Astrocyte contribution to the excessive glutamate release in the spinal cord of the SOD1G93A mouse model of amyotrophic lateral sclerosis

Marco Milanese, PhD
Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized by degeneration of cortical and spinal motor neurons.

**Incidence:** 2-3/100,000 person/year  
**Prevalence:** 6-8/100,000 persons  
**Higher incidence in men:** M/F ratio 2  
**Onset:** 55-70 years  
**Survival:** 3-4 years (>5 years in 20% and >10 years in 10% of cases)  

Death occurs for respiration failure or respiratory tract infections.
Sporadic (sALS, about 90%) and familiar (fALS, about 10%) forms of ALS have been described. In about 25% of familial cases, the disease is linked to single mutations in the gene encoding for the superoxide dismutase type 1 (SOD1).

More than 100 distinct SOD1 mutations have been found in human. It is generally accepted that mutations in SOD1 produce the disease by an aberrant gain of function.

Other mutated genes: TDP-43, FUS/TLS, OPTN, UBQLN-2, C9ORF72.
The pathogenesis of ALS is complex and multi-factorial.
Glutamate excitotoxicity is one major cause for motorneuron death.
Glial cells play an active role for motoneuron degeneration and death.
Glutamate release is increased in the spinal cord of SOD1\textsuperscript{G93A} mouse model of ALS

Abnormal exocytotic release of glutamate in a mouse model of amyotrophic lateral sclerosis

Group I metabotropic glutamate autoreceptors induce abnormal glutamate exocytosis in a mouse model of amyotrophic lateral sclerosis

Exocytosis regulates trafficking of GABA and glycine heteroporters in spinal cord glutaantergic synapses: a mechanism for the excessive heteroporter-induced release of glutamate in experimental amyotrophic lateral sclerosis

Altered mechanisms underlying the abnormal glutamate release in amyotrophic lateral sclerosis at a pre-symptomatic stage of the disease
Glutamate release is increased in the spinal cord of SOD1\textsuperscript{G93A} mouse model of ALS.

1. Spontaneous and depolarization evoked Glu release [mainly exocytotic]

2. Receptor induced Glu release [mGluR1 & mGluR5]

3. Glu release induced by the activation of plasma membrane transporters expressed on neurons [Glycine and GABA transporters]
Dissection the role of glial cells for the aberrant glutamate release in the spinal cord of SOD1\textsuperscript{G93A} mice

3. Glu release induced by the activation of plasma membrane transporters expressed on neurons

Which is the effect on glutamate release by activating GABA transporter (GAT1) expressed on astrocytes?

GLIOSOMES
Purification of spinal cord astroglial particles

Spinal cord

Homogenization

Gliosome

Percoll® gradient

Membranes
Gliosomes
Synaptosomes & glia particles
Synaptosomes
Mitochondria & debris
Purification of spinal cord astroglial particles

Spinal cord

Homogenization

Gliosome

Percoll® gradient

Membranes

Gliosomes

Synaptosomes & glia particles

Synaptosomes

Mitochondria & debries
Enriched glial preparation is highly purified and functional.

Glia re-sealed particles freshly prepared from adult rat brain are competent for exocytotic release of glutamate

Sara Stigliani,* Simona Zappettini,* Luca Raiteri,* Mario Passalacqua,†§ Edon Melloni,†§ Consuelo Venturi,‡‖ Carlo Tacchetti,‡‖ Alberto Diaspro,** Cesare Usai†† and Giambattista Bonanno*§
Which is the effect on glutamate release by activating GABA transporter (GAT1) expressed on astrocytes?
Spinal cord gliosomes express astroglial markers and are purified from neuronal contamination.
GAT-1 and other neurotransmitter transporters co-exists on the same gliosomal particles
Gliotransmitter release experiment exploiting the superfusion technique

Mouse spinal cord

Gliosome

[3H]D-Asp incubation

Drug stimulation

Collection of superfusion samples
GABA induces glutamate release from spinal cord gliosomes in a concentration dependent manner

GABA-evoked \[^{3}H\]D-ASP release (% of potentiation)

GABA concentration (µM)

Milanese et al., 2010, J. Neurochem. 113:489-501
Pharmacological characterization of the GABA-induced glutamate release in gliosomes


<table>
<thead>
<tr>
<th>Concentration (µM)</th>
<th>GABA</th>
<th>Muscimol</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>-</td>
<td>100</td>
</tr>
<tr>
<td>30</td>
<td>30</td>
<td>-</td>
</tr>
<tr>
<td>30</td>
<td>30</td>
<td>-</td>
</tr>
<tr>
<td>30</td>
<td>30</td>
<td>-</td>
</tr>
<tr>
<td>30</td>
<td>30</td>
<td>-</td>
</tr>
<tr>
<td>30</td>
<td>30</td>
<td>-</td>
</tr>
</tbody>
</table>

**GABAa**
Pharmacological characterization of the GABA-induced glutamate release in gliosomes

GABA-evoked $[^{3}H]$D-Asp release (% of potentiation)

<table>
<thead>
<tr>
<th>Concentration (µM)</th>
<th>GABA</th>
<th>Muscimol</th>
<th>(-)baclofen</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>-</td>
<td>100 -</td>
<td>-</td>
</tr>
<tr>
<td>30</td>
<td>30</td>
<td>- 100 -</td>
<td>-</td>
</tr>
<tr>
<td>30</td>
<td>30</td>
<td>30 100 -</td>
<td>-</td>
</tr>
<tr>
<td>30</td>
<td>30</td>
<td>30 30 -</td>
<td>-</td>
</tr>
<tr>
<td>30</td>
<td>30</td>
<td>30 30 30</td>
<td>-</td>
</tr>
<tr>
<td>30</td>
<td>30</td>
<td>30 30 30</td>
<td>30</td>
</tr>
</tbody>
</table>

**GABAa**

**GABAb**
Pharmacological characterization of the GABA-induced glutamate release in gliosomes


<table>
<thead>
<tr>
<th>Concentration (µM)</th>
<th>GABA</th>
<th>Muscimol</th>
<th>(-)baclofen</th>
<th>SR 95531</th>
<th>CGP 52432</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>-</td>
<td>100</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>30</td>
<td>-</td>
<td>-</td>
<td>100</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>30</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>10</td>
<td>-</td>
</tr>
<tr>
<td>30</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>10</td>
</tr>
</tbody>
</table>

GABAa and GABAb are indicated by red crosses.
Pharmacological characterization of the GABA-induced glutamate release in gliosomes

GABA-evoked [3H]D-Asp release (% of potentiation)

<table>
<thead>
<tr>
<th>Concentration (µM)</th>
<th>30</th>
<th>30</th>
<th>30</th>
<th>30</th>
<th>30</th>
<th>30</th>
<th>30</th>
</tr>
</thead>
<tbody>
<tr>
<td>GABA</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Muscimol</td>
<td>100</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>(-)baclofen</td>
<td>-</td>
<td>100</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>SR 95531</td>
<td>-</td>
<td>-</td>
<td>10</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CGP 52432</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>10</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>SKF 89976A</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>3</td>
<td>10</td>
<td>30</td>
</tr>
</tbody>
</table>

GABA transporter 1

Forward Transport
Pharmacological characterization of the GABA-induced glutamate efflux

Not a Ca\(^{2+}\) -dependent mechanism
Pharmacological characterization of the GABA-induced glutamate efflux

Not a Ca$^{2+}$-dependent mechanism

Not a carrier-mediated release

EAAT1 or EAAT2

Drug concentration (µM)


<table>
<thead>
<tr>
<th>Drug concentration (µM)</th>
<th>GABA</th>
<th>Ca$^{2+}$ (mM)</th>
<th>Mg$^{2+}$ (mM)</th>
<th>EGTA (µM)</th>
<th>BAPTA (mM)</th>
<th>DL-TBOA (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 30 30 30 30 30 30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.2 - - - 1.2 1.2 1.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.2 10 1.2 10 1.2 1.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- - 100 - - -</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- - 1 - - -</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- - - 10 - -</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Pharmacological characterization of the GABA-induced glutamate efflux

<table>
<thead>
<tr>
<th>Drug concentration (µM)</th>
<th>GABA</th>
<th>Ca$^{2+}$ (mM)</th>
<th>Mg$^{2+}$ (mM)</th>
<th>EGTA (µM)</th>
<th>BAPTA (mM)</th>
<th>DL-TBOA (µM)</th>
<th>niflumic acid (µM)</th>
<th>NPPB (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>10</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>1.2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1.2</td>
<td>10</td>
<td>1.2</td>
<td>10</td>
<td>1.2</td>
<td>1.2</td>
<td>1.2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>10</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>300</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>100</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

GABA-evoked $[^3]$HJ-D-ASP release (% of potentiation)

Diagram: Not a Ca$^{2+}$-dependent mechanism

EAAT1 or EAAT2

VSOAC
Is the GABA-induced glutamate release altered in amyotrophic lateral sclerosis?
The GABA-induced glutamate release is increased in spinal cord synaptosomes purified from SOD1\textsuperscript{G93A} mice

The GABA-evoked \[^{3}H\]D-Asp release (% of potentiation)

\[ \begin{align*}
0.1 & \quad 1 & \quad 10 & \quad 100 & \quad 1000 \\
\text{GABA concentration (\textmu M)} &
\end{align*} \]

- SOD1
- SOD1/G93A

*\textit{Milanese et al., 2015, Neurobiol of Dis.74:314-324}
*\textit{Raiteri et al., 2005, Neurotoxicology 26:883-892}
The GABA-induced glutamate release in SOD1^{G93A} spinal cord synaptosomes is triggered by GAT-1 and operated through the VSOACs.
GABA evokes glutamate release in spinal cord gliosomes in purified from SOD1 control animals.
The GABA-induced glutamate release is increased in spinal cord gliosomes purified from SOD1\textsuperscript{G93A} mice.
The GABA-induced glutamate release is triggered by GAT-1 activation.
The GABA-induced glutamate outflow is operated through the volume sensitive anion channels (VSOACs).


Drug concentration (μM)

- 30
- 30
- 30
- 30

GABA
Niflumic acid

SOD1
SOD1/G93A

* 
** 

VSOAC

Glutamate (Glu)
GABA evokes the release of glutamate from astrocytes in a concentration dependent manner by the activation of specific GAT-1 transporters and occurs through the volume activated anion channels (VSOAC).

The GABA-evoked release of glutamate is more pronounced gliosomes from SOD1\textsuperscript{G93A} mice.

The same phenomenon is also present in synaptic terminals from SOD1G93A mice even in early stage of the disease.

1. Neuronal-supported Glutamate excitotoxicity

2. Astrocyte-supported Glutamate excitotoxicity
ACKNOWLEDGEMENTS:

Pharmacology and Toxicology Unit “The Ghianda Group”

Prof. Giambattista Bonanno
Tiziana Bonifacino
Elena Gallia
Luca Cattaneo
Francesca Provenzano

Our undergraduate students:
Mirna Nitro
Martina Gullace
Roberta Roncallo

Confocal microscopy analysis:
Prof. Cesare Usai
Inst. of Biophysics
National Research Council
Genova

THANK YOU ALL FOR YOUR ATTENTION!
GABA-induced glutamate release requires the presence of sodium and chloride ions.


Concentration (mM)

- GABA: 0.03
- Na$^+$: 146.2, 6.2
- Cl$^-$: 144.2, 4.2
The GABA-induced glutamate release is not blocked by riluzole.