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Ceftriaxone, a GLT-1 transporter activator, disrupts hippocampal learning in rats

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Abstract

Glutamate transporters (GluTs) are important for maintaining optimal glutamate concentrations at the synapse. This allows proper synaptic response, plasticity and prevents neurotoxicity. It has been shown that the β -lactam antibiotic ceftriaxone (Rocephin) induces an up-regulation of the glutamate transporter GLT-1. This GLT-1 up-regulation blocks the metabotropic glutamate receptor (mGluR) dependent long-term depression (LTD) at the mossy fiber (MF)-CA3 hippocampal synapse. It also has negative effects on long-term potentiation (LTP). However, the effects of GLT-1 up-regulation on hippocampal learning in rats are not known. In this study, we examine the role of chronic administration of ceftriaxone on novel object recognition, which is a hippocampal-dependent spatial learning task. Male Sprague Dawley rats (2–3 months old) were administered ceftriaxone (via i.p. injections, 200 mg/kg) for 8 consecutive days prior to training and testing on a standard novel object recognition task. We found that rats administered ceftriaxone display memory impairments in novel object recognition, when compared to control rats ($p < 0.05$). Our findings show that a potential up-regulation of GLT-1 via ceftriaxone administration has detrimental effects on spatial learning and memory in rats. Our results further support the notion that glutamate transporters provide an essential regulatory role in hippocampal learning and memory.

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Keywords

ceftriaxone; GLT-1; hippocampus; learning; memory

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1. Introduction

Glutamate transporters (GluTs) are important for maintaining optimal glutamate concentrations at the synapse, allowing proper synaptic response, plasticity, and prevention of neurotoxicity. Deletion of glutamate transporter-1 (GLT-1) appears to exacerbate the deposition of β -amyloid plaques in animal models of Alzheimer's disease (Mookherjee, Green et al. 2011). It has been shown that the β -lactam antibiotic ceftriaxone (Rocephin) induces an up-regulation of GLT-1 (Rothstein, Patel et al. 2005), disrupting mGluR2-mediated long-term depression (Omrani, Melone et al. 2009). Previous work from our lab showed that GluTs are differentially expressed during different stages of a spatial learning task (Fratice-Torres, Matos-Ocasio et al. 2010), with a significant down-regulation of GluTs during the early stages of a Morris Water Maze task. Also, GLT-1 modulation affects different disorder models, in which a down-regulation of the transporter (Huntington's disease) (Miller, Dorner et al. 2008; Sari, Prieto et al. 2010) or an excess glutamate release (drug addiction relapse) (Sari, Smith et al. 2009) is the cause. GLT-1 up-regulation also blocks the mGluR-dependent LTD at the MF-CA3 synapse (Omrani, Melone et al. 2009). However, the behavioral effects of GLT-1 up-regulation on normal rats are not known. In this study, we will assess the effects of chronic administration of ceftriaxone on hippocampal learning, using a novel object recognition (NOR) task. Our findings will provide additional information regarding the effects of GLT-1 up-regulation on hippocampus-dependent memory mechanisms. We hypothesize that a GLT-1 up-regulation, induced by administration of ceftriaxone, will have detrimental effects on normal rats' learning and memory (hippocampal processes). This will further support the notion that fine tuning needs to be maintained for proper synaptic plasticity to occur.

2. Materials and methods

The experiments reported here were performed in accordance with the principles described in the "Guide for the Care and Use of Laboratory Animals", Publication No. DHMS (NIH) 86-23.

2.1 Animals

Male Sprague Dawley rats (approximately 2–3 months old, 280–380g, obtained from Southern Veterinary Services, PSMHS, PR) were housed in pairs on a 12 hour light-dark cycle. Rats were randomly assigned to a group (control or experimental), but there was one of each group per cage. Food and water was provided *ad libitum*. To reduce stress-mediated effects on behavior, animals were handled for five days prior the novel object recognition (NOR) task. All experimental protocols were approved by the Institutional Animal Care and Use Committee at Ponce School of Medicine and Health Sciences.

2.2 Drugs

We used the β -lactam antibiotic ceftriaxone (NDC 63323-345-10, APP Pharmaceuticals, LLC, Schaumburg, IL 60173), which is known to cause a GLT-1 up-regulation. The drug was diluted in 0.9% sodium chloride (saline) and administered intraperitoneally (IP) for eight consecutive days, once a day, using a dosage of 200mg/kg (Rothstein, Patel et al. 2005), prior to training and testing on a standard novel object recognition (NOR) task to measure cognition.

2.3 Novel object recognition task

The Novel Object Recognition (NOR) task is used to evaluate cognition, particularly recognition memory, in rodents (Ennaceur and Delacour 1988). Rodents have an innate tendency to spend more time exploring a novel object than a familiar one (Ennaceur and Delacour 1988; Antunes and Biala 2012). The choice to explore the novel object (rather than the familiar one) reflects the use of learning and recognition memory.

Since the inception of the NOR protocol (Ennaceur and Delacour 1988) many modifications to the original protocol have occurred (Hale and Good 2005; Williams, Herring et al. 2007; Ennaceur 2010; Oliveira, Hawk et al. 2010; Antunes and Biala 2012; Heyser and Chemero 2012). Our NOR test occurred in an open field arena (16" $\times$ 19", Fig. 1) placed in a sound attenuated room, with a masking white noise of approximately 70 dB above human threshold (Ennaceur and Delacour 1988). Illumination consisted of two 60w light bulbs, which corresponds with lighting used in other NOR experiments (Clark, Zola et al. 2000). The bulbs (lamps) were covered with dark plastic bags to diffuse the light and avoid light reflection on the camera. After each trial, the open field arena and objects were cleaned using 70% ethanol to avoid any preference due to scent.

During habituation, the animals were allowed to explore an empty arena. Twenty-four hours after habituation, the animals were exposed to the familiar arena with three identical objects (3.7" Nylon bones). The next day, the rats were allowed to explore the open field in the presence of two familiar objects and a novel object (50 ml vial) to test long-term recognition memory (retention memory) (Taglialatela, Hogan et al. 2009). The time spent exploring each object and the discrimination index percentage were recorded for each trial. Each trial was recorded using a ceiling mounted video camera connected to a computerized tracking/imaging analyzer system (EthoVision XT, v.8.5.614, Noldus Information Technology) (Benice and Raber 2008).

2.4 Experimental design

Rats were divided into groups, either ceftriaxone or saline. Drugs were administered as previously described for eight consecutive days. As seen in Figure 1, during those eight days, the first five consisted of handling and the last three of habituation. This was done to reduce any possible behavioral effects due to stress. On day nine (Acclimation) rats were exposed to three identical objects (3.7" Nylon bones) attached to the walls of the box for three trials (1 minute duration per trial) with a one hour intertrial interval. On day ten (Novel Object Recognition), one of the objects was changed to a new one (50ml vial) and the exploration time was measured. After the last trial each rat was deeply anesthetized with

sodium Nembutal solution (100 mg/kg; ip) and euthanized by decapitation. The brain was removed and stored for further processing.

2.5 Statistical analysis

Statistical analysis was performed using GraphPad Software (Prism v.5.02). Two-tailed t-test or two-way analysis of variance (ANOVA) followed by a Bonferroni posttest were performed and a $p < 0.05$ was used for an acceptable significant difference. Data was presented as mean $\pm$ SEM.

3. Results

3.1 Ceftriaxone treated rats showed less anxiety than control rats

Our studies show that rats treated with ceftriaxone spent significantly less time close to the walls and more time in the center of the box when compared to controls [$F(1,46) = 1269$, $p < 0.01$] (Fig. 2.a). On an open field arena, rats that spend more time close to the walls and less time crossing the center are the most anxious or stress.

3.2 Rats showed impairment in learning after ceftriaxone administration

We used the novel object recognition task to test the rat's learning and memory. During habituation both the control and ceftriaxone rats did not show any preference for a particular quadrant [$F(3,92) = 2.462$, $p > 0.05$] (Fig 2.b). During acclimation both groups spent equals amounts of time exploring each object, meaning that there was no preference [$F(2,69) = 0.073$, ns] (Fig. 3). While both groups manage to recognize the new object (Fig. 4.b), ceftriaxone treated rats showed an impairment when compared to controls [$F(2,69) = 80.74$, $p < 0.05$]. During the novel object recognition phase, the ceftriaxone group seems to spend less time exploring when compared to controls but this difference was non-significant [$t(23) = 1.418$, ns] (Fig. 4.a).

4. Discussion

Our results clearly support the notion that glutamate transporters play an important role in the mechanisms underlying hippocampal learning in rats.

Our initial finding is that chronic exposure to ceftriaxone appears to result in less anxious behavior when compared to controls, given that ceftriaxone treated rats spent significantly less time close to the walls of the open arena. Typically, rats prefer closed environments rather than open ones, and how much time they spend close to the walls during an open field task is routinely used as a measurement for anxiety. Interestingly, previous work has shown that reduced expression of the vesicular glutamate transporter-1 (VGLUT1) enhanced anxiety, depressive-like behavior and impaired recognition memory in mice (Tordera, Totterdell et al. 2007). Also, mice lacking the VGLUT1 have been proposed as a genetic model of deficient glutamate function linked to depressive-like behavior (Tordera, Garcia-Garcia et al. 2011). These results clearly indicate that reductions in glutamate transporter expression affect the behavioral presentation of anxiety and depressive-like behavior in rodents. Given that ceftriaxone typically results in increased GLT-1 expression, it is

probable that such increases account for the reduction in anxiety-like behavior observed in our ceftriaxone treated rats.

Second, rats that received ceftriaxone seem to spend less time exploring when compared to controls. This finding could be important given the frequency with which ceftriaxone is administered clinically. In fact, recent work shows that astrocytic clearance and destination of glutamate in the synaptic cleft might be altered in some of the clinical symptoms of autism, such as social interaction, and repetitive and stereotyped behaviors, all of which involve several areas of the central nervous system (CNS), including hippocampus (Bristol Silvestrin, Bambini-Junior et al. 2013). Alterations in glutamate transporter modulation (as induced by ceftriaxone administration) could potentially account for the apparent reduced lack of exploration observed in our rats.

Our main finding is that ceftriaxone-treated rats display a significant impairment in novel object recognition, which is consistent with a hippocampal deficit. It has been shown that ceftriaxone induces an up-regulation of the GluT GLT-1 (Rothstein, Patel et al. 2005), and this up-regulation may be detrimental under normal neuronal activity. In fact, other studies have shown that protein synthesis inhibitor antibiotics produce amnesia, perhaps by inhibiting general or global mRNA translation. For example, the antibiotic anisomycin interferes with protein synthesis by inhibiting peptidyl transferase, resulting in deficits in hippocampal memory consolidation that could be chemically reversed (Jiang, Belforte et al. 2010). Also, hippocampal glutamate levels and glutamate aspartate transporter (GLAST) expression were shown to be up-regulated in rats displaying isoflurane-induced spatial learning/memory impairments (Qu, Xu et al. 2013). Chronic administration of ceftriaxone disrupted skill learning and motor functional outcome after ischemic cortical damage in rats (Kim and Jones 2013). In addition, the combination of MK-801 and ceftriaxone impairs the acquisition and reinstatement of morphine-induced conditioned place preference, and delays morphine extinction in rats (Fan, Niu et al. 2012). These findings suggest that, although ceftriaxone may be extremely effective when administered acutely, caution must be taken as to the potential implications of chronic administration.

Interestingly, another study (Karaman, Kizilay-Ozfidan et al. 2013) found that ceftriaxone administered to mice did not impair spatial memory, using a water maze task. These contrasting results are not surprising, given the well documented inter-species differences between mice and rats (Whishaw 1995; Whishaw and Tomie 1996; Stranahan 2011). The Karaman et al. (2013) study used a Morris water maze task, instead of the Novel Object recognition task used in the present experiments. Clear anatomical differences in hippocampal volume exist between mice and rats, and more subtle differences in intracortical connectivity have also been shown (van Groen, Kadish et al. 2002). Also, rats and mice show species differences in some water maze testing paradigms, but perform comparably on dry land mazes, such as the radial arm maze (Whishaw and Tomie 1996). Hence, inherent differences in the tasks tested (dry versus water) could also account for the conflicting results.

Overall, our results further support the notion that glutamate transporters provide an essential regulatory role in hippocampal learning and memory.

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Highlights

- We study the effects of chronic administration of ceftriaxone on learning.
- We test male Sprague Dawley rats on a novel object recognition task.
- Ceftriaxone treated rats showed less anxiety than control rats.
- Rats showed impairment in learning after ceftriaxone administration.

Fig. 1. Experimental Protocol

A schematic description of the NOR protocol used for these experiments. The open field arena (plastic container) was 16" × 19" and the center area was 13" × 16".

Fig. 2. Cef rats display less anxious-like behavior than controls during habituation

(a) Cef rats spent less time close to the walls (index of thigmotaxis) when compared to controls, which indicates that they were less anxious. (b) None of the groups showed a significant preference for any of the quadrant. (* $p < 0.01$)

Fig. 3.

No differences were noted between groups during acclimation. None of the groups showed a significant preference for any of the objects. All objects were the same (a cross made out of two 3.7" Nylon Bones). (Obj A on Q2; Obj B on Q3; Obj C on Q4).

Fig. 4. Ceftriaxone-treated rats display moderately impaired novel object recognition. (a) Total exploration during the 3min trial. (b) Cef rats showed impaired learning compared to controls. They spent significantly less time exploring the new object (Obj D, a 50ml Vial) than controls. (* $p < 0.05$)