Neuroscience 2013

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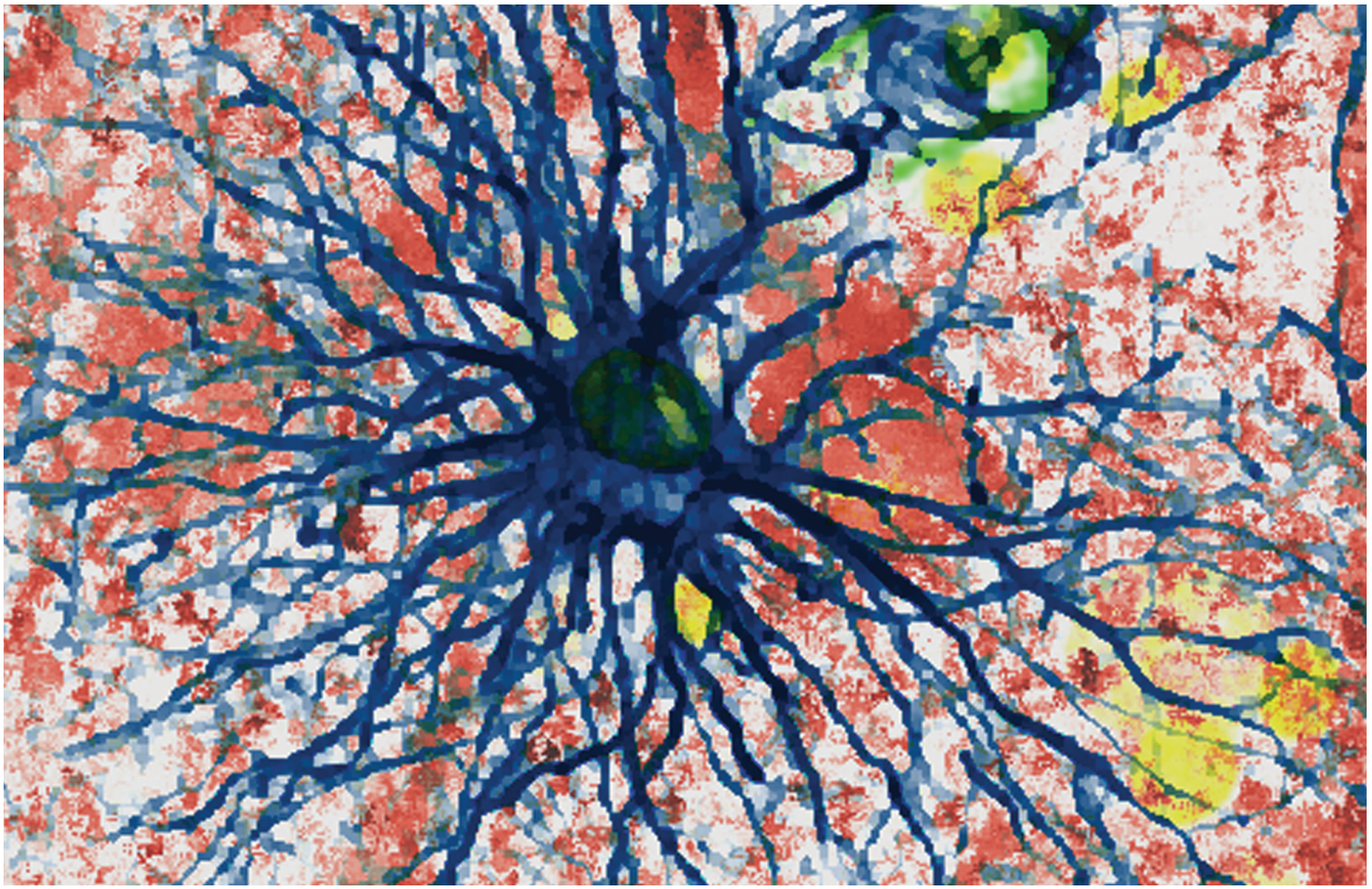
# Hot Topics



Society for Neuroscience

## OPTOGENETIC GLIAL ALKALIZATION RELIEVES ISCHEMIC BRAIN DAMAGE

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**Program Number:** 521.19
**Session Date/Time:** Tuesday, Nov.12, 8:00 AM
**Room Number:** Halls B-H
**Board Number:** G23
**Presentation Time:** 10:00 - 11:00 AM
**Session Title:** Astrocytes: Injury and Disease

Our research shows that excess release of glutamate from non-neuronal cells, the glial cells, leads to brain deterioration upon ischemia. The key trigger of this release is glial acidification and the release can be effectively stopped by countering the acidification with optical activation of a transgenetically expressed proton pump.
Clogging of brain blood vessel can happen anytime on healthy individuals, which often leads to brain damage with severe consequences. Reperfusion of the vessels with thrombolytic agents is the only currently available therapy to save the brain cells from oxygen and glucose deprivation (OGD); however, careful use of thrombolytic agents is required on patients with possible cerebral aneurysm and reperfusion itself could also cause secondary wave of injury. Excess liberation of glutamate upon ischemia is known to be the direct cause of neuronal cell death; however, the mechanisms of release, let alone the possible method to stop this release have never been sought.
We focused on the astrocytes of the glial cell population as they rapidly respond to ischemic stress. When aerobic metabolism shuts down as a consequence of OGD, lactate production from glycogen stored predominantly in astrocytes continues, which leads to severe acidosis. Neuronal cell death via glutamate excitotoxicity follows. We simply connected these two events and hypothesized that glial acidosis triggers glial glutamate release.
Based on this hypothesis, we sought of an intervention of ischemic brain damage by optogenetically controlling glial pH. Light-sensitive channels or pumps, originally found in algae or archaebacteria, can be exogenously expressed in mammalian cells and are used in recent studies as tools for controlling membrane potential. However, the fact largely ignored by the neuroscientists applying the optogenetic technique is that the major cation that flows through the widely used channelrhodopsin-2 (ChR2) and archaerhodopsin-T (ArchT) is proton. Thus, ChR2 and ArchT could be regarded as optogenetic tools for instant intracellular acidification and alkalization, respectively. We used ChR2 to bypass the OGD process and directly manipulate the intracellular pH and ArchT to counter with the intracellular acidification upon OGD.
Optical activation of ChR2 expressed in glial cells led to glial acidification and to the release of glutamate. Although ChR2 activation leads to depolarization in addition to acidification, depolarization alone by local application of potassium was insufficient to cause glial glutamate release. We also found that acid-induced glutamate release is mediated through DIDS-sensitive anion channel. On the other hand, glial alkalization via optogenetic activation of a proton pump, ArchT, led to cessation of glutamate release and to the relief of ischemic brain damage in vivo.
Evidence for glia modulating neuronal activity and glutamate being used as gliotransmitter is accumulating; however, the mechanism of its release remains to be elucidated. Several reports have indicated that the release is Ca2+-dependent, although functional significance of glial Ca2+ has also been questioned. Here, we propose that glial pH changes are the major regulator of glutamate release. As pH-dependent glial ATP release has also been shown, pH could be a key glial intracellular signal, which is as pivotal as Ca2+, especially in pathological conditions.
Direct application of our findings in patients is limited as it would require preemptive gene therapy before ischemia. However, we do show the mechanism leading to the excess glutamate release upon ischemia and the importance of controlling the glial pH. Based on our findings, methods aimed at delivering strong intracellular pH buffer, development of drugs that enhances H+ extrusion mechanisms, or designing of glutamate-releasing anion channel blocker could be sought to alleviate brain damage upon ischemia.