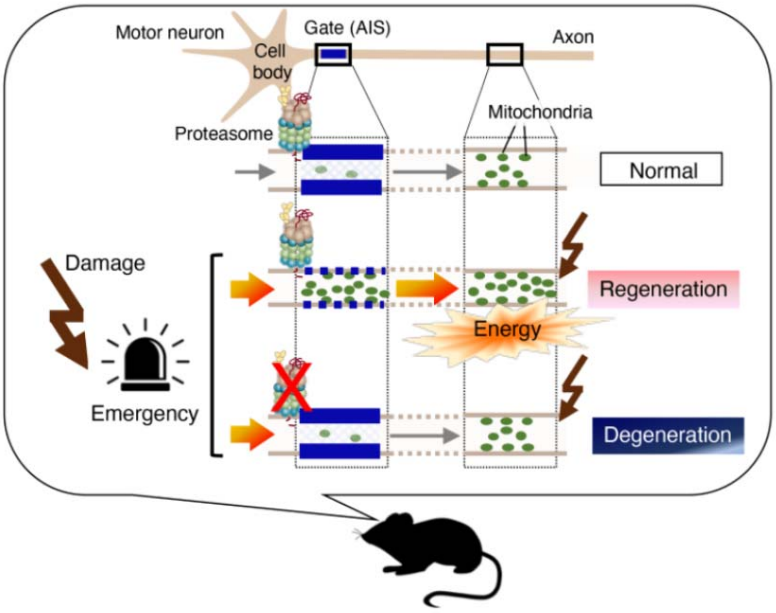


# News Release

## Title

**Transient disassembly of the axon initial segment is critical for damaged motor neurons to protect axons: a novel mechanism to avoid neurodegeneration**

## Key Points

- Researchers have established a unique, genetically engineered mouse wherein *cre* recombinase expression and mitochondrial labeling occur simultaneously in response to damage.
- While studying these unique mice, it became clear that proteasome-dependent axon initial segment (AIS) disassembly is critical to the response of motor neurons to damage, enabling them to meet axonal energy demands and so avoid degeneration.
- The finding could be of interest as a new therapeutic target in neurodegenerative diseases.

## Summary

Researchers at Nagoya University, Kyoto University, and Osaka University have shown that transient, proteasome-mediated disassembly of the axon initial segment (AIS) is crucial for damaged motor neurons to avoid degeneration.

Physiological responses in the cell are spatiotemporally regulated by protein degradation. The proteasome is the main player in protein degradation. The proteasome is thought to be involved in protein degradation for the stress responses to protect damaged motor neurons and promote axon regeneration. Intriguingly, the proteasome dysfunction has been implicated in the motor neuron pathology of amyotrophic lateral sclerosis (ALS), a progressive neurodegenerative disease. However, it remains unclear how proteasome function influences the stress resilience of damaged motor neurons.

To clarify the proteasome-mediated stress responsive mechanism of damaged

motor neurons, the researchers developed a unique mouse system in which mitochondrial labeling and proteasome ablation co-occur in damaged motor neurons specifically. This newly established mouse system has revealed that the gate at the entrance to the axon (the AIS structure) is a crucial target of the proteasome and is actively disrupted in response to damage. The AIS resides between the cell body and axon and acts as a gatekeeper for the axonal transport under normal condition. Upon damage, the transient disassembly of the AIS allows the entry of quick and large number of healthy mitochondria from the cell body into damaged axons. This unexpected mechanism ensures the damaged axons are able to acquire enough energy from mitochondria to prevent degeneration. However, motor neurons damaged by disease in a mouse model of ALS are unable to activate this emergency mechanism owing to proteasome dysfunction. The team, led by Associate Professor Sumiko Kiryu-Seo and Professor Hiroshi Kiyama from Nagoya University, expects that these findings will contribute to the understanding of the molecular basis of neuronal damage that occurs prior to neurodegeneration and present new therapeutic targets for neurodegenerative diseases.

The study was published in *The EMBO Journal* on August 25, 2022.

**Research Background**

Effective treatments for motor neurodegenerative diseases such as ALS have not yet been established. This research team believes that understanding the molecular basis of motor neuron responses to damage could provide new insights into the pathogenesis of the disease. It is widely accepted that proteasomes are dysfunctional in many neurodegenerative diseases, including ALS. However, it is unclear how proteasome function positively impacts the resilience of damaged motor neurons.

Neurons demand large amounts of energy to maintain their functions and integrity. Mitochondria play pivotal roles in energy supply and metabolism. By monitoring the behavior of fluorescent-labeled mitochondria, the researchers attempted to clarify the proteasome-dependent stress response in motor neurons soon after damage.

**Research Results**

The researchers first developed *Atf3*:BAC Tg mice, in which mitochondrial labeling and *cre* recombinase expression co-occur in damaged motor neurons specifically. Three-dimensional images of the transparent brain of *Atf3*:BAC Tg mouse showed fluorescent-labeled mitochondria in the cell body, axon and dendrites of damaged neurons specifically. This mouse system also enabled them to measure the movement of fluorescent-labeled mitochondria within damaged neurons in living mice. Here, researchers used motor axon injury as a simple motor neuron damage model. In this model, motor neurons show resilience to axon injury in that they survive and regenerate. The combination of this model with a unique, genetically engineered mouse has identified an emergency stress-resilience mechanism in damaged motor neurons. In this mechanism, the AIS structure, at the juncture between the cell body and the axon, is targeted by the proteasome for protein degradation upon damage, thereby allowing a rapid influx of large number of mitochondria into the damaged axon to provide sufficient energy to it. However, damage-induced proteasome-deficient motor neurons fail to activate this emergency mechanism, resulting in ALS-like neurodegeneration. It is remarkable that many other early-stage

damage responses occur normally owing to compensatory protein degradation by autophagy.

The researchers further hypothesized that ALS motor neurons may be unable to activate this proteasome-mediated mechanism in response to disease damage, because ALS motor neurons dysfunction proteasome. When ALS mice were crossed with *Atf3*:BAC Tg mice, mitochondria in vulnerable (i.e., stress-sensitive) motor neurons were selectively labeled by fluorescence at a pre-symptomatic stage. These motor neurons showed the signature of a damage response, but were not able to dismantle the AIS structure, and therefore failed to increase mitochondrial supply to their axons.

**Research Summary and Future Perspective**

This work on the response to neuronal damage has uncovered a fundamental mechanism of neurodegeneration. The mouse model system established here is a powerful tool that expands our understanding of the molecular basis of neuronal damage. As such, their use may open up new pathways for therapeutic intervention in neurodegenerative diseases.

**Publication**

**Title:**

Impaired disassembly of the axon initial segment restricts mitochondrial entry into damaged axons

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