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The β -lactam antibiotic ceftriaxone as a treatment for the symptoms of Parkinson's disease and L-DOPA-induced dyskinesia in 6-OHDA-lesioned rats.

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by
Caroline Neville

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Running Head: CEFTRIAXONE IN PARKINSON'S DISEASE AND LID

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Abstract

Parkinson's disease (PD) is a progressive neurodegenerative disorder caused by the loss of dopamine (DA) neurons in the substantia nigra. The most effective treatment is DA replacement therapy using the DA precursor L-DOPA, which can unfortunately often result in L-DOPA-induced dyskinesia (LID). Animal studies in hemi-Parkinsonian rats have shown glutamatergic NMDA receptor antagonists to be effective in treating both PD symptoms and LID. However, the cognitive side effects prevent these drugs from passing clinical trials. Upregulation of GLT-1, the primary glutamate transporter that removes glutamate from the synapse, could be an alternative to direct receptor antagonism. The β -lactam antibiotic ceftriaxone has been shown to substantially increase GLT-1 protein expression and activity in the brain without side effects. In Experiment 1 it was found that sub-chronic injections of 100 mg/kg ceftriaxone in unilaterally 6-OHDA-lesioned rats caused a 44% increase in impaired forepaw stepping, a measure of bradykinesia, that lasted at least 30 days after the last ceftriaxone injection. In Experiment 2, sub-chronic injections of 50 mg/kg ceftriaxone resulted in a 41% increase in impaired forepaw stepping that was found to be equivalent to that produced by 10 mg/kg L-DOPA. However unlike ceftriaxone, treatment with L-DOPA resulted in the development of L-DOPA-induced dyskinesia. Ceftriaxone was able to slow the development of LID, but not decrease the expression of pre-established LID. Indicating that the effects of ceftriaxone on forepaw stepping were due to enhanced GLT-1 function, injections of the selective GLT-1 inhibitor, dihydrokainate (DHK) reduced the improvement in stepping produced by ceftriaxone. Collectively, these data indicate that ceftriaxone may be a superior treatment for Parkinson's disease than L-DOPA.

The β -lactam antibiotic ceftriaxone as a treatment for the symptoms of Parkinson's disease and L-DOPA-induced dyskinesia in 6-OHDA-lesioned rats.

Parkinson's disease (PD) is the second-most common progressive neurodegenerative disorder, second to Alzheimer's disease, affecting 2% of the population over 65. Parkinson's is characterized by the progressive loss of dopaminergic (DA) input from the substantia nigra pars compacta to the striatum of the basal ganglia (Massie et al., 2010). Loss of striatal DA causes five typical motor dysfunctions: akinesia, muscle rigidity, resting tremors, gait disturbances, and postural imbalance (Cenci & Lundblad, 2006). Symptoms typically begin after the loss of 50-60% of DA neurons and 70-80% of the striatal extracellular DA (Cenci & Lundblad, 2006).

At this point there is no cure, prevention, or slowing of Parkinson's disease, only symptom management. Symptoms are most commonly treated with the dopamine precursor levodopa (L-DOPA), which remains the most effective anti-parkinsonian drug 40 years after its development (Calon, Morissette, Raiput, Hornykiewicz, Bedard, & Di Paolo, 2003). L-DOPA treatment causes a mild to dramatic reduction of motor deficits (Cenci & Lundblad, 2006) by increasing the synthesis of dopamine (Carta, Carlsson, Kirik, & Bjorklund, 2007). However, as the disease progresses, there are fewer DA neurons available to synthesize DA. Interestingly, serotonin (5-HT) neurons are able to convert L-DOPA into DA and, as the disease progresses and there are fewer DA neurons, these 5-HT neurons are responsible for the majority of the DA release in the striatum (Miller & Abercrombe, 1999). Because the 5-HT neurons are less able to regulate release, extracellular DA concentrations fluctuate depending on L-DOPA availability (Carta et al., 2007). This sporadic activation of DA receptors can lead to reduced L-DOPA efficacy, motor fluctuations, and, ultimately, prevalent side effects such as L-DOPA-induced

dyskinesia (LID; Mouradian, Heuser, Baronti, & Chase, 1990). Both motor fluctuations and LID develop in 40% of patients after 5 years and in 90% of patients after 9 years of L-DOPA treatment (Cenci & Lundblad, 2006; Kobylecki, Cenci, Crossman, Ravenscroft, 2010). Because of these undesirable side effects to the primary PD treatment, it is clear that better therapies are needed.

Recently it has become widely accepted that the pathophysiology of PD and LID also involves increased glutamatergic transmission within the striatum. With the loss of dopaminergic projections onto glutamatergic corticostriatal afferents, these striatal inputs are disinhibited, causing an increased release of glutamate into the striatum (Nash et al., 2000; Chung, Chen, Chan, & Yung, 2008). Moreover, this glutamate release appears to be further enhanced during LID (Jonkers et al., 2002; Robelet et al., 2004). Indicating the causal nature of increased glutamate in PD, antagonists, especially of the NMDA receptor, have been shown to improve PD-like motor symptoms (Kelsey, Maque, Pijanowski, Harris, Kleckner, & Matthews, 2004; Nash et al., 2000), potentiate L-DOPA's therapeutic effects (Kelsey et al., 2004; Klockgether & Turski, 1990), and suppress LID in animal models (Blanchet, Konitsiotis, & Chase, 1998; Kobylecki et al., 2010). Although NMDA antagonists have shown therapeutic effects in human PD patients (Snow, Macdonald, Mcauley, & Wallis, 2000), they have a very narrow therapeutic index (Metman, Blanchet, Munckhof, Dotto, Natte, & Chase, 1998). Moreover, most NMDA receptor antagonists have failed clinical trials due to intolerable side effects, suggesting the need for alternative therapies (Chu et al., 2007).

More recently, upregulation of the primary glutamate transporter GLT-1 (EAAT-2 in humans) is being explored as an alternative to direct glutamate antagonism in many neurodegenerative diseases other than PD. In 2005 Rothstein et al. discovered that β -lactam

antibiotics, such as ceftriaxone, increase GLT-1 protein expression by 300-600% (Rothstein et al., 2005) and decrease extracellular glutamate by 50% (Rasmussen, Baron, Kim, Unterwald, & Rawls, 2011). Thus, far ceftriaxone has proven useful in reducing neurodegeneration and behavioral deficits in animal models of amyotrophic lateral sclerosis (ALS), stroke, and Huntington's disease (Miller et al., 2008; Rothstein et al., 2005; Thone-Reineke et al., 2008). Conveniently, the dose of ceftriaxone required to upregulate GLT-1 protein expression to a level that is neuroprotective falls within the range approved by the FDA to treat meningitis (Nau et al., 1993; Rothstein et al., 2005).

In this study I will test the hypothesis that the upregulation of GLT-1 by ceftriaxone will also improve Parkinson-like symptoms and decrease LID in the 6-OHDA-lesioned hemiparkinsonian rat. In this introduction, I will first examine the pathophysiology of PD and LID. I will then focus on the role of glutamate. I will finish by exploring the actions of ceftriaxone in other neurodegenerative disorders as a background for its use in the treatment of PD and LID.

Parkinson's Disease

Typically Parkinson's disease first presents with a resting tremor in a limb on the side contralateral to the greatest deterioration (Guttman, Kish, & Furukawa, 2003). Bradykinesia then begins to develop, causing slowing of movement and difficulty initiating action. Rigidity of muscles at rest, postural imbalance, and gait disturbances are also very common (Cenci & Lundblad, 2006; Guttman, 2003). Often motor symptoms occur concurrently with non-motor symptoms, whose etiology is less understood. These typically include REM sleep disorder and autonomic dysfunction (Braak, Del Tredici, Rub, de Vos, Steur, & Braak, 2003). In the later stages of the disease some patients develop dementia (Braak et al., 2003). However, no two patients are alike in the type or progression of symptoms (Halliday & McCann, 2010). Diagnosis

is typically made based on motor symptoms and response to DA replacement therapy, as there are no specific changes associated with PD that can be detected in scans. However, a definitive diagnosis can only be made through autopsy (Guttman et al., 2003).

Etiology and pathology of PD. The cause of Parkinson's disease remains elusive. It is currently thought that most cases of idiopathic PD are due to a combination of environmental and genetic factors that cause a predisposition for the disease (Guttman et al., 2003). Although the causes are typically unknown there is some understanding of the mechanism behind neuronal death and disease progression. In the substantia nigra cell death is thought to be caused by a combination of a dysfunction of the α -synuclein protein, mitochondrial dysfunction, inflammation, oxidative stress, disruption of calcium homeostasis, and excitotoxicity caused by excess glutamate (Duty, 2010; Greenamyre & Hastings, 2004; Schulz, 2007). A common occurrence is the aggregation of the α -synuclein protein in Lewy bodies, which are thought of as a hallmark of the disease of both idiopathic and the less common hereditary form of the disease (Greenamyre & Hastings, 2004). α -synuclein proteins are susceptible to environmental influence, causing them to unfold, aggregate and form fibrils that make up the main component of Lewy bodies (Schulz, 2007). Environmental factors typically associated with the disease include overexposure to carbon monoxide, heavy metals, and pesticides (Guttman et al., 2003; Murdoch, Cheer, & Wagstaff, 2004).

Basal Ganglia circuitry and PD. The motor symptoms of Parkinson's disease are caused by the degeneration of the dopamine neurons of the substantia nigra pars compacta, which results in the loss of striatal DA and resultant disruption in function of the basal ganglia (Massie et al., 2010). In order to understand the changes in the basal ganglia caused by the depletion of dopamine in the striatum, it is imperative to understand the connections and interactions between

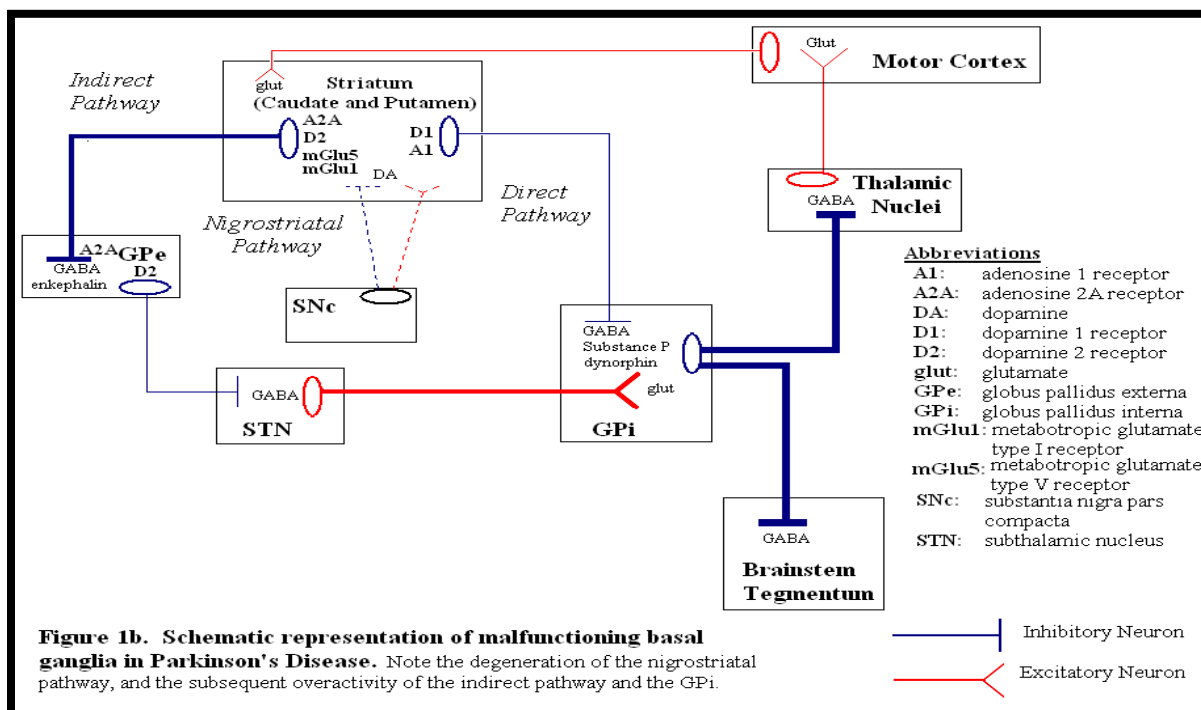
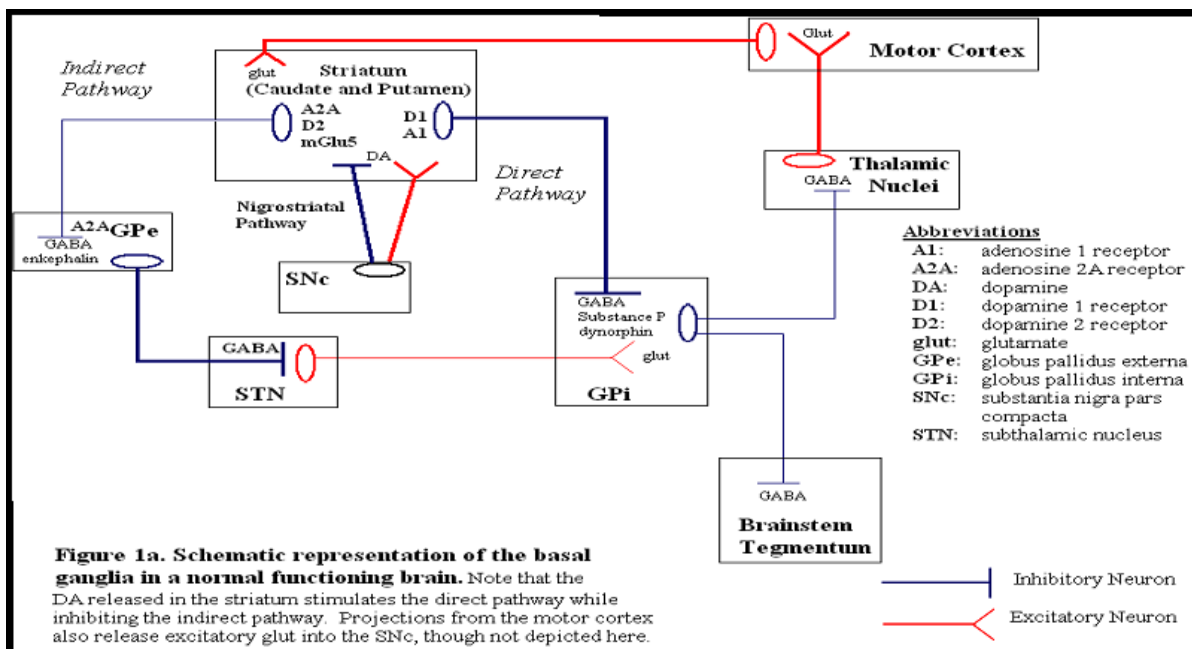


Figure 1a and 1b. Normal and Abnormal Function of the Basal Ganglia. In normal functioning basal ganglia, both direct and indirect pathways coordinate input to produce refined movement. In PD, the direct pathway has decreased input, whereas the indirect pathway becomes overactive.

the structures of the basal ganglia. The substantia nigra pars compacta is the major dopaminergic afferent to the medium spiny GABAergic neurons of the striatum (Albin, Young, & Penny, 1989; Exposito, Del Arco, Segovia, & Mora, 1999). The striatum, which is the major afferent structure of the basal ganglia, consists of the medial caudate, containing inhibitory D2 receptors of the indirect pathway, and lateral putamen, containing excitatory D1 receptors of the direct pathway (Albin et al., 1989; Doig, Moss & Bolam, 2010; Feyder, Bonito-Oliva, & Fisone, 2011). In the direct pathway, neurons from the putamen release inhibitory GABA onto the internal globus pallidus (GPi), which decreases the release of GABA by the GPi onto the thalamic nucleus, disinhibiting it (Castle, Aymerich, Sanchez-Escobar, Gonzalo, Obeso, & Lanciego, 2005). The disinhibited thalamus then releases excitatory glutamate onto the motor cortex, increasing movement (Albin et al., 1989). In contrast, activation of the indirect pathway causes inhibition of the motor cortex, effectively opposing the direct pathway (Feyder et al., 2011). However dopamine is inhibitory when acting on the D2 receptors of the indirect pathway, thereby inhibiting the GABA projections from the medial caudate nucleus to the external globus pallidus (GPe), disinhibiting it, which causes the GPe to release GABA onto the subthalamic nucleus (STN; Albin et al., 1989; Doig et al., 2010). Inhibition of the STN reduces glutamate release onto the GPi, preventing the GPi from inhibiting the thalamic nucleus. The disinhibited thalamic nucleus can then excite the motor cortex, eliciting movement (Feyder et al., 2011).

In Parkinson's disease, because of the degeneration of the dopamine neurons of the substantia nigra, there is a decrease in dopaminergic input to both the caudate and putamen of the striatum which decreases activity in the direct pathway (Blandini, Porter, & Greenamyre, 1996; Feyder et al., 2011) and increases activity of the indirect pathway (Albin et al., 1989; Klockgether & Turski, 1990), both resulting in increased activation of the STN (Nash & Brotchie,

2002; Rylander, Recchia, Mela, Dekundy, Danysz, & Cenci, 2009) and decreased activation of the motor cortex and, thus, decreased movement (Blandini et al., 1996; Duty, 2010).

L-DOPA

Because there is still no cure for Parkinson's and no prevention, as its causes are unknown, the only relief that can be brought to patients is symptom management. The most commonly used and most effective treatment of PD symptoms is levodopa (L-DOPA), although direct dopamine agonists are also effective (Miller & Abercrombe, 1999). L-DOPA is a dopamine precursor that works to increase dopamine synthesis and, therefore, transmission in the striatum (Carta et al., 2007). When L-DOPA is given to a less impaired person, the L-DOPA easily crosses the blood-brain-barrier and is transported into dopaminergic neurons and converted to DA by aromatic L-amino acid decarboxylase (AADC) (Miller & Abercrombe, 1999). The DA is then transported into synaptic vesicles by vesicular monoamine transporter-2 (VMAT-2; Cenci & Lundblad, 2006). Once the neuron is activated, the vesicles release the DA into the synapse where it can either bind to DA receptors, be taken back into the DA neuron by the high-affinity dopamine transporter (DAT), or broken down by monoamine oxidase (MAO) or catechol-O-methyl transferase (Cenci & Lundblad, 2006; Miller & Abercrombe, 1999).

In Parkinson's, however, because of the degeneration of the dopaminergic neurons of the substantia nigra, there are fewer DA neurons available to take up the L-DOPA and convert it to DA. Recent evidence suggests that serotonergic (5-HT) neurons projecting to the striatum from the raphe nucleus also take up the excess L-DOPA. Because 5-HT cells also contain AADC and VMAT-2, DA is able to be synthesized and released from serotonergic as well as dopaminergic neurons into the striatum (Miller & Abercrombe, 1999). This allows L-DOPA to continue to be therapeutic despite the degeneration of the majority of the dopaminergic neurons in the

substantia nigra (Carta et al., 2007). Although this enhanced release of DA from 5-HT neurons can be helpful in treating advanced cases of PD with severe DA degeneration, this release is less effectively regulated, resulting in decreased efficacy and side effects. For example, 5-HT neurons lack DAT, thereby decreasing the clearance of dopamine from the synapse (Cenci & Lundblad, 2006). In addition, because serotonergic neurons lack DA autoreceptors, there is no feedback mechanism to regulate the amount of DA being released. Finally, because the DA is taking up room in the vesicles, there is less vesicular space available for 5-HT, leading to a decrease in extracellular 5-HT, and, therefore, reduced activation of the serotonergic autoreceptors further increasing extracellular DA (Carta et al., 2007). Because the DA is, thus, released in large quantities over a short period of time, there are large fluctuations in DA release depending on L-DOPA availability (Cenci & Lundblad, 2006). This non-physiological, unregulated, intermittent release of DA is thought to be a major contributor to the development of levodopa-induced dyskinesia (Carta et al., 2007; Mouradian et al., 1990). Consequently, the application of continuous L-DOPA in patients with severe PD and LID decreases the magnitude of LID and increases L-DOPA's therapeutic window (Mouradian et al., 1990).

Furthermore, this unregulated, pulsatile release of large amounts of DA is almost certainly responsible for the development of a number of motor fluctuations, most commonly expressed as a decreased duration of effect (wearing-off) or unpredictable swings in efficacy (on-off; Hametner, Seppi, & Poewe, 2010). Wearing-off is thought to be caused by the use of all the L-DOPA over a short period of time due to the over-release of DA by 5-HT neurons (Cenci & Lundblad, 2006). The on-off phenomenon, seen in the random appearance and disappearance of PD symptoms, is much less understood but is probably also related to the large fluctuations in DA release (Hametner et al., 2010).

Levodopa-induced dyskinesia. Although L-DOPA is the most effective method of symptom management, as Parkinson's disease gets worse, efficacy decreases and negative side effects begin to develop, the most common of which is levodopa-induced dyskinesia (LID) (Mouradian et al., 1989). Over one third of patients develop dyskinesias within the first five years of treatment, usually beginning in the limb or side most affected by PD (Calon et al., 2003; Kobylecki et al., 2010). There are three types of dyskinesias seen most commonly in LID: ON dyskinesias, biphasic dyskinesias, and OFF period dyskinesias (dystonia; Hametner et al., 2010). ON dyskinesias are caused by peak levels of L-DOPA and are characterized by chorea (brief irregular movement) or ballistic movements (Fabbrini, Brotchie, Grandas, Nomoto, & Goetz, 2007). Biphasic dyskinesias occur as L-DOPA concentrations rise and fall past threshold level and are often comprised of stereotypic, rapid, repeating movements. Dystonia, or sustained muscle contractions that result in twisting motions or postures, occurs due to subthreshold L-DOPA concentrations (Hametner et al., 2010).

The unregulated fluctuations of DA in L-DOPA-treated patients are assumed to produce dyskinesia, in large part, by overactivation of DA receptors in the striatum, specifically the D1 receptors, which leads, in turn, to an amplification of D1-dependent intracellular signaling (Darmopil, Martin, De Diego, Ares, & Moratalla, 2009). Indeed, LID is reduced in mice lacking the D1, but not D2, receptors, suggesting D1 receptors are essential in LID development (Darmopil et al., 2009). When 6-OHDA lesioned dyskinetic rats were treated with SCH23390, a specific D1 receptor antagonist, there was a reduction in L-DOPA-induced rotational behavior, which further suggests LID is mainly due to alterations in D1 receptor function (St. Hilaire, Landry, Levesque, & Rouillard, 2005). Consistent with this hypothesis, Konradi et al. (2004) found increased D1 receptor mRNA in dyskinetic 6-OHDA lesioned rats. However, others have

found no change in mRNA expression, but instead have found increased localization of the D1 receptors at the cell membrane surface thought to be caused by impaired receptor internalization and trafficking (Aubert et al., 2005; Berthet et al., 2009).

Interestingly, there is also an upregulation of D3 receptor mRNA in the direct pathway, seen in both dyskinetic 6-OHDA-lesioned rats and MPTP-lesioned nonhuman primates (Bezard et al., 2003; Van Kampen & Stoessl, 2003). D3 receptors are thought to interact with D1 receptors, increasing sensitization and decreasing receptor internalization (Bezard et al., 2003; Van Kampen & Stoessl, 2003). Treatment with the D3 receptor antagonist ST 198 decreases LID expression and normalizes D1 receptor concentration in the plasma membrane (Feyder et al., 2011).

Closely associated with the sensitization of D1 receptors in the striatum is an increased coupling of D1 receptors with the $G_{s/olf}$ protein, leading to an amplification of the downstream intracellular pathway (Aubert et al., 2005). The overactivation of this G-protein causes increased activation of extracellular signal-regulated kinases 1 and 2 (ERK1/2) (Darmopil et al., 2009; Westin, Vercammen, Strome, Konradi, & Cenci, 2007), inactivation of which prevents the induction of LID (Santini, Valjent, & Fisone, 2007). Additionally, D1 receptor sensitization causes an increase in DARPP-32 phosphorylation by PKC, resulting in the inhibition of protein phosphatase-1, which decreases the dephosphorylation of ERK1/2, NMDA receptor subunits, and AMPA receptor subunits. Increasing evidence suggests that phosphorylation of these glutamate receptors are an important event in LID development (Cenci, 2010; Santini et al., 2007), and additional evidence now suggests that dysfunction of DA transmission in the striatum during PD leads to the increased activity of the glutamate system in the basal ganglia, accounting

for many of the manifestations of both PD and LID. These data are discussed further in the next section.

Glutamate

Although the pathology of the motor aspects of Parkinson's disease and L-DOPA-induced dyskinesia is primarily due to the loss of dopamine in the striatum and attempts to restore this loss, respectively, it has become increasingly evident that enhanced glutamate transmission, especially at the NMDA receptor, plays a large role in the development and expression of both disorders and has therefore been found to be a promising target for the alleviation of symptoms.

Glutamate and Parkinson's disease. The depletion of dopaminergic input to the striatum is thought to cause an increase in corticostriatal glutamatergic transmission onto the indirect pathway (Gardoni, Ghiglieri, Luca, & Calabresi, 2010). These afferents are regulated by inhibitory D2 presynaptic dopamine receptors on glutamate terminals (Jonkers, Sarre, Ebinger, & Michotte, 2002; Rowlands & Roberts, 1980; Yamamoto & Davy, 1992). Without the inhibitory input of dopamine in Parkinson's disease there is an increase in activity of these afferents resulting in the increased release of glutamate (Calabresi, Mercuri, Sancassario, & Bernardi, 1993; Chung et al., 2008; Lievens, Salin, Nieoullon, & Kerkerian-Le Goff, 2001; Nash & Brotchie, 2002) shown through the increase in glutamate binding to the striatum in patients and animal models of PD (Nash & Brotchie, 2002; Weihmuller, Ulas, Nguyen, Cotman, & Marshall, 1992). Increased activation of the glutamate receptors of the indirect pathway by excess extracellular glutamate would cause a downstream disinhibition of the STN, as seen in Figure 1 (Duty, 2010). Overactivation of the STN, as discussed previously, causes a downstream lack of excitation of the motor cortex, resulting in a loss of movement (Blandini et al., 1996; Duty,

2010). Additionally, the STN has glutamatergic projections that synapse in the substantia nigra. In PD when the STN is overactive, excess glutamate is dumped onto the substantia nigra, which may exacerbate degeneration via excitotoxicity (Duty, 2010; Plaitakis & Shashiaran, 2000; Rodriguez, Herrera, Koistinaho, & Hokfelt, 1999).

Although excess glutamate has been implicated in excitotoxicity, substantial evidence from animal models indicate that glutamate antagonists, especially at the NMDA receptor, can reduce the symptoms of PD well after degeneration has occurred. For example, NMDA receptor antagonists, such as MK-801, CPP, and the NMDA receptor subunit NR2B specific drug Ro 25-6981, have been found to decrease the motor symptoms of Parkinson's disease as shown by improvements in forepaw stepping and increases in contralateral turning in 6-OHDA-lesioned rats (Kelsey et al., 2004; Loschmann et al., 2004), and by increases in the median mobility scores in MPTP-lesioned non-human primates (Nash et al., 2000). Additionally, NMDA receptor antagonists have been shown to potentiate the effects of L-DOPA in non-dyskinetic 6-OHDA lesioned rats (Kelsey et al., 2004; Klockgether & Turski, 1990; Morelli, Fenu, Pinna, & Chiara, 1992). Antagonism of the metabotropic glutamate receptor mGluR5 with MPEP has also been shown to potentiate the ameliorative effects of L-DOPA on PD motor symptoms in 6-OHDA-lesioned mice (De Leonibus et al., 2009). Direct injections of the NR2B NMDA receptor subunit antagonist ifenprodil into the striatum (Nash & Brotchie, 2002) and the NMDA receptor antagonist MK-801 into the STN have also been shown to decrease PD motor deficits in 6-OHDA-lesioned rats (Blandini, Greenamyre, Fancellu, & Nappi, 2001). Further indicating that excess glutamate transmission at NMDA receptors may be causal of PD motor symptoms, injections of the direct agonist NMDA into the striatum have been shown to induce Parkinsonian motor deficits in normal rats (Nash & Brotchie, 2002). Treatment of Parkinson's disease patients

with NMDA receptor antagonist have had mixed results. A trial of memantine was able to decrease Parkinsonian symptoms and significantly decrease “off” time, but several subjects dropped out of the study due to the development of side effects such as abdominal pain, psychomotor agitation, confusion and dizziness (Rabey, Nissipeanu, & Korczyn, 1992).

Glutamate and LID. Given that PD and LID are assumed to represent opposite effects on DA transmission, it is surprising to find that glutamate antagonists are successful in reducing LID as well as the symptoms of PD (Kobylecki et al., 2010; Nash et al., 2000). NMDA antagonists such as amantadine are highly successful in decreasing motor symptoms of LID in the MPTP-lesioned nonhuman primate model of PD (Blanchet et al., 1998). Additionally, the AMPA receptor antagonist IEM 1460 was able to decrease abnormal involuntary movements (AIMs), a standard measure of LID in animals (Winkler, Kirik, Bjorklund, & Cenci, 2002), without decreasing the anti-PD effects of L-DOPA in both the 6-OHDA-lesioned rat and the MPTP-lesioned nonhuman primate (Kobylecki et al., 2010). The combination of a low dose of the AMPA receptor antagonist GYKI-47261 and the NMDA receptor antagonist MK-801 was also effective at reducing AIMs in 6-OHDA-lesioned rats (Bibbiani, Oh, Kielaitė, Collins, Smith, & Chase, 2005). The NMDA antagonist amantadine has also been found to reduce the severity of LID without reducing L-DOPA’s effects on PD in human trials (Snow et al., 2000).

Recently more attention has been paid to metabotropic Group I receptors such as mGluR5s, antagonism of which decreases the motor dysfunctions and cellular alterations associated with LID in 6-OHDA-lesioned rats (Mela, Marti, Dekundy, Danysz, Morari, & Cenci, 2007; Rylander et al., 2009) and MPTP-lesioned non-human primates (Johnston, Fox, McIlldowie, Piggott, & Brotchie, 2010). Similarly, agonists of Group II and III mGlu autoreceptors have been

shown to be beneficial in the treatment of LID and PD (Cuomo et al., 2009), although not all studies have found these effects (Murray et al., 2001; Rylander et al., 2009).

As discussed previously, chronic L-DOPA sensitizes dopamine receptors, especially the D1 receptors of the direct pathway (Aubert et al., 2005). Activation of the sensitized D1 receptors in the striatum appears to result in increased phosphorylation of NMDA and AMPA receptors, causing an increase in open-state probability of the ion channels and, thus, increased transmission (Bagetta, Ghiglieri, Sgobio, Calabresi, & Picconi, 2010; Kobylecki et al., 2010; Oh, Russell, Vaughan, & Chase, 1998). As described earlier, this increased phosphorylation is thought to be caused by a D1-dependent increase in cAMP-stimulated protein kinases (Kobylecki et al., 2010; Oh et al., 1998) and DARPP-32 mediated inhibition of dephosphorylation (Cenci, 2010; Santini et al., 2007). Indeed, DARPP-32 knock-out mice do not undergo AMPA receptor phosphorylation and do not develop LID (Santini et al., 2007). This L-DOPA-induced sensitization of these glutamate receptors combined with the L-DOPA-induced increase in glutamate release (Jonkers et al., 2002; Robelet, Melon, Guillet, Salin, & Goff, 2004) is presumed to result in the increased activity-independent movement seen in LID (Cenci & Lundblad, 2006).

Limitations of glutamate receptor antagonists. Although glutamate antagonists have been shown to be effective in ameliorating both PD-like symptoms and LID in animal models of Parkinson's disease and in human patients, there are some serious drawbacks that limit their use. Some NMDA antagonists have worsened LID (Bibbiani et al., 2005) or decreased the anti-Parkinsonian effects of L-DOPA (Goetz et al., 2007). Additionally, NMDA receptor antagonists have low therapeutic indexes in humans (Bibbiani et al., 2005; Metman et al., 1998) and often cause sedation, ataxia, cognitive impairment, and memory loss (Caraci et al., 2012). Because of

the severity of these side effects, these drugs have generally failed to pass clinical trials (Chu et al., 2007). Consequently, other means of decreasing excess glutamate transmission are needed. The next sections focus on the possibility that this may be more safely and efficaciously done by enhancing the activity of GLT-1, the main glutamate transporter.

GLT-1. Glutamate is not broken down in the synapse but is removed by a member of high affinity excitatory amino acid transporters (EAATs) (Vemuganti et al., 2001). Within the basal ganglion the most prevalent transporter is EAAT-2, known as GLT-1 in non-human animals (Rothstein et al., 1996), which is located primarily on astrocytes (Anderson & Swanson, 2000). Once inside astrocytes, glutamate is broken down into the less toxic glutamine and transported back into glutamate neurons (Anderson & Swanson, 2000; Chu et al., 2007). Although there is variation depending on brain region and presence of other transporters, in most regions, including the striatum, the majority of synaptic glutamate clearance occurs via GLT-1 (Anderson & Swanson, 2000; Rothstein et al., 1996).

The ability of GLT-1 to clear glutamate from the synapse is essential for proper neuronal functioning. Many degenerative diseases, such as amyotrophic lateral sclerosis (ALS), Alzheimer's, and Huntington's disease, are thought to involve a substantial loss of GLT-1 (Beart & O'Shea, 2007). Without properly functioning GLT-1 there is a massive increase in extracellular glutamate, as demonstrated through GLT-1 knockout, antisense oligonucleotide, and pharmacological blockade studies, which causes neuronal death by excitotoxicity (Rothstein et al., 1996; Selkirk et al., 2005; Vemuganti et al., 2001). Consequently, an increasing number of researchers have begun to focus on using GLT-1 upregulation to alleviate the symptoms and degeneration potentially caused by increased glutamate transmission and/or GLT-1 downregulation in several neurodegenerative disorders.

Ceftriaxone

After an extensive screening of 1040 FDA-approved drugs, Rothstein et al. (2005) discovered that β -lactam antibiotics cause a massive upregulation of GLT-1 expression, and the most clinically useful and effective β -lactam antibiotic was ceftriaxone. Although other β -lactam antibiotics such as penicillin caused a greater increase in GLT-1, ceftriaxone more easily crosses the blood brain barrier and has minimal side effects (Nau et al., 1993). Conveniently, the concentration of ceftriaxone in the cerebrospinal fluid required to upregulate GLT-1 falls within the range approved by the FDA for treatment of meningitis, making it a very promising candidate for passing clinical trials (Rothstein et al., 2005). In fact, ceftriaxone has recently begun stage III clinical trials for the treatment of ALS (Traynor et al., 2006).

Behavioral effects. Ceftriaxone has been used successfully in a number of studies on animal models to decrease extracellular glutamate and act as a neuroprotective agent. In their original study, Rothstein et al. (2005) found that daily injections of 200 mg/kg ceftriaxone beginning at the time of symptom onset in a mouse model of ALS were able to delay loss of strength and body weight in addition to significantly increasing survival by 10 days. Additionally, 5 days of pretreatment with either 100 or 200 mg/kg ceftriaxone has been found to be neuroprotective in the transient focal cerebral ischemia model of stroke, significantly decreasing cell death (Chu et al., 2007). This effect was blocked by an injection of 10 mg/kg dihydrokainate (DHK), a specific GLT-1 blocker, indicating that the neuroprotective effects of ceftriaxone were indeed due to increased glutamate uptake by GLT-1 (Chu et al., 2007). Further indicating that ceftriaxone is effective because it enhances GLT-1 expression and/or activity, antibiotics that do not have these effects on GLT-1 are not neuroprotective in animal models in which ceftriaxone is neuroprotective (Rothstein et al., 2005). When tested in the R6/2 mouse model of Huntington's

disease, 5 days of 200 mg/kg ceftriaxone was able to decrease early stage Huntington's associated motor dysfunctions, such as paw claspings, twitching, and motor inflexibility for the duration of treatment (Miller et al., 2008). Moreover, some effects on motor symptoms lasted for at least a week following ceftriaxone cessation. Because the motor symptoms of Huntington's disease in this model develop before there is significant cell death in the striatum (Miller et al., 2008), it is possible that ceftriaxone decreases these symptoms not just by decreasing excitotoxicity, but also by normalizing the effects on neural transmission produced by excess glutamate.

Further indicating that ceftriaxone can be effective in normalizing some behaviors in the absence of excitotoxicity, daily injections of 50, 100, and 200 mg/kg ceftriaxone were able to dose-dependently decrease morphine analgesic tolerance in rats. These effects were blocked by an injection of 10 mg/kg DHK, showing this effect was due to an increase in glutamate uptake by GLT-1 (Rawls, Zielinski, Patel, Sacavage, Baron, & Patel, 2010). Similarly, 100 and 200 mg/kg ceftriaxone given daily over 5 days of extinction training following cocaine self-administration were able to decrease subsequent cocaine-induced relapse in rats (Sari, Smith, Ali, & Rebec, 2009). Thus, it is quite clear that ceftriaxone, by enhancing GLT-1 function, can normalize behaviors caused by excess glutamate transmission as well as reduce glutamate-induced excitotoxicity. As a consequence, ceftriaxone should be helpful in reducing some of the motor disturbances associated with Parkinson's disease. Interestingly, there was no change in behavior in any of the control animals given ceftriaxone compared to those given saline (Miller et al., 2008; Sondheimer & Knackstedt, 2011), suggesting that it may not produce many side effects.

Mechanisms. Several studies have shown that ceftriaxone dose-dependently increases GLT-1 expression (Rothstein et al., 2005) and that its actions are specific to GLT-1, as the levels

of the other common glutamate transporters, GLAST and EAAC1, are unchanged by ceftriaxone treatment (Lee et al., 2008; Lievens et al., 2001; Rothstein et al., 2005). GLT-1 upregulation has been shown by the upregulation of GLT-1 mRNA (Lee et al., 2008; Rothstein et al., 2005), total GLT-1 protein (Miller et al., 2008; Sari, Prieto, Barton, Miller, & Rebec, 2010; Sondheimer & Knackstedt, 2011), and GLT-1 expression on the cell surface, with the maximum GLT-1 expression occurring following injections of 200 mg/kg (Rothstein et al., 2005). GLT-1 mRNA upregulation typically reaches a maximum after 3 days of ceftriaxone injections while protein expression is typically maximized after 5 days of injections (Chu et al., 2007). These increases in GLT-1 have been seen to last from 20 days to 3 months after the last injection of ceftriaxone (Rasmussen et al., 2011; Rothstein et al., 2005). More critically, these increases in GLT-1 expression have been associated with long lasting decreases in extracellular glutamate (Rasmussen et al., 2011). In fact, evidence indicates that ceftriaxone can increase glutamate uptake in the absence of increased GLT-1 expression, presumably reflecting an increase in GLT-1 activity. For example, Thone-Reinke et al. (2008) found that a single acute injection of 200 mg/kg ceftriaxone 90 min after transitive focal ischemia was neuroprotective without increasing GLT-1 mRNA or protein expression. However, the acute injection did increase glutamate clearance. Although Lipski et al. (2007) found no increase in GLT-1 after five injections of 200 mg/kg ceftriaxone, they did find evidence for increased GLT-1 function.

Increased GLT-1 expression may be due to an increase in the activation of the GLT-1 promoter fragment (Rothstein et al., 2005). Subsequent research by Lee et al. (2008) showed ceftriaxone increased binding of nuclear factor- κ B to the GLT-1 promotor at position -272. Ceftriaxone does this by causing the degradation of the NF- κ B inhibitor protein I κ B α . Once NF-

κ B is disinhibited it can then be translocated into the nucleus. Inhibition of NF- κ B with Ad.I κ B α -mt32 blocks the effects of ceftriaxone (Lee et al., 2008).

However, these effects do not explain the action of ceftriaxone when there is no increase in GLT-1 protein. Recently it has been suggested that a palmitic acid molecule must be attached to GLT-1 through the process of palmitoylation in order to function correctly. Palmitoylation is thought to cause the increased tethering of GLT-1 to the membrane through the attachment of a palmitic acid molecule to a cysteine residue on the protein (Huang et al., 2010). It is thus possible that ceftriaxone may increase GLT-1 activity (with or without increased expression) by directly increasing palmitoylation, which, for example, is disrupted in some Huntington's disease models (Huang et al., 2010). Additionally, ceftriaxone may increase GLT-1 activity through its ability to chelate metals, which would reduce the toxic effect of some metal ions and the associated oxidative stress that would normally decrease GLT-1 activity and expression (Ji, Shen, & Zhang, 2005; Lipski et al., 2007).

Rationale for Experiments

These studies effectively indicate that ceftriaxone can reduce glutamate-induced excitotoxicity and other behavioral effects of excess glutamate transmission in a variety of animal models by increasing glutamate clearance from the synapse through upregulation of the expression and/or activity of GLT-1. Thus, in so far as some of the behavioral effects of PD and LID are due to excess glutamate transmission (Jonkers et al., 2002; Robelet et al., 2004), it seems reasonable to suggest that ceftriaxone would be effective in reducing both of these motor effects, perhaps without the side effects of glutamate receptor antagonists. Thus the intent of my study is to determine if injections of ceftriaxone will ameliorate the contralateral forepaw stepping deficits produced by unilateral medial forebrain injections of 6-OHDA in rats and the subsequent

increase in LID produced by repeated injections of 10 mg/kg L-DOPA. Because the degeneration produced by 6-OHDA occurs quickly, it is likely that any effects of ceftriaxone in this model will be on symptom expression rather than on ongoing excitotoxic degeneration.

In Experiment 1, I examined the effects and timecourse of daily injections of 100 mg/kg ceftriaxone on stepping with the impaired contralateral forelimb, which is a measure of bradykinesia (Olsson, Nikkah, Bentlage, & Bjorklund, 1995). A dose of 100 mg/kg ceftriaxone was chosen because prior studies have indicated that its effects on glutamate transport (Rasmussen et al., 2011) and behavior are similar to those of the more commonly used 200 mg/kg (Chu et al., 2007; Rawls et al., 2010b; Sari et al., 2009). Given the success of 100 mg/kg ceftriaxone in Experiment 1, in Experiment 2 I examined the effectiveness of acute and sub-chronic injections of 50 mg/kg ceftriaxone in the treatment of PD in half the animals, as in Experiment 1. I then induced LID in these animals with daily injections of 10 mg/kg L-DOPA to determine if concurrent ceftriaxone could slow the development of LID without decreasing the effects of L-DOPA on forepaw stepping. The severity of LID was measured using the Abnormal Involuntary Movement (AIM) Scale which evaluates four AIM subtypes: contralateral turning, axial dystonia, forelimb dyskinesia, and orolingual dyskinesia (Lee et al., 2000; Lundblad, Andersson, Winkler, Kirik, Wierup, & Cenci, 2002; Winkler et al. 2002). In the other half of the animals, LID was induced before ceftriaxone treatment began to determine if ceftriaxone could reduce the expression of established LID. I then determined if the GLT-1 antagonist DHK could block the effect of ceftriaxone on forepaw stepping.

Experiment 1: Effect of 100 mg/kg Ceftriaxone on Forepaw Stepping.

Method

Subjects

Eleven Long Evans rats from Charles River Laboratory (Wilmington, MA) were individually housed in plastic cages (45 x 25 x 20 cm) with corncob bedding in a room with a 12-hour light cycle. Rats had unlimited access to food and water except 24 hr before surgery and during behavioral testing. All procedures were approved by Bates College Institutional Animal Care and Use Committee.

Surgery

Rats were anesthetized with an intraperitoneal (i.p.) injection of a 1 ml/kg combination of ketamine (100 mg/ml; Sigma Chemicals, St. Louis, MO) and xylazine (10 mg/ml; Astra Pharmaceutical Products, Westborough, MA) in water. They were then placed in a stereotax (Kopf Instruments, Tujunga, CA) with the incisor bar 3.3 mm below interaural line. Noradrenergic neurons were protected from 6-OHDA by an i.p. injection of desipramine (1 ml/kg of 25 mg/kg in 0.9% saline; Sigma Chemicals, St. Louis, MO) given shortly after placement of the rat in the stereotax. A flat-tipped 5- μ l Hamilton syringe was used to inject 2 μ l of 6-OHDA (6 μ g/ μ l dissolved in a 0.9% saline solution) at a rate of 0.25 μ l/min. 6-OHDA (Sigma Chemicals, St. Louis, MO) was thawed from frozen aliquots immediately before injection into the left medial forebrain bundle 4.7 mm posterior to bregma, 7.8 mm ventral to skull surface, and 1.4 mm left of midline. The syringe was left in place for 5 min post injection. Following surgery, rats were injected subcutaneously (s.c.) with 5 mg/kg Rimadyl (1 ml/kg; Hospira, Lake Forest, IL) and 5 ml Lactate Ringers Solution (Bimeda-MTC Animal Health, Ontario Canada).

Measurement of Forepaw Stepping

Motor impairment was measured by counting medial and backward steps taken with each forepaw over 1 m on a canvas belt treadmill moving at a speed of 0.1 m/s. One forepaw was

restrained while the rat was held at an angle so that its hind legs were approximately 5 cm above the treadmill surface such that the forepaw was placed on the moving belt. Backward stepping was measured by holding the rat's long axis parallel to the treadmill, whereas medial stepping was measured while holding the rat perpendicular to the treadmill, with the belt moving away from the rat in both cases. Testing consisted of three trials for both forepaws in both directions. The starting position was rotated after each trial, with trial one starting with ipsilateral (left) backward, contralateral (right) backward, ipsilateral medial, contralateral medial. Trial two began with contralateral backward, ended with ipsilateral backward, and so forth.

Procedure

Table 1. Timeline of Experiment 1. The abbreviations at the bottom refer to the corresponding days in Figure 2: B = baseline, 6 LD = 6 mg/kg L-DOPA, C = 100 mg/kg ceftriaxone, and P = post-. The trailing numbers refer to the day(s) in that condition during which testing occurred.

Day							
1-4	5	7-13	14	15	16-45	46	47
Baseline	6 mg/kg L-DOPA	Ceft	6 mg/kg L-DOPA & Ceft	Ceft	Post Ceft	Acute Ceft	Post Ceft
B4	6 LD	C1-C7	6 LDC8	C9	PC1-PC30	Acute C	C

See Table 1 for timeline of Experiment 1. Forepaw stepping was initially measured for 4 days beginning around 1 week post-surgery to determine baseline impairment caused by the lesion. Two rats were excluded from further testing due to a lack of impairment in stepping; their impairment in right forepaw stepping compared to left forepaw stepping was less than 15%. The acute effect of an i.p. 1 mg/kg injection of 6 mg/kg L-DOPA (dissolved in 0.1% sodium metabisulfite in water with 6.25 mg/ml benserazide; all drugs were from Sigma Chemicals, St. Louis, MO) on forepaw stepping was then measured in the nine impaired rats 30 min after the injection. Two of these rats were also tested 24 hr post-L-DOPA, prior to the injection of ceftriaxone (see below), to determine the duration of L-DOPA's effects.

Ceftriaxone (100 mg/kg in 0.9% saline; Sigma Chemicals, St. Louis, MO) was then administered i.p. at 1 ml/kg daily for 9 days to all rats to determine the effect of subchronic administration of ceftriaxone on forepaw stepping. Animals were tested 24 hr after each injection and were given subsequent injections immediately after testing.

On the eighth day of ceftriaxone testing (day 14), animals were also injected with 6 mg/kg L-DOPA (1 ml/kg) 30 min before testing to determine if a moderate dose of L-DOPA has an additive effect with ceftriaxone on forepaw stepping. On the ninth day, the rats then received their last dose of ceftriaxone.

Forepaw stepping after cessation of ceftriaxone was measured periodically over 30 days to observe the time course of the decrease in ceftriaxone's effects. On post-ceftriaxone day 31 (day 46), 100 mg/kg ceftriaxone was given acutely 45 min before forepaw step testing, and stepping was also measured 24 hr later with no additional injections.

Results

Stepping

Figure 2 shows the changes in mean (\pm SEM) steps with both forepaws throughout the 47 days of Experiment 1.

6 mg/kg L-DOPA. On the day following the 4th baseline day, the rats were tested after an acute injection of 6 mg/kg L-DOPA. The Forepaw (right, left) \times Condition (fourth day of baseline [B4], L-DOPA [6 LD]; see Figure 2) repeated measures analysis of variance (ANOVA) showed a main effect of forepaw, $F(1, 8) = 281.63, p < .001$, indicating that the rats stepped less with their right forepaw than their left forepaw. There was also a main effect of condition, $F(1, 8) = 23.75, p < .005$, indicating that 6 mg/kg L-DOPA improved stepping. Finally, there was also a Forepaw \times Condition interaction $F(1, 8) = 57.79, p < .001$, and subsequent simple effects tests

showed the animals stepped less with the right forepaw irrespective of condition, $F_s(1, 16) > 161.95$, $p < .001$, and that 6 mg/kg L-DOPA increased stepping with the impaired right forepaw, $F(1, 16) = 74.32$, $p < .001$, but not the left forepaw.

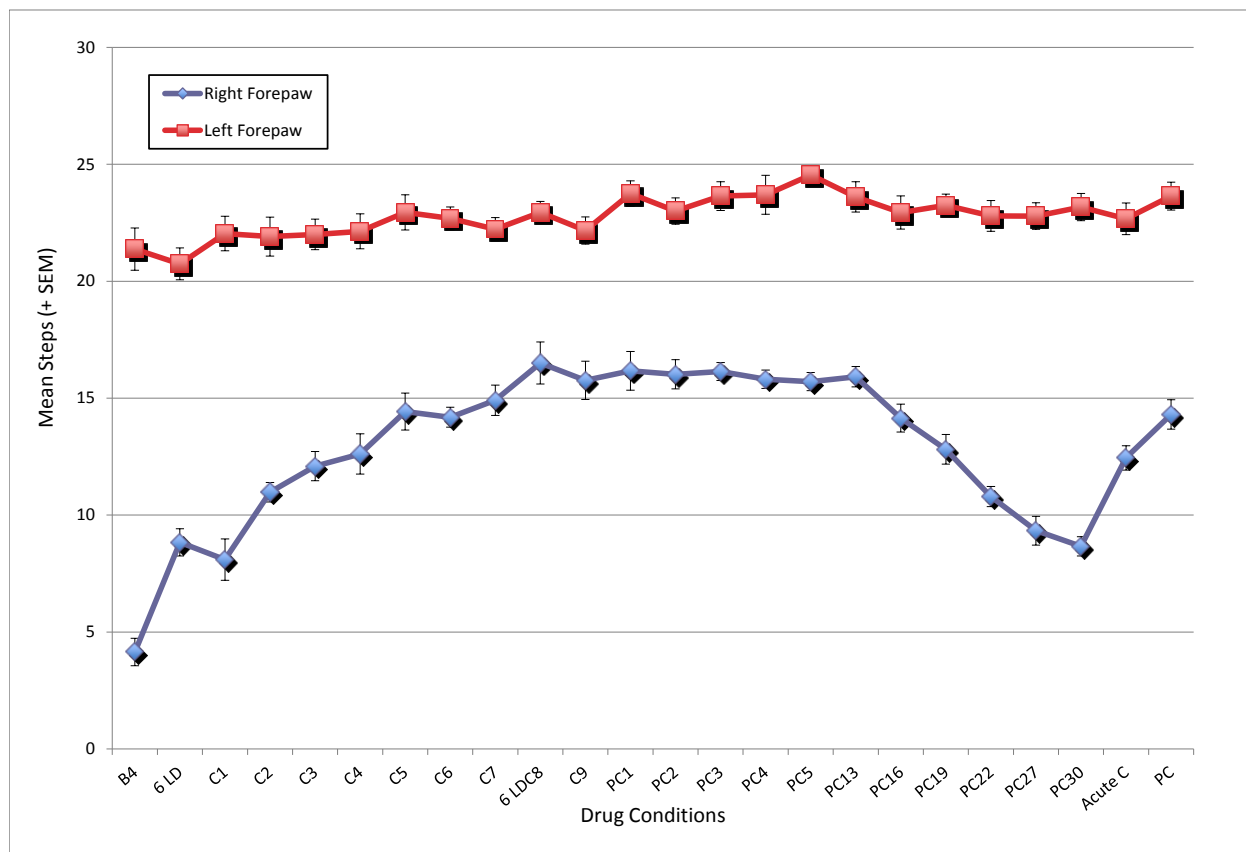


Figure 2. The change in mean steps (\pm SEM) taken with both left and right forepaws throughout the course of Experiment 1. The letters refer to the drug condition (B = baseline; 6 LD = 6 mg/kg L-DOPA; C = 100 mg/kg ceftriaxone; P = post-), and the trailing numbers refer to the day in that condition.

100 mg/kg ceftriaxone. The rats were then injected with 100 mg/kg ceftriaxone for 7 days, and stepping was compared to baseline. The Forepaw (2) \times Day (baseline [B4] and 7 days of ceftriaxone [C1 – C7]; see Figure 2) repeated measures ANOVA showed main effects of forepaw, $F(1, 8) = 166.75$, $p < .001$, and day, $F(7, 56) = 35.46$, $p < .001$, and a Forepaw \times Day interaction, $F(7, 56) = 24.99$, $p < .001$. Simple effects tests showed the animals stepped less with their right forepaw than with their left forepaw across all days, $F_s(1, 64) > 49.96$, $p < .001$. More

critically, simple effects tests indicated that, compared to baseline, repeated injections of 100 mg/kg ceftriaxone progressively increased right forepaw stepping, $F(7, 112) = 59.84, p < .001$, which seemingly reached asymptote after five injections, as pairwise comparisons indicated that stepping on the last 3 days (C5 - C7) did not differ. There was no effect of ceftriaxone on left forepaw stepping.

6 mg/kg L-DOPA combined with 100 mg/kg ceftriaxone. The rats were then given an acute injection of 6 mg/kg L-DOPA combined with ceftriaxone, and the data were compared to the prior and succeeding days of ceftriaxone alone. The Forepaw (2) \times Condition (seventh injection of ceftriaxone [C7], 6 mg/kg L-DOPA [6 LDC8], post-L-DOPA [PLDC9]; see Figure 2) repeated measures ANOVA showed only a main effect of forepaw, $F(1, 8) = 95.20, p < .001$. Although 6 mg/kg L-DOPA tended to improve right forepaw stepping even further, this additive effect was not significant.

Ceftriaxone abstinence. The ceftriaxone injections were then stopped for 30 days to determine the duration of its effects, and the rats were tested periodically. The Forepaw (2) \times Day (baseline [B4] and 11 post-ceftriaxone tests [PC1 – PC30]; see Figure 2) repeated measures ANOVA showed main effects of forepaw, $F(1, 5) = 187.00, p < .001$, and day, $F(11, 55) = 48.77, p < .001$, and a Forepaw \times Day interaction, $F(11, 55) = 27.90, p < .001$. Subsequent simple effects tests showed that the animals stepped less with their right than left forepaw on all days, $F_s(1, 60) > 53.89, p_s < .001$, and that right forepaw stepping decreased across days, $F(11, 110) = 74.71, p < .001$, while left forepaw stepping slightly increased, $F(11, 110) = 2.28, p < .02$. As can be seen in Figure 2, pairwise comparisons indicated that the effects of ceftriaxone did not begin to decrease until the 16th day of abstinence, $F(1, 110) = 4.57, p < .05$. Although stepping decreased progressively thereafter, it had still not returned to baseline by the 30th day of

abstinence, $F(1, 110) = 37.83, p < .001$. Left forepaw stepping was greater than baseline through the 19th day of abstinence, $F_s(1, 110) > 4.96, p_s < .05$, and no significant changes occurred during the course of abstinence.

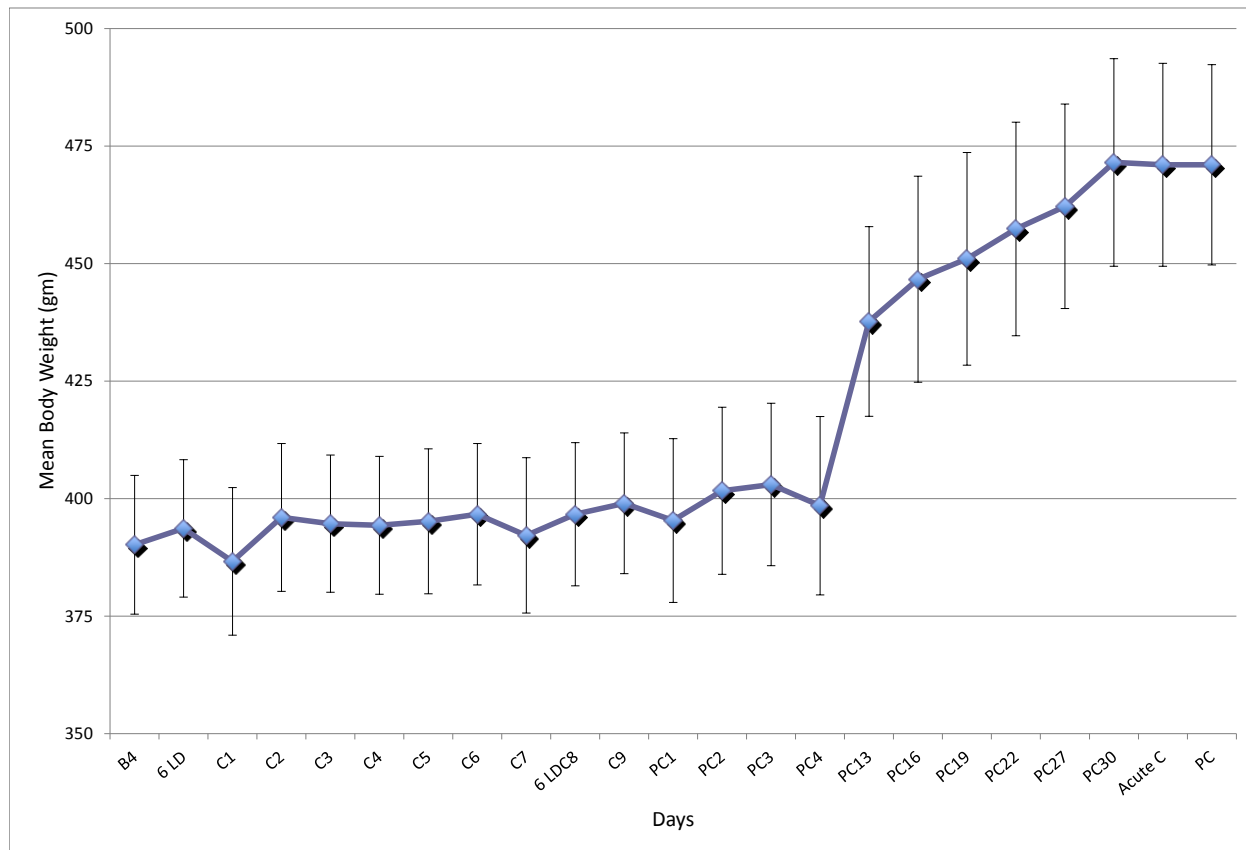


Figure 3. The change in mean body weight (\pm SEM) in gm over the course of the experiment. As in Figure 2, the letters on the x-axis refer to the drug condition (B = baseline; 6 LD = 6 mg/kg L-DOPA; C = 100 mg/kg ceftriaxone; P = post-), and the trailing numbers refer to the day in that condition.

Acute 100 mg/kg ceftriaxone. The animals were then re-tested 45 min following an acute injection of ceftriaxone, and the data were compared to the preceding and succeeding days without ceftriaxone. The Forepaw (2) \times Condition (post-ceftriaxone day 30 [PC30], acute ceftriaxone [Acute C], 24 hr post-ceftriaxone [PC]; see Figure 2) repeated measures ANOVA showed main effects of forepaw, $F(1, 5) = 1268.79, p < .001$, and condition, $F(2, 10) = 26.18, p < .001$, and Forepaw \times Condition interaction, $F(2, 10) = 47.60, p < .001$. Simple effects tests

showed the animals stepped less with their right forepaw on all days, $F_s(1, 15) > 417.77$, $p < .001$, and that ceftriaxone increased right forepaw stepping, $F(2, 20) = 63.82$, $p < .001$, without affecting left forepaw stepping. Pairwise comparison showed the acute dose of ceftriaxone increased right forepaw stepping compared to post-ceftriaxone day 30, $F(1, 20) = 55.19$, $p < .001$, and that this effect was even larger 24 hr later, $F(1, 20) = 13.37$, $p < .005$.

Weight. A one-way repeated measures ANOVA on the weights from the last day of baseline through the end of the experiment for the six animals for whom all weights were available revealed a main effect, $F(25, 125) = 30.15$, $p < .001$ (see Figure 3). Subsequent pairwise comparisons indicated that weights did not change from baseline until the 13th day of abstinence from ceftriaxone, $F(1, 125) = 36.70$, $p < .001$, suggesting that ceftriaxone suppressed weight gain.

Discussion

Unilateral 6-OHDA lesions

Lesions to the left medial forebrain bundle (MFB) caused an 81% decrease in forepaw stepping in the impaired right forepaw, contralateral to the lesion, compared to the unimpaired ipsilateral left forepaw during baseline testing. This level of impairment is consistent with previous data from our lab showing the same lesion causes a 68-87% decrease in forepaw stepping (Kelsey et al., 2004). This degree of impairment is usually found in MFB lesions that produce greater than 80% depletion of dorsal striatal DA (Chang, Wachtel, Young, & Kang, 1999), although it has been found to occur with less DA depletion (Kelsey et al., 2004). Although a neurochemical assessment of lesion damage has not been performed yet, it is likely that these lesions have produced an average of 80% decrease in dorsal striatal DA and its metabolites compared to the unlesioned side.

6 mg/kg L-DOPA

The acute injection of 6 mg/kg L-DOPA caused a 23% increase in right forepaw stepping compared to baseline, without affecting left forepaw stepping. Percent improvement was computed as the percentage of steps taken with the right forepaw compared to those taken with the left forepaw when injected with L-DOPA minus the same percentage on the preceding baseline day. This finding is also consistent with previous findings that this and similar doses of L-DOPA improved, but did not normalize, forepaw stepping (Chang et al., 1999; Kelsey et al., 2004). Although the data are not shown, right forepaw stepping had returned to baseline in the two animals tested on the day following this L-DOPA injection, also consistent with prior findings from our lab (Kelsey et al., 2004; Kelsey, Langelier, Oriel, & Reedy, 2009).

100 mg/kg ceftriaxone

One day after the first injection of 100 mg/kg ceftriaxone, right forepaw stepping had significantly increased by 18% compared to baseline. Right forepaw stepping progressively improved with subsequent injections, reaching an apparent maximum improvement of 44% after five injections, although still not normalizing stepping. Furthermore, this improved stepping was not further enhanced by an injection of 6 mg/kg L-DOPA, consistent with data indicating that there appears to be a ceiling above which impaired forepaw stepping cannot be further improved (Kelsey et al., 2004; Kelsey et al., 2009).

Moreover, in contrast to the therapeutic effects of L-DOPA, which disappear within 24 hr, the therapeutic effects of ceftriaxone are long lasting. After ceftriaxone treatment ceased, the ameliorative effects of ceftriaxone on right forepaw stepping continued until at least 16 days after the last injection. At this point stepping began to progressively decrease, although stepping still had not returned to baseline after 30 days of abstinence. Finally, although stepping had not

yet returned to baseline, right forepaw stepping still improved by 18% 45 min after an acute injection of ceftriaxone and by an additional 6% the following day. Although Miller et al. (2008) found that the behavioral effects of ceftriaxone decreased to the level of saline animals within 7 days of the last injection of ceftriaxone in an animal model of Huntington's, the progressive degeneration that occurs in the HD model may limit the ability of ceftriaxone to maintain its effectiveness.

In summary, acute injections of 100 mg/kg ceftriaxone increased right forepaw stepping in this model as effectively as 6 mg/kg L-DOPA, and daily injections further increased right forepaw stepping without affecting left forepaw stepping. Moreover, this impressive therapeutic effect did not decline until 16 days of ceftriaxone abstinence and was still apparent after 30 days of abstinence. The rapid onset of these therapeutic effects in the absence of apparent side effects and the long duration of these effects suggest that ceftriaxone may be a superior treatment to the short lasting effect of L-DOPA and other approved therapies.

Although similar doses of ceftriaxone have been found to be effective in reducing symptoms in models of other degenerative disease such as ALS and Huntington's disease (Miller et al., 2008; Rothstein et al., 2005), this appears to be the first study to examine its effects on an animal model of PD. Moreover, in contrast to those studies, the improvement in PD-like symptoms that I observed is not likely to be due to a decrease in ongoing neurodegeneration as ceftriaxone was not administered until 14 days after the 6-OHDA injection, well after degeneration occurred. Rather, these data suggest an effect on symptom expression.

Because there is no significant upregulation of GLT-1 protein until 2 days after the first injection (Chu et al., 2011; Rothstein et al., 2004), it can be hypothesized that the effect of ceftriaxone on forepaw stepping seen after the acute and first couple of injections is most likely

not due to GLT-1 upregulation, and is probably due to an increase in GLT-1 activity (Lipski et al., 2007; Thone-Reineke et al., 2008). However, the subsequent increase in stepping with a maximum effect after five injections corresponds to the finding that GLT-1 protein expression reaches a maximum at that time (Chu et al., 2007). Similarly, the findings that GLT-1 upregulation lasts for 3 months following the final injections (Rothstein et al., 2005) and that extracellular glutamate was decreased for at least 20 days (Rasmussen et al., 2011) are consistent with my finding that stepping was still elevated 30 days after ceftriaxone removal. Thus, I propose that it is most likely that ceftriaxone's ability to decrease motor impairment in 6-OHDA-lesioned rats is due to its ability to decrease extracellular glutamate (Rasmussen, et al., 2011), consistent with the finding that blocking glutamate transmission with NMDA and AMPA receptor antagonists is ameliorative in animal models of PD and human PD patients (Kelsey et al., 2004; Loschmann et al., 2004; Nash et al., 2000).

Body Weight

It would appear as though chronic injections of 100 mg/kg ceftriaxone suppressed weight gain until 2 weeks after treatment had ceased. As far as I can tell, this effect has not been noted consistently in humans, although it is listed as a rare side effect.

Experiment 2: Effect of 50 mg/kg Ceftriaxone on Forepaw Stepping and L-DOPA-Induced Dyskinesia.

Experiment 2 was designed to clarify and extend the findings of Experiment 1. Although 50 mg/kg ceftriaxone has failed to show behavioral or GLT-1 effects in other studies (Rawls et al., 2010), given the substantial effects of 100 mg/kg ceftriaxone in Experiment 1, I chose to examine the effects of 50 mg/kg ceftriaxone in this experiment. Additionally, I wanted to determine if an acute effect of ceftriaxone could be demonstrated more cleanly. I also chose to

use a second group that initially received saline in order to demonstrate that the ability of ceftriaxone to improve stepping was not merely an effect of practice. Both groups were subsequently repeatedly injected with 10 mg/kg L-DOPA to induce LID, which enabled me to determine if ceftriaxone could reduce both the development and expression of LID. Most importantly I wanted to determine if the effects of ceftriaxone were due to an increase in GLT-1 function by seeing if selectively blocking GLT-1 with dihydrokainate (DHK) would decrease the therapeutic effects of ceftriaxone on stepping.

Method

Subjects

Seventeen rats were purchased from Charles River were used and housed as in Experiment 1. All procedures were approved by the Bates College Institutional Animal Care and Use Committee.

Surgery

Surgery was conducted as in Experiment 1.

Drugs

All drugs were obtained and prepared as in Experiment 1 unless otherwise noted.

Behavioral Measures

Stepping. Stepping was measured as in Experiment 1.

Abnormal involuntary movements (AIMs) rating. Each rat was observed individually for levodopa-induced dyskinesia (LID) in a clear plastic cage (45 x 25 x 20 cm) with corncob bedding 30 and 60 min after the injection of L-DOPA. LID was quantified using the rat AIMs rating scale (Winkler, Kirik, Bjorklund, & Cenci, 2002). Four different subtypes of rat AIMs were measured for severity: limb dyskinesia, axial dystonia, orolingual dyskinesia, and

contralateral rotation (see Table 2 for details and scoring). In addition, the amplitude of forelimb and axial AIMS were also measured (see Table 2 for scoring). The scales for the two tests were added, and the maximum attainable score each day was 48.

Table 2. Rating scale for abnormal involuntary movements (AIMs) in the rat.
AIMs subtypes criteria

Limb	Repetative, rhythmic jerky movements or dystonic posturing of the forelimb on the side contralateral to the dopamine-denervated striatum, opening/closing of digits and pronation/supination of the wrist.
Axial	Lateral deviation or torsion of the head, neck, trunk towards the side contralateral to the dopamine-denervated striatum.
Orolingual	Repetitive chewing movements of the jaw, tongue protrusion, facial grimacing, biting (either injurious or noninjurious) of the fur and skin on the forelimb contralateral to the lesion.
Contralateral Rotation	Circular locomotion contralateral to the dopamine-denervated striatum in which all four paws contacted the floor during turning.

Severity Scale

0	Absent
1	Occasional- present less than 50% of observation time
2	Frequent- present more than 50% of observation time
3	Continuous but interrupted by strong sensory stimuli (ie., loud tap on cage)
4	Continuous, can not be interrupted by strong sensory stimuli

(Adapted from Winkler et al., 2002)

Amplitude Scale

Axial AIMS	Angle is estimated with respect to the deviation from (or torsion around) the longitudinal axis of the body
0	Absent
1	Consistent lateral deviation of head and neck at ~30 degree angle
2	Lateral deviation of head and neck to an angle between 30 and 60 degrees
3	Lateral deviation and/or torsion of head, neck and upper trunk to an angle between 60 and 90 degrees
4	Torsion of head, neck and trunk at > 90 degree angle, causing the rat to lose balance

Forlimb AIMS	
0	Absent
1	Tiny oscillatory movements of the paw and distal forelimb around a fixed point
2	Movements of low amplitude but causing visible contraction of the shoulder muscles
3	Translocation of the whole limb with visible contraction of shoulder muscles
4	Vigorous limb and shoulder movements of maximal amplitude, may have a ballistic character

(Adapted from Winkler et al., 2002)

Procedure

Table 3. Timeline of Experiment 2. The abbreviations at the bottom of each row refer to the corresponding days in Figures 2 and 3: B = baseline, S = saline, C = 50 mg/kg ceftriaxone, LD = 6 and 10 mg/kg L-DOPA, and DHK = 10 mg/kg DHK. The trailing numbers refer to the day(s) in that condition during which testing occurred.

Group	Day								
	1-4	5	7-16	18	19-30	31-37	38-39	40	41
Saline	Baseline B4	Saline S1	Saline S2-S10	6 mg/kg L-DOPA & Saline 6 LD1S12	10 mg/kg L-DOPA & Saline LD4S15- LD10S21	L-DOPA & Ceft LD12C1- LD16C5	Ceft C7	Ceft & DHK C9DH K	None C10
Ceftriaxone	Baseline B4	Acute Ceft Acute C	Ceft C2-C10	6 mg/kg L-DOPA & Ceft 6 LD1C12	10 mg/kg L-DOPA & Ceft LD4C15- LD10C21	L-DOPA & Ceft LD12C23- LD16C27	L-DOPA & Ceft LD18C29		

See Table 3 for a timeline of Experiment 2. As in Experiment 1, baseline forepaw stepping was measured in 17 rats for 4 days, beginning approximately 1 week after surgery. One rat was excluded due to insufficient impairment. The rats were then divided into two equally impaired groups: a ceftriaxone group and a saline group. In eight of the rats, the acute effect of 50 mg/kg ceftriaxone (1 ml/kg i.p. in 0.9% saline; Sigma Chemicals, St. Louis, MO) on forepaw stepping was measured 45 min after injection, whereas eight others were tested after an injection of 0.9% saline. On the following day, the rats began receiving daily injections of either 50 mg/kg ceftriaxone or 0.9% saline for 10 additional days, at which point an asymptote appeared to be reached in the ceftriaxone animals. In order to accommodate all 16 animals, forepaw stepping was measured every other day, for a total of 6 out of 11 days. As in Experiment 1, on test days, the rats received their saline or ceftriaxone injections immediately following testing.

The acute effect of 6 mg/kg L-DOPA was measured on forepaw stepping and AIMs in all rats on day 18, as in Experiment 1. The following day all rats began receiving daily 1 ml/kg i.p. injections of 10 mg/kg L-DOPA (dissolved in 0.1% sodium metabisulfite in water with 6.25

mg/ml benserazide; Sigma Chemicals, St. Louis, MO) along with injections of saline or ceftriaxone for 12 days in order to induce LID. Testing now occurred every third day for a total of four tests. On testing days, AIMS were measured 30 and 60 min after the L-DOPA injection, and forepaw stepping was measured between the two AIMS tests. On test days, saline or ceftriaxone injections were given immediately after the second AIMS test.

AIMs in the saline group appeared to reach asymptote after 13 days (4 tests) of L-DOPA treatment, at which point 50 mg/kg ceftriaxone was given daily for 7 days, instead of 0.9% saline, to determine if ceftriaxone could decrease the expression of pre-established LID. Testing continued as before except that it occurred 45 minutes after the first ceftriaxone injection and every other day after that for a total of three tests. Beginning on the day following the last test (LD16C5), injections of L-DOPA were discontinued, but daily ceftriaxone injections continued, and the rats were retested 24 hr after L-DOPA removal (C7). The original ceftriaxone group continued to receive daily L-DOPA and ceftriaxone injections through day 38 at which point they were not tested further.

After a third day without L-DOPA (day 40), the original saline-injected rats were given an acute injection of 10 mg/kg the GLT-1 antagonist dihydrokainic acid (DHK; 1 ml/kg i.p. in 0.1 M phosphate buffered saline; Tocris, Minneapolis, MN) 45 min before forepaw stepping to see if selective blockade of GLT-1 would reverse the anti-Parkinsonian effects of ceftriaxone. Immediately following this test, these rats received their last injection of ceftriaxone, and the rats were tested for forepaw stepping on the following day and then periodically thereafter to determine the duration of ceftriaxone's effects.

Results

Stepping

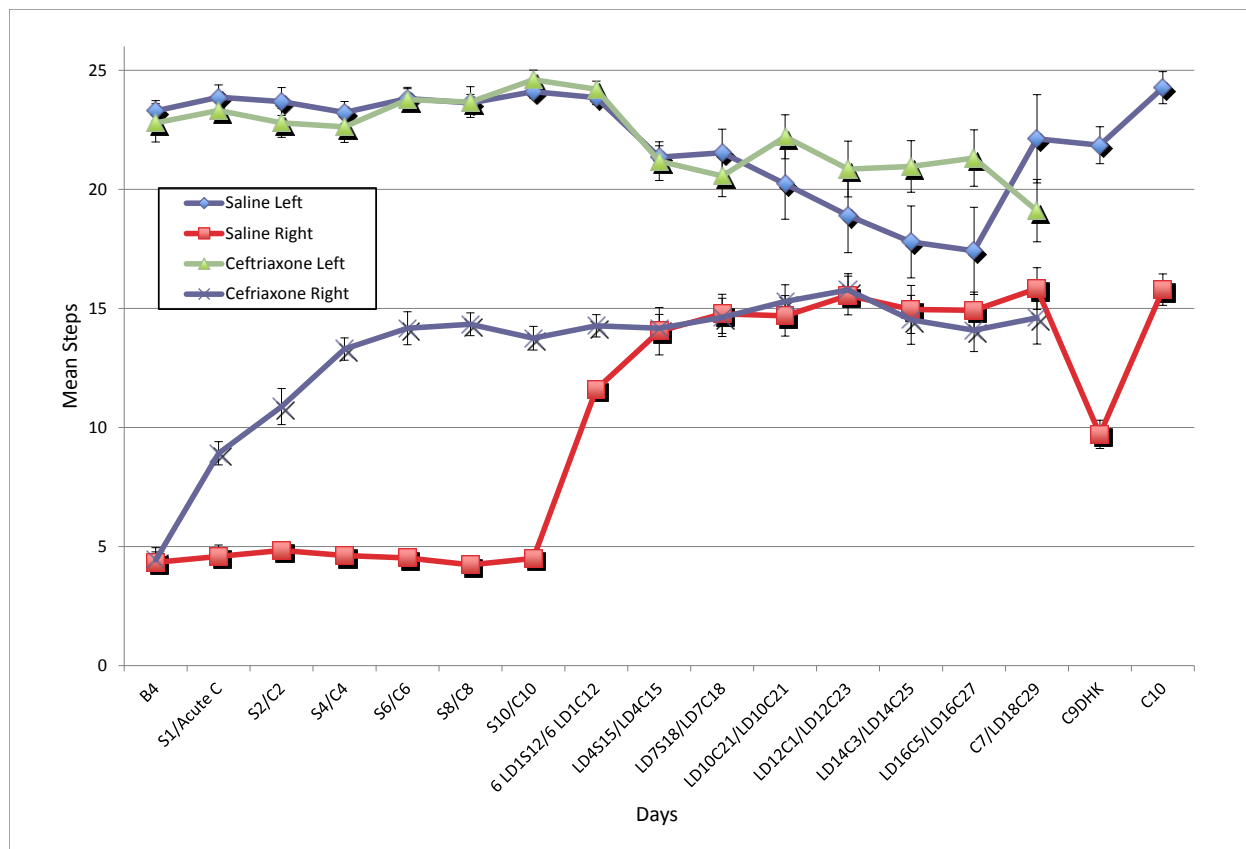


Figure 4. The change in mean steps (\pm SEM) taken with both left and right forepaws throughout the course of Experiment 2. The letters refer to the drug condition (B = baseline; C = 50 mg/kg ceftriaxone; LD = 6 and 10 mg/kg L-DOPA; DHK = 10 mg/kg DHK), and the trailing numbers refer to the day in that condition during which testing occurred. The symbols to the left of the backslash refer to the original saline-injected group, and the numbers to the right refer to the original ceftriaxone-injected group.

50 mg/kg ceftriaxone. Following the last baseline test (B4), the eight saline-injected rats received daily injections of saline during the first 10 days of this experiment (S1 – S10) while the ceftriaxone-injected rats received daily injections of 50 mg/kg ceftriaxone (acute C1– C10).

Testing occurred every other day. The Forepaw (2) \times Day (fourth day of baseline and 6 tests during the saline or ceftriaxone injections [B4 – S10/C10]) \times Group (saline, ceftriaxone)

ANOVA with repeated measures on forepaw and day showed a main effects of forepaw, $F(1, 14) = 2384.94$, $p < .001$, day, $F(6, 84) = 37.14$, $p < .001$, and group, $F(1, 14) = 82.95$, $p < .001$ (see Figure 4). All of the two-way interactions were significant, as was the triple interaction, $F(6, 84)$

= 22.96, $p < .001$. Subsequent simple, simple effects tests indicated that both groups of rats stepped less with their right forepaw than with their left on all days, $F_s(1, 98) > 207.14$, $p_s < .001$, and that 50 mg/kg ceftriaxone increased stepping with both forepaws, $F_s(6, 168) > 4.04$, $p_s < .001$, while saline injections had no effect on either forepaw. Subsequent pairwise comparisons indicated that acute ceftriaxone increased right forepaw stepping above baseline, $F(1, 168) = 81.07$, $p < .001$, and that right forepaw stepping progressively increased until reaching asymptote after the sixth injection. As a consequence, the ceftriaxone-injected rats were already stepping more with their right forepaw than the saline-injected rats by the first acute injection of ceftriaxone, $F_s(1, 196) > 42.28$, $p_s < .001$. Left forepaw stepping gradually increased across days in the ceftriaxone-treated rats such that they were stepping more with their left forepaw on the 10th day of treatment than they were on baseline through the fourth injection of ceftriaxone, $F_s(1, 168) > 6.74$, $p_s < .02$. There were no differences between the saline- and ceftriaxone-treated rats on left forepaw stepping.

L-DOPA. The daily saline or ceftriaxone injections were continued, but all rats were also injected with 6 mg/kg L-DOPA once and then 10 mg/kg L-DOPA daily for 10 days and were tested every third day. The Forepaw (2) \times Day (saline/ceftriaxone day 10 [S10/C10] through four L-DOPA tests [6 LD1S12/6 LD1C12 - LD10S21/LD10C21]) \times Group (L-DOPA combined with saline or ceftriaxone) ANOVA with repeated measures on forepaw and day showed a main effect of forepaw, $F(1, 14) = 165.14$, $p < .001$, day, $F(4, 56) = 4.39$, $p < .005$, and group, $F(1, 14) = 7.16$, $p < .02$ (see Figure 4). All two-way interactions with day were significant as was the triple interaction, $F(4, 56) = 7.87$, $p < .001$. Subsequent simple, simple effects showed that both groups of rats stepped less with their right forepaw than with their left on all days, $F_s(1, 70) > 17.01$, $p_s < .001$, and that L-DOPA increased right forepaw stepping in the saline-injected rats, $F(4, 112) =$

43.02, $p < .001$, and decreased left forepaw stepping in both groups, $F_s(4, 112) > 6.52$, $ps < .001$. Pairwise comparisons indicated that an acute injection of 6 mg/kg L-DOPA (6 LD1) increased right forepaw stepping in the saline-injected rats compared to the prior saline injection (S10), $F(1, 112) = 57.79$, $p < .001$, and that the third injection of 10 mg/kg L-DOPA (10 LD4) further and asymptotically increased right forepaw stepping, $F(1, 112) = 6.80$, $p < .02$, such that right forepaw stepping increased to the level of that in the ceftriaxone group. L-DOPA had no effect on right forepaw stepping in the ceftriaxone-injected rats. In contrast, 10 mg/kg L-DOPA decreased left forepaw stepping in both groups, $F_s(1, 112) > 6.57$, $ps < .02$.

50 mg/kg ceftriaxone combined with 10 mg/kg L-DOPA. Daily L-DOPA and ceftriaxone injections continued as above. In addition, on the 11th day of L-DOPA injections, the saline rats began receiving daily injections of 50 mg/kg ceftriaxone for 6 days. Testing now occurred every 2 days beginning the day after the first ceftriaxone injection. The Forepaw (2) \times Day (the 10th day of L-DOPA [LD10S21/LD10C21] and three tests with ceftriaxone [LD12C1/LD12C23 – LD16C5/LD16/C27]) \times Group (saline, ceftriaxone) ANOVA with repeated measures on forepaw and day showed only a main effect of forepaw, $F(1, 14) = 20.18$, $p < .001$, indicating that adding ceftriaxone to L-DOPA did not alter stepping in the animals that had previously received only L-DOPA injections (the original saline-injected group; see Figure 4). The triple interaction was not significant, $F(3, 42) = 1.91$, $p < .15$, but simple, simple effects tests showed that L-DOPA progressively decreased left paw stepping in the original saline-injected group, $F(3, 84) = 3.70$, $p < .02$, such that these rats were stepping less with their left paw than were the original ceftriaxone-injected rats by the 16th day of L-DOPA injections, $F(1, 112) = 5.12$, $p < .03$.

Removal of L-DOPA. One day later, the L-DOPA injections were stopped for the original saline-injected group, while the ceftriaxone injections were continued. The ceftriaxone and L-

DOPA injections continued as before in the original ceftriaxone-injected group. The data collected on the second day of L-DOPA removal were compared to those on the preceding 16th injection of L-DOPA for all rats. The Forepaw (2) × Condition (the 16th day of L-DOPA [LD16C5/LD16C27], L-DOPA removal [C7/LD18C29]) × Group (saline, ceftriaxone) ANOVA with repeated measures on forepaw and condition revealed a main effect of forepaw, $F(1, 14) = 26.14, p < .001$, a Day × Group interaction, $F(1, 14) = 8.07, p < .02$, and a triple interaction, $F(1, 14) = 9.74, p < .01$ (see Figure 4). Simple effects tests indicated that right forepaw stepping was less than left forepaw stepping, $F_s(1, 28) > 7.91, p_s < .02$, except for the original saline-injected rats on the 16th day of L-DOPA. These simple effects tests also indicated that the removal of L-DOPA from the saline-injected rats increased left forepaw stepping, $F(1, 28) = 16.13, p < .001$. In contrast, the additional 18th injection of L-DOPA tended to decrease left forepaw stepping in the original ceftriaxone-injected rats, $F(1, 28) = 3.55, p < .08$.

DHK and ceftriaxone. Two days later, the original saline-injected rats were given an injection of 10 mg/kg of the GLT-1 inhibitor DHK in addition to the daily injection of ceftriaxone, and on the following day were retested without DHK. The Forepaw × Condition (ceftriaxone [C7], DHK [C9DHK], post-ceftriaxone [PC]) repeated measures ANOVA on the eight saline rats revealed main effects of forepaw, $F(1, 7) = 115.03, p < .001$, and condition, $F(2, 14) = 84.85, p < .001$, and an interaction, $F(2, 14) = 23.39, p < .001$ (see Figure 4). Simple effects tests indicated that the rats stepped less with the right forepaw than with their left during all three tests, $F_s(1, 21) > 41.70, p_s < .001$, and that the drug conditions affected stepping with both forepaws, $F_s(2, 28) > 11.59, p_s < .001$. Pairwise comparisons indicated that DHK decreased right forepaw stepping, $F(1, 29) = 123.99, p < .001$, and that right forepaw stepping increased back to the level of ceftriaxone alone the following day. In contrast, left forepaw stepping was

not affected by DHK, but did increase on the following day, $F(1, 28) = 19.30$, $p < .001$, back to the level seen before L-DOPA administration. Although it is possible that the failure to see an increase in left forepaw stepping during DHK reflected a depressant effect of DHK, I found no suppression of stepping when 10 mg/kg DHK was subsequently re-administered to two animals (I ran out of drug) whose left forepaw stepping had returned to pre-L-DOPA baseline by the day following the initial DHK injection.

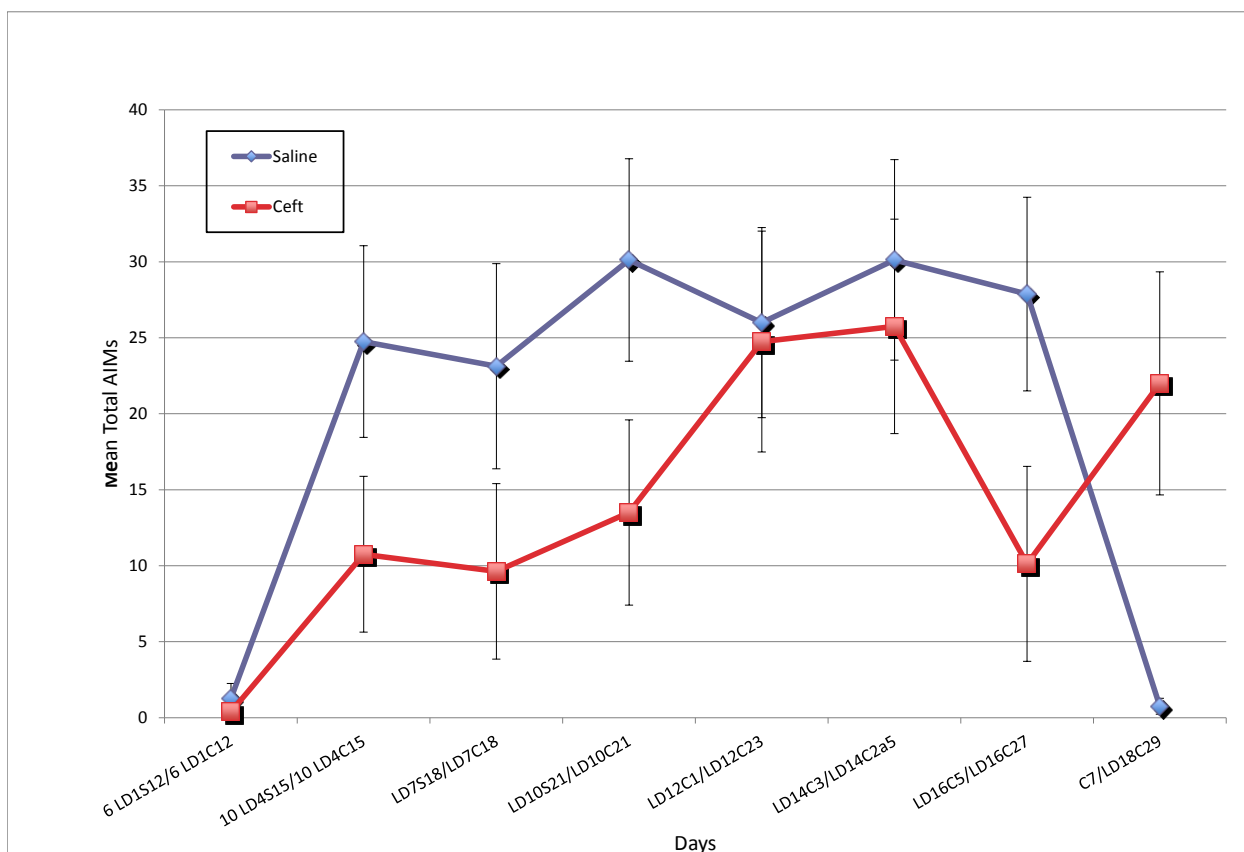


Figure 5. The change in mean total AIM scores (\pm SEM) during the injections of L-DOPA in Experiment 2. As in the prior figures, LD = 6 and 10 mg/kg L-DOPA, S = saline, C = ceftriaxone, and the trailing numbers refer to the day in that condition during which testing occurred. The symbols to the left of the backslash refer to the original saline-injected group, and the numbers to the right refer to the original ceftriaxone-injected group.

AIMs

The Group (L-DOPA combined with ceftriaxone or saline) x Day (8 days of testing [6 LD1/6 LD1C12 – C7/LD19C30]) ANOVA with repeated measures on day revealed a main effect of day, $F(7, 98) = 7.47, p < .001$, and an interaction, $F(7, 98) = 4.27, p < .001$, for total AIMS. Subsequent simple effects tests showed that AIMS changed across days for both groups, $F_s(7, 98) > 4.00, p_s < .001$ (see Figure 5). Although 6 mg/kg L-DOPA had no effect, 10 mg/kg L-DOPA increased AIMS by the first day of testing (LD4) in the saline-injected rats, $F(1, 98) = 14.17, p < .001$, an increase that was not affected by subsequent injections of L-DOPA or the addition of ceftriaxone. LID never developed in two of these animals, but the remaining six consistently averaged 34-40 total AIMS from LD10 on. Total AIMS returned to zero in all saline rats on the day following discontinuation of L-DOPA (C7).

In contrast, 10 mg/kg L-DOPA did not significantly increase total AIMS in the ceftriaxone-injected rats until the fourth day of testing (LD10), $F(1, 98) = 4.42, p < .05$. These AIMS reached a peak on the fifth and sixth days of testing (LD12 and 14), $F_s(1, 98) = 5.03, p_s < .05$, decreased to insignificance on the seventh day of testing (LD16), and were restored by the last day of testing (LD18), $F(1, 98) = 12.00, p < .001$. Only six of the eight animals developed AIMS, and only one expressed more than 35 total AIMS consistently. Among these six rats, there were 15 days on which total AIMS was less than 6, whereas there was only 1 such day in the saline-injected rats. Further indicating that subchronic ceftriaxone reduced the development of LID, simple effects tests showed that the ceftriaxone group expressed fewer total AIMS than the saline group on LD10, $F(1, 112) = 4.03, p = .05$, and LD16, $F(1, 112) = 4.59, p < .02$.

Body weight. The body weight data for the testing days are shown in Figure 6. The Group (2) x Day (15) ANOVA with repeated measures on day revealed only a main effect of day, $F(1, 14) = 49.47, p < .001$ (see Figure 6). Although it appears as though injections of ceftriaxone

slowed the increase in body weight initially in the ceftriaxone-treated rats and later in the saline rats, these effects were not significant.

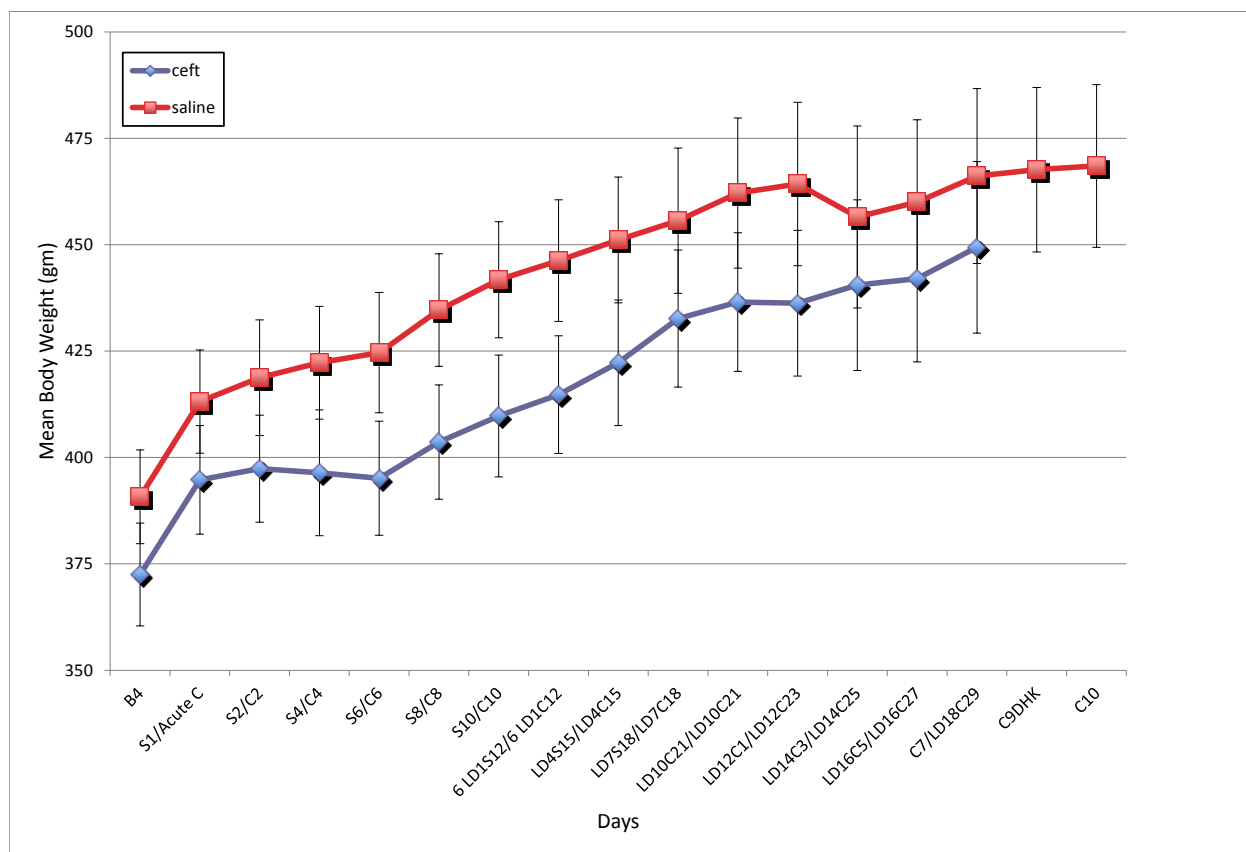


Figure 6. The change in mean body weight (\pm SEM) in gm over the course of the experiment. As in Figures 4 and 5, the letters on the x-axis refer to the drug condition (B = baseline; C = 50 mg/kg ceftriaxone; LD = 6 and 10 mg/kg L-DOPA; DHK = 10 mg/kg DHK), and the trailing numbers refer to the day in that condition during which testing occurred. The symbols to the left of the backslash refer to the original saline-injected group, and the numbers to the right refer to the original ceftriaxone-injected group.

Discussion

Unilateral 6-OHDA lesions

As in Experiment 1, the unilateral MFB lesions decreased right forepaw stepping by 80% in the eight saline-injected animals and by 81% in the ceftriaxone-injected rats, presumably reflective of approximately 80% depletion of DA in the contralateral striatum (Chang et al., 1999).

50 mg/kg ceftriaxone

The first acute injection of 50 mg/kg ceftriaxone improved impaired right forepaw stepping by 19% when tested within 45 min of the injection, an effect that is remarkably similar to the increase seen in Experiment 1 when the effects of an acute injection of 100 mg/kg ceftriaxone was tested on stepping that had not yet returned to baseline following ceftriaxone removal. Both effects are likely caused by an increase in GLT-1 function rather than expression, as GLT-1 expression takes more time to occur (Chu et al., 2007; Lipski et al., 2007). Subsequent injections further increased, but did not normalize, stepping, which appeared to reach asymptote (41% improvement compared to baseline) after the sixth injection. The exceptional similarity in the time course and magnitude of effects of 50 mg/kg ceftriaxone in this experiment and 100 mg/kg in Experiment 1 is somewhat surprising as other experiments have shown that 100 mg/kg has a larger effect on behavior, GLT-1 expression, and glutamate clearance (Rasmussen et al., 2011; Sari et al., 2009). This suggests that in the 6-OHDA model of PD, where there is no ongoing neurodegeneration, only a moderate upregulation in GLT-1 is required to improve symptoms. Given that 50 mg/kg ceftriaxone appears to be sufficient in this model, it is interesting to note that 100 mg/kg and the presumed excess GLT-1 does not cause any noticeable side effects, as are typically seen with higher doses of L-DOPA and NMDA antagonists (Chu et al., 2007). This finding and others would seem to make ceftriaxone an ideal alternative to NMDA antagonists that have low therapeutic indexes (Bibbiani et al., 2005; Metman et al., 1998).

L-DOPA With and Without Ceftriaxone

An acute injection of 6 mg/kg L-DOPA caused a 30% increase in right forepaw stepping in the previously saline-injected group, somewhat larger than the 18% improvement seen in

Experiment 1. Subsequent injections of 10 mg/kg L-DOPA caused a significantly larger 48% increase in stepping, bringing stepping to the level produced by 50 mg/kg ceftriaxone. This L-DOPA-induced increase in right forepaw stepping was expected, as previous research has shown similar results (Chang et al., 1999; Kelsey et al., 2004). As in Experiment 1, there was, however, no additive effect of L-DOPA, at either dose, on right forepaw stepping in the rats receiving ceftriaxone, which may be due to a ceiling effect on impairment (Kelsey et al., 2004; Kelsey et al., 2009).

In contrast to ceftriaxone, the addition of 10 mg/kg L-DOPA caused a significant decrease in left forepaw stepping, an effect that increased with repeated injections, and is most likely due to the development of LID (see below). This effect is often seen when the dyskinetic movements in the unimpaired limb interfere with stepping, as shown previously in our lab (Chen, 2009). The fact that ceftriaxone is as good as a highly therapeutic dose of L-DOPA at improving stepping but does not affect the unimpaired forepaw further indicates that ceftriaxone may prove to be a better treatment for the motor impairments associated with L-DOPA.

Just as the addition of L-DOPA did not alter the effects of ceftriaxone on right forepaw stepping, the addition of ceftriaxone did not alter the effects of L-DOPA on right forepaw stepping. Nor did it stop the progressive decrease in left forepaw stepping produced by L-DOPA in the previously saline-injected rats. However, the finding that left forepaw stepping was less impaired in the original ceftriaxone group, indicates that prior treatment with ceftriaxone may slow the development of this impairment, a point that will be elaborated on in the section on AIMs. Not surprisingly, left forepaw stepping significantly improved within 24 hr of the cessation of L-DOPA injections in the original saline group. The finding that left forepaw stepping did not fully return to pre-L-DOPA levels immediately suggests that L-DOPA caused

some lasting effects. Because the DA receptors become highly sensitized during LID (Darmopil et al., 2009), it is likely that this sensitization remains for some time after cessation of L-DOPA treatment.

DHK

Acute treatment of the rats still on ceftriaxone with 10 mg/kg dihydrokainate (DHK), a selective GLT-1 blocker, caused a significant reduction in the ameliorative effects of ceftriaxone on right forepaw stepping, without affecting left forepaw stepping. Although this dose did not eliminate the effects of ceftriaxone, it does indicate that at least the bulk of the effects of ceftriaxone on forepaw stepping are, as I have suggested, due to increased GLT-function. In the absence of direct measures of GLT-1 mRNA and protein and glutamate clearance before and after DHK administration, we can't be certain precisely where the effects lie.

AIMs

Daily injections of 10 mg/kg L-DOPA caused the development of LID by the fourth day of treatment in rats given saline, at which point AIMs had seemingly reached an asymptote. This procedure has become rather standard for producing LID in this animal model (Cenci & Ohlin, 2009; Lundblad, Anderson, Winkler, Kirik, Wierup, & Cenci, 2002), and my results are similar. It is of interest to note that two of the eight animals were resistant to the development of LID, while the other six consistently expressed almost maximal AIMs. The subsequent addition of 50 mg/kg ceftriaxone did not decrease the expression of AIMs. This is apparently contradictory to findings of Miller et al. (2008), who found a decrease in HD-associated paw claspings, which is similar to dyskinesia, after ceftriaxone treatment. However, they used 200 mg/kg ceftriaxone, suggesting that ceftriaxone may be able to decrease the expression of AIMs at higher doses.

Nevertheless, pretreatment with ceftriaxone did slow the development of LID and the associated impairment in left forepaw stepping in the ceftriaxone group. Additionally, although six of the eight rats in this group did eventually express as much LID as the rats in the saline group, they did so far less consistently. These findings suggest that the reduction of glutamate transmission produced by ceftriaxone may reduce the sensitization of the NMDA and AMPA receptors typically seen in LID, but are unable to reverse this sensitization once it has developed (at least at the dose I used) (Bagetta et al., 2010; Kobylecki et al., 2010; Oh et al., 1998).

Body Weight

Although none of these effects were statistically significant, the initial injections of 50 mg/kg ceftriaxone caused a minor suppression of weight gain in both groups that was transient. This observation is consistent with the findings in Experiment 1 that ceftriaxone is able to suppress weight gain. However, the suppression appears to be longer lasting in the previous experiment, most likely due to the larger dose (100 mg/kg) of ceftriaxone. The observation that ceftriaxone appears to slow weight gain is an important consideration if ceftriaxone is to be used for a prolonged period in people for the treatment of any disease. However, this small effect was the only negative side noted in either experiment, demonstrating the almost complete lack of side effects of ceftriaxone.

General Discussion

As of yet, there is no way to prevent or even slow the progressive neurodegeneration of Parkinson's disease. The only therapies that exist focus on symptom management. The most effective drug for decreasing the motor symptoms of PD is the dopamine precursor L-DOPA which acts to increase DA synthesis, and thus transmission, in the dopamine-depleted striatum. However, after a few years of L-DOPA therapy, the therapeutic effects of L-DOPA decline, and

the majority of patients develop L-DOPA-induced dyskinesia as a side effect. Based on data that one of the key problems behind PD and LID pathology is increased glutamate transmission (Calabresi et al., 1993; Chung et al., 2008; Lievens et al., 2001; Nash & Brotchie, 2002), I hypothesized that the upregulation of the primary glutamate transporter GLT-1, known to decrease extracellular glutamate, by ceftriaxone might be beneficial to the treatment of the motor symptoms associated with both PD and LID.

The data from my experiments show that repeated injections of both 50 and 100 mg/kg ceftriaxone are as effective as 10 mg/kg L-DOPA at improving impaired forepaw stepping in the 6-OHDA-lesioned hemi-Parkinsonian rat, and, in contrast to L-DOPA, produce no detectable dyskinesia. Also in contrast to L-DOPA, the effects of ceftriaxone are long-lasting and are still apparent even 30 days after the termination of ceftriaxone. Given that ceftriaxone has been proven safe in humans, it would appear as though it would offer several substantial advantages over traditional L-DOPA therapy. Although the data strongly support the use of ceftriaxone as monotherapy, it is possible that ceftriaxone could be useful as adjunctive therapy. While ceftriaxone does not reduce the expression of already established LID, it does reduce the development. Because the doses of each produced maximal therapeutic effects, I was unable to determine if subthreshold doses of both would produce additive effects and reduce the development of LID even further.

By blocking GLT-1 function with DHK, I was able to show that the therapeutic effects of ceftriaxone on forepaw stepping were due, at least in large part, to ceftriaxone's action on GLT-1. Given that ceftriaxone had therapeutic effects acutely and at a dose (50 mg/kg) that has not been shown to upregulate GLT-1 expression (Chu et al., 2007), it is likely that at least some of these effects are due to an ability of ceftriaxone to increase the activity of GLT-1.

Limitations

The biggest limitation of this study was the inability to determine the exact mechanism by which ceftriaxone acts to improve the motor deficits of Parkinson's disease. Because the effects of ceftriaxone were not completely eliminated by DHK, mechanisms other than enhanced GLT-1 activity are possible. Additionally, the injection of DHK did not determine exactly how ceftriaxone acted on GLT-1, and there is no one clear answer to be found in the literature. Although these data would not have been decisive, this study would have benefited from a determination of GLT-1 mRNA and protein expression and especially from measures of extracellular glutamate uptake and concentration. It would also be interesting to examine ceftriaxone's effects on other physiological changes typically associated with PD and LID such as striatal enkephalin and dynorphin levels and D1 and D2 receptor function.

The study of ceftriaxone's effects on LID was limited by the expense of ceftriaxone. I would have liked to determine if a higher dose (200 mg/kg) used to improve the motor symptoms of Huntington's disease would have decreased LID expression. Because 50 mg/kg slows LID development, it seems possible that higher doses of ceftriaxone might reduce LID expression.

Future Directions

There are a number of additional studies that could clarify or enhance the results found in this study. As discussed in the introduction, the mechanism of ceftriaxone action is not well understood. Although it is thought that ceftriaxone acts to decrease glutamate through GLT-1, it is not clear if this is mediated through an increase in protein expression and/or function. The results of this study suggest that ceftriaxone acts acutely by increasing GLT-1 function, but less is known about the effects of multiple doses. The finding that DHK did not completely reverse the effects of ceftriaxone raise the possibility that ceftriaxone may not act completely through

GLT-1 modification in this model. Thus, it would be of value to determine if larger doses of DHK would produce a more complete blockade, and it would be extremely useful to determine if these effects can be blocked by injections of DHK into various locations within the basal ganglia as that is the presumed site of GLT-1 modification.

The long lasting effects of ceftriaxone in this and other studies, consistent with an upregulation of a protein, suggest that ceftriaxone would not need to be injected every day and that the therapeutic effects of ceftriaxone could be achieved with far less frequent injections. This implication should be tested, and, if confirmed, would provide yet another advantage over L-DOPA, which must be taken daily. Fewer administrations would also be useful, as ceftriaxone is only currently available to humans as an IV injection (Nau et al., 1993).

Although it seems unlikely that ceftriaxone is effective in this study because it has decreased ongoing neurodegeneration, the fact that it can reduce such degeneration in animal models of other disorders (Rothstein et al., 2005) suggests that it is possible that ceftriaxone could also reduce ongoing degeneration in PD. Thus, it is possible that ceftriaxone could reduce the progression or perhaps even prevent the occurrence of PD in addition to ameliorating the symptoms. Consequently, it would be useful to determine if pretreatment with ceftriaxone would reduce the degeneration and behavioral effects of 6-OHDA injections in our model or in the MPTP-treated mouse model, as MPTP appears to produce a more sustained degeneration.

Because of ceftriaxone's success in animal models of ALS, it is now being tested in stage III clinical trials for this disorder (Traynor et al., 2006). Because of the lack of side effects when used to treat meningitis at similar doses (Nau et al., 1993), its efficacy in neurodegenerative studies (Rothstein et al., 2005; Thone-Reinke et al., 2008), and the behavioral effects found in this study, it seems likely that ceftriaxone may be a good candidate for clinical trials in

Parkinson's disease to both treat the motor symptoms more effectively than L-DOPA and perhaps even reduce degeneration. On a more modest level, it certainly seems that ceftriaxone could be a viable alternative to NMDA antagonists which, although efficacious at reducing LID in human patients (Snow et al., 2000), have a number of undesirable cognitive side effects (Caraci et al., 2012).

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