Published in final edited form as:

Restor Neurol Neurosci. 2013 ; 31(1): 87–97. doi:10.3233/RNN-2012-120245.

The effects of ceftriaxone on skill learning and motor functional outcome after ischemic cortical damage in rats

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Abstract

Purpose—Ceftriaxone, a β -lactam antibiotic, can selectively enhance the expression of glutamate transporter 1 (GLT1), the most abundant astrocytic glutamate transporter expressed in the cortex. It has been found to have neuroprotective effects when administered prior to brain ischemic damage or during the acute phase post-stroke, but its effects in chronic period have not been examined.

Methods—We examined the effects of ceftriaxone on the acquisition of motor skill and the functional outcome after focal ischemic cortical lesions. In adult male rats, ceftriaxone (200mg/kg) or vehicle was intraperitoneally injected daily for 5 days, a treatment regime previously established to upregulate GLT-1. This preceded 28 days of skilled reach training in intact animals or began 3 days following lesions, followed by 5 weeks of rehabilitative reach training.

Results—In intact rats, ceftriaxone did not affect skill learning rate or final performance. Following ischemic lesions, though there was no significant difference in lesion sizes between groups, ceftriaxone exacerbated initial deficits in reaching performance.

Conclusion—These findings of detrimental effects on motor functional outcome suggest that ceftriaxone may be more useful for neuroprotection during the acute phase of ischemia than for functional recovery in the post-acute period after ischemic damage.

Keywords

stroke; functional outcome; skilled reaching; astrocytic glutamate transporter; GLT1; β -lactam antibiotics

1. Introduction

Astrocytes regulate extracellular milieu around synapses by maintaining extremely low concentrations of glutamate, the primary excitatory neurotransmitter in the central nervous

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system. High affinity glutamate transporters on astrocytes quickly remove excess amounts of released glutamate from the synaptic cleft, which is critical for fine-tuning synaptic transmission and limiting intersynaptic crosstalk between neighboring synapses (Danbolt, 2001). Glutamate transporter 1 (GLT1, or also known as excitatory amino acid transporter 2, EAAT2, in humans) is located primarily on astrocytes and responsible for most glutamate transport in the adult brain (Danbolt et al., 1992; Tanaka et al., 1997).

Astrocytic ensheathment around synapses appears to enable such astrocytic control of extracellular glutamate concentrations and thereby mediate synaptic transmission and glutamate spillover. For example, in the supraoptic nucleus (SON) of the rat hypothalamus, reductions in the ensheathment of astrocytic processes around synaptic clefts of oxytocin-releasing neurons results in reduced glutamate uptake by astrocytes during lactation (Oliet et al., 2001; Oliet and Bonfardin, 2010). Glutamate spillover then occurs, and the excess glutamate diffuses to neighboring synapses via volume transmission (Piet et al., 2004), leading to suppression of inhibitory γ -aminobutyric acid (GABA) ergic synaptic transmission and, as a consequence, massive hormonal secretion. Moreover, direct astrocytic contacts with postsynaptic dendritic spines can control synaptic function and plasticity by altering astrocytic glutamate transport. Contact-mediated Eph-ephrin signaling between dendritic spines and perisynaptic astrocytes modulates spine morphology (Murai et al., 2003) and changes astrocytic glutamate transport (Carmona et al., 2009), thereby altering synaptic function (Filosa et al., 2009) in the adult mouse hippocampus. These findings suggest that structural relationships between astrocytes and synapses are tightly associated with astrocytic glutamate uptake from synaptic clefts, and that this relationship strongly influences synaptic function and plasticity.

Astrocytic contact with synaptic elements can change following brain damage and in response to behavioral experience. For example, rats reared in a complex environment have more astrocytic contacts with synapses in the primary visual cortex (Jones and Greenough, 1996). Intensive whisker stimulation in mice induces synaptogenesis (Genoud et al., 2004) along with increased astrocytic coverage around synapses and upregulation of astrocytic glutamate transporters in the barrel cortex (Genoud et al., 2006). We found that synapse numbers are positively correlated with astrocytic volume in the contralateral homotopic cortex after unilateral ischemic SMC lesions in middle-aged rats (Kim and Jones, 2010). The proportion of astrocytic membrane apposed to synapses in layer V of the perilesion motor cortex increases following both reach training and ischemic lesions and the fraction of synapses with direct astrocytic contacts is positively correlated with functional recovery following lesions (Kim et al., 2009). Given that astrocytic glutamate uptake can influence synaptic cross talk and efficacy (Oliet et al., 2001; Piet et al., 2004; Sykova and Vargova, 2008), it is likely that alterations in astrocytic glutamate transporters can affect both experience-dependent plasticity in intact animals and functional outcome following lesions.

Ceftriaxone, a β -lactam antibiotic, selectively increases GLT1 expression and the functional capacity of glutamate uptake (Rothstein et al., 2005). Ceftriaxone has been shown to have a potent neuroprotective effect in animal models of amyotrophic lateral sclerosis (Rothstein et al., 2005), Huntington's disease (Rothstein et al., 2005; Miller et al., 2008), and spinal muscular atrophy (Hedlund, 2011; Nizzardo et al., 2011). In experimental stroke,

ceftriaxone injected prior to the onset of middle cerebral artery occlusions (MCAo) has been found to reduce infarct volume (Chu et al., 2007). It also reduces neuronal death in the peri-infarct zone and the mortality rate when injected 90 minutes after MCAo (Thone-Reineke et al., 2008). These previous studies in animal models were focused on neuroprotection prior to or during the acute phase after stroke, when glutamate uptake by astrocytes can mitigate excitotoxicity-mediated neuronal death. Ceftriaxone is also being tested clinically as a preventive treatment for post-stroke infections (Nederkoorn et al., 2011). However, the effect of ceftriaxone had not been tested on functional recovery during later time periods after stroke, when brain remodeling plays a key role in improving behavioral outcome (Jones et al., 2009).

The present study tested the effects of ceftriaxone treatment on motor skill acquisition in intact animals and in those re-establishing skills after ischemic injuries. Ceftriaxone (200mg/kg per day) or vehicle (sterile saline) was intraperitoneally injected daily for five days, as in previous studies (Rothstein et al., 2005; Chu et al., 2007). Protein expression of GLT1 peaks after 5 days of ceftriaxone treatment (Chu et al., 2007). Reach training in both intact and injured rats began on the last day of treatment to coincide with this peak. Given that astrocytic glutamate uptake plays an important role in the synaptic activity and plasticity, we postulated that ceftriaxone would influence motor learning rates and possibly enhance functional outcome by increasing GLT1 function at a time of training-induced plasticity of neuronal circuits. We found little effect of GLT1 in intact rats and a transient detrimental effect in animals with ischemic lesions.

2. Materials and methods

2.1 Animals and experimental designs

Twenty-three male 4 month-old Long-Evans hooded rats were used. Rats were received from Harlan Laboratories three months before the onset of experimental procedures, and made tame by handling to minimize stress during training. Animals were housed in pairs on a 12:12 hour light:dark cycle, receiving water *ad libitum*. All animal use was in accordance with a protocol approved by the Animal Care and Use Committee of the University of Texas at Austin.

To investigate the effect of ceftriaxone on motor skill learning (Experiment 1, Fig. 1A), rats were divided into two groups: (1) ceftriaxone (CEF), $n=12$ and (2) vehicle (VEH), $n=11$. Reach training on a single pellet retrieval task was carried out for 14 days and, in a subset of animals, this was extended to 28 days ($n=7$ in CEF, $n=6$ in VEH). There were no obvious signs of sickness resulting from ceftriaxone injections. Body weights were similar between treatment groups before and after treatment. On the day before treatment, the day after treatment and one week later, body weights (g) in CEF vs. VEH treated rats were 478 ± 15 vs. 456 ± 9 , 481 ± 15 vs. 466 ± 8 , and 475 ± 14 vs. 464 ± 8 , respectively (mean $\pm$ S.E.).

To investigate the effect of ceftriaxone on rehabilitative training induced functional improvements after ischemic lesions, 10 of the rats (6 from CEF, 4 from VEH) that underwent training for 28 days in Experiment 1 were used (Experiment 2, Fig. 2A). Thus, Experiment 1 of the study also served to establish the skill so that lesion-induced

impairments and functional improvements in the skill could be studied. Lesions were made from 35 to 45 days after CEF or VEH treatment in Experiment 1. Rats from the two pre-injury injection groups were randomly assigned in equal proportions to post-injury treatment group: (1) CEF, n=5 and (2) VEH, n=5, with the exception that they were matched as closely as possible for pre-operative reaching performance. This was done because there were no differences in pre-operative reaching performance between CEF and VEH groups, as described below. The injections were administered beginning 3 days after lesion induction and for the subsequent 5 days. All ten rats then underwent rehabilitative tray reach training for 5 weeks. Reaching performance was probed weekly using a single pellet-retrieval task because it more sensitively measures reaching performance than the tray reaching task.

2.2 Single pellet reach retrieval task

The single pellet retrieval task was used for skilled reach training in intact animals and to measure lesion-induced deficits and rehabilitative training-induced functional improvements following ischemic lesions. Rats were given banana flavored food pellets (45 mg Dustless Precision pellets, Bioserve, Inc.) for about two weeks before the start of reach training to avoid neophobic responses to the food reward during shaping and training. Scheduled feeding (15–18 g per rat, once per day) were initiated 2 days before the onset of reaching training to ensure that animals were not sated at the time of training. The single pellet retrieval task was performed using an apparatus modified from those used in previous studies (Withers and Greenough, 1989; Miklyaeva and Whishaw, 1996; McKenna and Whishaw, 1999). A Plexiglas chamber with a tall narrow window in the center of the front wall was used, as previously described (Allred et al., 2005; Hsu and Jones, 2005; Maldonado et al., 2008). Rats were shaped for several days on the task and allowed to use either limb so that a preferred limb for reaching could be determined. The preferred limb was determined when a rat used the same limb for more than 70% of its reach attempts and made more than 20 reaches over a 15-minute period. After a preferred limb was established, ceftriaxone or vehicle was injected for five consecutive days. On the last day of injections, the first training session started. A wall was placed 1.5 cm from the center window to force an animal to use the preferred limb for pellet retrieval. Skilled reach training consisted of 30 trials in which rats could make up to 5 reach attempts per trial for a pellet located in a shallow well outside the front window. A trial ended when (1) the pellet was successfully retrieved from the well and eaten (success); (2) more than 5 reach attempts were made without retrieving the pellet; (3) the food pellet was knocked from its well; or (4) the pellet was dropped inside the chamber before the animal could place it in its mouth. The percentage of successful reaches out of the total number of reach attempts was calculated.

2.3 Post-operative motor rehabilitation and assessment of functional recovery

Animals received ischemic lesions in the hemisphere opposite to the trained forelimb. Three days after surgeries, rats were given either ceftriaxone (200mg/kg, i.p.) or vehicle (sterile saline) injections daily for 5 days (Fig. 2A). On the last day of injection (a week after lesions), all animals received a probe test on the single pellet retrieval task that consisted of 30 reaching trials. Pre-CEF treatment probe trials were omitted because the onset of treatment (3 days post-lesion) was too early to permit the full return of appetitive task

motivation. Subsequent probe tests were performed on two consecutive days after every fifth day of rehabilitative reach training. The post-ischemic treatment groups had very similar pre-operative reaching performance (CEF, $60.0 \pm 5.3\%$; VEH, $57.8 \pm 2.5\%$; $F_{(1,8)} = 0.15$, $p = 0.71$, one-way ANOVA). Therefore, post-ischemic probe trial data were analyzed as a change from pre-operative performance using the formula: (%successes per reach attempt on a post-operative probe test) – (%pre-operative reaching performance). Data from the two consecutive probe tests were averaged to simplify the presentation.

Motor rehabilitation of the paretic forelimb consisted of five weeks of daily practice in tray reaching task for 5 days per week (Whishaw et al., 1986; Gharbawie et al., 2005; Maldonado et al., 2008). After being placed in the reaching chamber, rats were allowed to reach for up to 100 pellets (45 mg per pellet, 4.5g total) in an inclined ($\sim 25^\circ$) tray until all were retrieved, or for 20 minutes, whichever came first. As in the single pellet task, a wall was positioned in the chamber to discourage the use of the non-paretic forelimb during motor rehabilitation. The time required for each rat to retrieve all of the pellets from the tray was recorded. When the rat did not eat all of the pellets during the given 20 minutes, the pellets left in the tray were weighed to calculate the number of pellets retrieved per minute during the session.

2.4 Ischemic lesion induction

Unilateral ischemic lesions to the forelimb representation region of the sensorimotor cortex were made in the hemisphere opposite to the trained forelimb using endothelin-1 (ET-1), as described previously (Adkins et al., 2004). ET-1 results in focal and potent vasoconstriction, resulting in ischemia followed by gradual reperfusion over a 24 hour period (Biernaskie and Corbett, 2001). Rats were anesthetized with a cocktail of ketamine (90–100 mg/kg) and xylazine (9–10 mg/kg). A craniectomy was made between 1.5 mm posterior to 2.5 mm anterior to bregma and 3.0 and 4.5 mm lateral to midline and the underlying dura was removed. Ischemic lesions were produced by placing 3.5 μL of endothelin-1 (ET-1, 0.2 $\mu\text{g}/\mu\text{L}$ in sterile saline, American Peptide, Inc.), a vasoconstricting peptide, directly onto the pial surface. ET-1 was divided into two drops (2 μL and 1.5 μL), applied 4 minutes apart. The cortex was left undisturbed for an additional 6 minutes after the second application of ET-1. The craniectomy was then filled with gel foam and dental cement to reduce the tissue distortion from cortical upwelling through the craniectomy.

2.5 Histological tissue processing

One day after the last probe test, animals were anesthetized with a lethal dose of sodium pentobarbital (>100 mg/kg) and transcardially perfused with 0.1 M phosphate buffer followed by 4 % paraformaldehyde in the same buffer. Brains were extracted and sliced with a Leica VT 1200S after 24 hours of post-fixation. 50 μm sections were Nissl stained and used for lesion reconstruction and volume estimation.

2.6 Lesion analyses

Lesion size was estimated to assess the possibilities that (1) ceftriaxone treatment affected lesion size, and/or (2) behavioral differences between groups were related to differences in lesions, since the size of sensorimotor cortical lesions affects the magnitude of behavioral deficit and lesion-induced brain plasticity (Hsu and Jones, 2006; Kim and Jones, 2010).

Lesion size was defined as the volume difference between injured and intact cortex. Perimeters of Nissl-stained coronal sections were traced using NeuroLucida (MicroBrightField, Inc.), beginning approximately 2.7 mm anterior to bregma and continuing through six caudal sections (800 μ m apart), to focus the sampling in the sensorimotor cortical region. Volume was then calculated using the Cavalieri principle (Gundersen et al., 1988) by the formula: $V = \sum A \times T$, where $\sum A$ is the sum of the area of all the sections and T is the distance between section planes. Lesion placement was assessed by reconstructions onto schematic coronal section templates (Fig. 3C).

2.7 Statistical analysis

Behavioral data were analyzed using the Statistical Package for the Social Science (SPSS, IBM Inc.) general linear model procedure (GLM) for repeated measures analyses of variance (ANOVA) for the effects of treatment, time, and treatment by time interaction. To determine the effect of ceftriaxone on functional recovery after ischemic lesions, post-operative data following injections were analyzed. The post-operative effects of CEF and VEH treatment did not vary significantly with pre-operative exposure ($F_{(2,6)}$'s = 0.32 and 0.67, p 's = 0.74 and 0.60). Post hoc analyses were performed when warranted by significant treatment by time interaction using one-way ANOVAs for group at each post-operative time point. Lesion size data were also analyzed using one-way ANOVA.

3. Results

3.1 In intact rats, ceftriaxone did not significantly change skill learning rate or final level of reaching performance

Ceftriaxone injections did not affect the rate of learning or plateau level of performance on the reaching task (Fig. 1B). During the first 14 days of training, though there was a significant effect of training day ($F_{(13, 273)} = 19.3$, $p < 0.001$), there was neither main effect of treatment (CEF vs. VEH, $F_{(1, 21)} = 0.55$, $p = 0.47$) nor an interaction effect of treatment by time ($F_{(13, 273)} = 1.46$, $p = 0.13$). When training was extended to 28 days ($n=7$ in CEF, $n=6$ in VEH), there was also a significant day effect ($F_{(13, 143)} = 2.02$, $p < 0.05$) in this later training period. However, there was neither a treatment ($F_{(1, 11)} = 1.46$, $p = 0.25$) nor treatment by time interaction effect ($F_{(13, 143)} = 0.75$, $p = 0.71$).

3.2 Ceftriaxone exacerbated initial deficits in reaching performance after ischemic lesions

Eight days after ischemic lesions, the tray reaching task was used for motor rehabilitation of the impaired limb. Reaching performance in the single pellet retrieval task was probed prior to the onset of rehabilitative training and weekly thereafter. As measured in reaching probe trials, ceftriaxone injections exacerbated deficits prior to the onset and after a week of rehabilitative training (PostOp weeks 1 and 2, Fig. 2B). In repeated measures ANOVA, there was no main effect of treatment ($F_{(1,8)} = 1.47$, $p = 0.26$), but there was a significant effect of treatment by time interaction ($F_{(5,40)} = 2.51$, $p < 0.05$). In *post hoc* analyses, the early performance of the CEF group was significantly lower than the VEH group at week 1 (prior to the onset of rehabilitation, $F_{(1,8)} = 5.88$, $p < 0.05$) and week 2 (after 7 days of rehabilitative training, $F_{(1,8)} = 10.9$, $p < 0.05$; Fig. 2B). This difference subsequently disappeared and the final level of reaching performance was similar between groups. There

were no significant differences between groups in the number of reach attempts made in probe trials at week 1 and 2 (mean $\pm$ S.E.; 39 ± 2 in CEF, 40 ± 3 in VEH, $p = 0.69$).

3.3 Ceftriaxone interfered with acquisition of the rehabilitative training task

On the tray reaching task used for rehabilitative training, performance was assessed by the average number of pellets retrieved per min. There were significant effects of time ($F_{(4, 32)} = 9.37$, $p < 0.001$) and group by time ($F_{(4, 32)} = 3.43$, $p < 0.05$), but there was no main effect of treatment group ($F_{(1, 8)} = 1.12$, $p = 0.32$). In *post hoc* analyses, there was a tendency for the CEF group to retrieve fewer pellets per min than the VEH group, but this did not reach statistical significance ($p = 0.13$, Week 3; Fig. 2C). This tendency was diminished after 2 weeks of rehabilitation training.

3.4 Lesion size and placement were similar between ceftriaxone and vehicle treated groups

As measured by volume difference, there was no significant difference in lesion sizes between ceftriaxone and vehicle treatment groups (Fig. 3). Furthermore, lesion placement was similar between the two treatment groups, as shown in lesion reconstructions overlaid on schematic coronal sections (Fig. 3C).

4. Discussion

Ceftriaxone, a β -lactam antibiotic, selectively induces the expression of the astrocytic glutamate transporter, GLT1, and improves its functional capacity (Rothstein et al., 2005). Ceftriaxone's potential neuroprotective effects as a therapeutic strategy is supported by studies administering it during the acute phase of a stroke (Rothstein et al., 2005; Chu et al., 2007; Lipski et al., 2007; Lee et al., 2008; Thone-Reineke et al., 2008). The present study found no evidence to suggest that ceftriaxone may be useful for enhancing motor rehabilitation and long-term functional recovery following cortical ischemic damage. We found that (i) ceftriaxone had no significant effect on motor learning in intact rats and that (ii) despite the absence of treatment effects on lesion size, ceftriaxone treatment beginning 3 days after an ischemic stroke initially worsened motor performance as measured by a skilled reaching task and a decrement in function persisted for a week after the end of treatment. The latter findings indicate that ceftriaxone, possibly as a result of its upregulation of astrocytic glutamate transporters, can at least transiently worsen function. This suggests that the post-stroke timing of ceftriaxone treatment may need to be carefully considered to avoid potential adverse effects on motor behavior.

The neuroprotective effects of earlier ceftriaxone treatment that were found in previous studies were presumably a result of its ability to alleviate excitotoxicity-mediated neuronal damage, during the acute phase of a stroke (Hazell, 2007). Its neuroprotective effects vary with the timing of ceftriaxone administrations. For example, ceftriaxone had no effect on infarct size when administered either 30 min (Chu et al., 2007) or 2 h (Verma et al., 2010) after MCA occlusion in rats. However, when administered before the onset of occlusion, it was found to reduce infarct size in both studies. Though a single injection of ceftriaxone 90 min after transient MCA occlusion showed neuroprotective effects in rats, as seen by a

greater number of remaining cells in the peri-infarct area compared with vehicle treatment, it did not significantly reduce infarct size in rats (Thone-Reineke et al., 2008). In the present study, using a different experimental model of stroke (ET-1 induced focal ischemic lesions), ceftriaxone administered 3 days after ischemic onset did not affect lesion size or placement compared to the vehicle treatment. These findings suggest that the neuroprotective effects of ceftriaxone, in particular its ability to reduce infarct volume, may be maximized when ceftriaxone is injected prior to ischemia, enabling astrocytic glutamate uptake to be enhanced at the onset of the stroke. In the present study all groups have similar lesion sizes, and it is therefore likely that any behavioral differences found between groups reflect how ceftriaxone treatment and the upregulation of astrocytic glutamate uptake directly affect the functional activity and ongoing reorganization of remaining brain regions.

The ceftriaxone administered 3 days after ischemic lesions in the present study exacerbated initial deficits. We did not assay GLT1 expression changes in the present study, but several previous studies have shown that similar ceftriaxone treatment regimens upregulate GLT1 expression (Chu et al., 2007; Lee et al., 2008; Miller et al., 2008; Rothstein et al., 2005; Sari et al., 2010). Thus, it is possible that ceftriaxone treatment in the present study disrupted synaptic transmission via upregulation of GLT1 and that this contributed to the decrement in motor behavioral function. Indeed, previous findings support that excessive glutamate transport by astrocytes can disrupt synaptic transmission and plasticity. Contact-mediated EphA4/ephrin-A3 signaling between postsynaptic dendritic spines and astrocytes in the hippocampus is required for proper spine morphological maturation (Murai et al., 2003) and spine dynamics at excitatory synapses (Haber et al., 2006). Ephrin-A3 null mice have impaired hippocampus-dependent learning in a fear-conditioning context and remarkable upregulation of astrocytic glutamate transporters, GLT1 and GLAST (Carmona et al., 2009). Lack of either dendritic EphA4 or ephrin-A3 in the hippocampus is also associated with defective long-term potentiation (LTP) at CA1-CA3 synapses, and was linked with the greater abundance of astrocytic glutamate transporters (Filosa et al., 2009). Pharmacological inhibition of astrocytic glutamate uptake was found to reverse these effects, suggesting that excessive removal of glutamate in the vicinity of synapses may lead to insufficient activation of synaptic glutamate receptors, and result in impaired LTP (Filosa et al., 2009). Ceftriaxone-induced GLT1 upregulation was also found to impair long-term depression (LTD) as well as LTP at mossy fibre-CA3 synapses by reducing activation of perisynaptic metabotropic receptors (Omrani et al., 2009). Furthermore, ceftriaxone-induced upregulation of GLT1 impairs prepulse inhibition, an index of sensorimotor gating (Bellesi et al., 2009). Ceftriaxone treatment also reduces theta power in electroencephalography (EEG) and increases motor activity in intact rats (Bellesi et al., 2012), indicating that the activity of the sensorimotor system is very sensitive to the effects of ceftriaxone treatment. Taken together, these findings support the possibility that the detrimental motor behavioral effects of ceftriaxone observed in the present study are due, at least in part, to alteration of synaptic transmission by excessive glutamate uptake.

The detrimental effects of ceftriaxone administration on functional outcome are reminiscent of the previous findings that the glutamate receptor antagonist, MK801 can be beneficial when administered early after lesions (12–16 hours) but detrimental when administered later, following behavioral recovery (Barth et al., 1990). Thus, timing of manipulation of

glutamate transmission might be a critical factor for mediating functional outcome. Further investigation of the effect of ceftriaxone on different time points (e.g., later on functional recovery) can provide some insight into this issue.

It also seems important to further characterize how altered astrocytic glutamate uptake affects synapses in the motor cortex and, the structural association between astrocytes and synapses. Reach training positions astrocytic process around synapses but not at the vicinity of synaptic cleft (Kim et al., 2009). The avoidance by astrocytic processes of the synaptic cleft could enable cross talk between synapses by permitting glutamate and other substances to diffuse into the extracellular space, i.e., 'volume transmission' (Oliet et al., 2001; Piet et al., 2004; Sykova and Vargova, 2008). If so, it seems likely that ceftriaxone diminishes such cross talk between synapses by excessive glutamate uptake.

The effect of ceftriaxone on functional outcome following ischemic lesions persisted for a week after the discontinuation of injections in the present study. Similar durations of ceftriaxone effects have been found in other studies. For example, ceftriaxone attenuated the symptoms of the Huntington's disease phenotype in transgenic R6/2 mice, indicating that impaired astrocytic glutamate uptake in striatum is involved in the pathophysiology of the disease (Miller et al., 2008). The effect of ceftriaxone on most of the behavioral symptoms of the Huntington's disease was seen at one day following the final injection but had disappeared a week later. Moreover, a previous study about ceftriaxone effects on prepulse inhibition (PPI) in rats showed that the ceftriaxone-enhanced expression of GLT1 in various regions of brain returned back to basal levels, along with the impaired PPI, eight days following the cessation of injections (Bellesi et al., 2009). These findings suggest that the effect of ceftriaxone on GLT1 persists for a week after discontinuing injections, consistent with the findings in the present study.

In contrast to its effects after ischemic lesions, the effect of ceftriaxone was not obvious during motor skill learning in intact animals. The difference in its effects after lesions suggests that ischemic damage creates a hypersensitivity to deleterious effects of ceftriaxone, at least on motor performance. For example, it has been found that there is a chronic increase in tonic inhibition in peri-infarct cortex and that reducing GABA-mediated tonic inhibition induces early and sustained functional recovery (Clarkson et al., 2010). Activation of astrocytic glutamate transporters can induce GABA release from astrocytes (Heja et al., 2009). Therefore, it is possible that activation of astrocytic glutamate uptake induced by ceftriaxone may increase astrocytic GABA release and result in excessively triggered GABA-mediated tonic inhibition in peri-infarct cortex that can interfere with functional recovery. Further investigations of the mechanisms underlying the detrimental effects of ceftriaxone on motor function following lesions are needed.

Numerous previous studies have shown the robust effect of ceftriaxone on astrocytic GLT1 expression or function (Chu et al., 2007; Lipski et al., 2007; Miller et al., 2008; Sari et al., 2010). It has been found that ceftriaxone specifically increases GLT1 expression by promoter activation (Lee et al., 2008) but has no effect on the expression of a neuronal glutamate transporter, excitatory amino acid carrier 1 (EAAC1, Rothstein et al., 2005). However, GLT1 and its splice variant, GLT1b, are found in neurons (Chen et al., 2002;

2004). Ceftriaxone increases the expression of GLT1b (Rothstein et al., 2005) and of neuronal GLT1 (Omran et al., 2009). Thus, though ceftriaxone clearly can influence astrocytic GLT1, it is also possible that the effect of ceftriaxone in the present study is due in part to changes in neuronal GLT1 expression.

Although the present study used ceftriaxone primarily as a pharmacological manipulation of glutamate transporters, ceftriaxone is one of the most commonly used antibiotics in patients with infection. Infections are strongly associated with stroke, both as a risk factor (Ameriso et al., 1991; Macko et al., 1996), and as a post-stroke complication that is associated with poorer recovery (Emsley and Hopkins, 2008). Ceftriaxone is an effective antibiotic treatment for pneumonia and urinary tract infections, which are among the most common types of post-stroke infections (Nederkoorn et al., 2011). Given this and its neuroprotective effects, ceftriaxone has received attention as a possible combination prophylactic and neuroprotective stroke treatment (Nederkoorn et al., 2011). However, the present findings of detrimental effects of ceftriaxone on motor behavioral outcome indicate the need for a more thorough characterization of dose-response and temporal profiles of post-ischemic treatment effects on behavioral function, and for careful consideration of the timing of its administration post-stroke. (Bellei et al., 2012)

Acknowledgments

The authors thank Dr. DeAnna L. Adkins for helpful advice and Cole Husbands for editing this manuscript. This study was supported by NS056839 and William S. Livingston Fellowship.

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Fig. 1.

Experiment 1 tested the effect of ceftriaxone on motor skill learning in intact animals (A). Ceftriaxone was administered 200mg/kg per day, for 5 days and training on a skilled reaching task (the single pellet retrieval task) began on the last day of injection, at a time when the GLT1 expression level has been found to peak (Chu et al., 2007). Performance on the single pellet reaching task over days of training in Experiment 1 was examined. In intact rats, ceftriaxone administration failed to influence the rate of learning or plateau level of performance on the reaching task (B). On Day 14, training ceased for 10 of animals (n=5 per each group). VEH, vehicle (sterile saline); CEF, ceftriaxone.

Fig. 2.

Experiment 2 tested ceftriaxone effects on motor functional outcome and rehabilitation efficacy after ischemic sensorimotor cortex lesions (A). The ceftriaxone regimen (200mg/kg/day for 5 days) was initiated 3 days after the lesions and rehabilitative training began on the last injection day (8 days post-lesion). Ceftriaxone worsened functional deficits in skilled reaching, as measured in probe trials on the single pellet retrieval task (B). Functional outcome was measured as the difference in the % successful pellet retrieval per reach attempt between post- and pre-operative performance. Ceftriaxone injections worsen functional deficits prior to (PostOp week1, $*p < 0.05$) and a week after motor rehabilitative training onset (PostOp week 2, $*p < 0.05$). Ceftriaxone also hindered performance of the rehabilitative tray reaching task as measured by the average number of pellets successfully retrieved per min during the rehabilitative tray reaching sessions (C). There was a significant interaction effect of treatment group by time ($p < 0.05$) but *post hoc* group comparisons at individual time points were nonsignificant. Each week is the mean of 5 days of tray reaching performance. VEH, vehicle ($n=5$); CEF, ceftriaxone ($n=5$).

Fig. 3.

Ceftriaxone injections beginning 3 days after ischemic lesions did not affect lesion size and placement. (A) A representative Nissl-stained coronal section showing an ischemic lesion in the right hemisphere. Scale bar = 1mm. (B) There were no effects on lesion size as defined as the volume difference between cortices (contra- minus ipsilateral cortex to lesion). (C) Lesion reconstructions on schematic coronal sections. Numbers are coordinates in mm relative to bregma. Lesion reconstructions are overlaid so that the darker color indicates greater overlap of lesion territory between animals. VEH, vehicle (n=5); CEF, ceftriaxone (n=5).