COMMITTEE CERTIFICATION OF APPROVED VERSION

The committee for Song Liu certifies that this is the approved version of the following dissertation:

ROLES OF INTERLEUKIN-1 BETA IN GLUTAMATE-INDUCED SPINAL CORD INJURY

Committee:

David J. McAdoo (Supervisor)

Ping Wu

Raymond J. Grill

Allan R. Brasier

Alexander Kurosky

Dean, Graduate School

ROLES OF INTERLEUKIN-1 BETA IN GLUTAMATE-INDUCED SPINAL CORD INJURY

by
Song Liu, M.D.

Dissertation
Presented to the Faculty of The University of Texas Graduate School of Biomedical Sciences at Galveston
in Partial Fulfillment of the Requirements
for the Degree of

DOCTOR OF PHILOSOPHY

Approved by the Supervisory Committee

David J. McAdoo, Ph. D.
Ping Wu, M. D., Ph. D.
Raymond J. Grill, Ph. D.
Allan R. Brasier, M. D.
Alexander Kurosky, Ph. D.

November, 2006
Galveston, Texas

Key words: glutamate, IL-1β, IL-1ra, spinal cord injury, MAPKs

To my husband, my parents, my sister and brother-in-law and all my friends.

ACKNOWLEDGMENTS

I extend my sincerest gratitude to my mentor Dr. David J. McAdoo, for sharing his ideas, knowledge and experience with me all of the time. Without his patience, encouragement and support, it would have been impossible for me to finish my project. I also thank the members in my supervisory committee: Dr. Ping Wu, Dr. Raymond J. Grill, Dr. Allan R. Brasier and Dr. Alexander Kurosky, for sharing their minds and time with me.

I would like to thank all of my co-wokers: Dr. Claire E. Hulsebosch, Dr. Guo-Ying Xu, Dr. Linghui Nie, Dr. Lisa Cain, Dr. Clement O. Echetebu, Mike Hughes, Dr. Charles Hudson, Kathia Johnson, Dr. Zaiming Ye and Geda C. Unabia. They all lent their hands to me when I needed them, and their support was so important in my project. I also express my gratitude to Debbie A. Pavlu, who provided secretary assistance to me.

I also thank the graduate program director of the Department of Biochemistry and Molecular Biology, Dr. Lillian L. Chan and program coordinator, Ms. Debora M. Botting, for all the things they did for me.

Thanks to my family are always too much to say in words. Thanks for my parents to bring me to this world and then give me such a great care. They always support me when I am weak. Thanks to my sister and brother-in-law for sharing my tears when I am frustrated and standing by my side whenever I need them.

Thanks to all my friends in China and U.S. They gave me a wonderful time which is an unforgettable memory.

Last but not least, I would like to thank my husband for his company and support.

ROLES OF INTERLEUKIN-1 BETA IN GLUTAMATE-INDUCED SPINAL CORD INJURY

Publication No._____

Song Liu, Ph. D
The University of Texas Graduate School of Biomedical Sciences at Galveston, 2006

Supervisor: David. J. McAdoo

Glutamate release contributes to the impairments caused by spinal cord injury (SCI). This study addresses the mechanisms of glutamate toxicity involving activation of interleukin-1β (IL-1β). To assess the effects of glutamate on IL-1β and its natural blocking agent interleukin-1 receptor antagonist (IL-1ra), ELISA assays were used to measure the responses of endogenous IL-1β and IL-1ra to glutamate administered to the spinal cord. Levels of activated IL-1β and IL-1ra changed in a reciprocal fashion starting 1 hour after glutamate exposure. Exposure to glutamate initially increases IL-1β expression while it decreases IL-1ra. IL-1β then decreases and IL-1ra increases. IL-1β and IL-1ra change reciprocally in the same fashion in a contused spinal cord. To check whether this mutual effect is due to actions of IL-1β and IL-1ra on each other, IL-1β was applied on the spinal cord and then IL-1ra was measured. IL-1ra was applied onto the cord and IL-1β was measured as well. The results show that administration of IL-1β stimulates the production of IL-1ra and administration of IL-1ra suppresses the activation of IL-1β. To identify subtypes of glutamate receptors involved in this phenomenon, NMDA and AMPA receptor agonists were separately applied to the spinal cord and IL-1β and IL-1ra expression in the cord were measured with ELISA assays. Activation of both the AMPA and the NMDA receptors also induced reciprocal changes between the IL-1β and IL-1ra levels. To determine the effects of activating AMPA and NMDA receptors on IL-1β and IL-1ra, MK801 and NBQX were applied individually on the spinal cord with glutamate. The results show that both AMPA and NMDA receptors are involved in glutamate-induced reciprocity between IL-1β and IL-1ra. To determine if this

reciprocity between the expression of IL-1β and IL-1ra affected post-SCI locomotor function, recombinant IL-1β and IL-1ra were administered to glutamate-exposed spinal cords. The Basso-Beattie-Bresnahan (BBB) test of functional recovery demonstrated that IL-1β impaired rat locomotive ability and that IL-1ra improved the recovery of the rats from glutamate-induced locomotor impairment. To explore the mechanism of this IL-1β involvement in excitotoxicity, MAPK activities responding to glutamate were measured and the results showed that both ERK1/2 and p38 are involved in IL-1β induced SCI. Cell death assays showed that apoptosis is caused by glutamate-induced SCI. Overall, we established the following pathway from spinal cord injury to functional impairment: SCI → glutamate release → IL-1β and IL-1ra changes → ERK1/2 and p38 activation → cell death → functional impairment. This is the first pathway traced from glutamate release to functional impairments.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF ABBREVIATIONS

AD	Alzheimer's disease
AMPA	Amino-3-hydroxy-5-methyl-4-isoxazolepropanic acid
ANOVA	Analysis of variance
AP-1	Activator protein-1
ASK-1	Apoptosis signal-regulating kinase 1
ATP	Adenosine triphosphate
BBB	Basso, Beattie and Bresnahan
CNS	Central nervous system
COX-2	Cyclooxygenase-2
DTT	Dithiothreitol
EAA	Excitatory amino acids
IH	Infinite Horizons
IL-1	Interleukin-1
IL-1ra	Interleukin-1 receptor antagonist
IL-1RAcP	IL-1 receptor accessory protein
IL-1R1	Type I IL-1 receptor
IL-1R2	Type II IL-1 receptor
MAPK	Mitogen-activated protein kinase
MEK	MAPK kinase
MKK	MAP kinase kinase
MNK	MAPK-interacting kinases
MP	Methylprednisolone
NASCIS	National Acute Spinal Cord Injury Studies
NF-κB	Nuclear Factor-κB
NMDA	N-methyl-D-aspartate
NOS	Nitric oxide synthase
PG	Prostaglandins
PRAK	P38-regulated kinase
RT	Room temperature
SCI	Spinal cord injury
SEM	Standard error of the mean
TAK-1	TGFβ-activated kinase 1

CHAPTER 1: INTRODUCTION

About 12,000 to 15,000 spinal cord injuries (SCI) occur per year in the United States. More than 50% of the victums are permanently paralyzed, and many of them die as a result of their spinal cord injuries (Data from National Spinal Cord Injury Association). Survivors suffer permanent locomotion impairment, bladder problems and respiratory system dysfunctions. Most of the cases occur in male, young and healthy people. The injuries are classified as complete injury and incomplete injury. A complete injury means that there is no function below the level of the injury, no sensation and no voluntary movement. Both sides of the body are equally affected. An incomplete injury leaves some function below the primary level of the injury. Both incomplete and complete injuries need emergency care and long term treatment. Surgery may be essential to remove the bone fragments, tissue or fluid from around the spinal cord. Spinal traction is important to reduce dislocation and can be used to immobilize the spine. Long term physical and occupational therapy may be needed to help the patients to cope with the disability caused by the spinal cord injury. The drugs that can be used to treat spinal cord injury are still limited in number and effectiveness. Corticosteroids, such as dexamethasone or methylprednisolone, are used to reduce swelling that may damage the spinal cord. The National Acute Spinal Cord Injury Studies (NASCIS I, II and III), large and randomly designed clinical trials to study the effect of steroid administration in patients with acute spinal cord injury, demonstrated the effectiveness if steroids when administered less than 8 hour post trauma (Bracken et al., 1984; Bracken et al., 1985; Bracken et al., 1990; Bracken et al., 1992; Bracken et al., 1997, Bracken et al., 1998), provided conflicting results regarding the effectiveness of methylprednisolone.

Glutamate is a major central nervous system (CNS) neurotransmitter. Extensive studies show that glutamate is involved in spinal cord injury (SCI), although the mechanisms are incompletely explored. Studies demonstrated that actions of glutamate cause cell death, inflammation and excitoxity. The goal of this study is to explore

pathways from glutamate to apoptosis and functional deficits through interleukin-1 beta (IL-1β) and mitogen activated protein kinases (MAPKs).

PATHOPHYSIOLOGY OF SPINAL CORD INJURY

To find better treatments for SCI, it is necessary to understand its pathophysiology. There are four general types of SCI (Hulsebosch et al., 2002): 1) spinal cord maceration, in which the cord is totally destroyed, 2) spinal cord laceration, which can be caused by gunshot or knife wound, 3) cord contusion, which is responsible for a major part of human spinal cord injury, and 4) solid spinal cord injury. The first two types of spinal cord injury are usually more severe than the last two types because an entire cross section of the cord is destroyed (Bunge et al., 1993; Bunge et al., 1997). In each category, the degree of damage determines the outcome of SCI. Generally speaking, the recovery from incomplete injury can be significant, but there is none from complete injury.

There are three phases of the response to SCI after the initial injury: acute, secondary and chronic stages. In the acute phase, which happens from the moment of injury to a couple of days later, mechanical and chemical damage to the spinal cord and surrounding soft tissues causes apoptosis and necrosis of cells and edema of the surrounding tissue. Secondary injury takes place over minutes to weeks after SCI. Many secondary processes exacerbate the initial damage: glutamate release is substantial for the first hour, vascular defects lead to hypoxia, and energy is depleted due to the failure of ATP regeneration, and calcium influx, which mediates excitotoxicity and production of free radicals. All of these responses trigger inflammation and cell death, which lead to further tissue loss. The chronic phase starts days after injury and lasts for years. It is characterized by ongoing demyelination, necrosis and apoptosis of cells in the spinal cord and brain (Hulsebosch et al., 2002; Klussmann et al., 2005).

Even in the best medical circumstances, getting from the time and place of injury to the medication takes 1 hour or more. In addition, cell death of the affected neurons and tissues is immediate. So the acute phase of SCI is not an ideal target to treat. Secondary

damage happens over hours to days, quite a feasible time to give treatment. Because of the importance of secondary damage in clinical outcomes and its potential as targets for treatment, secondary damage has been widely studied during the last decades.

As we discussed before, secondary damage happens minutes to weeks after SCI. In the 1970's, people believed that free radicals were the leading factors causing secondary damage (Milvy et al., 1973; Demopoulos et al., 1980). In the 1980's scientists started to focus on calcium influx and lipid peroxidation (Hall et al., 1986; 1988; Young et al., 1992). From the 1990's, glutamate excitotoxicity was widely studied and showed significant involvement in secondary damage (Xu et al., 1998; 2000; McAdoo et al., 2000; 2005). Astrocytes release adenosine triphosphate (ATP) upon mechanical injury. The released ATP binds to P2X7 purine receptors and triggers high-frequency spiking, which leads to the influx of calcium into the cytosol. Following the increase in cytosolic calcium within the first several minutes after the initial injury, the extracellular concentrations of glutamate and other excitatory amino acids reach cytotoxic concentrations as a result of cell lysis and cell death resulting from mechanical damage and synaptic and non-synaptic released and transport processes. This elevation of the glutamate concentration also triggers more calcium release, lipid peroxidation and free-radical production. Neutrophil infiltration occurs within 24 hours after initial injury, which is a signal of inflammation. At the same time, inflammatory responses are evoked, and IL-1 and TNF-α expression and activation are elevated (Nesic et al., 2001).

Several studies link IL-1 to spinal cord injury, but the mechanism of how that occurs is not clear (Gelderd et al., 1996; Hayashi et al., 1997; Wang et al., 1997; Klusman et al., 1997; Tonai et al., 1999; Lescovar et al., 2000; Nesic et al., 2001; Pan et al., 2002; Kenney et al., 2002; Tonai et al., 2002; Yang et al., 2004; Perrin et al., 2005; Fu et al., 2005; Wang et al., 2005; Wang et al., 2006). In this study I explored the hypotheses that IL-1β activation after spinal cord injury (SCI) is a contributor to glutamate-induced excitotoxicity and that IL-1β dependent activation of MAPKs also contributes to glutamate-induced SCI.

MOLECULAR TARGETS AND STRATEGIES IN SCI

Based on the molecular events happening after SCI, possible targets for treating SCI can be classified as follows: energy failure, calcium influx, free radical production, lipid peroxidation, glutamate excitotoxicity, inflammation and apoptosis. Strategies to treat SCI have been devised by targeting these molecular events. However, only methylprednisolone treatment has reached routine clinical application.

ATP depletion, calcium influx and free radicals

Upon traumatic injury, released ATP binds to the P2X7 receptor and increases calcium influx and excitotoxic cell death. Blockage of the P2X7 receptor in a rat model with an antagonist (PPADS) decreased cell death in the injured cord and improved function significantly (Wang et al., 2004).

Disruption of calcium homeostasis after injury causes defects in mitochondrial respiration electron transport. Reactive oxygen species and nitric oxide (NO) are the major types of free radicals present post-SCI. Different free radical scavengers have been tested, and among them Vitamin E decreased damage after SCI (Bozbuga et al., 1998). Targeting nitric oxide synthase (NOS) decreased tissue loss (Pearse et al., 2003). Similar effects were observed in mice deficient in neuronal NOS (Farooque et al., 2001).

Hemorrhage, edema and thrombosis occurring after SCI cause local ischemia in the lesion area. Insufficient oxygen supply prevents the further production of ATP. As a result, excess adenosine is released and binds to adenosine receptors. Treating ischemia in a rabbit with ATL-146e, an adenosine A2A receptor analogue, increased neuronal survival and improved function (Cassada et al., 2002).

The depletion of ATP allows collapse of the essential cation concentration gradients. One of the critical outcomes is the influx of calcium. Several studies with calcium channel blockers showed protective effects after SCI (Guha et al., 1987; Black et al., 1988; Guha et al., 1989; He et al., 1991; Anghelescu et al., 1994; Du et al., 1999; Haller et al., 2003; Winkler et al., 2003; Levendoglu et al., 2004; Brand-Schieber et al., 2004).

Glutamate excitotoxicity

Triggered by calcium influx and cell rupture following SCI, the extracellular glutamate concentration reaches a toxic level. A variety of glutamate receptor antagonists have been used to help recovery after spinal cord injury. N-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and the kainate receptor have been widely studied. Both NMDA receptor antagonists (MK801) and AMPA receptor antagonists (NBQX) have shown beneficial effects on animal models of SCI (Wrathall et al., 1994; 1997; Kocaeli et al., 2005; Li et al., 1999; Yoshiyama et al., 1999; Wada et al., 1999; Haghighi et al., 1996; Hao et al., 1992; Mu et al., 2002; Liu et al., 1997; Teng et al., 1996).

Inflammation

Inflammation is a component of secondary injury. Methylprednisolone (MP) is approved clinically to treat for inflammation and edema. It is effective against SCI when administered in a high dose within the first eight hours after initial injury. Many pro-inflammatory cytokines, such as TNF-α, IL-1β and IL-6, increase within several hours after initial injury. Neutralization of TNF-α strongly reduces neutrophil infiltration and decreases apoptosis (Martin-Villalba et al., 2001). Blocking the IL-1 receptor prevents inflammation (Nesic et al., 2001). Systemic administration of anti-inflammatory IL-10 reduced tissue damage (Bethea et al., 1999). Prostaglandins (PGs) and their derivatives are another group of mediators of inflammation. Cyclooxygenase-2 (COX)-2 is responsible for the synthesis and regulation of PGs. Application of COX-2 inhibitors reduces pain and improves functional recovery in SCI animal models (Hains et al., 2001; Resnick et al., 1998; Yamamoto et al., 1996).

Apoptosis

Within hours and days after initial injury, neurons and glia at the site of an SCI lesion undergo apoptosis. The cord 6mm away from the lesion center (both rostral and caudal sides) undergoes apoptosis as well (Xu, et al., 2006). Caspases, a family of

cysteine-aspartate proteases, are major participants in apoptosis. Caspase-3 is activated after SCI and leads to apoptosis. Caspase inhibitors are neuroprotective in a SCI animal model (Nicholson et al., 2000). Calpains are calcium-dependent cysteine proteases which calcium influx activates after SCI. Several calpain inhibitors have been tested on SCI animal models and showed neuro-protective effects (Ray et al., 2003; Schumacher et al., 2000). Bcl-2 and Bax family members are another group of proteins that is involved in apoptosis after SCI. The ratio between Bcl-2 and Bax is critical to outcome following SCI. Overexpression of the anti-apoptotic Bcl-2 gene decreased tissue damage after SCI (Saavedra et al., 2000; Seki et al., 2003). Inhibition of the pro-apoptotic Bax decreased oligodendrocyte death after SCI (Dong et al., 2003).

The losses of function after SCI are partly due to cell loss. Therefore cell replacement strategies may regenerate loss of spinal cord function caused by SCI. A variety of tissue and cells have been implanted into injured spinal cords, including stem cells, olfactory ensheathing cells, Schwann cells, dorsal root ganglia, adrenal tissue, hybridomas, peripheral nerves to improve outcomes (Hulsebosch et al., 2002; Jones et al., 2001; Zompa EA et al., 1997; Whittemore et al., 2002; Gao et al., 2005). These trials showed that some patients had improved recovery (Zompa et al., 1997).

Taken together, many studies are going on to clarify the mechanisms of spinal cord injury in order to find better treatments for it; the treatments are quite limited now.

INTERLEUKIN-1 AND SPINAL CORD INJURY

General review

As we described above, inflammation plays a very important role in secondary injury. Although inflammation is usually a defense mechanism after insult, most of the inflammatory cascade-producing pathways are degradative. Activation of interleukins is one of the characteristics of inflammation. Although the use of an IL-1β inhibitor on SCI is beneficial (Nesic et al., 2001), the mechanism of that protection is still not clear.

Inflammation accompanying SCI is characterized by infiltration of circulating immune cells, which can express, release and respond to pro-inflammatory mediators,

such as interleukins and complement. In the CNS, one of the most widely studied mediators of inflammation is the pro-inflammatory cytokine IL-1β.

The IL-1 family has an important role in inflammation and host defense in many inflammatory diseases (Arend et al., 1998). IL-1 was discovered 50 years ago and was originally described as the "endogenous pyrogen" because of its fever-inducing properties. There are two IL-1 ligands, IL-1α and IL-1β. These two ligands share about 60% sequence homology, although they are transcribed by different genes, and their distributions in tissues and organs are different. There are many IL-1 producing cells, including lymphocytes and monocytes. Although transcribed by different genes, IL-1α and IL-1β have similar biological effects. Both IL-1α and IL-1β are produced as pro-forms. Pro-IL-1α is cleaved by calpain to generate a smaller mature protein. Pro-IL-1β (34kd) is similarly cleaved by caspase-1 to generate a smaller mature protein (17kd).

IL-1β is the major form of IL-1 in the CNS. There are two types of IL-1 receptors, type I IL-1 receptor (IL-1R1) and type II IL-1 receptor (IL-1R2). IL-1β binds to the membrane-bound IL-1R1, which is associated with the IL-1 receptor accessory protein (IL-1RAcP), to form a complex, and then triggers intracellular signals. IL-1 can bind to IL-1R2 as well. However, IL-1R2 lacks an intracellular-signaling domain, so no signaling can be triggered when IL-1 binds to IL-1R2. So IL-1R2 is called a decoy IL-1 receptor. All three receptor molecules, IL-1R1, IL-1R2 and IL-1RAcP, can be shed from the cell membrane and exist as soluble forms. Most studies showed that sIL-R1 also functions as a decoy receptor. sIL-1R2 and sIL-1 RAcP both inhibit IL-1 mediated signal transduction. sIL-1R2 can associate with IL-1RAcP to prevent the formation of the IL-1-IL-1R1-IL-1RAcP complex (Subramaniam et al., 2004).

There is another endogenous IL-1 receptor ligand, IL-1ra, which is a natural antagonist of the IL-1 receptor. IL-1ra binds to the IL-1R1 receptor competitively with IL-1α or IL-1β, but does not trigger signal transduction.

Regulation of IL-1

All IL-1 group members are produced under physiological conditions. The balance between each member is finely regulated to maintain normal physiological activities. There are multiple levels of regulation of IL-1: transcription, translation and cleavage activation. A variety of stimuli can affect these processes and the expression of IL-1, both at the mRNA and the protein level.

The expression of genes that encode IL-1α and IL-1β is induced by many pro-inflammatory stimuli, injury and other cytokines. The promoter region of the gene that encodes IL-1β has a TATA box to which transcription factors can bind. In addition, the promoter region of the gene that encodes IL-1β has a CAAT box, which controls the efficiency of transcription initiation. The transcription efficiency of IL-1 is controlled by many transcription factors, such as cyclic AMP response element, the activator protein-1 (AP-1) and NF-kB. Several other stimuli can accelerate the transcription of IL-1, such as complement components and prostaglandin E. To finely tune the transcription of IL-1, inhibitors of transcription are essential as well. Glucocorticoid and lipocortin-1 play inhibitory roles in IL-1 transcription, which are possibly neuroprotective in the CNS (Cover et al., 2002; Philip et al., 2001; Newman et al., 1994).

To produce signaling by means of IL-1, regulation of post-transcription and translational is essential. The production of IL-1 can be regulated positively or negatively. For example, translation of IL-1 can also be increased by epidermal growth factor, ICAM1 and corticotrophin-releasing hormone. Dexamethasone can regulate IL-1 production negatively.

The release of mature IL-1 requires the cleavage of the pro-form, so the factors that can affect the cleavage process can regulate the release of IL-1 as well. Caspase-1 is responsible for the cleavage of pro-IL-1β, so the activity of caspase-1 is important in IL-1β release. For example, cytoplasmic calcium ionophores increase potassium efflux through the purinergic ligand-gated ion channel P2X7, which leads to the cleavage of pro-caspase-1, which is essential for the maturation and activation of caspase-1.

IL-1 in neurotrauma

Expression of IL-1 in the central nervous system is detectable under normal physiological conditions. IL-1β is the dominant form of IL-1 compared to IL-1α in the CNS. IL-1β expression is quickly elevated following CNS insults, such as trauma, ischemia and oxidative damage. mRNA of IL-1β increases in minutes and the level of IL-1β is increased in the hours after trauma (Nesic et al., 2001; Vezzani et al., 1999; Streit et al., 1998; Bartholdi et al., 1997; Eriksson et al., 1998; Friedlander et al., 1997; 1996; Hamada et al., 1996; Hopkins et al., 1995; Klusman et al., 1997, Wang et al., 1997; Zhang et al., 1998). Many types of cells produce IL-1β, including neurons, oligodendocytes, microglia and astrocytes (Yang et al., 2004). Invading immune cells are also important to the late expression of IL-1β in neurotraumas.

Although most studies show that the administration of IL-1β itself into healthy rat brains does not cause brain damage (Allan et al., 2005), a recent study showed that the administration of IL-1β into rat brains killed dopamine-producing neurons. Co-administration of IL-1β with other insulting agents led to a greater damage to the CNS. Co-administration of IL-1β with other cytokines showed synergistic effects that led to a greater degree of neurotoxicity (Gadient et al., 1990). Furthermore, administration of IL-1β with traumatic or ischemic stimuli led to a greater degree of damage compared with traumatic or ischemic stimuli alone. Study of the inhibition of IL-1β in the CNS has added evidence regarding the role of IL-1β in the CNS. IL-1ra, a natural antagonist to the IL-1 receptor, was used to study the effect of IL-1 (Nesic et al., 2001; Lawrence et al., 1998; Loddick et al., 1996; Lundkvist et al., 1999; Martin et al., 1994; Panegyres et al., 1998; Toulmond et al., 1995; Plata-Salaman et al., 1995; Relton et al., 1996; Sanderson et al., 1999). The studies showed that the administration of IL-1ra after ischemia or trauma in the CNS markedly protects against trauma or ischemia. The administration of IL-1ra protected the CNS in NMDA-induced damage. The administration of exogenous or over-expression of endogenous IL-1ra markedly reduced neuronal injuries caused by ischemia or excitotoxin-induced CNS damage (Oprica et al., 2004). IL-1ra limits epileptic seizures

in rodents and brain injury induced by heat stroke in rabbits (Vezzani et al., 2004). These effects of IL-1ra strongly support the mediation of damage of neuronal insults by IL-1β.

Besides IL-1 and IL-1ra administration, the usage of antibodies that neutralize IL-1β protected the CNS as well. The inhibition of IL-1β cleavage, such as by inhibition of caspase-1, protected against trauma as well (Hara et al., 1997; Schielke et al., 1998).

MAPKS, GLUTAMATE AND SPINAL CORD INJURY

General review

The release of excitatory amino acids (EAA) after SCI activates glutamate receptors, which is followed by activation of protein kinase A, protein kinase C, calmodulin kinase and adenylyl cyclase. The activation of these cascades leads to the activation of MAPKs (Ji et al., 2003; Crown et al., 2006). The activation of MAPKs in turn leads to phosphorylation of transcription factors and changes gene expression (Ji et al., 2003).

The MAP kinase pathways are multi-functional pathways that are conserved in all eukaryotic cells. Three MAPKs, ERK, p38 and JNK, have been identified (Takeda et al., 2003; Kyriakis et al., 2001). The activation of each MAPK is a chain reaction. MKK kinase activates MAP kinase kinase (MKK), and the activation of MKK leads to the activation of MAPK kinases (MEKs). The activation of MAPK is a site-specific phosphorylation. The phosphorylation of MAPKs requires MEKs. The MEKs are an evolutionarily conserved family of protein serine/threonine kinases. In the CNS, ERK MAPK is involved in cellular responses to oxidative stress or glutamate receptor activation (Fiore et al., 1993; Rosen et al., 1994; Aikawa et al., 1997). p38 MAPK is associated with signaling pathways that are involved in inflammation (Kyriakis et al., 1996; Herlaar et al., 1999; Mielke et al., 2000; Ono et al., 2000). In addition, ERK1/2 and p38 are activated in contused rat spinal cords (Crown et al., 2006). Our second goal is to study the roles of ERK1/2 and p38 in glutamate-induced spinal cord injury.

ERK1/2 MAPKs

ERK1/2 are serine threonine kinases that are localized in cytoplasm. The activation of ERK1/2 involves dual phosphorylation on both serine and threonine residues (Chen et al., 1992; Sanghera et al., 1992; Seth et al., 1992; Gonzalez et al., 1993; Raingeaud et al., 1995). ERK1/2 is activated by MEK1 and MEK2 (Crews et al., 1992). The activation of MEK1 and MEK2 is regulated by Raf proteins, A-Raf, B-Raf and C-Raf. Raf proteins are phosphorylated by various kinases including Ras, c-Src protein tyrosine kinase and protein kinase C (Sugden et al., 1997). The activation of ERK1/2 is triggered by a series of kinases, and this chain reaction is well-regulated in cells. The activation and inactivation of these pathways are related to multiple cell processes, including proliferation, differentiation and apoptosis.

The two related ERK MAPKs, ERK1 and ERK2, are distributed and expressed at high levels in the brain and spinal cord. Activation of these MAPKs leads to phosphorylation of other protein kinases and transcription factors. The downstream targets of ERK1/2 include many nuclear and cytosol signaling proteins. MAPK-interacting kinases (MNKs) are involved in transcription and translation regulating of the expression of MAPKs. The main transcription factor that is regulated by ERK1/2 MAPKs is Elk1. The activation of Elk1 leads to the activation of the serum response element on the fos promoter, and then the activation of fos leads to target protein synthesis (Marais et al., 1993; Whitmarsh et al., 1995; Kyriakis et al., 2001; Adams et al., 2002).

Activation of ERK1/2 MAPKs is involved in many CNS diseases. Microglial activation of ERK1/2 MAPKs is found in most chronic neurodegenerative diseases, such as Parkinson's disease, Alzheimer's disease (AD) and amyotrophic lateral sclerosis. TNF-α and NO release can be induced by the activation of ERK1/2 MAPKs in AD (McDonald et al., 1998; Pyo et al., 1998; Combs et al., 2001). In CNS injuries, such as stroke, brain injuries and spinal cord injuries, there is evidence that ERK1/2 MAPKs are involved in the inflammatory reaction in response to the injuries. The activation of

ERK1/2 MAPKs mediates inflammatory cytokine production (D'Aversa et al., 2002). Increased activation of ERK1/2 MAPKs was found in almost all kinds of cells in the CNS in areas surrounding cerebral infarcts in patients who died after acute ischemic attack (Slevin et al., 2000). In response to excitotoxic reagents, phosphorylated ERK1/2 MAPKs were detected in neurons and glial cells (Ferrer et al., 2001). ERK 1/2 signaling is involved in nicotine-mediated neuroprotection in spinal cord neurons (Toborek et al., 2006). ERK1/2 MAPKs are activated in excitotoxic spinal cord injury (Yu et al., 2005). A recent study showed directly that contusion of the spinal cord activated ERK1/2 MAPKs (Crown et al., 2006). In this study we focused on the role of ERK1/2 MAPKs in the inflammation caused by glutamate-induced spinal cord injury.

p38 MAPKs

P38 MAPKs have four isoforms that are distributed in different tissues and organs. In the CNS, α and β are the major forms. Activation of p38 MAPKs leads to phosphorylation of their downstream molecules, such as p38-regulated kinase (PRAK). Nuclear targets of p38 MAPKs include many components of the AP-1 complex. In inflammation, p38 MAPKs phosphorylate histone H3, leading to the production of interleukin-8 and monocyte chemoattractant protein 1, which results in NF-γB-induced transcription. Activation of p38 MAPKs has essential roles in induction of IL-1, IL-6, TNF-α and cyclooxygenase-2 (COX-2) (Lee et al., 1994; Ridley et al., 1997; Da Silva et al., 1997; Bhat et al., 1998; Guan et al., 1998 ; Miyazawa et al., 1998).

Similarly to ERK1/2 MAPKs, p38 MAPKs undergo phosphorylation on both serine and threonine residues (Chen et al., 1992; Sanghera et al., 1992; Seth et al., 1992; Gonzalez et al., 1993; Raingeaud et al., 1995). P38 MAPKs have the Thr-Gly-Tyr dual phosphorylation motif on which phosphorylation occurs (Hanks et al., 1995). P38 MAPKs are selectively activated by MEK3 or MEK6. Like ERK1/2 MAPKs, the phosphorylation of MEK3 or MEK6 is regulated by multiple kinases. The best known players in this pathway are apoptosis signal-regulating kinase 1 (ASK1), and TGFβ-activated kinase 1 (TAK1) (Kyriakis et al., 2001).

P38 MAPKs are involved in many chronic and acute CNS diseases. Similarly to ERK1/2 MAPKs, they are involved in most chronic neurodegenerative diseases, such as AD, Parkinson's disease and amyotrophic lateral sclerosis. P38 MAPK activity was associated with the formation of neuritic plaques, tangle-bearing neurons in neurodegenerative diseases (Hensley et al., 1999). In acute CNS injuries, such as stroke, spinal cord injury and brain injury, p38 MAPKs are involved in the microglial stress response (Koistinaho et al., 2002), and this activation contributes to the chronic pain caused by SCI (Hains et al., 2006). Activation of p38 MAPKs is also associated with apoptosis after SCI (Zhang et al., 2005). Induction of interleukin-1 in SCI is p38 MAPK-dependent (Wang et al., 2005). Continuous intrathecal infusion of SB203580, a selective inhibitor of p38 mitogen-activated protein kinase, reduces the damage of hind-limb function after thoracic spinal cord injury in the rat (Horiuchi et al., 2003). The second part of this study is focused on the role of p38 MAPKs in glutamate-induced SCI.

HYPOTHESIS

Previous studies primarily emphasized the involvement of increased intracellular ion concentrations and the roles of reactive oxygen and nitrogen species in secondary damage due to excitotoxicity following spinal cord injury. Present work is designed to characterize a pathway from spinal cord injury through cell death by excitotoxicity by way of cytokines and kinases. A series of experiments were performed to test our hypothesis that IL-1β is involved in the loss of function following SCI caused by the exposure of the rat spinal cord to glutamate. We also explored whether the role of IL-1β in glutamate-induced SCI includes the activation of MAPKs (diagram shown below).

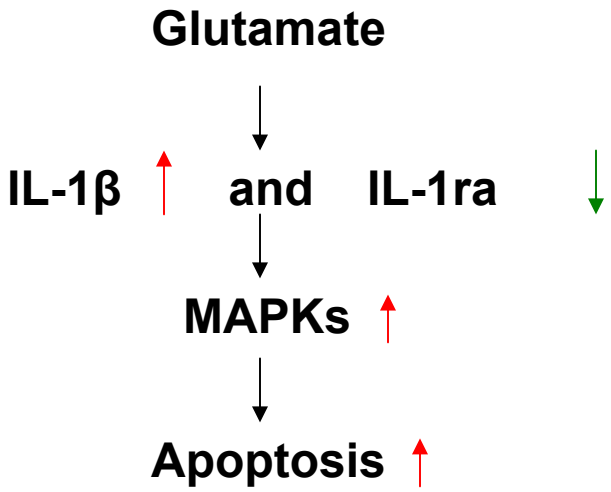
Glutamate was administered to the spinal cord and the expression of IL-1β, IL-1ra and MAPKs were measured. IL-1ra and MAPKs inhibitors were administered to the spinal cord to study the mutual role between glutamate, IL-1β and MAPKs. In this work I tested the following hypothesis.

Hypothesis 1: Elevation of glutamate in the spinal cord activates IL-1β and suppresses the expression of IL-1ra.

Hypothesis 2: The activation of IL-1β and decrease of IL-1ra play important roles in loss of rat locomotor function that is caused by glutamate.

Hypothesis 3: The activation of MAPKs (ERK and p38) in response to glutamate administration is mediated by IL-1β.

Hypothesis 4: The activation of MAPKs and IL-1β by glutamate is important in glutamate-induced apoptosis and impairment of locomotor function.



My results show that administration of glutamate to the cord increased IL-1β activation and decreased IL-1ra activation. The activation of IL-1β stimulated the activation of MAPKs, which in turn led to apoptosis (Shown in the diagram above). Red and green arrows indicate increases or decreases in levels.

CHAPTER 2: MATERIALS AND METHODS

ANIMALS

Male Sprague Dawley (Harlan, Indianapolis, IN) rats (200-250 g) were used in all experiments. Rats were housed in the Animal Resource Center at the University of Texas Medical Branch at Galveston and fed regularly. All procedures complied with the recommendations in the *NIH Guide for the Care and Use of Laboratory Animals* and were approved by the *UTMB Animal Care and Use Committee.*

ANIMAL PREPARATION

Rats (200-250g) were anesthetized with pentobarbital (50mg/kg i.p.) with buprenorphine added (0.1 mg/kg, subcutaneously) for pain relief. Anesthesia was considered complete when there was no flexor withdrawal in response to noxious foot pinch. Following the establishment of anesthesia, the back of the rat was shaved and sanitized by iodine. Cord segment T10 was exposed by removal of muscle and according to our research goals.

Contusion injury model

After complete anesthesia, the spinal cord was injured with the Infinite Horizons (Precision Systems, and Instrumentation, Louisville, KY) spinal cord contusion device, which was set to administer a maximal force of 150 kDynes onto the cord at spinal segment T10 with zero second dwell time. Following injury, muscle and fascia were sutured, and the skin was fastened with surgical staples to close the wound (McAdoo et al., 1999). The rats received the analgesic buprenorphine (0.1mg/kg) subcutaneously twice a day for 3 days. They were also administered the antibiotic baytril (2.7mg/kg) subcutaneously twice a day until bladder function returned to normal. Animals were transcardially perfused with 100ml of heparinzed 0.9% saline at different time points, depending on the goal of the experiment.

Glutamate-induced injury model

Following the establishment of anesthesia, the back of the rat was shaved and sanitized by iodine. Cord segment T10 was exposed by removal of muscle and a laminectomy at vertebrae T9-T11. 100µl of glutamate (10mM) or ACSF (control) was placed on the top of the cord at T10 for 1 hour after the dura was opened. The maximum glutamate concentration after SCI is around 500 µM (McAdoo et al., 1999). Glutamate put on the top of the cord diffuses into the cord, but the concentration decreases dramatically with distance. To reach the glutamate concentration in cords following injury, we put 10 mM glutamate on top of the cord. The muscle and bone around the exposed cord restricted the glutamate from flowing away. The glutamate solution was removed after 1hour of cord exposure, and the rats were transcardially perfused at different time points, depending on our research goals.

NMDA and AMPA models

To study the role of different glutamate receptors in SCI, we applied specific glutamate receptor agonists or antagonists on the cord. To get an effect equal to that of glutamate (10 mM) on glutamate receptors, 10 mM NMDA or AMPA receptor agonists were applied. To study NMDA receptors, 100µl of NMDA (*N*-methyl-D-aspartic acid) (10 mM) was applied instead of glutamate on the top of T10 for 1 hour after the dura was opened. To study AMPA receptors, 100µl of S-AMPA (alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) (10 mM) was applied on the top of the cord at segment T10 for 1 hour.

We also applied glutamate and glutamate-receptor antagonists together onto the cord. To study further the role of AMPA receptors, we applied glutamate (10 mM) plus NBQX (3-dihydroxy-6-nitro-7-sulfamoyl-benzo(f)quinoxaline) (5 mM) onto the top of the cord. To study the role of NMDA receptors, we applied glutamate (10 mM) and MK801 (5 mM) onto the top of the cord.

IL-1β and IL-1ra models

To study the reciprocal relationships of IL-1β and IL-1ra, recombinant IL-1β (1ng/ml) or IL-1ra (1.25 mg/ml) was applied onto the cord for 1 hour and then removed from the cord. Rats were transcardially perfused with 0.9% saline and IL-1β exposed cords were collected at 1h, 6h and 24h after the exposure. The IL-1ra concentration of the cord was measured with an IL-1ra specific ELISA kit (Invitrogen. Inc.). Cords similiarly exposed to IL-1ra were collected at the same time points after the exposure, and the IL-1β concentration in the cords was measured with an IL-1β specific ELISA kit (Invitrogen, Inc.).

Drug application

To determine if the administration of IL-1β alone could cause rat locomotor function loss, recombinant IL-1β was applied intrathecally using a micro-osmotic infusion pump (Alzet, Durect Co., Cupertino, CA, USA). This pump releases fluid at a rate of 1ul/hr for as long as three days. A thin polyethylene tube (0.28 mm in diameter) was inserted into the subarachnoid space of the spinal cord in the T10 area. The pump filled with IL-1β was connected to the subarachnoid tube and inserted into the subcutaneous space.

To study the protective effect of IL-1ra on glutamate-induced SCI, recombinant IL-1ra (1.25 mg/ml) was applied intrathecally, as described for IL-1β. The amount of IL-1ra given to rats is 100 times lower than that to human beings considering the rat weight. To study the protective effect of ERK and p38 inhibitors, 400 μM PD 98059 or SB 203580 was administered to the cord, concentrations chosen based on previous studies (Cuenda et al., 1995; Borsch-Haubold et al., 1998).

PROTEIN EXTRACTION

After perfusion, spinal segment T10 was taken out and put on dry ice for quick freezing. Then the tissue was stored at -70 ˚C until we extracted its proteins. 100 μl of extraction buffer (10 mM HEPES, 10 mM KCl, 0.1 mM EDTA, 0.1 mM EGTA, 1 mM

DTT, 0.5 mM PMSF, protease inhibitor cocktail) was added to each tissue and the tissue was sonicated and then centrifuged at 8000rmp for 15 minutes at 4 ˚C. Supernants were collected and protein concentrations were measured by a Bio-Rad protein assay (#500-0006, Bio-Rad Laboratories).

PROTEIN CONCENTRATION DETERMINATION

To load equal amounts of proteins in biochemistry assays, protein concentrations were determined. Bovine serum albumin was diluted with distilled water to get a standard curve with concentrations of 0.5 mg/ml, 1.0 mg/ml, 2.5 mg/ml, 5.0 mg/ml, 7.5 mg/ml, and 10.0 mg/ml. 20 µl of 0.12N HCl was added to each standard and sample. 5 µl of standard or sample was added to each tube. 875 µl of filtered dilute dye (Bio-Rad #500-0006) was added to each tube. Tubes were gently vortexed and the tubes were placed on the bench for 5 minutes. Then the absorbance at 595 nm was read on a Quant plate reader.

ELISA IL-1 BETA DETECTION ASSAY

An ELISA (Invitrogen, Camarillo, CA) was used to measure IL-1β protein levels in cytosolic fractions of spinal cords. The assay was performed according to the protocol for a solid phase sandwich ELISA using antibodies specific for rat IL-1β (17 kd). The sample absorbance was read with an ELISA plate reader adjusted to a wavelength of 450 nm, and the concentration was determined based on a standard IL-1β curve established with recombinant rat IL-1β. The protocol is briefly described as follows: Samples were incubated on the plate for 3 hours at room temperature (RT). Each well was washed 4 times after the incubation and 100 µl of Biotin conjugate was added to each well and incubated for 1 hour at RT. Each well was washed 4 times and then 100 µl of Streptavidin-HRP working solution was added and incubated for 30 minutes at RT. After 4 washes, 100 µl of stabilized chromogen was added to wells for 30 minutes at RT. 100 µl of stop solution was added, and the absorbance at 450 nm was read on the µQuant.

ELISA IL-1RA DETECTION ASSAY

An ELISA (Invitrogen, Camarillo, CA) was used to measure IL-1ra protein levels in cytosolic fractions of spinal cords. The assay was performed according to the protocol for the solid phase sandwich ELISA using antibodies specific for rat IL-1ra. The sample absorbance was read with an ELISA plate reader at 450 nm, and the concentration was determined based on a standard IL-1ra curve established with recombinant rat IL-1ra. The protocol is briefly described as follows: Samples were incubated on the plate for 2 hours at 37 ^{o} centigrade. Each well was washed 4 times after the incubation, and 100 µl of the Biotin conjugate was added to each well and incubated for 1 hour at RT. Each well was washed 4 times and then 100 µl of Streptavidin-HRP working solution was added and incubated for 30 minutes at RT. After 4 washes, 100 µl of stabilized chromogen was added to the wells for 30 minutes at RT. 100 µl of stop solution was added and the absorbance at 450 nm was read on the µQuant.

ELISA CELL DEATH DETECTION ASSAY

Apoptosis was measured by an ELISA assay that is specific for nucleosome-associated cytosolic DNA. The apoptosis ELISA was performed by the Roche Cell Death Detection ELISA kit (Roche, Germany). The assay is based on the sandwich-enzyme-immunoassay principle. Mouse monoclonal antibodies specific to DNA fragments and histones were used. This allows the specific determination of mono and oligonucleosomes in the cytoplasmic fraction of the spinal cord. Briefly, each well of the 96-well plate was coated with 100µl of anti-histone antibody solution overnight at 4°C. After removing the antibody solution from the well, 200µl of incubation solution was added, followed by 30 minute incubation at room temperature. The wells were washed three times with 250-300µl of washing buffer. 100µl of sample solution and 100 µg of cytoplasmic fraction proteins, which were isolated as described above, were added to the wells and incubated for 90 minutes at RT. 100µl of incubation buffer alone was used as a background control. After washing the wells three times with 250-300µl of washing buffer, 100µl of conjugate buffer was added for a 90 minute incubation at RT. After

incubation, the wells were washed three times with 250-300µl of washing buffer. 100µl of substrate buffer was then added. The plate was placed on a plate shaker at 250 rpm for 10-20 minutes. The sample absorbance was read with an ELISA plate reader (µQuant) at 450nm wavelength.

WESTERN BLOT ANALYSIS

Each sample (50 µg) was boiled for 5 minutes in an equal volume of sample buffer (100 mM Tris, pH 6.8, 250 mM 2-mercaptoethanol, 4% SDS, 0.01% bromophenol blue, 20% glycerol), placed on ice, and then loaded into a 10% polyacrylamide gel. Samples were separated by electrophoresis in a Tris-glycine buffer (25mM Tris, 250mM glycine, 0.1% SDS) at 200 volts for 70 minutes. Then proteins were transferred overnight to polyvinylidene difluoride (PVDF) membranes (Bio-Rad) at 4ºC, 100 volts for 2.5 hours, in a transfer buffer containing 20% methanol, 20mM Tris, and 150 mM glycine at pH 8. Membranes were incubated for one hour at room temperature in a blocking buffer containing 5% powdered milk in TBS-Tween (20mM Tris, 137mM NaCl, 0.1% Tween-20), followed by incubation in primary antibody solution diluted 1:2000 in 50% blocking buffer in TBS-Tween overnight, and then washed 3 times (5 minutes each) in TBS-Tween. Membranes were subsequently incubated in horseradish peroxidase-conjugated IgG, diluted 1:5000 in 50% blocking buffer for one hour, and then washed three times in TBS-Tween for 15 minutes. Peroxidase activity was detected using the Amersham enhanced chemiluminescence lighting system (ECL, Amersham, Buckinghamshire, England) and exposure to X-ray film. Protein bands were quantitated using a densitometer. All antibodies were purchased from Cell Signaling Inc. The density of bands was analyzed with a densitometer.

IMMUNOHISTOCHEMISTRY

Rats were anesthetized and fixed by intracardiac perfusion with 4% paraformaldehyde in 0.1 M PBS (pH 7.5). The spinal cords were removed and kept in 4% paraformaldehyde for 6 hour after fixation. Then the spinal cords were immersed in 25% sucrose overnight at 4 ^{o}C. The cords were next cut into 30µm thick sections with a sliding

microtome. Cord sections were collected in a glass Petri dish with cold 0.05 M TBS. The sections were washed with 0.05 M TBS three times, 10 minutes each, with shaking at RT. Then the sections were washed with 0.05 M TBS/Triton X-100 twice, 10 minutes each, with shaking at RT. The sections were blocked with 0.05 M TBS + 0.15% Triton-X100 + 5 NGS + 0.3% BSA for 45 minutes at RT with shaking. After the blocking step, the sections were incubated with either anti-Neurofilament (Oncogen Inc.) or Anti-GFAP (Oncogen Inc.). For double staining, anti-IL-β (Serotec Inc.) was added at the same time for overnight gentle shaking at RT. The next day, the sections were washed with 0.05 M TBS three times at RT. Then the sections were incubated with 1:500 Alexa Fluor 488 (green) goat anti-mouse IgG for about two hours at RT (For double staining, 1:500 Alexa Fluor 568 (red) goat anti-rabbit IgG was added at the same time). During the incubation, the sections were wrapped with foil to keep them away from light. After the incubation, the sections were washed with 0.05 M TBS three times at RT; then the sections were mounted on slides and left to dry. After the slides were dry, they were covered with a coverslip using hard-set fluorescence mounting medium with DAPI. The slides were allowed to sit at RT for about 20-30 minutes. Then they were refrigerated in a -70^{o}C freezer until viewing.

The stained sections were thawed and photographed using a Bio-Rad Radiance 2100 confocal analysis system combined with a Nikon Eclipse E 800 microscope and software. Images were collected from two channels, individual excitation wavelengths being 488 nm for green and 568 nm for red.

LOCOMOTOR FUNCTION TESTING

Basso, Beattie and Bresnahan (BBB) test

Locomotor function was evaluated using the Basso, Beattie and Bresnahan (BBB) open-field locomotor test (Basso et al., 1995). Briefly, the BBB scale ranges from 0 (no hind limb movement) to 21 (normal movement-coordinated gait with parallel paw placement). Scores in the range 0-7 indicate the return of isolated movements in the 3

hindlimb joints (hip, knee, and ankle). Scores from 8-13 indicate the return of paw placement and coordinated movements with the forelimbs. Scores 14-21 show the return of toe clearance during stepping, predominant paw position, trunk stability, and tail position. BBB scores were measured on various days post contusion. The BBB test was performed starting 24 hours after injury. The rats were individually placed in an open area while the viewer watched the rat hindlimb movements for 5 minutes. The viewer evaluated the rat hindlimb function and recorded the BBB score. To avoid subjective bias, BBB tests were done double-blinded. To control for possible asymmetric injuries, left and right hind limbs were scored individually. Reported BBB scores for an individual subject represent the averages from both hind limbs. The tests were done on post injury days 1, 7, 14, 21 and 35. The data were analyzed using two-way repeated measures analysis of variance (ANOVA) based on the factors time and group.

Photobeam activity system (PAS)

Photobeam activity system (PAS) was also used to assess rat hindlimb recovery. We (Xu et al., 2005) previously showed a good correlation between BBB and activity chamber results. PAS data were collected using the software and hardware provided by the PAS with FLEXFIELD software (San Diego Instruments, Inc.). Two chambers were used simultaneously with one rat in each chamber. Rat activities were measured continuously for 30 minutes. All PAS measurements were done in the morning (8:00 AM to 12:00 AM) during each day. One rat per chamber was tested at a time, so two rats were tested per time interval. Before the glutamate exposure, control activities were established for each rat as baselines. The activity chambers contain 16 photobeams parallel to the x-axis and 16 photobeams parallel to the y-axis (32 total). These photobeams are 4 cm above the chamber floor. The obstruction of these beams is recorded as movements along the x and y-axes. Obstruction of another set of photobeams (12 cm above the chamber floor) records the movement in the z direction (rearing events). Six parameters are recorded by the chambers: rearing time (the total amount of time the animal spends up on its hindlimbs), number of rearing events (the total number

of times the animal gets up on its hindlimbs), distance traveled (the distance that the rat travels during the monitoring time), resting time (the total amount of time the rat is motionless for 1 second or longer), active time (the total amount of time that the rat spends moving around the cage or in place) and total beams broken (the number of times that the animal blocks two beams in succession). PAS measurements were done on post injury days 1, 7, 14 and 28. Changes in each parameter were evaluated separately. Two way repeated measures ANOVA was used to compare change over time for each group.

STATISTICAL ANALYSIS

Mean values (±SEM) for each experiment were determined. For two-group comparisons, values were compared using a two-tailed student t-test. For multiple-group comparison of ELISA results, data were analyzed using one-way analysis of variance (ANOVA). For behavior results, data were analyzed by repeated measures two-way analysis of variance (two-way ANOVA). Bonferroni tests were for pairwise comparisons following ANOVA. *p* values less than 0.05 was considered significant.

CHAPTER 3: RESULTS

Glutamate release contributes to the apoptosis and impairments caused by spinal cord injury (SCI). The administration of IL-1ra prevented apoptosis after SCI in rats (Nesic et al., 2001). One issue this study addresses is the mechanisms of glutamate toxicity involving activation of interleukin-1β (IL-1β). To assess the effects of glutamate on IL-1β and its natural blocking agent interleukin-1 receptor antagonist (IL-1ra), ELISA assays were used to measure the responses of endogenous IL-1β and IL-1ra to glutamate administration to the spinal cord. Levels of activated IL-1β and IL-1ra changed in a reciprocal fashion starting 1 hour after glutamate exposure. Exposure to glutamate initially increases IL-1β expression while IL-1ra decreased. At longer times IL-1β decreased and IL-1ra increased. IL-1β and IL-1ra also changed reciprocally in a contused spinal cord. To check whether these mutual effects were due to IL-1β and IL-1ra on each other, IL-1β was applied on the spinal cord and then IL-1ra was measured. IL-1ra was applied onto the cord and IL-1β was measured as well. To identify subtypes of glutamate receptors involved in this phenomenon, NMDA and AMPA receptor agonists were separately applied to the spinal cord and IL-1β and IL-1ra expression in the cord were measured with ELISA assays. To determine the effects of AMPA and NMDA receptor antagonists on IL-1β and IL-1ra, MK801 and NBQX were applied individually on the spinal cord with glutamate. Activation of both the AMPA and the NMDA receptors also induced reciprocal changes between the expressions of IL-1β and IL-1ra levels. To determine if this reciprocity between the expression of IL-1β and IL-1ra affected post-SCI locomotor function, recombinant IL-1β and IL-1ra were administered to the glutamate-exposed spinal cords. The Basso-Beattie-Bresnahan (BBB) assessment of function demonstrated that IL-1β impaired rat locomotive ability and that IL-1ra improved the recovery of the rats from glutamate-induced locomotor impairment. To explore the mechanism of this IL-1β involvement in excitotoxicity, MAPK activities were measured and the results showed that both ERK1/2 and p38 are involved in this IL-

1β induced SCI. Cell death assays showed that apoptosis is caused by this glutamate-induced SCI.

GLUTAMATE EXPOSURE UP-REGULATED IL-1 BETA AND DOWN-REGULATED IL-1RA IN THE SPINAL CORD

Rat spinal cords were exposed to glutamate (10 mM) or ACSF (sham) as described in Methods and Materials. T10 segments were collected at 1 hour, 6 hours and 24 hours after the exposure of the spinal cord to glutamate. Cytosol proteins (200 ug) were analyzed with an IL-1β-specific ELISA kit according to the protocol provided by Invitrogen, Inc. The results showed that IL-1β (Fig. 1) expression started to increase at 1 hour after glutamate exposure and reached a peak at 6 hours. At about 24 hours after glutamate exposure, the IL-1β expression level was back to the sham level. These results are similar to previous observations of increased IL-1β expression after SCI (Nesic et al., 2001; Yang et al., 2004).

I measured levels of IL-1ra with a rat IL-1ra specific ELISA kit from Invitrogen, Inc. The expression of IL-1ra (Fig. 2) started to decrease at 1 hour after the termination of glutamate exposure. At about 24 hours after glutamate exposure, IL-1ra returned to its sham level. Thus levels of IL-1β and IL-1ra changed reciprocally following exposure to glutamate. Previous study demonstrated that the administration of IL-1ra to the rat after contusion SCI prevented apoptosis (Nesic et al., 2001) and improved recovery of rat locomotor function. However, the expression pattern of endogenous IL-1ra following SCI was not previously measured. I measured the endogenous IL-1ra and showed that the levels of IL-1β and IL-1ra change in approximately reciprocal patterns after exposure to glutamate.

CONTUSION OF THE SPINAL CORD LED TO THE UP-REGULATION OF IL-1 BETA AND DOWN-REGULATION OF IL-1RA

To investigate if the reciprocity of IL-1β and IL-1ra expression elicited by glutamate is also present in the contusion spinal cord injury model, we also measured the expression of IL-1β and IL-1ra in the contused spinal cord. T10 segments were collected

at 1h, 6h and 24h after contusion. IL-1β (Fig. 3) expression started to increase at 1 hour after contusion and peaked at 6. At about 24 after contusion, IL-1β expression returned to near the sham level. The expression of IL-1ra (Fig. 4) decreased markedly at 1 after contusion. At about 24 after contusion, the IL-1ra expression level was significantly above the sham level. Thus levels of IL-1β and of IL-1ra also changed reciprocally in response to contusion SCI. Patterns of IL-1ra changes after SCI and exposure to glutamte (Figs. 2 and 4) are quite similar.

ACTIVATION OF BOTH NMDA AND AMPA RECEPTORS GENERATED RECIPROCAL RESPONSES OF IL-1 BETA AND IL-1RA LEVELS IN GLUTAMATE-INDUCED SPINAL CORD INJURY

To identify which glutamate receptor subtypes were involved in the glutamate-induced reciprocity of IL-1β and IL-1ra, rats were exposed to NMDA, AMPA or ACSF, as described in Materials and Methods. T10 segments were collected at 1h, 6h and 24h after exposure to the glutamate receptor agonists. The results showed that both NMDA and S-AMPA triggered reciprocal changes between IL-1β and IL-1ra levels (Fig. 5, 6, 7 and 8). To confirm the roles of NMDA and AMPA receptors, we tested the effect of their antagonists, MK801 (NMDA receptor antagonist) and NBQX (AMPA receptor antagonist) on IL-1β and IL-1ra levels. Both MK801 and NBQX reversed the up-regulation of the IL-1β level caused by glutamate (Fig. 9) and the down-regulation of IL-1ra caused by glutamate (Fig. 10), demonstrating involvement of both of the corresponding receptors in the responses to IL-1β and IL-1ra.

IL-1 BETA INDUCED IL-1RA PRODUCTION AND IL-1RA SUPPRESSED ACTIVATION OF IL-1 BETA

The reciprocity of IL-1β and IL-1ra was found in all SCI models that I tested, including the glutamate model, the contusion model, the NMDA model and the S-AMPA model (Fig. 11). Based on these findings, I hypothesized that IL-1β and IL-1ra act on the expressions and/or activations of each other. To test this hypothesis, rat spinal cords were exposed to recombinant IL-1β or IL-1ra. Cords that were exposed to recombinant IL-1β were taken out at 1h, 6h and 24h after IL-1β exposure and analyzed with an IL-1ra

specific ELISA kit. This showed that the administration of IL-1β to the cord induced the production of IL-1ra, starting hours after the IL-1β exposure. This induction lasted to at least 24 hours after IL-1β administration (Fig. 13).

To study the effect of IL-1ra on IL-1β concentrations, rat spinal cords were exposed to recombinant IL-1ra alone. Tissues were collected at 1h and 24 h after IL-1ra exposure. IL-1β was measured with an IL-1β specific ELISA kit. IL-1β activation was suppressed by the administration of IL-1ra. The decrease of IL-1β started from 1 hour after IL-1ra administration and lasted until at least 24 hours after it (Fig. 12). These results indicate that the administration of IL-1β induced the production of IL-1ra, while the administration of IL-1ra suppressed the activation of IL-1β. These results are consistent with my previous data showing the IL-1β and IL-1ra reciprocity in glutamate-induced SCI.

ADMINISTRATION OF A CASPASE-1 INHIBITOR PREVENTED THE CLEAVAGE OF IL-1 BETA

The activation of IL-1β involves the cleavage of pro-IL-1β to IL-1β by caspase-1. Z-YVAD-FMK, a caspase-1 inhibitor, was used in experiments to test if glutamate exposure activates IL-1β. 50μM of Z-YVAD-FMK was applied on the top of the cord together with 10mM glutamate and removed after 1h. The cord was taken out 6h later and IL-1β was measured with an ELISA kit. The administration of Z-YVAD-FMK to block the activity of caspase-1 significantly reduced the activation of IL-1β induced by exposure to glutamate (Fig. 14), evidence that glutamate activates IL-1β through caspase-1.

ADMINISTRATION OF IL-1RA HELPED RATS RECOVER FROM GLUTAMATE-INDUCED INJURY

The administration of glutamate to spinal cords led initially to up-regulation of IL-1β and down-regulation of IL-1ra. The next question was if the reciprocity between IL-1β and IL-1ra after glutamate exposure was important in rat functional impairment induced by glutamate. Several behavior experiments were done to test the importance of

IL-1β in glutamate excitotoxicity. Three groups of rats were checked. A sham group consisted of rats which had ACSF administered to their cords. The glutamate group consisted of ten rats which had glutamate administered onto the cord, and osmotic pumps filled with ACSF was placed subcutaneously in the rats in this group. The glutamate +IL-1ra group had ten rats whose cords were treated with glutamate, as described in Materials and Methods. At the same time, osmotic pumps filled with recombinant IL-1ra were placed subcutaneously in the rats in this group and recombinant IL-1ra was directly pumped onto the cord at segment T10, where glutamate exposure occurred. IL-1ra was pumped at 1μl/hr for three days. BBB tests were done on day 1, day 7, day 14 and day 28 after surgery (Fig. 15). The BBB scores of rats in the Glutamate + IL-1ra group are higher than those of rats in the Glutamate group on all days, demonstrating a beneficial effect of IL-1ra in glutamate-induced SCI.

Another behavior test using the photobeam activity chamber (PAS) was done to determine if the administration of IL-1ra to the cord affected the outcome of rat locomotor function. The PAS includes a computer, and the computer records the rat activity in these chambers by recording photobeams that are broken by rat movements (details in Materials and Methods). Several parameters were measured by activity chambers: rearing time (the total amount of time the animal spends up on its hindlimbs), number of rearing events (the number of times the animal gets up on its hindlimbs), distance traveled (the distance that the rat travels during the monitoring time), resting time (the amount of time the rat is motionless for 1 second or longer), active time (the total amount of time that the rat spends moving around the cage or in place) and total beams broken (the number of times that the animal blocks two beams in succession). Rats in the Glutamate+IL-1ra group traveled longer than rats in the Glutamate group on all measuring days (Fig. 16). Rats in the Glutamate+IL-1ra group had more rearing events compared to rats in the Glutamate group on day 7 and day 28 after surgery. On day 7, rats in the Glutamate+IL-1ra group showed significantly longer rearing times than rats in the Glutamate group.

The BBB results and activity chamber results together indicate that the administration of IL-1ra to rats in addition to glutamate helps rats to recover from the injury caused by glutamate, suggesting that the decrease of IL-1ra after rat cords were exposed to glutamate contributes to the rat's loss of locomotor function.

THE ADMINISTRATION OF IL-1 BETA ALONE LED TO RAT LOCOMOTIVE FUNCTION LOSS

IL-1ra administration helps rats to recover from glutamate-induced SCI. Our next goal was to check whether IL-1β alone caused rats to lose part of their locomotive function. Two groups of rats were tested. The control group consisted of 10 rats which were given ACSF by an osmotic pump for 3 days. Rats in the IL-1β group were administered IL-1β via an osmotic pump for 3 days. BBB tests were done on day 1, day 7, day 14 and day 28 after the surgery (Fig. 17). The administration of IL-1β significantly impaired rat locomotive function on all of the measuring days.

The behavioral results showed that the administration of IL-1ra helped the rat recovery from glutamate-induced SCI, while the administration of IL-1β impairs the rat locomotive function. All of the results indicate that the increase of IL-1β and decrease of IL-1ra are important contributors to glutamate-induced SCI.

IL-1 BETA PRODUCING CELLS - NEURONS AND ASTROCYTES

Different cells in the CNS are usually tightly connected to specific physiological functions. Knowing the IL-1β producing cells would very helpful in further understanding the role of IL-1β and IL-1ra in glutamate induced SCI. To attain the IL-1β cellular location, we performed immunohistochemistry with an IL-1β specific antibody and different cell marker antibodies.

To check if IL-1β is resident in neurons, spinal cord slices which were fixed previously were incubated with an anti-IL-1β antibody and an anti-neurofilament antibody, the latter of which is a cell marker for neurons. Then the slides were viewed under a Bio-Rad Radiance 2100 confocal analysis system combined with a Nikon Eclipse E 800 microscope and software (Fig. 18). The green staining in the pictures is IL-1β

staining and the red staining in the pictures is neurofilament staining. When the two pictures were merged, the yellow color shows the overlay of red and green. Yellow means IL-1β and neurofilaments are co-localized in the same cells. Our staining produced much yellow, indicating that IL-1β and neurofilaments are co-localized (Fig. 18). The co-localization of IL-1β and neurofilaments demonstrates that IL-1β is in neurons.

To check if IL-1β is also resident in astrocytes, spinal cord slides which were fixed previously were incubated with anti-IL-1β antibody and anti-GFAP antibody (a cell marker for astrocytes). Then the slides were viewed under a confocal system, as described above. The green staining in the pictures is IL-1β staining and the red staining in the pictures is GFAP staining (Fig. 19). My results show that majority of IL-1β is not localized in cells that express GFAP. The IL-1β staining is mostly in the nuclei of cells that don't have GFAP in their cytosol, indicating that IL-1β may not also be located in GFAP positive cells, astrocytes.

The immunohistochemistry results show that most IL-1β is located in neurons. IL-1β in neurons could be produced by those cells, or transported into them by other mechanisms. The residency of IL-1β in neurons provides further information regarding the role of IL-1β in glutamate-induced SCI.

ERK1/2 WAS ACTIVATED AFTER CORDS WERE EXPOSED TO GLUTAMATE

ERK is a well-known kinase that is activated by phosphorylation, and the activation of ERK is involved in many inflammatory processes. In this study I hypothesized that ERK is involved in the IL-1β-mediated, glutamate-induced SCI. Rats were treated with ACSF (control) or 10mM glutamate. Cords were taken out at 1h, 6h and 24h after glutamate exposure and protein was extracted. Western-blotting was done with an anti-p-ERK antibody (Fig. 20). The western-blotting results showed that the exposure of cords to glutamate activated ERK1/2. The activation started from 1 hour after glutamate exposure and lasted until 24 hours after glutamate exposure. Total ERK1/2 was

blotted for a loading control. The ratio of phospho-ERK to total ERK was used to quantitate ERK activation.

ERK1/2 ACTIVATION WAS DECREASED BY ADDITION OF IL-1RA TO THE CORD

To determine if ERK1/2 activation is IL-1β dependent, IL-1ra was added to the cord with glutamate. Two groups of rats were used; the glutamate group (exposed cords to 10 mM glutamate at T10 for 1 hour) and the glutamate+IL-1ra group (exposed T10 cords to 10mM glutamate and 1.25mg/ml IL-1ra for 1 hour). Cords were taken out at 6 hours after drug exposure because 6 hour is the peak time for IL-1β production and ERK1/2 activation after glutamate exposure. Western-blotting was done with an anti-p-ERK antibody. The results showed that adding IL-1ra to glutamate suppressed the activation of ERK1/2 after glutamate exposure (Fig. 21). Total ERK1/2 was blotted for a loading control. The ratio of phospho-ERK to total ERK was used to determine the ERK activation.

ERK1/2 CO-LOCALIZED WITH IL-1 BETA

My results show that the administration of IL-1ra partially reverses the ERK activation caused by glutamate-exposure. To further explore the functional connection between ERK and IL-1β, we performed immunohistochemistry experiments to check if ERK and IL-1β are co-localized. The cords that were previously treated with glutamate were taken out at 6 hours after glutamate exposure and fixed. The slides were incubated with anti-p-ERK antibody and anti-IL-1β antibody and then viewed under a microscope associated with a confocal system (Fig. 22). The green staining is IL-1β and the red staining is p-ERK. When we merged them together, the yellow staining showed the co-localization of IL-1β and p-ERK. Our results show a lot of yellow, indicating that IL-1β and p-ERK were co-localized in cells in the spinal cord after glutamate exposure.

ERK INHIBITION IMPROVED THE RAT FUNCTIONAL OUTCOME AFTER GLUTAMATE EXPOSURE

My data showed that ERK was activated by exposure of the spinal cord to glutamate, and that this activation is blocked by adding IL-1ra with the glutamate. The next question is if the activation of ERK contributes to the functional losses caused by glutamate. BBB tests were done to measure the effects of the ERK inhibitor, PD 98059, on locomotor recovery. Three groups of rats were used: a control group, treated with ACSF; a Glutamate group, treated with 10 mM glutamate for 1 hour on the top of the cord T10 segment; a PD 98059 group, treated with 10 mM glutamate on the top of the cord at T10 and injected with PD 98059 via an osmotic pump for three days (described in Materials and Methods). BBB scores were recorded on day 1, day 7, day 14 and day 28 after the surgery (Fig. 23). The rats in the PD 98059 group showed better recovery compared to the glutamate only group on day 1, day 14 and day 28. The differences between the glutamate group and PD 98059 group are statistically significant based on repeated measures two-way ANOVA tests.

P-38 WAS ACTIVATED AFTER CORDS WERE EXPOSED TO GLUTAMATE

Similarly to ERK, p-38 is involved in many inflammatory processes. My data support a role of ERK in glutamate-induced SCI. Now I also hypothesize that p-38 is involved in IL-1β involving glutamate-induced SCI. Rats were treated with ACSF (control) or glutamate. Cords were taken out at 1h, 6h and 24 h after glutamate exposure, and proteins were extracted. Western-blotting was done with an anti-p-p-38 antibody. The western-blotting results showed that the exposure of cords to glutamate activated p-38 (Fig. 24). The activation started from 1 hour after glutamate exposure and lasted until 24 hours after glutamate exposure. Total p-38 was blotted for a loading control. The ratio of phospho- p-38 to total p-38 was used to compare p-38 activation.

P-38 ACTIVATION WAS DECREASED BY ADDITION OF IL-1RA

To study if the p-38 activation is IL-1β dependent, IL-1ra was added with glutamate. Two groups of rats were used; a control group (exposed T10 cords to ACSF

for 1 hour) and a glutamate+IL-1ra group (expose T10 cords to 10mM glutamate and 1.25mg/ml IL-1ra for 1 hour). Cords were taken out at 1h, 6h and 24 h after drug exposure. Western-blotting was done with an anti-p-p-38 antibody (Fig. 25). Adding IL-1ra to glutamate suppressed the activation of p-38 after glutamate exposure. Total p-38 was blotted for a loading control. The ratio of phospho- p-38 of total p-38 was used to determine the p-38 activation.

P-38 WAS CO-LOCALIZED WITH IL-1 BETA

Our results showed that the administration of IL-1ra could partially reverse the p-38 activation caused by glutamate-exposure. To further understand the functional connection between p-38 and IL-1β, we performed immunohistochemistry experiments to check if p-38 and IL-1β are co-localized. The cords that were previously treated with glutamate were taken out at 6 hours after glutamate exposure and fixed. The slides were incubated with an anti-p-p-38 antibody and an anti-IL-1β antibody and then viewed under a three-channel confocal system (Fig. 26). The green staining is IL-1β and the red staining is p-p-38. When we merged them, the yellow staining showed the co-localization of IL-1β and p-p-38. Our results showed a lot of yellow, indicating that IL-1β and p-p-38 were co-localized in cells in the spinal cord after glutamate exposure.

A P-38 INHIBITOR IMPROVED THE RAT FUNCTIONAL OUTCOME AFTER GLUTAMATE EXPOSURE

Previous data showed that p-38 was activated by exposure of the spinal cord to glutamate, and this activation is blocked by adding IL-1ra to glutamate. The next question is if the activation of p-38 contributes to the loss of function caused by glutamate. BBB tests were done to measure the effects of the p-38 inhibitor, SB203580, on effects of glutamate. Three groups of rats were used: a control group, administered with ACSF; a Glutamate group, 10 mM glutamate administered for 1 hour on the top of the cord at T10 cord and a SB203580 group, administered with 10 mM glutamate on the top of T10 cord and injected with SB203580 via an osmotic pump for three days (described in Materials and Methods). BBB scores were recorded on day 1, day 7, day 14

and day 28 after the surgery (Fig. 26). The rats in the SB203580 group showed better recovery compared to the glutamate group on day 1 and day 28. The differences between the glutamate group and the SB 203580 group are statistically significant based on repeated measured two-way ANOVA tests.

EXPOSURE OF CORDS TO GLUTAMATE LED TO APOPTOSIS

Glutamate exposure on cords decreases rat locomotor function and involves IL-1β, ERK and p38. In SCI, apoptosis plays important roles in loss of locomotor function. In addition, IL-1β, ERK and p38 are widely involved in apoptosis. So we tried to determine if the glutamate-induced SCI is related to apoptosis. A cell death detection ELISA (Roche, Germany) was used to determine if apoptosis followed glutamate exposure. This kit allows detecting mono and oligonucleosomes formed during apoptosis. The results showed (Fig. 27) that apoptosis reached its peak at 6 hours after the cords were exposed to glutamate. Then at 24 hours after glutamate exposure, the cytoplasmic histone-associated DNA fragments decreased to control levels.

IL-1RA PD 98059 AND SB 203580 DECREASED APOPTOSIS INDUCED BY GLUTAMATE

The above results show that glutamate induces apoptosis. We wanted to explore if IL-1β, ERK and/or p38 are involved in this apoptosis pathway. One group of rats was administered 10mM glutamate onto their cords. Other groups of rats were administered glutamate plus IL-1ra, or glutamate plus PD98059 or glutamate plus SB203580. Cords were taken out 6 hours after exposure to reagents. A cell death detection ELISA was used to measure the amount of apoptosis (Fig. 28). The results show that IL-1ra, PD98059 and SB203580 decreased apoptosis induced by glutamate, demonstrating the involvement of IL-1β, ERK and p38 in glutamate-induced apoptosis.

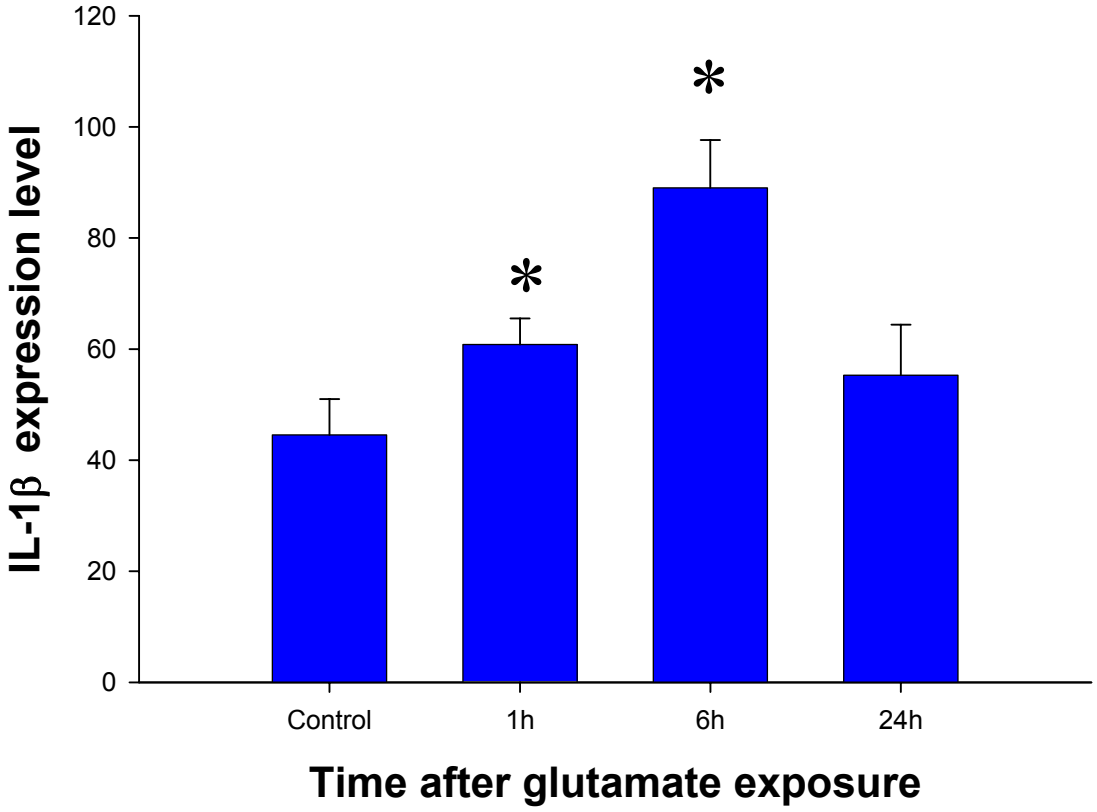


Fig. 1. The time course of ELISA measurements of IL-1β levels following glutamate administration. The level of IL-1β (20pg/ml) in sham rats (control) was normalized to 1. All other IL-1β levels were normalized to the level of IL-1β in sham rats. A one-way ANOVA ($p<0.05$) followed by Bonferroni t-test was used to determine the statistics. * Statistically different from control ($p<0.05$)

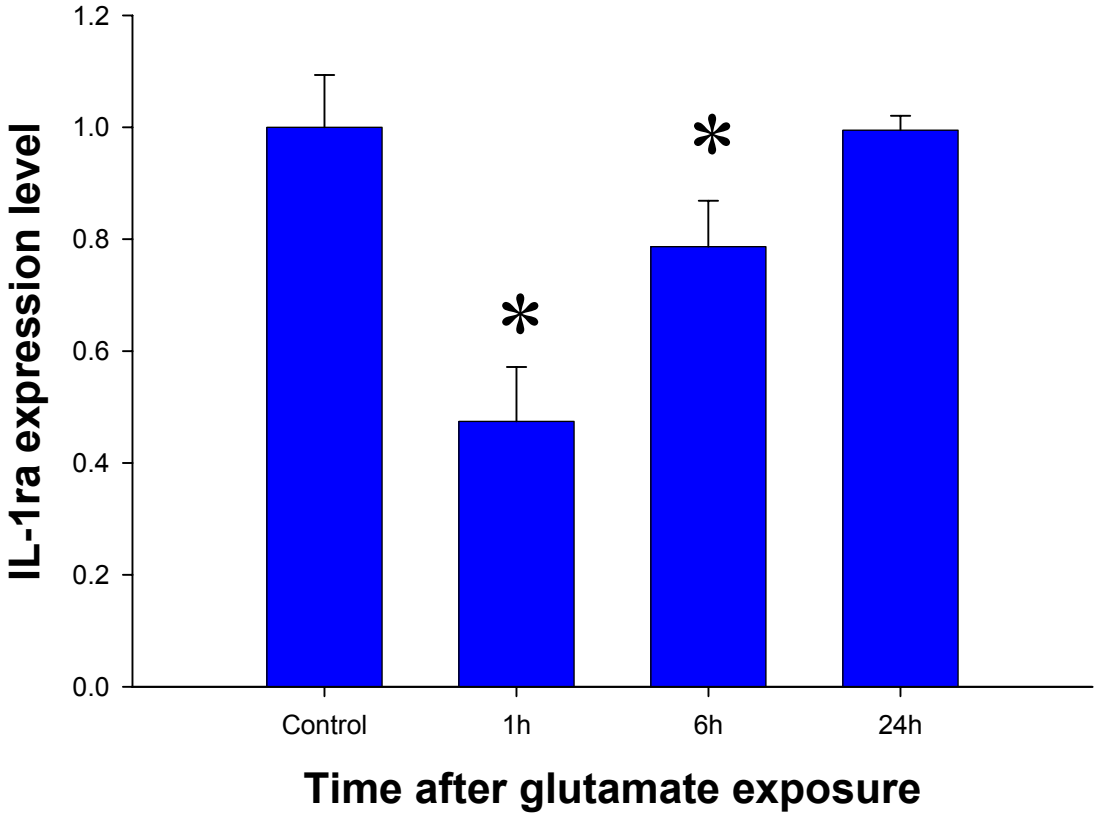


Fig. 2. The time course of IL-1ra levels measured by ELISA after glutamate exposure. The IL-1ra level (200pg/ml) in sham rats (control) was normalized to 1. All other IL-1ra levels were normalized to the IL-1ra level in sham rats. A one-way ANOVA ($p<0.05$) followed by Bonferroni t-test was used to determine the statistics. * Statistically different from control ($p<0.05$)

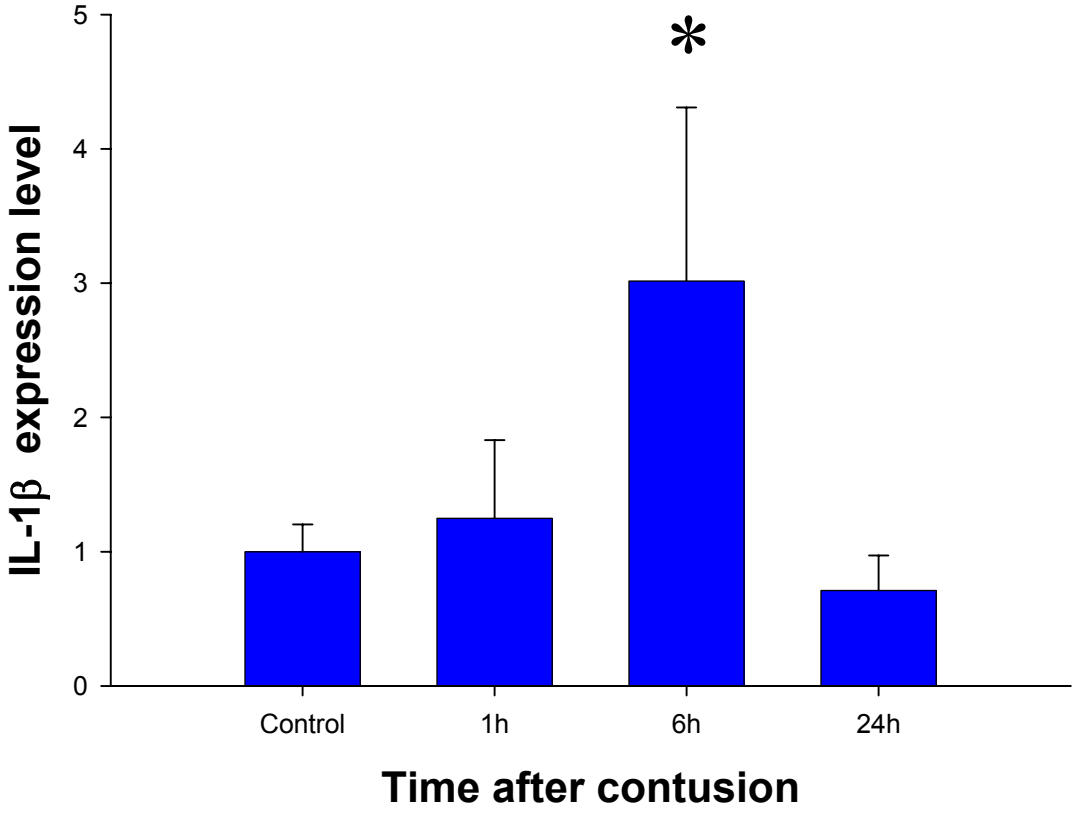


Fig. 3. The measurement of the time course of IL-1β expression in a contusion model. The T10 segments were contused with a 150 kdyne force and were taken at various time points after contusion. A rat IL-1β specific ELISA kit was used to determine the IL-1β level. A one-way ANOVA ($p<0.05$) followed by Bonferroni t-test was used to determine the statistics. * Statistically different from control ($p<0.05$)

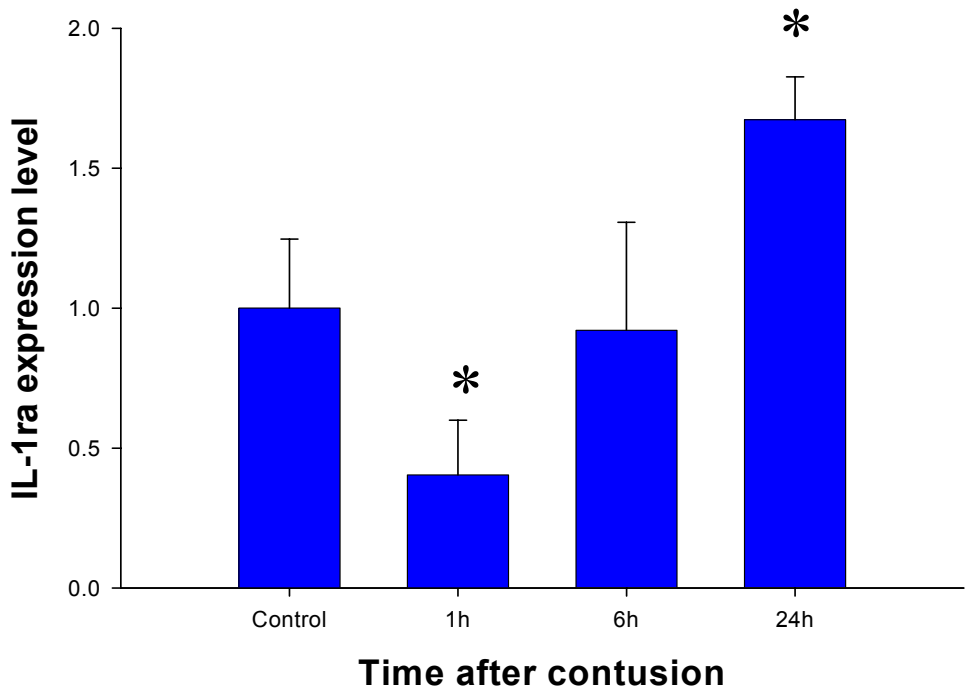


Fig. 4. The time course of IL-1ra expression following contusion injury. The IL-1ra level was determined by a rat specific IL-1ra ELISA kit. A one-way ANOVA ($p<0.05$) followed by Bonferroni t-test was used to determine the statistics. * Statistically different from control ($p<0.05$)

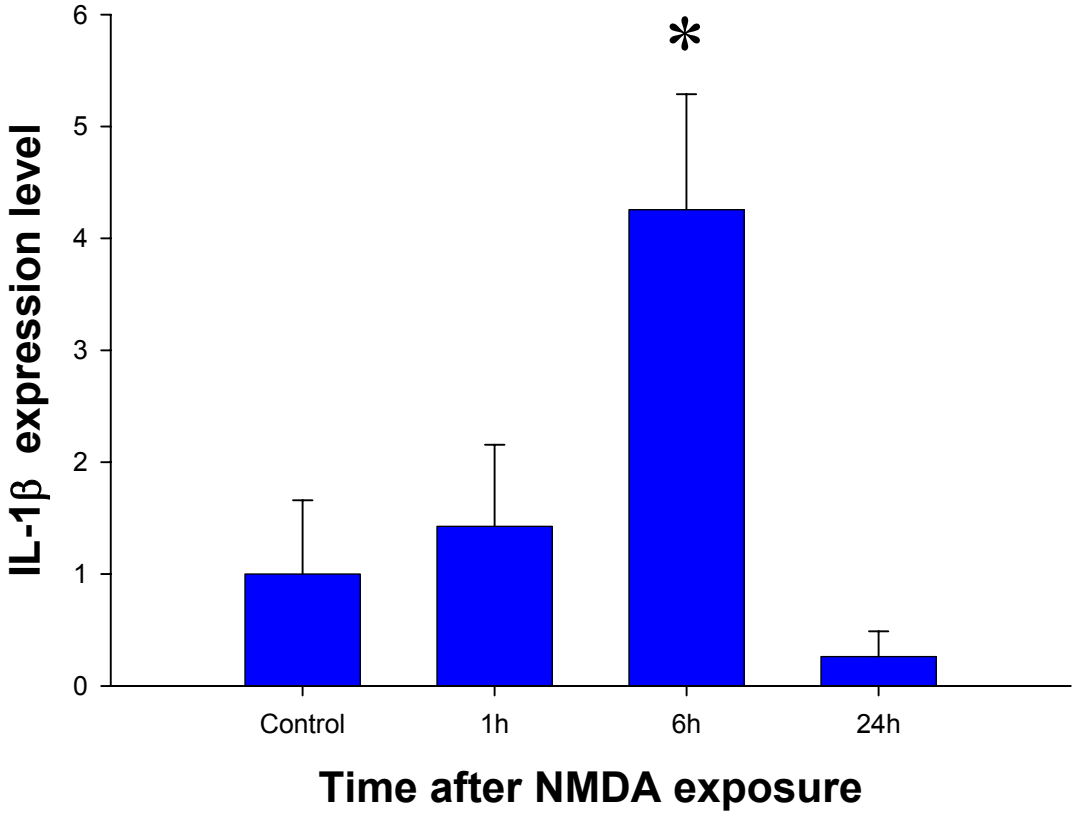


Fig. 5. The time course of ELISA measurements of IL-1β levels following NMDA administration. The level of IL-1β in sham rats (control) was normalized to 1. All other IL-1β levels were normalized to the level of IL-1β in sham rats. A one-way ANOVA ($p<0.05$) followed by Bonferroni t-test was used to determine the statistics. * Statistically different from control ($p<0.05$)

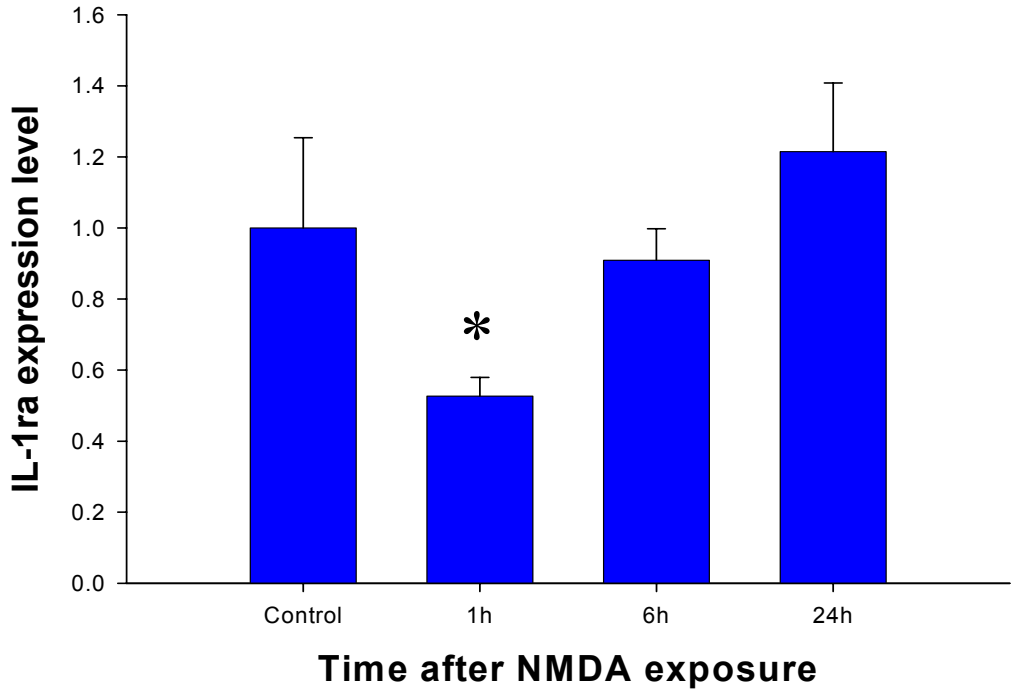


Fig. 6. The time course of IL-1ra levels measured by ELISA after NMDA exposure. The rats were administered NMDA (15mM) or ACSF (sham). The IL-1ra level in sham rats was normalized to 1. All other IL-1ra levels were normalized to the IL-1ra level in sham rats. A one-way ANOVA ($p<0.05$) followed by Bonferroni t-test was used to determine the statistics. * Statistically different from control ($p<0.05$)

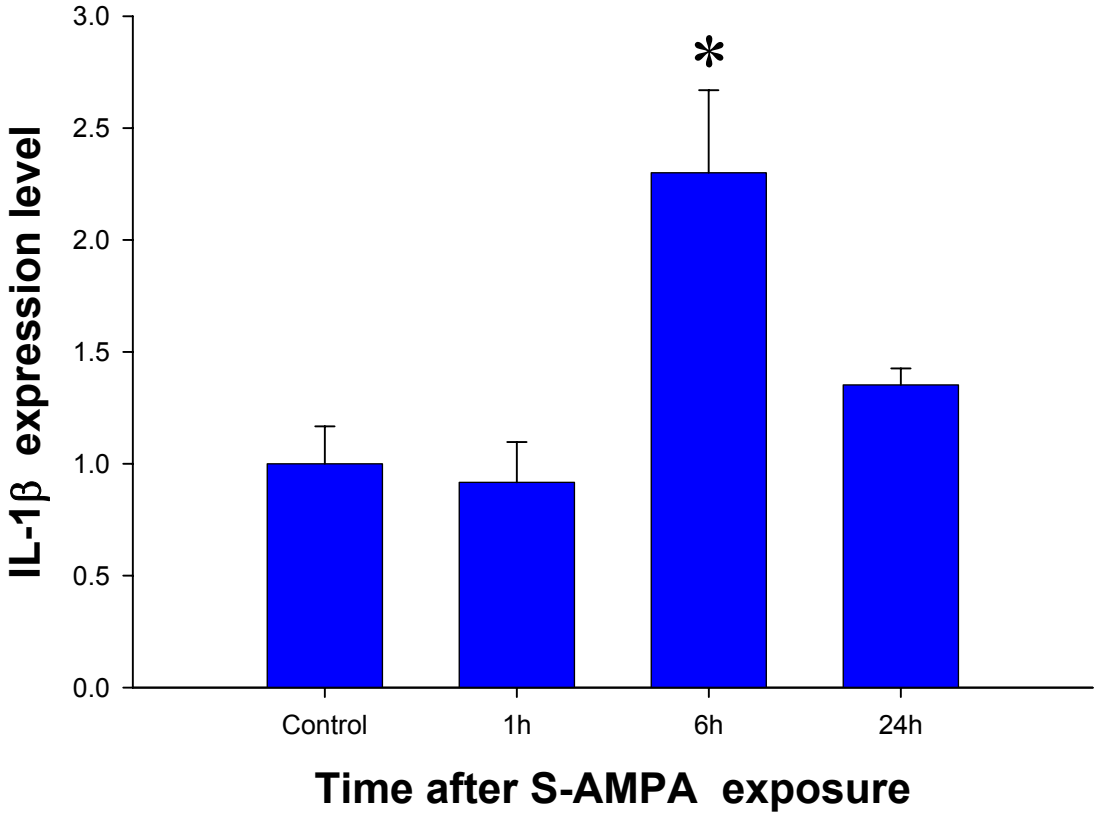


Fig. 7. The time course of IL-1β levels following S-AMPA administration. The level of IL-1β in sham rats (control) was defined as 1. All other IL-1β levels were normalized to the level of IL-1β in sham rats. A one-way ANOVA ($p<0.05$) followed by Bonferroni t-test was used to determine the statistics.* Statistically different from control ($p<0.05$)

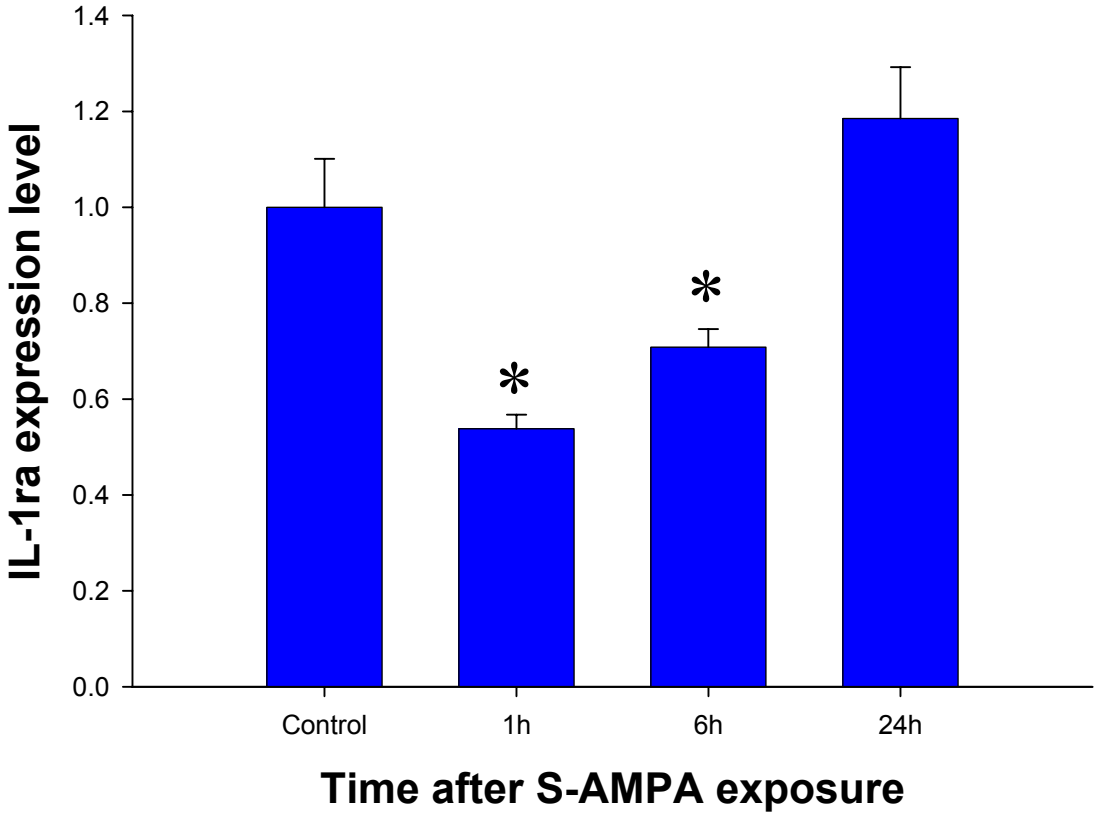


Fig. 8. The time course of IL-1ra levels after S-AMPA exposure by ELISA. The rats were administered S-AMPA (15mM) or ACSF (sham). The IL-1ra level in sham rats was normalized to 1. All other IL-1ra levels were normalized to the IL-1ra level in sham rats. A one-way ANOVA ($p<0.05$) followed by Bonferroni t-test was used to determine the statistics.* Statistically different from control ($p<0.05$)

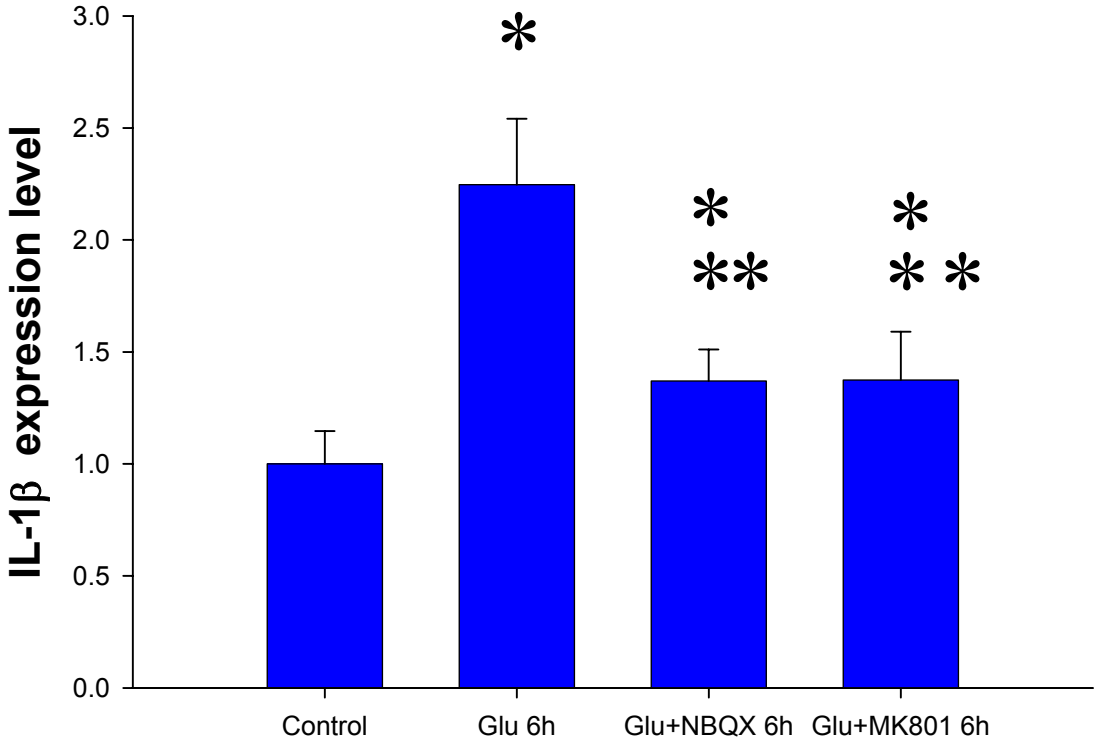


Fig. 9. Effects of MK801 and NBQX on IL-1 expression. MK801 and NBQX were administered to determine if the usage of glutamate receptor antagonists can block the increase of IL-1β expression. Spinal cords were taken at 6 hour after they were exposed to glutamate or glutamate plus MK801 or glutamate plus NBQX. IL-1β was measured with an IL-1β specific ELISA kit. A one-way ANOVA followed ($p<0.05$) by Bonferroni t-test was used to determine the statistics.* Statistically different from control ($p<0.05$) and ** statistically different from glutamate ($p<0.05$)

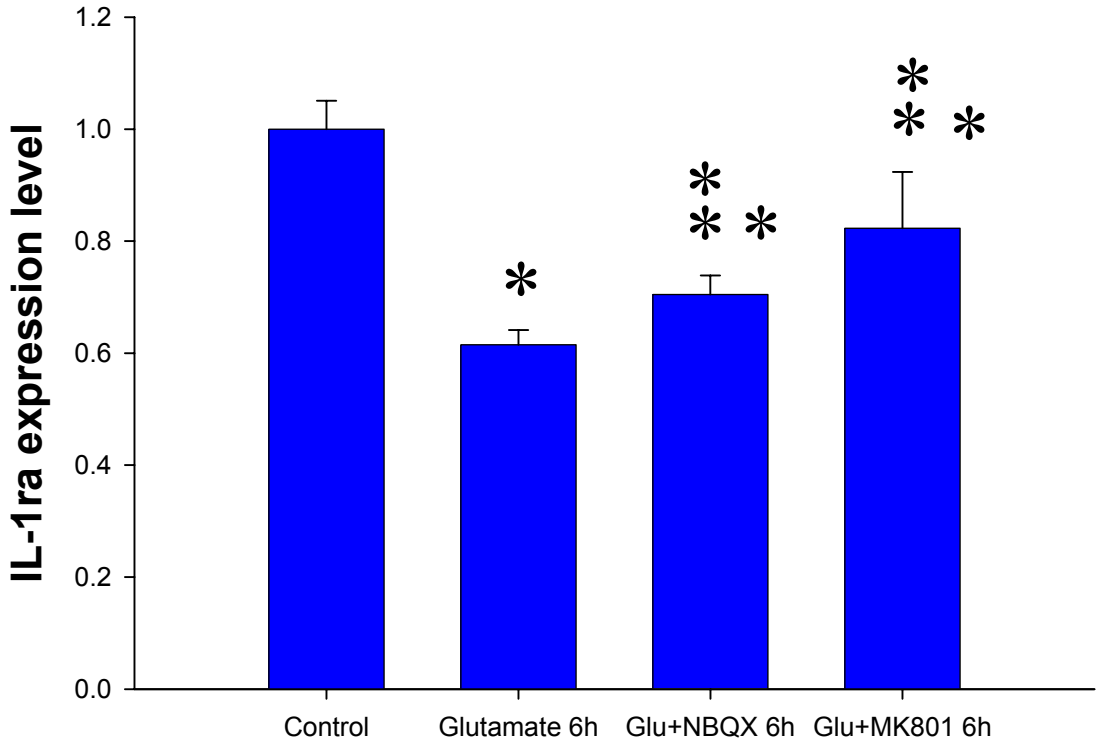


Fig. 10. Effects of MK801 and NBQX administration on IL-1ra expression. MK801 and NBQX were administered to determine if the usage of glutamate receptor antagonists can block the increase of IL-1ra expression. Spinal cords were taken at 6 hour after they were exposed to glutamate or glutamate plus MK801 or glutamate plus NBQX. IL-1ra was measured with an IL-1ra specific ELISA kit. A one-way ANOVA ($p<0.05$) followed by Bonferroni t-test was used to determine the statistics.* Statistically different from control or glutamate ($p<0.05$) and ** statistically different from glutamate ($p<0.05$)

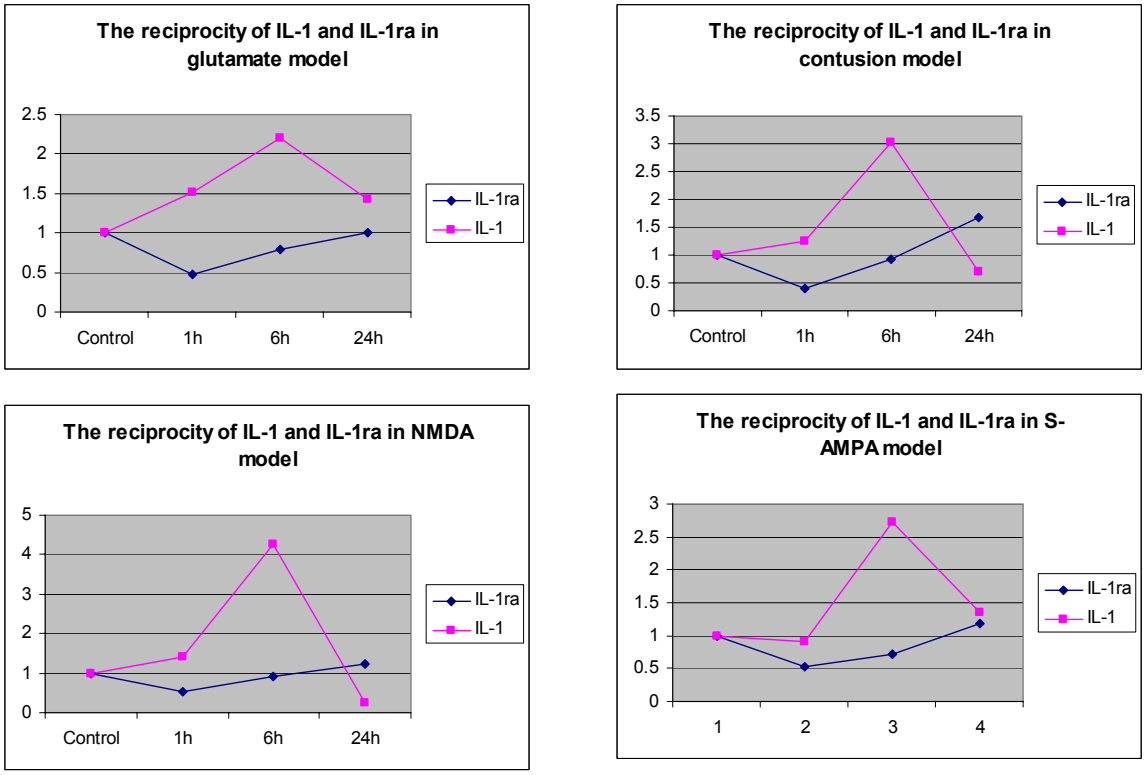


Fig. 11. The reciprocal pattern of IL-1β and IL-1ra levels in different glutamate agonist models and contusion spinal cord injury model. All models have similar patterns of IL-1β and IL-1ra expression. Data are from Figures 5-12. The levels of IL-1 and IL-1ra in control rats are normalized to 1. The actual concentration of IL-1 in control tissue is around 1.5 pg/200μg protein. The actual concentration of IL-1ra in control tissue is around 20pg/200μg protein.

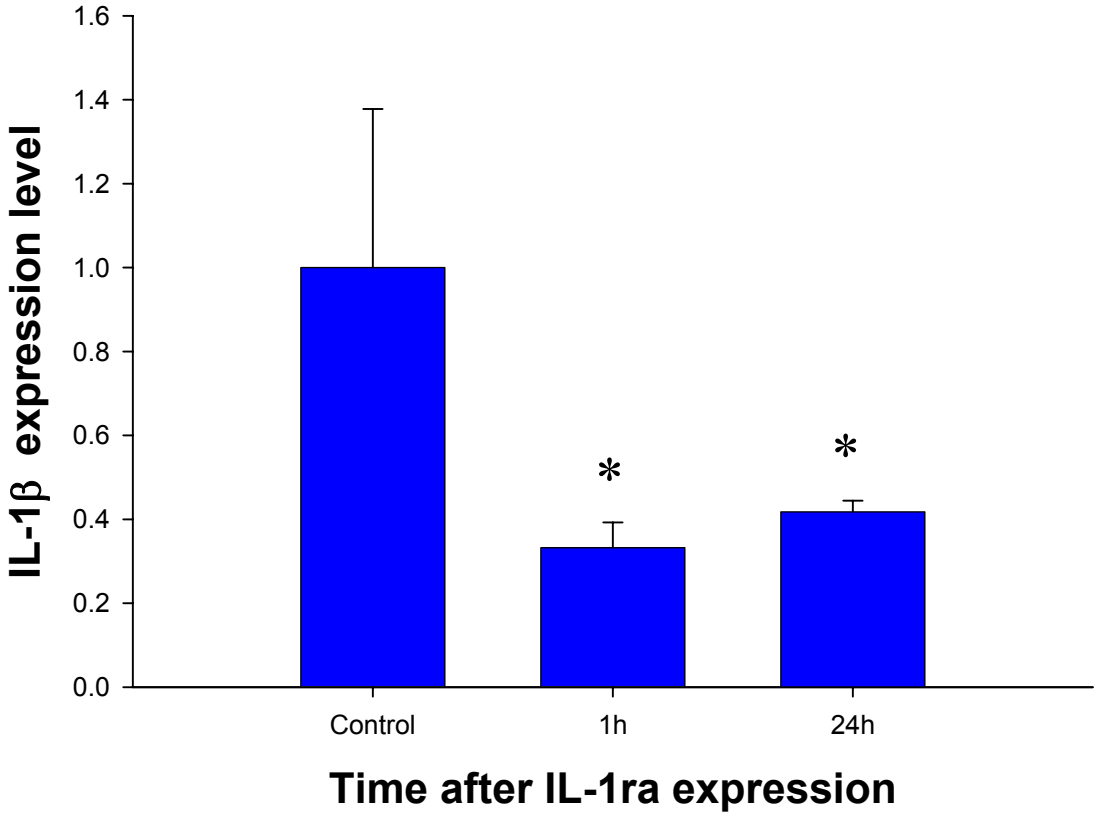


Fig. 12 The time course of ELISA measurements of IL-1β levels following IL-1ra administration. The level of IL-1β in sham rats (control) was defined as 1. All other IL-1β levels were normalized to the level of IL-1β in sham rats. A one-way ANOVA ($p<0.05$) followed by Bonferroni t-test was used to determine the statistics.* Statistically different from control ($p<0.05$)

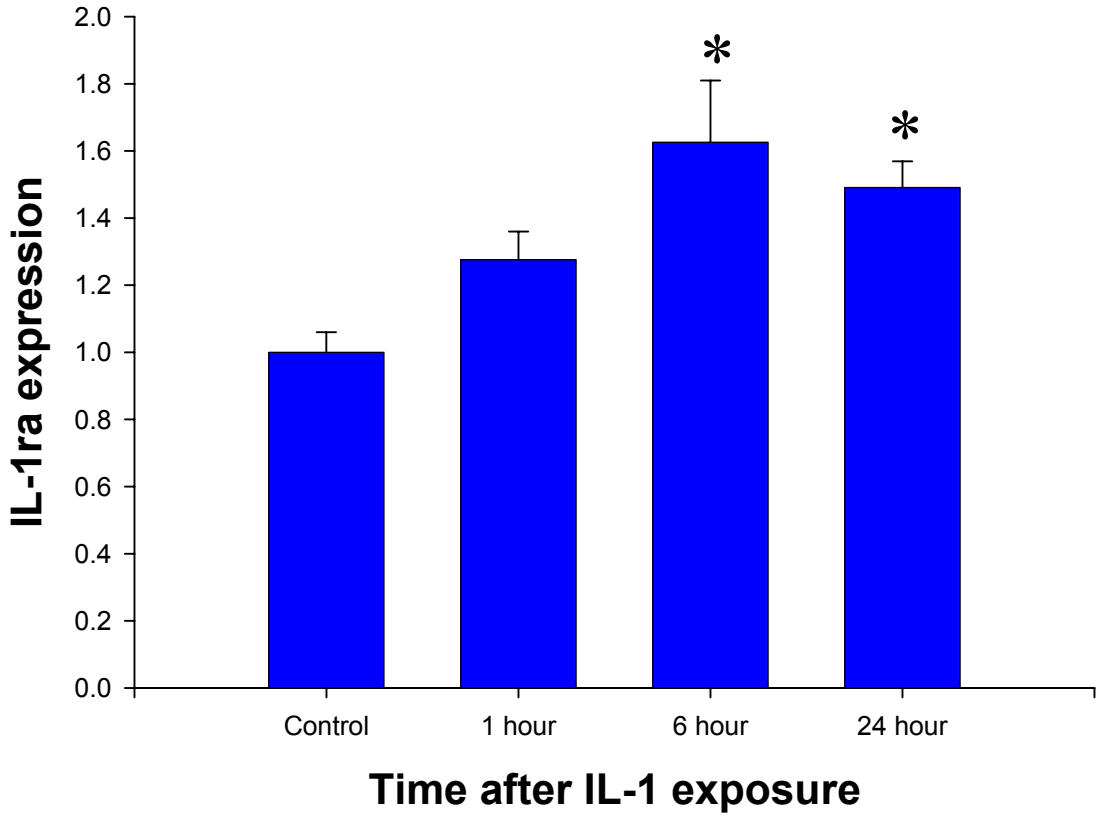


Fig. 13. The time course of measurement of the IL-1ra level after IL-1β exposure by ELISA. The rats were administered IL-1β or ACSF (control) for 1 hour. The IL-1ra level in sham rats was defined as 1. All other IL-1ra levels were normalized to the IL-1ra level in sham rats. A one-way ANOVA ($p<0.05$) followed by Bonferroni t-test was used to determine the statistics.* Statistically different from control ($p<0.05$)

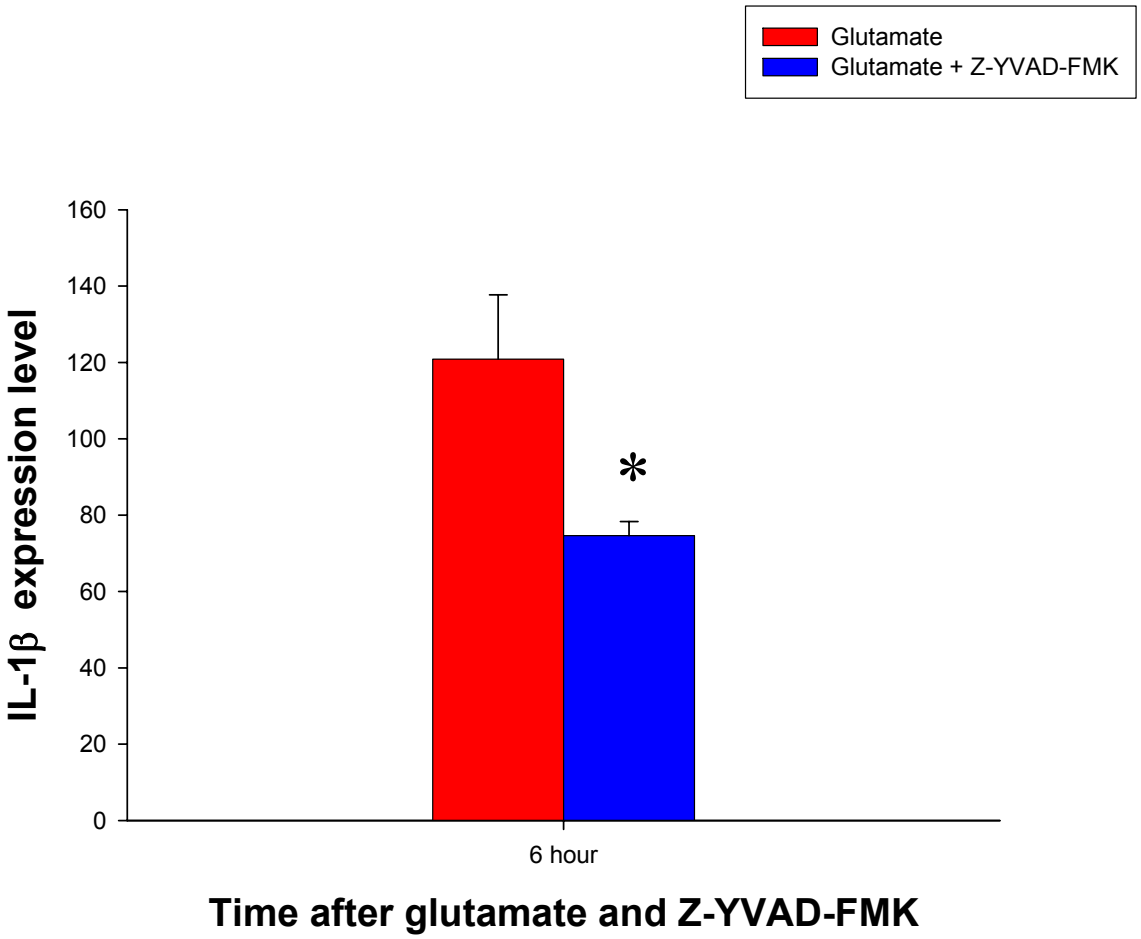


Fig. 14. Effect of Z-YVAD-FMK on IL-1β activation. The cord was exposed to glutamate together with a caspase-1 inhibitor, Z-YVAD-FMK. The administration of Z-YVAD-FMK blocks the activity of caspase-1, inhibiting the cleavage of IL-1β, which is essential to form the active form. The units of IL-1β expression are pg/ml tissue. The blockage of caspase-1 inhibits the formation of active IL-1β. The IL-1β expression levels are significantly different ($p<0.05$, t-test).

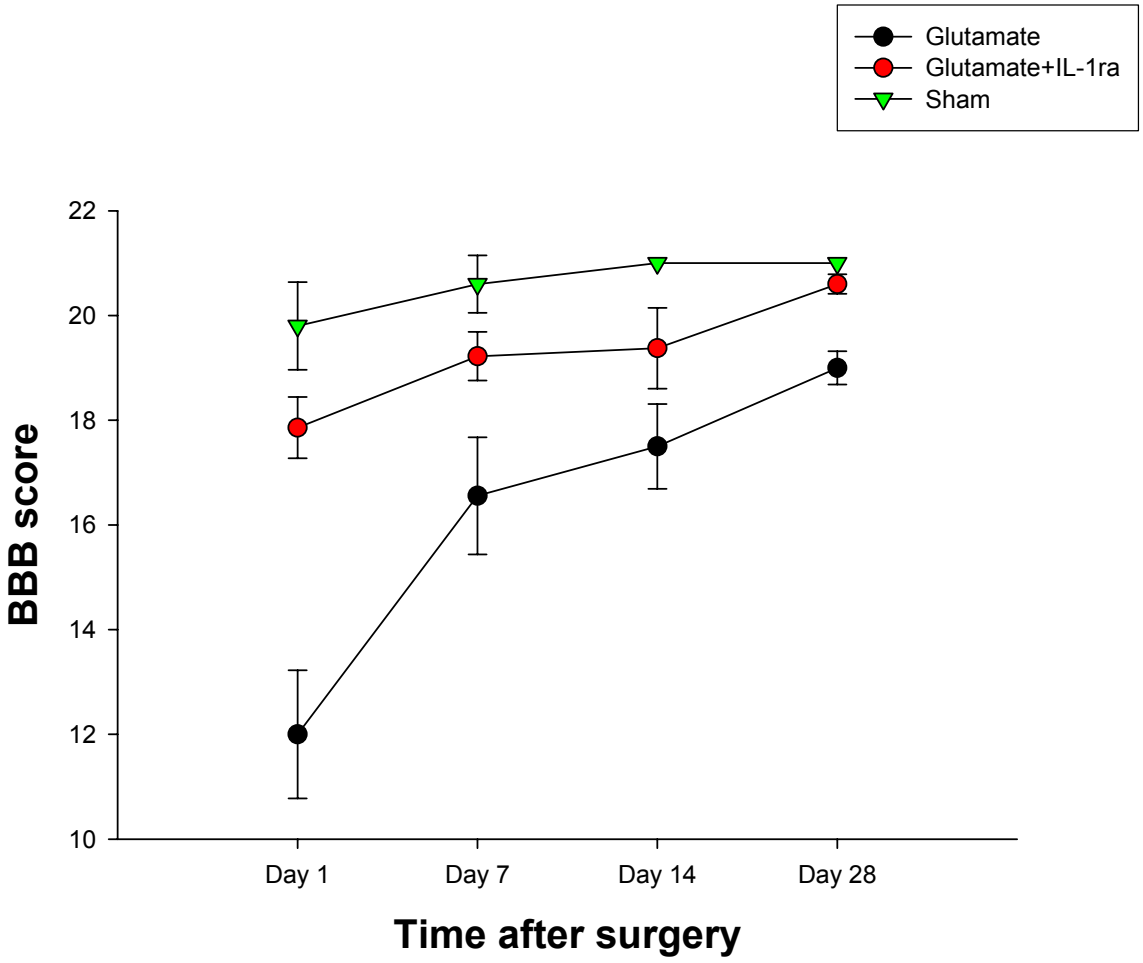


Fig. 15. BBB score showing the effect of IL-1ra on glutamate-induced SCI. BBB tests were done to study the effect of IL-1ra on glutamate-induced rat locomotive ability impairment. Repeated measures two-way ANOVA tests followed by Student-Newman-Keuls Method were used to determine statistics. The IL-1ra group showed significantly better recovery (values of all Glutamate+ IL-1ra are significantly different from the corresponding Glutamate time points, $p<0.05$).

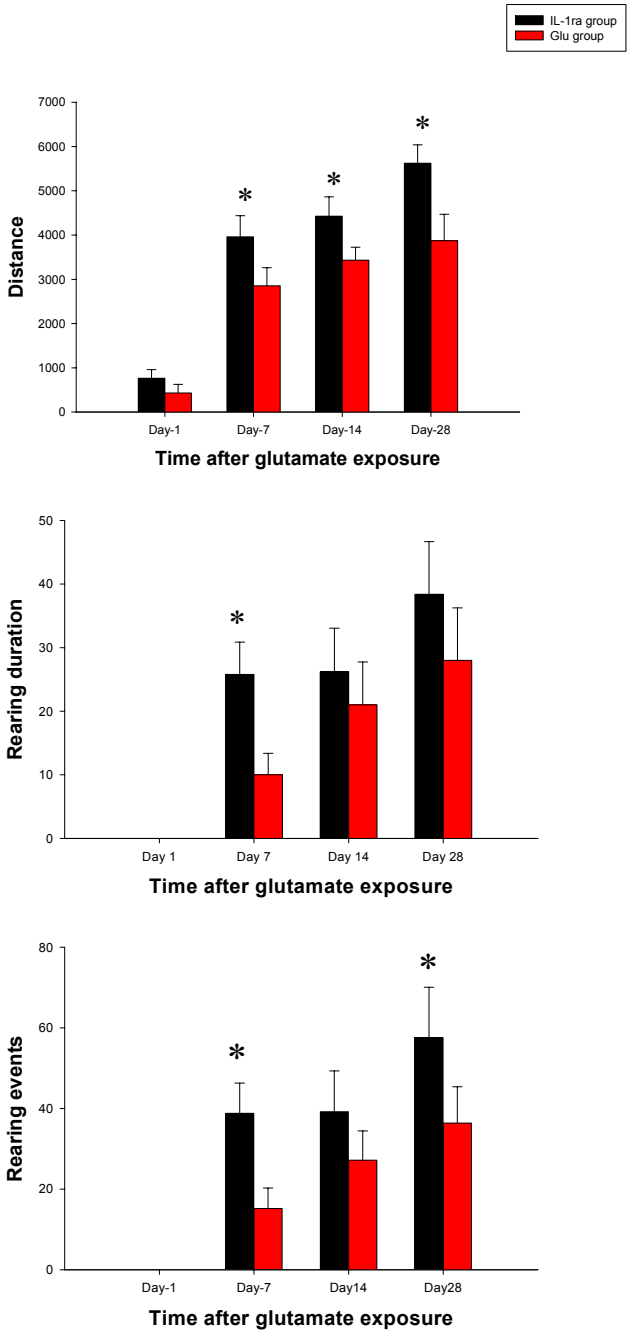


Fig. 16. The measurements of rat activity via PAS. The glutamate group was compared with the Glutamate+IL-1ra group. Three parameters are shown here: total distance traveled, rearing events and rearing time. Repeated measures two-way ANOVA tests followed by Student-Newman-Keuls Method were used to determine statistics. * statistically different on the same day ($p<0.05$)

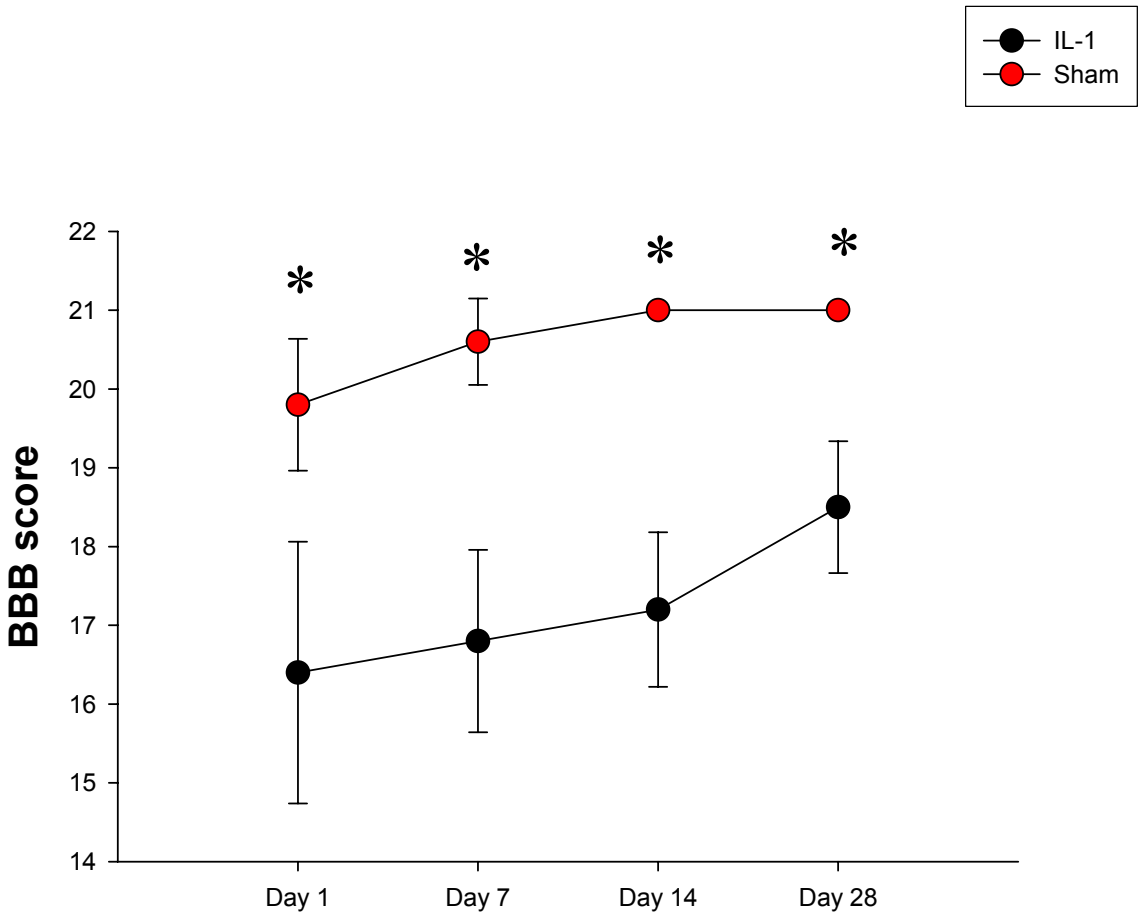
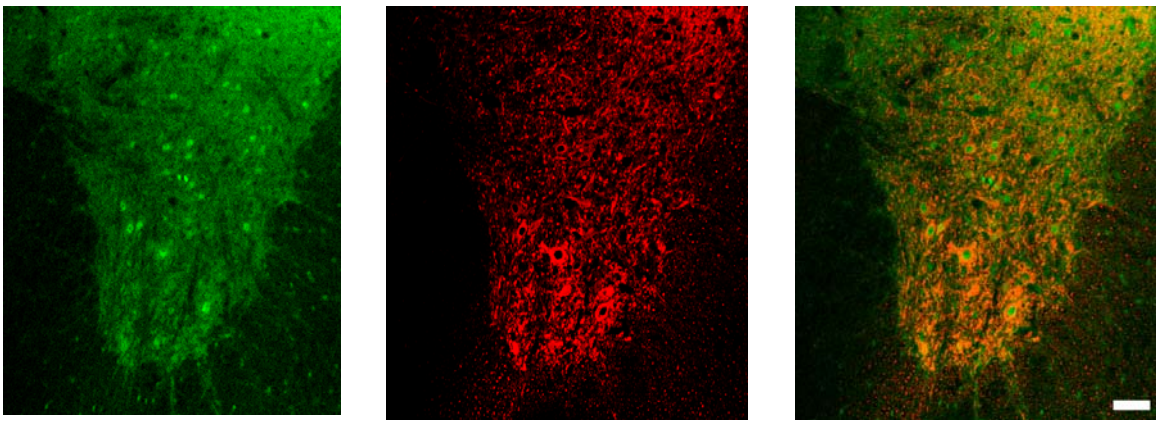
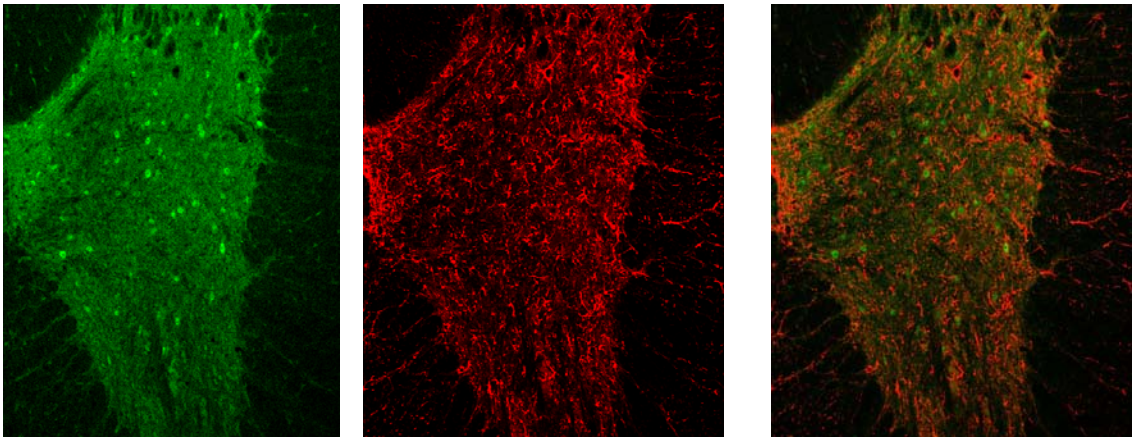


Fig. 17. BBB tests of the effect of IL-1β on rat locomotion. BBB tests were done to test whether IL-1β causes locomotor impairment. The results showed the administration of IL-1β itself caused rat function impairment (all time points are significantly different between treated and sham, $p<0.05$). Repeated measures two-way ANOVA tests followed by Student-Newman-Keuls Method were used to determine statistics.



IL-1β Neurofilament Merge

Fig. 18. Co-staining of IL-1β and neurons. Cord T10 that was exposed to 10mM glutamate was taken out at 6 hours after glutamate exposure. The cord slides were double-stained with anti-IL-1β antibody (left) and anti-neurofilament antibody (Middle). Merging the two images showed that IL-1β is in neurofilament staining positive cells, which are neurons.



IL-1β GFAP Merge

Fig. 19. Co-staining of IL-1β and astrocytes. Cord segments around T10 that were exposed to 10mM glutamate was taken out at 6 hours after glutamate exposure. The cord slides were double-stained with anti-IL-1β antibody (left) and anti-GFAP antibody (middle). Merging the two images showed that IL-1β is in GFAP-staining positive cells, which are astrocytes.

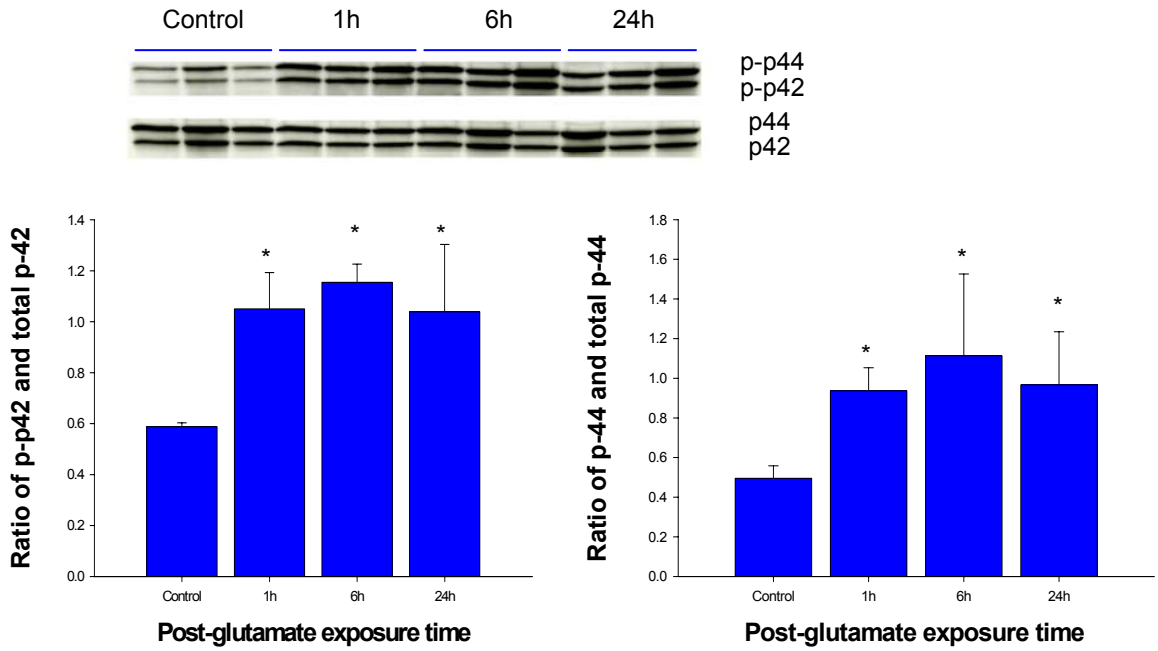


Fig. 20. Activation of ERK1/2 by glutamate. ERK (p44/42) were activated after glutamate exposure. The activation started from 1 hour after the exposure and lasted until 24 hours after the glutamate exposure. The bottom diagrams show the ratio of activated forms of p44/42 to total p44/42. T-test was used to determine the statistics.* Statistically different from control ($p<0.05$).

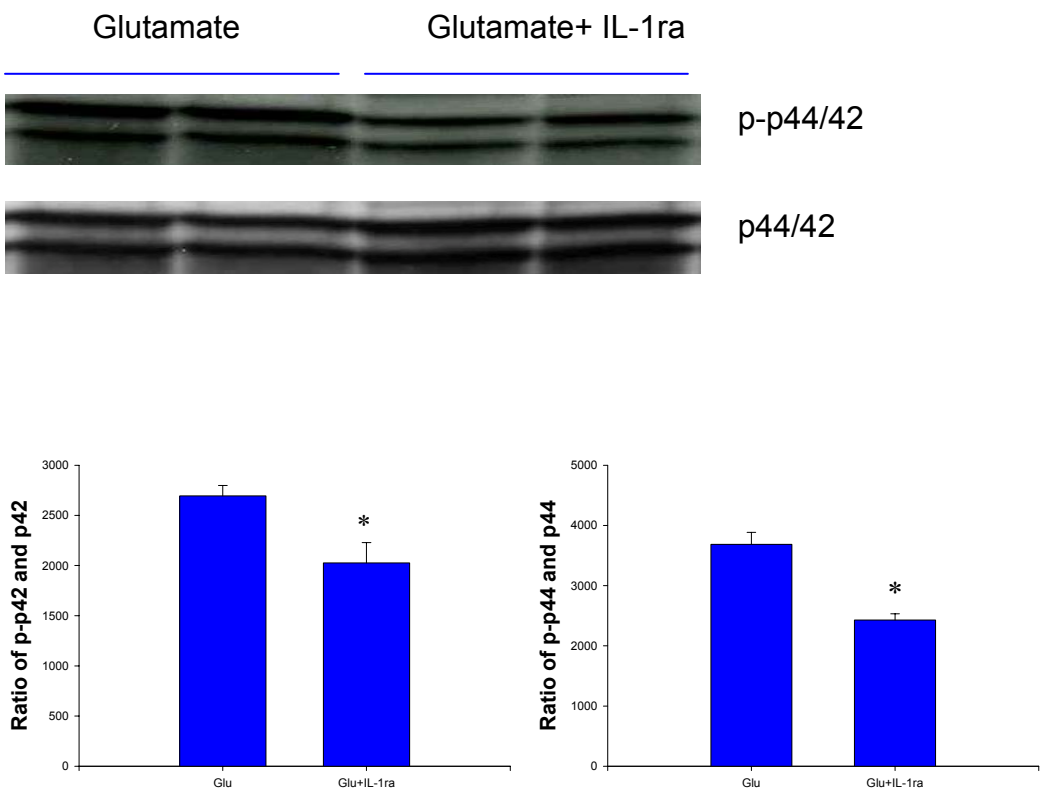
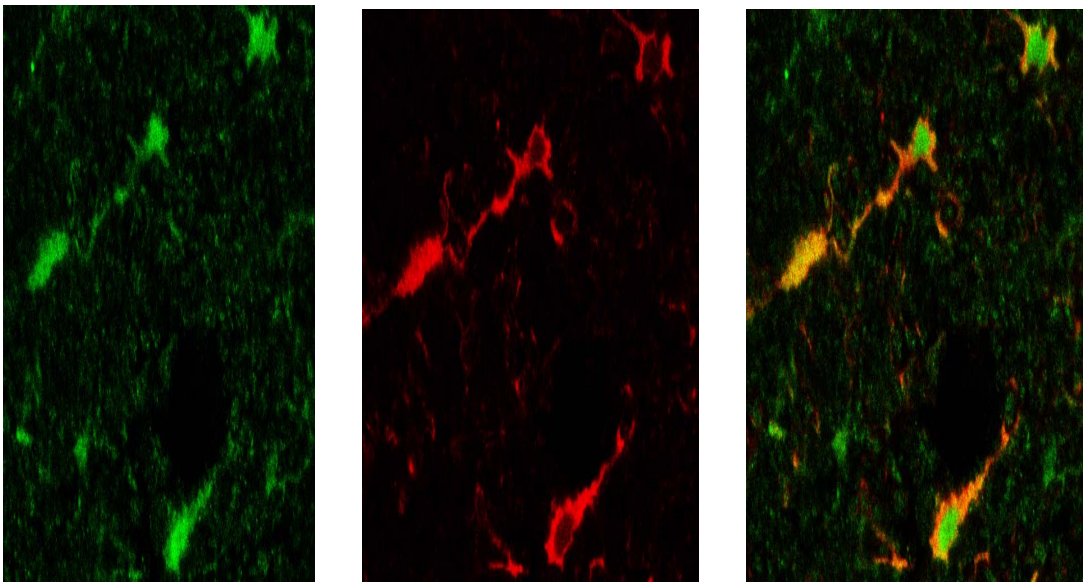


Fig. 21. Effects of IL-1ra on ERK activation. IL-1ra was applied on the cord together with glutamate. The administration of IL-1ra reversed the ERK activation induced by glutamate. * Statistically different from glutamate.



IL-1β **p-ERK** **Merge**

Fig. 22. Co-localization of IL-1β and p-ERK. Cords T10 that were exposed to 10mM glutamate were taken out at 6 hour after glutamate exposure for immunohistochemistry. Green shows the staining of IL-1β and red shows the staining of p-ERK. When they are merged, the yellow shows the co-localization of IL-1β and p-ERK.

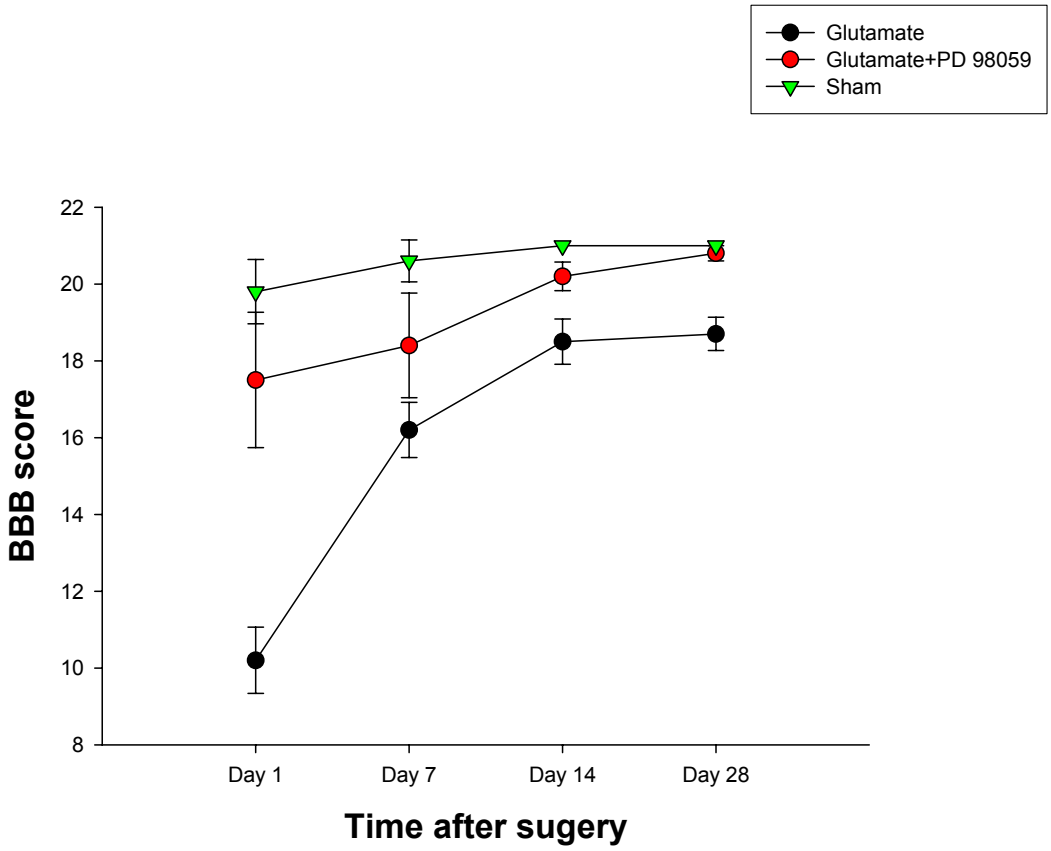


Fig. 23. BBB tests of the effect of PD 98059 on rat behavior. BBB tests were done to establish if the administration of ERK inhibitor (PD 98059) helps the rat recover from glutamate-induced locomotor function loss. Repeated measures two-way ANOVA tests followed by Student-Newman-Keuls Method were used to determine statistics. BBB scores on day 1 and day 28 show significant differences between glutamate group and PD 98059 group ($p<0.05$).

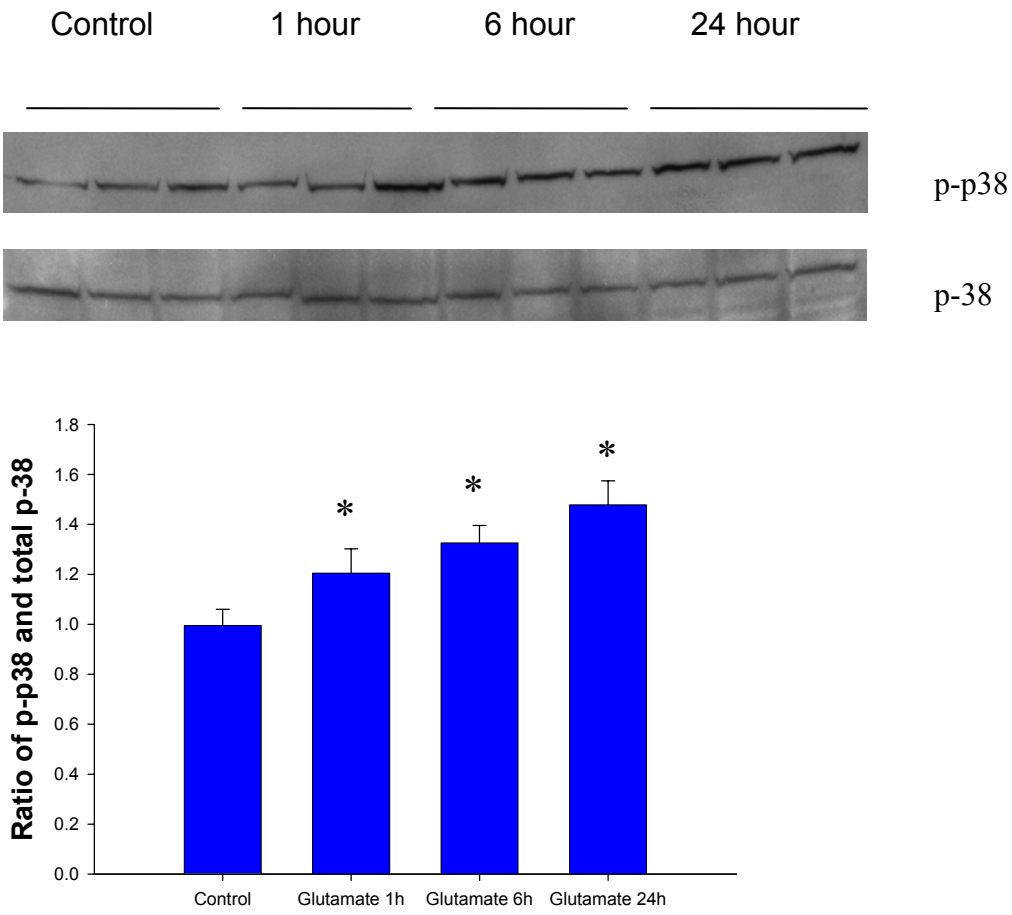
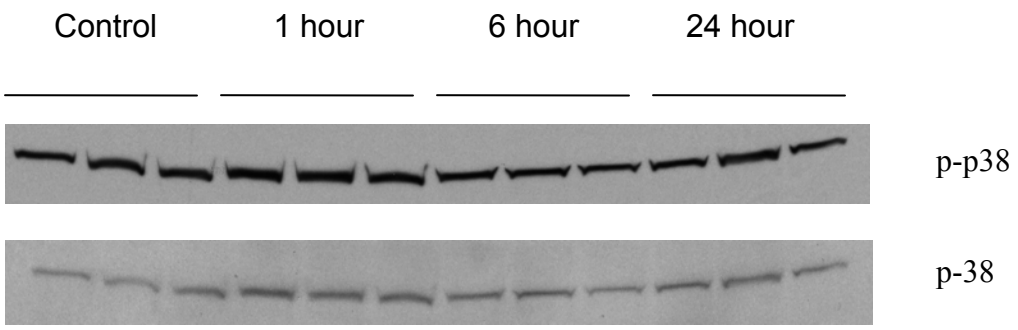


Fig. 24. Activation of p38 by glutamate exposure. p38 was activated after glutamate exposure. The activation started from 1 hour after the exposure and lasted until beyond 24 hours after the glutamate exposure. The bottom graphs show the ratio of activated forms of p-p38 to total p38. * Statistically different from control.



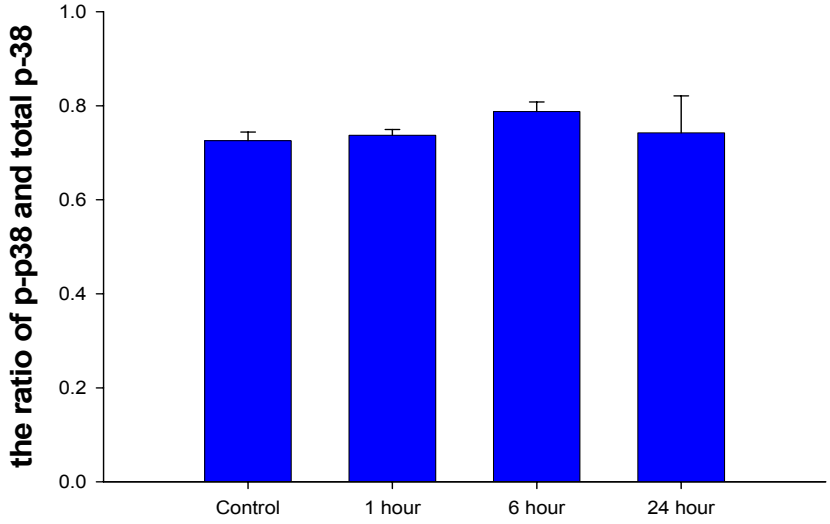
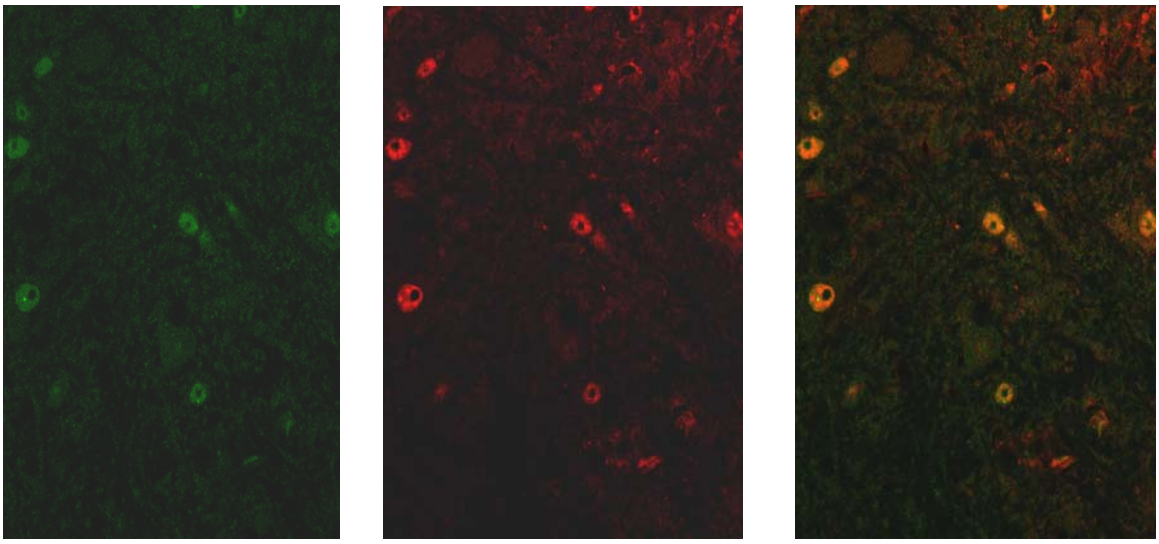


Fig. 25. Effect of IL-1ra on glutamate-induced p38 activation. IL-1ra was administered to the cord together with glutamate. When IL-1ra was co-administered with glutamate, there was no increase of p-p38, in contrast to the effect of administration of glutamate alone (Fig. 27). This indicates that IL-1ra blocked the p38 activation induced by glutamate.



IL-1β p-p38 merge

Fig. 26. Co-localization of IL-1β and p-p38. Cords T10 that were exposed to 10mM glutamate were taken out at 6 hour after glutamate exposure for immunohistochemistry. IL-1β and p-p38 are co-stained with an anti-IL-1β antibody and an anti-p-p38 antibody. The green staining is IL-1β and the red staining is p-p38. The merged picture shows the co-localization of these two molecules.

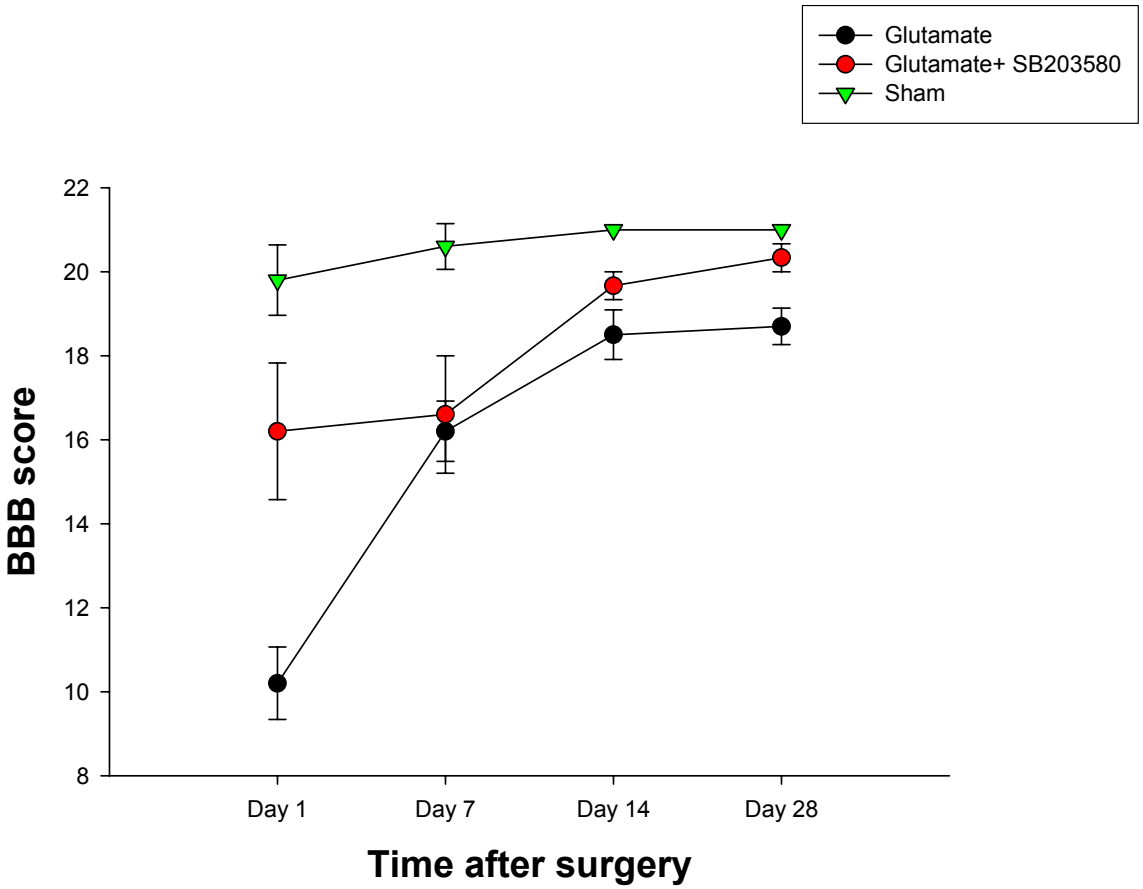


Fig. 27. BBB tests of the effect of SB 203580 on glutamate-induced SCI. The BBB test was done to test the effect of the p38 inhibitor (SB 203580) on glutamate-induced locomotor function loss. Repeated measures two-way ANOVA tests followed by Student-Newman-Keuls Method were used to determine statistics. The SB 203580 group showed significantly better performance than the glutamate group on day 1 and day 28 ($p<0.05$).

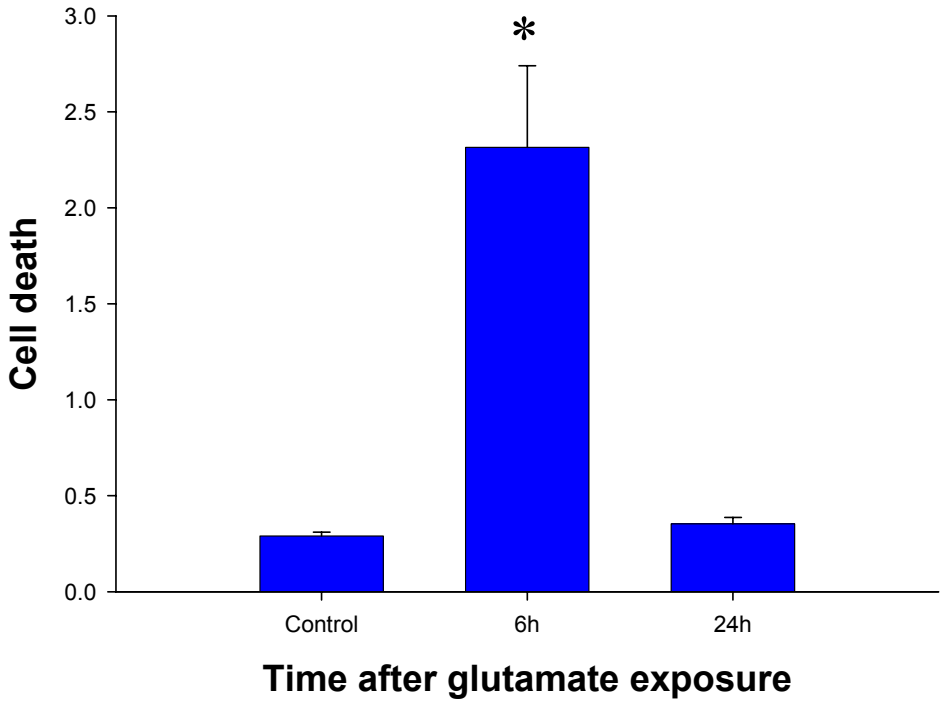


Fig. 28. Apoptosis after glutamate exposure. Cell death after glutamate exposure was tested with a cell-death detection kit. This kit measures the apoptosis that followed glutamate exposure. The results showed that at 6 hours after glutamate administration, apoptosis reached its peak and then decreased substantially by 24 hours. A one-way ANOVA ($p<0.05$) followed by Bonferroni t-test was used to determine the statistics.* statistically different from control ($p<0.05$)

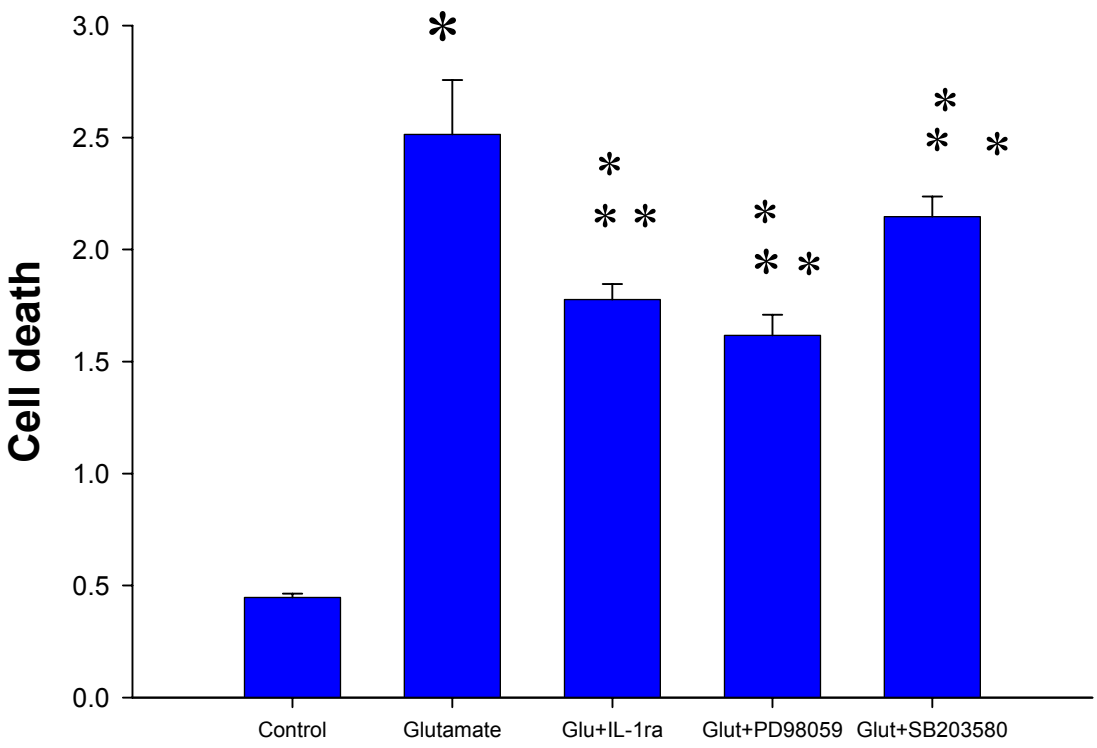


Fig. 29. Effect of IL-1ra, PD 98059 and SB 203580 on apoptosis induced by glutamate. To explore the mechanism of apoptosis after glutamate exposure, several pathways were targeted. IL-1ra, PD98059 or SB203580 were applied with glutamate. All three agents significantly reduced glutamate-induced cell death. These results showed that IL-1ra, PD98059 and SB203580 can reduce the apoptosis caused by glutamate, evidence that IL-1, ERK and p38 are involved in the pathways that lead to apoptosis from glutamate exposure. A one-way ANOVA ($p<0.05$) followed by Bonferroni t-test was used to determine the statistics.* Statistically different from control or glutamate ($p<0.05$) and ** statistically different from glutamate ($p<0.05$)

CHAPTER 4: DISCUSSION

SELECTION OF A SPINAL CORD INJURY MODEL

Most human spinal cord injuries involve contusions, so most investigators use contusion models to study SCI. Contusion injuries to rats, mice, cats and sheep have all been used in SCI studies. Among them, rats are most widely used presently (Yeo et al., 1975; Fawcett, 1998; Taoka and Okajima, 1998; Basso, 2000; Rosenzweig and McDonald, 2004; Stokes and Jakeman, 2002; Wrathall, 1992). Weight-drop contusion animal models are commonly used to study the pathophysiology of and genetic responses to spinal cord injury (Gruner et al., 1992). However, recently researchers are switching to the IH impactor model which controls the impact due to its better reproducibility, greater precision and more flexible adjustability of injury parameters (Scheff et al., 2003).

Toxic concentrations of glutamate are released into the extracellular space of the white and gray matter (Xu et al., 2000; Liu et al., 1991; 1999) of the spinal cord following SCI. Extensive evidence shows that glutamate released after SCI contributes substantially to secondary damage and permanent impairments (Li, et al., 2000; McAdoo et al., 1999, 2000, 2005; Liu et al., 1991, 1999; Panter et al., 1990; Xu et al., 2004). Antagonists (NBQX or MK801) to glutamate receptors protect after SCI (McDonald et al., 1998; Rosenberg et al., 1999; Li et al., 1999; Wrathall et al., 1994, 1996, 1997, Faden et al., 1988; 2001), further evidence for contributions of glutamate to the damage resulting from SCI. To improve treatments for SCI, it is urgent to further understand the mechanisms of the actions of glutamate in SCI, particularly how exposure of the spinal cord to elevated glutamate causes functional impairments. In the contusion model, mechanical damage as well as a variety of chemical insults occurs, confounds that make that model inappropriate to study the effects of glutamate. Administering glutamate into the spinal cord by microdialysis at a concentration and duration that approximate closely the glutamate concentration released after SCI is a glutamate model that has been used in our lab (Xu et al., 1998; 2000). BBB tests showed impairment of locomotor function after administration of glutamate (Xu et al., 1998; 2000). However, the disadvantage of this

microdialysis model is that the insertion of the microdialysis fiber produces mechanical damage to the spinal cord, although a sham control is included to deduct the fiber damage. Therefore, to study the role of glutamate in SCI, we introduced a new glutamate administration model. In this study I put solutions of glutamate and other agents in a depression on the top of the exposed cord after the dura was opened. Compared to our previous microdialysis fiber model, this model provides exposure to glutamate and other administered agents, but avoids the mechanical damage of the former. The peak extracellular glutamate concentration in the spinal cord after SCI is about 500 μM (Xu et al., 1998). Glutamate diffuses in the cord with a concentration decrease of about 6 fold per millimeter (McAdoo et al., 1999). The diameter of the spinal cord at T10 is around 3 mm. To reach the glutamate concentration present in cords following injury (ca. 500 mM), we put 10 mM glutamate on top of the cord. Based on the concentration gradient of glutamate administered into the cord (McAdoo et al., 1999), the concentration of glutamate achieved at about 1.5 mm into the cord, i.e., about the middle of the sections taken, by administration of 10 mM glutamate to the top of the cord equals that released by injury. The peak release of glutamate maximizes in the first few minutes after injury (McAdoo et al., 1999). By one hour after SCI, the glutamate concentration is substantially decreased, but remains higher than the control level for as much as a few hours. Considering that the peak of glutamate in SCI occurs within 1 hour after the time of impact, we put 10 mM glutamate on top of the cord for 1 hour and then removed it to mimic the actual time course of glutamate elevation in response to contusion.

EFFECTS OF GLUTAMATE AND ITS AGONISTS AND ANTAGONISTS ON IL-1 BETA AND IL-1RA

The involvement of IL-1β in inflammatory diseases has been widely studied (Wood et al., 1985; Pettipher et al., 1986; Farahat et al., 1993; Arend et al., 2002). Several reports indicate that IL-1β also has important roles in SCI. IL-1β has elevated expression of both its mRNA and protein levels following SCI (Hayashi et al., 1997; Nesic et al., 2001; Wang et al., 1997; Bartholdi et al., 1997; Tonai et al., 1999). The

increased expression of IL-1β contributes to the inflammatory response in SCI (Pan et al., 2002) and to cycloxygenase-2 production (Tonai et al., 2002). Up-regulation of the IL-1β receptor type 1 in SCI (Wang et al., 2006) is another indication that IL-1β is important in SCI, as this would amplify the effects of IL-1β. Methylprednisolone, an agent which ameliorates SCI clinically, inhibits production of IL-1β in the spinal cord following compression injury in rats (Fu et al., 2005). In human SCI case studies, increased IL-1β was detected immunohistochemically in neurons beginning at a half hour after initial injury and returned to normal at the end of two days (Yang et al., 2004). IL-1ra is a natural IL-1 receptor antagonist with anti-inflammatory characteristics (Arend et al., 2002). IL-1ra has been used in rheumatoid arthritis treatment and other inflammatory diseases because of its anti-inflammation characteristics, which suggest that it has a protective role. The balance between IL-1 and IL-1ra is very important in maintaining normal physiological circumstances (Arend et al., 2002; Mee et al., 2005). Administration of IL-1ra to the cord prevents apoptosis and caspase-3 activation after SCI (Nesic et al., 2001), further evidence for a protective function for IL-1ra. Previous study of IL-1ra in SCI is restricted to the exogenous administration of IL-1ra to check the effects of IL-1ra on outcomes of SCI (Nesic et al., 2001). IL-1ra is a natural antagonist of IL-1 receptors, so exploring its endogenous functions would be very helpful in understanding the role of IL-1β in SCI, including secondary damage by glutamate. Therefore I also investigated changes in IL-1ra in response to SCI.

My results placed the peak of IL-1β protein concentration at around 6 hours after glutamate administration, with return to near normal at 24 hours (Fig. 1). IL-1ra expression decreased to a minimum at about 1 hour after glutamate was removed from the spinal cord and increased to normal by 24 hour (Fig. 2). This demonstrates that the administration of glutamate alone induces IL-1β production and suppresses IL-1ra production, evidence for a role of IL-1β in the glutamate-mediated components of the inflammatory response to SCI.

To extend our understanding of the roles of IL-1β and IL-1ra in SCI and establish the relevance of the effects of glutamate on IL-1β and IL-1ra to SCI, I also measured the levels of IL-1β and IL-1ra in a contusion SCI model. My results showed that the peak of the IL-1β protein level in the contusion model was around 6 hours after SCI with return to normal at 24 hours (Fig. 3), which is consistent with previous findings (Nesic et al., 2001). IL-1ra expression again decreased starting 1 hour after contusion injury to the spinal cord, and returned to normal at 24 hour (Fig. 4), which is quite similar to the results for glutamate-induced IL-1ra expression. Thus the effects of SCI on the expression of IL-1β and IL-1ra parallel those of glutamate, consistent with glutamate-induced IL-1β expression contributing to the former.

Extensive studies show the involvement of NMDA glutamate receptors in SCI (Agrawal et al., 1997; Bennett et al., 2000; Eide et al., 1995; Faden et al., 1988; Grossman et al., 2000; Haghighi et al., 1996). NMDA receptors are activated following SCI (Ye et al., 1996; Grossman et al., 2000). The administration of MK801 (an antagonist of NMDA receptors) to the cord improves rat behavior and biochemical outcome following SCI or brain injury (Faden et al., 1988; Mcintosh et al., 1999; Lucas et al., 1990; Hao et al., 1992; Hashishi et al., 1996). Systemic administration of MK801 to the cat improved motor recovery after SCI and significantly reduced edema formation at the injured site without altering spinal cord blood flow or vascular permeability at that site (Yanasi et al., 1995). A NMDA receptor ion-channel blocker, aptiganel hydrochloride, has been developed to protect from acute CNS injury (McBurney et al., 1997). NMDA receptors are also involved in chronic pain following SCI (Willis et al., 2001; Woods et al., 2006; Lipton et al., 2006). In addition, central dysesthesia after traumatic spinal cord injury is dependent on N-methyl-D-aspartate receptor activation (Eide et al., 1995). Systemic administration of MK801 ameliorates a chronic allodynia-like response in spinally injured rats (Hao et al., 1996). I found that the administration of glutamate to the cord induced the production of IL-1β. Since the NMDA receptor is a glutamate receptor, I determined if it is involved in the activation of IL-1β and

suppression of IL-1ra. One way to test this is to administer a NMDA agonist to the spinal cord and see if it affects the expression of IL-1β and IL-1ra. Administration of NMDA, an agonist of the NMDA receptor, to the cord increased the production of IL-1β and decreased that of IL-1ra (Fig. 5 and 6), similar to such changes in response to contusion injury and glutamate administration, consistent with those insults producing damage through the NMDA receptor. I also applied MK801 to the cord together with glutamate to check if blocking the NMDA receptor reduces the induction of IL-1β and opposes the suppression of IL-1ra by glutamate. Administration of MK801 to the cord partially reversed the glutamate-induced IL-1β activation and IL-1ra suppression (Figs. 9 and 10). This experiment confirms that activation of the NMDA glutamate receptor by glutamate contributes to the SCI-induced elevation of IL-1β and decrease in IL-1ra, and thereby also to secondary injury.

The importance of the NMDA receptor in SCI has been studied for a long time. However, recently attention has also been drawn to the roles of AMPA receptor in SCI (Agarawal et al., 1997; Liu et al., 1997; Wrathall et al., 1997; Abraham et al., 2001; Li et al., 2000; Bennett et al., 2000). The AMPA/kainate antagonist NBQX reduces tissue loss and functional impairment after spinal cord trauma in a dose-dependent manner (Wrathall et al., 1994). Systemic administration of NBQX ameliorates rat functional deficits caused by spinal cord trauma (Wrathall et al., 1996), suggesting clinical potential. In my study I measured the expression of IL-1β after exposure of the cord to S-AMPA, an agonist of the AMPA receptor. Administration of S-AMPA to the cord initially increased the production of IL-1β and decreased the level of IL-1ra (Fig. 7 and 8), consistent with previous findings that the AMPA receptor is important in the toxicity of glutamate in SCI. Administration of NBQX to the cord together with glutamate partially blocked the induction of IL-1β and suppression of IL-1ra by glutamate (Figs. 9 and 10). The latter results confirm that the AMPA receptor is also involved in the glutamate-induced IL-1β activation, indicating a role of the AMPA as well as the NMDA receptor in the

inflammatory responses induced by glutamate. This is consistent with previous demonstrations that the AMPA receptor is also important in SCI.

The secondary injury that follows initial SCI is generated by ischemia, free radical release, inflammation, glutamate excitotoxicity, cytoskeletal degradation and apoptosis (Park et al., 2004). None of these events happen independently, and they interact. The failure of the production of sufficient ATP after SCI leads to a calcium influx, which in turn triggers the rise of the extracellular concentration of glutamate (Klussmann et al., 2005; Hulsebosch et al., 2002). The production of free radicals and reactive oxygen species leads to lipid peroxidation, which contributes to apoptosis or necrosis caused by glutamate excitotoxicity (Klussmann et al., 2005; Hulsebosch et al., 2002). My results connect glutamate excitotoxicity to IL-1β activation, which in turn connects it to inflammation. The changes in IL-1β and IL-1ra in response to glutamate receptor agonists and antagonists, indicate roles of the IL-1 system in the contributions of glutamate to SCI. This extends the range of the known mechanisms of glutamate toxicity. These results also extend our detailed knowledge of the roles of endogenous IL-1ra in SCI, which was little explored previously. The reduction of IL-1β in response to IL-1ra (Fig. 12) and increase in IL-1ra in response to increased IL-1β (Fig. 13) demonstrate the operation of a natural neuroprotective system in response to SCI.

RECIPROCITY OF IL-1 BETA AND IL-1RA PRODUCTION IN RESPONSE TO CONTUSION, GLUTAMATE AND GLUTAMATE RECEPTOR AGONISTS

To further investigate the endogenous neuroprotection against IL-1β and glutamate toxicity in SCI, I compared the expression patterns of IL-1β and IL-1ra induced by contusion SCI, by glutamate and by glutamate agonists by plotting those concentrations together following the same treatments (Fig. 11). A reciprocal pattern of opposite changes was found between the IL-1β and IL-1ra expression levels. In all experiments (Fig. 11) IL-1β started to rise while IL-1ra was declining and began to decline after IL-1ra had began to rise. IL-1ra always started to rise about an hour after IL-1β did. Thus IL-1ra appears to increase in response to elevation of IL-1β and IL-1β to

decrease in response to rising IL-1ra, further evidence for feedback regulation of the level of IL-1β by IL-1ra. Counter-regulation of IL-1α and IL-1ra in murine keratinocytes has been reported (Mee et al., 2005). However, reciprocity between IL-1β and IL-1ra in SCI has not been reported previously, nor to our knowledge has such reciprocity been reported in regard to any CNS trauma.

The balance between IL-1β and IL-1ra in local tissues or organs is important in the susceptibility to severe diseases and the maintenance of normal function (Arend et al., 2002). The reciprocity between IL-1β and IL-1ra levels in my results demonstrates the operation of mechanisms to restore and maintain a balance between these two molecules and indicate opposed roles of IL-1β and IL-1ra in glutamate-induced SCI.

The results in Figs. 12 and 13, decreases in IL-1β in response to IL-1ra administration and increases in IL-1ra in response to IL-1β administration, are further evidence for reciprocal interactions between IL-1β and IL-1ra and confirm that there is mutual regulation between IL-1β and IL-1ra. The administration of IL-1β induced the production of IL-1ra (Fig. 12), probably as part of a negative feedback mechanism that reduces the production of IL-1β following SCI. The suppression of the expression of IL-1β by IL-1ra (Fig. 12) is likely part of the mechanism by which administration of exogenous IL-1ra prevents apoptosis and caspase-3 activation (Nesic et al., 2001). After the cord was exposed to glutamate, the IL-1ra concentration decreased to a minimum at 1 hour, in turn reducing the suppression of IL-1β production, allowing IL-1β production to reach its maximum at 6 hours after the administration of glutamate. The increase of IL-1β stimulates the production of IL-1ra, which in turn helps IL-1β production to return to normal (Fig. 12, 13 and 14). For about the first hours after SCI, IL-1β production increases and IL-1ra production is decreased. As a result, more IL-1β binds to IL-1 receptors in that period, which should enhance inflammatory responses in SCI. The decrease of IL-1ra soon after glutamate administration also probably contributes to the damage induced by glutamate. However, beyond 6 hours IL-1ra is increasing and IL-1β decreasing, changes that should reduce the damage due to IL-1β. These results add more

information about how the IL-1β system works in SCI and help to reveal why administration of IL-1ra after SCI decreases impairment caused by SCI.

My results show that IL-1β contributes to SCI by actions on its receptor. Up-regulation of the IL-1β receptor type 1, which is the only functional IL-1 receptor (Wang et al., 2006) in SCI, could also contribute to the IL-1β-mediated inflammatory response after SCI. The IL-1β receptor type 2 could also play a protective role in SCI, because the presence of this decoy receptor prevents the binding of IL-1β to its receptors, i. e., the IL-1 receptor also act in opposition to each other (Oprica et al., 2003). Therefore like IL-1ra, the IL-1 type 2 receptor may be a natural regulator of IL-1β. IL-1ra counteracts the actions of IL-1β; thus the actions of IL-1β are naturally attenuated both by IL-1ra blocking the access of IL-1β to its receptor and by IL-1ra controlling the expression of IL-1β. This work demonstrates the operation of endogenous protective systems following SCI, potentially fruitful processes for future investigation including translational applicability.

ADMINISTRATION OF IL-1 BETA IMPAIRED RAT LOCOMOTOR FUNCTION AND ADMINISTRATION OF IL-1RA IMPROVED RAT LOCOMOTOR FUNCTION

As just described, reciprocal interactions between IL-1β and IL-1ra occur in contusion and glutamate-induced SCI. I performed behavior tests after administering exogenous IL-1β and IL-1ra to different groups of rats to reveal the significance to SCI impairments of the IL-1β increase and IL-1ra decrease and their reverse after contusion injury and glutamate exposure. The BBB test is a standardized locomotor rating scale that is widely used in SCI research (Basso et al., 1995). My BBB results show that the administration of IL-1β impairs rat locomotor function, and that this impairment lasts for at least 28 days after IL-1β exposure (Fig. 17). The administration of IL-1ra along with glutamate improves rat locomotor function impaired by glutamate (Fig. 15). My BBB results are consistent with previous findings that IL-1β is a mediator of SCI and IL-1ra is protective against neuronal damage (Nesic et al., 2001).

Motor neurons around spinal cord segment T10 innervate muscles are involved in rearing, so their loss upon glutamate exposure may be involved in the diminition of rearing time and rearing events. Therefore measuring the numbers of rearing events or rearing time of rats provides additional indicators of impaired function. The PAS measurements are totally objective methods to evaluate rat recovery. They provide six parameters: total beams broken, rearing time, rearing events, total distance traveled, active time and resting time. I did PAS measurements on two groups of rats, a glutamate treated group and a glutamate + IL-1ra group, to study the effect of IL-1ra on glutamate-induced SCI. Rats that were administered IL-1ra with glutamate showed more rearing events and increased rearing times compared to rats which were treated with glutamate alone (Fig. 16). These results indicate the improvement of rat locomotor function by administering IL-1ra, consistent with my BBB results. In addition to the better rearing performance, rats in the glutamate-IL-1ra group traveled farther than rats in the glutamate group (Fig. 16), confirming a protective effect of IL-1ra administration after exposure to glutamate.

Intrathecal IL-1β administration induces thermal hyperalgesia by activating expression of inducible nitric oxide synthase (Sung et al., 2004). Injection or overexpression of IL-1ra significantly inhibits neuronal damage induced by focal cerebral ischemia in traumatic brain injury (Touzani et al., 1999). Intrathecal IL-1ra, in combination with the soluble tumor necrosis factor receptor, shows an anti-allodynic action in a rat neuropathic pain model (Sweitzer et al., 2001). Thus my results regarding deleterious effects of IL-1β and protective effects of IL-1ra are generally consistent with those obtained by others.

IL-1 BETA CELLULAR LOCALIZATION

Different cells in the CNS have different functions. Therefore it is important to identify the IL-1β-producing cells. To establish the cellular localization of IL-1β, I performed immunohistochemistry with an anti-IL-1β antibody and cell marker antibodies to neurofilament proteins or GFAP. My results show that IL-1β is co-localized with

neurofilaments, demonstrating the localization of IL-1β to neurons. Neurons play major roles in locomotor function, so this localization is consistent with our finding that administration of IL-1β impairs rat locomotor function. Astrocyte activation plays an important role in SCI (Gordh et al., 2006; Emirandetti et al., 2006). My results show that low levels of IL-1β may be present in GFAP positive cells, demonstrating the existence of IL-1β in astrocytes, which is consistent with previous findings (Hebert et al., 2005; Churchill et al., 2005; Zhang et al., 2006). IL-1RI has been reported in neurons, oligodendrocytes, astrocytes, and activated microglia, and IL-1R1 mediates the response of cells to IL-1β (Wang et al., 2006). Taking my results and previous findings together, IL-1β and IL-1R1 reside in the same types of cells, suggesting an "autocrine" mechanism (a cell secretes a chemical messenger that signals the same cell), which was proposed previously (Wang et al., 2006).

ACTIVATION OF MAPKS IN GLUTAMATE-INDUCED SCI WAS IL-1 BETA DEPENDENT

MAPKs are major regulators of apoptosis in SCI (Namgung and Xia et al., 2001; Nath et al., 2001; Nozaki et al., 2001; Zou et al., 2002). They are activated in SCI by glutamate release, free radical generation and inflammation (Wang et al., 2006; Nath et al., 2001; Nozaki et al., 2001; Zou et al., 2002). ERK1/2 and p38 mitogen-activated protein kinases mediate iNOS-induced spinal neuron degeneration after acute SCI (Xu et al., 2006). Increases in the activated forms of ERK 1/2, p38 MAPK and CREB are correlated with the expression of mechanical allodynia (Pain from stimuli which are not normally painful) following SCI (Crown et al., 2006). ERK activation contributes to the regulation of bladder function in SCI (Cruz et al., 2006). P38 MAPK is reported to be involved in inflammatory responses in the progression of neurodegenerative diseases (Barone et al, 2001; Park et al., 2001). IL-1β up-regulates the activation of p38 in hepatic stellate cells (Zhang et al., 2006). ERK is essential for IL-1β-induced nuclear factor kappa B activation (Larsen et al., 2005). IL-1β regulates cyclooxygenase-2 activation through MAPKs. Several studies in different systems indicate that IL-1β exerts its effects

by activating MAPKs. Therefore, my hypothesis is that the activation of MAPKs in glutamate-induced SCI is mediated by IL-1β. To test it, I studied the role of MAPKs in glutamate-induced SCI to add new understanding about the roles of MAPKs in excitotoxicity. My results show that both ERK1/2 and p38 are activated after the cord is exposed to glutamate (Figs. 20 and 24), evidence for the involvement of ERK1/2 and p38 in glutamate-induced SCI. This is consistent with previous findings that activation of MAPKs in SCI is induced by stimulation of glutamate receptors (Wang et al., 2006). My results also show that the activation of both ERK1/2 and p38 is blocked by adding IL-1ra (Fig. 21 and 25), which further supports my above hypothesis. IL-1β-induced apoptosis in SCI is p38 dependent (Wang et al., 2005), so it is important to know if activation of MAPKs modulates IL-1β activation. Administration of PD98059 (an ERK inhbitor) did not block the activation of IL-1β (data not shown), but blockage of IL-1β did block the activation of ERKs (Fig. 21). My IL-1ra experiments together with the effects of PD98059 demonstrate that MAPKs are downstream of IL-1β in glutamate-induced SCI. This further identifies the cascades that are involved in glutamate excitotoxicity. Taken together, my results show the involvement of IL-1β in glutamate-induced SCI and the activation of MAPKs by IL-1ra (Figs. 21 and 25). Immunohistochemistry results show that both ERK1/2 and p38 are co-localized with IL-1β (Figs. 22 and 26), but the mechanisms of how IL-1β activates MAPKs in SCI still need to be determined.

BLOCKAGE OF ERK1/2 OR P38 IMPROVED RECOVERY OF RAT LOCOMOTOR FUNCTION

Several studies have demonstrated that contusion SCI generates MAPKs. Intrathecal infusion of SB 203580, an inhibitor of p38, reduces the damage to hindlimb function after thoracic spinal cord injury in the rat (Horiuchi et al., 2003). Blocking ERK1/2 or p38 cascades by administering their inhibitors showed beneficial effects after SCI (Xu et al., 2006). I investigated the roles of MAPKs in glutamate-induced impairment of locomotor function, using BBB tests. In these tests, PD 98059 or SB 203580 was intrathecally infused into the spinal cord for 3 days. The administration of

PD 98059 or SB 203580 both reduced the rat locomotor impairments induced by glutamate, indicating important roles of MAPKs in glutamate-induced SCI (Figs. 23 and 27). Combined with my previous behavior results (Figs. 15, 16 and 17), the effects of MAPK blockers show that glutamate activates IL-1β, and the activation of IL-1β induces the activation of the MAPKs, ERK1/2 and p38. This cascade is important in the impairment of rat locomotor function, so it provides potential treatment targets for agents such as IL-1ra.

GLUTAMATE ADMINISTRATION TO THE CORD INDUCED APOPTOSIS WHICH IS INHIBITED BY IL-1RA, PD 98059 AND SB203580

The occurrence of apoptosis after SCI is well established (Li et al., 1996; Crowe et al., 1997; Emery et al., 1998; Liu et al., 1997; Schuman et al., 1997). However, what directly triggers it is not clear. Blocking the NMDA receptor with an antagonist inhibits apoptosis after SCI, indicating a role of that glutamate receptor in apoptosis (Wada et al., 1999). Changes of AMPA receptor expression after spinal cord injury in glial cell white matter also reflect apoptotic cell death (Park et al., 2003). My results show that administration of glutamate to the cord induces apoptosis starting around 6 hours later (Fig 28), which is consistent with previous findings that glutamate receptors are involved in SCI-induced apoptosis. Administration of IL-1ra to the cord after SCI inhibits apoptosis induced by SCI (Nesic et al., 2001), additional evidence for the involvement of IL-1 in SCI-induced spinal cord injury. Administration of IL-1ra to the cord after glutamate exposure inhibits apoptosis induced by exposure to glutamate (Fig. 29), indicating the involvement of IL-1 in glutamate-induced apoptosis. This has not been explored before and adds new information to the understanding of apoptosis and SCI. ERK1/2 contributes to glutamate-induced cell death in HT 22 cells (Choi et al., 2006). ERK1/2 and p38 expression are up-regulated in SCI (Crown et al., 2006). However, the role of ERK1/2 in glutamate-induced cell death in SCI was not previously tested. Administration of PD 98059 or SB203580 with glutamate inhibited apoptosis induced by

glutamate exposure to the cord, indicating important roles of ERK1/2 and p38 in glutamate-induced apoptosis, extending previous in vitro finding (Choi et al., 2006) to SCI. The inhibition of apoptosis by IL-1ra or MAPKs inhibitors is not complete, indicating the involvement of other signaling in apoptosis induced by glutamate exposure, such as reactive oxygen species or calcium influx.

SUMMARY

In summary, the series of experiments described in this dissertation tested the hypotheses that IL-1β and MAPKs play important roles in glutamate-induced SCI. I established the following pathway from glutamate release to functional impairment following spinal cord injury: SCI → glutamate release → IL-1β and IL-1ra changes → ERK1/2 and p38 activation → cell death → functional impairment. This is the first cascade traced from glutamate release to functional impairments, and suggests potentially fruitful processes for future investigation in SCI, including the search for therapeutic targets.

REFERENCES

1. Abraham KE, McGinty JF, Brewer KL. (2001) The role of kainic acid/AMPA and metabotropic glutamate receptors in the regulation of opioid mRNA expression and the onset of pain-related behavior following excitotoxic spinal cord injury. Neuroscience. 104: 863-74.

2. Adams JP, Sweatt JD. (2002) Molecular psychology: roles for the ERK MAP Kinase cascade in memory. Annu Rev Pharmacol Toxicol 42: 135–163.

3. Agrawal SK, Fehlings MG. (1997) Role of NMDA and non-NMDA ionotropic glutamate receptors in traumatic spinal cord axonal injury. J Neurosci. 17: 1055-63.

4. Aikawa R, Komuro I, Yamazaki T, Zou Y, Kudoh S, Tanaka M, Shiojima I, Hiroi Y, Yazaki Y. (1997) Oxidative stress activates extracellular signal-related kinases through Src and Ras in cultured cardiac myocytes of neonatal rats. J Clin Invest 100: 1813-1821.

5. Allan SM, Rothwell NJ. (2000) Cortical death caused by striatal administration of AMPA and interleukin-1 is mediated by activation of cortical NMDA receptors. Journal of cerebral blood flow and metabolism 20: 1409-1413.

6. Allan SM, Tyrrell PJ, Rothwell NJ (2005) Interleukin-1 and neuronal injury. Nature reviews Immunology 5: 629-640.

7. Anghelescu N, Petrescu A, Alexandrescu I (1994) The effect of calcium channel blockers in experimental spinal cord injury. Rom J Neurol Psychiatry 32: 101-9.

8. Arend WP, Malyak M, Guthridge CJ (1998) Interleukin-1 receptor antagonist: role in biology. Ann. Rev. Immuno 16: 27-55.

9. Barone FC, Irving EA, Ray AM, Lee JC, Kassis S, Kumar S, Badger AM, White RF, McVey MJ, Legos JJ, Erhardt JA, Nelson AH, Ohlstein EH, Hunter AJ, Ward K, Smith BR, Adams JL, Parsons AA. (2001) SB 239063, a second-generation p38 mitogen-activated protein kinase inhibitor, reduces brain injury and neurological deficits in cerebral focal ischemia. J Pharmacol Exp Ther 296: 312-21.

10. Bartholdi D, Schwab ME (1997) Expression of pro-inflammatory cytokine and chemokine mRNA upon experimental spinal cord injury in mouse: an in situ hybridization study. Eur. J. Neurosci 9: 1422-1438.

11. Basso DM, Beattie MS, and Bresnahan JC. (1995) A sensitive and reliable locomotor rating scale for open field testing in rats. J. Neurotrauma 12: 1-21.

12. Basso DM. (2000) Neuroanatomical substrates of functional recovery after experimental spinal cord injury: implications of basic science research for human spinal cord injury. Phys. Ther. 80: 808-817.

13. Bennett AD, Everhart AW, Hulsebosch CE. (2000) Intrathecal administration of an NMDA or a non-NMDA receptor antagonist reduces mechanical but not thermal allodynia in a rodent model of chronic central pain after spinal cord injury. Brain Res. 859: 72-82.

14. Bethea JR, Nagashima H, Acosta MC, Briceno C, Gomez F, Marcillo AE, Loor K, Green J, Dietrich WD (1999) Systemically administered interleukin-10 reduces tumor necrosis factor-alpha production and significantly improves functional recovery following traumatic spinal cord injury in rats. J. Neurotrauma 16: 851-863.

15. Bhat NR, Zhang P, Lee JC, Hogan EL. (1998) Extracellular signalregulated kinase and p38 subgroups of mitogen-activated protein kinases regulate inducible nitric oxide synthase and tumor necrosis factor-alpha gene expression in endotoxin-stimulated primary glial cultures. J Neurosci 18: 1633–1641.

16. Black P, Markowitz RS, Finkelstein SD, McMonagle-Strucko K, Gillespie JA. (1988) Experimental spinal cord injury: effect of a calcium channel antagonist (nicardipine). Neurosurgery. (1 Pt 1): 61-6.

17. Borsch-Haubold AG, Pasquet S, Watson SP. (1998) Direct inhibition of cyclooxygenase-1 and -2 by the kinase inhibitors SB 203580 and PD 98059. SB 203580 also inhibits thromboxane synthase. J Biol Chem. 273: 28766-72.

18. Bozbuga M, Izgi N, Canbolat A (1998) The effects of chronic alpha-tocopherol administration on lipid peroxidation in an experimental model of acute spinal cord injury. Neurosurg Rev 21(1): 36-42.

19. Bracken MB, Collins WF, Freeman DF, Shepard MJ, Wagner FW, Silten RM. (1984) Efficacy of methylprednisolone in acute spinal cord injury. JAMA 251: 45-52.

20. Bracken MB, Shepard MJ, Hellenbrand KG, Collins WF, Leo LS, Freeman DF (1985) Methylprednisolone and neurological function 1 year after spinal cord injury. Results of the National Acute Spinal Cord Injury Study. J Neurosurg 63: 704-13.

21. Bracken MB, Shepard MJ, Collins WF, Holford TR, Young W, Baskin DS. (1990) A randomized, controlled trial of methylprednisolone or naloxone in the treatment of acute spinal-cord injury. Results of the Second National Acute Spinal Cord Injury Study. N Engl J Med 322: 1405-11.

22. Bracken MB, Shepard MJ, Collins WF Jr, Holford TR, Baskin DS, Eisenberg HM. (1992) Methylprednisolone or naloxone treatment after acute spinal cord injury: 1-year follow-up data. Results of the second National Acute Spinal Cord Injury Study. J Neurosurg 76: 23-31.

23. Bracken MB, Shepard MJ, Holford TR, Leo-Summers L, Aldrich EF, Fazl M. (1997) Administration of methylprednisolone for 24 or 48 hours or tirilazad mesylate for 48 hours in the treatment of acute spinal cord injury. Results of the Third National Acute Spinal Cord Injury Randomized Controlled Trial. National Acute Spinal Cord Injury Study. JAMA 277: 1597-604.

24. Bracken MB, Shepard MJ, Holford TR, Leo-Summers L, Aldrich EF, Fazl M. (1998) Methylprednisolone or tirilazad mesylate administration after acute spinal cord injury: 1-year follow up. Results of the third National Acute Spinal Cord Injury randomized controlled trial. J Neurosurg 89: 699-706.

25. Brand-Schieber E, Werner P. (2004) Calcium channel blockers ameliorate disease in a mouse model of multiple sclerosis. Exp Neurol 189: 5-9.

26. Bruce JC, Oatway MA, Weaver LC. (2002) Chronic pain after clip-compression injury of the rat spinal cord. Exp. Neurol 178: 33-48.

27. Bunge RP, Puckett WR, Becerra JL, Marcillo A, and Quencer RM. (1993) A review and classification of 22 new cases with details from a case of chronic cord compression with extensive focal demyelination. dv Neurol 59: 75–89.

28. Bunge RP, Puckett WR, and Hiester ED. (1997) Observations on the pathology of several types of human spinal cord injury, with emphasis on the astrocyte response to penetrating injuries. Adv Neurol 72: 305–315.

29. Cassada DC, Tribble CG, Young JS, Gangemi JJ, Gohari AR, Butler PD, Rieger JM, Kron IL, Linden J, Kern JA (2002) Adenosine A2A analogue improves neurologic outcome after spinal cord trauma in the rabbit. J. trauma 53: 225-229.

30. Chen RH, Sarnecki C, Blenis J. (1992) Nuclear localization and regulation of erk- and rsk-encoded protein kinases. Mol Cell Biol 12: 915–927.

31. Choi BH, Hur EM, Lee JH, Jun DJ, Kim KT. (2006) Protein kinase Cdelta-mediated proteasomal degradation of MAP kinase phosphatase-1 contributes to glutamate-induced neuronal cell death. J Cell Sci. 119: 1329-40. Epub 2006 Mar 14.

32. Churchill L, Yasuda K, Yasuda T, Blindheim KA, Falter M, Garcia-Garcia F, Krueger JM. (2005) Unilateral cortical application of tumor necrosis factor alpha induces asymmetry in Fos- and interleukin-1beta-immunoreactive cells within the corticothalamic projection. Brain Res. 1055: 15-24.

33. Combs CK, Karlo JC, Kao SC, Landreth GE. (2001) beta-Amyloid stimulation of microglia and monocytes results in TNFalpha-dependent expression of inducible nitric oxide synthase and neuronal apoptosis. J Neurosci 21: 1179–1188.

34. Cover PO, Baanah-Jones F, John CD, Buckingham JC. (2002) Annexin 1 (lipocortin 1) mimics inhibitory effects of glucocorticoids on testosterone secretion and enhances effects of interleukin-1beta. Endocrine 18: 33-9.

35. Crews CM, Alessandrini A, Erikson RL. (1992) The primary structure of MEK, a protein kinase that phosphorylates the ERK gene product. Science 258: 478–480.

36. Crowe MJ, Bresnahan JC, Schuman SL. (1997) Apoptosis and delayed degeneration after spinal cord injury in rats and monkeys. Nat. Med. 3: 73-76.

37. Crown ED, Ye Z, Johnson KM, Xu GY, McAdoo DJ, Hulsebosch CE (2006) Increases in the activated forms of ERK 1/2, p38 MAPK, and CREB are correlated with the expression of at-level mechanical allodynia following spinal cord injury. Exp Neurol 199: 397-407.

38. Cruz CD, McMahon SB, Cruz F. (2006) Spinal ERK activation contributes to the regulation of bladder function in spinal cord injured rats. Exp Neurol. 200: 66-73. Epub 2006 Mar 2.

39. Cuenda A, Rouse J, Doza YN, Meier R, Cohen P, Gallagher TF, Young PR, Lee C. (1995) SB 203580 is a specific inhibitor of a MAP kinase homologue which is stimulated by cellular stresses and interleukin-1. FEBS Lett. 364: 229-33.

40. Da Silva J, Pierrat B, Mary JL, Lesslauer W. (1997) Blockade of p38 mitogen-activated protein kinase pathway inhibits inducible nitricoxide synthase expression in mouse astrocytes. J Biol Chem 272: 28373–28380.

41. D'Aversa TG, Weidenheim KM, Berman JW. (2002) CD40-CD40L interactions induce chemokine expression by human microglia: implications for human immunodeficiency virus encephalitis and multiple sclerosis. Am J Pathol 160: 559–567.

42. Davies JE, Huang C, Proschel C, Noble M, Mayer-Proschel M, Davies SJ. (2006) Astrocytes derived from glial-restricted precursors promote spinal cord repair. J Biol 5:7. Epub 2006 Apr 27.

43. Demopoulos HB, Flamm ES, Pietronigro DD, Seligman ML. (1980) The free radical pathology and the microcirculation in the major central nervous system disorders. Acta Physiol Scand Suppl. 492: 91-119.

44. Dong H, Fazzaro A, Xiang C, Korsmeyer SJ, Jacquin MF, Mcdonald JW (2003) Enhanced oligodendrocyte survival after spinal cord injury in Bax-deficient mice and mice with delayed wallerian degeneration. J Neurosci 23: 8682-8691.

45. Du S, Rubin A, Klepper S, Barrett C, Kim YC, Rhim HW, Lee EB, Park CW, Markelonis GJ, Oh TH. (1999) Calcium influx and activation of calpain I mediate acute reactive gliosis in injured spinal cord. Exp Neurol. 157: 96-105.

46. Eide PK, Stubhaug A, Stenehjem AE. (1995) Central dysesthesia pain after traumatic spinal cord injury is dependent on N-methyl-D-aspartate receptor activation. Neurosurgery. 37: 1080-7.

47. Emery E, Aldana P, Bunge MB. (1998) Apoptosis after traumatic human spinal cord injury. J. Neurosurg. 89: 911-920.

48. Emirandetti A, Graciele Zanon R, Sabha M Jr, de Oliveira AL. (2006) Astrocyte reactivity influences the number of presynaptic terminals apposed to spinal motoneurons after axotomy. Brain Res. 1095: 35-42. Epub 2006 May 22.

49. Eriksson, C.; Winblad, B. and Schultzberg, M. (1998) Kainic acid induced expression of interleukin-1 receptor antagonist mRNA in the rat brain. Mol. Brain. Res. 58: 195-208.

50. Faden AI, Lemke M, Simon RP, Noble LJ. (1988) N-methyl-D-aspartate antagonist MK801 improves outcome following traumatic spinal cord injury in rats: behavioral, anatomic, and neurochemical studies. J Neurotrauma. 5: 33-45.

51. Faden AI, O'Leary DM, Fan L, Bao W, Mullins PG, Movsesyan VA. (2001) Selective blockade of the mGluR1 receptor reduces traumatic neuronal injury in vitro and improvesoOutcome after brain trauma. Exp Neurol. 167: 435-44.

52. Farahat MN, Yanni G, Poston R, Panayi GS. (1993) Cytokine expression in synovial membranes of patients with rheumatoid arthritis and osteoarthritis. Ann Rheum Dis 52: 870-875.

53. Farooque M, Isaksson J, Olsson Y (2001) Improved recovery after spinal cord injury in neuronal nitric oxide synthase-deficient mice but not in TNF-alpha-deficient mice. J Neurotrauma 18: 105-114.

54. Fawcett JW. (1998). Spinal cord repair: from experimental models to human application. Spinal Cord. 36: 811-817.

55. Ferrer I, Blanco R, Carmona M. (2001) Differential expression of active, phosphorylation-dependent MAP kinases, MAPK/ERK, SAPK/JNK and p38, and specific transcription factor substrates following quinolinic acid excitotoxicity in the rat. Brain Res Mol Brain Res 94: 48–58.

56. Fiore RS, Murphy TH, Sanghera JS, Pelech SL, Baraban JM. (1993) Activation of p42 mitogen-activated protein kinase by glutamate receptor stimulation in rat primary cortical cultures. J Neurochem 61: 1626-1633.

57. Friedlander RM, Gagliardini V, Hara H. (1997) Expression of a dominant negative mutant of interleukin-1 converting enzyme in transgenic mice prevents neuronal cell death induced by trophic factor withdrawal and ischaemic brain injury. J. Exp. Med.185: 933-940.

58. Friedlander RM, Gagliardini V, Rotello RJ (1996) Functional role of interleukin-1 in IL-1 converting enzyme mediated apoptosis. J. Exp. Med. 184: 717-724.

59. Fu ES, Saporta S. (2005) Methylprednisolone inhibits production of interleukin-1beta and interleukin-6 in the spinal cord following compression injury in rats. J Neurosurg Anesthesiol. 17: 82-5.

60. Gadient RA, Cron KC, Otten U. (1990) Interleukin-1 beta and tumor necrosis factor-alpha synergistically stimulate nerve growth factor (NGF) release from cultured rat astrocytes. Neurosci Lett. 117: 335-40.

61. Gao J, Coggeshall RE, Tarasenko YI, Wu P. (2005) Human neural stem cell-derived cholinergic neurons innervate muscle in motoneuron deficient adult rats. Neuroscience. 131: 257-62.

62. Gelderd JB, Hall NR, O'Grady MP, Oliver J, Ferrer C, Anderson JA. (1996) The effects of interleukin-1 receptor antagonist protein (IRAP) infusion following spinal cord transection in rats. Mol Chem Neuropathol. 27: 167-83.

63. Gonzalez FA, Seth A, Raden DL, Bowman DS, Fay FS, Davis RJ. (1993) Serum-induced translocation of mitogen-activated protein kinase to the cell surface ruffling membrane and the nucleus. J Cell Biol 122: 1089–1101.

64. Gordh T, Chu H, Sharma HS. (2006) Spinal nerve lesion alters blood-spinal cord barrier function and activates astrocytes in the rat. Pain. 124: 211-21. Epub 2006 Jun 27.

65. Grossman SD, Wolfe BB, Yasuda RP, Wrathall JR. (2000) Changes in NMDA receptor subunit expression in response to contusive spinal cord injury. J Neurochem. 75: 174-84.

66. Gruner,J.A. (1992). A monitored contusion model of spinal cord injury in the rat. J. Neurotrauma 9: 123-126.

67. Guan Z, Buckman SY, Pentland AP, Templeton DJ, Morrison AR. (1998) Induction of cyclooxygenase-2 by the activated MEKK13SEK1/ MKK43p38 mitogen-activated protein kinase pathway. J Biol Chem 273:12901–12908.

68. Guha A, Tator CH, Piper I. (1987) Effect of a calcium channel blocker on posttraumatic spinal cord blood flow. J Neurosurg. 66: 423-30.

69. Guha A, Tator CH, Smith CR, Piper I. (1989) Improvement in post-traumatic spinal cord blood flow with a combination of a calcium channel blocker and a vasopressor. J Trauma. 29: 1440-7.

70. Haghighi SS, Johnson GC, de Vergel CF, Vergel Rivas BJ. (1996) Pretreatment with NMDA receptor antagonist MK801 improves neurophysiological outcome after an acute spinal cord injury. Neurol Res. 18: 509-15.

71. Hains BC, Yucra JA, Hulsebosch CE (2001) Reduction of pathological and behabioral deficits following spinal cord contusion injury with the selective cyclooxygenase-2 inhibitor NS-398. J Neurotrauma 18: 409-423.

72. Hains BC, Waxman SG. (2006) Activated microglia contribute to the maintenance of chronic pain after spinal cord injury. J Neurosci. 26: 4308-17.

73. Hall ED, Braughler JM, McCall JM. (1988)New pharmacological treatment of acute spinal cord trauma. J Neurotrauma. 5: 81-9.

74. Hall ED, Braughler JM. (1986) Role of lipid peroxidation in post-traumatic spinal cord degeneration: a review. Cent Nerv Syst Trauma. 3: 281-94.

75. Haller H, Leblhuber F, Trenkler J, Schmidhammer R. (2003) Treatment of chronic neuropathic pain after traumatic central cervical cord lesion with gabapentin. J Neural Transm. 110: 977-81.

76. Hamada Y, Ikata T, Katoh S. (1996) Involvement of an intracellular adhesion molecule 1-depenent pathway in the pathogenesis of secondary changes after spinal cord injury in rats. J. Neurochem. 66: 1525-1531.

77. Hanks SK, Hunter T. (1995) Protein kinases 6. The eukaryotic protein kinase superfamily: kinase (catalytic) domain structure and classi- fication. FASEB J 9: 576–596.

78. Hao JX, Xu XJ, Aldskogious H. (1991) Allodynia-like effects in rat after ischemic spinal cord lesion photochemically induced by laser irradiation. Pain 45: 175-185.

79. Hao JX, Watson BD, Xu XJ, Wiesenfeld-Hallin Z, Seiger A, Sundstrom E. (1992) Protective effect of the NMDA antagonist MK-801 on photochemically induced spinal lesions in the rat. Exp Neurol. 118: 143-52.

80. Hao JX, Xu XJ. (1996) Treatment of a chronic allodynia-like response in spinally injured rats: effects of systemically administered excitatory amino acid receptor antagonists. Pain. 66: 279-85.

81. Hara H, Friedlander RM, Gagliardini V, Ayata C, Fink K, Huang Z, Shimizu-asamata M, Yuan J, Moskowitz MA. (1997) Inhibition of interleukin 1beta converting enzyme family proteases reduces ischemic and excitotoxic neuronal damage. Proc Natl Acad Sci U S A. 94: 2007-12.

82. Haghighi SS, Johnson GC, de Vergel CF, Vergel Rivas BJ. (1996) Pretreatment with NMDA receptor antagonist MK801 improves neurophysiological outcome after an acute spinal cord injury. Neurol Res. 18: 509-15.

83. Hayashi M, Ueyama T, Tamaki T, Senba E. (1997) Expression of neurotrophin and IL-1 beta mRNAs following spinal cord injury and the effects of methylprednisolone treatment Kaibogaku Zasshi. 72: 209-13.

84. He FC, Liu YB (1991) Experimental spinal cord injuries treated by calcium channel blockers Sheng Li Ke Xue Jin Zhan. 22: 173-5.

85. Hebert G, Mingam R, Arsaut J, Dantzer R, Demotes-Mainard J. (2005) Cellular distribution of interleukin-1alpha-immunoreactivity after MPTP intoxication in mice. Brain Res Mol Brain Res. 138: 156-63.

86. Hensley K, Floyd RA, Zheng NY, Nael R, Robinson KA, Nguyen X, Pye QN, Stewart CA, Geddes J, Markesbery WR, Patel E, Johnson GV, Bing G. (1999) p38 kinase is activated in the Alzheimer's disease brain. J Neurochem 72: 2053–2058.

87. Herlaar E, Brown Z. (1999) p38 MAPK signalling cascades in inflammatory disease. Mol Med Today 5: 439–447.

88. Hopkins SJ and Rothwell NJ. (1995) Cytokines and the nervous system. Expression and recognition. Trends. Neurosci. 18: 83-88.

89. Horiuchi H, Ogata T, Morino T, Chuai M, Yamamoto H (2003) Continuous intrathecal infusion of SB203580, a selective inhibitor of p38 mitogen-activated protein kinase, reduces the damage of hind-limb function after thoracic spinal cord injury in rat. Neurosci Res. 47: 209-17.

90. Ji RR, Kohno T, Moore KA, Woolf CJ. (2003) Central sensitization and LTP: do pain and memory share similar mechanism? Trends Neurosci. 26: 696-705.

91. Jones LL, Oudega M, Bunge MB and Tuszynski MH. (2001) Neurotropic factors, cellular bridges and gene therapy for spinal cord injury. J physiol 533: 83-89.

92. Kenney MJ, Blecha F, Wang Y, McMurphy R, Fels RJ. (2002) Sympathoexcitation to intravenous interleukin-1beta is dependent on forebrain neural circuits. Am J Physiol Heart Circ Physiol. 283: H501-5.

93. Klussmann I, and Schwab ME. (1997) Effects of pro-inflammatory cytokines in experimental spinal cord injury. Brain res. 762: 173-184.

94. Klussmann S, Martin-Villalba A. (2005) Molecular targets in spinal cord injury J Mol Med 83: 657–671.

95. Kocaeli H, Korfali E, Ozturk H, Kahveci N, Yilmazlar S. (2005) MK-801 improves neurological and histological outcomes after spinal cord ischemia induced by transient aortic cross-clipping in rats. Surg Neurol. 64 Suppl 2: S22-6; discussion S27.

96. Koistinaho M; Koistinaho J. (2002) Role of p38 and p44/42 Mitogen- Activated Protein Kinases in Microglia GLIA 40: 175–183.

97. Kyriakis JM, Avruch J. (1996) Sounding the alarm: protein kinase cascades activated by stress and inflammation. J Biol Chem 271: 24313–24316.

98. Kyriakis JM, Avruch J. (2001) Mammalian mitogen activated protein kinase signal transduction pathways activated by stress and inflammation. Physio Rev 81: 807-69.

99. Lawrence CB, Allan SM and Rothwell NJ. (1998) Interleukin-1 and the interleukin-1 receptor antagonist act in the striatum to modify excitotoxic brain damage in the rats. Eur. J. Neurosci. 10: 1188-1195.

100. Lee JC, Laydon JT, McDonnell PC, Gallagher TF, Kumar S, Green D, McNulty D, Blumenthal MJ, Heys JR, Landvatter SW, Strickler SE, McLaughlin MM, Seimens I, Fisher S, Livi GP, White JR, Adams JL, Young PR. (1994) A protein kinase involved in the regulation of inflammatory cytokine biosynthesis. Nature 372: 739–746.

101. Leskovar A, Moriarty LJ, Turek JJ, Schoenlein IA, Borgens RB. (2000) The macrophage in acute neural injury: changes in cell numbers over time and levels of cytokine production in mammalian central and peripheral nervous systems. J Exp Biol. 203: 1783-95.

102. Levendoglu F, Ogun CO, Ozerbil O, Ogun TC, Ugurlu H. (2004) Gabapentin is a first line drug for the treatment of neuropathic pain in spinal cord injury. Spine. 29: 743-51.

103. Li GL, Brodin G, Farooque M, Funa K, Holtz A, Wang WL, Olsson Y. (1996) Apoptosis and expression of Bcl-2 after compression trauma to rat spinal cord. J Neuropathol Exp Neurol. 55: 280-9.

104.Li GL, Garooque M, Holtz A, Olsson Y. (1999) Apoptosis of oligodendrocytes occurs for long distances away from the primary injury after compression trauma to rat spinal cord. Acta Neuropathol. 98: 473-480.

105.Li S, Tator CH. (1999) Effects of MK801 on evoked potentials, spinal cord blood flow and cord edema in acute spinal cord injury in rats. Spinal Cord. 37: 820-32.

106.Li S, Tator CH. (2000) Action of locally administered NMDA and AMPA/kainate receptor antagonists in spinal cord injury. Neurol Res. 22: 171-80.

107.Li S, Stys PK. (2000) Mechanisms of ionotropic glutamate receptor mediated excitotoxicity in isolated spinal cord white matter, J. Neurosci. 20: 1190-1198.

108.Lipton SA. (2006) NMDA receptors, glial cells, and clinical medicine. Neuron. 50: 9-11.

109.Liu D, Thangnipon W, McAdoo DJ. (1991) Excitatory amino acids rise to toxic levels upon impact injury to the rat spinal cord. Brain Res. 547: 344-348.

110.Liu D, Xu G.-Y, Pan E, McAdoo D J. (1999) Neurotoxicity of glutamate at the concentration released upon spinal cord injury. Neuroscience 93: 1383-1389.

111.Liu S, Ruenes GL, Yezierski RP. (1997) NMDA and non-NMDA receptor antagonists protect against excitotoxic injury in the rat spinal cord. Brain Res. 756: 160-7.

112.Liu XZ, Xu XM, Hu R. (1997) Neuronal and glial apoptosis after traumatic spinal cord injury. J. Neurosci. 17: 5395-5406.

113.Loddick SA and Rothwell NJ. (1996) Neuroprotective effects of human recombinant interleukin-1 receptor antagonist in focal cerebral ischaemia in the rat. J. Cereb. Blood Flow Metab. 16: 932-940.

114.Love R. (2006) Stem-cell derived astrocytes promote repair of spinal cord. Lancet Neurol. 5: 474-5.

115.Lucas JH, Wang GF, Gross GW. (1990) NMDA antagonists prevent hypothermic injury and death of mammalian spinal neurons. J Neurotrauma. 7: 229-36.

116.Lundkvist J, Sundgren-anderson A, Tingsborg S. (1999) Acute-phase responses in transgenic mice with CNS overexpression of IL-1 receptor antagonist. Am, J, Physiol. 276: R644-651.

117. Marais R, Wynne J, Treisman R. (1993) The SRF accessory protein Elk-1 contains a growth factor-regulated transcriptional activation domain. Cell 73: 381–393.

118. Martin D, Chinooksoswong N and Miller G. (1994) The interleukin-1 receptor antagonist protects against cerebral infarction in rat model of hypoxia-ischemia. Exp. Neurol. 130: 362-367.

119. Martin-Villalba A, Hahne M, Kleber S, Vogel J, Falk W, Schenkel J, Krammer PH (2001) Therapeutic neutralization of CD95L and TNF attenuates brain damage in stroke. Cell Death Differ 8: 679-686.

120. McAdoo DJ, Xu G-Y, Robak G, Hughes MG. (1999) Changes in amino acid concentrations over time and space around an impact injury and their diffusion through the rat spinal cord. Exp. Neurol. 159: 538-544.

121. McAdoo DJ, Xu G-Y, Robak G, Hughes MG, Price EM. (2000). Evidence that reversed glutamate uptake contributes significantly to glutamate release following experimental injury to the rat spinal cord, Brain Res. 865: 283-285.

122. McAdoo DJ, Hughes MG, Nie L, Shah B, Clifton C, Fullwood S, Hulsebosch CE. (2005) The effect of glutamate receptor blockers on glutamate release following spinal cord injury. Lack of evidence for an ongoing feedback cascade of damage—glutamate release—damage—glutamate release—etc., Brain Res. 1038: 92-99.

123. McBurney RN. (1997) Development of the NMDA ion-channel blocker, aptiganel hydrochloride, as a neuroprotective agent for acute CNS injury. Int Rev Neurobiol.40: 173-95.

124. McDonald DR, Bamberger ME, Combs CK, Landreth GE. (1998) beta- Amyloid fibrils activate parallel mitogen-activated protein kinase pathways in microglia and THP1 monocytes. J Neurosci 18:4451– 4460.

125. Mcintosh TK, Vink R, Soares H, Hayes R, Simon R. (1989) Effects of the N-methyl-D-aspartate receptor blocker MK-801 on neurologic function after experimental brain injury. J Neurotrauma. 6: 247-59.

126. Mee JB, Antonopoulos C, Poole S, Kupper TS, Groves RW. (2005) Counter-regulation of interleukin-1alpha (IL-1alpha) and IL-1 receptor antagonist in murine keratinocytes. J Invest Dermatol. 124: 1267-74.

127. Mielke K, Herdegen T. (2000) JNK and p38 stress kinases—degenerative effectors of signal-transduction-cascades in the nervous system. Prog Neurobiol 61: 45–60.

128. Milvy P, Kakari S, Campbell JB, Demopoulos HB. (1973) Paramagnetic species and radical products in cat spinal cord. Ann N Y Acad Sci. 222: 1102-11.

129. Miyazawa K, Mori A, Okudaira H. (1998) Regulation of interleukin- 1beta-induced interleukin-6 gene expression in human fibroblastlike synoviocytes by glucocorticoids. J Biochem (Tokyo) 124: 1130– 1137.

130. Mu X, Azbill RD, Springer JE. (2002) NBQX treatment improves mitochondrial function and reduces oxidative events after spinal cord injury. J Neurotrauma. 19: 917-27.

131. Namgung U, Xia Z. (2001) Arsenic induces apoptosis in rat cerebellar neurons via activation of JNK3 and p38 MAP kinases. Toxicol Appl Pharmacol. 174: 130-8.

132. Nath R, McGinnis K, Dutta S, Shivers B, Wang KK. (2001) Inhibition of p38 kinase mimics survival signal-linked protection against apoptosis in rat cerebellar granule neurons. Cell Mol Biol Lett. 6: 173-84.

133. Nesic O, Xu GY, McAdoo D, High KW, Hulsebosch C, Perez-Pol R. (2001) IL-1 receptor antagonist prevents apoptosis and caspase-3 activation after spinal cord injury J Neurotrauma. 18: 947-56.

134. Newman SP, Flower RJ, Croxtall JD. (1994) Dexamethasone suppression of IL-1 beta-induced cyclooxygenase 2 expression is not mediated by lipocortin-1 in A549 cells. Biochem Biophys Res Commun. 202: 931-9.

135. Nicholson DW (2000) From bench to clinic with apoptosis-based therapeutic agents. Nature 407: 810-816.

136. Nozaki K, Nishimura M, Hashimoto N. (2001) Mitogen-activated protein kinases and cerebral ischemia. Mol Neurobiol. 23: 1-19.

137. Ono K, Han J. (2000) The p38 signal transduction pathway: activation and function. Cell Signal 12: 1–13.

138. Oprica M, Eriksson C, Schultzberg M. (2003) Inflammatory mechanisms associated with brain damage induced by kainic acid with special reference to the interleukin-1 system. J. Cell. Mol. Med. 7: 127-140.

139. Oprica M, Van Dam AM, Lundkvist J, Iverfeldt K, Winblad B, Bartfai T, Schultzberg M. (2004) Effects of chronic overexpression of interleukin-1 receptor antagonist in a model of permanent focal cerebral ischemia in mouse. Acta Neuropathol (Berl). 108(1): 69-80. Epub 2004 May 8.

140. Ovelmen-Levitt J, Gorecki J, Nguyen K. (1995) Spontaneous and evoked dysesthesias observed in the rat after spinal cordotomies. Sterotact Funct Neurosurg 65: 157-160.

141. Pan JZ, Ni L, Sodhi A, Aguanno A, Young W, Hart RP. (2002) Cytokine activity contributes to induction of inflammatory cytokine mRNAs in spinal cord following contusion. J Neurosci Res. 68: 315-22.

142. Panegyres, PK and Hughes, J (1998) The neuroprotective effects of the recombinant interleukin-1 receptor antagonist rhIL-1ra after excitotoxic stimulation with kainic acid and its relationship to the amyloid precursor protein gene. J. Neurol. Sci. 154: 123-132

143. Panter SS, Yum SW, Faden AI. (1990) Alteration in extracellular amino acids after traumatic spinal cord injury, Ann. Neurol. 27: 96-99.

144. Park E, Liu Y, Fehlings MG. (2003) Changes in glial cell white matter AMPA receptor expression after spinal cord injury and relationship to apoptotic cell death. Exp. Neurol. 182: 35-48.

145. Park E, Velumian AA, Fehlings MG. (2004) The role of excitotoxicity in secondary mechanisms of spinal cord injury: a review with an emphasis on the implications for white matter degeneration. J Neurotrauma. 21: 754-74.

146. Park SY, Lee H, Hur J, Kim SY, Kim H, Park JH, Cha S, Kang SS, Cho GJ, Choi WS, Suk K. (2002) Hypoxia induces nitric oxide production in mouse microglia via p38 mitogen-activated protein kinase pathway. Brain Res Mol Brain Res. 107: 9-16.

147. Pearse DD, Chatzipanteli K, Marcillo AE, Bunge MB, Dietrich WD (2003) Comparison of INOS inhibition by antisense and pharmacological inhibitors after spinal cord injury. J. Neuropatho Exp Neurol 62: 1096-1107.

148. Perrin FE, Lacroix S, Aviles-Trigueros M, David S. (2005) Involvement of monocyte chemoattractant protein-1, macrophage inflammatory protein-1alpha and interleukin-1beta in Wallerian degeneration. Brain. 128: 854-66. Epub 2005 Feb 2

149. Pettipher ER, Higgs GA, Henderson B. (1986) Interleukin-1 induces leukocyte infiltration and cartilage proteoglycan degradation in the synovial joint, Proc Natl Acad Sci USA 31: 8749-8753.

150. Philip JG, John CD, Cover PO, Morris JF, Christian HC, Flower RJ, Buckingham JC. (2001) Opposing influences of glucocorticoids and interleukin-1beta on the secretion of growth hormone and ACTH in the rat in vivo: role of hypothalamic annexin 1. Br J Pharmacol. 134: 887-95.

151. Plata-Salaman, CR; Kelly, G; Agresta, C (1995) Interleukin-1 beta enhances spinal cord blood flow after intrathecal administration in the normal rat. Am.J. Physiol. 269: R1032-1037.

152. Pyo H, Jou I, Jung S, Hong S, Joe EH. (1998) Mitogen-activated protein kinases activated by lipopolysaccharide and beta-amyloid in cultured rat microglia. NeuroReport 9: 871– 874.

153. Raingeaud J, Gupta S, Rogers JS, Dickens M, Han J, Ulevitch RJ, Davis RJ. (1995) Pro-inflammatory cytokines and environmental stress cause p38 mitogen-activated protein kinase activation by dual phosphorylation on tyrosine and threonine. J Biol Chem 270: 7420–7426.

154. Ray SK, Hogan El, Banik NL (2003) Calpain in the pathophysiology of spinal cord injury: neuroprotection with calpain inhibitors. Brain Res Rev 42: 169-185.

155. Relton, JK; Martin, D; Thompson, RC. (1996) Peripheral administration of interleukin-1 receptor antagonist inhibits brain damage after focal cerebral ischemia in the rat. Exp. Neurol. 138: 206-213.

156. Rosen LB, Ginty DD, Weber MJ, GreenbergME. (1994) Membrane depolarization and calcium influx stimulate MEK and MAP kinase via activation of Ras. Neuron 12: 1207-1221.

157. Rosenberg LJ, Teng YD, Wrathall JR. (1999) 2,3-Dihydroxy-6-nitro-7-sulfamoyl-benzo(f)quinoxaline reduces glial loss and acute white matter pathology after experimental spinal cord contusion. J. Neurosci. 19: 464-475.

158. Resnick DK, Graham SH, Dixon CE, Marion DW (1998) Role of cyclooxygenase 2 in acute spinal cord injury, J. Neurotrauma 15: 1005-1013.

159. Ridley SH, Sarsfield SJ, Lee JC, Bigg HF, Cawston TE, Taylor DJ, DeWitt DL, Saklatvala J. (1997) Actions of IL-1 are selectively controlled by p38 mitogen-

activated protein kinase: regulation of prostaglandin H synthase-2, metalloproteinases, and IL-6 at different levels. J Immunol 158: 3165–3173.

160. Rosenzweig ES and McDonald JW. (2004) Rodent models for treatment of spinal cord injury: research trends and progress toward useful repair. Curr. Opin. Neurol. 17: 121-131.

161. Rothwell NJ (1999) Cytokines-killers in the brain? J Physiol 514,1: 3-17.

162. Saavedra RA, Murray M, deLacalle S, Tessler A (2000) In ivo neuroprotectgion of injured CNS neurons by a single injection of a DNA plasmid encoding the Bcl-2 gene. Prog Brain Res 128: 365-372.

163. Sanderson, KL; Raghupathi, R; Saatman, KE (1999) Interleukin-1 receptor antagonist attenuates regional neuronal bell death and cognitive dysfunction after experimental brain injury. J. Cereb. Blood Flow Metab. 19: 1118-1125.

164. Sanghera JS, Peter M, Nigg EA, Pelech SL. (1992) Immunological characterization of avian MAP kinases: evidence for nuclear localization. Mol Biol Cell 3: 775–787.

165. Scheff,S.W., Rabchevsky,A.G., Fugaccia,I., Main,J.A., and Lumpp,J.E., Jr. (2003) Experimental modeling of spinal cord injury: characterization of a force-defined injury device. J. Neurotrauma 20: 179-193.

166. Schielke GP, Yang GY, Shivers BD, Betz AL. (1998) Reduced ischemic brain injury in interleukin-1 beta converting enzyme-deficient mice. J Cereb Blood Flow Metab. 18: 180-5.

167. Schumacher PA, Siman RG, Fehlings MG. (2000) Pretreatment with calpain inhibitor CEP-4143 inhibits calpain I activation and cytoskeletal degradation, improves neurological function, and enhances axonal survival after traumatic spinal cord injury. J Neurochem 74: 1646-1655.

168. Seki T, Hida K, Tada M, Koyannagi I, Iwasaki Y. (2003) Role of the Bcl-2 gene after contusive spinal cord injury in mice. Neurosurgery 53: 192-198.

169. Seth A, Gonzalez FA, Gupta S, Raden DL, Davis RJ. (1992) Signal transduction within the nucleus by mitogen-activated protein kinase. J Biol Chem 267: 24796–24804.

170. Shuman SL, Bresnahan JC and Beattie MS. (1997) Spoptosis of microglia and oligodendrocytes after spinal cord contusion in rats. J. Neurosci. Res. 50: 798-808.

171. Slevin M, Krupinski J, Slowik A, Rubio F, Szczudlik A, Gaffney J. (2000) Activation of MAP kinase (ERK-1/ERK-2), tyrosine kinase and VEGF in the human brain following acute ischaemic stroke. NeuroReport 11: 2759–2764.

172. Stokes BT and Jakeman LB. (2002) Experimental modelling of human spinal cord injury: a model that crosses the species barrier and mimics the spectrum of human cytopathology. Spinal Cord. 40: 101-109.

173. Streit WJ, Semple-Rowland S, Hurley S. (1998) Cytokine mRNA profiles in contused spinal cord and axotomized facila nucleus suggests a beneficial role for inflammation and glisosis. Exp. Neurol. 152: 74-87.

174. Subramaniam S, Stansberg C and Cunningham C. (2004) The interleukin 1 receptor family. Dev. Comp. Immunol. 28: 415-428.

175. Sugden PH, Clerk A. (1997) Regulation of the ERK subgroup of MAP kinase cascades through G protein-coupled receptors. Cell Signal 9: 337–351.

176. Sung CS, Wen ZH, Chang WK, Ho ST, Tsai SK, Chang YC, Wong CS. (2004) Intrathecal interleukin-1beta administration induces thermal hyperalgesia by activating inducible nitric oxide synthase expression in the rat spinal cord. Brain Res. 1015: 145-53.

177. Sweitzer S, Martin D, DeLeo JA. (2001) Intrathecal interleukin-1 receptor antagonist in combination with soluble tumor necrosis factor receptor exhibits an anti-allodynic action in a rat model of neuropathic pain. Neuroscience.103: 529-39.

178. Takahashi JL, Giuliani F, Power C, Imai Y, Yong VW. (2003) Interleukin-1beta promotes oligodendrocyte death through glutamate excitotoxicity. Ann Neurol. 53: 588-95.

179. Takeda K, Marasuzawa A, Nishitoh H, Ichijo H. (2003) Roles of MAPKKK ASK1 in stress-induced cell death. Cell struct funct 28:23-29.

180. Taoka Y and Okajima K. (1998) Spinal cord injury in the rat. Prog. Neurobiol. 56: 341-358.

181. Teng YD, Wrathall JR. (1996) Evaluation of cardiorespiratory parameters in rats after spinal cord trauma and treatment with NBQX, an antagonist of excitatory amino acid receptors. Neurosci Lett. 209: 5-8.

182. Toborek M, Son KW, Pudelko A, King-Pospisil K, Wylegala E, Malecki A. (2006) ERK 1/2 signaling pathway is involved in nicotine-mediated neuroprotection in spinal cord neurons. J Cell Biochem. [Epub ahead of print].

183. Tonai T, Taketani Y, Ueda N, Nishisho T, Ohmoto Y, Sakata Y, Muraguchi , Wada K, Yamamoto S. (1999) Possible involvement of interleukin-1 in cyclooxygenase-2 induction after spinal cord injury in rats. J Neurochem. 72: 302-9.

184. Tonai T, Taketani Y, Ohmoto Y, Ueda N, Nishisho T, Yamamoto S. (2002) Cyclooxygenase-2 induction in rat spinal cord injury mediated by proinflammatory tumor necrosis factor-alpha and interleukin-1. Adv Exp Med Biol. 507: 397-401.

185. Toulmond S and Rothwell NJ. (1995) Interleukin-1 receptor antagonist inhibits neuronal damage caused by fluid percussion injury in the rat. Brain Res. 671: 261-266.

186. Touzani O, Boutin H, Chuquet J, Rothwell N. (1999) Potential mechanisms of interleukin-1 involvement in cerebral ischaemia. J Neuroimmunol. 100: 203-15.

187. Vezzani A, Conti M, Deluigi A. (1999) Interleukin-1 beta immunoreactivity and microglia are enhanced in the rat hippocampus by focal kainate application: functional evidence for enhancement of electrographic seizures. J. Neurosci. 19: 5054-5065.

188. Wada S, Yone K, Ishidou Y, Nagamine T, Nakahara S, Niiyama T, Sakou T. (1999) Apoptosis following spinal cord injury in rats and preventative effect of N-methyl-D-aspartate receptor antagonist. J Neurosurg. 91: 98-104.

189. Wang CX, Olschowka JA and Wrathall JR. (1997) Increase of interleukin-1 beta mRNA and protein in the spinal cord following experimental traumatic injury in the rats. Brain Res. 759: 190-196.

190. Wang X, Arcuino G, Takano T, Lin J, Peng WG, Wan P, Li P, Xu Q, Liu QX, Goldman SA, Nedergaard M (2004) P2X7 receptor inhibition improves recovery after spinal cord injury. Nat Med 10: 821-827.

191. Wang XF, Huang LD, Yu PP, Hu JG, Yin L, Wang L, Xu XM, Lu PH. (2006) Upregulation of type I interleukin-1 receptor after traumatic spinal cord injury in adult rats. Acta Neuropathol (Berl). 111: 220-228. Epub 2006 Feb 3.

192. Wang XJ, Kong KM, Qi WL, Ye WL, Song PS. (2005) Interleukin-1 beta induction of neuron apoptosis depends on p38 mitogen-activated protein kinase activity after spinal cord injury. Acta Pharmacol Sin. 26: 934-42.

193. Whitmarsh AJ, Shore P, Sharrocks AD, Davis RJ. (1995) Integration of MAP kinase signal transduction pathways at the serum response element. Science 269: 403–407.

194. Whittemore SR, Zhang YP, Shields CB, Morassutti DJ, and Magnuson DS. (2002) Optimizing stem cell grafting into the CNS. Methods Mol Biol 198: 319-325.

195. Willis WD. (2001) Role of neurotransmitters in sensitization of pain responses. Ann N Y Acad Sci. 933: 142-56.

196. Winkler T, Sharma HS, Stalberg E, Badgaiyan RD, Gordh T, Westman J. (2003) An L-type calcium channel blocker, nimodipine influences trauma induced spinal cord conduction and axonal injury in the rat. Acta Neurochir Suppl. 86: 425-32.

197. Wood DD, Ihrie EJ, Hamerman D. (1985) Release of interleukin-1 from human synovial tissue in vitro. Arthritis Rheum 28: 853-862.

198. Woods AS, Kaminski R, Oz M, Wang Y, Hauser K, Goody R, Wang HY, Jackson SN, Zeitz P, Zeitz KP, Zolkowska D, Schepers R, Nold M, Danielson J, Graslund A, Vukojevic V, Bakalkin G, Basbaum A, Shippenberg T. (2006) Decoy peptides that bind dynorphin noncovalently prevent NMDA receptor-mediated neurotoxicity. J Proteome Res. 5: 1017-23.

199. Wrathall JR. (1992) Spinal cord injury models. J. Neurotrauma 9 Suppl 1: S129-S134.

200. Wrathall JR., Choiniere D, Teng YD (1994) Dose-dependent reduction of tissue loss and functgional impairment after spinal cord trauma with the AMPA/Kainate antagonist NBQX. J Neurosci 14: 6598-6607.

201. Wrathall JR, Teng YD, Choiniere D. (1996) Amelioration of functional deficits from spinal cord trauma with systemically administered NBQX, an antagonist of non-N-methyl-D-aspartate receptors. Exp. Neurol. 137: 119-126.

202. Wrathall JR, Teng TD, Marriott R. (1997) Delayed antagonism of AMPA/kinate receptors reduces long-term functional deficits resulting from spinal cord trauma. Exp. Neurol. 145: 563-573.

203. Xu G-Y, McAdoo DJ, Hughes MG, Robak G, de Castro Jr R. (1998) Considerations in the determination by microdialysis of resting extracellular amino acid concentrations and release upon spinal cord injury. Neuroscience 86: 1011-1021.

204. Xu G-Y, Hughes MG, McAdoo DJ. (2000) Oligodendrocyte death after glutamate exposure in the rat spinal cord: do apoptotic features predict cell death? Proceedings of the 34th annual meeting, Socidety for Neuroscience, 2004, abstract 232.15.

205. Xu Z, Wang BR, Wang X, Kuang F, Duan XL, Jiao XY, Ju G. (2006) ERK1/2 and p38 mitogen-activated protein kinase mediate iNOS-induced spinal neuron degeneration after acute traumatic spinal cord injury. Life Sci. [Epub ahead of print].

206. Yamamoto T, Nozaki-Taguchi N (1996) Analysis of the effects of cyclooxygenase (COX)-1 and COX-2 in spinal nociceptive transmission using indomethacin, a non-selective COX inhibitor, and NS-398, a COX-2 selective inhibitor. Brain Res 739: 104-110.

207. Yanase M, Sakou T, Fukuda T. (1995) Role of N-methyl-D-aspartate receptor in acute spinal cord injury. J Neurosurg. 83: 884-8.

208. Yang L, Blumbergs PC, Jones NR, Manavis J, Sarvestani GT, Ghabriel MN. (2004) Early expression and cellular localization of proinflammatory cytokines interleukin-1 beta, interleukin-6, and tumor necrosis factor-alpha in human traumatic spinal cord injury. Spine. 29: 966-71.

209. Ye X, Zhou J, Li J. (1996) Changes of N-methyl-D-aspartate receptor in spinal cord after trauma Zhonghua Wai Ke Za Zhi. 34: 569-71.

210. Yeo JD, Payne W, Hinwood B and Kidman AD. (1975) The experimental contusion injury of the spinal cord in sheep. Paraplegia 12: 279-298.

211. Yoshiyama M, Nezu FM, Yokoyama O, Chancellor MB, de Groat WC. (1999) Influence of glutamate receptor antagonists on micturition in rats with spinal cord injury. Exp Neurol. 159: 250-7.

212. Young W. (1992) Role of calcium in central nervous system injuries. J Neurotrauma. Suppl 1: S9-25.

213. Yu CG, Yezierski RP. (2005)Activation of the ERK1/2 signaling cascade by excitotoxic spinal cord injury. Brain Res Mol Brain Res. 138: 244-55.

214. Zhang W, Petrovic JM, Callaghan D, Jones A, Cui H, Howlett C, Stanimirovic D. (2006) Evidence that hypoxia-inducible factor-1 (HIF-1) mediates transcriptional activation of interleukin-1beta (IL-1beta) in astrocyte cultures. J Neuroimmunol. 174: 63-73. Epub 2006 Feb 28.

215. Zhang XY, Zhou CS, Kuang ZD. (2005) Changes of p38 mitogen-activated protein kinase and apoptosis after spinal cord injury. Chin Med Sci J. Dec; 20: 241.

216. Zhang YP, Yao XX, Zhao X. (2006) Interleukin-1 beta up-regulates tissue inhibitor of matrix metalloproteinase-1 mRNA and phosphorylation of c-jun N-terminal kinase and p38 in hepatic stellate cells. World J Gastroenterol. 12: 1392-6.

217. Zhang, Z; Chopp, M; Goussev, A. (1998) Cerebral vessels express interleukin-1 beta after focal cerebral ischemia. Brain Res. 784: 210-6

218. Zompa EA, Cain LD, Everhart AW, Moyer MP, and Hulsebosch CE. (1997) Transplant therapy: recovery of function after spinal cord injury, J Neurotrauma 14: 479-506.

219. Zou W, Zeng J, Zhuo M, Xu W, Sun L, Wang J, Liu X. (2002) Involvement of caspase-3 and p38 mitogen-activated protein kinase in cobalt chloride-induced apoptosis in PC12 cells. J Neurosci Res. 67: 837-43.

VITA

Song Liu was born on February 28th, 1978 in Weifang, P.R. China. She graduated from Shandong University, School of Medicine in 2002. She began her pursuit of a Ph. D at the University of Texas Medical Branch at fall of 2002. She is married to Tiejun Hu.

Education

M.D., August, 2002, Shandong University, School of Medicine, Jinan, P.R. China.

Publications

1. Song Liu, Jason B. Dunsmore, David J. McAdoo, Ubiquitin and proteasome system in spinal cord injury, abstract, and presented at the annual meeting of Society for Neuroscience, Oct. 2004

2. Song Liu, Jason B. Dunsmore, David J. McAdoo, A proteomics study of ubiquitin and proteasome system in spinal cord injury, abstract, and presented in annual meeting of American Society of Mass Spectrometry, 2005

3. Song Liu, Kristina M. Jantz, Lisa Cain, Jason B. Dunsmore, Clement Echetebu, David J. McAdoo, A proteomics study of the effects of glutamate on spinal cord cells, abstract, presented at the annual meeting of Society for Neuroscience, Oct. 2005

4. Guoying Xu, Song Liu, Michael G. Hughes and David J. McAdoo. Glutamate-induced losses of oligodendrocytes and neurons and time courses of apoptotic features in the rat spinal cord. Submitted to Neuroscience, 2006.

5. Song Liu, Guoying Xu, Kathia M. Johnson, Clair E. Hulsebosch, David J. McAdoo. The reciprocity of IL-1β activation and IL-1ra expression in glutamate-induced spinal cord injury. In preparation and will submit to Journal of the Neurochemistry this month.