Nets on Neurons

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One possible function of perineuronal nets is to protect nerve cells

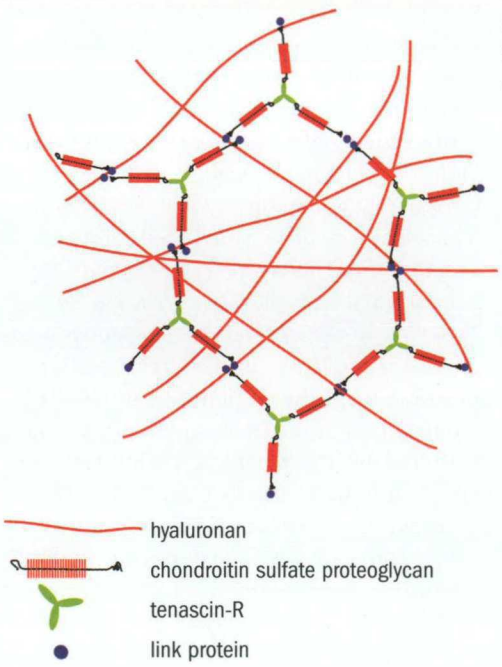
The discovery of perineuronal nets is attributed to Camillo Golgi, the father of contemporary neuroanatomy. In 1873, in a tiny laboratory in his kitchen, the Italian doctor and scientist devised a method of dyeing nervous tissue with a silver nitrate solution, allowing him to observe many hitherto unknown cellular structures. One of his discoveries was a delicate network present on the surface of certain neuron cell bodies and proximal dendrites. The discovery was initially met with a cool reception; the outstanding Spanish neuroanatomist Santiago Ramón y Cajal, one of Golgi's rivals, dismissed it as an artifact of the method, and halted his own research for a number of years. It was not until the 1950s and the development of new histological techniques and electronic microscopy that researchers were able to confirm the existence of these structures in the brain.

Hexagonal structure

Today's scientific opinion regards perineuronal nets as a structural form of the extracellular matrix – the substance filling the space between neurons. The extracellular matrix in the brain mostly comprises hyaluronan (a polymer of disaccharides) and chondroitin sulfate proteoglycans. However, in contrast to other tissues, it does not contain collagen or elastin. Perineuronal nets, with hexagonal eyelets, are located on the external surface of the neuron's cellular membrane; they comprise hyaluronan, whose long chains form the edges of the net, and a core, to which proteoglycans are bound. The specific hexagonal structure of the net is maintained by link proteins connecting proteoglycan molecules to hyaluronan and tenascin-R, linking individual proteoglycan molecules (Fig. 1). Due to the high content of carboxyl and sulfate groups in their polysaccharide chains, perineuronal nets carry a high negative charge. The most common proteoglycan in perineuronal networks is aggrecan. Together with hyaluronan, aggrecan is one of the key proteins in cartilage. In fact, there are striking chemical similarities between the extracellular matrix in the brain and cartilaginous tissue.

Perineuronal net markers

The number of animal species that have been studied to look for the presence of perineuronal nets in their brains is rather low, with rodents – mice and rats – having been studied the most closely. Such nets have also been detected in the brains of



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Hypothetical structure of a perineuronal net

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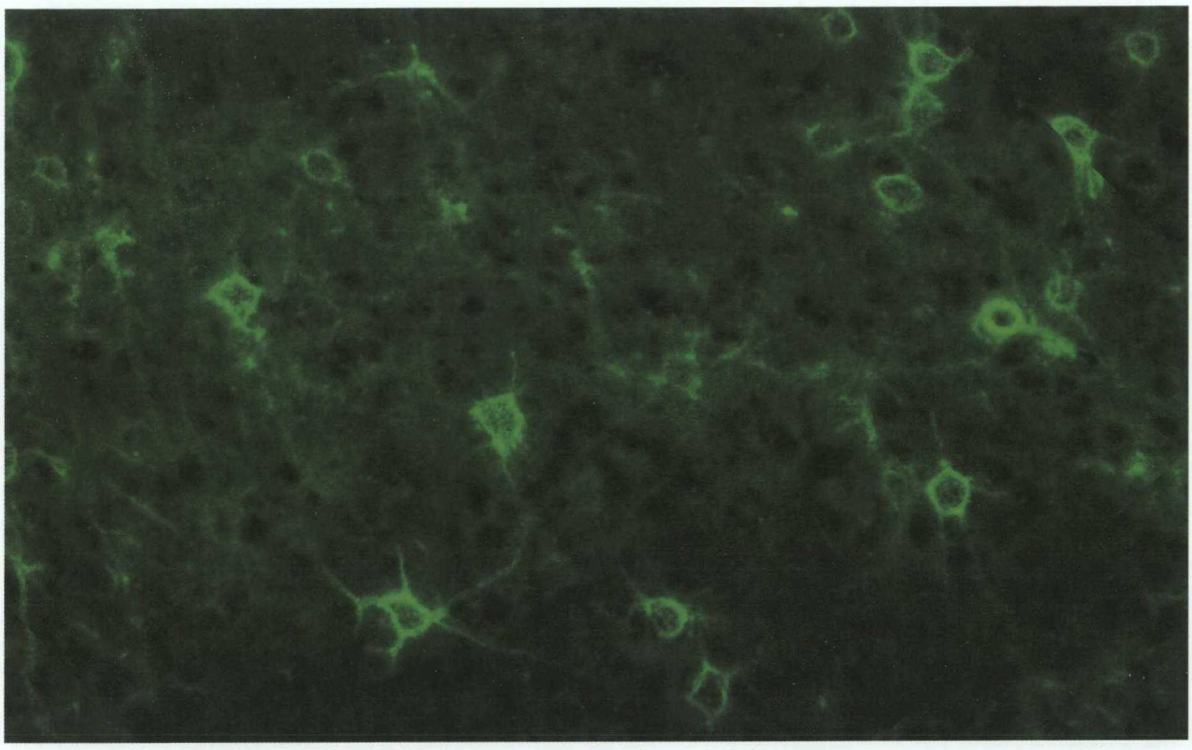


A group of neurons surrounded with perineuronal nets in a mouse cerebral cortex

gerbils, guinea pigs, cats, dogs, sheep, North American bison and raccoons. They have also been found in the brains of more exotic animals, such as the lesser hedgehog tenrec (*Echinops telfairi*), endemic to Madagascar, the gray short-tailed opossum (*Monodelphis domestica*) and the elegant fat-tailed mouse opossum (*Thylamys elegant*). Additionally, many cells in the brains of primates, including humans, are surrounded with perineuronal nets. Among non-mammal vertebrates, perineuronal nets have been found in birds and reptiles. Similar structures have been also observed in a representative of the amphibians, the European edible frog (*Rana esculenta*). It seems likely, then, that perineuronal nets do occur commonly in vertebrates, although there is currently no data on the presence of similar structures in non-vertebrates.

Many methods are currently available to researchers observing perineuronal nets. The structures can be detected using specific lectins - plant proteins able to recognize and bind to sugar molecules. They can be also visualized with antibodies specific to individual proteins or sugar epitopes. When conjugated with a fluorescent marker, lectins and antibodies glow under UV light and can be observed under a microscope (Fig. 2). A brain tissue sample washed with a solution containing those markers reveals that perineuronal nets are only present on the surface of some neurons (Fig. 3). They are most likely to be inhibitory (GABAergic) neurons that secrete gamma-aminobutyric acid (GABA) as a neurotransmitter. Even though these neurons form a low (10-20%) proportion of all the neurons in the brain, they play an important role in maintaining brain excitation at optimal levels. Many different types of such cells have been discovered, although only some are surrounded with nets. Interestingly, not all cells within a certain type are surrounded with a net, although the reasons for this are unknown. Some studies indicate that certain excitatory neurons that secrete glutamate as a neurotransmitter can also be surrounded with perineuronal nets. Data is limited, since there are insufficient methods for reliable histochemical identification of glutamatergic neurons, and researchers need to rely on morphological observations.

Cells surrounded with perineuronal nets are not distributed evenly throughout the brain; they are present at the highest density in regions of the cerebral cortex reached by information from the external environment: the visual, auditory and sensory cortexes. They are also found in the hippocampus, thalamus, cerebellum, and the spinal cord.

Why do neurons need nets?

As yet researchers have not been able to produce a satisfactory answer to this question. It seems that the polyanionic nature of the carbohydrate component of perineu-

ronal nets, in particular that forming part of proteoglycans, is extremely important in determining the nets' biophysical properties. The negative charge accumulated on the surface of the net facilitates the retention of water and cations. For neurons with a fast-changing membrane potential, maintaining the correct ionic concentration near the cellular membrane is especially important. Perineuronal nets can also store ions required to generate membrane potential, as well as act as a buffer absorbing their excess. The hypothesis is confirmed by the observation that perineuronal nets are frequently bound to fast-spiking neurons. In vitro studies show that components of perineuronal nets also bind other molecules, such as trophic factors, signaling proteins, extracellular proteases, chemokines, cytokines, and so on. Perineuronal nets can act as reservoirs of these substances, which are released following the action of an appropriate stimulus.

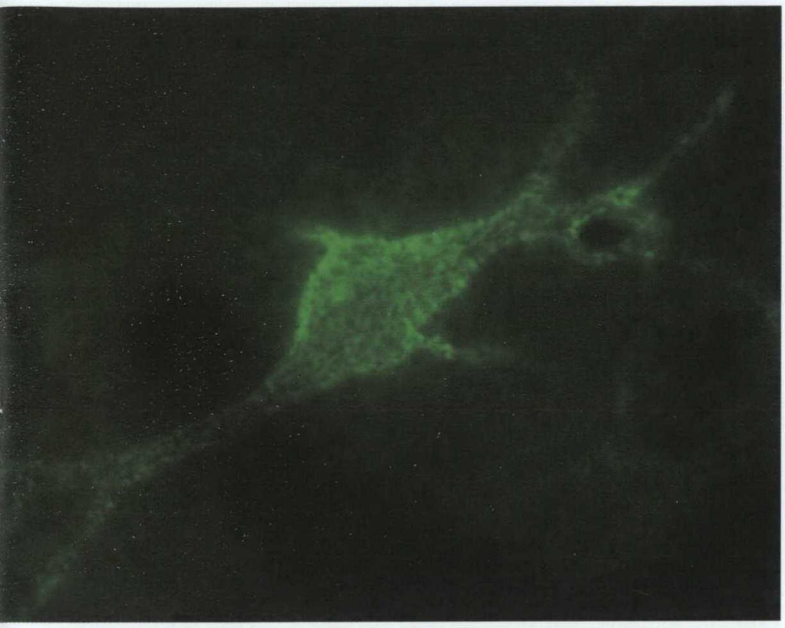
Perineuronal nets are also believed to have a neuroprotective function. In vitro-grown neurons that form networks are selectively resistant to free radicals and excessive stimulation caused by the introduction of a stimulating factor – glutamate. In humans, neurons surrounded by perineuronal nets do not degenerate during Alzheimer's disease, when many other neurons undergo atrophy. In vitro studies also confirm that neurons surrounded with nets are resistant to the presence of beta-amyloid and tau protein – proteins present in Alzheimer's pathology.

Nets and learning

The brain's ability to modify its function as a result of external stimuli, learning, or damage is known as neuroplasticity. It appears to be an extremely complex process, regulated on several levels, from genetic to structural. Neuroplasticity works by changing the number, properties and location of synapses; in effect it involves a restructuring of synaptic connections. The presence of perineuronal nets on the surface of neurons makes it difficult for new synapses to access the surface of the neuronal membrane; the net structure stabilizes existing connections and partially or completely prevents neuroplasticity.

The first evidence supporting the hypothesis of the involvement of nets in neuroplasticity was provided by studies into the developmental plasticity of the visual cortex in rats. This is a process that is known to occur in stages. In young animals, the visual cortex is extremely plastic during a certain stage of development. This can be demonstrated experimentally by monocular deprivation, where the function and structure of the visual cortex is adjusted by reducing the visual stimulus reaching one eye. After a few days, the cortex no longer responds to visual stimuli from the closed eye (even when it opens), while generating a stronger response to the signal from the non-deprived eye. The time window during which the cortex is susceptible to such changes is known as the critical period. At the end of the critical period, the visual cortex loses its sensitivity to manipulation of visual stimuli. It turns out that the closing of the critical period is closely correlated in time with the appearance and maturation of perineuronal nets in the visual cortex. Their presence stabilizes the formation of the network of connections, and future visual deprivation is unable to disturb it.

The function of perineuronal nets in limiting the plasticity of the visual cortex has been confirmed through deprivation experiments conducted in older animals; their visual cortex was injected with a bacterial enzyme, chondroitinase ABC, which digests the chondroitin sulfate residues of proteoglycans forming a part of the net. Eliminating the net made it possible to change the way the cortex functions, as observed during the critical period. The significance of perineuronal nets in plasticity was also confirmed in models based on research into memory, such as fear conditioning based on creating associations between a neutral stimulus and a negative one (usually pain). In young animals, such a reaction can be reversible (fear extinction), since the regions in the brain involved in the process (the amygdala) are still plastic. However, in adults the response is very durable. Eliminating perineuronal nets in adult animals using chondroitinase ABC made it possible to extinguish the response and thus return neuroplasticity. The participation of nets in developmental plasticity has also been confirmed in birds,



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Neuron from a rat sensory cortex surrounded with a perineuronal net, visualized with a fluorescent marker

where perineuronal nets have been shown to be involved in the process of birdsong learning in chicks.

Nets and memory

The brains of adult animals, including elderly individuals, maintain a certain neuroplasticity. Perineuronal nets are not fully permanent, and they can be modified by various factors. One is neuronal activity – the level of neuronal stimulation in a given region of the brain.

Keeping mice in an enhanced environment, providing a higher number of stimuli (such as cages fitted out with numerous toys), results in a reduction in the number of nets in the cerebellum and the visual cortex. A reduction in the number of nets in regions on the boundary with areas that have sustained damage, for example due to stroke or injury, has also been observed. Our research indicates that in regions of the cerebral cortex adjacent to the location of a stroke, the number of perineuronal nets is significantly reduced (by up to 80%). We believe that this may explain the mechanism responsible for the reorganization the synaptic network and the surviving cortex's assumption of the function of the damaged regions. However, rehabilitation of damage to the spinal cord (stimulation of a defined group of neurons) results in the formation of higher numbers of perineuronal nets near the lesion. It appears that the nature of changes to the number and structure of nets due to neuronal activity depends on the region of the brain these changes are studied, as well as the animal's age and the specific neuronal system.

This does not change the fact that networks do undergo modification. External factors that affect their number and structure include proteolytic enzymes, such as extracellular matrix metalloproteinases (MMP), and glycolytic enzymes such as hyaluronidases which digest hyaluronan.

While our understanding of the role of proteolysis in the process of degradation of extracellular matrix and perineuronal nets is well documented, little is known about the enzymes that are able to digest the carbohydrate components that give the nets their properties. This creates a new field of research into the regulation of the nets. Experiments using chondroitinase ABC indicate that eliminating the carbohydrate elements of nets returns neuroplasticity to the brain. Our research indicates that the number of perineuronal nets increases with age, at least in certain regions of the brain. This increase may be responsible for the reduction of cognitive abilities, observable after the age of forty. The ability to manipulate perineuronal nets and improve the cognitive abilities and memory in older people is a tempting prospect. Chondroitinase ABC delivered to damaged spinal cords in rats makes it possible to for synaptic connections to be reorganized, and significantly improves rehabilitation outcomes. However, the method is considered to be too invasive to use in humans. Identifying endogenic factors that modify perineuronal nets (such as glycolytic enzymes) and substances that affect their activity in a selective and controlled manner, and formulating them into easy to deliver drugs, could revolutionize treatment of brain disorders.

Pills that boost one's memory, treat dementia, improve rehabilitation outcomes for brain and spinal cord injuries... Who knows, perhaps this Holy Grail will one day be discovered. ■

Further reading:

Karetko-Sysa M., Skangiel-Kramska J., Nowicka D. (2011). Disturbance of perineuronal nets in the perilesional area after photothrombosis is not associated with neuronal death. *Experimental neurology,* 231(1):113-26.