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Japan Science and Technology Agency (JST)
Keio University
Aichi Medical University

A New Synthetic Molecule Repairs Damage to the Brain and Spinal Cord

—A Bridge over Troubled Synapses in Neuropsychiatric and Neurological Disorders—

Research Highlights

- A broad range of neuropsychiatric and neurological disorders are thought to be caused by abnormalities in synapses, which are the connections between nerve cells. However, there are currently no therapeutic methods for directly controlling the synaptic structure.
- We have developed a synthetic synaptic connector, which successfully restores synapses and improve pathological conditions associated with mouse models of cerebellar ataxia, Alzheimer's disease, and spinal cord injury.
- Structure-based design of synthetic synaptic connectors that bridge distinct pre- and postsynaptic molecules may pave the way for new treatments for neuropsychiatric or neurological disorders caused by synaptic abnormalities

Through a JST Strategic Basic Research Program, a research team led by Professor Michisuke Yuzaki and Assistant Professor Kunimichi Suzuki of the Department of Physiology, Keio University School of Medicine, have developed a novel synthetic synaptic organizer that can quickly connect a neural circuit.

Synapses[1], which are the connections between nerve cells, are formed, maintained, and remodeled not only during organism development, but also throughout life by synaptic organizers[2]. The onset of many neuropsychiatric or neurological disorders, such as autism spectrum disorders, schizophrenia, and Alzheimer's disease, is considered to be due in part to an imbalance of excitatory and inhibitory synaptic signaling. However, there are currently no therapeutic methods for directly controlling the synaptic structure.

In a previous study, Professor Yuzaki's research team discovered a synaptic connector[2], cerebellin (Cbln1)[3], which is a type of synaptic organizer that mainly connects pre- and postsynaptic membrane proteins in the cerebellum. Here, the team has developed a new synthetic synaptic connector, CPTX, by combining structural elements from Cbln1 and another synaptic organizer protein, neuronal pentraxin-1 (NP1)[4]. Application of CPTX to mouse models of cerebellar ataxia[5], Alzheimer's disease, and spinal cord injury could successfully restore synapses and improve motor coordination[6], spatial and contextual

memories, and locomotion associated with each of these disease models, respectively.

Structure-guided design of synthetic synaptic organizers targeting distinct pre- and postsynaptic molecules may inspire the development of a variety of innovative molecular tools that can repair or remodel a wide range of neural circuits. These tools are expected to lead to the elucidation of the mechanism of synapse formation and maintenance as well as the development and application of new therapeutic treatments for neuropsychiatric or neurological disorders.

This research is the result of an international collaboration between Keio University and research teams led by Professor Alexander Dityatev of the German Center for Neurodegenerative Diseases (DZNE), Professor Radu Aricescu of the MRC Laboratory of Molecular Biology, UK, and Professor Kosei Takeuchi and Assistant Professor Hiroyuki Sasakura of Aichi Medical University.

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Study: "New optogenetic tools to decipher synaptic plasticity underlying learning and memory in vivo"
Research Director: Michisuke Yuzaki (Professor, Department of Physiology, Keio University School of Medicine)
Research Period: October 2018 – March 2024

JST Exploratory Research for Advanced Technology (ERATO) Research Funding Program
Study: "HAMACHI Innovative Molecular Technology for Neuroscience"
Research Director: Itaru Hamachi (Professor, Graduate School of Engineering, Kyoto University)
Research Period: October 2018 – March 2024

1. Research Background

In our daily lives, we are constantly remembering and learning new things, and we freely move our bodies without much thought. While this may sound simple at first, the underlying mechanisms behind these actions are quite complex. The central nervous system, which includes the brain and spinal cord, plays a particularly important role in these actions. In the central nervous system, a large number of neuronal cells gather and connect with each other, forming a large and complex network that is not random, but rule-based. The special junctions that allow these nerve cells to form networks are called synapses. Synapses are where information is transferred from one neuron to another. Presynaptic neurons that send information release neurotransmitters, which are received by postsynaptic neurons to transmit the information. The molecules that create the bridges for this transfer to take place are collectively known as synaptic organizers. The synaptic organizer functions as a synapse connector that forms a "molecular bridge" between the presynaptic and postsynaptic sites and gathers molecules involved in the transfer of information at the synapse.

Although many synapses are made during an organism's developmental stage, synapses are removed when activity decreases, and conversely, the number and functions of synapses increase in active neural circuits, and are formed, maintained, and remodeled throughout life. It is believed that such synaptic homeostasis and dynamic development are the essence of our ability to remember things, forget things, and are what allow us to improve motor coordination through repeated practice. (See Fig. 1)

In recent years, dysfunction and mutation of synaptic organizers have been reported in neurodevelopmental disorders such as autism and in neuropsychiatric and neurological disorders such as Alzheimer's disease, leading them to be regarded as "synapse disorders." It has been strongly suggested that some neuropsychiatric and neurological disorders are caused by a decrease in the number of synapses, and the elucidation of the molecular mechanism that forms and maintains synapses as well as the development of methods that control these processes have become extremely important for effective therapeutic drug development. However, the synapses lost in neuropsychiatric and neurological disorders are diverse, and until now there had been no effective way to restore them.

Professor Yuzaki's research team has since discovered and studied a synapse organizer known as cerebellin (Cbln1). (Matsuda et al., *Science*, 2010) Cbln1 is a synaptic connector predominantly expressed in the cerebellum. Cbln1 strongly promotes synapse formation and maintenance by connecting presynaptic and postsynaptic molecules. The research team hypothesized that if synaptic connectors such as Cbln1 could work in other areas of the brain, they could improve the pathology of neuropsychiatric and neurological disorders caused by synaptic dysfunction and loss of synapses. This led the team to begin developing a new synthetic synaptic organizer protein based on the structural information of Cbln1 and one other synaptic organizer protein in order to connect specific molecules and restore the desired synapses.

2. Research Details

Cbln1 is a synaptic organizer protein that forms synapses by simultaneously binding to

presynaptic neurexin (Nrx)[7] and postsynaptic delta-type glutamate receptor (GluD)[8] (See Fig. 2 left). Nrx is ubiquitously present at presynaptic sites, while GluD is present in certain excitatory postsynaptic terminals, such as in the cerebellum. In other words, Cbln1 is an endogenous synaptic connector that bridges Nrx and GluD to form synapses in certain regions of the brain.

As a substitute for GluD, the research team focused on AMPA-type glutamate receptors (GluA),[9] a receptor that receives glutamate as a neurotransmitter and transmits information to the postsynaptic site. GluA is present at most excitatory postsynaptic sites. An endogenous synaptic organizer, NP1, is known to induce clustering of postsynaptic GluA. However, unlike CPTX, NP1 does not bridge pre- and postsynaptic sites because it does not bind Nrx (See Fig. 2, right). If Cbln1 were to bind to GluA, like NP1, it would function as a synaptic connector for a wide range of applications. This led the research team to clarify the crystal structure of the pentraxin domain where NP1 binds to GluA, and connected with the Nrx-binding domain of Cbln1 to create CPTX (**C**bln1 + neuronal **p**en**t**ra**x**in-1) (See Fig. 3). We then investigated whether CPTX would function as a synthetic synaptic organizer that would bridge Nrx and GluA to connect synapses (See Fig. 4).

First, we conducted experiments on binding properties using cultured cells. When Nrx, GluA, and GluD were expressed on the cell surface and CPTX was added, CPTX bound to Nrx and GluA as expected. Next, we investigated whether CPTX would induce synapse formation in neurons, we administered CPTX to cultured neurons and confirmed that Nrx and GluA were bridged to form excitatory synapses (See Fig. 5). Finally, we examined whether CPTX could act as a synaptic connector in living animal models. CPTX was administered to mouse models for cerebellar ataxia, Alzheimer's disease, and spinal cord injury, each disorders of three different parts of the central nervous systems, (1) cerebellum, (2) hippocampus, and (3) spinal cord, and we investigated whether neural circuits and synaptic connectivity would be restored (See Fig. 6 and 7).

① The cerebellum is an important structure in the brain responsible for motor coordination, examples of which include walking in a balanced manner, reaching for a cup, and riding a bicycle. It is also associated with neurological disorders such as spinocerebellar degeneration. Genetically modified mice with a reduced number of cerebellar synapses show symptoms of cerebellar ataxia, including stride irregularity and the inability to alternate between legs while walking. When CPTX was administered to the cerebellum of these mice, synaptic formation was restored within days, and they were again able to walk in a balanced and stable manner (See Fig. 6, lower left).

② The hippocampus is an important region of the brain responsible for learning and memory. In dementia such as Alzheimer's disease, hippocampal atrophy and synapse loss are observed. When CPTX was injected into the hippocampus of mouse models for Alzheimer's disease, synaptic formation was restored within days, and results for activities such as maze learning and memory recall were restored to the same level as those observed in healthy mice (See Fig. 6, lower middle).

③ The spinal cord is the nerve pathway that allows the brain to output motor information. If the spinal cord is damaged, as the result of a road traffic accident for example, serious trouble such as paralysis can occur. In mouse spinal cord injury models, synaptic connections around the

injured region decreased and the hind limbs were unable to move well, but administration of CPTX showed restoration of synaptic connection and motor function within days. (See Fig. 6, lower right and Fig. 7) Notably, injection of CPTX one week after spinal cord injury proved most effective for recovery, with continuous recovery of locomotion observed for more than 8 weeks following the injection.

These results have shown that CPTX, a synthetic synaptic connector that combines structural features of Cbln1 and NP1, is an unprecedented and innovative tool for restoring excitatory synapse loss in the central nervous system and promoting functional recovery of damaged neuronal circuits.

3. Future Development

This study proved a new concept that, by combining binding regions based on the structural features of different synaptic organizer proteins, synaptic function could be restored and applied to the treatment of disorders caused by synaptic dysfunction and loss of synapses. This shows that there is potential in this new approach of restoring neuronal circuits, which is completely different from conventional treatments in neuropsychiatric or neurological disorders.

There is also the potential to develop next-generation synthetic synaptic connectors that could repair or remodel neuronal circuits by using proteins other than CPTX. Going forward, we will continue to confirm the safety and characterization of synthetic synaptic connectors using animal models to help inform the future treatment of neuropsychiatric and neurological disorders in humans.

The design of synthetic synaptic connector CPTX is based on the knowledge of synapses that has been clarified through the accumulation of basic research in neuroscience. However, many details remain unclear, such as what kind of molecules form synapses and how complex neuronal circuits are wired. Synthetic synaptic connectors, including CPTX, are expected to drive development in basic neuroscience research.

4. Notes

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- Strategic Research Program for Brain Sciences (SRPBS):
"Development of new classification and treatment for neuropsychiatric disorders based on AMPA receptor levels"
- Project Promoting Support for Drug Discovery "The iD3 Booster":

5. Reference Images

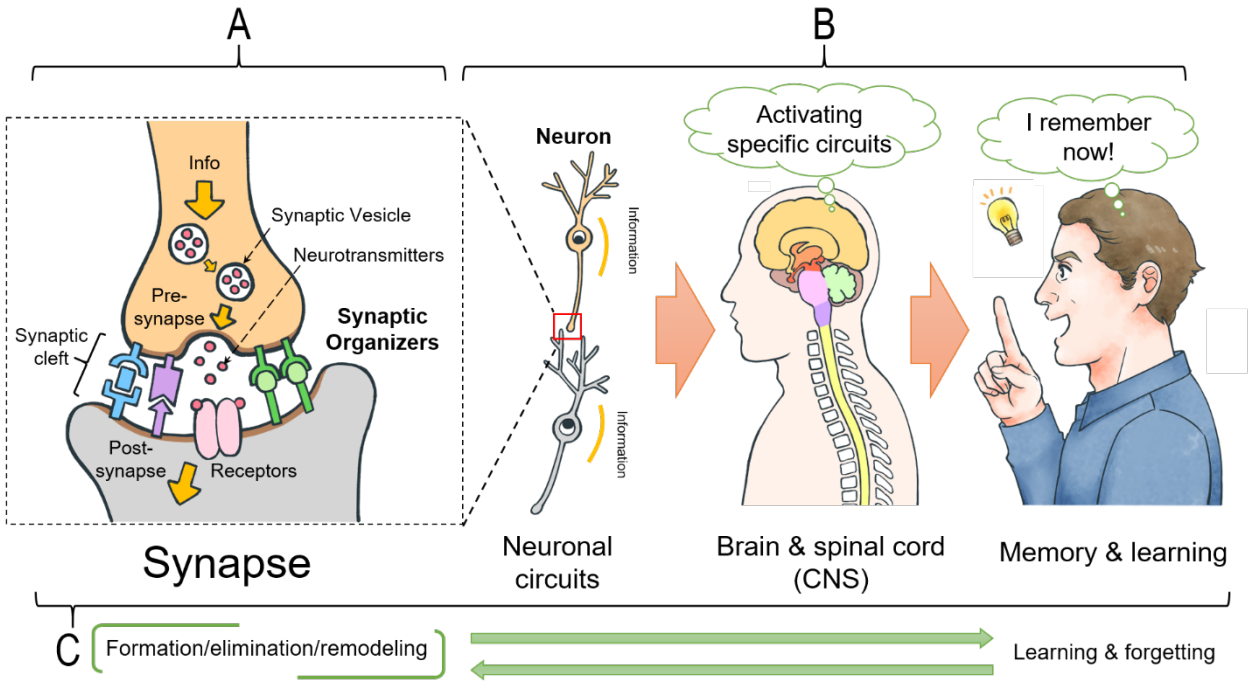

Fig. 1: Synapses are responsible for higher functions such as memory and movement in the human brain

A: Synapses, which are the point of connection between neuronal cells, are formed by synaptic organizers. When information reaches the presynaptic neuron, neurotransmitters are released into the synaptic cleft, and the receptors in the postsynaptic neuron receive them to transmit the information.

B: Most neuronal cells interconnect through synapses to form neural circuits, and these neural circuits further integrate to form the central nervous system. Neural circuits carry out specific functions such as memory and movement in different areas of the body.

C: The structure and function of synapses with increased activity are strengthened, while synapses where activity has ceased are removed. It is thought that as a result of this, we remember and forget things, and that motor coordination is improved with repeated practice.

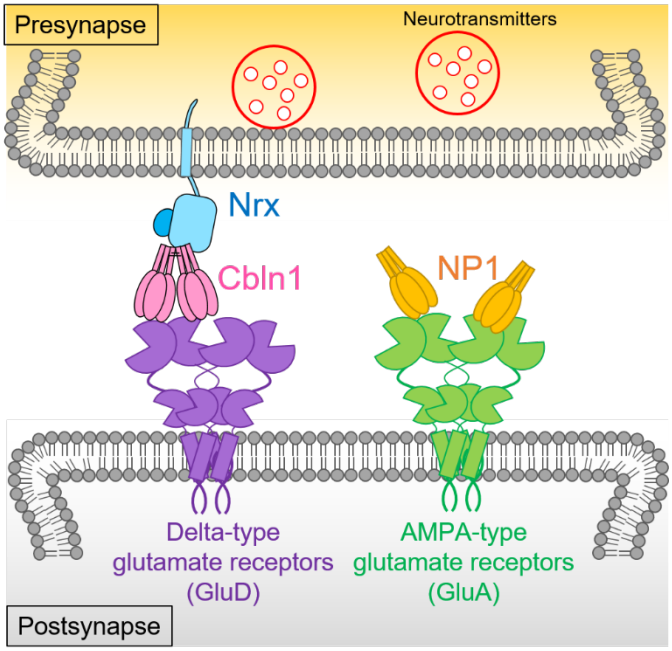

Fig. 2: Structure and function of synaptic organizers

Cbln1 is a synaptic organizer that acts as a synaptic connector by bridging presynaptic Nrx and postsynaptic GluD. NP1, on the other hand, does not function as a synaptic organizer for binding to Nrx, but it does function as a synaptic organizer for binding to postsynaptic GluA.

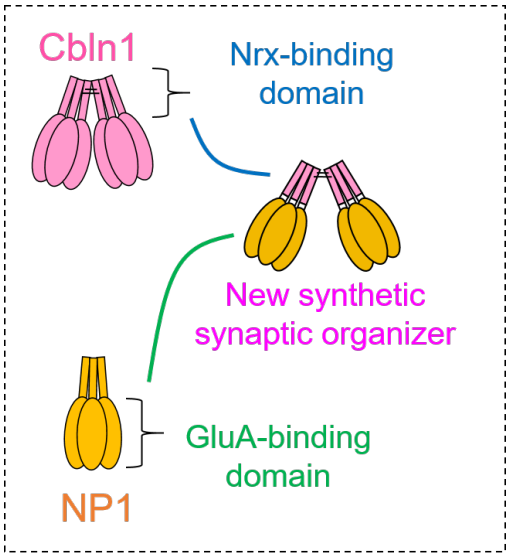

Fig. 3: Manufacturing process for synthetic synaptic organizer CPTX

The structures of the natural synaptic organizers Cbln1 and NP1 are divided into a rod-shaped region and a globular region. The rod-shaped region of Cbln1 can bind to Nrx and the globular region of NP1 can bind to GluA. We also knew that Cbln1 had a cherry-like hexameric structure. We developed the synthetic synaptic connector CPTX by designing a molecule that combined the Nrx binding region of Cbln1 and the GluA binding region of NP1 while retaining this hexameric structure.

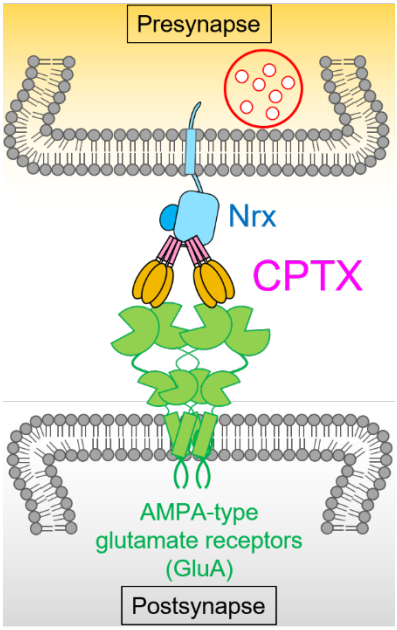

Fig. 4: Synaptic membranes connected by CPTX

We expected that CPTX would form synapses by bridging presynaptic Nrx and postsynaptic GluA. Since GluA exists at most excitatory postsynaptic sites, we thought that CPTX could induce the formation of excitatory synapses and re-establish the neural circuits damaged by the disease.

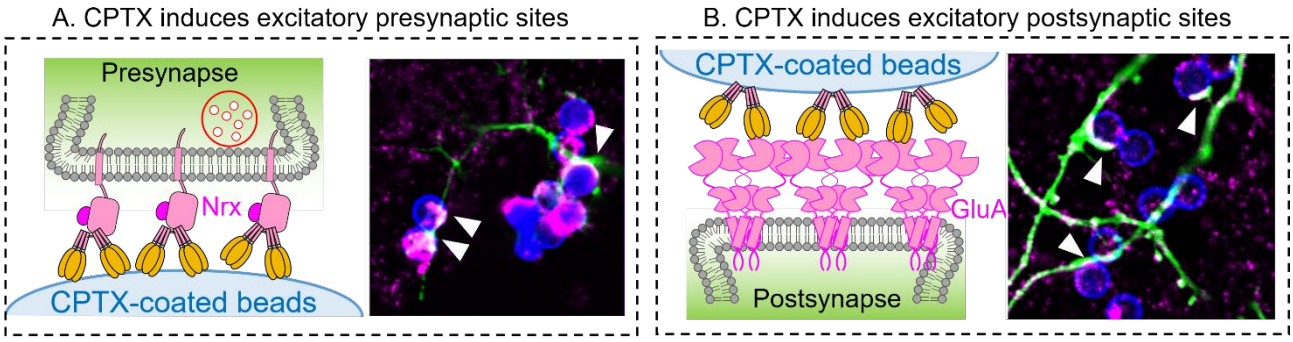

Fig. 5: CPTX induces synapse formation in cultured neurons

When beads (blue) coated with CPTX mixed with cultured neurons, they appeared to bind to Nrx (magenta) at the presynaptic site (green) (A, arrowhead), and to GluA (magenta) at the postsynaptic site (green) (B, arrowhead). The beads were 2 micrometers in diameter.

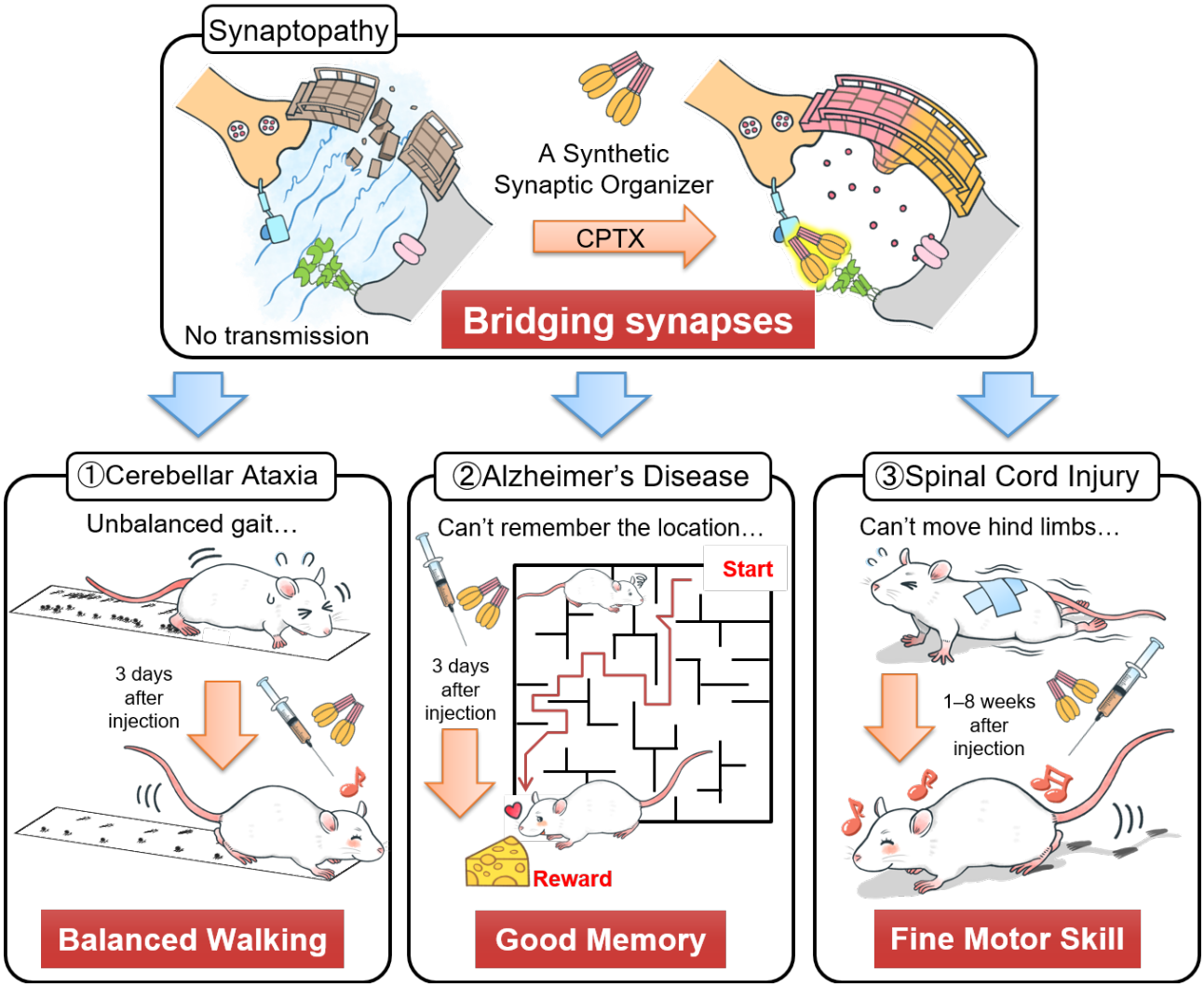

Fig. 6: CPTX restores neural circuits and improves motor coordination and spatial memory.

When synapses are damaged, information cannot be transmitted, resulting in loss of motor coordination and the inability to make memories. CPTX, which can induce functional synapses, was shown to restore synapses when administered to mouse models of neurological diseases with abnormal synapses (upper figure). Furthermore, (1) mouse models for cerebellar ataxia were able to walk in a balanced and stable manner, (2) mouse models for Alzheimer's disease were able to accurately remember the shortest route from the starting point to the reward point, and (3) mouse spinal cord injury models exhibited rapid recovery of motor performance, including the ability to move previously paralyzed legs.

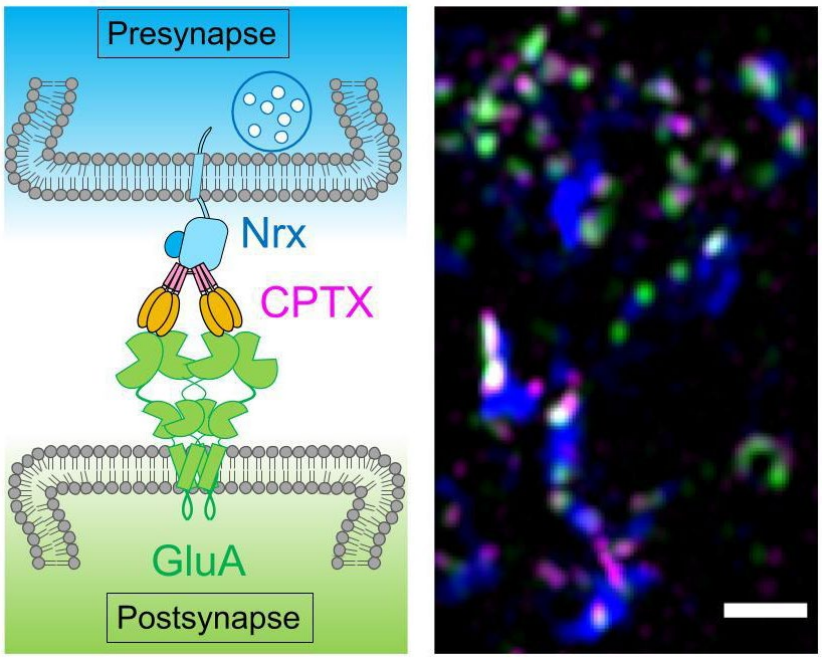

Fig. 7 CPTX restores synaptic connectivity

CPTX was administered to the spinal cords of mouse spinal cord injury models, and the state of synapses was observed using super-resolution microscopy. We observed that presynaptic (blue) and postsynaptic neurons (green) were bound by CPTX (magenta). Scale bars, 1 μm.

Glossary

[1] Synapse

A synapse is the specialized junction where information is transmitted between neurons. Signals are transmitted from the presynaptic neuron where neurotransmitters are stored and released to the postsynaptic cell where receptors are ready to receive them. In excitatory synapses, glutamate is released from the presynaptic neuron and excites nerve cells via glutamate receptors in the postsynaptic cell. The presynaptic and postsynaptic sites of the synapse are separated by a cell membrane, and groups of molecules that play a role in organizing the transmission of information here are called synaptic organizers.

[2] Synaptic Organizer/Synaptic Connector

Molecules that regulate the construction of synapses and the transmission of information between them are collectively referred to as synapse organizers. Within synaptic organizers are synaptic connectors, molecules that act as a bridge between presynaptic and postsynaptic sites.

[3] Cerebellin (Cbln1)

Cbln1 is a synaptic organizer that is abundant in the cerebellum and functions as a synaptic connector by binding to presynaptic neurexin and postsynaptic GluD[8].

[4] Neuronal Pentraxin-1 (NP1)

NP1 is one of the synaptic organizers that promotes information transmission by binding to GluA[9] and accumulating at the postsynaptic site.

[5] Cerebellar Ataxia

Cerebellar ataxia is a disorder in which the function of the cerebellum is impaired. The cerebellum is an important structure in the brain responsible for motor coordination[6]. Symptoms of cerebellar ataxia include impaired motor function, such as staggering when walking and the inability to move quickly and with precision.

[6] Motor Coordination

Motor coordination involves the combination of multiple muscles at the right time with proper intensity. Examples of motor coordination include walking in a balanced manner, reaching for a cup, and riding a bicycle. Mice that cannot perform coordinated movements may not be able to alternate legs while walking, or their stride may become unstable.

[7] Neurexin (Nrx)

Nrx is one of the synaptic organizers found in presynaptic neurons. In addition to being involved in synaptic bridging, Nrx also gathers the machinery required for neurotransmitter release.

[8] Delta-type Glutamate Receptor (GluD)

Delta-type glutamate receptors (GluD) are one of the synaptic organizers that are selectively expressed in places such as the excitatory postsynaptic membranes of the cerebellum and are involved in synaptic bridging.

[9] AMPA-type Glutamate Receptor (GluA)

AMPA-type glutamate receptors (GluA) are the main glutamate receptor type located at the postsynaptic site of most excitatory synapses. They receive glutamate released from the presynaptic neuron and transfer information to the neurons of the postsynaptic site.

Research Paper

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Authors	Kunimichi Suzuki†, Jonathan Elegheert†, Inseon Song†, Hiroyuki Sasakura†, Oleg Senkov, Keiko Matsuda, Wataru Kakegawa, Amber J. Clayton, Veronica T. Chang, Maura Ferrer-Ferrer, Eriko Miura, Rahul Kaushik, Masashi Ikeno, Yuki Morioka, Yuka Takeuchi, Tatsuya Shimada, Shintaro Otsuka, Stoyan Stoyanov, Masahiko Watanabe, Kosei Takeuchi, Alexander Dityatev*, A. Radu Aricescu*, Michisuke Yuzaki* († Lead Author, * Corresponding Author)
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Contact Information

Inquiries regarding this research:
Michisuke Yuzaki
Professor, Department of Physiology, Keio University School of Medicine
35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582 Japan
Tel: 03-5363-3749 Fax: 03-3359-0437
E-mail: myuzaki@keio.jp

Inquiries regarding JST projects:
Mutsuko Yasuda
Japan Science and Technology Agency (JST)
Department of Strategic Basic Research
Life Innovation Group
K's Gobancho, 7 Gobancho, Chiyoda-ku, Tokyo, 102-0076 Japan
Tel: 03-3512-3524 Fax: 03-3222-7348 E-mail: crest@jst.go.jp

Go Kato
Japan Science and Technology Agency (JST)
Department of Research Project
Green Innovation Group
K's Gobancho, 7 Gobancho, Chiyoda-ku, Tokyo, 102-0076 Japan
Tel: 03-3512-3528 Fax: 03-3222-2068 E-mail: eratowww@jst.go.jp

Press

Japan Science and Technology Agency (JST)
Department of General Affairs Public Relations Division
5-3 Yonbancho, Chiyoda-ku, Tokyo 102-8666 Japan
Tel: 03-5214-8404 Fax: 03-5214-8432 E-mail: jstkoho@jst.go.jp

Keio University Shinanomachi Campus Office of General Affairs
35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582 Japan
Tel: 03-5363-3611 Fax: 03-5363-3612 E-mail: med-koho@adst.keio.ac.jp

Aichi Medical University School of Medicine
Administrative Affairs Department, Miscellaneous Affairs Section
1-1 Karimata, Yazako, Nagakute-shi, Aichi 480-1195 Japan
Tel: 0561-61-5396 Fax: 0561-62-6690 E-mail: syomu@aichi-med-u.ac.jp