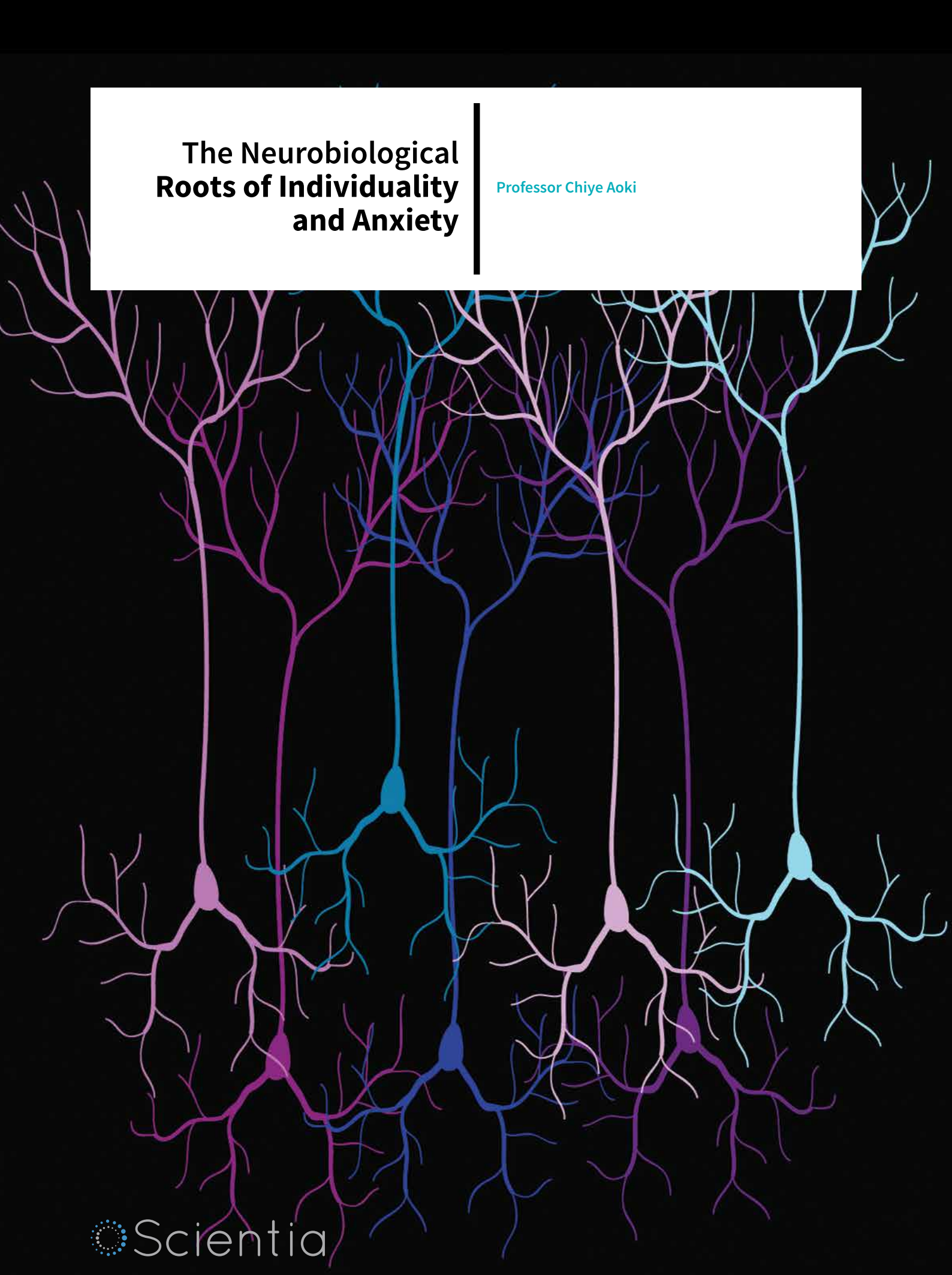
The Neurobiological **Roots of Individuality and Anxiety**

Professor Chiye Aoki



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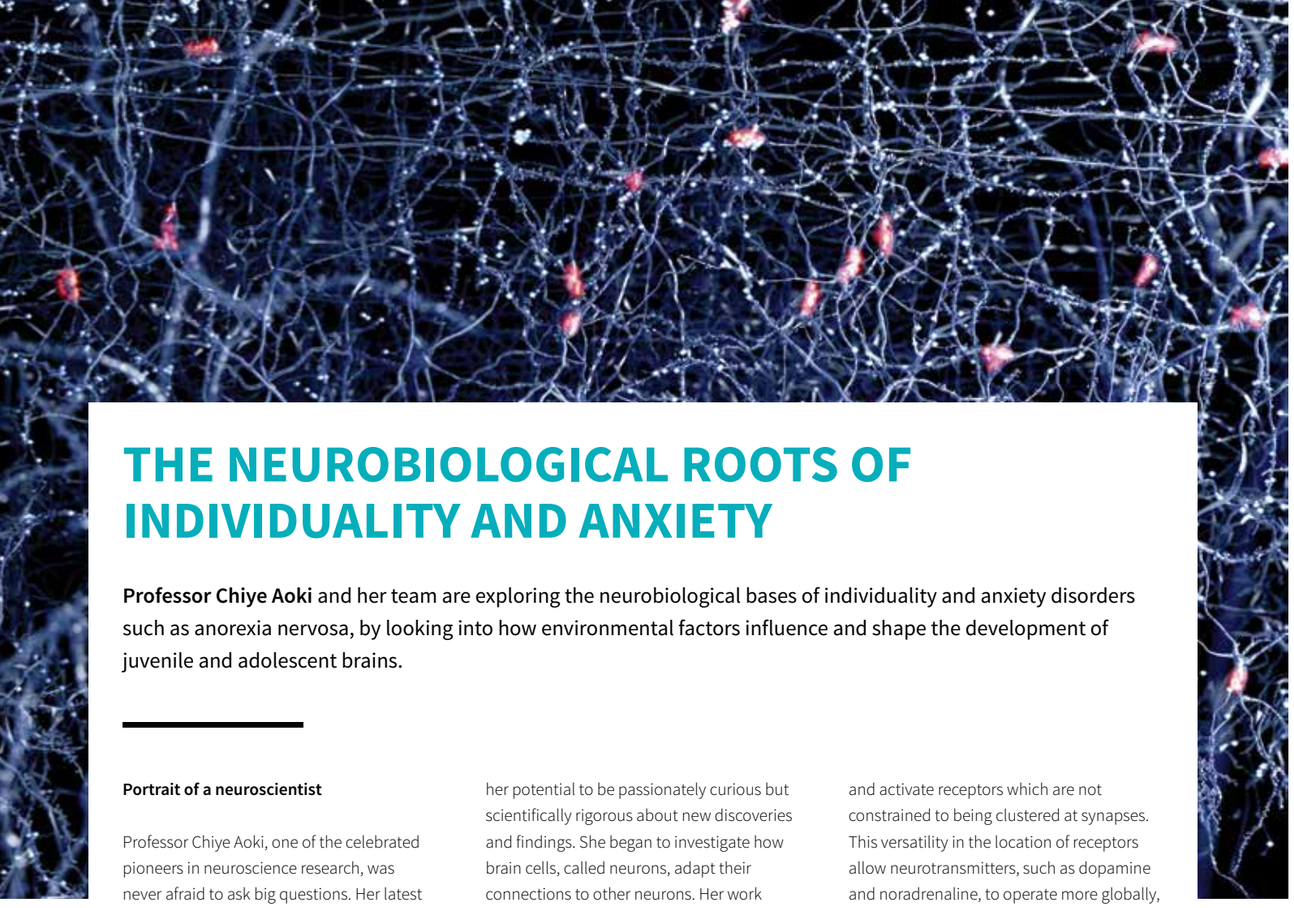


THE NEUROBIOLOGICAL ROOTS OF INDIVIDUALITY AND ANXIETY

Professor Chiye Aoki and her team are exploring the neurobiological bases of individuality and anxiety disorders such as anorexia nervosa, by looking into how environmental factors influence and shape the development of juvenile and adolescent brains.

Portrait of a neuroscientist

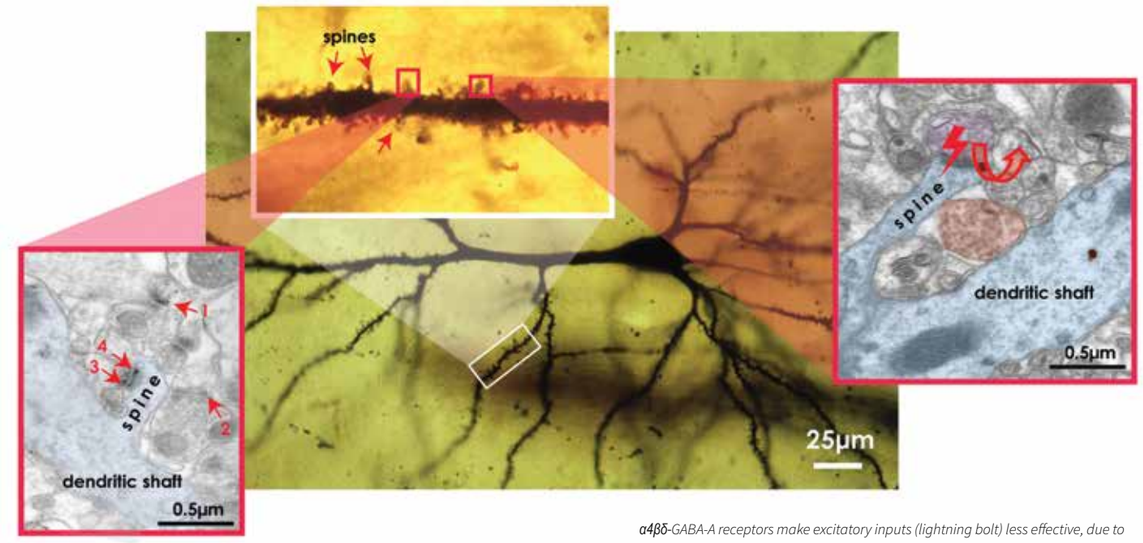
Professor Chiye Aoki, one of the celebrated pioneers in neuroscience research, was never afraid to ask big questions. Her latest research focuses on how changes at the synapse relate to resilience or vulnerability to anorexia. However, her interest in neuroscience was spiked by an interesting observation she made regarding her childhood years. She first learned to speak in the United States but she later moved to Japan for a few years. Much to her surprise, she had to relearn English upon her return to the US. However, this adaptation was much easier for her, still a child, than it is for adults. To her it became clear that changes within the maturing brain must be the cause of such differences. This observation placed her onto the path of asking deeper questions, regarding how human individuality – the self – is shaped by experiences and what might be the neurobiological basis of this process. By asking such questions, she seeks to understand the physical changes in the brain and the mechanisms that shape the character of the sense of self.

During her undergraduate years, Professor Aoki thrived in a climate where neuroscience was a still developing science. She enjoyed being able to challenge the existing knowledge and make new discoveries about neuroscience. This approach shaped her view of the field and helped her understand her potential to be passionately curious but scientifically rigorous about new discoveries and findings. She began to investigate how brain cells, called neurons, adapt their connections to other neurons. Her work led to finding a correlation between how malleable the shape of a neuron is and how adaptable a neuron's connectivity to other neurons is – a term called neuronal plasticity. This functional link is particularly interesting because it acts during a period critical for the development of the visual cortex and language acquisition regions, thus disruptions in neural activity in these areas have life-long consequences. For her discovery, she received her PhD degree in 1985 from The Rockefeller University.

During her postdoctoral fellowship with the Cornell University Weill Medical College in the laboratory of Dr Virginia Pickel, Professor Aoki learned how to view molecular interactions at synapses, which are structures specialised for neuron-to-neuron connections. Synapses, although so vital for brain function, are extremely small, requiring an electron microscope to magnify the structure at least 20,000 fold to be viewable. The techniques involving the use of electron microscopes enabled her to observe the behaviour of new or established synapses and helped her make some interesting discoveries. One of her findings was that neurotransmitters such as dopamine and noradrenaline propagate over the gaps between neurons and activate receptors which are not constrained to being clustered at synapses. This versatility in the location of receptors allow neurotransmitters, such as dopamine and noradrenaline, to operate more globally, rather than through pathways specified by point-to-point synaptic connections between neurons. Such global actions of neurotransmitters may be the key to their role in affecting mood or vigilance, rather than relaying specific events or features that an individual also experiences through synaptic activity.

Another discovery she made involved a separate group of brain cells called glia, which enshroud, protect, and feed neurons. She found that these cells participate in the conversion of glutamate, an excitatory neurotransmitter, into its non-toxic form, glutamine, which plays a role in the synthesis of proteins and production of cellular energy. Later on, she researched nitric oxide, a very important molecular compound with a role in cardiovascular signalling and an extremely short lifetime, which makes it difficult to study. In fact, nitric oxide was so difficult to find that its discoverer was awarded the 1998 Nobel Prize in Physiology. Professor Aoki found that nitric oxide can be generated by neurons with glutamate receptors – called NMDA receptors. Following this line of research, she found that glutamate receptors can be found on both ends of a synapse and that they aggregate in clusters on the main

Spines, seen by light microscopy at a magnification of 1,000x. Both light micrographic images were created by Professor Dominick P. Purpura, Professor Emeritus of Neuroscience at Albert Einstein Medical College and past president of the Society for Neuroscience



The presence of NMDA receptors at excitatory synapses is revealed by first using antibodies that specifically recognize only those proteins that form these receptors. The binding of antibodies to the NMDA receptors at excitatory synapses are then detected by electron microscopy at a magnification of 40,000x, which enables detection of colloidal gold particles that have been linked to these antibodies (tiny dark spots 1, 2, 3 and 4).

α4βδ-GABA-A receptors make excitatory inputs (lightning bolt) less effective, due to shunting inhibition (arrow, indicating leakage of the excitatory synaptic current). The presence of α4βδ-GABA-A receptors at excitatory synapses is revealed by using a similar antibody-colloidal gold method for electron microscopy, but this time, using antibodies that specifically recognize only those proteins that form α4βδ-GABA-A receptors (dark spot under the arrow).

Professor Aoki's research paves the way for new treatments for anorexia nervosa, and contributes to a better understanding of the neurobiological bases of plasticity.

trunks of neurons, called dendritic shafts, during early life, clearly before they specialise into forming a synapse.

To the present day, her research uses electron microscopy to gaze into the processes going on between transmitters and receptors – processes having potential roles in synaptic plasticity. The phenomenon of synaptic plasticity is a response to the level of activity of neurons. In one of the many types of plasticity, synapses can strengthen when they are used and weaken when the activity level decreases. This variation of the synapse strength with activity level is what makes cognition a 'use it or lose it' type of process and forms the basis of the importance of education and repetition in improving intellectual ability. Through observations made using electron microscopy, Professor Aoki was able to confirm that neurotransmitter receptors are brought to the neural membranes on both ends of a synapse in a way that depends on activity. Moreover, neurons show a measurable change in response to activity in less than ten minutes in adult brains.

More recently, Professor Aoki and her team have been exploring the hypothesis that the proteins that form filaments to maintain the shape of cells have a role in the circulation of synaptic molecules within dendritic spines, the small protrusions of a dendrite specialised to receive input from other neurons. For this reason, the team is researching what happens when two molecules are present or absent from specific neural sites. These molecules, drebrin and neurexin, help recruit synaptic molecules and enroll them into forming new synapses. Neurexin is a protein mostly found in the cell membrane of the sending axon terminal – the presynaptic membrane – while drebrin is hypothesised to play a role in plasticity on the receiving, dendritic side of synapses.

Professor Aoki's research is also focused on the molecular and cellular mechanisms involved in acetylcholine and gamma-aminobutyric acid (GABA) regulation. Acetylcholine is a neurotransmitter present in both the central and peripheral nervous systems, which activates muscles and plays a key role in the regulation of attention and memory within the brain. GABA, on the other hand, functions as an inhibitor of neuronal excitability in the brain, which helps to refine brain function by counter-balancing excitation and also prevents epilepsy and

uncontrolled anxiety. Professor Aoki's interest is to understand why there are so many types of GABA and acetylcholine receptors in the brain. Moreover, she wants to understand how these receptors regulate anxiety through modulation of neuronal pathways in three key regions of the brain - the hippocampus, prefrontal cortex and amygdala. One possibility she has considered is that GABA and acetylcholine receptors are distributed along neuronal pathways formed by excitatory neurons. The neuronal pathways formed by excitatory neurons enable specific processes in each region of the brain, such as for the motor cortex to generate very precise visually guided behaviour, such as a baseball player to hit a homerun. If so, variations in GABA and acetylcholine receptor distribution along an excitatory pathway would allow for modulation (boosting or dampening) of that pathway, thereby providing much more flexibility and individuality to our thought processes, perception, behaviour, and decision-making.

To test her working hypothesis, Professor Aoki and her team are feeding neuronal sites with neurotransmitters that modulate neuron activity. To analyse their findings, the scientists are statistically comparing the measured response in neural activity with the map showing the distribution of receptors across neurons belonging to the activated pathway.

Measuring the impact of stress on the young brain

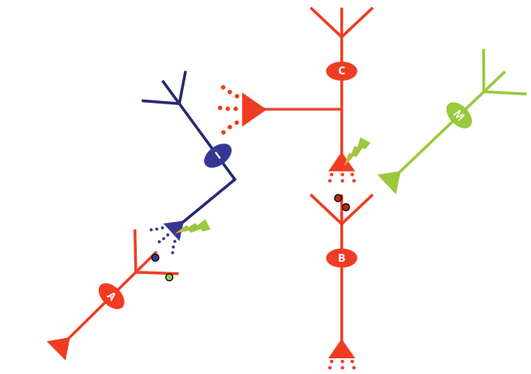
As Professor Aoki explains, plasticity is a term widely used in neuroscience to describe many types of phenomena. For example, plasticity is used to describe the reorganisation of neuron-to-neuron connections in large areas of the cortex, or axons' regeneration, the refresh rate of neurotransmitter receptors, creation of new neurons, regulation of neurotransmitter secretion, and so on. This wide applicability of the term is due to the realisation that the brain actively adapts to the environment and its own activities, or acts such that it can compensate for damage or prevent overdrive of the nervous system.

The phenomenon of plasticity is extremely useful in learning, because it allows for the creation of new pathways and strengthens existing ones – for example when learning a new language, which is an example particularly interesting for Professor Aoki. Yet the plasticity of the brain can be hijacked by harmful processes, such as addiction or stress, and can be vulnerable to the action of hormones. When a behavioural pattern becomes fixed in the brain, it can lead to stress reactions even in the absence of a stressful stimulus, based only on previous experience. Traumatic stress, for example, can determine individuals to overreact to otherwise innocuous environments and triggers.

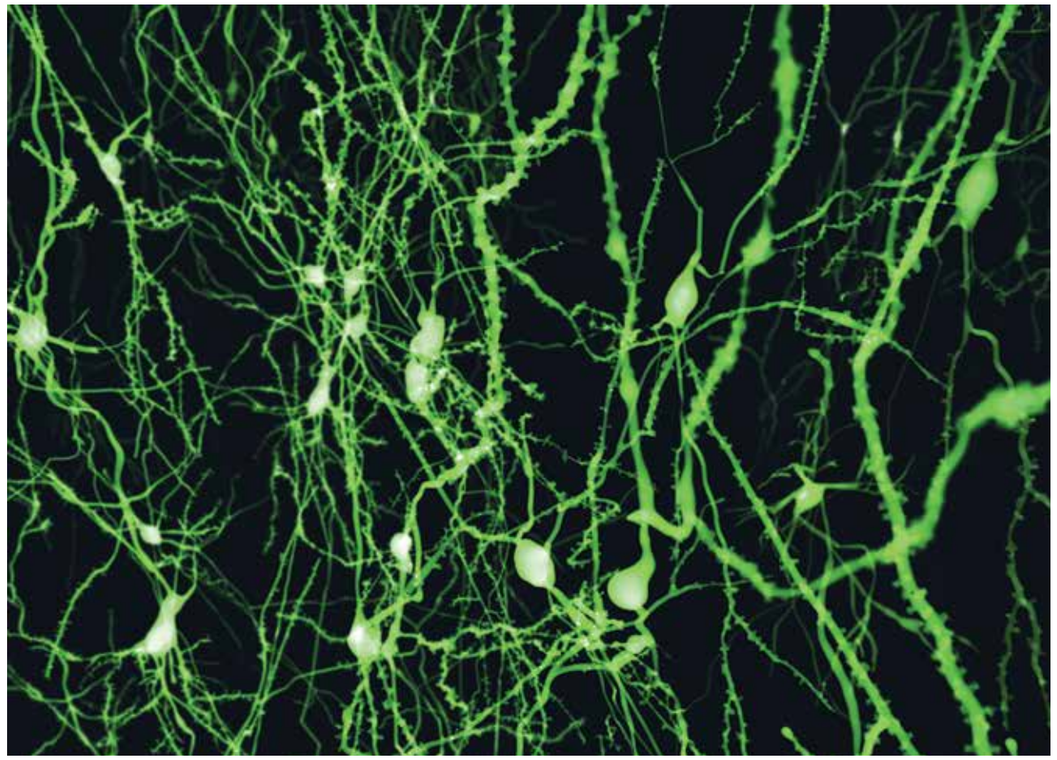
Brain plasticity is more prominent in young individuals. Moreover, it plays an important role in the formation of the future adult brain. For this reason, Professor Aoki used a clever experimental setup to study the effects of the vicious circle of stress and anorexia nervosa. She published her results in a paper called 'Synaptic changes in the hippocampus of adolescent female rodents associated with resilience to anxiety and suppression of food restriction-evoked hyperactivity in an animal model for anorexia nervosa'. Her working hypothesis was that a GABA-dependent mechanism within the hippocampus controls the regulation of anxiety in individuals – anxiety which has a strong influence on the vulnerability of the respective individual to activity-based anorexia (ABA).

Anorexia nervosa is a mental illness affecting mostly adolescents, with a mortality rate 200 times larger than that of suicide. The disease is known to affect females nine times more often than it affects men, suggesting that sex hormones may play a role in this condition. The current research points to a paramount importance of the prefrontal cortex, amygdala, striatum, and hypothalamus regions of the brain, and anomalies in the function of neurotransmitters such as dopamine and serotonin as most prominent causes of the disease. Professor Aoki's contribution has been to hypothesise GABA action in the hippocampus and prefrontal cortex as additional important factors in the risk of developing anorexia nervosa.

Anorexia nervosa manifests through heightened anxiety, extreme abstaining from food despite hunger, intense fear of gaining weight, and a preference for excessive exercise. Since there is no pharmacological cure for this condition and the relapse rate is larger than 25%, Professor Aoki decided to use an animal model of the illness so that she was able to study brain changes in her subjects, which were adolescent female rodents. The ingenuity of her setup was that it exposed rodents to two bouts of activity-based anorexia separated by a period of recovery, thus mimicking the natural course of the illness. In the first stage, the rodents were introduced to a running wheel. After that, their access to food was restricted to only 1-2 hours per day. The researchers made two important observations. Firstly, the rodents became extremely anxious and began preferring to run excessively on the running wheel even when they had access to food, instead of eating. Secondly, many, but, importantly, not all of the rodents became excessive runners: those that did were the more anxious types that lost the greatest amount of body weight.



Red neurons are excitatory, providing a neuronal pathway through synaptic connections that generate behavior. The blue neuron is inhibitory. The green neuron is modulatory. In this schematic, Excitatory Neuron A generates a simple response, whenever excited. However, this response can be suppressed through a higher command neuron, C, that activates an inhibitory neuron, I, that is set in place to inhibit Neuron A. The command neuron also excites an alternative excitatory neuron B that generates an alternative response. The neuromodulator can activate one type of receptor (green lightning bolt) to boost neurotransmitter release, thereby favoring the elicitation of the alternative response. The same neuromodulator can active another type of receptor (green ball on neuron A) to suppress excitability of Neuron A. Receptors for neuromodulators are not constrained to reside at synapses. This allows for neuromodulators to operate more widely and in a varied fashion, sculpting overall behavior of an individual.



Although the setup cannot capture the complex social causes leading humans to anorexia nervosa, such as peer pressure to be thin, it is extremely valuable because the neurobiological changes in the rodents' brains should capture changes that are similar in human brains triggered by self-imposed food restriction and excessive exercise. Even though previous studies creating animal models of anorexia are available, the novelty of this research is that it specifically considers the role of anxiety, plasticity, and chemical regulation during the development of the young hippocampus and prefrontal cortex.

For a better understanding of the results, we should recall that GABA inhibits neural excitability, whereas the hippocampus plays a central role of enabling memory to form for long periods. Besides that, the amygdala, together with the hippocampus, processes emotional reactions while prefrontal cortex is involved in decision-making processes. Professor Aoki found that one type of GABA receptor, called α4βδ-GABA-A receptor, is particularly well-correlated with the rodents' propensity to give up running on the wheel, so as to feed more efficiently during the limited hours of food availability. It is a type of GABA receptor that is not receptive to benzodiazepines, like Valium. This may explain why benzodiazapines have not been so effective in treating anorexia nervosa, even though they are usually effective in reducing anxiety. Her work also suggests that drugs favouring the recruitment of the special type of GABA receptors found in brains of the more efficient feeders may help treat the more vulnerable individuals.

Another finding was that the number of NMDA-type glutamate receptors associated with excitatory neurons in the hippocampus varies proportionally with the weight loss. Therefore, Professor Aoki and her team were able to propose that, at least in some cases, the excitability of glutamate receptors associated with anorexia can be dampened with the help of GABA.

Many have asked Professor Aoki why rodents choose to run, especially when they are hungry. This seems paradoxical. Why don't animals quiet down, so as to conserve energy? Professor Aoki speculates that these hungry rodents might be exhibiting a form of foraging behaviour. Foraging is an adaptive innate behaviour, enhancing the chance for an individual to locate a new source of food, rather than perishing in a food-scarce environment. Recall that some, but not all, rodents become hyperactive when hungry. Could it be that a select number of animals and people are born to become foragers, to ensure that its gene pool survives famine? If so, Professor Aoki continues to surmise, then the individuals that run less on a wheel are doing so, because they have learned to suppress a powerful innate behaviour, achieved through active inhibition of key brain areas, such as the prefrontal cortex and the hippocampus. Professor Aoki's research paves the way for new treatments for anorexia nervosa in particular, and contributes to a better understanding of the neurobiological bases of plasticity. In the following years, the resourcefulness and ingenuity shown by Professor Aoki's research will help her uncover additional mechanisms that shape the developing brain.

Meet the researcher

Professor Chiye Aoki

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Professor Chiye Aoki is currently leading a lab with the New York University Center for Neural Science. She and her team explore neurotransmitter expression and stress modulation in the brain, as well as neuronal plasticity. She received her PhD in 1985 from The Rockefeller University and continued with a postdoctoral fellowship at Cornell. In 1999, she was appointed as Director of the CNS Summer Undergraduate Research Experience Program and her work has been funded since 1985 through a variety of sources, including the NIH FIRST Award, the NSF Presidential Faculty Fellowship Award, The International Human Frontiers Science Program and the Klarman Family Foundation Grants Program in Eating Disorders Research. Throughout her career, she has written more than 100 papers and received almost 7,500 citations. She has been an invited speaker at over 50 conferences.

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REFERENCES

C Aoki, T Chowdhury, G Wable and Y-W Chen, Synaptic changes in the hippocampus of adolescent female rodents associated with resilience to anxiety and suppression of food restriction-evoked hyperactivity in an animal model of anorexia nervosa, Brain Research Special Issue on Adolescent Plasticity, 2016, DOI: 10.1016/j.brainres.2016.01.019

Y-W Chen, G Wable, TG Chowdhury and C Aoki, Enlargement of axo-somatic contacts formed by GAD-immunoreactive axon terminals onto layer V pyramidal neurons in the prefrontal cortex of adolescent female mice is associated with suppression of food restriction-evoked hyperactivity and resilience to activity-based anorexia, Cerebral Cortex, 2015, 26, 2574–2589.

H Nedelescu, C Kelso, M Purpura, G Lazaro Munoz, CK Cain, JE LeDoux and C Aoki, Endogenous GluR1-containing AMPA receptors translocate to asymmetric synapses in the lateral amygdala during the early phase of fear memory formation: an electron microscopic immunocytochemical study, J. Comp. Neurol., 2010, 518, 4723–39.

G Wable, Y-W Chen and C Aoki, Exogenous progesterone exacerbates running response of adolescent female mice to repeated food restriction stress by changing α4-GABA$_A$ receptor activity of hippocampal pyramidal cells, Neuroscience, 2015, 310, 322–41.

C Aoki, DS Bredt, S Fenstemaker and M Lubin, The subcellular distribution of nitric oxide synthase relative to the NR1 subunit of NMDA receptors in the cerebral cortex, In Prog in Brain Res 118 Nitric Oxide in Brain Development, Plasticity, and Disease, RR Mize, TM Dawson, VL Dawson and MJ Friedlander, eds., 1998, 83–100.