



Calcium Channel Agonists and Dystonia in the Mouse

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Summary: Systemic administration of the L-type calcium channel agonists \pm Bay K 8644 or FPL 64176 causes a characteristic pattern of motor dysfunction in normal C57BL/6J mice that resembles generalized dystonia. There is no associated change in the electroencephalogram, confirming that the motor disorder does not reflect epileptic seizures. However, the electromyogram reveals an increase in baseline motor unit activity with prolonged phasic discharges consistent with dystonia. The duration and severity of dystonia is dependent on the dose administered and the age of the animal at testing. The effects are transient, with the return of normal motor behavior 1–4

hours after treatment. Similar effects can be provoked by intracerebral administration of small amounts of the drugs, indicating a centrally mediated response. Dystonia can be attenuated by co-administration of dihydropyridine L-type calcium channel antagonists (nifedipine, nimodipine, and nitrendipine) but not by non-dihydropyridine antagonists (diltiazem, verapamil, and flunarizine). These results implicate abnormal function of L-type calcium channels in the expression of dystonia in this model. **Key Words:** Animal model—Seizure—Epilepsy—EEG—EMG.

Dystonia is a disorder of movement characterized by excessive involuntary co-contraction of agonist and antagonist muscles leading to twisting movements and sustained abnormal postures.^{1,2} Although some cases of dystonia are associated with damage or dysfunction of the basal ganglia or its connections, in most cases the pathogenesis is poorly understood.^{2–5} In comparison with other movement disorders, basic research directed toward elucidating the biologic basis of dystonia has been challenging, in part because of the limited number of available animal models.^{4,6}

Only a few genetic models of dystonia in rodents have been described. The *dystonia musculorum* mouse carries a mutation in the dystonin gene and displays progressive dystonia from an early age in association with pathologic changes in the peripheral nervous system, red nucleus, and basal ganglia.^{7–9} An inbred strain of rats without any obvious neuropathology also has generalized dystonia, although the responsible genetic mutation has not yet

been identified.^{10,11} An inbred strain of hamsters with paroxysmal dystonia in response to stress or other stimuli has been extensively studied, although again the genetic mutation has not yet been identified.^{8,12} Most recently, dystonia has been associated with mutations of genes encoding sodium or calcium channels in mice.^{13,14}

\pm Bay K 8644 functions as an L-type calcium channel agonist^{15–17} and induces a characteristic neurobehavioral syndrome in rodents. Prior reports of the motor abnormalities associated with this drug have described impaired ambulation, twisting and stretching movements, transient limb extension, back arching, spasticity, ataxia, catatonia, barrel-rolling, and tonic or tonic-clonic motor activity.^{18–25} It was suggested that the motor phenomena represent epileptic seizures, but electroencephalogram (EEG) correlates have been identified only for the tonic-clonic motor activity seen in the audiogenic seizure-prone DBA/2 mouse.¹⁸ The current studies demonstrate that the motor abnormalities provoked by \pm Bay K 8644 in normal mice are best characterized as dystonia rather than epilepsy. A similar motor syndrome was also found to be provoked by another L-type calcium channel agonist, FPL 64176.^{16,26} These drugs may therefore provide valuable new tools for the investigation of the pathogenesis of dystonia and suggest a novel treatment strategy.

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METHODS

Animals

Normal C57BL/6J, C3H, and Swiss-Webster mice were obtained from Jackson Laboratories (Bar Harbor, ME, USA) and maintained in the Johns Hopkins Medical Institutions animal care facilities for at least 5 days prior to testing. They were kept on a 12-hour light–dark cycle with free access to food and water. All animal procedures were conducted in strict accordance with published guidelines from the National Institutes of Health.

Materials

±Bay K 8644, +Bay K 8644, –Bay K 8644, diltiazem, flunarizine, FPL 64176, nifedipine, nimodipine, nitrendipine, and verapamil were obtained from Research Biochemicals International (Natick, MA, USA). The dihydropyridines are poorly soluble in aqueous solutions; for systemic administration, all drugs were dissolved in ethanol at a concentration of 20 mg/mL, then mixed with an equal volume of Tween-80 (Sigma Chemical Co, St Louis, MO, USA). This preparation was diluted at least 10-fold with distilled water and injected subcutaneously at a final volume of 10 mL/kg. For intracerebral microinjection, the drugs were dissolved in dimethylsulfoxide and injected in a total volume of 2 mL.

Behavior Testing

To assess the influence of age, dose-response profiles were initially determined for three separate groups of mice at 3–4, 4–5, and 6–8 weeks of age. The groups consisted of 10 animals that each received 2 mg/kg, 4 mg/kg, 8 mg/kg, and 12 mg/kg at daily intervals. To assess the influence of repeated dosing, one group of 10 mice were given the same drug dose on five separate occasions. To verify that the behavior was mediated by the central nervous system, another group of animals was examined after direct injection of the drug into the brain. For the intracerebral injections, animals were anesthetized with methoxyflurane and the surface of the skull was partially exposed with a 1-cm midline longitudinal incision. Vehicle or drug was administered by inserting a 28-g needle through the skull into the lateral ventricle using coordinates estimated from an atlas of the mouse brain.²⁷ The needle was blunted and shortened to provide a consistent injection depth of 3 mm from the surface of the skull. Injections were 2.5 mm anterior to bregma and 1 mm lateral to the sagittal suture. Animals began to awaken 5 minutes after discontinuation of methoxyflurane anesthesia and were usually ambulatory within 10 minutes.

TABLE 1. Rating scale for motor disability

Score	Behavior
D0	Normal motor behavior
D1	No impairment; slightly slowed or abnormal motor behavior
D2	Mild impairment; limited ambulation unless disturbed, transient abnormal postures, infrequent falls
D3	Moderate impairment; limited ambulation even when disturbed, frequent abnormal postures, frequent falls but upright for majority of time
D4	Severe impairment; almost no ambulation, sustained abnormal postures, not upright for majority of time

After injection with vehicle or drug, mice were placed singly in 20 × 30 cm clear plastic boxes similar to the home cage. They were observed for 1 minute each at 10-minute intervals for 1 hour using a behavioral sampling method.²⁸ Motor disability was rated at each time point on a 4-point scale (Table 1) similar to that developed for a dystonic hamster.¹² ±Bay K 8644 sometimes caused self-biting that could lead to serious injury.²⁹ To prevent unnecessary pain or suffering, any animal exhibiting self-injurious behavior was anesthetized with methoxyflurane and injected subcutaneously with 20 mg/kg nifedipine to terminate the behavior. Self-biting behavior sufficiently severe to require termination of the test session was markedly dependent on ±Bay K 8644 dose and the age of animals at testing.²⁹ These animals received motor scores equal to the last scoring interval for the remainder of the test session. Motor scores for each mouse were averaged over the entire recording period to give a single score, and scores for all mice per experimental group were then compared by analysis of variance with subsequent Tukey tests when appropriate.

Electrophysiology

For EEG recordings, mice were first anesthetized with 2,2,2-tribromoethanol, and a 6 mm longitudinal incision was made on the scalp to expose the underlying skull. Three electrodes were attached to the skull with the aid of a 3-mm screw. Two of the electrodes were placed approximately 2 mm anterior to bregma and 2 mm to either side of the sagittal suture. The third electrode was placed 3 mm posterior to bregma. A 24-hour recovery period was allowed before recording. One electrode was used as a ground, and the other two electrodes were used for bipolar EEG recordings with an analog Grass model 8–16 electroencephalographic instrument. Recordings were made with both low-frequency (1 Hz) and high-frequency (50 Hz) filters. Sensitivity varied from 7–50 mV/mm with a fixed paper speed of 30 mm/sec.

For electromyogram (EMG) recordings, mice were placed in a plastic restraining device and allowed to

accommodate for 10 minutes. A 28-g monopolar recording needle was inserted into the hamstring muscles, with a reference needle inserted more proximally at the knee and a grounding needle inserted into the tail. Continuous recordings were made from the same electrodes with a Nicolet Viking (Madison, WI, USA) instrument before and after drug administration.

RESULTS

Motor Behavior After \pm Bay K 8644

When placed in the test cage, untreated or vehicle-treated mice engaged in their typical normal behaviors, including ambulation, rearing, sniffing, manipulating bedding, and occasional grooming (videotape segment 1). Mice treated with 2 mg/kg \pm Bay K 8644 demonstrated obvious changes in motor behavior. Rearing stopped and ambulation became slower. The motion of lifting a paw and placing it forward was visibly slowed. Sometimes a trailing limb would appear stuck to the cage bottom or get hung up in transit for a few seconds before being placed down (Fig. 1A; videotape segment 2). Animals also occasionally demonstrated ptosis, hyperre-activity to auditory or tactile stimuli, or tonic dorsal extension of the tail (Straub tail). At a dose of 4 mg/kg \pm Bay K 8644, hypokinetic and bradykinetic motor dysfunction became more obvious, with more frequent hyperre-activity to sensory stimuli and Straub tail. The animals walked in a hunched posture with the trunk abnormally flexed and elevated from the cage bottom (videotape segment 2).

At doses of 8–12 mg/kg \pm Bay K 8644, ambulation nearly stopped, and the truncal flexion was exaggerated (videotape segment 2). The mice often reared into a peculiar “tripod” sitting posture, with support being supplied anteriorly by splaying the hindlimbs forward and posteriorly by the extended tail (Fig. 1B). The head was flexed toward the abdomen and the forelimbs held close to the body, often scissored with the paws clenched. After maintaining this posture for a few seconds to a few minutes, the animals would often fall sideways or backward. After falling, the animal would rapidly right itself, its trunk would slowly begin to flex again, it would rear up to the tripod posture, and fall again.

In addition to the exaggerated truncal flexion, the ability to remain upright was impaired in many animals and they would assume unnatural postures for extended periods of time. Abnormal flexion, extension, or twisting movements were evident in the trunk and limbs as they attempted to move (Fig. 1C, D). Limb movements were asymmetric and asynchronous, and muscle tone was increased. This behavior did not fit typical definitions of catatonia in which animals maintain abnormal postures that are externally imposed. These postures were internally generated and could not be imposed by the examiner. Despite profound impairment of their spontaneous motor behavior, the animals could dart quickly from one end of the cage to the other if disturbed by the examiner or by an unexpected loud noise.

In more than 100 trials of doses ranging from 2–12 mg/kg \pm Bay K 8644, typical tonic or tonic-clonic motor behavior suggestive of motor seizures was observed

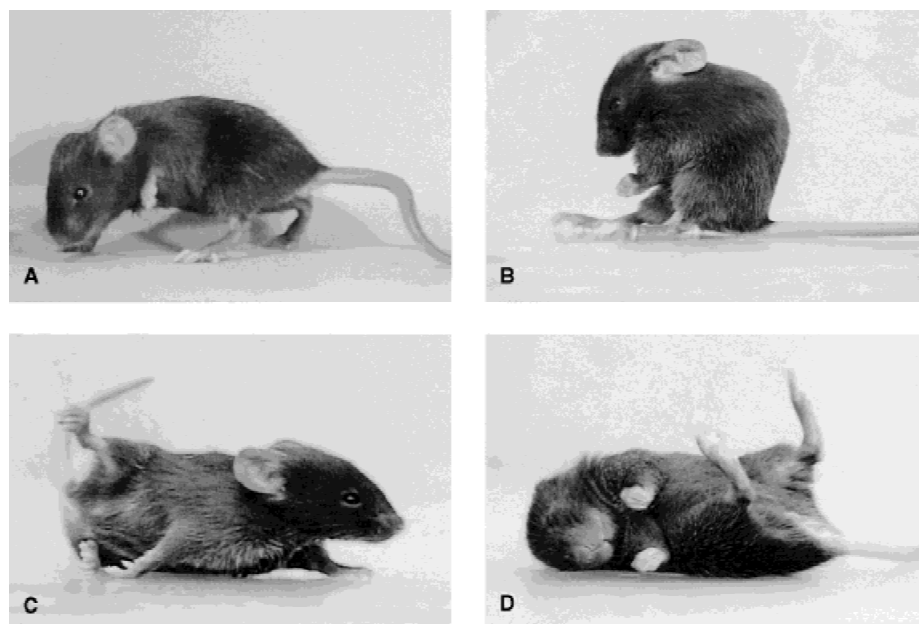


FIG. 1. Abnormal motor behavior associated with \pm Bay K 8644. (A) Ambulation with nose down, arching of the back, and a forelimb that is transiently stuck in an abnormally elevated position. (B) Tripod posture, with fisted forepaws, extreme flexion of the trunk and neck, and atypical sitting posture. (C) Fixed posture with truncal torsion and abnormal limb position. (D) Loss of the righting reflex with truncal torsion, fisting of the forelimbs, and forced eye closure.

fewer than five times. These events typically occurred in older animals at high doses, often when the animal was disturbed physically or by an unexpected loud noise. The lack of tonic-clonic seizures in the C57BL/6J mice contrasts with the high incidence of lethal seizures previously observed with DBA/2 mice,¹⁸ a finding that might reflect the known susceptibility of DBA/2 mice to audiogenic seizures together with the apparent acoustic hypersensitivity provoked by the drug. The motor syndrome resulting from \pm Bay K 8644 was not limited to C57BL/6J mice, because it could also be provoked in outbred Swiss-Webster and inbred C3H mice (results not shown).

EEG Recordings

To demonstrate the typical motor and EEG changes associated with tonic-clonic seizures in the mouse, recordings were obtained from three normal adult mice before and after treatment with the epileptogenic drug pentylentetrazole at a dose of 50 mg/kg. The normal mouse baseline EEG consisted of a medium-voltage mixture of frequencies in the 3–15-Hz range (Fig. 2A), and characteristic motor and EEG abnormalities evolved after administration of pentylentetrazole. Initially, the recordings slowed to the 2–4-Hz range in association with generalized motor hypoactivity. Next, single high-voltage spikes emerged in association with rapid myoclonic-like jerks. Still later, the EEG demonstrated high-voltage polyspike activity along with generalized tonic and tonic-clonic motor behavior characteristic of an epileptic seizure (Fig. 2B). After the seizure, which gener-

ally lasted less than 10–20 seconds, the tracing became slow with a clear voltage decrement.

The EEG was then recorded from four normal mice before and after administration of 8 mg/kg \pm Bay K 8644. No changes in the baseline activity were detected during the slow and twisting movements associated with \pm Bay K 8644 (Fig. 2C). These observations demonstrate that the generalized motor phenomena associated with this drug are not accompanied by epileptiform activity on the EEG.

EMG Recordings

The EMG was recorded continuously from monopolar needles fixed in the thigh muscles of three normal mice before and after the administration of \pm Bay K 8644. The pretreatment EMG demonstrated a baseline resting activity of 20–40 mV with intermittent phasic bursts of activity in the 100–400-mV range associated with movement (Fig. 3A). Two changes became evident 20 minutes after administration of 8 mg/kg \pm Bay K 8644. First, the resting baseline increased two- to threefold (Fig. 3B). Second, movement-related phasic bursting appeared prolonged and of higher amplitude. Anesthetizing the same animal with methoxyflurane, with no change in the positions of the recording needles, resulted in an obvious decrease in the amplitude of resting activity and cessation of all phasic bursting (Fig. 3C). The high resting EMG activity with prolonged phasic bursting returned (Fig. 3D) after full recovery from methoxyflurane anesthesia approximately 40 minutes after \pm Bay K 8644 administration. These studies demonstrate that \pm Bay K

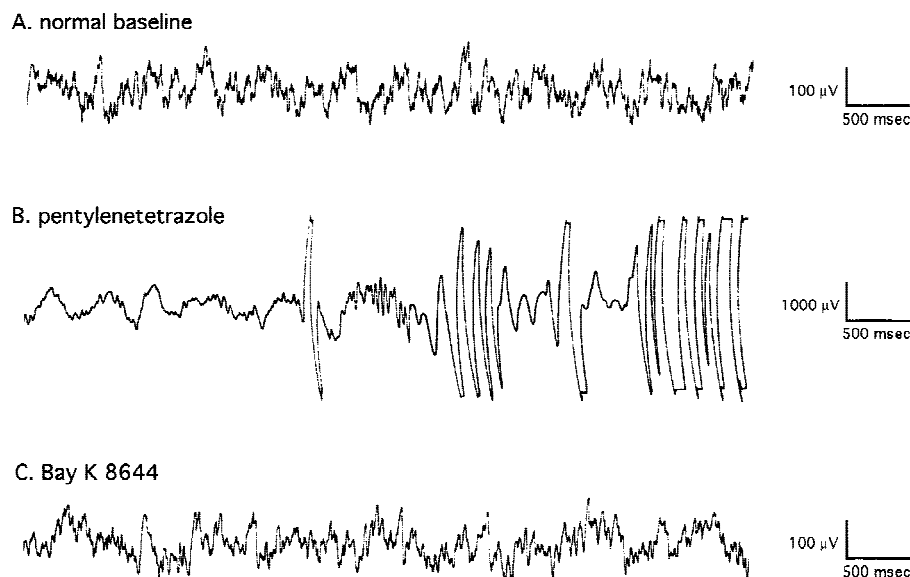


FIG. 2. Electroencephalographic recordings. This figure shows electroencephalographic activity from two representative mice. (A) Resting baseline from a normal mouse. (B) Recording associated with myoclonic jerks and tonic-clonic motor activity in a mouse treated with 50 mg/kg pentylentetrazole. (C) Recording associated with abnormal motor behavior of a mouse treated with 8 mg/kg \pm Bay K 8644.

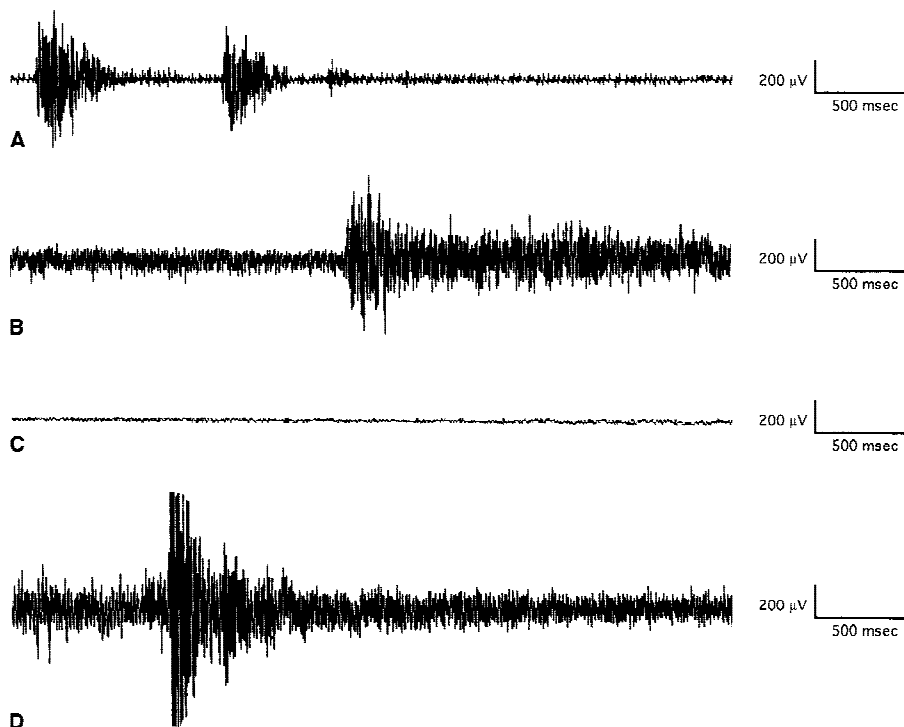


FIG. 3. Electromyographic recordings. This figure shows representative electromyographic activity in hamstring muscles from continuous recording of a single animal under different conditions. (A) Normal baseline with phasic discharges associated with movement at the start of recording. (B) Increase in baseline activity and exaggerated phasic discharges 20 minutes after administration of 8 mg/kg \pm Bay K 8644. (C) Flattened baseline with absent phasic discharges after anesthetizing the same animal with methoxyflurane. (D) Return of increased baseline activity and exaggerated phasic discharges after recovery from methoxyflurane anesthesia.

8644 causes significant changes in both resting and activated motor unit activity.

Mouse Age and Drug Dose

The motor effects of escalating doses of \pm Bay K 8644 were quantified in mice of different ages. The drug pro-

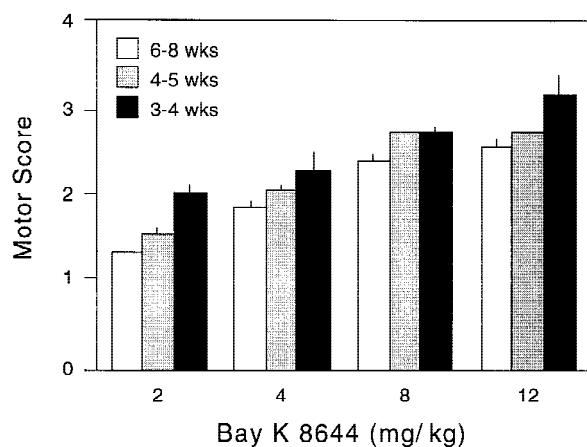


FIG. 4. Influence of \pm Bay K 8644 dose at different ages. Three groups of 10 mice at different ages were treated with escalating doses of \pm Bay K 8644 at daily intervals. Data show the average motor scores \pm standard error of mean for 10 mice per group. Ages tested include 3–4 weeks (black bars), 4–5 weeks (medium gray bars), and 6–8 weeks (light gray bars). A two-way analysis of variance revealed significant main effects for both age ($F = 24.3$, $p < 0.001$) and dose ($F = 79.4$, $p < 0.001$), but no significant interactive effect between these two variables ($F = 1.2$, $p > 0.1$).

duced a dose-dependent increase in the severity of motor disability at all ages tested (Fig. 4). The disability appeared age-dependent, with weanling mice (3–4 weeks of age) being slightly more susceptible than adult mice (6–8 weeks of age). Older mice, 6 and 12 months of age, showed responses similar to those observed in adults.

Time Course and Influence of Repeated Dosing

At a dosage of 2 mg/kg \pm Bay K 8644, motor dysfunction became evident within 5 minutes, peaked at 20–40 minutes, began to wane by 60 minutes, and disappeared by 90 minutes. Higher doses were associated with slightly earlier onset of motor dysfunction, reduced time to peak response, and a longer duration of response. The temporal profile of motor dysfunction during a typical 60-minute recording period is shown in Figure 5.

The responsiveness of mice previously treated with \pm Bay K 8644 was examined to determine if there might be any long-lasting behavioral effects of the drug. One day after administration of 8 mg/kg \pm Bay K 8644, the mice appeared entirely normal. However, another trial with the same dose of \pm Bay K 8644 was associated with slightly less motor dysfunction than that seen with the first dose (Fig. 6). A third trial a day later was associated with a further decrement in motor abnormalities. The severity of the motor disorder appeared at least partly restored in a fourth trial after a 1-week recovery period and fully restored after a 1-month recovery period (Fig.

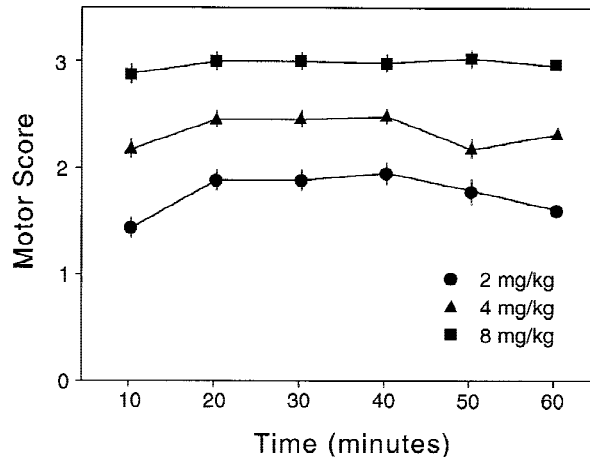


FIG. 5. Time course of motor dysfunction after subcutaneous administration of \pm Bay K 8644. Three separate groups of 50 mice were treated with one of three doses of \pm Bay K 8644, and motor dysfunction was rated every 10 minutes for 60 minutes. Data show average motor disability scores \pm standard error of mean.

6). These results demonstrate reversible desensitization of the behavioral responsiveness with repeated treatment with \pm Bay K 8644.

Anatomic Locus of Drug Action

To determine if the motor effects of \pm Bay K 8644 were the result of an effect of the drug on the central nervous system, direct intracerebral injections were performed after brief methoxyflurane anesthesia. Administration of dimethylsulfoxide vehicle alone into the lateral ventricle was associated with slightly slowed or unsteady

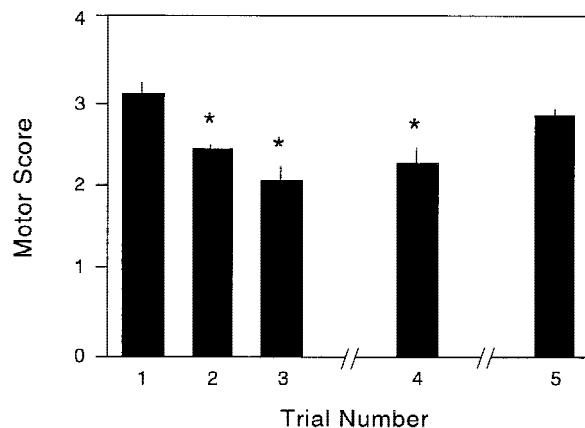


FIG. 6. Influence of repeated doses of \pm Bay K 8644. One group of 10 adult mice was treated with 8 mg/kg \pm Bay K 8644 by subcutaneous injection on five separate occasions. The first three trials were conducted at daily intervals. The fourth trial was conducted after 1 week of recovery, and the fifth trial was conducted after 1 month of recovery. Data show the average motor disability scores \pm standard error of mean. Analysis of variance revealed significant differences between the groups; asterisks denote significant differences from the first trial.

ambulation in the initial test period for some animals as they recovered from the methoxyflurane anesthesia. Administration of 12.5–100 μ g \pm Bay K 8644 dose-dependently reproduced all of the motor abnormalities observed with subcutaneous administration of the drug (Fig. 7). These results imply that the motor effects of \pm Bay K 8644 are centrally mediated.

To confirm that the motor effects of \pm Bay K 8644 were not the result of a direct effect of the drug on muscle (myotonia) or the spinal motor neurons (neurotonia), thoracic spinal cord transections were performed in three animals to eliminate descending inputs to motor neurons of the rear limbs. After a 24-hour recovery period, treatment with 4 mg/kg \pm Bay K 8644 produced the typical motor abnormalities in the forelimbs whereas the rear limbs remained motionless. These results indicate that the abnormal movements are not consistent with myotonia or neurotonia.

Pharmacologic Specificity

Three experiments were performed to verify that the behaviors observed with \pm Bay K 8644 were the result of activation of L-type calcium channels and not the result of some nonspecific side effect. If the motor effects of \pm Bay K 8644 are the result of L-type calcium channel activation, then they should be provoked selectively by the $-$ Bay K 8644 enantiomer, which is the active agonist of L-type calcium channels. In contrast, the $+$ Bay K 8644 enantiomer, which is actually a weak antagonist, should have no effect. In fact, motor dysfunction was evident with all doses of $-$ Bay K 8644 tested, whereas the $+$ Bay

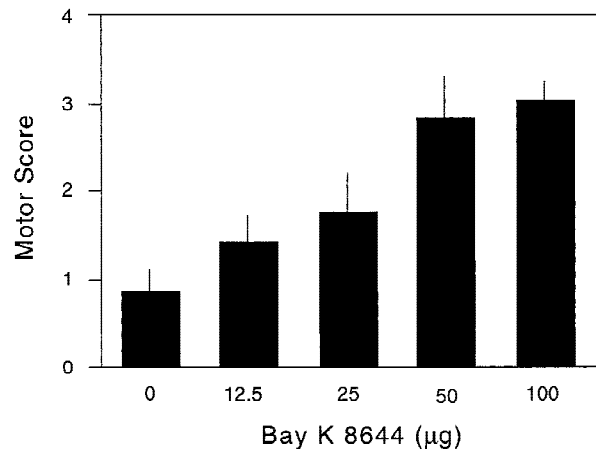


FIG. 7. Intracerebral delivery of \pm Bay K 8644. Mice were anesthetized briefly with methoxyflurane, and different quantities of \pm Bay K 8644 dissolved in 2 mL dimethylsulfoxide were delivered into the lateral ventricle by direct injection. After allowing 10 minutes for recovery from anesthesia, motor scores were recorded every 10 minutes for 60 minutes. Data show the average motor scores \pm standard error of mean for five mice per group.

TABLE 2. Bay K 8644 enantiomers*

Motor score	-Bay K 8644	+Bay K 8644
2 mg/kg	1.4 ± 0.1	0.0 ± 0.0
4 mg/kg	1.8 ± 0.2	0.1 ± 0.1
8 mg/kg	1.9 ± 0.1	0.0 ± 0.0

* Data represent average values from eight mice ± standard error of mean.

K 8644 enantiomer had virtually no effect (Table 2). The combined racemic ±Bay K 8644 preparation is likely to function as an agonist, because the agonist is approximately 16-fold more potent than the antagonist.³⁰

If the motor effects of ±Bay K 8644 are the result of L-type calcium channel activation, then a structurally distinct L-type calcium channel agonist should produce similar effects. Indeed, the L-type calcium channel activator FPL 64176^{16,26} provoked motor abnormalities essentially identical to those produced by ±Bay K 8644 (Fig. 8; videotape segment 3). In comparison with ±Bay K 8644, equivalent doses of FPL 64176 appeared to produce a steeper dose-response curve. The steeper curve might reflect the fact that FPL 64176 is a full agonist, whereas racemic ±Bay K 8644 functions as a partial agonist. FPL 64176 also appeared to be significantly more toxic than ±Bay K 8644, because more than half of the animals did not survive treatment after the highest dose. However, those that survived appeared behaviorally normal 4–6 hours after treatment.

If the motor effects of ±Bay K 8644 are the result of L-type calcium channel activation, then calcium channel antagonists should block its effects. To verify this prediction, adult mice were pretreated with one of six calcium channel antagonists 5 minutes before receiving

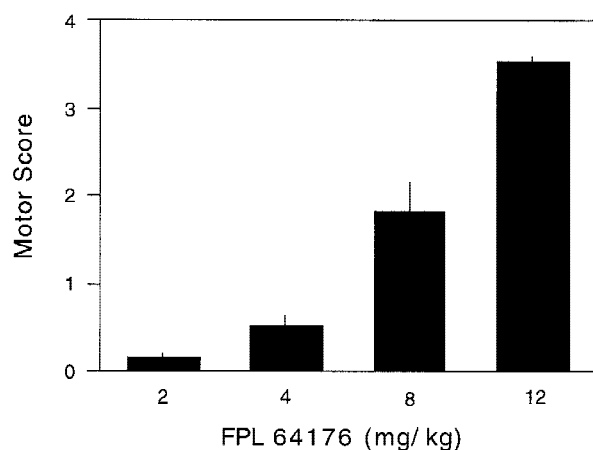


FIG. 8. Influence of FPL 64176. A group of 10 adult mice were treated with escalating doses of FPL 64176 by subcutaneous injection at daily intervals. Data show the average motor scores ± standard error of mean.

±Bay K 8644. Nifedipine, nimodipine, and nitrendipine are dihydropyridine L-type calcium channel antagonists that bind to the same molecular site as ±Bay K 8644. Although these drugs produced no obvious behavioral effects when administered alone, each markedly attenuated the motor dysfunction caused by ±Bay K 8644 (Fig. 9). In contrast to the effects of the dihydropyridine blockers, diltiazem (a benzothiazepine), flunarizil (a piperazine), and verapamil (a phenylalkylamine) antagonize L-type calcium channels by binding to distinct molecular sites and had no significant protective effect (Fig. 9). These results demonstrate that the motor dysfunction associated with ±Bay K 8644 can be attenuated with some, but not all, L-type calcium channel antagonists.

DISCUSSION

The current studies show that ±Bay K 8644 produces a characteristic pattern of motor dysfunction in normal mice. Although the motor syndrome has previously been described using a number of different labels, it has most frequently been considered a form of epilepsy and studied extensively in the audiogenic seizure-prone DBA/2 mouse.^{18–25} However, several lines of evidence now suggest that the motor disorder in normal mice is better characterized as dystonia rather than epilepsy. First, the observed phenomenology, with sustained and asynchronous twisting postures, is more characteristic of dystonia

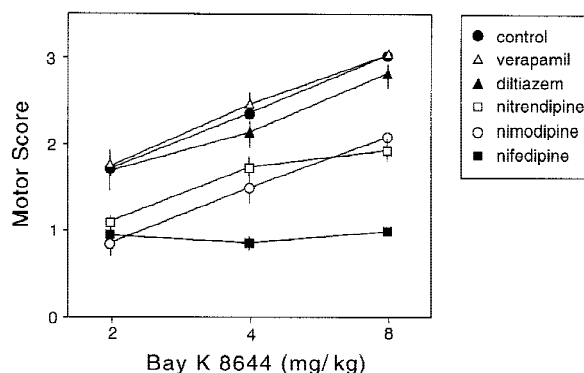


FIG. 9. Inhibition of ±Bay K 8644 induced motor disability with calcium channel antagonists. Independent groups of drug-naïve mice were pretreated with saline (closed circles), 20 mg/kg diltiazem (closed triangles), 20 mg/kg nifedipine (closed squares), 20 mg/kg nimodipine (open circles), 20 mg/kg nitrendipine (open squares), 20 mg/kg verapamil (open triangles), or 20 mg/kg flunarizine (not shown). They then received 2, 4, or 8 mg/kg of ±Bay K 8644. Data show average motor disability scores of 8–10 mice ± standard error of mean. A two-way analysis of variance with ±Bay K 8644 dose and pretreatment condition as the main variables revealed a significant main effect for ±Bay K 8644 dose ($F = 42.8$, $p < 0.001$) and pretreatment condition ($F = 55.5$, $p < 0.001$), as well as a significant interaction between the variables ($F = 2.9$, $p < 0.001$). Pretreatment with nifedipine, nimodipine, or nitrendipine significantly attenuated motor disability in comparison with the control group ($p < 0.001$), whereas pretreatment with diltiazem, flunarizine, or verapamil did not.

than motor seizures. Second, the duration of 20–120 minutes is consistent with dystonia, but quite unusual for a seizure which typically lasts only 1–60 seconds. Third, the motor phenomena do not respond to traditional anti-convulsants such as carbamazepine, diphenylhydantoin, phenobarbital, or valproic acid.^{18,22,24,25} Fourth, despite apparent generalization with involvement of the entire trunk and all limbs, the motor abnormalities are not associated with epileptiform activity on the EEG. Finally, EMG recordings demonstrate an increase in baseline resting activity and prolonged movement-related bursts of activity, two changes often associated with dystonia in people.³¹

Extensive prior pharmacologic studies demonstrating that \pm Bay K 8644 functions as a potent and selective activator of L-type calcium channels,^{15–17} the reproduction of a qualitatively similar motor syndrome with another L-type calcium channel activator (Fig. 8), and the protection afforded by L-type calcium channel antagonists (Fig. 9) strongly suggest that activation of these channels underlies the ability of \pm Bay K 8644 to provoke dystonia in mice. Dystonia is likely to result from activation of L-type calcium channels in the brain, because microinjection of the drug directly into the lateral ventricles reproduces the motor disorder at smaller doses than required for subcutaneous administration. The reason why only some calcium channel antagonists are capable of attenuating the effects of \pm Bay K 8644 remains unclear. The inability of verapamil to pass through the blood-brain barrier could explain its lack of efficacy, but cannot explain the behavior of diltiazem and flunarizine which do pass through the blood-brain barrier. The antagonists block calcium channel activity by binding to three distinct but allosterically linked sites. The effective antagonists (nifedipine, nimodipine, and nitrendipine) all share the property of binding to the dihydropyridine site, where they competitively antagonize the binding of both \pm Bay K 8644 and FPL 64176. In contrast, diltiazem, flunarizine, and verapamil bind to distinct sites on the channel. Although the molecular mechanisms responsible for the functional differences among these antagonists remain to be determined, it is noteworthy that these six antagonists showed the same pattern of efficacy (or lack of efficacy) against tonic-clonic seizures in the audiogenic seizure-prone DBA/2 mouse, providing further evidence that these calcium channel antagonists are not functionally equivalent.¹⁸

Although many of the motor features observed with \pm Bay K 8644 or FPL 64176 appear dystonic, it should be emphasized that both drugs cause additional behaviors that are not classified as dystonia. For example, the self-biting behavior observed in young animals is not likely

to be a manifestation of dystonia.³² Similarly, the hypersensitivity to auditory stimuli and Straub tail are suggestive of the serotonin syndrome rather than dystonia. Ataxia could account for clumsy behavior and frequent falling; but careful observation suggests that the stiff and twisting movements of the trunk and limbs, together with the slow and impaired postural reflexes, account for falling better than true cerebellar ataxia. In view of these considerations, it is clear that the calcium agonist drugs do not provoke a pure syndrome of dystonia, but rather a more extensive neurobehavioral syndrome in which dystonia is a major component.

The dystonia provoked by \pm Bay K 8644 and FPL 64176 provides a valuable new model to investigate the molecular, neuroanatomic, and neuropharmacologic basis of dystonia. Although mutant dystonic rats and hamsters have been extensively evaluated as animal models for dystonia, the elucidation of early molecular events responsible for pathogenesis is difficult to study with these models because the responsible genetic mutations have not yet been identified.^{8,10–12} Mutations of dystonin, a hemidesmosomal protein involved with structural interactions between cells, has been identified as the cause of dystonia in the *dystonia musculorum* mouse, but the mechanisms responsible for dystonia are difficult to distinguish against the background of extensive central and peripheral nervous system pathology in this model.^{7–9} Dystonia has only recently been recognized as a consequence of mutations in sodium and calcium channels in mice, and the pathogenesis of dystonia has not been studied extensively.^{13,14} The induction of dystonia with L-type calcium channel agonists provides a novel, precisely defined, and easily controlled experimental paradigm for studying the pathophysiology and treatment of dystonia.

Ion channels provide intriguing potential candidates as mediators of human dystonia. As previously noted, most cases of idiopathic dystonia are associated with little or no overt neuropathologic abnormality. Ion channel mutations have been identified as the cause of several neurologic diseases in which, like most cases of idiopathic dystonia, profound motor impairment may be associated with little or no neuroanatomic pathology.^{33,34} Abnormal calcium channel function is associated with hypokalemic periodic paralysis, episodic ataxia type 2, spinocerebellar atrophy type 6, and the Eaton-Lambert myasthenic syndrome. Defects in sodium channel function are responsible for hyperkalemic period paralysis, paramyotonia congenita, and myasthenia gravis. Potassium and chloride channels are associated with episodic ataxia type 1 and myotonia congenita, respectively. These observations demonstrate that functional disruption of ion

channel function can cause significant neurologic dysfunction, often without any obvious neuroanatomic pathology.

Calcium channels also may be good candidates as mediators of dystonia because they regulate several aspects of neurotransmission, including neurotransmitter synthesis, release of neurotransmitters, and postsynaptic intracellular signaling.³⁵ Although there may be few neuropathologic changes in idiopathic dystonia, pervasive abnormalities in the content and turnover of multiple neurotransmitters have repeatedly been identified in both human and animal studies.^{2,8,11,36} Thus, a defect in calcium channel function could provide a potential explanation for the apparent changes in neurotransmitter function in the absence of underlying anatomic pathology.

If abnormal calcium channel activity can cause dystonia, then calcium channel antagonists might provide a novel treatment strategy for dystonia. In fact, L-type calcium channel antagonists delayed the onset and reduced the severity of attacks in a hamster with inherited paroxysmal dystonia.³⁷ L-type calcium channel antagonists also prevented dystonic spasms in tottering mutant mice, which carry a point mutation in the $\alpha 1A$ subunit of the P/Q-type calcium channel.¹⁴ A review of the literature disclosed no controlled trials of calcium channel drugs for the treatment of dystonia in people, but one patient with myoclonic dystonia was reported to respond favorably to verapamil.³⁸ Multiple case reports and small clinical trials have suggested that calcium channel antagonists might be effective in the treatment of tardive dyskinesia, which often includes prominent dystonic features. A recent review of these reports has concluded that nifedipine may be the most promising agent, particularly if used at high doses in specific subpopulations of affected individuals.³⁹ Further support that calcium channel blockers can have a significant influence on the human extrapyramidal motor system is provided by multiple studies implicating this class of drugs as a cause of parkinsonism. Parkinsonism has been repeatedly documented with a variety of calcium channel antagonists, including amlodipine, cinnarizine, diltiazem, flunarizine, manidipine, and verapamil.⁴⁰⁻⁴⁷ Although there are no direct studies of calcium channel antagonists for the treatment of human dystonia, these observations provide strong indirect evidence that calcium channel drugs should be considered as potential therapeutic agents.

LEGENDS TO THE VIDEOTAPE

Segment 1: This segment shows the typical motor behaviors of a normal mouse to provide a frame of reference for assessing the drug-treated mice. Ambulation is fluid and quick as it explores the test cage. It

sniffs frequently and occasionally rears up onto its hindlimbs to explore the corners. When held by the tail, it adopts a characteristic posture and struggles occasionally in anticipation of being released. The neck is slightly extended, the back is slightly arched, the forelimbs extend rostrally toward the floor, and the hindlimbs extend caudally.

Segment 2: This segment provides examples of the typical motor dysfunction observed at the peak of the response with different doses of \pm Bay K 8644. At 2 mg/kg there is a reduction in spontaneous motor activity and ambulation is slightly slowed. At 4 mg/kg movements are even slower with a stiff, dystonic, and sometimes athetoid quality. Severe impairments are observed at 8 mg/kg, when the animal has difficulty maintaining the upright posture because of near-continuous twisting movements; and the mouse frequently assumes unnatural twisted postures for extended periods.

Segment 3: This segment provides examples of the typical motor dysfunction observed with different doses of FPL 64176. The characteristics of the motor syndrome are similar to those observed with \pm Bay K 8644.

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