Studying the mechanisms of anxiety

Deciphering Fear

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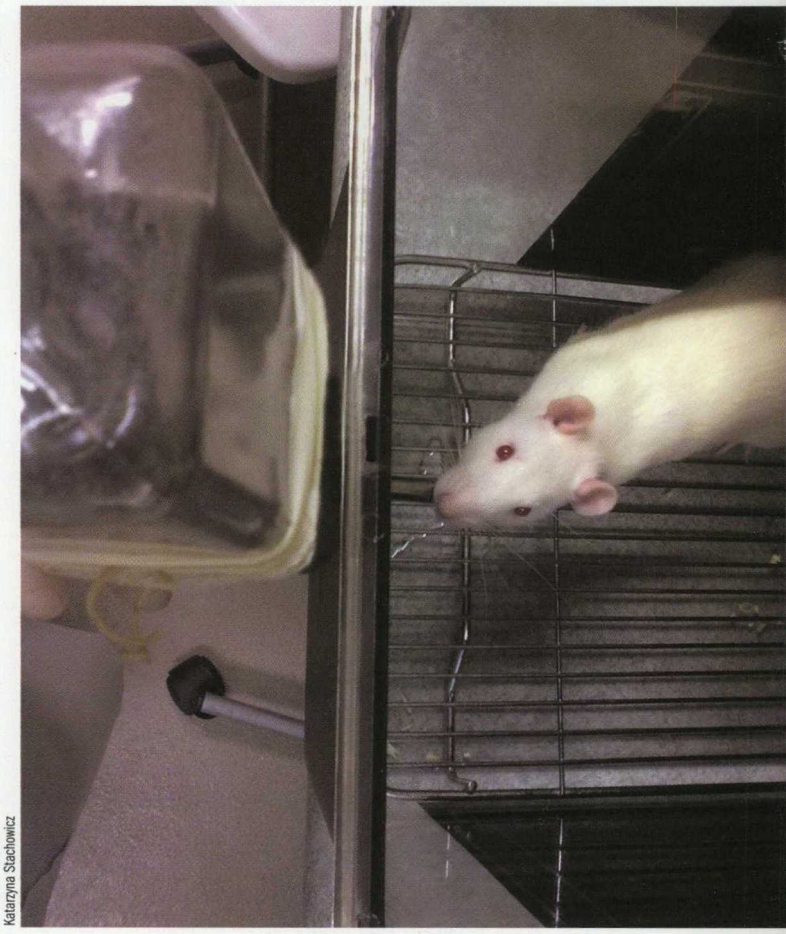
Current research into fear indicates that there is no single mechanism for triggering fear. However, neuropharmacologists are getting closer to unraveling the mystery

We've all experienced fear at various stages of our lives. We feel the emotions known as fear from infancy through childhood, adolescence and adulthood to old age. It is a natural physiological state that allows us to learn about the world and set up boundaries for our behavior and safety. Frequently, it is a driving force for our actions; it also allows us to develop correctly. A young child is afraid to be left home alone; a mother worries about her offspring throughout her life. Fear is written into many of our emotions – it even accompanies us when we love and care for others. However, when this feeling loses a sense of reality and we start experiencing it persistently and for no obvious reason, we are no longer dealing with fear, but with a pathological state of anxiety. According to textbook definitions, anxiety is a feeling of unfounded worry or dread with varying degrees of intensity and duration. It is a common component of neuroses and numerous psychoses, and may persist for many years. Its symptoms include unfounded worry, irritability, outbursts of anger, inability to concentrate, depersonalization, and motor agitation. Anxiety disorders are common and constitute a major clinical problem. Approximately 40% of all psychoactive drugs currently on the market are anxiolytics. The **DSM-IV** (Diagnostic and Statistical Manual of Mental Disorders) has classified anxiety, listing the following types: generalized anxiety disorder, which is chronic and presents with varying degrees of intensity; panic disorder, which presents with frequent attacks of intense terror and apprehension with no obvious trigger; phobias, in which fear and anxiety are triggered by specific stimuli, disproportionately to actual danger levels; obsessive-compulsive disorder (OCD) characterized by persistent intrusive thoughts and urges to perform specific acts or rituals; post-traumatic stress disorder (PTSD), which may affect anyone following a traumatic experience; and various other anxiety disorders.

There are many different predisposing factors to anxiety disorders. Of particular importance are biological factors affecting



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The Vogel test (1971) describes a model of generalized anxiety

the central nervous system (CNS), physical condition, inherited and innate temperament, social pressures including those from family or professional life, traumatic experiences and mental trauma, and chronic stress. Scientists have suggested there may be a genetic predisposition to anxiety in individuals with a functional polymorphism of the serotonin transporter (SERT) gene (5-HTTLPR region), resulting in a reduced serotonin (5-HT) reuptake into presynaptic cells.

Decoding: step one

In their search for drugs to treat humans, researchers frequently perform experiments on animals. Early studies into anxiety required an introduction of experimental models combining the human and animal worlds to represent a given nosological entity in humans. Contemporary scientists working in behavioral neuropsychopharmacology use experimental models every day, with several important behavioral tests being used in anxiety research.

In 1971, John R. Vogel described a test for modeling generalized anxiety in the journal *Psychopharmacology*. Now known as the Vogel Test or the Vogel Conflict Drinking Test, it is a basic screening tool used to search for potential anxiolytic properties of substances being studied. It utilizes the basic physiological need to quench thirst by drinking. Rats are deprived of access to water for 48 hours preceding the test, except for two intervals during these adaptation days when they can drink unlimited water for half an hour. On the day of the experiment the animals are placed in cages for a 5 minute long test, during which their attempts to drink are "punished" with a mild electrical impulse (0.5 mA, duration 1 second, interval between impulses 2 seconds). Animals experiencing "anxiety" abandon their attempts to drink water despite experiencing powerful thirst, while animals treated with active anxiolytics continue drinking in spite of the discomfort they experience. The experimenters analyze the numbers of "punished" attempts at drinking.

Mazes and glass spheres

In 1986, Sandra E. Pellow and Sharon File described the elevated plus maze test for rats in the journal *Pharmacology Biochemistry and Behavior*. The test setting consists of a plus-shaped apparatus with two open and two enclosed arms, each with an open roof, elevated 40-70 cm from the floor and placed in a darkened room. The open arms are dimly lit from above. The experimental animals are placed in the room with the maze an hour prior to the test. At the end of the adaptation period, the animal is placed in the centre of the maze, and the number of times it enters the open and enclosed arms is recorded, together with the time spent there. Anxiety reduction is indicated by an increased proportion of entries to and time spent in the open arms.

Another test, for anxiety-induced hyperthermia in mice, is carried out following a method described by Van der Heyden et al. in 1997. The test takes advantage of animals' physiological ability to raise body temperature in stressful situations. Mice that were previously kept as a group are placed in individual cages 24 hours prior to the test to create a stress-inducing situation. On the day of the experiment, the animals' body temperature (T1) is taken prior to placing the mice in a "strange" cage. Their temperature is taken again after 15 minutes (T2). The T2-T1 difference is described as stress-induced hyperthermia. Anxiolytic activity is described as an absence of a temperature difference in mice exposed to stress induced by being moved to a new cage. Mice that were not isolated from the rest of the group do not exhibit an increased body temperature when exposed to the same treatment.

Another fascinating experiment, described by Millan et al, in 2000, is the marble burying test. The behavior it induces in mice is reminiscent of OCD in humans. The animals are placed individually in experimental cages

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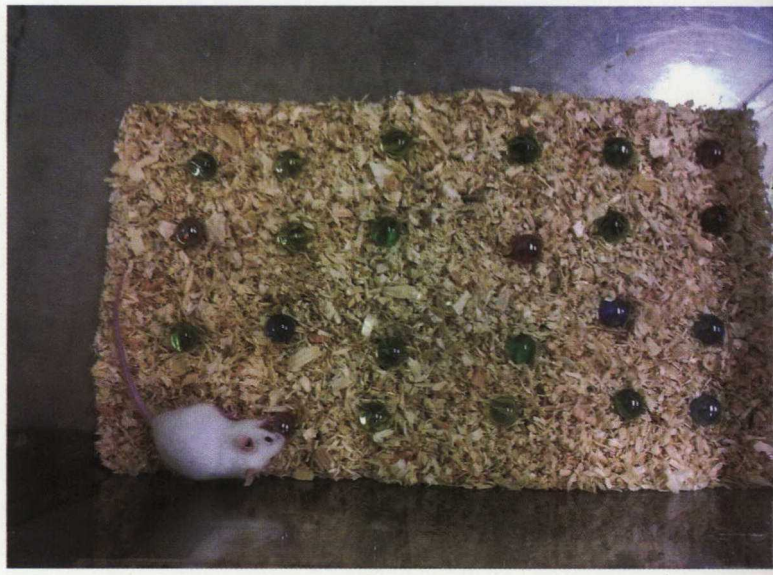
Model of anxiety-induced hyperthermia in mice (Van der Heyden et al., 1997). Mice that were previously kept in a group are placed in individual cages 24 hours prior to the test to create a stress-inducing situation

in which marbles are laid out in regular rows. The marbles are a foreign addition to the previous safety and familiarity of the cages. Mice left in those conditions for half an hour bury the marbles deep in their bedding so they become invisible. When mice are given anxiolytics, the number of buried marbles is reduced. In this case anxiolytic activity is described as a reduction in the number of buried spheres.



Decoding: step two

The search for effective anxiolytics has been ongoing for several decades. The pharmacotherapy of anxiety is a fast-growing area of psychopharmacology. In recent decades, the important role of almost all neurotransmitter systems in the pathogenesis of anxiety and the mechanism of action of many anxiolytic drugs have been shown. The first known substances with anxiolytic properties were barbiturates and carbamates. However, they caused numerous side effects, and since they were characterized as toxic and ineffective, they have been replaced by benzodiazepines. Benzodiazepines, discovered by the Polish scientist Leo Sternbach, are mainly used in the treatment of generalized anxiety. They also cause numerous adverse effects, such as dizziness, sedation, and memory problems, as well as interacting dangerously with alcohol. Long-term use of benzodiazepines may lead to increased tolerance and dependence. Agonists of benzodiazepine receptors show slightly milder adverse effects. Another compound used in the pharmacotherapy of anxiety is buspirone, a serotonin 5-HT_{1A} receptor partial agonist. It is characterized by high efficacy, similar to that of diazepam, with none of the adverse effects exhibited by benzodiazepines. However, it may cause dizziness, headaches, lightheadedness, and indigestion. Clinical treatment of anxiety (especially anxiety attacks), phobias and OCD mainly includes antidepressants, such as serotonin and noradrenaline reuptake inhibitors, selective serotonin reuptake inhibitors (SSRIs), and monoamine oxidase inhibitors. They must be taken for at least 4-6 weeks to achieve full therapeutic effect. Motivating patients to take medication that needs to be taken systematically and long term, but which does not produce an improvement for such a long time, constitutes a major clinical problem. Treatment of performance anxiety mainly includes beta-adrenergic antagonists (beta blockers) such as propanolol and oxprenolol. Their usage may cause a reduction in blood pressure. Other anxiolytics include herbal treatments such as valerian, kava, and blue lotus.

This wide variety of effective compounds with different mechanisms of action indicates that the pharmacotherapy of anxiety operates on several neurotransmitter systems. Researchers are especially interested in compounds that inhibit glutamate transmission by blocking ionotropic receptors, in particular the NMDA complex, and metabotropic receptors. Finding a new drug able to work quickly without causing adverse effects is an important goal for research into anxiety treatment.

Researchers' worst nightmare

The third step towards deciphering anxiety is finding the mechanism responsible for triggering it in the first place. For scientists it is essential to discover the "how and why," that is, the mechanism of action. This continues to present us with the greatest headache in our search. So far we remain defeated... And so in this 21st century we still do not understand the mechanism of many disorders (or, more precisely, we grasp hardly any at all). One of those that continue to elude us is anxiety.

We have now reached the point when scientists should bring together their knowl-

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A mouse busy burying stress-inducing "foreign" marbles added to a familiar environment: this "marble burying test" is used as a mouse model of human obsessive-compulsive behavior

edge and research methods and delve deep into the cell, to look at receptors and study the inner workings of the genome. Current research indicates that there is no single anxiety-triggering mechanism. The activation of the CNS is linked to the system of excitory amino acids - glutamic (Glu) and aspartic (Asp) - which remains in dynamic equilibrium with the system inhibiting neuronal processes, that is the gamma aminobutyric acid (GABA) system. It is likely that a disruption of this equilibrium in favor of glutamate and an increased glutamatergic transmission, that is, a hyperfunction of the glutamatergic system, is a direct trigger of the anxiety response and may induce changes in brain plasticity, resulting in long-term effects. Stress alone causes an inhibition of glutamate reuptake, most likely by inactivating transporters of excitory amino acids. This has led to the hypothesis that anxiolytic properties of substances inhibit, weaken, or modulate glutamatergic transmission.

Unraveling the secret?

The amygdala are the brain structures responsible for recognizing, processing, and storing information regarding anxiety responses in all studied mammal species. Researchers have discovered that overgrowth or hyperactivity of the amygdala, or disturbed mechanisms regulating its activity via the hippocampus and the cerebral cortex (the hippocampus is responsible for regulating our emotions), are present in anxiety disorders. The significance of this discovery has given rise to the glutamatergic theory of anxiety. Ligands of the metabotropic glutamate receptors (mGluR) play a part in the modulation of synaptic excitability. This brings hope of researchers finding substances with anxiolytic properties that have affect the basic excitory transmitters while lacking the adverse effects characteristic of other compounds. Research shows that substances that block glutamatergic activity display anxiolytic properties. Scientists currently believe that anxiolytic properties are shown by antagonists and negative allosteric modulators of group I mGluRs; antagonists, agonists and positive allosteric modulators of group II mGluRs; and antagonists, agonists and positive allosteric modulators of group III mGluRs. And while we are still a long way from unraveling the mystery, we are getting closer every day and can repeat after Albert Einstein: "The important thing is not to stop questioning; curiosity has its own reason for existing. (...) It is enough if one tries merely to comprehend a little of the mystery every day. The important thing is not to stop questioning; never lose a holy curiosity." ■

Further reading:

Stachowicz K. (2009). Potencjalne przeciwlękowe działanie ligandów metabotropowych receptorów glutaminianergicznych I i III grupy. PhD Thesis.

Pilc A., Chaki S., Nowak G., Witkin J. M. (2008). Mood disorders: regulation by metabotropic glutamate receptors. *Biochem Pharmacol.*, 75, 997-1006.