# Anesthetic activation of $GABA_A$ receptors in astrocytes triggers a persistent increase in cell-surface expression of $\alpha5GABA_A$ receptors in neurons via IL-1β in mice

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**Introduction:**
Many patients develop delirium and cognitive deficits in the postoperative period, which is associated with poor long-term outcomes (1,2). Preclinical studies have shown that even a brief exposure to commonly used general anesthetic drugs triggers a sustained increase in inhibitory tonic current generated by α5 subunit–containing $GABA_A$ receptors ($\alpha5GABA_ARs$) in mouse hippocampal neurons (3). The resulting increase in inhibition causes subtle, yet sustained postanesthetic cognitive deficits. Interestingly, astrocytes are necessary for this effect and express anesthetic sensitive $GABA_A$ receptors. Furthermore, the proinflammatory cytokine IL-1β also increases tonic current in neurons via a p38 mitogen-activated protein kinase (p38 MAPK) signaling pathway (4). Additionally, inhibiting the IL-1 receptor or p38 MAPK function in neurons prevents the anesthetic-induced increase in tonic current (5). However, how the release of IL-1β is stimulated, the cellular source of IL-1β, and if

this promotes the persistent phosphorylation of p38 MAPK, are currently unknown. Synthesizing these observations, we hypothesize that anesthetic drugs activate $GABA_A$Rs in astrocytes to trigger the release of IL-1β which acts on neurons through p38 MAPK to induce an increase in the cell-surface expression of α5$GABA_A$Rs.

**Methods:**
Studies were approved by the local animal ethics committee. Cortical astrocytes and hippocampal neurons were isolated from fetal CD1 mice and grown in cell cultures. Astrocyte cultures were treated with etomidate (1 μM) +/- the $GABA_A$ receptor antagonist bicuculline (20 μM) plus etomidate for 1 hour, then the drug was washed out and the cultures were incubated for further 2 hours. The conditioned medium was then transferred to hippocampal neuronal cultures, and 24 hours later the cell-surface expression of α5$GABA_A$Rs was assessed via biotinylation and Western blot. The protein levels of IL-1β and p38 MAPK were measured with Western blot in etomidate-treated astrocytes and conditioned medium-treated neurons. Furthermore, the protein levels of IL-1β in the treated conditioned media was measured via ELISA. The detected IL-1β concentration was then used to treat neurons, and 24 h later tonic current was recorded with voltage clamp techniques.

**Results:**
Treating astrocytes with etomidate induced a significant increase in neuronal α5$GABA_A$R cell-surface expression. Furthermore, cotreating the astrocytes with bicuculline prevented the etomidate-induced persistent increase in α5$GABA_A$R surface expression in neurons. Following etomidate treatment, mature IL-1β protein was increased in astrocytes and the phosphorylation of p38MAPK was increased in neurons. A significant increase in IL-1β protein was also detected in etomidate-treated astrocyte conditioned media. The detected concentration of IL-1β was sufficient to drive a persistent increase in tonic current in neurons.

**Conclusion:**
These results suggest that anesthetic activation of $GABA_A$Rs in astrocytes triggers the maturation and release of IL-1β from astrocytes. The released IL-1β then acts on neurons through the phosphorylation of p38 MAPK, driving an increase in α5$GABA_A$R surface expression and function. Our results identify a novel cross-talk mechanism between astrocytic $GABA_A$Rs and neuronal $GABA_A$Rs that might be targeted to mitigate postanesthetic cognitive deficits.

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**Abstract Category:**
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Preclinical Study

**Meeting Selection:**
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**Subspecialty Categories:**
Neuroscience in Anesthesiology and Perioperative Medicine

## Affirmations

**The study was approved by the appropriate IRB or other local review board.**
Yes