Medical Xpress

Aggression causes new nerve cells to be generated in the brain

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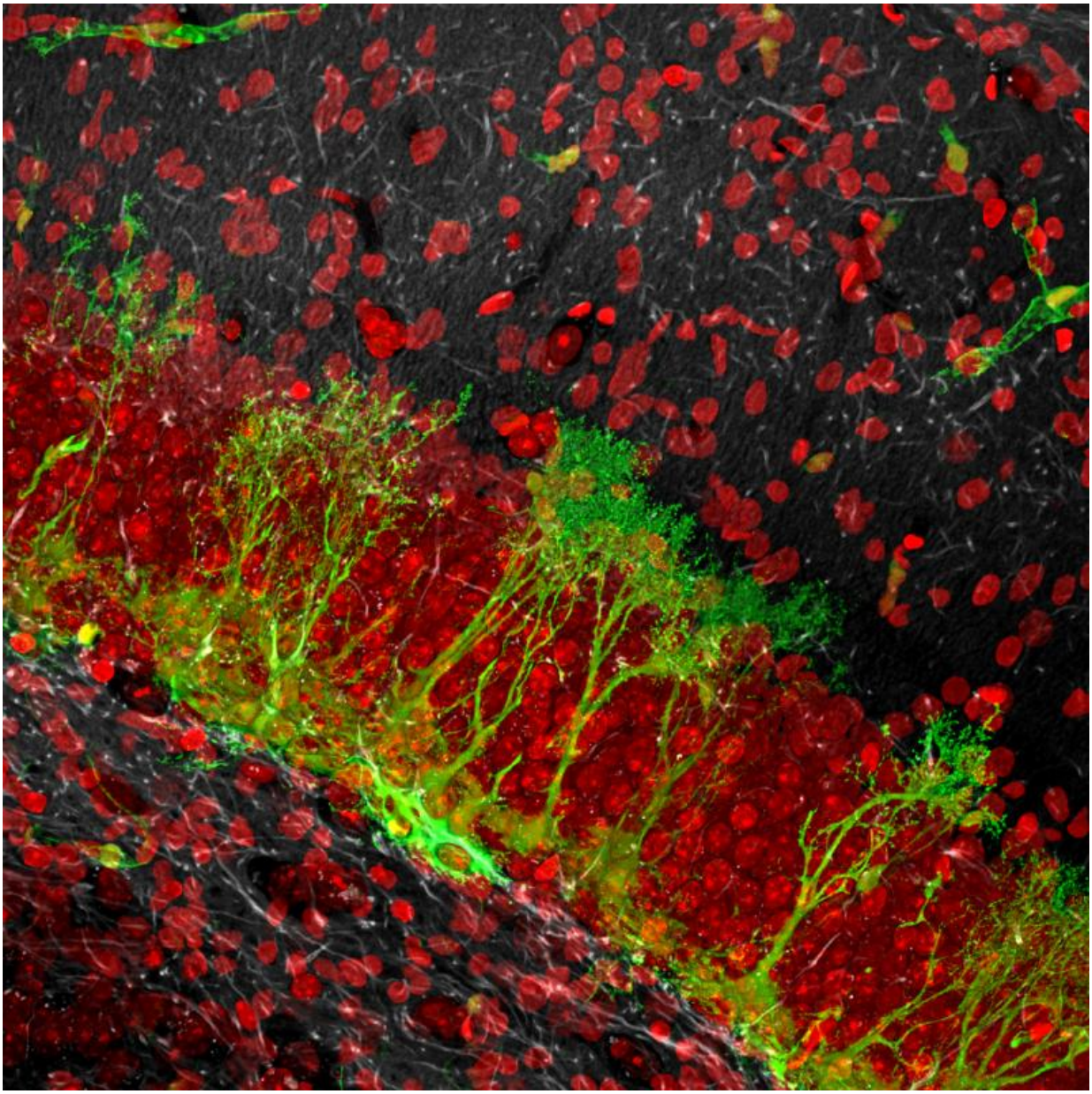


The photo has received an award at BioArt and is featured in the "Developmental Biology" by Scott Gilbert; image courtesy of Grigori Enikolopov.

A group of neurobiologists from Russia and the USA, including Dmitry Smagin, Tatyana Michurina, and Grigori Enikolopov from Moscow Institute of Physics and Technology (MIPT), have proven experimentally that aggression has an influence on the production of new nerve cells in the brain. The scientists conducted a series of experiments on male mice and published their findings in the journal *Frontiers in Neuroscience*.

Researchers studied the changes that occurred in the brains of mice demonstrating aggressive behaviour. These mice attacked other mice and won in fights. After a win, they became even more aggressive, and new neurons appeared in their hippocampus, a key brain structure. In mice that were allowed to continue fighting, certain changes were observed in the activity of their nerve cells. The scientists hope that the new information on the neurobiological bases of aggression will not only help in understanding this important phenomenon, but will also encourage research in other areas – and even help in finding causes of autism and other similar disorders in humans.

In order to explain exactly how aggression affects the formation of new neurons, how it alters the functioning of the brain and what autism has to do with all of this, we need to take a careful look at various aspects of the recently published study.

"Once again I am amazed at how the basic building blocks that make up complex behaviour are similar in different organisms and it is truly fascinating how they can be combined with other blocks to create an enormous variety of behavioural reactions in animals and humans," said

Grigori Enikolopov, the head of MIPT's Laboratory of Brain Stem Cells and corresponding author of the study.

C57BL male laboratory mice. Credit: Alexei Timoshenko

At the behavioural level

For the experiment, pairs of male mice were placed in a cage bisected by a partition. The partition allowed the animals to see, hear and smell each other, but did not allow physical contact. Every day, in the early afternoon, the partition was removed and the observations began. It did not normally take long for fights to break out. After two or three

encounters, the winner was established. Aafter three minutes, or sometimes less to avoid injuries to the defeated male, the mice were separated from their neighbours again. After repeating the process for three days in a row, the scientists changed the mice in the cages, randomly placing defeated males with a new neighbour—but most importantly, each time a defeated male was placed in the same cage as another winning male. In one group, after three weeks of these rotations, winners were prevented from entering into confrontation, and in another group, the mice continued to fight with one another.

The scientists also conducted a series of tests to demonstrate the effect of aggression on behaviour. For example, the mice were placed in a cross-shaped maze (plus-maze) where one corridor was closed and the other was an open space. The more time that the mice preferred to spend in the dark, closed space, the more their behaviour could be described as "avoiding risk".

The mice were placed in a cage with a transparent partition and another male on the other side – the more time the mice spent close to the barrier, the higher the level of potential aggression. This interpretation is consistent with the fact that the active animals in the study tend to attack their partners if the opportunity arises (tests were also performed to prove this).

Line is a more rigorous concept than "species". A line describes all the mice produced by the inbreeding of the offspring of one pair of mice with the same genotype. The C57BL line is one of the most common. And incidentally, BL stands for black – so laboratory mice are not typically white.

All the tests showed that males who won a number of fights display a more "brazen" attitude – they approach the transparent partition more often and initiate an attack on their opponents more quickly. If the mice

were deprived of fighting for a period of time before the test, they became even more aggressive: The latency to the first attack was almost three times less, and the fights themselves lasted for longer periods. But what is particularly interesting is that at the same time, their level of anxiety increased – a male who succeeded in tearing out patches of hair from the back of a weaker mouse would rather avoid open spaces, preferring to sit in the dark wherever possible!

Mice of different lines may even exhibit different behaviour when fighting. In a confrontation, C57BL mice normally pull out patches of hair from their opponent's back. The fights are rarely fatal, but deaths have been known to occur.

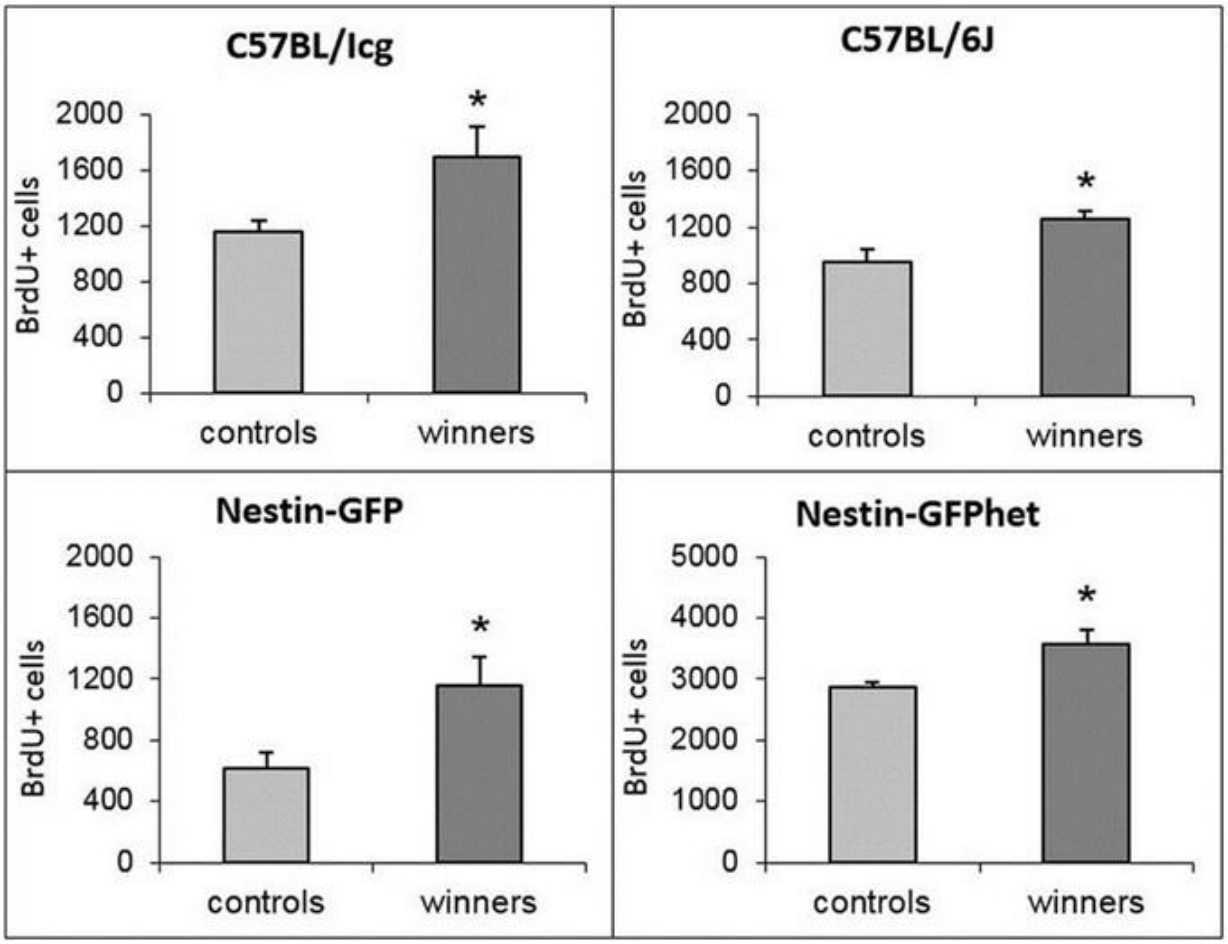
The methods used in the experiments were not chosen by chance. Natalia Kudryavtseva, one of the authors of the study, is an internationally recognised leader in the study of the biological bases of aggression, and the behavioural model and method of studying aggression in mice has been developed over a period of decades.

At the cellular level

The study of aggression in the context of the function of the brain at the level of individual cells was made possible as a result of the progress achieved in neuroscience in recent decades. Three statements are now considered to be reliably proven:

- Behaviour has an influence on the function of the brain and may cause long-term changes;
- Contrary to the previously accepted view, new neurons *can* be generated in a mature brain and this process plays a key role in learning;
- In order to initiate long-term changes at cellular level, cells need to activate certain genes and suppress the activity of others.

Despite the fact that DNA is the same in all cells, different sections (different genes) have different status. If the DNA is chemically modified, or the proteins that combine with DNA to form chromosomes are modified, it is no longer possible to read information from the gene and synthesize molecules encoded by that gene. The cell stops the production of unnecessary proteins, e.g., a neuron does not synthesize the muscle fibres required by myocytes, muscle tissue cells. By controlling the activity of genes, neurons can also rebuild themselves, and activating stem cells in the brain can lead to the generation of new nerve cells in order to build the neural networks that play an essential role in memory, for example.



The four diagrams correspond to four different lines of animals, and the dark columns distinguish the winners from the defeated mice in the control group. Image from the researchers' paper: Front. Neurosci., 01 December 2015 | http://dx.doi.org/10.3389/fnins.2015.00443

Studies in the field of the neurobiology of memory, which were first conducted in the mid-20th century, have shown that learning or even simply encountering something new sets off a series of molecular changes in neurons – and certain genes, which scientists call immediate early genes (IEGs), are activated to produce long-term transformations in the brain. If a test sample of an animal's brain is taken shortly after a learning experiment and combined with special labels of the protein encoded by c-fos, scientists are able to observe the changes triggered by the experiment. This is exactly what the authors of the paper did to trace the effects of aggression at cellular level – monitoring c-fos levels is one of the standard methods of actively searching for changes in nerve cells.

Neuroscience – the study of the brain often requires knowledge in unexpected fields, such as optics, game theory, economics, or sociology. Practical skills employed by neuroscientists often include the ability to manipulate, program, and control rats and make statistical calculations. This is why this interdisciplinary field has been classified separately by British and American scientists as neuroscience.

The hippocampus and amygdala

Simply observing individual neurons, or even groups of neurons, does not give a complete picture. The location of the cells needs to be taken into account. The activity of neurons in different regions of the brain may vary significantly, as these regions perform different functions.

In this particular study, the scientists examined the hippocampus and the amygdala. It is often said that the amygdala is associated with emotions, and the hippocampus with memory, and this is generally true – but it should be clarified that despite this, memory is not localized in the hippocampus, and to experience emotions, even mice need more than just the amygdala.

Many structures in the brain perform multiple tasks, in the same way that in computers, a processor or RAM chip are used for a wide variety of tasks: There are no individual components that are only used for games, or only used for office programs. The hippocampus is used in the formation of long-term memory, and in navigating mazes – and the amygdala is responsible for fear, aggression, and also anxiety. A number of studies involving people even showed that the amygdala is linked to alcoholism, and also political views. This extensive list is easy to explain if we take into account the fact that memories themselves come in different forms: A mental "map" of an area, the ability to balance when riding a bicycle, and a traumatic experience are all stored differently in the brain.

The amygdala is involved in the memory of unpleasant stimuli – it is the reason why a mouse freezes when it is placed in a cage where on a previous occasion the floor was electrified. The hippocampus is also linked to memory, but, as demonstrated in the 1950s in the case of the patient H.M. who underwent an unsuccessful operation, it stores information on entirely conscious events. Henry Molaison (widely known as H.M.), who had his hippocampus removed due to severe epilepsy, began to forget things that had happened to him only a few minutes ago! He did develop an ability to solve certain puzzles, although whenever he did them, he was certain that he had never seen them before.

A cage with an electrified floor is a standard method of forming

memory. We would like to note that a representative of the MIPT Press Office personally experienced a similar environment and confirmed that it is not a serious electric shock, but an entirely tolerable, although slightly uncomfortable sensation. The best way to describe it would be to say that it was as if the floor had suddenly become a rubber foot massage mat.

Comparing the activity of the amygdala and the hippocampus enabled scientists to trace the influence of the aggression experiment on two key structures at once. Past evidence suggested that in aggressive and socially active mice, more new neurons are produced in the hippocampus, and in specially bred lines of mice with increased aggressive behaviour, the level of neurogenesis is also higher than those who were selected on the basis of reduced aggression.

In this experiment, scientists discovered that with repeated fights the level of the c-fos protein increases in the hippocampus, but decreases in the amygdala. And if the mouse is prevented from being involved in further fights, these changes do not occur in the function of immediate early genes, although new neurons still develop. The researchers also conducted a number of additional tests and experiments to interpret the observations made.

Neurogenesis is the process by which neurons are generated. It is interesting to note that it has not yet been possible to see this process in all areas of the brain, however in the dentate gyrus of the hippocampus neurogenesis has been reliably proven.

In relative terms, the effect varied from around 10 percent to double the amount of new neurons and for all four lines of mice used in the experiments, the effect was statistically significant. This means that it is unlikely to be a coincidence; obtaining such a result exclusively due to the individual differences of the animals has a very low level of

probability (no more than a few percent).

What does this mean?

The new publication confirms a previous theory – mice that are accustomed to fighting not only behave differently, but exhibit altered brain functionality. The number of new cells of the hippocampus increases, and if the mice are allowed to continue fighting, the activity of existing cells also changes. New cells seem to be one of the key mechanisms of the increase in aggression and, perhaps, also anxiety – although scientists are not yet certain of this: the winning reputation of an aggressive and dominant mouse would almost certainly need to be backed up by new fights, but this is not something that will help to reduce anxiety.

Compared to previous data, the new results are slightly confusing in some areas. It was previously demonstrated that increased anxiety is normally accompanied by a reduction in neurogenesis, but in this case it is the other way around – males with more new neurons in the hippocampus preferred to avoid going out into open, lit areas. It could be that a win produced an effect opposite to the effect of anxiety, it could also be that the researchers have come across a new phenomenon. Further tests will be needed to find out the truth.

However, the conclusion regarding the activity of cells of the amygdala is interesting not only in the context of fundamental principles of behaviour in mice. The scientists note that in humans, the amygdala is involved in a number of pathological processes, including the formation of autism. Increased anxiety, stereotypical repetitive behaviour, impaired ability to communicate with others – these symptoms were observed in the mice from the experiments described above and are partially similar to the symptoms of autism. Perhaps this may be a link that will eventually lead to progress not only for scientists, but also for doctors.

More information: Dmitry A. Smagin et al. Altered Hippocampal Neurogenesis and Amygdalar Neuronal Activity in Adult Mice with Repeated Experience of Aggression, *Frontiers in Neuroscience* (2015). DOI: 10.3389/fnins.2015.00443