Cannabinoid CB₁ Receptor Agonists Produce Cerebellar Dysfunction in Mice

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Received October 2, 2000; accepted January 13, 2001 This paper is available online at http://jpet.aspetjournals.org

ABSTRACT
The purpose of these studies was to characterize the effects of agonists of the CB₁ cannabinoid receptor on cerebellar function: gait analysis and the bar cross test. CB₁ receptor agonists CP55940, Win 55212-2, Δ⁹-tetrahydrocannabinol, arachidonylethanamide (AEA), and two AEA analogs with high affinity for the CB₁ receptor (arachidonyl-2-chloroethylamide and arachidonylcyclopropylamide) all produced increases in gait width, a measure of truncal ataxia. All of the CB₁ agonists tested significantly increased the number of slips on the bar cross test, which is consistent with motor incoordination. Pretreatment with the CB₁ receptor antagonist SR141716 attenuated both the change in gait width and number of slips induced by CP55940 and AEA. Neither cannabidiol nor Win 55212-3 affected these measures, further evidence that this effect is mediated by the CB₁ receptor. Pretreatment with the dopamine receptor agonists apomorphine or bromocriptine did not attenuate the diminished performance on the bar cross or the gait abnormality induced by CP55940. These data indicate that the assays used in this study are specific for cerebellar-mediated behavioral deficits, and that these deficits are not mediated by the basal ganglia or cannabinoid-induced alterations in nigrostriatal dopaminergic transmission. Other well known effects of cannabinoids in mice, such as hyperreflexia exemplified by jumping or “popcorn” behavior and postural hypotonia are discussed in relationship to cerebellar dysfunction and a working model of the effects of CB₁ receptor activation on cerebellar circuitry is presented.

The behavioral effects of Δ⁹-tetrahydrocannabinol (THC) and other cannabinoids have been studied extensively during the past four decades, and include pronounced motor deficits such as catalepsy, hypolocomotion, and static ataxia (Dewey, 1986; Chaperon and Thiebot, 1999). Both the basal ganglia and the cerebellum have long been acknowledged as probable sites of action for these cannabinoid-induced motor deficits (Gough and Olley, 1977; Dewey, 1986). It has been proposed that cannabinoid-induced catalepsy and other manifestations of extrapyramidal deficits are due primarily to inhibition of nigrostriatal dopaminergic transmission (Sanudo-Pena et al., 1999). There is less known about the contribution of cannabinoid effects on the cerebellum to motor deficits seen after their administration. In this study, we used behavioral tests specific for cerebellar function to characterize cannabinoid-induced cerebellar deficits in mice.

Physiological symptoms of cerebellar dysfunction include ataxia, side-to-side truncal tremor, and an abnormal wide stance base in the gait. To evaluate the ability of various cannabinoids to produce cerebellar dysfunction in mice, we used two specific physiological measures: gait analysis and the bar cross test. Gait analysis includes quantification of various parameters of gait such as step width and length, alternation coefficient, and linear movement. Alterations of these parameters, especially gait width, are hallmarks of cerebellar dysfunction (Gilman et al., 1981). In the bar cross test, the mouse crosses a narrow bar with several obstacles. Crossing the bar without falling requires smooth, accurate, and coordinated movement of several muscle groups; an animal with cerebellar ataxia will slip or fall multiple times while completing the task. Since both of these tests are analyses of animal movement, it is unlikely that the extrapyramidal effects of cannabinoids (in which difficulty in initiating rather than accurately completing movements is the predominant symptom) will report false positive results.

The cerebellum influences descending motor systems by evaluating disparities between intended and actual movement. The Purkinje cell of the cerebellum receives excitatory inputs from the inferior olivary nucleus via climbing fibers, and cortical and peripheral sensory information via mossy fibers. Mossy fiber input is disynaptic; mossy fibers synapse on granule cells whose axon terminals (parallel fibers) activate Purkinje cell dendrites in the molecular layer of the cerebellar cortex. A third major input to the Purkinje cell is the basket cell, an inhibitory GABAergic interneuron. Basket
cell terminals synapse with several Purkinje cells, forming a peri-cellular basket surrounding the axon hillocks of these cells. Purkinje cells send inhibitory projections to the deep cerebellar nuclei, which in turn project to postural and motor structures such as the vestibular nucleus and motor cortex.

It has long been hypothesized that symptoms of cerebellar dysfunction arise when the excitatory output of the deep cerebellar nuclei is reduced or interrupted. For example, lesions of the deep cerebellar nuclei result in symptoms of cerebellar dysfunction in primates (Poirier et al., 1974). In addition, abnormal Purkinje cell activation, which results in inhibition of the deep cerebellar nuclei, induces symptoms of cerebellar dysfunction. For example, indole alkaloids such as harmaline and ibogaine, increase Purkinje cell activation subsequent to an increase in synchronous firing of the inferior olive and produce profound symptoms of cerebellar dysfunction (O’Hearn and Molivar, 1997). We hypothesize that cannabinoids produce cerebellar deficits by a similar mechanism, i.e., cannabinoids induce abnormal Purkinje cell activation and subsequent inhibition of the deep cerebellar nuclei and, therefore, disruption of normal posture and movement.

Recent anatomical and functional studies have identified two populations of CB1 receptors within the rodent cerebellum. Immunohistochemistry has revealed a high density of CB1 receptors on the axon terminals of GABAergic basket cells (Tsou et al., 1998). Activation of this CB1 receptor population in cerebellar slice preparations results in inhibition of GABA release at the basket cell-Purkinje cell synapse (Takahashi and Linden, 2000). High CB1 receptor density has also been demonstrated in the molecular layer of the cerebellum (Herkenham et al., 1991; Tsou et al., 1998). It is likely that the CB1 receptor of the cerebellar molecular layer is present on granule cell axons (Herkenham et al., 1991). Electrophysiological experiments have demonstrated that cannabinoids also inhibit transmitter release at the parallel fiber-Purkinje cell synapse (Levenes et al., 1998; Takahashi and Linden, 2000). These data suggest that cannabinoids may alter cerebellar function via inhibition of synaptic transmission at either the parallel fiber-Purkinje cell or basket cell-Purkinje cell synapse, or both. It is the purpose of this study to quantify the effects of various cannabinoids on cerebellar function in mice using standard behavioral tests and to determine the most likely cellular mechanism for these effects.

Materials and Methods

Animals and Drugs. Male ICR albino mice (Harlan, Madison, WI) weighing between 21 and 24 g were used for all experiments. Animals were housed five to a cage with ad libitum access to food and water under a 12-h light/dark cycle with lights on at 6:00 AM. All experiments were carried out in accordance with the Guide for the Care and Use of Laboratory Animals as promulgated by the National Institutes of Health.

All drugs, except apomorphine, were administered in Emulphor/ethanol vehicle containing 0.9% saline, Emulphor, and 100% ethanol in an 18:1:1 ratio (Craddock et al., 1973). Apomorphine was dissolved in 0.9% saline. In most experiments, drugs were administered by intraperitoneal injection in a volume of 10 μl/g of body weight. AEA, arachidonyl-2-chloroethanamide (ACEA), and arachidonylecyclopentylamide (ACPA) were administered intravenously via the tail vein in a volume of 4 μl/g of body weight.

Emulphor (Alkamuls EL-620) was provided by Rhone-Poulenc (Cranbury, NJ). CP55940 was a generous gift from Pfizer Central Research (Groton, CT). AEA was purchased from Cayman Chemical Company (Ann Arbor, MI). ACEA and ACPA (Hillard et al., 1999) were a kind gift from Tocris Cookson (Ballwin, MO). Win 55212-2, Win 55212-3, apomorphine, and bromocriptine were purchased from Sigma/Research Biochemicals International (Natick, MA). THC and cannabidiol were obtained from National Institute on Drug Abuse (Rockville, MD). SR141716 was a kind gift from Sanofi Research (Montpellier, France). All other materials were purchased from standard commercial sources.

Behavioral Tests. All behavioral tests were conducted between 12:00 PM and 3:00 PM, with animals given several hours to acclimate to the testing room before the beginning of each experiment. The room was quiet with no distractions and maintained at a temperature of 21°C. The observer was not blinded to the treatment.

The bar cross test is carried out using a wooden bar 100 cm in length and 2 cm in width with eight low obstacles averaging 0.7 cm in height (Goldowitz et al., 1992). The bar is just wide enough for mice to stand on with their hind feet hanging over the edge such that any slight lateral misstep will result in a slip. The bar was elevated 12 inches from the bench surface so that animals did not jump off; yet were not injured upon falling from the bar. To eliminate the novelty of the task as a source of slips, all animals were given four trials on the bar at the beginning of the testing session. By the fourth trial, control animals ran the bar with no more than two slips. In an experimental session, the number of hind limb lateral slips and falls from the bar was counted on four consecutive trials. If an animal fell, it was placed back on the bar at the point at which it fell and was allowed to complete the task. The bar was cleaned with ethanol after each animal.

Gait analysis was carried out on footprints obtained by painting the hind feet of mice with nontoxic black paint and having them walk on paper along a 30-cm-long, 9-cm-wide runway, with 16-cm-high walls on either side (Jolicoeur et al., 1979). Trials were repeated until seven consecutive steps were recorded on the paper covering the floor of the runway. Gait width was obtained by drawing lines connecting the middle toe of each consecutive step of both feet, and then drawing a perpendicular line from the middle toe of one foot to the line connecting two steps of the other foot (Fig. 1A). Width (in cm) was measured at all five possible locations of a seven-step unit and the average gait width was recorded for each animal. Average step length was obtained by measuring the distance from the first step to the seventh step and dividing by seven. The alternation coefficient, which describes the uniformity of step alternation, was calculated by determining the mean of the absolute values of 0.5 minus the ratio of (right to left) to (right to right) step distance. A perfect tandem alternating gait, in which each step falls equidistant from the preceding and succeeding opposite steps, would have a score of 0. A shuffle gait, in which all alternate steps fall exactly beside the preceding opposite step, would have a score of 0.5. Linear movement, which is an indication of weaning, was calculated by drawing a line perpendicular to the line of travel starting from the first footprint. The angles between this perpendicular line and each subsequent right footprint were determined. The absolute values of the angle differences between consecutive steps were summed and divided by the number of steps scored. A large value for this parameter is indicative of nonlinear movement along the runway (Clark et al., 1997).

Behavioral Observations. We observed mice for the specific cerebellar symptoms of postural hypotonia and hyperreflexia. Postural hypotonia is characterized by animals lying flat on their abdomens with fore and hind limbs splayed laterally (Modianos and Pfaff, 1976). This effect is known to be caused by inhibition of postural muscle tone (Gilman, 1969). Hyperreflexia is manifested as jumping or popcorn behavior (Dewey, 1986). This involved exaggerated and synchronous contraction of fore and hind limb extensors, which could...
be elicited by auditory stimulus or disturbing the animals cage. In addition, animals suspended by their tails were observed for exaggerated bilateral limb extension.

Data Analysis. For the bar cross test, the mean number of slips was recorded for each group at each time point tested. A one-way ANOVA factoring number of slips and treatment group, or a one-way repeated measures ANOVA factoring slips and treatment were used to determine significance. Post hoc tests for individual groups included Dunnett’s, paired, and unpaired t tests as indicated.

For the gait analysis, a one-way ANOVA factoring either gait width, step length, alternation coefficient, or linear movement and treatment group, or a one-way repeated measures ANOVA factoring one of the gait parameters and treatment was used to determine statistical significance, followed by post hoc Dunnett’s, paired, or unpaired t tests as indicated.

Results

Effects of CP55940 on Cerebellar Function. Mice were injected intraperitoneally with the CB₁ receptor agonist CP55940 at 0.3, 1, or 3 mg/kg. Mice treated with 1 or 3 mg/kg CP55940 slipped significantly more on the bar cross test than mice treated with vehicle (p < 0.05 and p < 0.01, respectively) at all time points tested (Fig. 2A). When placed on the horizontal bar for testing, CP55940-treated mice exhibited symptoms of truncal ataxia, including oscillations of the trunk when attempting to balance and walk on the bar. Doses of CP55940 higher than 3 mg/kg elicited significant catalepsy, which prevented the mice from completing the bar cross.

CP55940, at doses of 1 and 3 mg/kg, significantly increased
1. **Effects of Other Cannabinoids on Cerebellar Function.** Administration of THC at 100 mg/kg significantly increased the number of slips on the bar cross (p < 0.01) and significantly increased gait width (p < 0.01) compared with measurements taken before administration of THC (Table 2). THC did not significantly affect either measure at 30 mg/kg i.p. Animals treated with 100 mg/kg THC showed some symptoms of truncal ataxia as indicated by mild oscillatory motions when attempting to balance or cross the bar. These effects were much less dramatic than those seen with CP55940. Mice also displayed postural hypotonia, but only a few mice exhibited mild hyperreflexive movements.

2. **Administration of the CB1 agonist Win 55212-2 (10 mg/kg) significantly increased the number of slips (p < 0.01) on the bar cross and increased gait width (p < 0.01) compared with values obtained before treatment (Table 2). Mice behaved similarly and showed symptoms comparable to mice treated with THC with the exception of hyperreflexive movements, which were more pronounced after administration of Win 55212-2 than THC.

3. The arachidonate amides AEA, ACEA, and ACPA (3–10 mg/kg i.v.) all significantly increased the number of slips on the bar cross (p < 0.01) and increased gait width (p < 0.01) compared with measurements taken before drug administration (Table 2). Mice treated with AEA, ACEA, or ACPA were initially hyperactive and hyperreflexive; this was followed by a brief period of sedation with animals returning to normal behavior within 10 min of administration. Like CP55940-treated animals, these mice exhibited symptoms of truncal ataxia upon attempting to walk the bar. The performance on the bar cross and gait width were not significantly different from control values within 5 min of administration, although the sedative effects of ACPA were of longer duration than AEA or ACEA.

4. AEA can be rapidly converted to arachidonic acid through the action of fatty acid amide hydrolase in the brain and liver (Willoughby et al., 1997). To ensure that the effects of AEA were CB1 receptor mediated, mice were pretreated with SR141716 (5 mg/kg). Pretreatment of mice with SR141716 significantly attenuated the diminished performance on the bar cross (p < 0.01) and increased gait width (p < 0.01) induced by administration of 3 mg/kg CP55940 (Fig. 3). The exaggerated bilateral limb extension produced by CP55940 was also inhibited by pretreatment with SR141716 (Table 1).

5. **Effects of SR141716 on Cerebellar Dysfunction.** To evaluate the contribution of the CB1 receptor to the effects of CP55940 on cerebellar function, animals were pretreated with the CB1 receptor antagonist SR141716 30 min before the administration of 3 mg/kg CP55940. Treatment of mice with SR141716 alone at 1 mg/kg did not significantly alter performance on the bar cross or gait width (Fig. 3). However, SR141716 at a dose of 5 mg/kg significantly increased gait width compared with vehicle-treated animals (Fig. 3A). Pretreatment of mice with SR141716 at both doses significantly attenuated the diminished performance on the bar cross (p < 0.01) and increased gait width (p < 0.01) induced by administration of 3 mg/kg CP55940 (Fig. 3). The exaggerated bilateral limb extension produced by CP55940 was also inhibited by pretreatment with SR141716 (Table 1).

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### Table 1

<table>
<thead>
<tr>
<th>Drug Treatment</th>
<th>No. of Animals Exhibiting Bilateral Limb Extension</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>0/4</td>
</tr>
<tr>
<td>CP55940 (3 mg/kg)</td>
<td>4/4</td>
</tr>
<tr>
<td>SR141716A (5 mg/kg)</td>
<td>0/4</td>
</tr>
<tr>
<td>SR141716 + CP55940</td>
<td>1/5</td>
</tr>
</tbody>
</table>

# Fig. 2

Mean number of slips on the bar cross (A) and average gait width (B) after administration of vehicle (●) or CP55940 at 0.3 (□), 1 (○), and 3 mg/kg (●). Statistical significance determined by repeated measures ANOVA (A) or one-way ANOVA (B) followed by post hoc Dunnett's test (n = 4–7/group). *Significantly different from vehicle-treated group with p < 0.05.
Effects of cannabinoids on gait width and bar slips in male mice

Gait width and number of slips were determined in untreated mice as described under Materials and Methods; then mice were treated with cannabinoid with the dose and route of administration indicated. Gait width was determined again 1 to 2 min after i.v. drug administration or 15 min after i.p. drug administration and number of slips on the bar cross was determined 5 min after i.v. drug administration or 10 min after i.p. drug administration. Differences between predrug and postdrug measures are reported as mean ± S.E.M., numbers in parentheses indicate number of replications.

<table>
<thead>
<tr>
<th>Drug Treatment</th>
<th>Change in Gait Width</th>
<th>Change in Slips</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle (3)</td>
<td>±0.8 ± 1.4</td>
<td>±0 ± 0.6</td>
</tr>
<tr>
<td>CP55940, 3 mg/kg i.p. (3)</td>
<td>±9.7 ± 1.3*</td>
<td>±23.0 ± 3.5*</td>
</tr>
<tr>
<td>Win 55212-2, 10 mg/kg i.p. (5)</td>
<td>±7.5 ± 1.2*</td>
<td>±18.2 ± 4.6*</td>
</tr>
<tr>
<td>THC, 100 mg/kg i.p. (5)</td>
<td>±8.2 ± 1.1*</td>
<td>±7.4 ± 1.8*</td>
</tr>
<tr>
<td>Win 55212-3, 10 mg/kg i.p. (5)</td>
<td>±0.7 ± 0.8</td>
<td>±0.2 ± 0.2</td>
</tr>
<tr>
<td>Cannabidiol, 100 mg/kg i.p. (5)</td>
<td>±0.4 ± 0.6</td>
<td>±0.2 ± 0.7</td>
</tr>
<tr>
<td>ACEA, 10 mg/kg i.v. (4)</td>
<td>±11.4 ± 1.7*</td>
<td>±19.8 ± 3.8*</td>
</tr>
<tr>
<td>ACPSA, 3 mg/kg i.v. (3)</td>
<td>±10.3 ± 1.2*</td>
<td>±21.7 ± 3.2*</td>
</tr>
</tbody>
</table>

*Significantly different from vehicle treated mice at p < 0.01 (Dunnett’s t test).

Effects of Dopamine Agonists on CP55940-Induced Cerebellar Dysfunction. To evaluate the contribution of cannabinoid-induced inhibition of nigrostriatal dopaminergic transmission to the diminished performance on the bar cross and abnormal gait, we pretreated animals with apomorphine (15 min) and bromocriptine (3 h) before the administration of CP55940. Neither administration of apomorphine alone at 4 mg/kg, nor bromocriptine alone at 8 mg/kg, significantly altered any parameters of gait or performance on the bar cross, although there was a trend toward motor incoordination after administration of apomorphine at 4 mg/kg. Pretreatment of mice with apomorphine or bromocriptine did not attenuate the effects of 3 mg/kg CP55940 on either the bar cross or gait width (Fig. 5). In fact, mice pretreated with both dopamine agonists showed a significant increase in the number of slips on the bar cross test at 30- and 50-min time points for apomorphine and 10-, 30-, and 50-min time points for bromocriptine compared with mice treated with CP55940 alone (Fig. 5A). Bromocriptine also potentiated the effects CP55940 on gait width (Fig. 5B).

Mice treated with apomorphine and bromocriptine alone were hyperactive. Mice pretreated with apomorphine followed by CP55940 exhibited a prolonged hyperactive stage in which they displayed hyperreflexive jumping movements and attempted to rear with the support of the cage wall. These effects were less pronounced in animals pretreated with bromocriptine followed by CP55940. This period was followed by a hypoactive period during which mice displayed postural hypotonia, followed by sleep. Animals in both pretreatment groups displayed symptoms of truncal ataxia such as truncal oscillations when attempting to balance on the bar.
Discussion

Early research into the behavioral effects of cannabinoids noted cerebellar deficits such as static ataxia in dogs and incoordination and hyperreflexia in primates (Conrad et al., 1972; Ho et al., 1972; Stark and Dews, 1980; Dewey, 1986). In spite of these early observations, little is known about the cellular mechanism(s) underlying these cannabinoid effects. The purpose of this study was to use well characterized tests of cerebellar function to determine the role of the CB₁ receptor in, and to begin to understand the neuroanatomical bases for, these cannabinoid effects.

All of the CB₁ receptor agonists tested were found to induce cerebellar motor incoordination as measured by the bar cross test. These results are in agreement with the recent report of decreased latency to fall from a roto-rod apparatus in mice.
given intracerebellar injections of THC (Dar, 2000). In addition to cerebellar motor incoordination, all of the CB1 receptor agonists tested induced symptoms of profound truncal ataxia, including increased base of support while walking (revealed as an increase in gait width) and truncal oscillations when attempting to balance or walk on the bar (Poirier et al., 1974). Postural hypotonia was also induced by all of the CB1 receptor agonists, although the duration of the deficit was variable among the various compounds. Symptoms of postural hypotonia have been described previously (Dewey, 1986).

The CB1 receptor agonists also induced alterations in reflexes that are consistent with a cerebellar locus of action. The CB1 receptor agonists produced hyperreflexia as manifest by pronounced jumping behavior as a result of synchronous bilateral contraction of both fore and hind limb extensors. This effect is consistent with alterations in the vestibulo-limb reflex pathway that is under the control of cerebellar circuits (Lindsay et al., 1976; Lindsay and Rosenberg, 1977). In addition, mice exhibited exaggerated bilateral extension of fore and hind limbs when suspended by their tails. During normal postural adjustments, animals exhibit ipsilateral shortening and contralateral lengthening of limb extensors; however, animals with cerebellar/vestibular lesions exhibit bilateral shortening of limb extensors under similar circumstances (Lindsay et al., 1976; Lindsay and Rosenberg, 1977).

The motor incoordination, truncal ataxia, and exaggerated bilateral limb extension induced by administration of CP55940 and AEA were attenuated by the CB1 receptor-selective antagonist SR141716 (Rinaldi-Carmona et al., 1995). SR141716 has also been shown to attenuate THC- and AEA-induced static ataxia in dogs (Lichtman et al., 1998). Similarly, Dar (2000) reported that the motor deficits seen after intracerebellar THC infusion were eliminated by pretreatment with an antisense deoxyoligonucleotide sequence for the CB1 receptor. The negative controls used in the present study, two compounds structurally similar to active cannabinoids, without affinity for the CB1 receptor [cannabidiol (Devane et al., 1988) and Win 55212-3 (Kuster et al., 1993)], did not produce any cerebellar motor deficits. These data further support a CB1 receptor-mediated mechanism.

The structure-activity profile for the CB1 receptor agonists tested is similar between the two cerebellar tests with the exception of THC, which has significantly lower efficacy in the bar slip assay than the other compounds. In vitro studies demonstrate that THC is a low-efficacy, partial agonist of the CB1 receptor compared with CP55940 and Win 55212-2 (Sim et al., 1996). We suggest that the lower efficacy of THC is apparent in the bar slip assay because there is no upper limit of impairment (i.e., there is theoretically no limit to the number of times an animal can slip). On the other hand, the gait width assay has a ceiling, the mice can only spread their limbs a certain distance, such that even a partial agonist is fully effective. The ratio of equieffective doses of CP55940, Win 55212-2, and THC in the gait width assay is approximately 1:3:10, which agrees well with the relative potencies of these compounds to produce other physiological and behavioral effects in mice, including hypothermia, hypolocomotion, and antinociception (Little et al., 1988; Compton et al., 1993; Wiley et al., 1998).

We have also compared the potencies of three ethanol-amides of arachidonic acid: the endocannabinoid AEA and two high-affinity, CB1 receptor-selective derivatives, ACPA and ACEA (Hillard et al., 1999). These CB1 agonists were administered by i.v. injection because of their rapid metabolism and short half-lives (Willoughby et al., 1997; Hillard et al., 1999). In spite of the fact that ACPA and ACEA bind to the CB1 receptor with Ki values 25- to 50-fold lower than AEA, all three arachidonamides affected cerebellar function in a similar dose range. This is in agreement with the effects of these agonists on body temperature in mice (Hillard et al., 1999) and likely reflects the low bioavailability of these compounds.

Increased GABAergic tone and subsequent inhibition of nigrostriatal dopaminergic projection neurons have been proposed to mediate the extrapyramidal deficits seen after administration of CB1 receptor agonists (for review, see Ameri, 1999). It has been demonstrated that dopamine agonists attenuate cannabinoid-induced catalepsy (Ghosh et al., 1980). The dopamine agonist apomorphine has also been used to rule out extrapyramidal effects in the cerebellar neurotoxicity of 3-acetyl pyridine (De Michelle et al., 1980). To eliminate cannabinoid-induced inhibition of nigrostriatal dopaminergic transmission as a possible cause of the motor incoordination and ataxia, mice were pretreated with D1/D2 receptor agonist apomorphine or the D2 receptor agonist bromocriptine before challenge with CP55940. Neither apomorphine nor bromocriptine attenuated the effects of CP55940 on either bar slips or increased gait width. Since CP55940 was able to produce significant cerebellar deficits as measured by gait analysis and the bar cross test in the presence of dopamine agonists, it is unlikely that the extrapyramidal deficits induced by cannabinoids contribute to the motor deficits observed in this study. These results are consistent with our hypothesis that the CB1 receptor agonists produce direct effects on cerebellar function, and the behavioral deficits measured by these tests are not a consequence of inhibition of dopaminergic function in the extrapyramidal system.

Interestingly, both dopamine agonists tested produced a potentiation of CP55940-induced motor incoordination as measured by the bar cross; bromocriptine also potentiated the effects of CP55940 on gait width. The mechanism by which dopamine agonists interact with cannabinoids to potentiate the cerebellar deficits remains unclear. However, both dopamine agonists increase spontaneous locomotion (Dogrul and Yesilyurt, 1999), which could increase the number of slips in the bar cross test.

The CB1 receptor is localized at two synapses within the cerebellum: the granule cell/Purkinje cell synapse and the basket cell/Purkinje cell synapse (Tsou et al., 1998). Activation of the CB1 receptor at either of these synapses results in an inhibition of neurotransmitter release (Takahashi and Linden, 2000). Since the granule cell input is excitatory, CB1 agonists would be expected to decrease Purkinje cell activity through effects on this synapse. Conversely, the basket cell synapse is inhibitory, so CB1 agonists acting here would increase Purkinje cell excitability. Based upon data discussed below, we hypothesize that the primary mechanism by which cannabinoids induce cerebellar deficits is via inhibition of GABAergic neurotransmission at the basket cell-Purkinje cell synapse (Fig. 6). Inhibition of GABAergic transmission at this synapse would lower the threshold for...
Purkinje cell is disinhibited as a result of the activation of the basket cell Purkinje cell synapse, which leads to an increased Purkinje cell output in response to climbing fiber excitation, and inhibition of the deep cerebellar nuclei. The reduced facilitatory output of these nuclei to projection sites such as the vestibular nucleus or motor cortex may underlie the cerebellar deficits seen after cannabinoid administration. BC, basket cell; CF, climbing fibers; DCN, deep cerebellar nuclei; LVN, lateral vestibular nucleus; PC, Purkinje cell; inf. oliv., inferior olivary nucleus.

Purkinje cell firing and result in inhibition of the deep cerebellar nuclei.

Acute treatment of mice with indole alkaloids produces cerebellar effects that are similar to the effects of the cannabinoids (O’Hearn and Molivar, 1997). In particular, these agents induce ataxia and extensor limb movements that propel the animals into the air. The proposed mechanism of action of these agents is increased Purkinje cell excitation via synchronous activation of climbing fibers, which leads to inhibition of the deep cerebellar nuclei and manifestation of motor deficits. It is our working hypothesis that CB1 receptor agonists also increase Purkinje cell excitation; however, the mechanism is a reduction in the threshold for Purkinje cell firing in response to normal levels of climbing fiber input. This model is further supported by data indicating lesions of the deep cerebellar nuclei or the vestibular nucleus or motor cortex may underlie the cerebellar deficits seen after cannabinoid administration. BC, basket cell; CF, climbing fibers; DCN, deep cerebellar nuclei; LVN, lateral vestibular nucleus; PC, Purkinje cell; inf. oliv., inferior olivary nucleus.

Other evidence that supports this hypothesis includes the demonstration, using functional magnetic resonance imaging, that THC decreases the activity of neurons in the deep cerebellar nuclei in humans (Bloom et al., 2000). Furthermore, cannabinoids increase glucose metabolism (Volkow et al., 1991) and blood flow (Mathew et al., 1998) in the cerebellar cortex, which is also predicted by the model since the Purkinje cell is disinhibited as a result of the activation of CB1 receptors on the basket cell (Fig. 6).

In summary, we have demonstrated that CB1 agonists produce cerebellar deficits such as motor incoordination, truncal ataxia, postural hypotonia, and hyperreflexia via a receptor-mediated mechanism. Additionally, these effects were not attenuated by pretreatment with dopamine agonists, indicating that cannabinoids produce motor deficits that are not dependent on cannabinoid-induced inhibition of nigrostriatal dopaminergic transmission. A mechanism of action that is consistent with the localization of the CB1 receptors within the functional anatomy of the cerebellum is that activation of the CB1 receptor of the basket cell results in inhibition of GABAergic transmission at the basket cell-Purkinje cell synapse. This leads to an increased Purkinje cell output and inhibition of neurons in the deep cerebellar nuclei. In addition, we suggest that the assays used in this study to quantify the effects of cannabinoids on cerebellar function be added to the classic behavioral measures currently being used to evaluate cannabinimetic activity.

Acknowledgment
We thank Alan S. Bloom, Ph.D., for helpful discussions and advice.

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Fig. 6. Functional diagram of normal cerebellar circuitry (A) and alterations we hypothesize are induced by cannabinoids (B). Line thickness represents strength of synaptic transmission. CB1 receptor agonists inhibit GABAergic transmission at the basket cell-Purkinje cell synapse, which leads to an increased Purkinje cell output in response to climbing fiber excitation, and inhibition of the deep cerebellar nuclei. The reduced facilitatory output of these nuclei to projection sites such as the vestibular nucleus or motor cortex may underlie the cerebellar deficits seen after cannabinoid administration. BC, basket cell; CF, climbing fibers; DCN, deep cerebellar nuclei; LVN, lateral vestibular nucleus; PC, Purkinje cell; inf. oliv., inferior olivary nucleus.
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