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# Effects of Knockdown SOCS3 on Glutamate Current in Astrocytes of C57BL/6 mice

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## Abstract

The JAK-STAT signaling pathway is associated with gene regulation during development and differentiation in the central nervous system (CNS). JAK/STAT pathway can inhibited the in a negative-feedback manner by the suppressors of cytokine signaling (SOCS). SOCS3, is expressed by immune cells and cells of CNS, and regulates activation of astroglial cells. Glutamate extracellular levels are regulated by specific transporters that are located in astrocytes, such as GLAST and GLT. JAK/STAT signaling is a target to GLAST expression and uptake of glutamate in brain development. Primary cultures of astrocytes were prepared with 10% (v/ v) fetal calf serum. The cultures were maintained at 37°C in a 95% incubator for 3 weeks in MEM with 10% air and 5% $CO_2$. Knockdown of endogenous SOCS3 preformed by transduction of lentiviral shRNA. The in vitro distribution of 6-Diazo-oxy-L-norleucine (glutamate analogue) in culture was evaluated with protein separation and with HPLC. HPLC didn't showed any decrease or increase in 6-Diazo-oxy-L-norleucine (a glutamate analogue) in culture of astroglial in comparison with the control.

***Keywords:*** *JAK-STAT; Suppressors of cytokine signaling; Glutamate/aspartate transporter*

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## Introduction

The janus kinase/signal transducer and activator of transcription (JAK/STAT) pathway is a signaling cascade that links the activation of specific cell membrane receptors to nuclear gene expression. The JAK-STAT signaling pathway is associated with gene regulation during development and differentiation in the central nervous system (CNS) [1]. JAK/STAT pathway can inhibited the in a negative-feedback manner by the suppressors of cytokine signaling (SOCS). The suppressors of cytokine signaling (SOCS) are a family of intracellular proteins, several of which have emerged as key physiological regulators of cytokine responses. The SOCS proteins seem to regulate signal transduction by combining direct inhibitory interactions with cytokine receptors and signaling proteins with a generic mechanism of targeting associated proteins for degradation. SOCS proteins function in a negative feedback loop to terminate signal transduction through the JAK/STAT pathway. SOCS3, is expressed by immune cells and cells of CNS, and regulates activation of astroglial cells [2].

Glutamate is the major excitatory neurotransmitter in the CNS. astrocytes make glutamate, which is transferred to glutamatergic neurons. Glutamate is released from glutaminergic neurons, and acts on glutamate receptors at the postsynaptic membrane, including N-methyl-D-aspartate (NMDA), and metabotropic glutamate receptors, resulting in $Ca^{2+}$ influx and depolarization of the postsynaptic membrane. Glutamate extracellular levels are regulated by specific transporters. Clearance of synaptically released glutamate, and hence termination of glutamatergic neurotransmission, is carried out by glutamate transporters, most especially glutamate transporter-1 (GLT-1) and the glutamate-aspartate transporter (GLAST) that are located in astrocytes. under different physiological and pathological conditions, changes in the function and expression of GLT-1 and GLAST occur [3].

GLAST and GLT (GLT-1 and GLT-2) are the glutamate transporters involved in maintaining extracellular glutamate concentrations in glial cells. The $Na^{+}$-dependent glutamate/aspartate transporter GLAST plays an important role in the uptake of glutamate from the synaptic cleft and regulation of neurotransmitter signals at excitatory synapses in central nervous system. GLAST is specific for L-glutamate and L-aspartate, which also named Excitatory Amino Acid Transporters1 (EAAT1) [4]. The glutamate transporter GLAST is localized on the cell membrane of mature astrocytes and is also expressed in the ventricular zone of developing brains. In addition, the glutamate transporter GLAST is expressed from glial cells through astrocytes during spinal cord development [5].

## Materials and Methods

### Primary cultures of astrocytes

Primary cultures of astrocytes were prepared according to previously established protocols. Astrocytes were then recovered by the repeated removal of dissociated cells and plated at a density of 1 × 105 cells/ml. Twenty-four hours after the initial plating, the medium was changed to preserve the adhering astrocytes and to remove neurons and oligodendrocytes. media were supplemented with 10% (v/v) fetal calf serum, 100 U/mL of penicillin and 100 µg/mL of streptomycin. The cultures were maintained at 37°C in a 95% incubator for 3 weeks in MEM with 10% air and 5% $CO_2$ [6].

### Incorporation of anti-GLAST IgG on lentiviruses

In order to characterization of morphological and immunochemical properties of GLAST expressing cells performe anti-mouse GLAST antibody. Incorporation of the monoclonal mouse anti-GLAST IgG or (Abcam, American) on to the lentivirus was performed by incubating viral particles with the soluble IgG on ice for 1h before transduction. Antibody concentrations used for in vitro and in vivo experiments were 1.5 µg/ml and 2 µg/ml, respectively. Optimal IgG incorporation was analyzed in vitro on primary glial cultures. Serial dilutions (0.5, 1, 1.5, 2 and 3 µg/ml antibody) were tested for optimal viral attachment to target cells. Transduction experiments were performed by incubating the viruses for 4.5h on cells, followed by washing with PBS, and adding new cell medium. Cells were then incubated for 48h [7]. SOCS3 deficiency cell lines were created by shRNA lentiviral particles in Iscove's Modified Medium.

### Evaluation of 6-Diazo-oxy-L-norleucine (a glutamate analogue)

The *in vitro* distribution of 6-Diazo-oxy-L-norleucine (a glutamate analogue) in culture was evaluated with protein separation and with HPLC [8].

## Results

HPLC didn't showed any decrease or increase in 6-Diazo-oxy-L-norleucine (a glutamate analogue) levels in culture of astroglial in comparison with the control.

## Discussion

JAK2 and STAT3 are involved in neural plasticity. JAK/STAT pathway is active in astrocytes and is important in astrocytes differentiation. JAK/STAT signaling is a target to GLAST expression and uptake of glutamate in brain development. In addition, plays a role in astrocyte pathology also in the immature brain [9]. Ubiquitylation and degradation of receptor-kinase complex mediated by SOCS-1.

Studeis demonstrated that Nedd4-2 (neuronal precursor cell expressed developmentally down-regulated 4-2) can mediate the ubiquitination of the glutamate transporters channels. Nedd4-2 knockdown decreases the ubiquitination of glutamate transporters, promotes glutamate uptake, and increases the expression of glutamate transporters *in vitro* and *in vivo* [10].

## Conclusion

In summary glial NMDA receptors may be potentially active at the resting membrane potential, which is in contrast to neurons. Nevertheless, many published and surely many more unpublished studies have failed to prove the expression of NMDA receptors on glial cells, a phenomenon that could, however, be connected to the clear regional specificity in the expression of NMDA receptors on glial cells in the CNS and moreover, also to their probable age-dependent expression. It should also be taken into account that before discovering the composition and properties of NMDA receptors on glial cells, many studies were considered negative due to the expectation of observing NMDA-specific responses typical of neurons, which are not, in fact, characteristic of glial cells. Under physiological conditions, astroglial NMDA receptors were shown to be involved in neuronal-glial signaling.

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