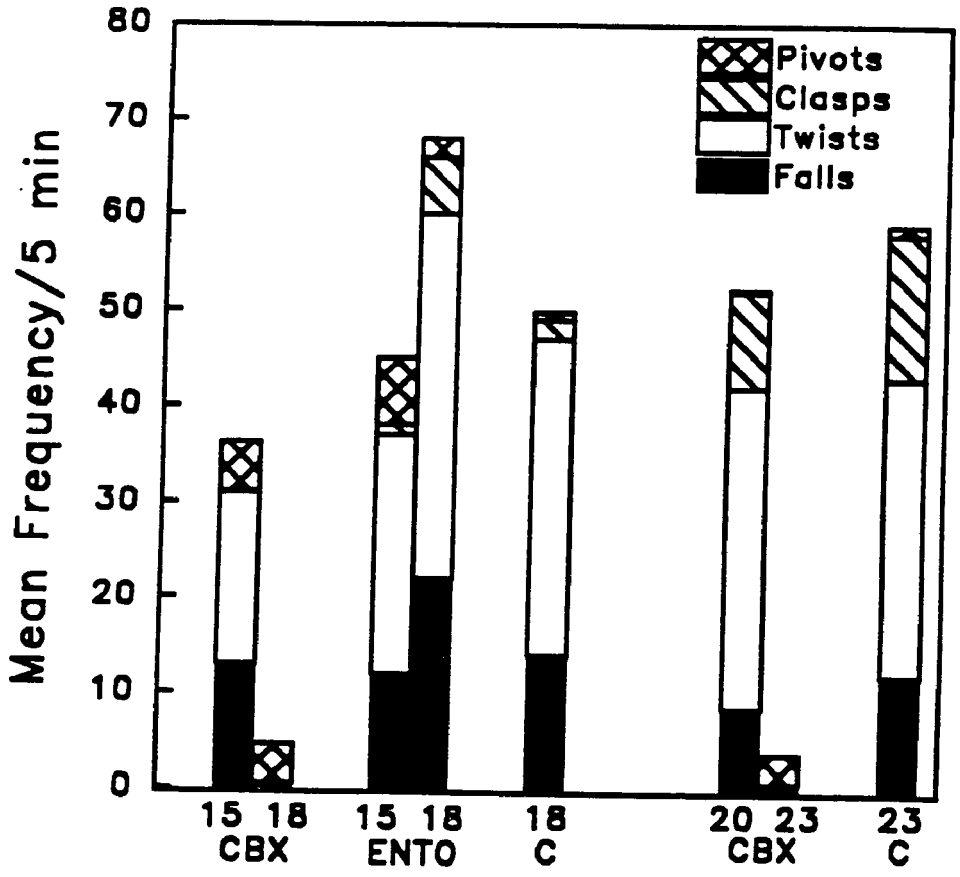
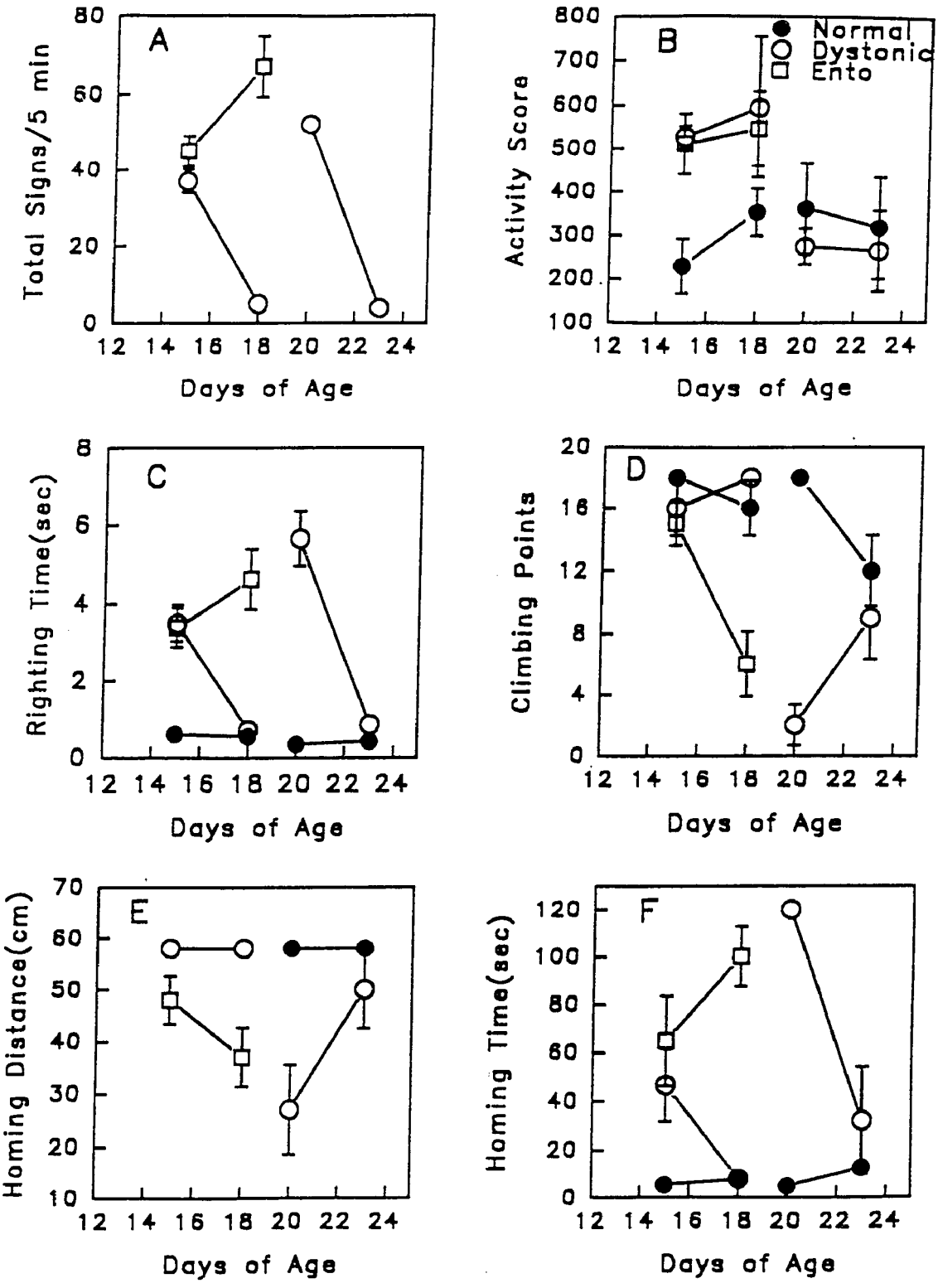


Figure 5. The mean frequency of 4 discrete motor signs characteristic of the dt rat counted before (Day 15 or 20) and after (Day 18 or 23) CBX or ENTO lesions. Unoperated dt controls (C) 18 or 23 days of age are also included for comparison. Postoperative reductions in motor signs in both CBX lesion groups are significant in comparison with preoperative values ($p < .001$). Rats in the ENTO group showed a significant increase in signs ($p < .025$). Comparisons between lesion groups and the age-matched unoperated controls at Day 18 indicated that there were differences between the CBX group and both the ENTO and control groups ($p < .0001$, for both). At Day 23, the CBX group also showed fewer motor signs ($p < .0001$) than the unoperated group (23C).

Figure 6. Pre- and postoperative performance of normal and dt rats that received CBX at Day 15 or Day 20 and dt rats that received ENTO lesions at Day 15. All values are mean ± SEM. Behavioral tasks are the same as those in Figure 4. The results for normal rats are not included in A because they did not exhibit abnormal motor signs. In addition, the results for normal rats at 15 and 18 days are excluded from E because of overlap with the dt CBX group. Clinical signs decreased in the dt CBX groups and increased in the ENTO group (A) ($p < .025$, for all). General activity levels were unaltered in any lesion group, normal or dt (B). In the young CBX dt rats, a decrease was seen in righting time (C) and homing time (F); however, only the decrease in righting time was statistically significant ($p < .02$). In the older CBX dt rats, a decrease was seen in righting time (C) and improvement was observed in climbing (D) and homing (E,F); all effects were significant in comparison with preoperative values ($p < .02$), except for homing distance. In normal rats, the only significant effect was a reduction in climbing ability ($p < .05$) after CBX on Day 20 (C). In the ENTO group, the frequency of motor signs increased (A) and climbing ability decreased (D) in comparison with preoperative performance ($p < .025$, for both).


A
Total Signs/5 min
Days of Age
B
Activity Score
Normal
Dystonic
Ento
Days of Age
C
Righting Time(sec)
Days of Age
D
Climbing Points
Days of Age
E
Homing Distance(cm)
Days of Age
F
Homing Time(sec)
Days of Age

($p < .02$, for all). By contrast, the ENTO group that also underwent surgery on Day 15 did not differ from the age-matched unoperated group on any task (Figure 6B-F). No significant effects on any task were observed in normal 15-day-old CBX rats.

In the older dt CBX group that received surgery on Day 20, significant improvement in comparison with preoperative performance was evident (Figure 6). The reduction in clinical signs seen in older CBX dt rats (Figure 6A) was paralleled by significant decreases in righting time (Figure 6C), and improvement in climbing (Figure 6D) and homing (Figure 6E-F). All effects were significant in comparison with preoperative values ($p < .02$), except for homing distance. Comparisons of the older CBX group at Day 23 with age-matched unoperated dt rats and CBX normal rats, indicated that the dt CBX group did not differ from normal rats on any measure but showed significant improvement in comparison with the unoperated 23-day-old dt rats on all measures ($p < .05$, for all), except homing distance. When the postoperative performance of young and old dt CBX rats was compared directly, they differed only on the performance of the climbing task ($p < .02$). Thus, the age at which dt rats underwent CBX had little effect on their final level of performance on the behaviors studied.

The data presented in Figure 6 document the finding that CBX eliminates the signs of the rat disease, prevents the progressive deterioration of motor performance in young

dt rats, and improves performance on several tasks in older dt rats. The long-term consequences of CBX were examined in a separate group of six dt rats that underwent CBX on Postnatal Day 15. These animals survived into adulthood without further treatment. As adults, they showed instability of gait. They used all limbs in locomotion and were able to raise the head, shoulders, and ventral surface of the trunk off the ground. This argues against a simple loss of muscle tone as an explanation for the decrease in clinical signs. The dramatic reduction in signs of the movement disorder seen three days postlesion persisted, even in animals followed for over 6 months. As in the immediate postlesion period, pivoting movements were still observed in animals with long survival times. All rats continued to grow, reaching normal adult weights. At no time was it necessary to provide hand feeding or other types of support. Both males and females were able to mate successfully and females were able to rear their offspring, showing many characteristic maternal behaviors such as pup retrieval.

Discussion

Cerebellectomy

CBX eliminated the signs of the movement disorder in the dt rat and produced a significant and permanent improvement in motor performance. The decrease in clinical signs observed in the CBX rats cannot be attributed to a non-specific effect such as akinesia. There was no evidence of hypoactivity in the CBX rats. Specific tests of motor

performance revealed improvement in function within 3 days of the lesion and long-term observations confirmed the enduring nature of the effect. These observations of improved performance with CBX in the dt rats are surprising in view of the motor impairments described in normal rats following complete or partial CBX (Manni & Dow, 1963; Gramsbergen, 1982; Auvray et al., 1989; Molinari et al., 1990). It is also evident from these studies that many of the effects of cerebellar lesions are age-dependent and when a variety of tasks is used to test CBX animals, not all motor functions are permanently impaired. Furthermore, the CBX dt rats do show some of the impairments seen following CBX in normal animals. Mostly notably, none of the CBX dt rats developed a normal gait and all continued to pivot. In the dt rat, however, the presence of a malfunctioning cerebellum is more debilitating than the complete absence of a cerebellum.

The locomotor abilities tested in this study were not seriously impaired by CBX in normal rats. The only significant change detected was a decrement in the climbing scores of phenotypically normal rats that underwent CBX on Day 20. More demanding skills acquired at later ages would certainly have revealed impairments in normal CBX rats; however, the goal of the study was to examine the effects of the lesion on dt rats. Studies by others (Auvray et al., 1989; Petrosini et al., 1990) on the effects of CBX during early development suggest that motor skills such as

balancing on a rotating rod are left relatively intact by CBX at Postnatal Day 15, if the animals have been preoperatively trained. In the absence of preoperative training, acquisition of this skill is seriously impaired. Figure 4 shows that by Day 15, the normal rats were already at an asymptotic level of performance on the tasks evaluated in the present study. The fact that these tasks were readily performed by normal rats prior to surgery may account for the fact that CBX had relatively little effect on their performance. For the dt rats, the data suggest that the ability to perform the behavioral tasks was not lost, but was masked by the increasing severity of the disease.

Olivo-cerebellar Abnormalities in the dt Rat

Behavioral changes that result from brain lesions are not necessarily an index of the function of the ablated structure. The fact that lesions of the cerebellum eliminate the signs of disease in the dt rats does not by itself indicate that the cerebellum is crucial in the production of the movement disorder. In this case, however, a substantial body of evidence suggests that the output of the cerebellum is abnormal.

GAD activity, glucose utilization, and neuronal firing rates are all increased in the DCN of dt rats (Brown & Lorden, 1989; Beales et al., 1990; Lorden et al., 1992). Furthermore, neuropharmacological evidence supports the argument that these effects are the result of a defect

within the olivo-cerebellar system, rather than secondary to abnormalities elsewhere in the brain. In response to harmaline administration, cells in the cMAO of the dt rat show an increase in firing rate and rhythmicity that is indistinguishable from that seen in normal rats (Stratton & Lorden, 1991). Activation of the cMAO cells by harmaline is conveyed to the cerebellar vermis by the climbing fibers. This results in a rhythmic increase in complex spike rate and the suppression of simple spikes in over 60% of vermal Purkinje cells in normal rats, but in fewer than 10% of the Purkinje cells in the dt rat (Stratton et al., 1988). Although these data do not specify the nature of the defect, the failure of the dt rat Purkinje cells to respond to harmaline-induced activation of the IO indicates a failure in neurotransmission within the system. Thus, the finding that removal of the cerebellum improves the condition of the dt rat strongly supports the conclusion that the abnormal output of the cerebellum makes a critical contribution to the motor syndrome.

Because the DCN have widespread projections to premotor and motor nuclei (Faull, 1978; Faull & Carmen, 1978), abnormal output from these nuclei has marked effects on motor function. In normal rats, postural asymmetries can be induced with unilateral chemical lesions of the fastigial nucleus (Imperato et al., 1984) or hemicerebellectomy (Gramsbergen et al., 1982; Manni & Dow, 1963; Petrosini et al., 1990). Alteration of cerebellar output by destruction

of the climbing fiber afferents with the neurotoxin 3-AP also produces a complex movement disorder that includes twisting movements of the axial musculature during the early postlesion period (Sukin et al., 1992). CBX in normal rats does not, however, reproduce the movement disorder of the dt rat and hemicerebellectomy produces postural asymmetries to one side (Manni & Dow, 1963; Gramsbergen, 1982; Petrosini et al., 1990), not the alternating, twisting movements seen in the dt rat. This suggests that the dt motor syndrome is the result of aberrant activity in the cerebellum or DCN, rather than a loss of activity as would occur with a lesion.

It is not possible to rule out the presence of abnormalities elsewhere in the brain of the dt rat. It is possible that the cerebellum is required for the behavioral expression of dysfunction at another site within the nervous system such as the basal ganglia. The long-term improvement induced by CBX in the behavioral capacity of the mutant indicates, however, that other defects are not sufficient to produce the movement disorder. In addition, the poor survival of untreated dt rats (Lorden et al., 1984) cannot be attributed to an unidentified defect. Instead, the dramatic increase in survival time suggests that the early death of untreated animals is a consequence of abnormal movements interfering with the ability of the rats to swallow food and/or breathe.

Relationship to Human Dystonia

Spontaneous mutations are natural experiments that provide opportunities to test novel hypotheses about mechanisms underlying pathological phenomena. In humans, dystonia is defined as a syndrome of sustained muscle contractions, frequently causing twisting and repetitive movements or abnormal postures (Fahn, 1988). Symptomatic dystonia occurs in association with a large number of diseases, including inflammatory, cerebrovascular, and metabolic disorders, and may appear during neuroleptic drug treatment (Calne & Lang, 1988). Both hereditary and nonfamilial cases of primary dystonia also occur (Fahn, 1988). Although the pathology in many cases of symptomatic dystonia has been localized to the basal ganglia (Calne & Lang, 1988), neither careful postmortem examination (Zeman, 1970; Zweig et al., 1987) nor MRI (Rutledge et al., 1988) have identified consistent abnormalities in patients with primary dystonia.

The observation that many cases of secondary dystonia are associated with pathology in the basal ganglia has led to the assumption that primary dystonia is also the result of basal ganglia dysfunction (Fahn, 1988; Fahn, 1989a; Marsden, 1992). Lesions of the basal ganglia do not always lead to dystonia (McGeer & McGeer, 1988). Furthermore, many years may pass between basal ganglia insult and the onset of symptoms, suggesting that additional changes may be needed to cause dystonias (McGeer & McGeer, 1988). The present

study suggests that the cerebellum may play a critical role in the pathophysiology of some human dystonias.

EXPERIMENT #2

Do all cerebellar nuclei and dorsal portions of the lateral vestibular nuclei contribute to the dt rat motor syndrome?

Previous studies have identified the cerebellum as a site of significant functional abnormality in the dt rat, whereas examination of the basal ganglia has been unrevealing (Lorden et al., 1984; Oltmans et al., 1986; Lorden et al., 1988; Brown & Lorden, 1989; Beales et al., 1990). More specifically, the cells of the MCN fire more rapidly and rhythmically than normal(Lorden et al., 1992). Measures of glucose utilization (Brown & Lorden, 1989), glutamic acid decarboxylase activity (Oltmans et al., 1986), and muscimol binding (Beales et al., 1990) also indicate the presence of abnormality in the DCN of the dt rat.

The dramatic effect of CBX strongly suggests that the DCN are critical to the expression of the dt rat motor syndrome. Because CBX included all DCN and the dLV, it was not possible to determine the relative importance of specific nuclei in the generation of the dt rat motor syndrome. Therefore, Experiment #2 was designed to assess the effects of eliminating output from individual nuclei. The effects of both electrolytic and axon-sparing lesions were examined.

Method

The motor function of 15-day-old homozygous (dt/dt) dystonic rats (Jfl:SD-dt) was tested before the creation of bilateral electrolytic or excitatory amino acid (EAA) lesions of specific cerebellar nuclei. Bilateral lesions of the MCN, INT, LCN, and dLV were made in separate groups of 15-day-old dt rats. Rats were tested again on Postnatal Day 20. Unoperated dt rats of the same age served as controls. The control group was not subjected to a sham surgical procedure so that the lesion effects could be compared to the undisturbed, natural progression of the dt rat motor syndrome. Pups were weighed on Day 15, before surgery, and again on Day 20. All pups were housed with their dams until sacrifice and removed from their cages only for behavioral testing and surgery.

Behavioral Testing

The frequency of four motor signs (falls, twists, clasps, pivots) occurring over a 5-min observation period with animals alone on a rubber mat was determined for each rat. Total signs were counted by summing falls, twists, clasps, and pivots. Falls were counted when movement resulted in an animal's lying on its flank with the forelimb and hindlimb on one side of the body lifted off the mat. Grossly abnormal rotations of the head, neck, and upper thorax about the longitudinal axis of the body were classified as twists. Paw clasps were defined as movements

in which an animal extended any two limbs and brought them into apposition, flexing the digits of one paw around those of the other. Pivoting occurred when an animal rotated at least 180 degrees about an adducted hindlimb.

To measure the amount of spontaneous movement displayed by an animal, activity scores were obtained by counting interruptions of infrared light beams spaced 3 cm apart in an 18- X 28.5-cm chamber. Counts were summed for a 15-min period, following 2 min of acclimation.

Rats were tested on several standard measures of neonatal motor performance (Altman & Sudarshan, 1975). Because of the severe constraints on motor control imposed by a dystonic motor syndrome and the age at which the animals were tested, a limited range of tasks proved suitable. For example, dt rats cannot balance on narrow paths or reach through holes for food pellets (Moroz & Bures, 1981), tasks that are not difficult for normal rats. However, the tests of homing, climbing, hanging and righting used in this study provide, for the dt rat, practical, age-appropriate measures of motor function. These tests were not intended, however, to specifically test the function of individual cerebellar nuclei.

To obtain righting reflex times, rats were placed in a supine position and released. The time required for all 4 limbs to contact the floor was measured on 5 trials and a median was obtained. In the climbing task, rats received scores of 1, 2, or 3 points, respectively, for climbing at

least 2 cm up a 1-cm wire mesh incline set at 30, 45, or 70 degrees from horizontal. Three 1-min trials were run at each angle for a maximum score of 18. The homing task was used as a measure of ambulatory ability. Rats were required to traverse a 58-cm long, corrugated plastic tube, 5 cm in diameter, to reach a home cage from a start box that contained a bed of crushed ice. The distance and time of the best performance in three trials of 120-sec duration were recorded. For the hanging task, a plastic-coated wire was strung 40 cm above a 5-cm thick foam pad. Rats were allowed to grasp the wire with their forelimbs and were then released to hang freely. The longest hanging time in three 120-sec trials was recorded. Right and left hindlimb use was recorded for each trial to yield a maximum total hindlimb use score of 6 for the three trials.

Surgery

Rats were anesthetized for surgery with intraperitoneal injections of ketamine hydrochloride (45 mg/kg) and xylazine (5 mg/kg). The stereotactic coordinates in mm for the four nuclei lesioned in this study are given with reference to lambda (AP), the midline (L), and the pial surface (D) with the skull flat between bregma and lambda: MCN (AP, -2.25; L, ±0.85; D, -3.70), INT (AP, -2.10; L, ±1.85; D, -3.60), LCN (AP, -2.10; L, ±2.80; D, -3.50), dLV (AP, -2.15; L, midline; D, -4.80 at an angle of ±20 degrees with the mid-sagittal plane). Electrolytic lesions were made with a

parylene-coated stainless steel electrode coupled to a Grass direct current lesion maker (1.5 mA for 20 sec).

Excitatory amino acid (EAA) lesions were made with either kainic acid (9.4 nM, Sigma Chemical Co.) or N-methyl-DL-aspartic acid (NMDA; 0.1 M, Sigma Chemical Co.) diluted in sterile normal saline. The EAA solution was injected into each nucleus over 1 min with a continuous infusion pump (KD Scientific Co., Model 100) through a 33-gauge cannula connected to a Hamilton syringe (Hamilton Syringe Co.). A volume of 0.50 µl was injected into each MCN, whereas 0.25 µl was used for each EAA-dLV lesion. Kainic acid consistently produced complete lesions, but was associated with poor postoperative weight gains and seizures in a minority of rats. Prolonged seizures were stopped by re-anesthetization with intraperitoneal injection of ketamine and xylazine. In an attempt to reduce the occurrence of seizures and improve postoperative weight gains, NMDA was used instead of kainic acid for some EAA-MCN and EAA-dLV lesions. However, cell loss after NMDA lesions was frequently incomplete and weight gains were not different from those seen after kainic acid injections. Ibotenic acid (Research Biochemicals International) did not produce DCN lesions in pilot studies.

After postoperative testing, rats were deeply anesthetized with sodium pentobarbital and transcardially perfused with normal saline followed by 10% buffered formalin. All brains were sectioned in the coronal plane at

40 µm and sections were stained with cresyl violet. Alternating sections from the EAA lesion cases were also stained with a modified Heidenhan protocol for myelin (Hutchins & Weber, 1983).

Statistics

A blind analysis of the histological material (150 cases) allowed for inclusion of only those cases with appropriately placed lesions. After histological analysis, a total of 78 cases remained in 7 groups: CTRL (control, $N = 12$), MCN ($N = 11$), INT ($N = 11$), LCN ($N = 10$), dLV ($N = 12$), EAA-MCN ($N = 11$), EAA-dLV ($N = 11$). The NMDA and kainic acid cases did not significantly differ on any measure for either the EAA-MCN or EAA-dLVN group. Therefore, NMDA and kainic acid cases were pooled. There were four NMDA and seven kainic acid cases in the EAA-MCN group and six NMDA and five kainic acid cases in the EAA-dLV group.

The data were screened for normality before choosing an analysis strategy for each variable. An analysis of covariance (ANCOVA) with Day 15 measures as covariates was used to determine group effects on weight, activity, twists, total signs and righting time. Tests of the assumptions of ANCOVA were performed on each covariate. There were no group differences on the covariates and there was no heterogeneity of regression coefficients. An analysis of variance on difference scores was used to determine group effects on falls and hanging time. The Kruskal-Wallis test

was used to detect group effects on the Day 15 to Day 20 change scores for clasps, pivots, homing time, homing distance, climbing points, and hindlimb use. Wilcoxon 2-sample tests were used for planned pairwise group comparisons on these measures.

All lesion groups were compared to the control group. Selected lesion effects were contrasted to assess the relative importance of individual nuclei to the dt rat motor syndrome. The effects of MCN and dLV lesions were compared because both regions play important roles in the control of axial musculature, posture and balance (Sprague & Chambers, 1953; Sprague & Chambers, 1954; Imperato et al., 1984; Marlinsky, 1992). In contrast, the LCN plays an important role in the control of voluntary movements and coordination of the distal extremities (Schwartz et al., 1987; Ivry et al., 1988; McKay, 1988). Therefore, the effects of MCN and LCN lesions were compared because of their relationships to different aspects of movement. In addition, electrolytic and EAA lesion groups were contrasted to assess the contribution of fiber damage to the effects of electrolytic lesions.

The Holm procedure was used to provide family-wise error control ($\alpha = 0.05$) for multiple comparisons of experimental groups (Holm, 1979; Seamen et al., 1991). Estimated omega squared ($\acute{\omega}^2$) was used as a measure of effect magnitude (Hays, 1988).

Results

Histology

As shown in Figures 7 through 10, cases chosen for statistical analysis were characterized by near complete destruction of cells within the nuclei of interest and minimal extension into adjacent nuclei and white matter. A single coronal section through representative MCN lesions is seen in Figure 7. The variability seen in the two MCN cases presented in Figure 8 is typical of the sample. An LCN lesion that extends into cerebellar hemispheric white matter and cortex is shown in Figure 9. The case reconstructed in Figure 9 depicts the largest lesions in the entire 78 rat sample.

EAAs were used to create discrete lesions without fiber damage as shown in Figure 10. Lesions of the lateral vestibular nucleus were limited to its dorsal half (Figures 10A and 10B). Myelin staining was used to demonstrate the preservation of fiber pathways with EAA lesions (Figures 10C and 10D). As with electrolytic lesions, extension into adjacent nuclei was also seen with EAA lesions (Figures 10A and 10B). However, only cases with minimal spread into adjacent nuclei were included in the final analysis.

Lesion Effects

The mean weight of all groups increased over Postnatal Days 15 to 20. However, using Day 15 weight as a covariate, the Day 20 weight of the control group ($\underline{M}$ = 41.6 gm) was significantly greater than that of the dLV [$\underline{M}$ = 36.4 gm,

Figure 7. Photomicrograph of coronal section through the cerebellum and brainstem demonstrates electrolytic MCN lesions. There is minimal extension into the interpositus nuclei and adjacent white matter. Bar = 1 mm.

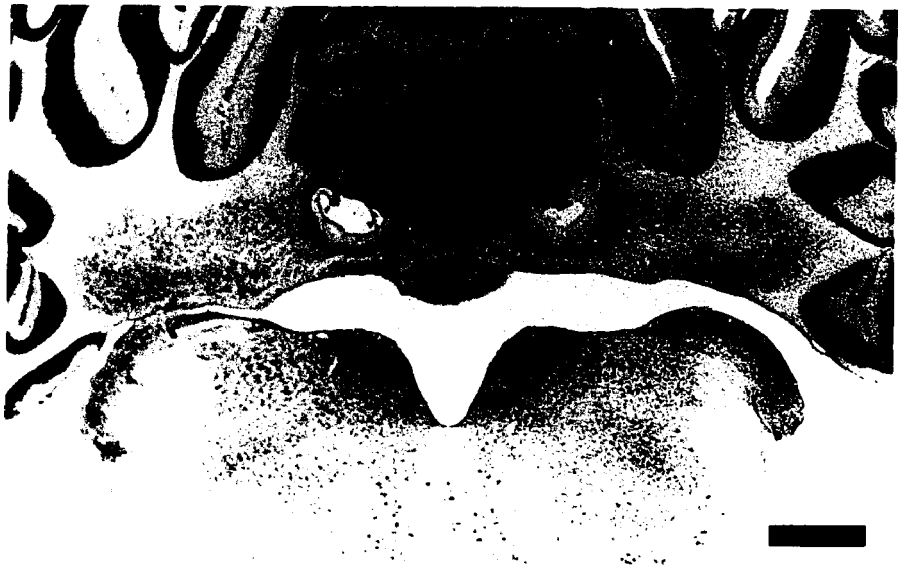
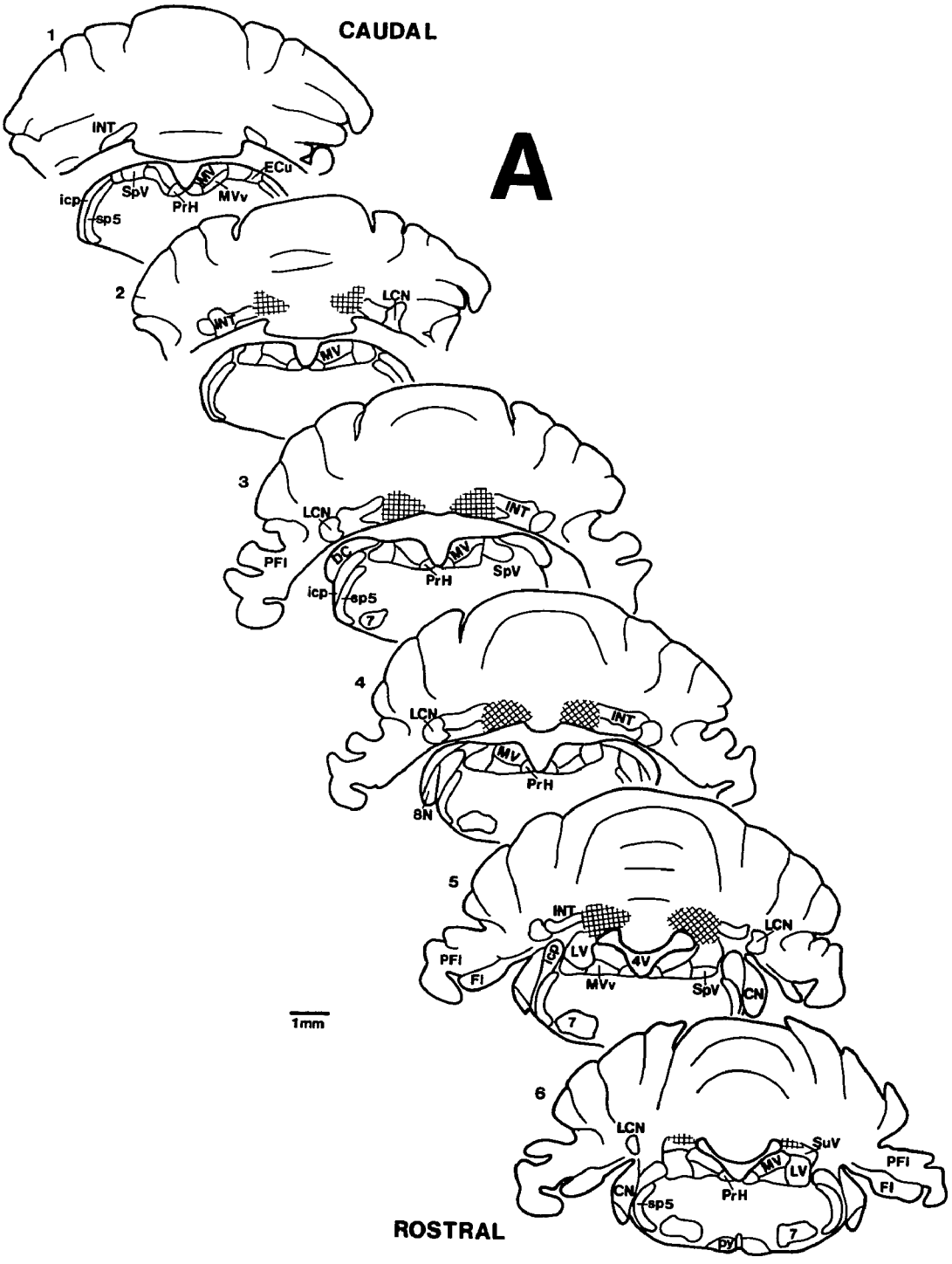


Figure 8. Serial reconstruction of the cerebellum and brainstem from electrolytic MCN lesion cases. These cases are representative of the variability within the sample of MCN lesions. Sections are 480 µm apart. A: the MCN is eliminated bilaterally. The lesion slightly extends into the INT and vestibular nuclei. B: this lesion extends through the roof of the 4th ventricle. The MCN is eliminated bilaterally. The lesion extends into the INT and vestibular nuclei.


CAUDAL
A
1
INT
ECu
icp
SpV
PrH
MVv
sp5
2
LCN
INT
MV
3
LCN
INT
PFl
DC
MV
PrH
SpV
icp
sp5
7
4
LCN
INT
MV
PrH
8N
5
INT
LCN
PFl
Fl
LV
4V
MVv
SpV
CN
7
1mm
6
LCN
SuV
MV
LV
PFl
CN
PrH
Fl
sp5
py
7
ROSTRAL


CAUDAL
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1
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MVv
PrH
ECu
icp
sp5
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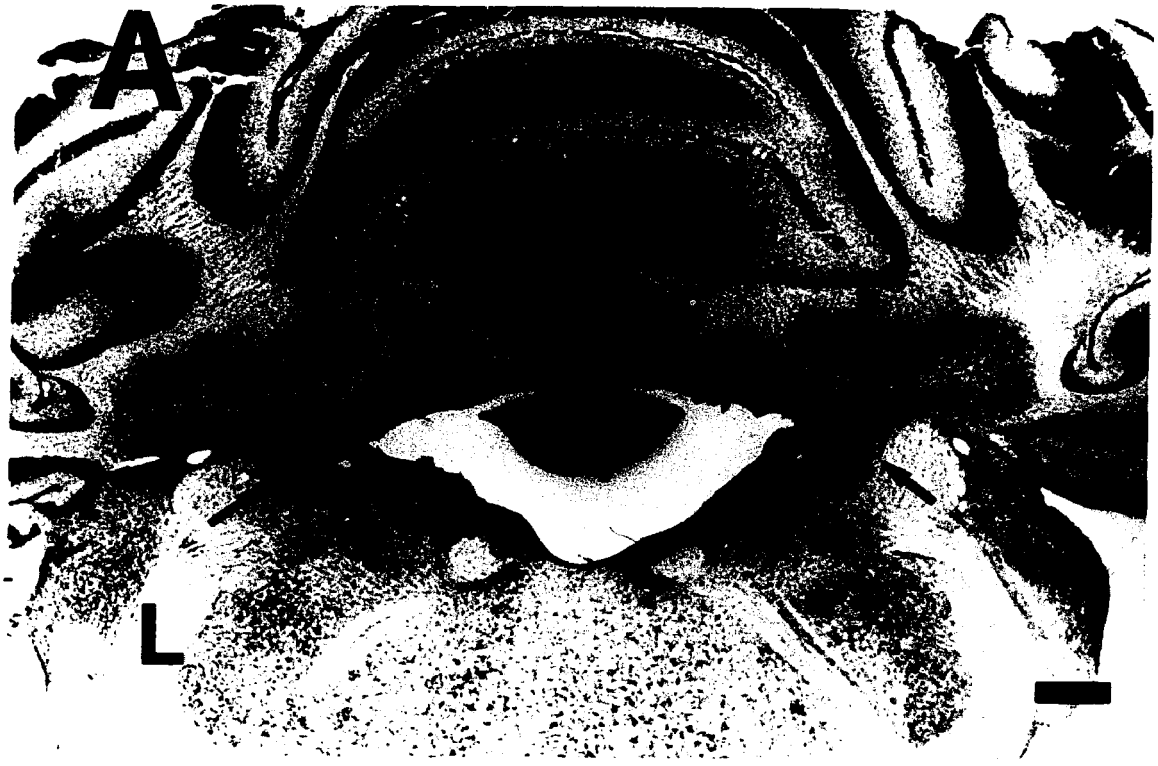
INT
LCN
SpV
3
INT
LCN
4V
MV
icp
PrH
sp5
4
LCN
PFl
SpV
MV
MVv
5
INT
LCN
LV
4V
MV
CN
PrH
sp5
6
LCN
SuV
INT
LV
MV
LV
SpV
7
7
1 mm
SuV
LCN
PFl
Fl
MV
LV
MVv
PrH
CN
sp5
7
ROSTRAL

Figure 9. The lesions seen in this case were the largest noted in the entire LCN sample. However, a small portion of the LCN remains caudally. Sections are 480 µm apart.


CAUDAL
1
INT
MCN
PFl
MV
4V
SpV
icp
sp5
PrH
MVv
2
MCN
INT
MV
DC
MVv
SpV
3
INT
MCN
DC
SpV
4V
MV
PrH
icp
sp5
4
MCN
INT
LV
LV
MV
SpV
PFl
MVv
5
SuV
INT
MV
4V
LV
CN
PrH
MVv
7
IC
1mm
6
SuV
PFl
Fl
LV
MV
MVv
CN
7
RPa
py
7
ROSTRAL

Figure 10. Characteristics of EAA-dLV lesions. A: bilateral EAA-dLV lesions (arrows) with minimal extension into ventral INT (arrowheads). L, left. Bar = 0.5 mm. B: enlargement of left EAA-dLV lesion shown in A. Note that large LV cells are still visible in ventral portions of the nucleus (arrow). Bar = 0.25 mm. C: myelin stain of coronal section through EAA-dLV lesions demonstrates preservation of fiber tracts that course through and adjacent to the dLV. Bar = 1 mm. D: myelin stain of coronal section at the level of the dLV from an unoperated control case is presented for comparison with C. Bar = 1 mm.


A
L


B


CY


D

$F(1, 70) = 20.06$, $p = .0001$, $\omega^2 = .118$] and MCN [$M = 34.7$ gm, $F(1, 70) = 11.58$, $p = .0011$, $\omega^2 = .078$] EAA lesion groups. There were no significant differences between the electrolytic lesion groups and the control group.

Analysis of the main effect of group on the Day 20 activity measure [$F(7, 70) = 1.83$, $p = .096$] suggested a tendency for the control group to be less active ($M = 292$) than the lesion groups ($M = 368$). However, there were no differences in activity among the lesion groups.

Figure 11 shows that all lesions produced a reduction in the total number of abnormal motor signs on Day 20 in comparison with the control group ($p < .008$, for all). The largest effect was produced by dLV lesions [$F(1, 70) = 32.33$, $p = .0001$, $\omega^2 = .30$]. This group differed significantly from the MCN lesion group [$F(1, 70) = 8.13$, $p = .0057$, $\omega^2 = .083$]. There were no other significant group differences. When components of the motor syndrome were analyzed separately, there were lesion effects on falls, clasps, and twisting, but not pivoting.

All lesions reduced the increase in the number of paw clasps normally seen in unoperated dt rats from Day 15 to Day 20 ($p < .005$, for all). The dLV lesions tended to be more effective than both MCN lesions ($S = 173.5$, $p = .011$) and EAA-dLV lesions ($S = 167.0$, $p = .033$) in reducing clasps. There were no other significant differences among the lesion groups.

The largest reduction of Day 20 twists was produced by dLV lesions [$F(1, 70) = 20.04$, $p = .0001$, $\omega^2 = .22$]. A significant reduction of Day 20 twists was also produced by EAA-dLV lesions [$F(1, 70) = 9.36$, $p = .0031$, $\omega^2 = .11$]. The dLV lesions were more effective than lesions of another midline structure, the MCN [$F(1, 70) = 9.18$, $p = .0034$, $\omega^2 = .10$]. Lesions of the other DCN did not produce significant effects when compared with the control group.

The dLV lesions significantly reduced the increase in the number of falls seen in unoperated dt rats from Day 15 to Day 20 [$F(1, 71) = 11.49$, $p = .0011$, $\omega^2 = .15$]. In addition, there was a tendency for INT [$F(1, 71) = 4.75$, $p = .033$, $\omega^2 = .067$] and EAA-MCN [$F(1, 71) = 4.06$, $p = .048$, $\omega^2 = .059$] lesions to do the same. No other lesions produced significant effects on the fall change score when compared with the control group.

Figure 12 shows the effects of DCN lesions on homing time, homing distance, righting time, and climbing points. The performance of unoperated dystonic control rats deteriorated on homing time, homing distance, righting, and climbing from Day 15 to Day 20. All lesions prevented deterioration on one or more measures of motor performance during this time. Overall, however, the dLV lesions had the largest and most widespread positive effects on motor performance.

All lesions reduced Day 20 righting time in comparison with the control group, $p < .01$ for all (Figure 12A). The

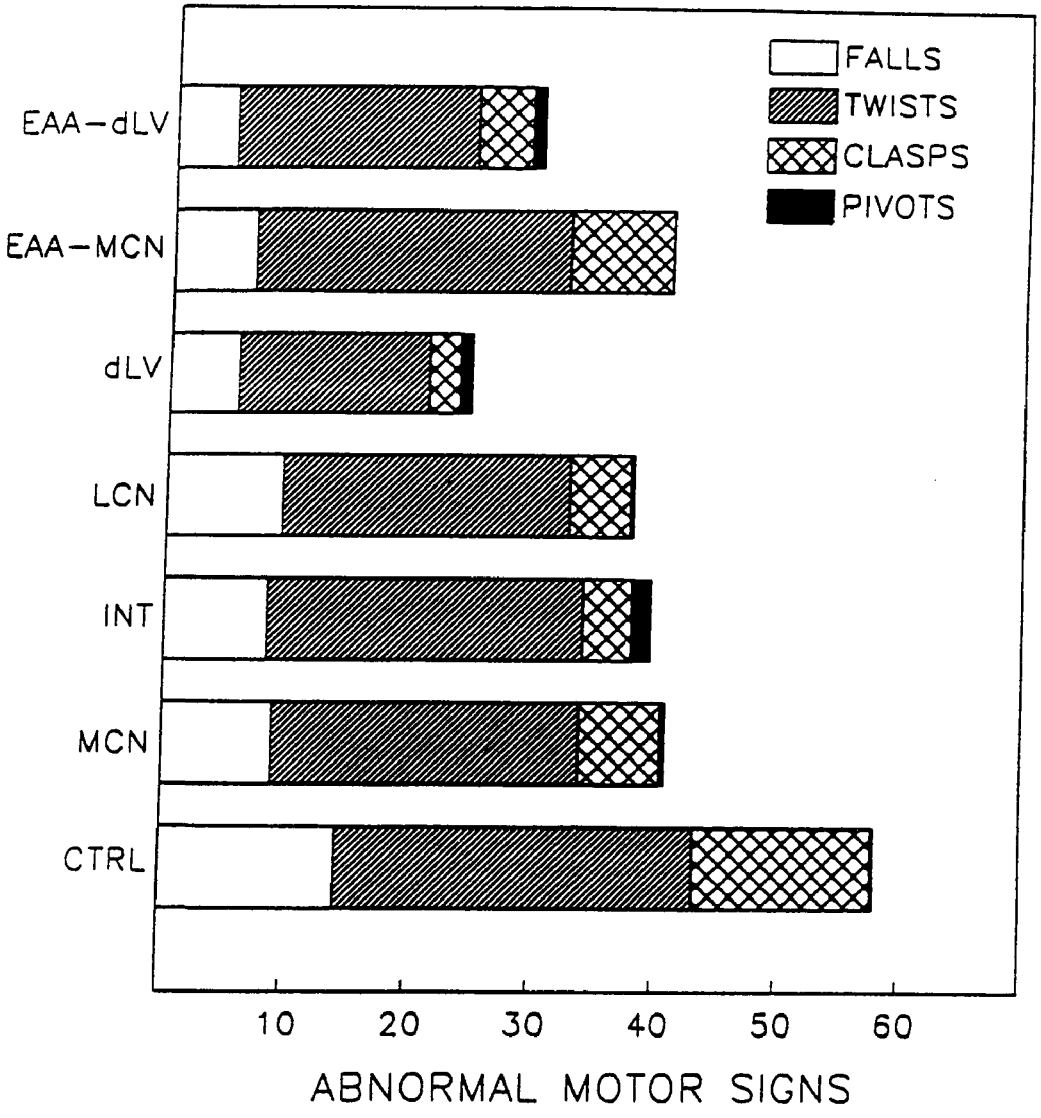
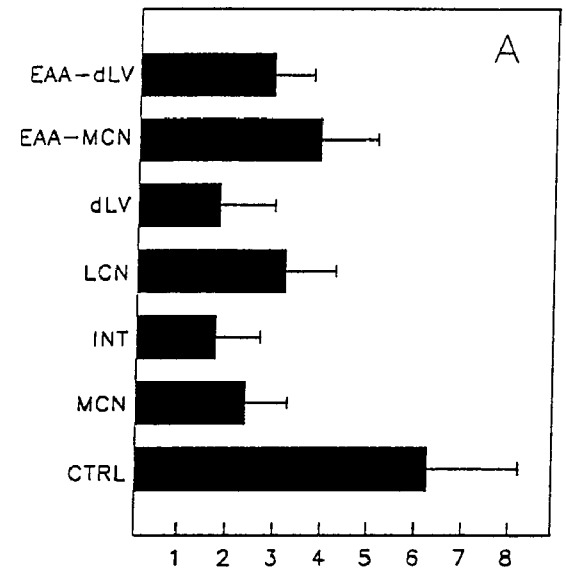
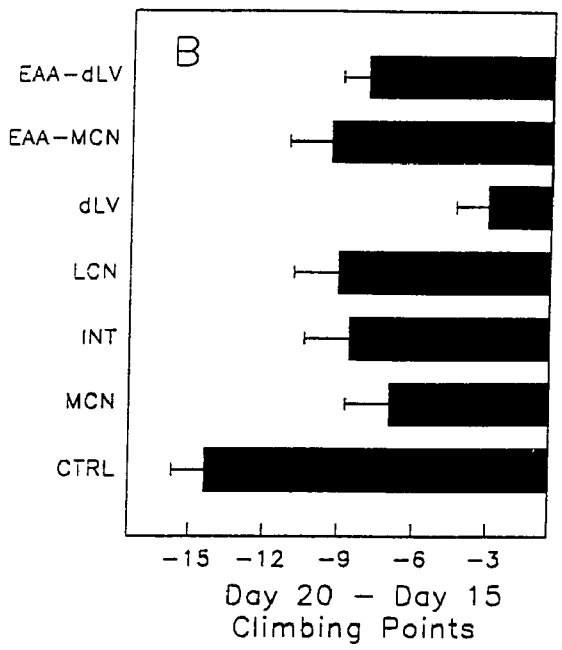
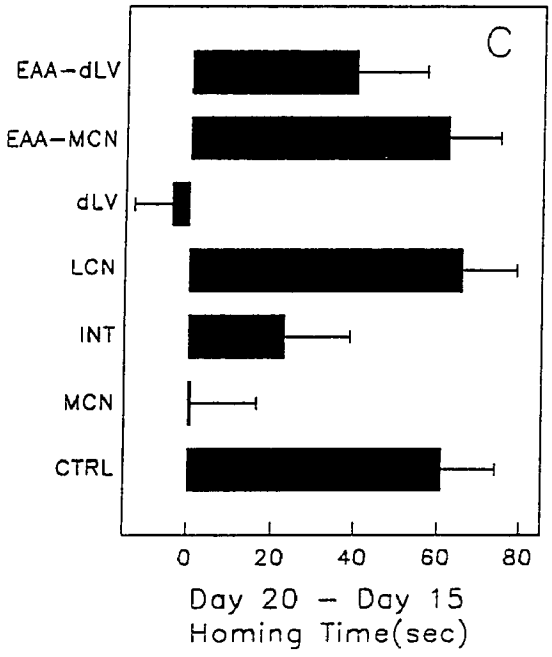


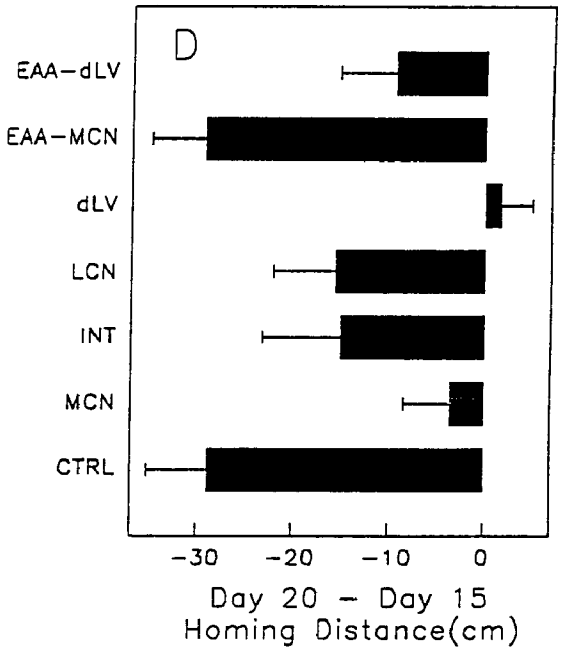
Figure 11. The number of Day 20 abnormal motor signs counted during a 5-min observation period. Four discrete motor signs were observed: falls, twists, clasps, pivots. All lesions produced a reduction in the total number of Day 20 abnormal signs in comparison to controls ($p < .008$, for all).

Figure 12. Lesion effects on four measures of motor performance. All values are mean ± SEM. All lesions reduced Day 20 righting time (A) in comparison to controls ($p < .01$, for all). The dLV group demonstrated the smallest deterioration in climbing performance (B), homing time (C), and homing distance (D).


A
EAA–dLV
EAA–MCN
dLV
LCN
INT
MCN
CTRL
1 2 3 4 5 6 7 8
Day 20 Righting Time(sec)


B
EAA–dLV
EAA–MCN
dLV
LCN
INT
MCN
CTRL
−15 −12 −9 −6 −3
Day 20 – Day 15
Climbing Points


C
EAA–dLV
EAA–MCN
dLV
LCN
INT
MCN
CTRL
0 20 40 60 80
Day 20 – Day 15
Homing Time(sec)


D
EAA–dLV
EAA–MCN
dLV
LCN
INT
MCN
CTRL
−30 −20 −10 0
Day 20 – Day 15
Homing Distance(cm)

largest effect was produced by dLV lesions [$F(1, 70) = 30.33$, $p = .0001$, $\omega^2 = .25$]. There were no significant differences among lesion groups.

All rats showed some loss in climbing ability between Days 15 and 20. The rats in the MCN, dLV, and EAA-dLV groups had the smallest decline in climbing scores between Days 15 and 20 in comparison with unlesioned control rats, $p < .002$ for all (Figure 12B). There was also a tendency for INT, LCN, and EAA-MCN lesions to prevent deterioration in climbing between Days 15 and 20 ($p < .05$, for all). The largest effect on climbing scores was seen in rats with dLV lesions ($S = 87.0$, $p = .0003$). In addition, the performance of the electrolytic dLV lesion group ($M = -3.08$) was better than that of the EAA-dLV lesion group ($M = -8.00$) ($S = 90.5$, $p = .011$). The remaining lesion groups did not differ from each other.

Homing time increased ($M = 60.7$ sec) and homing distance decreased ($M = -28.9$ cm) for the dystonic control group from Day 15 to Day 20 (Figures 12C and 12D). Only the dLV group demonstrated a statistically significant improvement in homing time ($S = 203.0$, $p = .0024$) and homing distance ($S = 102.0$, $p = .0044$) in comparison with the control group. However, MCN lesions tended to prevent deterioration in both homing time ($S = 87.0$, $p = .0061$) and homing distance ($S = 172.5$, $p = .0125$). Although there was no reliable difference between the electrolytic and EAA dLV groups, the deterioration in homing distance between Days 15

and 20 was smaller for the MCN lesion group than for the EAA-MCN lesion group (S = 170.5, p = .0031). No other statistically significant comparisons were noted.

Hanging time for the controls increased by a mean of 36.0 sec from Day 15 to Day 20. Hindlimb use by the control group increased by a mean of 0.9 during this time. There was no group effect on hanging time [$F(6, 71) = 0.10$, p = n.s.]. In addition, there were no statistically significant lesion effects on hindlimb use.

Discussion

From Postnatal Days 15 to 20, untreated dt rats show a progressive increase in the frequency of abnormal motor signs and progressive decrease on several measures of motor function including righting, climbing, and homing. Electrolytic and EAA lesions of the cerebellar nuclei and dLV improved motor performance and decreased the severity of the dt rat motor syndrome without impairing general activity. The performance of the dystonic rats on the hanging test suggests that the reductions in clinical signs and improvements in motor function demonstrated by the lesion groups were not the result of weakness or hypotonia.

No single lesion used in this study had effects as large as those obtained with CBX. Rats subjected to a total CBX showed a 90% reduction in abnormal motor signs with complete elimination of twisting and clasps. In the dt rat, CBX on Day 15 prevented deterioration in climbing, homing, and righting. Normal and dt rats that received CBX on Day

15 could not be distinguished on Day 20 righting or homing times. For example, after CBX on Day 15 or 20, righting times for dt rats were less than 1 sec. In this experiment, the largest effect on abnormal motor signs was the approximately 60% fewer signs in the electrolytic dLV lesion group in comparison to unoperated controls. Although selective lesions did not produce the profound effects of CBX, all selective lesions had a positive effect on the dt rat motor syndrome and reduced the deterioration in motor performance that occurs in unoperated dt rats. The finding that eliminating cerebellar output improves motor performance in mutants with an abnormal cerebellum is not unprecedented. Weaver mice, mutants with a clear morphological abnormality in the cerebellum, benefit from CBX (Grüsser-Cornehls, 1988). In these mice, lesions of the vermis are as effective as complete CBX.

The positive effect of the DCN and dLV lesions supports the hypothesis that the expression of the dt rat motor syndrome depends on abnormal cerebellar output, although no single region of the cerebellum appears to account for the entire motor syndrome. Several other lines of evidence suggest that cerebellar output is abnormal in the mutant. Glucose utilization (Brown & Lorden, 1989) and glutamic acid decarboxylase activity (Oltmans et al., 1986) is significantly increased in the dt rat MCN, INT, and LCN in comparison with phenotypically-normal controls. GABA receptor density in the dt rat MCN, INT, and LCN is

decreased compared to normal rats (Beales et al., 1990). Besides the biochemical findings, cells in the dt rat's MCN and INT fire more rapidly and rhythmically than those from normal rats; cells from the LCN were not sampled (Lorden et al., 1992). Although the dLV was not examined in any of these studies, similar abnormalities might be expected in view of the connections of this nucleus (Mehler & Rubertone, 1985; Voogd et al., 1985; Buisseret-Delmas, 1988a).

Cerebellar Functional Anatomy

Most functional descriptions of the cerebellum are based upon dividing it into three longitudinal zones with the flocculonodular lobe treated separately (Buisseret-Delmas, 1988a; 1988b; 1988c; Voogd et al., 1985). These three divisions each include cerebellar cortex, underlying white matter, and associated nuclei: (1) a medial zone projecting to the MCN and dLV; (2) an intermediate zone projecting to the INT; and (3) a lateral zone projecting to the LCN. The cerebellar nuclei or dLV provide the final output from each longitudinal zone.

The DCN demonstrate both divergence and convergence of projections (Bava et al., 1986; Rispal-Padel, 1987). The MCN projects to multiple mesencephalic sites and has bilateral, but mainly crossed, projections to the reticular and vestibular nuclei and spinal cord (Faull, 1978; Faull & Carman, 1978; Haroian et al., 1981; Voogd et al., 1985). Fiber sparing kainic acid lesions show that the MCN exerts a crossed inhibitory and an ipsilateral excitatory influence

on extensor postural tone (Imperato et al., 1984). The INT projects to the red nucleus, thalamus, pontine reticular tegmental nucleus, pretectal nuclei, zona incerta, medullary reticular formation, and superior colliculus (Faull, 1978; Faull & Carmen, 1978; Haroian et al., 1981; Voogd et al., 1985; Daniel et al., 1988). The LCN also projects to the red nucleus, thalamus, and medullary reticular formation (Faull, 1978; Faull & Carmen, 1978; Haroian et al., 1981; Voogd et al., 1985). Temporary cooling of the LCN in rats demonstrates that this nucleus is critical for preprogrammed voluntary movements, particularly those of the distal forelimbs (Moroz & Bures, 1981).

The lateral vestibulospinal tract originates mainly from the dLV and terminates in the anterior horn of the spinal cord (Mehler & Rubertone, 1985). In the rat, dLV projections to lumbosacral segments of the spinal cord predominate (Shamboul, 1980). The lateral vestibulospinal tract plays a critical role in the maintenance of tone in antigravity muscles so that balance can be preserved during various movements (Marlinsky, 1992). Unilateral lesions of the LVN can cause scoliosis in rats, thereby emphasizing the critical role of the LVN in the control of axial musculature (Barrios & Arrotegui, 1992). In view of the widespread projections from the DCN and dLV to important motor and premotor structures, an abnormal signal from these nuclei could have marked effects at many levels of the motor system.

dLV Lesions

In the present study, the dLV lesions were most effective in reducing clinical signs and improving motor performance. This suggests that the dLV, like the DCN, plays an important role in the expression of the dt rat motor syndrome. The EAA-dLV lesions were, however, less effective than electrolytic lesions. Because of the central location of the dLV, electrolytic lesions of this nucleus invariably and unavoidably damaged a larger portion of the output from adjacent nuclei than lesions of the DCN. Thus, the dLV lesions were probably more similar to CBX than DCN lesions in terms of extent. Although less effective than the electrolytic lesions, the EAA-dLV lesions were still more effective than the INT and LCN lesions. The particular effectiveness of electrolytic and EAA dLV lesions may be a consequence of the role that this structure plays in the maintenance of posture. Improvement in balance and posture would have a substantial impact on all the tests of motor performance used in this study.

MCN Lesions

On several measures, the MCN lesions were as effective or nearly as effective as the dLV lesions. This may be attributed to two factors. First, lesions of the MCN are likely to damage fibers of passage from the vermis to the dLV. It is possible that elimination of an abnormal signal from Purkinje cells is functionally better than leaving this connection intact. Second, the MCN plays an important role

in the maintenance of posture (Sprague & Chambers, 1953; 1954; Imperato et al., 1984). Lesions that improve the postural stability of the dt rats will produce a greater enhancement of their general performance on tests such as climbing, homing, and righting than lesions with effects that might be seen mainly in the distal extremities.

The results of the climbing and homing tests underscore the importance of extensor tone, balance, and posture as the framework upon which movement can occur. The largest effects on climbing and homing occurred in the dLV and MCN lesion groups. Similar but less marked effects were displayed by the EAA-dLV lesion group. However, the EAA-MCN group was only slightly better than controls on the climbing task and not significantly different from controls on the homing task. One possible explanation for the poor performance of the EAA-MCN group in comparison to the electrolytic MCN lesion group is that destruction of Purkinje cell projections to the dLV improves the motor function of dt rats.

The individual DCN and dLV operate in parallel so that spatially and temporally correct movements can occur (Thach et al., 1992). The inability of behavioral and motor testing to clearly differentiate lesion groups may be explained by the parallel processing of different movement components by individual nuclei. Because the motor tasks used in this study were functionally complex, it is likely that more than one nucleus was involved in each task.

Lesions of individual nuclei were associated with modest generalized improvements in motor function and decreases in abnormal motor signs rather than specific changes on a particular test or a decrease of one abnormal motor sign.

EXPERIMENT #3

What are the distinguishing features of the abnormal signal from the cerebellar nuclei?

Althought lesion studies established the DCN as critical to the dt rat motor syndrome, they did not reveal the mechanisms by which cerebellar output distorts normal motor function. Because the DCN have projections to multiple motor and premotor nuclei, an abnormality at the level of the DCN could have a profound influence on motor function. Experiment #3, was designed to characterize neuronal signals in the DCN of the awake dt rat. The results of this experiment form the framework for future studies to delineate the effects of abnormal cerebellar output on the motor system.

Awake recordings from the DCN were also designed to clarify the conflicting results of biochemical and electrophysiological studies of the olivo-cerebellar loop in the dt rat. When compared to normal rats, DCN GAD activity is increased (Oltmans et al., 1986), DCN muscimol binding is decreased (Beales et al., 1990), DCN glucose utilization is increased (Brown & Lorden, 1989), Purkinje cell firing rates are decreased (Stratton et al., 1988), and MCN firing rates are increased (Lorden et al., 1992) in the dt rat. In the

absence of electrophysiological information, the biochemical data would suggest that Purkinje cell firing rates are high and DCN firing rates are low in the dt rat. The discrepancy between firing rates and biochemical indexes may be clarified with awake recordings since measures of GAD activity, muscimol binding, and glucose utilization were not influenced by anesthetic drugs, whereas neuronal firing rates were obtained under urethane anesthesia.

Under urethane anesthesia, the firing rate of MCN cells in dt rats was nearly twice the rate seen in normals (Lorden et al., 1992). Interspike interval (ISI) histograms were normally distributed in unaffected pups compared to the leptokurtic pattern shown by MCN and INT cells from the mutants. The findings under urethane anesthesia may be significantly different from DCN neuronal activity in awake dt rats. The differences in MCN and INT neuronal activity between normal and dt rats may be caused by differential sensitivity to urethane. In addition, LCN neuronal activity has not been compared to MCN and INT neuronal activity in the dt rat.

There are several other reasons why the results of awake recordings from the DCN in awake dt rats may be important. First, glucose utilization could be correlated to an abnormality in neuronal activity within a circumscribed brain region. Correlates between glucose utilization and neuronal firing rates are not possible in human imaging studies. Second, the effects of urethane on

DCN neuronal firing properties can be determined. Third, overt differences in DCN neuronal firing patterns between normal and dystonic rats might provide impetus for microelectrode stereotactic localization in the treatment of intractable dystonia (Slaughter et al., 1970; Zervas, 1977).

Method

Single-unit activity was obtained from the MCN, INT, and LCN in awake normal (N = 25) and dt (N = 24; Jfl:SD-dt) rats between and including Postnatal Days 17 and 25. The 49 rats came from a total of 20 litters. All pups were housed with their dams until sacrifice and removed from their cages only for surgery and recording from the DCN. A total of 96 cells was isolated.

Surgery

Prior to surgery, indifferent electrodes, U-shaped metal skull posts, and circular acrylic dams were constructed. The skull posts were made by bending 1-cm sections of 28-gauge stainless steel tubing into a U-shape with the base of the "U" kept as linear as possible to limit dural deformation (see below). The indifferent electrode was made by soldering one end of a 2-cm section of 38-gauge copper wire (Cooner Wire Co.) to a filister head stainless steel screw (0-80 x 1/32, 3.5 mm long) and the other end to a male gold connector pin (Amphenol). The acrylic dams were made by cutting 23-gauge needle caps (Becton Dickinson & Co.) into 3 mm-diameter circular sections.

Rats were anesthetized for surgery with intraperitoneal injections of ketamine hydrochloride (45 mg/kg) and xylazine (5 mg/kg). A midline incision was made from the nasal bones to the mid-nuchal region. A monopolar cautery was used to dissect muscle attachments from the occipital bone and to limit bleeding from the scalp margins, muscle, and other soft tissues. A drill bit was used to make a hole in the posterior part of one parietal bone. The indifferent lead was screwed through this hole in the parietal skull and the underlying dura into posterior parietal cortex. Small burr holes were made in the frontal and caudal occipital bones and the U-shaped skull posts were tunneled extra-durally between the pairs of burr holes in each bone.

For access to the MCN and INT, a burr hole was made over the midline cerebellum at a point 2.15 mm posterior to lambda. For access to the LCN, burr holes were made 2.10 mm posterior to lambda and 2.80 mm lateral to the midline. An acrylic dam was placed around the cerebellar access site to prevent acrylic from occluding the burr hole. A 17.5 mm long, flat-head, machine screw (Elco Industries, Inc.) was placed, head down, on the midline, mid-parietal region and the entire assembly was secured to the skull with dental acrylic.

Microelectrode Recordings

Single-unit recordings were obtained at least 4 hours after full recovery from anesthesia or on the day following surgery. Rats were placed in a soft foam-lined container

and the head bolt was secured to a head restraint. The head restraint was designed to fit the dovetail slot on the nosepiece of a Kopf stereotactic frame (David Kopf, Inc.). Potentials were recorded extra-cellularly with parylene-coated tungsten microelectrodes (1-2 Mohms; Microprobe, Inc.), amplified (A.M. Systems, Inc.), displayed on an oscilloscope (Tektronix, Inc.) and played through an audio monitor (Grass Instrument Co.). Unit activity was digitized and stored on VHS tape for offline analysis using R.C. Electronics Computerscope software. Small electrolytic marking lesions (75 µamps, anodal, 10 sec) were created at each recording site with a direct current lesion maker (Grass Instrument Co.). Ratemeter histograms, ISI histograms, and autocorrelograms were generated for each cell. Between 3000 and 8200 spikes were obtained for each cell. There were no significant differences between normal and dt rats in the number of spikes recorded.

Data Analysis

Ratemeter histograms for the entire duration of each cell's spike train were constructed with consecutive 2-sec bins. The variance of the bin counts was used as a general measure of neuronal firing rate variability.

Autocorrelograms (Gerstein and Kiang, 1960; Perkel et al., 1967) were formed from 4-msec bins with a lag time of 524 msec (see Figures 13-17). Individual bin counts were divided by the total number of bin counts and the bin duration to determine the probability for each bin. The bin

probabilities were multiplied by 10,000 to simplify graphical presentation. Bursting Index 1 (BI1) was calculated by dividing the largest bin count at the largest autocorrelogram peak occurring after the 20-msec bin by the lowest bin count in the next valley. BI1 was designed to measure the tendency for bursts to occur at a particular interburst interval (IBI) to the exclusion of other IBIs.

Univariate analysis of ISIs generated measures of variance and skewness. An ISI histogram with 2-msec bins was created for each cell (Figures 13-17). The cumulative percentages for each ISI histogram were used to calculate Bursting Index 2 (BI2). BI2 was defined as the percentage of ISIs that were greater than twice as long as the ISI at the first peak of the ISI histogram. BI2 was designed as an approximate measure of the percentage of ISIs occurring between bursts of neuronal activity.

Nonlinear regression with SAS (SAS Research Institute) was used to model each cell's ISI histogram with a single gamma distribution (Johnson and Kotz, 1970) of the form

$$f(t) = \left(\frac{A}{C}\right)\left(\frac{t}{C}\right)^{(M/C)} e^{(-t/C)} \qquad (1)$$

The variable t represents time in milliseconds. Least-squares estimates of the parameters of the gamma distribution were generated by use of one or more of the following iterative algorithms: gradient method, Newton

method, modified Gauss-Newton method, Marquardt method, and multivariate secant method. The A parameter is a scaling factor and the C parameter helps to determine the overall shape of the function. The derivative of f(t), f'(t), defines the slope of f(t) at any time t > 0. The maximum value or mode of f(t) can be determined by setting f'(t) = 0 and solving for t. The mode of (1) will occur at time M. The squared correlation coefficient R^2 was determined for regression of (1) on each ISI histogram. Each ISI histogram with an R^2 less than 0.90 was then modeled with a double gamma distribution

$$f(t) = A*\left(\left(p*\left(\frac{1}{C1}\right)\left(\frac{t}{C1}\right)^{(M1/C1)}e^{(-t/C1)}\right) + \left((1-p)*\left(\frac{1}{C2}\right)\left(\frac{t}{C2}\right)^{(M2/C2)}e^{(-t/C2)}\right)\right) \quad (2)$$

The parameters C1 and M1 are associated with the first gamma component of the distribution. The parameters C2 and M2 are associated with the second gamma component. The parameter p determines, in part, the proportion of the distribution defined by each individual gamma component. An ISI histogram was considered bimodal if the increment in R^2 produced by the double gamma distribution was statistically significant ($p < .05$).

Seven dependent variables (DVs) were calculated or measured for each cell's spike train: frequency, ISI variance, ISI skewness, ratemeter variance, BI1, BI2, and the temporal location of the first autocorrelogram peak

occurring after 20 msec. Based on a priori hypotheses, four DVs (frequency, BI1, BI2, ratemeter variance) were used in a 2 x 3 between-"spike train" multivariate analysis of variance (MANOVA). Other DVs were excluded from the MANOVA because of significant violations of the homogeneity of variance-covariance assumption. Because of minor violations of this assumption by the DVs that were included in the MANOVA, Pillai's criterion was used instead of Wilks' Lambda to evaluate multivariate significance (Olsen, 1979). Independent variables were phenotype (dystonic and normal) and nucleus (MCN, INT, LCN). Specific comparisons of phenotypes across all cells were performed for significant univariate effects. The MCN and LCN cells from dystonic rats were compared to test the hypothesis that there is no difference between the medial and lateral parts of the DCN in terms of neuronal activity. Regression analysis of BI1 and BI2 with age as the independent variable was performed to test the hypothesis that bursting activity increases with age in the dt rat.

Results

Univariate statistics are presented in Tables 1 and 2. Correlation matrices for normal and dt rats appear in Tables 3 and 4. As determined by an analysis of variance separate from the MANOVA described above, there was no statistically significant postnatal age difference between the normal (M = 20.3) and dt (M = 21.2) rats used, although

Figure 13. Spike train characteristics of an MCN cell from a 20-day-old normal rat. A. Representative portion of spike train. B. Interspike interval histogram. C. Autocorrelogram. D. Ratemeter histogram.

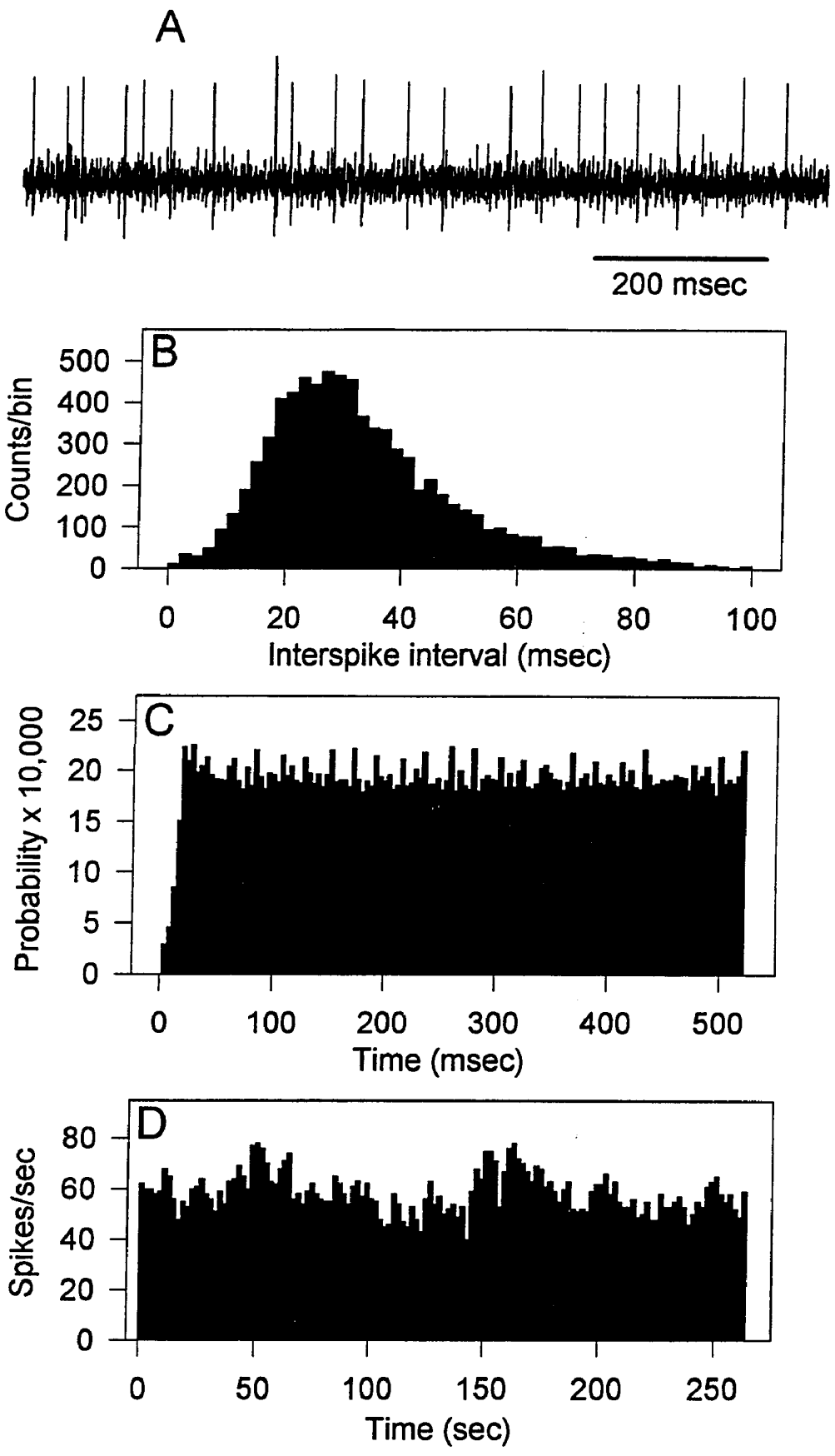

A
200 msec
B
Counts/bin
500
400
300
200
100
0
0
20
40
60
80
100
Interspike interval (msec)
C
Probability x 10,000
25
20
15
10
5
0
0
100
200
300
400
500
Time (msec)
D
Spikes/sec
80
60
40
20
0
0
50
100
150
200
250
Time (sec)

Figure 14. Spike train characteristics of an MCN cell from a 17-day-old dt rat. A. Representative portion of spike train. B. Interspike interval histogram. C. Autocorrelogram. D. Ratemeter histogram.

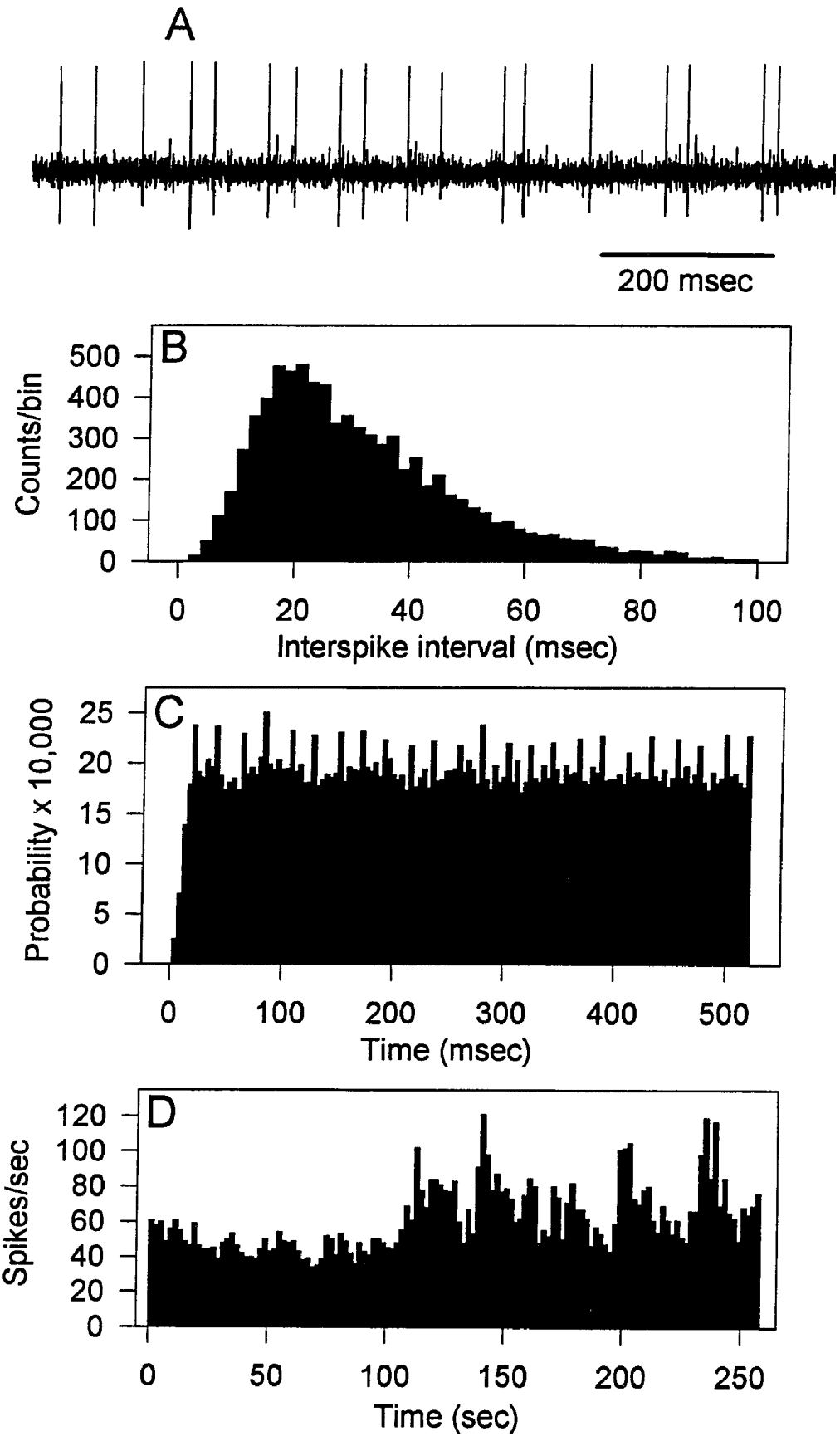

A
200 msec
B
Counts/bin
500
400
300
200
100
0
0
20
40
60
80
100
Interspike interval (msec)
C
Probability x 10,000
25
20
15
10
5
0
0
100
200
300
400
500
Time (msec)
D
Spikes/sec
120
100
80
60
40
20
0
0
50
100
150
200
250
Time (sec)

Figure 15. Spike train characteristics of an MCN cell from a 19-day-old dt rat. A. Representative portion of spike train. B. Interspike interval histogram. C. Autocorrelogram. D. Ratemeter histogram.

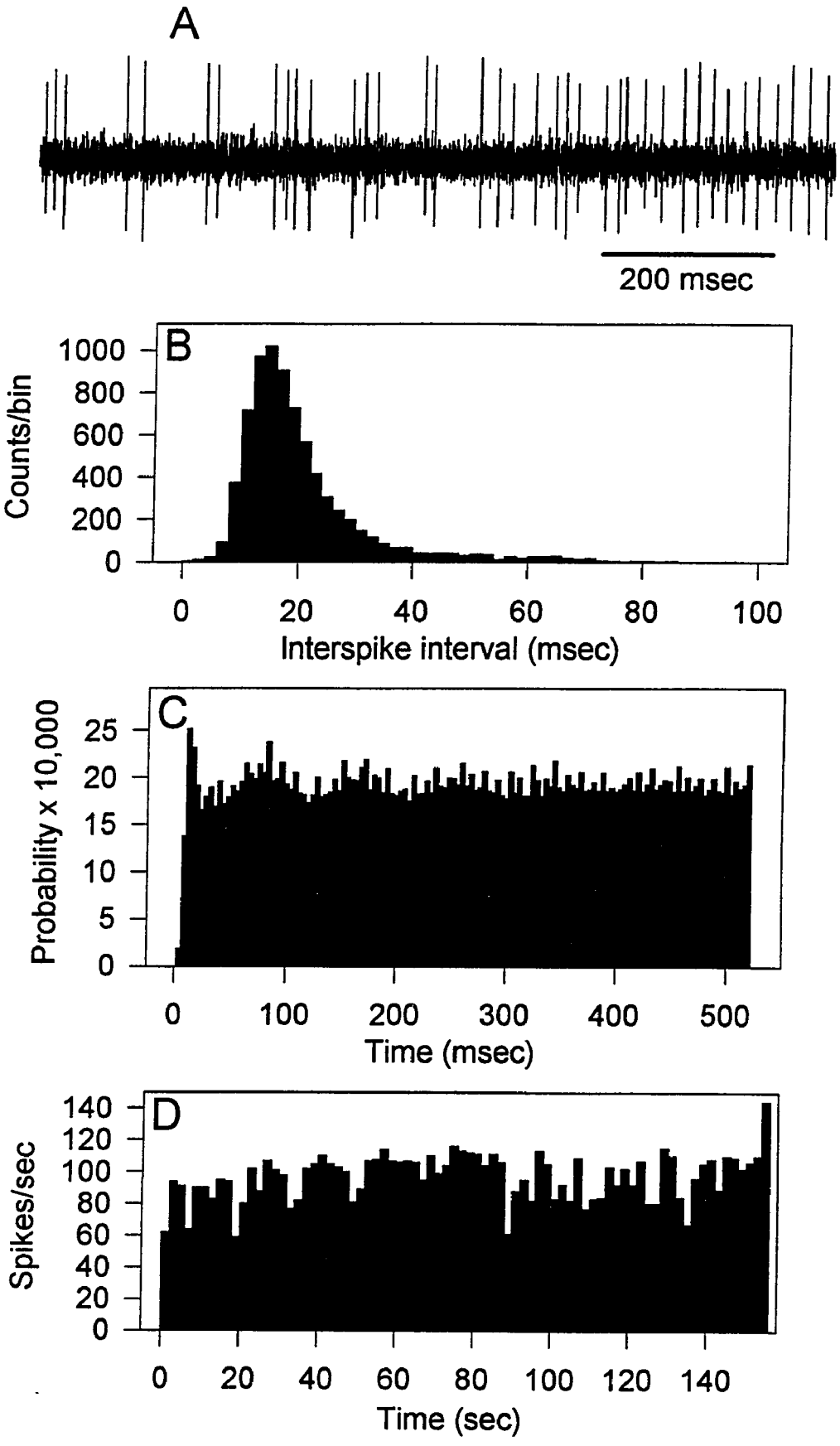

A
200 msec
B
Counts/bin
1000
800
600
400
200
0
0
20
40
60
80
100
Interspike interval (msec)
C
Probability x 10,000
25
20
15
10
5
0
0
100
200
300
400
500
Time (msec)
D
Spikes/sec
140
120
100
80
60
40
20
0
0
20
40
60
80
100
120
140
Time (sec)

Figure 16. Spike train characteristics of an LCN cell from a 23-day-old dt rat. A. Representative portion of spike train. B. Interspike interval histogram. C. Autocorrelogram. D. Ratemeter histogram.

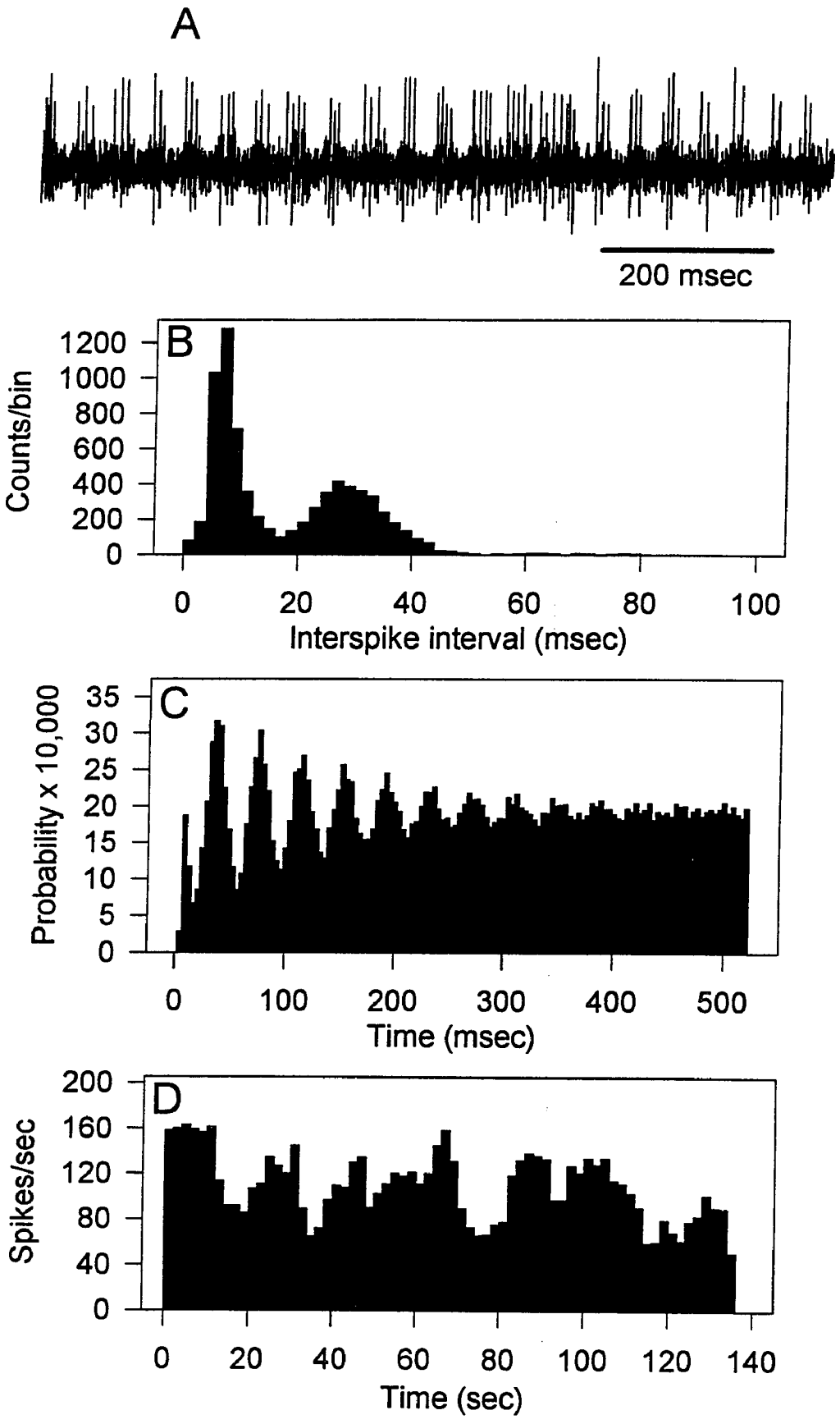
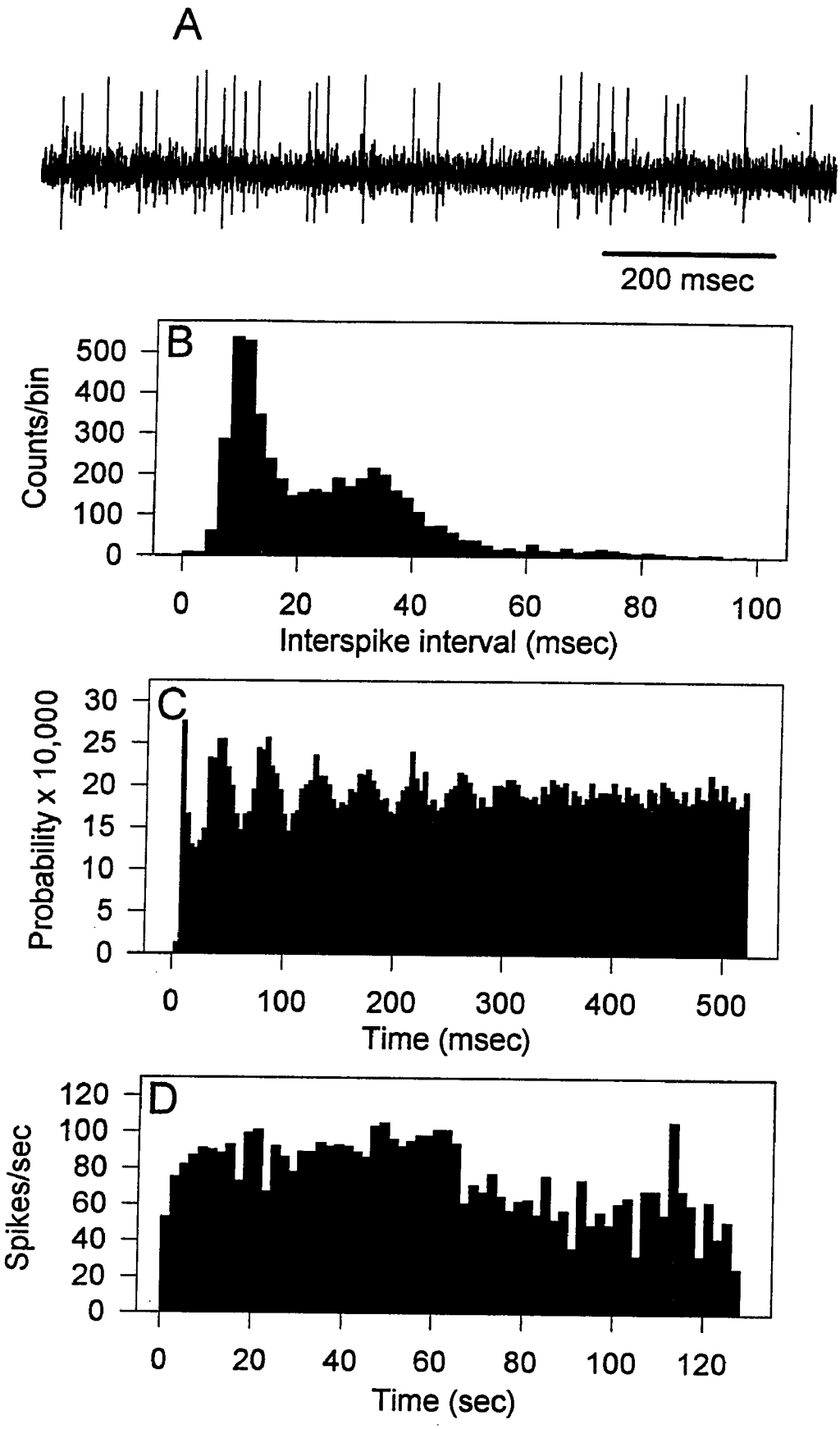

A
200 msec
B
Counts/bin
1200
1000
800
600
400
200
0
0
20
40
60
80
100
Interspike interval (msec)
C
Probability x 10,000
35
30
25
20
15
10
5
0
0
100
200
300
400
500
Time (msec)
D
Spikes/sec
200
160
120
80
40
0
0
20
40
60
80
100
120
140
Time (sec)

Figure 17. Spike train characteristics of an LCN cell from a 24-day-old dt rat. A. Representative portion of spike train. B. Interspike interval histogram. C. Autocorrelogram. D. Ratemeter histogram.


A
200 msec
B
Counts/bin
500
400
300
200
100
0
0
20
40
60
80
100
Interspike interval (msec)
C
Probability x 10,000
30
25
20
15
10
5
0
0
100
200
300
400
500
Time (msec)
D
Spikes/sec
120
100
80
60
40
20
0
0
20
40
60
80
100
120
Time (sec)

there was a trend for cells from dt rats to be from older rats ($F(1, 95) = 3.49$, $p = .065$).

The distinctiveness of DCN cells from dt rats is apparent from comparison of Figures 13-17. For both normal and dt rats, a burst was defined as a spike or group of spikes occurring after a relatively long ISI. The spike trains from normal rats exhibited occasional bursts of variable duration. In contrast, the number of spikes per burst varied over a narrow range for DCN cells from dt rats. For example, the DCN cell's spike train shown in Figure 16 has only one to three spikes per burst.

In Figure 16A, note the sinusoidal background noise with an approximate frequency of 25 Hz that corresponds to the frequency of bursting. This finding was common in DCN cells from older dt rats and suggests that the population of neurons around the isolated single-unit are firing in phase.

With the use of Pillai's criterion, the combined DVs (frequency, BI, BI2, ratemeter variance) were significantly affected by both nucleus ($F(8, 176) = 2.93$, $p < .005$) and phenotype ($F(4, 87) = 6.50$, $p < .0001$), but not by their interaction ($F(4, 176) = .72$, p = n.s.). Employing the Holm procedure (Holm, 1979; Seamen et al., 1991) to maintain family-wise error control ($\alpha = .05$), there were significant univariate effects of nucleus on BI2 ($F(2, 95) = 7.54$, $p < .001$) and of phenotype on both BI1 ($F(1, 95) = 12.45$, $p < .001$) and BI2 ($F(1, 95) = 13.66$, $p < .001$). There were

Table 1

Deep cerebellar nuclei neuronal spike train characteristics (mean ± SEM) for normal rats

	MCN	INT	LCN	All cells
Number of cells	18	15	14	47
Age (days)	20.6 ± 0.5	20.0 ± 0.6	20.3 ± 0.6	20.3 ± 0.3
Frequency	47.0 ± 2.9	39.7 ± 5.9	37.8 ± 5.5	41.9 ± 2.8
ISI variance	286.3 ± 60.3	719.7 ± 204.3	3866.6 ± 1526.1	1491.1 ± 503.4
ISI skewness	6.47 ± 1.80	7.37 ± 3.30	7.48 ± 1.84	7.06 ± 1.35
Ratemeter variance	437.5 ± 141.0	462.4 ± 191.0	531.7 ± 106.9	473.5 ± 85.7
Auto-correlogram peak (msec)	67.9 ± 12.5	66.7 ± 11.4	53.1 ± 6.9	63.1 ± 6.3
BI1	1.15 ± 0.02	1.20 ± 0.04	1.16 ± 0.02	1.17 ± 0.02
BI2	14.03 ± 1.87	16.44 ± 3.86	29.24 ± 4.00	19.33 ± 2.05
1st peak ISI histogram (msec)	18.26 ± 1.38	20.53 ± 2.29	13.05 ± 0.80	17.43 ± 1.01
Number bimodal	0	2	0	2
2nd peak bimodal ISI histograms (msec)	none	52.55 ± 4.38	none	52.55 ± 4.38

no univariate interaction effects. BI2 tended to increase from the medial-to-lateral DCN for both normal and dt rats.

Table 2

Deep cerebellar nuclei neuronal spike train characteristics (mean ± SEM) for dt rats

	MCN	INT	LCN	All cells
Number of cells	18	15	16	49
Age (days)	21.4 ± 0.6	21.2 ± 0.7	20.9 ± 0.4	21.2 ± 0.3
Frequency	50.3 ± 3.5	48.3 ± 4.6	36.2 ± 3.4	45.1 ± 2.4
ISI variance	956.0 ± 611.6	641.4 ± 270.1	3033.5 ± 983.7	1538.1 ± 422.1
ISI skewness	6.16 ± 1.75	7.37 ± 2.87	9.23 ± 2.14	7.54 ± 2.20
Ratemeter variance	942.6 ± 428.2	317.1 ± 72.0	830.0 ± 200.3	714.3 ± 173.2
Auto-correlogram peak (msec)	54.2 ± 6.2	56.0 ± 12.3	52.0 ± 5.4	54.0 ± 4.7
BI1	2.08 ± 0.40	1.62 ± 0.19	1.65 ± 0.19	1.80 ± 0.29
BI2	30.93 ± 5.08	24.63 ± 4.80	43.02 ± 4.80	32.95 ± 3.05
1st peak ISI histogram (msec)	10.79 ± 0.77	15.36 ± 1.85	11.74 ± 1.25	12.50 ± 0.79
Number bimodal	8	3	9	20
2nd peak bimodal ISI histograms (msec)	33.65 ± 2.67	30.76 ± 10.46	33.65 ± 2.70	33.22 ± 2.05

Both BI1 and BI2 were higher for DCN cells from dt rats in comparison to normal rats (Tables 1 and 2). There was no effect of nucleus or phenotype on ratemeter variance. There

was a trend for nucleus to affect frequency ($F(2, 95) = 3.56$, $p = .033$). For both normal and dt rats, MCN cells tended to fire more rapidly than INT cells which, in turn, tended to fire more rapidly than LCN cells. Phenotype did not affect average frequency. Nucleus did not affect BI1.

The cells from the dt rat's MCN and LCN differed on BI2 ($F(1, 95) = 4.24$, $p < .05$), but not on BI1. For the dt rat, BI2 was higher for the LCN ($M = 43.02$) than the MCN ($M = 30.93$). However, the difference in bursting activity between cells in the LCN and MCN may not be a specific manifestation of the dt rat's disordered olivo-cerebellar physiology since a similar difference between the MCN and LCN is also seen in normal rats (Table 1).

A step-down analysis was used to determine the unique contribution of BI1 to the separation of phenotypes with BI2 treated as a covariate. The multivariate analysis of covariance showed that BI1 uniquely contributed to the of the separation of phenotypes demonstrated by MANOVA ($F(1,95) = 4.02$, $p < .05$).

Linear regression analysis for the dystonic phenotype showed that there was no relationship of BI1 with age ($F(1,48) = 1.49$, $p = .23$). However, there was a significant increase in BI2 with age ($F(1, 48) = 5.016$, $p < .03$, $R^2 = .096$).

ISI skewness and variance increased from the medial-to-lateral DCN for both normal and dystonic rats. Because low average frequency is associated with longer ISIs, ISI

Table 3

Correlation matrix of dependent variables for normal rats

	Age	Frequency	ISI variance	ISI skewness	Ratemeter variance	BI1	BI2
Age	1	.21	-0.18	.36	.41*	-0.13	-0.21
Frequency		1	-0.37*	.27	.60*	-0.23	-0.56*
ISI variance			1	.33*	-0.06	.10	.44*
ISI skewness				1	.69*	-0.14	-0.03
Ratemeter variance					1	-0.33*	-0.04
BI1						1	-0.02
BI2							1

*p < .05

variance is negatively correlated with average frequency. Although positive skewness will initially increase as the tail of a distribution increases in area, it will decrease when the longer ISIs begin to form a distinct second peak. Therefore, ISI skewness, in isolation, is not useful for distinguishing DCN cells from normal and dt rats.

The median and mode of the autocorrelogram peak were at 40 msec for both dt and normal rats. Twelve out of 47 autocorrelogram peaks for normal rats and 16 out of 49 for dystonic rats occurred at 40 msec. The location of the autocorrelogram peak may reflect an intrinsic property of DCN cells.

Table 4

Correlation matrix of dependent variables for dt rats

	Age	Frequency	ISI variance	ISI skewness	Ratemeter variance	BI1	BI2
Age	1	.18	-0.09	-0.25	.33*	.18	.31*
Frequency		1	-0.56*	-0.03	.03	-0.15	-0.49*
ISI variance			1	.31*	.29*	-0.13	.45*
ISI skewness				1	.19	-0.25	-0.12
Ratemeter variance					1	-0.09	.09
BI1						1	.45*
BI2							1

*$p < .05$

Twenty of the 49 spike trains obtained from the DCN of dt rats exhibited statistically significant second peaks on their ISI histograms. In contrast, only 2 of 47 spike trains from normal rats had bimodal ISI histograms. The bursting activity of DCN cells from dt rats is also reflected in the locations of the ISI histogram peaks. For a bursting cell, the first peak of the ISI histogram represents the time between spikes within bursts. The second peak of the ISI histogram represents the time between the last spike of a burst and the first spike of the next burst. The first ISI histogram peak occurred earlier in cells from the dt rats' DCN (M = 12.50) than in normal rats'

DCN (M = 17.43). The average location of the second ISI histogram peak in dt rats is consistent with the most common location of the largest autocorrelogram peak.

Discussion

Advantages of Awake Physiology

The development of a method to record from awake neonatal rats may prove to be a crucial step in characterizing the abnormal physiology of the dt rat. In addition, awake recordings allow for more confident comparisons of biochemical and electrophysiological findings since the biochemical experiments previously performed on the dt rat were not influenced by anesthesia. Now it may be possible to directly relate the dt rat's dysfunctional olivo-cerebellar physiology to its expression as a dystonic motor syndrome.

The effects of urethane on DCN neuronal firing rates are striking when compared to the results from awake recordings. Under urethane anesthesia (Lorden et al., 1992), MCN firing rates were significantly higher in dt rats (M = 23.7) compared to normal rats (M = 12.8). INT firing rates were also higher in the dt rat than in normal rats although these differences were not statistically significant. In contrast to the results obtained under general anesthesia, MCN and INT firing rates were much higher in both normal and dt rats in the awake preparation. The average firing rate for MCN cells was 47.0 in normal rats and 50.3 in dt rats. The differential sensitivity of

normal and dt rats to urethane should now be considered in the interpretation of previous single-unit recordings obtained in the dt rat.

Bursting Activity

Although DCN neuronal firing rates were not different between normal and dt rats in the awake preparation, the pattern of firing was markedly different. DCN cells from dt rats showed frequent short bursts of activity. These bursts usually consisted of only one to five spikes and, in this regard, appear to be different from bursts of activity by DCN neurons that occur during normal motor activity. For example, in the oculomotor region of the fastigial nucleus in monkeys, bursts with at least five and frequently more than ten spikes are associated with saccadic eye movements (Ohtsuka & Noda, 1991). Phasic response cells in the DCN of monkeys also exhibit five or more spikes at specific points during an arm movement (MacKay, 1988).

Two indexes, BI1 and BI2, were developed to characterize the bursting activity of DCN neurons. BI1 measures the tendency for IBIs to occur at one frequency to the exclusion of other frequencies. Because there was no rhythmicity to the bursts shown by cells from normal rats, BI1 was able to distinguish DCN cells from normal and dt rats. However, because the increase of BI1 with age was not statistically significant in dt rats, BI1 does not correlate well with the severity of the dt rat motor syndrome. BI2 provides an estimate of the frequency of bursting activity

within a spike train. BI2 clearly distinguished normal from dt rats and had a positive linear relationship with age in the dt rats. Therefore, BI2 is a better measure than BI1 of the severity of the dt rat motor syndrome. Step-down analysis showed that BI1 and BI2 measure relatively distinct aspects of bursting activity.

The bimodal ISI histograms seen with 41% of cells from dt rats is a striking representation of bursting activity. However, the statistical demonstration of bimodality with nonlinear curve fitting is time consuming and cannot be done online. In contrast, BI2 is a simple, practical, approximate measure of bursting that could be used to characterize neurons during human microelectrode-guided stereotactic surgery.

Abnormal DCN Activity

The results of selective elimination of cerebellar output suggested that all of the individual DCN and the dLV contribute to the dt rat motor syndrome. Single-unit recordings from the MCN, INT, and LCN confirm the conclusions of the lesion studies by showing that the cerebellar output signal from each of the DCN is abnormal. The dt rat's disordered olivo-cerebellar physiology does not appear to spare any of the cerebellar output nuclei. In addition, no single nucleus stands out as being much more abnormal than its neighbors. The two measures of bursting used in this experiment were the primary indicators of cellular abnormality. There was no effect of nucleus on

BI1. The effect of nucleus on BI2 was not qualified by an interaction with phenotype. Thus, BI2 was greater for LCN than MCN cells in both normal and dt rats.

An analysis of in vitro recordings from the DCN (Jahnsen, 1986), the in vivo response of DCN neurons in the climbing fiber deafferented rat to iontophoretically applied GABA (Billard & Batini, 1991), the long-term consequences of climbing fiber deafferentation in the DCN and LVN (Karachot et al., 1987; Billard & Daniel, 1988) and DCN GABAergic activity in the dt rat have formed the framework for the hypothesis that pathological hyperpolarization of the DCN neuronal membrane is responsible for the highly distinctive DCN spike train characteristics seen in the dt rat. This hypothesis will form the basis for future in vitro investigations of the intrinsic electrophysiological properties of the DCN in the dt rat.

3-AP Treated Rat

The neurotoxin 3-AP destroys IO neurons (Desclin & Escubi, 1974) and eliminates the climbing fiber projection to Purkinje cells. The 3-AP treated rat develops a movement disorder with features similar to the dt rat motor syndrome (Lorden et al., 1984; Sukin et al., 1987). However, while the dt rat motor syndrome gets progressively worse with increasing postnatal age, the 3-AP treated rat typically shows some degree of motor recovery and change in the signs characteristic of its motor syndrome during the weeks and months following IO lesioning. Physiological studies of the

3-AP rat may provide considerable insight into the pathophysiological mechanisms operative in the dt rat.

The initial effects of climbing fiber destruction are an elimination of Purkinje cell complex spikes (Colin et al., 1980; Savio & Tempia, 1985), an increase in Purkinje cell simple spike rate (Colin et al., 1980; Savio & Tempia, 1985), increased glucose utilization in the DCN (Batini et al., 1984) and decreased neuronal firing rates in the DCN and LVN (Batini & Billard, 1985). The long-term consequences of climbing fiber deafferentation are much different: the Purkinje cell simple-spike firing rate is similar to that demonstrated by intact animals, and the firing rate of DCN neurons is increased and exhibits a bursting pattern (Billard & Daniel, 1988).

GABA was applied to rat DCN neurones several months after 3-AP IO destruction (Billard & Batini, 1991). Nearly all neuronal firing was dose-dependently depressed by GABA in control rats that did not receive 3-AP. In 3-AP treated rats, the sensitivity of DCN neurons to GABA was markedly reduced. Bicuculline increased DCN neuronal firing rates in control rats, but had little or no effect on DCN neurons from the 3-AP treated rats. These findings suggest that a long-term consequence of climbing fiber deafferentation is decreased post-synaptic sensitivity of DCN neurons to the inhibitory transmitter GABA. Intracellular recordings of synaptic potentials in the LVN of long-term 3-AP treated rats (Karachot et al., 1987) support the iontophoretic

studies in the DCN. One month after climbing fiber deafferentation, inhibitory postsynaptic potentials recorded from LVN neurons after stimulation of Purkinje cell axons were relatively small in size and increased in latency (Karachot et al., 1987).

Inhibition in the DCN

In the dt rat, GABA receptor density is decreased in the DCN (Beales et al., 1990). This presumed down-regulation of GABA receptors in the dt rat may be the consequence of increased GABAergic transmission at Purkinje cell terminals. Therefore, it is possible that the amount of inhibition produced by Purkinje cells is not directly related to Purkinje cell firing rate. Alternatively, Purkinje cell firing rates may be higher in the awake dt rat than in awake normal rats. Decreased activity in climbing fiber collaterals to the DCN could also contribute to decrease the ratio of excitatory to inhibitory inputs to the DCN in the dt rat (Van der Want et al., 1989). Decreased excitatory drive to the DCN could result in membrane hyperpolarization.

Jahnsen (1986) has shown that in DCN neurons there is a rebound train of spikes in response to injection of hyperpolarizing current. The rebound burst is both voltage and time dependent. With small hyperpolarizing pulses, the rebound does not reach the spike threshold. The number of spikes produced by hyperpolarizing pulses that cause the rebound to reach threshold is correlated with the size of

the hyperpolarizing pulse. In addition, longer hyperpolarizations increase the number and frequency of rebound spikes.

Llinás and Mühlethaler (1988) extended Jahnsen's work by showing that a depolarizing current step delivered at the resting membrane potential elicits tonic firing in DCN neurons while the same current step delivered from a hyperpolarized level elicits all-or-none burst responses. More important with regard to the intrinsic excitability of DCN neurons was the demonstration of a low-threshold inactivating calcium-dependent conductance that generates rebound excitation following transient membrane hyperpolarization. The maximum rate of rise of membrane potential in response to the low-threshold inactivating calcium conductance increases as holding potential decreases below resting membrane potential. The location of the autocorrelogram peaks in DCN cells may be related to the time course of this conductance. The bursting shown by DCN neurons in the dt rat may be the manifestation of the intrinsic excitability of DCN neurons superimposed on a hyperpolarized resting membrane potential. Voltage and current clamp *in vitro* examination of the dt rat's DCN will be necessary to test this hypothesis.

Glucose Utilization

Single-unit recordings from the DCN in the awake preparation provide a unique opportunity to examine the relative roles of somas and synapses in the generation of

regional metabolic rates of glucose utilization. Brown and Lorden (1989) showed that glucose utilization was increased in the DCN of dt rats in comparison to normal controls. Glucose utilization in the DCN or in other grey matter is directly related to the sum of metabolic activity in inhibitory synapses, excitatory synapses, neuronal cell bodies and interposed glial cells. There is no evidence that there are differences between normal and dt rats in activity of excitatory synapses or interposed glial cells in the DCN.

Awake recordings do not show a significant difference in DCN neuronal firing rates between normal and dt rats. Purkinje cell recordings from urethane anesthetized normal and dt rats show that firing rates are significantly lower in the dt rat. In addition, one excitatory input to the DCN, the climbing fiber collateral, is likely to be decreased in the dt rat as suggested by its low Purkinje cell complex spike rate and low spontaneous firing rate of IO neurons. Therefore, an overt source for the observed difference in glucose utilization between normal and dt rats is not present.

The maintanence of electrolyte gradients probably requires a large percentage of the adenosine triphosphate (ATP) produced by neurons (Clarke et al., 1989; Sokoloff, 1989). Maintainence of a cell in a hyperpolarized state requires increased activity by the enzyme Na^+-K^+ ATPase. Therefore, it is possible that a population of

hyperpolarized DCN neurons could increase glucose utilization. However, alternative explanations exist. For example, the relatively decreased Purkinje cell simple spike rate noted in the anesthetized dt rat may be an artifact of differential sensitivity to urethane. If the Purkinje cell simple spike rate in dt rats is greater than or equal to the simple spike rate seen in normal rats, a source for the differences in glucose utilization between normal and dt rats becomes apparent. Awake recordings from Purkinje cells will be required to determine the source or sources of increased glucose utilization in the dt rat's DCN.

GENERAL DISCUSSION

DCN Effects at Recipient Nuclei

The positive results of eliminating the DCN in the dt rat suggest that abnormal cerebellar output can have significant deleterious effects at multiple sites within the motor system. However, the electrophysiological representation of the dt rat's cerebellar output signal at recipient nuclei has not been determined. In addition, the precise mechanisms by which this signal produces a dystonic motor syndrome are not known. The dt rat is a unique vehicle for understanding the pathophysiological basis of a movement disorder not associated with gross or microscopic structural abnormalities of the brain or peripheral nervous system (McGeer & McGeer, 1988; Fahn, 1989b; Jankovic & Fahn, 1993). Continued study of the dt rat may provide critical insights into the functional organization of motor systems and pathophysiology of movement disorders in general.

The red nucleus receives massive inputs from the DCN, particularly the INT and LCN (Faull & Carmen, 1978; Carpenter, 1984; Ito, 1984; Daniel et al., 1988), and cerebral cortex (Carpenter, 1984; Jenny et al., 1991). In rats treated several months earlier with 3-AP, the rhythmic bursting seen in the DCN can also be recorded in the red

nucleus (Billard et al., 1988). This is strong evidence that abnormal DCN activity can have important effects on motor nuclei. It is possible that activity similar to that noted in the red nucleus of the 3-AP treated rat is also seen in other cerebellar receiving structures such as the thalamus and anterior horn of the cervical spinal cord. However, the magnitude and characteristics of abnormal DCN signals on recipient neurons will depend on the density and distribution of synapses of cerebellar origin. The red nucleus, for example, receives a very dense projection from the contralateral INT and LCN and many of these synapses are on the soma and proximal dendrites of red nucleus neurons (Shinoda et al., 1988). Recordings from red nucleus, thalamic, and other cerebellar receiving neurons will be required to determine the degree to which the DCN abnormalities detected in the dt rat influence the neurons of important motor and premotor nuclei.

Features of Human Dystonia

Secondary dystonia has been associated with lesions of a wide variety of neural structures including the basal ganglia (Calne & Lang, 1988), midbrain (Foltz et al., 1959; Jankovic & Patel, 1983; Krauss et al., 1992), pons (Gibb et al., 1988; Maraganore et al., 1992) and cerebellum (Fletcher et al. 1988; Tranchant et al., 1991; Lauterbach et al., 1994). However, the majority of structural lesions associated with dystonia involve the basal ganglia. In addition, other movement disorders such as Parkinson's

disease and Huntington's chorea have well-defined basal ganglia abnormalites. Therefore, many clinical neurologists believe that dysfunction of the basal ganglia is responsible for idiopathic dystonia despite the failure of imaging and postmortem studies to demonstrate basal ganglia pathology in this movement disorder (Fahn, 1989a; Marsden, 1992). Unfortunately, the electrophysiological findings in patients with dystonia have not been able to definitively localize the sites of dysfunction within the nervous system.

Consistent features of dystonia are excessive and inappropriately timed cocontraction of antagonist muscles during both voluntary and involuntary movements, overflow of contraction to muscle groups not normally involved in a particular movement, and spasms of cocontraction (Rothwell et al., 1983). A possible correlate of abnormal cocontraction is decreased spinal segmental reciprocal inhibitory activity demonstrated with H-reflex testing. The early disynaptic 1a pathway functions normally, but the late phase of reciprocal inhibition, which is under the influence of suprasegmental inputs, is defective (Rothwell et al., 1988).

At the level of the brainstem, patients with dystonia involving the head or neck exhibit increased excitability of the blink reflex (Tolosa et al., 1988). With paired-pulse stimuli at interstimulus intervals of less than 1 sec in normal subjects, the magnitude of the late blink component (R2) in response to the second pulse is attenuated in

comparison to R2 magnitude in response to the first pulse. The second blink shows significantly less attenuation in dystonic patients. In addition to abnormalities in segmental reflex control, an isometric tracking task showed that dystonic patients have difficulty making rapid transitions in muscle activity (Ghez et al., 1988). Many clinical researchers have attempted to ascribe these abnormalities to dysfunction of the basal ganglia. However, these electrophysiological abnormalities may be more closely related to dysfunction at the level of the cerebellar nuclei.

Basal Ganglia Versus Cerebellum

Recent studies of the oculomotor system and distal forelimb movements in awake primates have defined the relative roles of the basal ganglia and cerebellum in motor control. The caudate sends inhibitory projections to the medial globus pallidus which sends inhibitory projections to the thalamus (Young & Penny, 1993). Neurons in the central longitudinal zone of the caudate nucleus have a low rate of tonic discharge and show an increased rate of discharge with saccades to remembered targets but not with spontaneous saccades (Hikosaka, 1989). In the medial globus pallidus, neuronal activity is not temporally related to movement initiation and neurons do not encode movement parameters (Mink and Thach, 1991a; 1991b). Therefore, the basal ganglia may serve as a gate that opens for volitional activity, but remains closed for extraneous movements (Mink

and Thach, 1991c). For example, lesions of the subthalamic nucleus which normally provide excitatory drive to the inhibitory output of the medial globus pallidus are frequently associated with hemiballismus (Grossman & Hamilton, 1993).

In contrast to the basal ganglia, the cerebellum is more closely related to movement initiation and parameters (Thach et al., 1992). In the fastigial oculomotor region, neurons show presaccadic bursts during contralateral saccades and burst duration is highly correlated with saccade duration (Ohtsuka & Noda, 1991). The cerebellum also plays a critical role in setting the gain of the blink (Evinger & Manning, 1988) and vestibulo-ocular (Robinson, 1976) reflexes. Therefore, in comparison to the basal ganglia, the cerebellum is more closely related to the specific characteristics of both voluntary and involuntary movements.

In order to relate the normal physiology of the basal ganglia and cerebellum to the pathophysiology of dystonia, the distinguishing features of the most common movement disorder, Parkinson's disease, will be compared to those of human dystonia. The patient with Parkinson's disease can make accurate movements but takes an abnormally long time to complete tasks (Ashe & Georgopoulos, 1993). In patients with dystonia, movements are inaccurate and slightly slower than in normal subjects (Inzelberg et al., 1990). In addition, transitions between different movements are much

slower in dystonic than normal subjects (Ghez et al., 1988). In sequential arm movement tasks, Parkinsonian, but not dystonic patients, take progressively longer to complete individual components of a task sequence as the sequence length increases (Agostino et al., 1992).

Parkinsonian and dystonic patients have been compared on the kinematic properties of upper-limb trajectories both with and without visual feedback (Inzelberg et al., 1990). In dystonic patients, both movements with and without visual feedback were inaccurate. Velocity profiles showed that the deceleration but not the acceleration phase of movement was slowed in comparison with normal subjects. In Parkinsonian patients, only movements without visual feedback were inaccurate. Velocity profiles showed that both acceleration and deceleration phases were slow in comparison with normal subjects.

Voluntary movements can be divided into two main steps: (1) formation and assembly of the motor plan, and (2) execution of the motor plan (Delwaide & Gonce, 1993). Based on normal physiology and the results of the human psychophysical studies reviewed above, the basal ganglia can be considered as critical for step 1 of voluntary movements while the cerebellum is required for step 2. In Parkinson's disease, it appears that step 1 is abnormal. In dystonia, because step 2 is clearly abnormal, it is not possible to exclude abnormalities of step 1. Continued study of the normal and pathological physiology of the basal ganglia and

cerebellum will be required to further delineate the different movement disorders based on electrophysiological criteria.

Cerebellum and Human Dystonia

The dt rat model allows for invasive testing of novel hypotheses about the mechanisms that operate in dystonia. The dramatic effects of CBX and the demonstration that selective elimination of cerebellar output improves the motor syndrome of the dt rat suggest that cerebellar output may also be critical to the pathophysiology of human dystonia. Recent clinical reports support a potential role for the olivo-cerebellar network in the generation of dystonic syndromes (Fletcher et al., 1988; Lees, 1990; Khara & Calabrese, 1991; Tranchant et al., 1991; Adler et al., 1994). Dystonia can be a feature of olivopontocerebellar atrophy and other cerebellar degenerations (Fletcher et al., 1988; Lees, 1990; Khara & Calabrese, 1991). There is a high incidence of essential tremor in patients with dystonia (Lou & Jankovic, 1991). The fact that essential tremor has been eliminated by a unilateral cerebellar stroke (Dupuis et al., 1989) supports a critical role for the cerebellum in essential tremor and, by extension, a role for the cerebellum in dystonia. The presence of ataxia, dystonia, and essential tremor in single families is also suggestive (Adler et al., 1994).

The results of stereotactic surgery for medically intractable generalized dystonia provide additional clues in

the effort to delineate the pathophysiological mechanisms operative in dystonia. Thalamic lesions centered in the posterior ventral oral (VLp) nuclei of Jones (Hirai & Jones, 1993) are superior to lesions of other thalamic nuclei (Cooper, 1982; Yamashiro & Tasker, 1993). The VLp is the predominant thalamic cerebellar receiving zone (Strick, 1985; Hirai & Jones, 1993). Projections from the basal ganglia terminate more rostrally in the thalamus (Strick, 1985; Hirai & Jones, 1993). Thalamic VLp lesions generally have the greatest effect on dystonia of the extremities and little effect on axial dystonia (Cooper, 1982; Yamashiro & Tasker, 1993). During the 1960s and 70s, several centers performed dentatotomies in an effort to control severe dystonic movements. The outcomes of dentatotomies were good in some cases but marginal in most (Crosby et al., 1966; Fraioli et al., 1973; Zervas et al., 1977). If abnormal DCN output is critical to the pathophysiology of human dystonia, as it is in the dt rat, the results of stereotactic surgery for human, generalized dystonia are understandable. First, dentatotomies would be only marginally effective because the vast majority of output from the MCN, INT, and dLV remain intact. Second, VLp thalamotomies should be superior to more rostral thalamic lesions. Third, VLp lesions would not be effective for axial dystonia and would not completely correct extremity dystonia because they eliminate only a small part of MCN and INT output and do not disturb dLV projections to the spinal cord. Detailed functional

imaging, postmortem biochemical examination, and intraoperative microelectrode recordings will be required to determine whether the mechanisms operative in the dt rat model of dystonia are also critical to the pathophysiology of human dystonia.

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