Neurobiology

The Brain Under Pressure

We talk to **Dr. Emilia Rejmak-Kozicka** from the PAS Institute of Experimental Biology about how our bodies respond to chronic stress.

ACADEMIA: **You study the effects of chronic stress on cognitive function and brain structure. But what is stress, and when is it qualified as chronic?**

EMILIA REJMAK-KOZICKA: The term "stress" was coined by Hans Selye, the pioneering Austrian-Canadian physiopathologist and endocrinologist of Hungarian origin. He defined stress as the non-specific response of an organism to a certain type of stimuli known as stressors [the physiological aspects of stress were discussed by Dr. Magdalena Markowska in the 2/2016 issue of "Academia" – ed.].

There is currently no single, precise definition of stress in the literature. Stressors can be any stimuli which disrupt an organism's homeostasis. They fall into four main categories: physical stressors (e.g. immobilization, noise, extreme temperature), psychological ones (driven by emotional processes and resulting in anxiety, fear, frustration, etc.), social ones (e.g. an animal finding itself in another individual's territory) and stressors disturbing metabolic and circulatory homeostasis (e.g. physical exertion, hypoglycemia). They can also be further divided into two categories by duration: acute (one-off episodes) vs. chronic (prolonged episodes).

Why does your research focus on chronic rather than acute stress?

Because it affects synaptic plasticity – the ability of the nervous system to modify the activity and organization of the neural network in response to external or internal stimuli. In contrast to acute stress, chronic stress involves changes driven by disrupted functioning of the hypothalamus-pituitary-adrenal (HPA) axis and the resulting increased secretion of corticotropic-releasing hormone and excitotoxic activity of glutamic acid in the brain. This was the most important part of our research. Chronic stress is a significant factor in the development of neuropsychological disorders; one such condition is depression, described by the World Health Organization as the fourth leading cause of disease.

Are levels of matrix metalloprotease (MMP-9) – the extracellular enzyme that degrades proteins secreted by the synapses – subject to fluctuation under the influence of chronic stress?

Yes, the level of MMP-9 increases under the influence of chronic stress. MMP-9 is a proteolytic enzyme whose basic function is to degrade the intercellular matrix (as a result of which the medium is more susceptible to changes in strength in the synaptic connections) and to cleave many of the membrane proteins, leading to changes in the organization of the synapses. In the brain, MMP-9 is expressed on a low level and is released in a synapse only under the influence of neuronal stimulation, such as in chronic stress. MMP-9 is released as an inactive proenzyme into the intercellular matrix, the space between the cells in the brain, where it is subsequently activated. This results in the fragmentation of cell adhesion molecules – pro-

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teins situated on the surface of the cell allowing for various types of connections between cells, between cells and macromolecules in the intercellular matrix, and between components of the intercellular matrix and the actin cytoskeleton. This is one of the main mechanisms whereby metalloprotease influences the aforementioned synaptic plasticity.

How does MMP-9 cleave nectin-3 – a protein that interconnects two brain cells?

This reaction is initiated by a higher concentration of glutamate, an excitatory neurotransmitter, in the nervous system, resulting from chronic stress. Next the stimulation of NMDA receptors, engaged in mechanisms of synaptic plasticity, results in the secretion of MMP-9 into the intercellular matrix. There this leads to the activation of the protease and fragmentation of nectin-3, the protein linking the pre-and post-membrane. Our results showed increased nectin-3 cleavage in region CA1 of the hippocampus of rats subjected to chronic stress.

> Chronic stress is a significant factor in the development of such neuropsychological disorders as depression.

How does fragmentation of nectin-3 affect brain function?

Nectin-3 plays a key role in the formation and stabilization of synapses by interacting with the actin cytoskeleton and intracellular signal transmission pathways. Gaining an understanding of the mechanisms controlling proteolytic cleavage of adhesive molecules is extremely important, since they act as scaffolds for the synaptic gap as well as modulating functional and structural elements of synaptic plasticity under physiological and pathological conditions. Disruptions of expression and correct function of cellular adhesion molecules such as nectin-3 are at the root of morphological disorders, the loss of well-developed intercellular connections and disorganization of the actin cytoskeleton. Molecular changes resulting from chronic stress are reflected in animal behavior, such as increased aggression and abnormal social behavior. Interestingly, administering an MMP-9 inhibitor directly to the CA1 region of the hippocampus – the region where we found the highest increase of MMP-9 activity as a result of stress – prevents these disruptions.

Tell us about your in vitro research.

Our *in vitro* studies of neurons allowed us to observe the relationship between proteolysis (cleavage, fragmentation) of nectin-3 and MMP-9 activity, significantly reducing the number of animals which would have otherwise been used in experiments. The results confirm the involvement of this protease in mechanisms driving nectin-3 fragmentation. We also showed that nectin-3 proteolysis depends on the presence of Ca^{2+}/calmodulin (calcium-binding protein) activation of the NMDA receptor.

What are the animal models of chronic stress you also used in your research?

The model we used was selected in collaboration with Prof. Carmen Sandi from the Swiss Federal Institute of Technology in Lausanne, whose team is one of the leading groups conducting research into stress. We selected a 21-day long model of stress caused by immobilization since it is extremely well characterized in terms of functional and structural changes in the hippocampus. It has been shown that this type of stress causes significant changes in nerve cells; in particular it inhibits neurogenesis – the process whereby new neurons are formed.

Why did you focus on studying the cleaving of nectin-3 under the influence of chronic stress in the hippocampus?

This region of the brain plays a key role in stress responses and the development of depression. The hippocampus plays an important role in memory and it is highly vulnerable to stress, as shown by structural changes in neural networks and the changing number and shape of stimulating synapses. Some of these effects also influence how effectively glutamate receptors are stimulated, which in turn affects learning and memory processes and social behavior.

What kinds of mental disorders arise as a result of molecular changes driven by chronic stress?

Prolonged exposure to stress is a significant factor in the development of many brain disorders, such as depression. At certain stages of life depression can affect over 10% of the population and it carries vast costs; these aren't just in terms of treatment but the sufferers' inability to function in society and the resulting consequences, such as suicides or long-term disability resulting from unsuccessful suicide attempts. Depression is a major social problem, yet the mechanisms behind it remain unclear and existing treatments are slow to act and frequently carry serious side effects. Depression is also linked with other mental disorders, making treatment even more complex. As such, studying the molecular mechanisms of stress brings us closer to developing better drugs

and prescribing more effective treatment regimens for disorders such as depression.

You are also working on the effects of MMP-9 on the emergence and development of epilepsy in animals. What stage is this research at?

treat epilepsy. Our ongoing applied research, financed by the National Centre of Research and Development, analyses such substances as potential treatments for epileptogenesis. Even though many new drugs have been introduced in recent years, in around 40% of patients epilepsy remains resistant to treatment. In

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works at the Laboratory of Neurobiology, PAS Institute of Experimental Biology. She is the winner of the START grant awarded by the Foundation of Polish Science; she is also one of the leading authors of a paper on chronic stress published in *Nature Communications*. She studies synaptic plasticity involved in learning and memory processes under physiological and pathological conditions.

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Data indicates that MMP-9 plays an important role in epilepsy. The enzymatic and extracellular character of MMP-9 makes it highly attractive in therapeutic applications. Nonspecific MMP-9 inhibitors have been tested in other disease types such as cancers. Unfortunately there is a large pool of drugs which aren't used to their cases finding new treatments seems to be the only way to control epileptic attacks and improve their quality of life.

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