

and minimally impaired patients with amyloid deposits¹³, and it is thought to be one pathophysiological correlate of cognitive dysfunction. As β -amyloid is produced by neurons in an activity-dependent manner, a vicious cycle may be in place in which accumulation of β -amyloid and aberrant neuronal activity aggravate each other¹⁴.

These studies provide important evidence that discrete alterations in neuronal function occur in proximity to amyloid plaques early during the course of the disease. Thus, it is becoming clearer that therapy aimed at slowing or halting the disease progress must be administered

as early as possible. This leads to some important questions. At what point are the alterations actually reversible? Which comes first, β -amyloid or hyperactivity? These questions will be among those guiding future research on the treatment of Alzheimer's disease.

COMPETING FINANCIAL INTERESTS

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Knockout punch: cardiolipin oxidation in trauma

Robin B Chan & Gilbert Di Paolo

During brain injury, mechanical injuries to neural cells initiate an apoptotic cascade through cardiolipin oxidation. Mitochondrially targeted electron scavenger compounds block this process, suggesting new therapeutic avenues.

Traumatic brain injury (TBI) is a major global health burden that cuts across demographics. In North America and Europe alone, TBI may affect 3–4 million people annually, with a fatality rate of 3–6% and an even higher incidence of long-term disabilities¹. Furthermore, those afflicted by TBI may sustain an increased lifelong risk of amyotrophic lateral sclerosis, Parkinson's disease, dementia, substance abuse and other psychiatric disorders¹. TBI results from blows or jolts to the head, typically as a result of falls, contact sports, motor vehicle accidents or assaults, including those frequently experienced by soldiers in wartime (<http://www.ninds.nih.gov/disorders/tbi/tbi.htm>). If severe, the primary injury sets in motion a complex series of secondary detrimental molecular and cellular events that do further damage in neurons. In this issue of *Nature Neuroscience*, Ji *et al.*² provide elegant evidence for mitochondrial oxidative stress-associated lipid dysregulation as a landmark event that determines the balance between survival and death of neurons after TBI and suggest a new therapeutic avenue.

Mitochondria have diametrically opposed roles: on the one hand, they support life by generating ATP, and on the other hand, they

extinguish life by initiating apoptosis. The proverbial grease of this organelle's machinery is the mitochondrion-specific phospholipid cardiolipin, also known as diphosphatidylglycerol. The function of cardiolipin is determined by both structural diversity and intramitochondrial localization. Cardiolipin comprises a negatively charged hydrophilic head group, consisting of three glycerols linked by phosphates, and a hydrophobic portion consisting of four acyl chains³ (**Fig. 1a**). Notably, cardiolipin in brain mitochondria, but not in other tissues, displays a wide array of acyl chain combinations, with predominant species consisting of polyunsaturated fatty acids, such as linoleic acid (with 18 carbons and two double bonds, or 18:2), arachidonic acid (20:4) and docosahexaenoic acid (22:6)⁴. This makes these lipid species highly vulnerable to oxidation. In healthy mitochondria, most cardiolipin (75–90%, depending on cell type) is confined to the inner mitochondrial membrane (IMM), whereas a smaller fraction is found in the outer mitochondrial membrane (OMM), with exchange occurring via the inner-outer membrane interface^{5,6}. At the IMM, cardiolipin complexes with cytochrome *c* (cyt *c*) via electrostatic and hydrophobic forces, keeping cyt *c* closely associated with the electron transport chain, where it functions as an electron carrier (**Fig. 1b, left**)^{3,7}.

A change in the oxidative state of cardiolipin has been proposed to trigger the mitochondrial switch from ATP generation to apoptosis initiation^{3,6}. Changes in acyl chain composition

may also lead to cardiolipin reorganization into mitochondrial rafts that can amplify apoptotic signals^{3,8}. When cells are chemically injured using staurosporine or actinomycin D, oxidized cardiolipin (CLOx) is generated by the H₂O₂-induced peroxidase activity of cyt *c* that is still bound to native cardiolipin⁹. The initial generation of CLOx is believed to trigger a further surge of peroxidase activity by cyt *c* unfolding, which promotes further oxidation of cardiolipin⁷. CLOx has a greater propensity than cardiolipin to translocate to the OMM, where it subsequently recruits the proapoptotic Bcl2-related proteins tBID, BAK and BAX, leading to the formation of mitochondrial transition pores^{3,10}. At the same time, oxidation of cardiolipin weakens the interaction between cyt *c* and cardiolipin, resulting in the release of soluble cyt *c*, which then diffuses through the mitochondria transition pores into the cytoplasm¹¹, triggering the apoptosis cascade through sequential interaction with apoptosis activating protein-1 (Apaf-1) and caspases 9 and 3 (**Fig. 1b, center**)^{3,6}.

To determine whether TBI follows a similar molecular trajectory, Ji *et al.*² used a two-dimensional liquid chromatography–mass spectrometry (LC-MS) global lipidomics approach to detect CLOx products in brain tissue garnered from a rat controlled cortical impact (CCI) TBI model (**Fig. 1a**)¹². In total, they detected approximately 150 new CLOx molecules, whereas other, more abundant polyunsaturated phospholipids, such as phosphatidylcholine and phosphatidylethanolamine,

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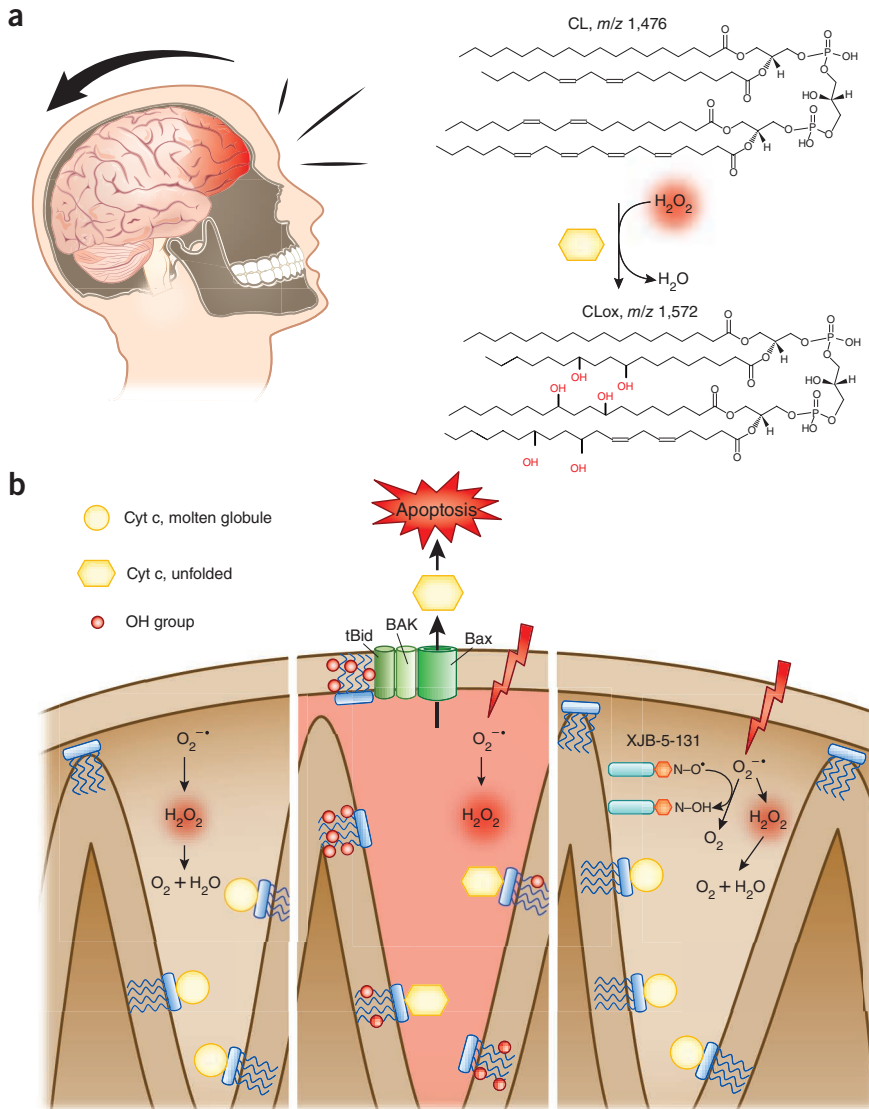


Figure 1 Blocking cardiolipin peroxidation during acute brain injury prevents apoptotic cell death. (a) Blows to the head result in a surge of reactive oxidative species production and oxidative stress. This is replicated in rats using CCI. During TBI-induced oxidative stress, major polyunsaturated cardiolipin (CL) species found in brain tissue such as CL 18:0,18:2,18:2,20:4 undergo oxidation by the peroxidase activity of cyt c. The CLOx molecule shown represents a possible resulting species. (b) Under normal circumstances (left), cardiolipin binds and retains cyt c in its native molten-globule structure at the inner mitochondrial membrane, keeping it close to the electron transport chain. Superoxide formation as a result of electron leakage from the electron transport chain or autooxidation reactions occurs at a sustainable rate, as the resulting dismutation intermediate, H_2O_2 , is enzymatically converted to water and oxygen. During TBI, however (center), a massive production of superoxide leads to accumulation of H_2O_2 and unfolding of cyt c, enhancing cyt c's peroxidase activity, which targets polyunsaturated acyl chains of CL. Oxidation of CL weakens its interaction with cyt c, which is now released and escapes to the cytosol via the mitochondria transition pore formed by the proapoptotic Bcl-2 family proteins that also interact with CLOx, triggering apoptosis. However, mitochondrially directed nitroxide conjugates (right) can scavenge electrons, reining in superoxide dismutation and avoiding the apoptosis pathway.

were not oxidized. The amount of CLOx increased approximately 20-fold, at the expense of non-oxidized cardiolipin. The authors also observed a comparable, although less marked, elevation in CLOx in the penumbra of contused brain tissue obtained from a

patient with TBI, although this milder increase may have been a result of differences in etiology and duration of the injury compared with the CCI in rat. Overall, these data confirm that acute brain injury caused by TBI activates cardiolipin oxidation.

However, is TBI-induced cardiolipin peroxidation an innocuous consequence of cell death or is it causative? To address this question, Ji *et al.*² turned to an *in vitro* model of mechanical stretch injury using rat primary cortical neurons. Consistent with the CCI results, there was a selective oxidation of cardiolipin accompanied by a marked increase in lactate dehydrogenase release, cytosolic cyt c levels, caspase3/7 activity and annexin V positivity, indicating the induction of apoptosis³. Furthermore, apoptosis could be induced by exposing primary neurons to CLOx, but not to non-oxidized cardiolipin, implicating CLOx as a direct initiator of the pathological cascade. The authors further validated the specificity of this phenomenon using RNA interference against cardiolipin synthase (*Crls1*) and cytochrome c (*Cytc*) to decrease cellular cardiolipin and cyt c levels, respectively. Both of these rescued cells from apoptosis induced by mechanical stretch.

From these data, the authors hypothesized that preventing cardiolipin oxidation could attenuate neuronal death caused by TBI and perhaps ameliorate neurological impairment. They tried a conjugate compound, XJB-5-131, which was designed by linking a stable nitroxide, 4-amino-TEMPO (4-AT), to a re-engineered hemi-gramicidin S pentapeptide sequence that was originally used to target antibiotics to the bacterial membrane¹³. Owing to the characteristic high levels of cardiolipin found in both IMM and bacterial membranes, the peptide region targets the 4-AT payload directly to the IMM, where 4-AT acts as an electron scavenger and exerts protection against chemically induced apoptosis¹³ (Fig. 1b, right). In the *in vitro* stretch model, XJB-5-131 prevented both superoxide formation and cyt c release into the cytosol. The efficacy of XJB-5-131 was mitochondria specific, as removing the mitochondria-targeting hemi-gramicidin S moiety from the compound abolished its protective capacity.

A major problem with drugs intended for the brain is poor penetrance. The presence of the hemi-gramicidin S moiety makes the conjugate compound highly hydrophobic, suggesting that XJB-5-131 may be able to cross the blood-brain barrier. Indeed, when the compound was injected intravenously into rats, the characteristic electron paramagnetic resonance signal of XJB-5-131 was detected in both cerebrospinal fluid and brain tissue. The presence of the compound in the brain was further proven by mass spectrometry imaging, thus providing irrefutable evidence that the compound can reach the brain. Furthermore, at the organismal level, XJB-5-131 inhibited cardiolipin oxidation in rats when administered

10 min after CCI, maintaining levels of cardiolipin and CLox similar to those in uninjured rats. The compound also restored the motor function and cognitive performance of rats across a battery of standard behavioral tests. Untreated rats subjected to CCI experienced prolonged cognitive deficits after injury, an outcome akin to the long-lasting effects of TBI in human subjects. This suggests that substantial and irreversible neuronal loss caused by TBI must be prevented before its onset by blocking oxidative stress, including the peroxidation of cardiolipin. The administration of XJB-5-131 appeared to block the CCI-induced increase in caspase-3/7 activity and prevent the degeneration of neurons *in vivo* as visualized by Fluoro-Jade C staining, strongly suggesting that maintaining a stable mitochondria oxidative state is critical for neuronal survival.

In summary, Ji *et al.*² identify cardiolipin oxidation as an important molecular mechanism by which the mitochondria switch from life givers to executioners after TBI. The mechanism depends on cardiolipin head group interaction with cyt c, which is in turn modulated by the oxidative status of cardiolipin acyl moieties. This provides further support for the idea that the structural diversity of fatty acyl composition of lipids in various cell types is not random. Instead, fatty acid diversity

has evolved to provide an additional layer of regulatory mechanisms involving specific lipid-lipid and lipid-protein interactions that are crucial for normal cellular function. This concept is conserved even in simpler organisms such as *Caenorhabditis elegans*, which requires ceramide and sphingomyelin species with 20- or 22-carbon fatty acyl profiles for effective response to anoxia¹⁴. The functional significance of fatty acyl carbon chain structure in lipid biology has been largely overlooked in the past owing to the lack of sensitive analytical methods. Pairing LC-MS lipidomic technology with the right experimental models holds promise for elucidating new lipid functions.

Unlike other cell types, neurons have a limited capacity for regeneration when the system is dealt severe insults, particularly in the CNS. The extent and site of neuronal loss is likely to correlate with behavioral and neurological disability experienced by TBI patients, even years after apparent recovery from the initial injury. Ji *et al.*² show that electron-scavenging compounds targeting mitochondria offer great hope in the prevention of oxidative stress damage after the primary injury has abated in TBI. As noted by the authors, these compounds may turn out to be helpful in other age-related neurodegenerative diseases that exhibit oxidative stress components, such as

Alzheimer's disease and Parkinson's disease¹⁵. Considering the dearth of effective treatments against neurodegenerative diseases in general, the continued development and evaluation of such compounds in CNS disorders should be strongly supported.

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Sleep tight, wake up bright

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Sleep consolidates memory. By cuing specific memories during sleep, a study now links this consolidation of memory to the replay of neuronal ensemble activity that occurs in the hippocampus during slow-wave sleep after learning.

The idea of learning during sleep is a common motif that occurs not only in science fiction literature, but also in oft-advertised suspicious learning methods. Although many of us are skeptical of the exaggerated promises found in these sources, recent findings of psychologists and neuroscientists shed new light on the effect of sleep on memory, the neural mechanisms behind it and even potential practical implications. It was recently found that cues such as smells or tones that are presented while learning a task can enhance memory of the task if they are then presented during sleep^{1–3}. The neuronal and network processes

that underlie sleep-induced memory enhancement are of great interest⁴. A strong candidate mechanism for this phenomenon is temporally sequenced replay, during sleep after the learning experience, of the neural activity patterns that occurred while awake during learning⁵. This replay occurs during slow-wave sleep, a phase of sleep in which learning-related cues can enhance memory consolidation, and it occurs in the hippocampus, which is an important structure for consolidating declarative memories about episodes and facts. If hippocampal replay underlies memory consolidation, then cues present while learning a task might enhance replay of task-related neuronal activity during sleep. Bendor and Wilson⁶ tested this hypothesis in a study in this issue of *Nature Neuroscience*. The authors report that, consistent with the hypothesis, acoustic stimuli associated with

neural activity during learning enhance replay of the same activity pattern if they are presented during sleep.

Bendor and Wilson⁶ trained rats on a simple task (Fig. 1). In a linear track, the rats learned to run to the right side of the track to get food reward in response to one sound (sound R) and to run to the left side of the track for the reward in response to another sound (sound L). During this task, the authors recorded the activity of hippocampal neurons. They observed that a distinct neuronal ensemble was activated during rightward runs (associated with sound R) and a different ensemble was activated during leftward runs (associated with sound L). Sounds R and L were also played to the rats, together with other sounds, during a sleep period following the training, and neural activity was again recorded. The sound modulated the replay

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