OPTOGENETICS

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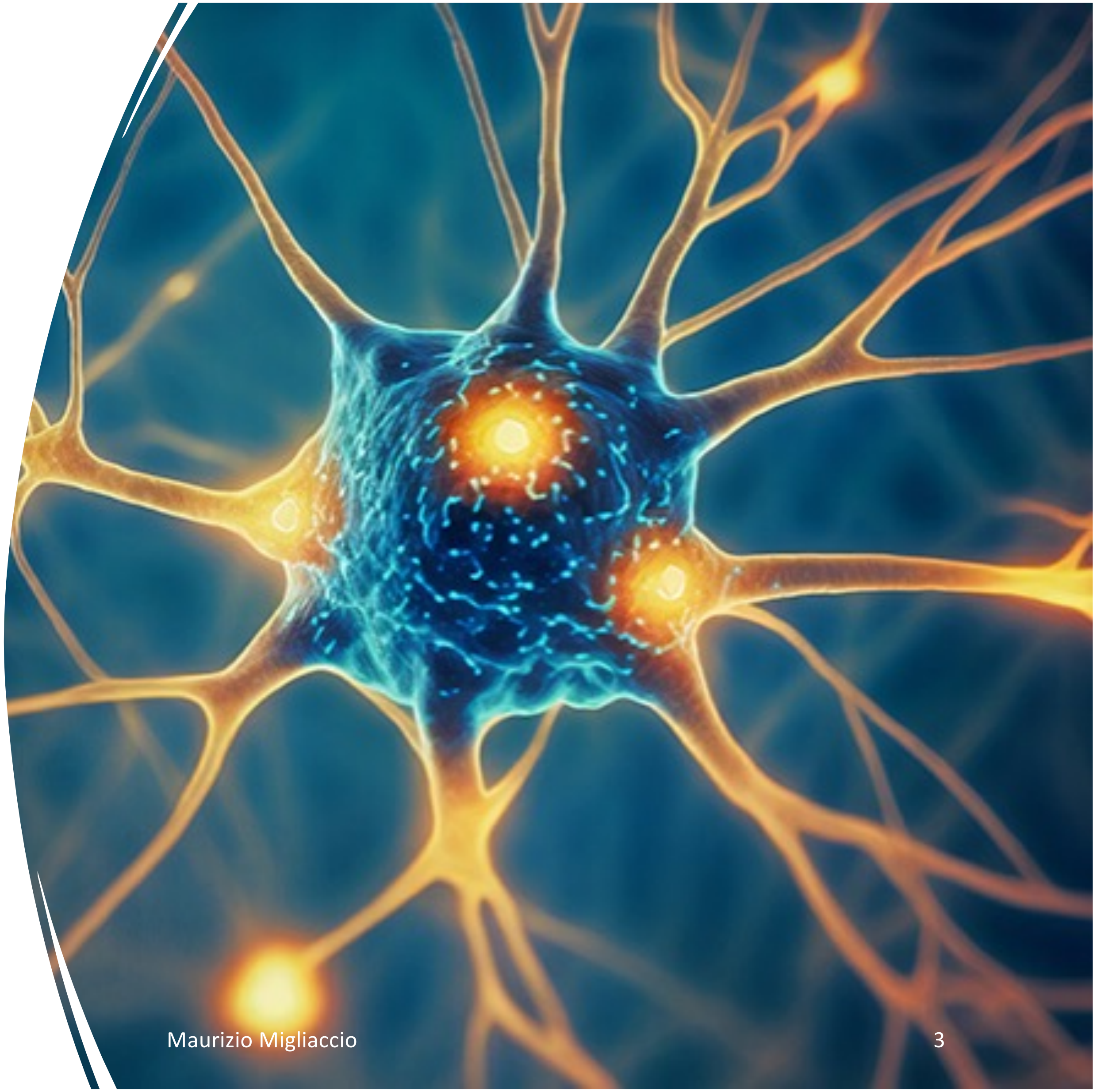
Summary

Definition

- The term “optogenetics” means a combination of genetic manipulation and optics.



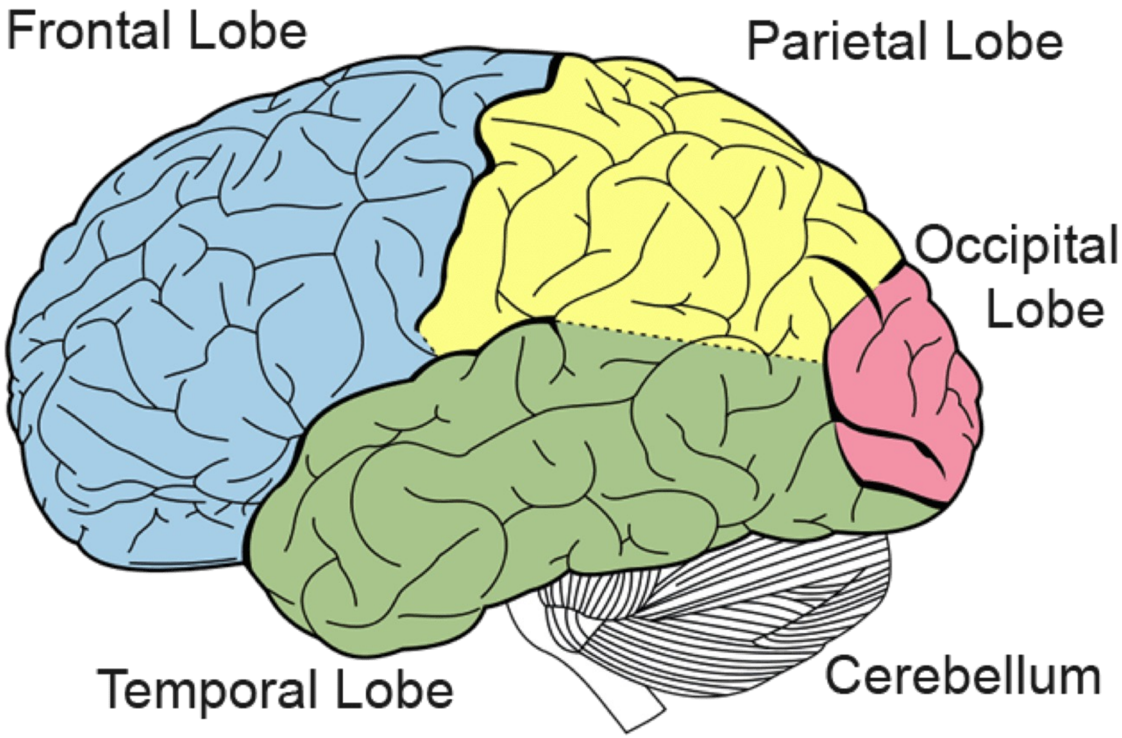
Maurizio Migliaccio

Definition

Optogenetics is an emerging science that combines optical and genetic sensing techniques with the aim of probing neuronal circuits within the intact brains of mammals and other animals, in times on the order of milliseconds, times necessary to understand the modalities of processing and transformation of information between neurons.

Neuroscience

- A central goal of neuroscience is to understand how the brain works in various behavioral conditions.
- For this, it is essential to uncover the functions of many types of neurons and neuronal circuits that are responsible for information processing in the brain.

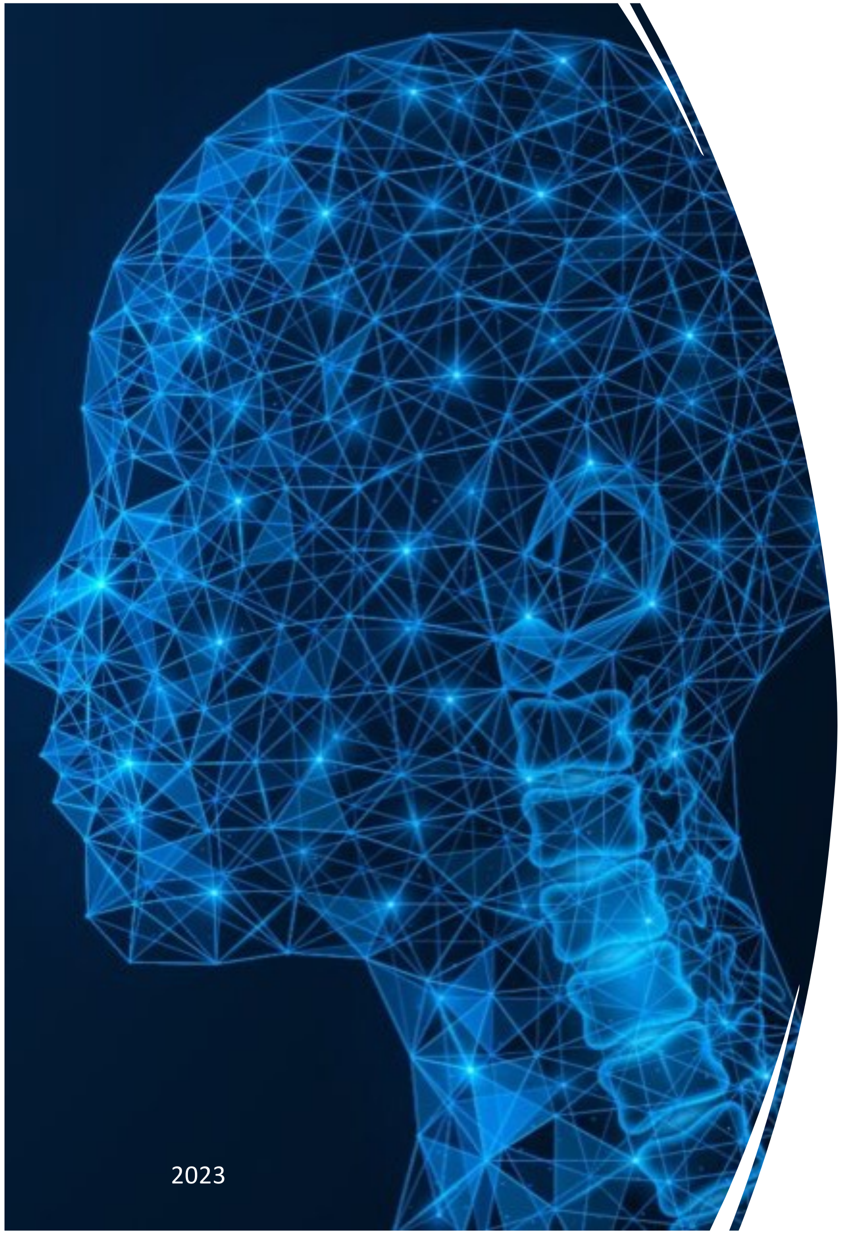


Neuroscience

- The brain consists of several discrete parts: most notably the cerebrum, the brain stem, and the cerebellum.
- The cerebrum consists of six brain areas that span two hemispheres. Four of these areas can be seen from the external view of the brain– the frontal, parietal, occipital, and temporal lobes.
- The two other lobes, the limbic and insular lobes, are found within the cerebrum.
- The outermost part of the cerebrum is called the cortex (or sometimes the neocortex). This contains a sheet of neurons that wraps around all the lobes of the brain, and is roughly 1.5 – 3mm thick.

Neuroscience

- Neurons are the principal communicators of the brain – they are involved in sending signals from one region to another, and ultimately triggering actions, encoding and retrieving memories, and creating the experience of being, well, alive.
- A dense collection of neurons are found within the cortex, and other areas within the limbic and insular lobes. Neurons send the messages, and a variety of other cells – called glial cells.



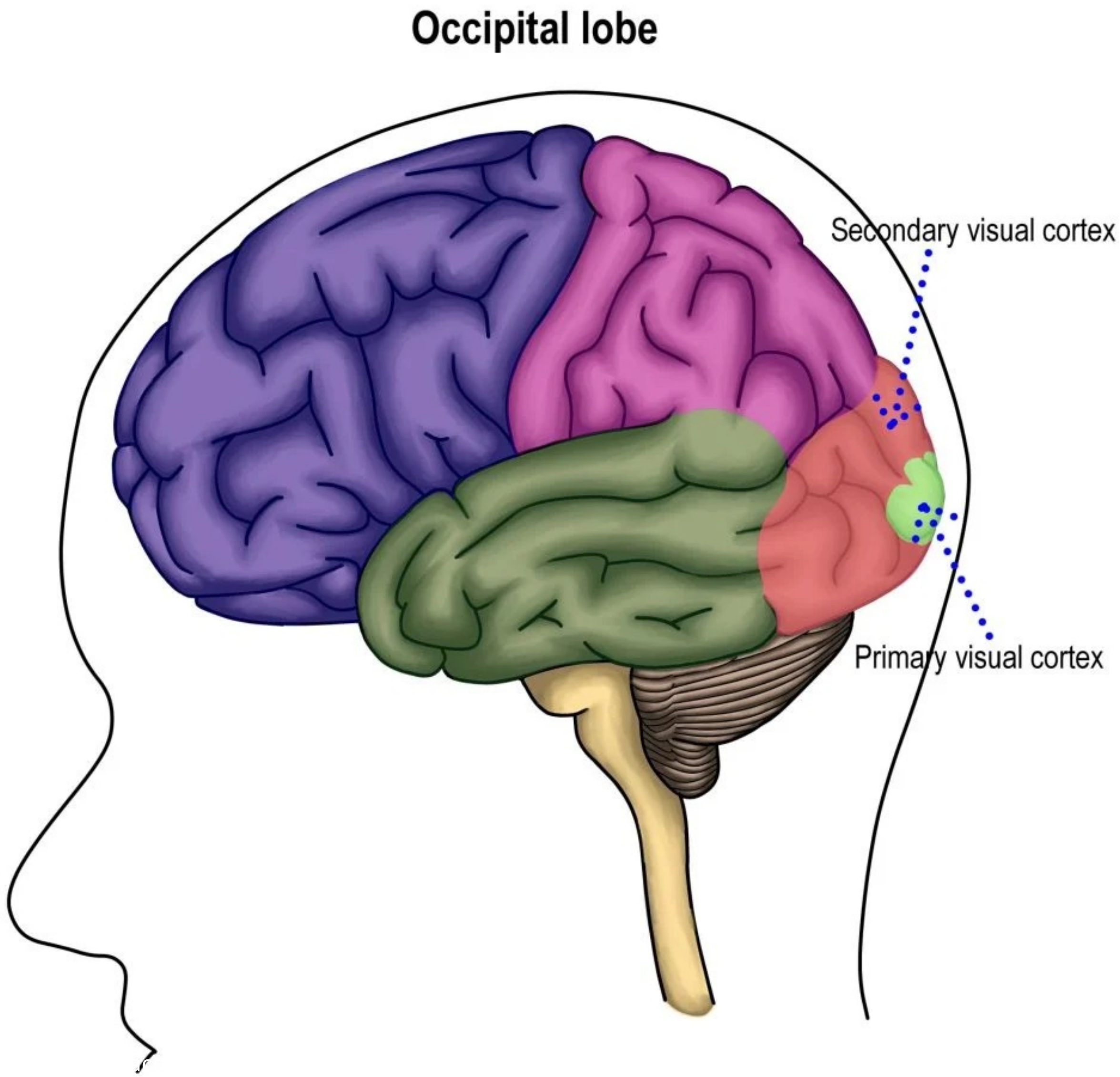
Neuroscience

Some of the most crucial and well-studied parts of the brain are:

- The **frontal cortex** (located at the very front of the frontal lobe) – plays role in a range of cognitive functions, including attention, decision making, planning complex behaviors, and regulating social actions. This is typically termed executive function.
- The **middle of the brain** (in the parietal lobe, roughly just after the border of the frontal cortex) is involved in both motor processing (the feeling of touch) and in motor coordination (movement) . These aren't the only brain areas involved in these processes, but they are the principal actors.

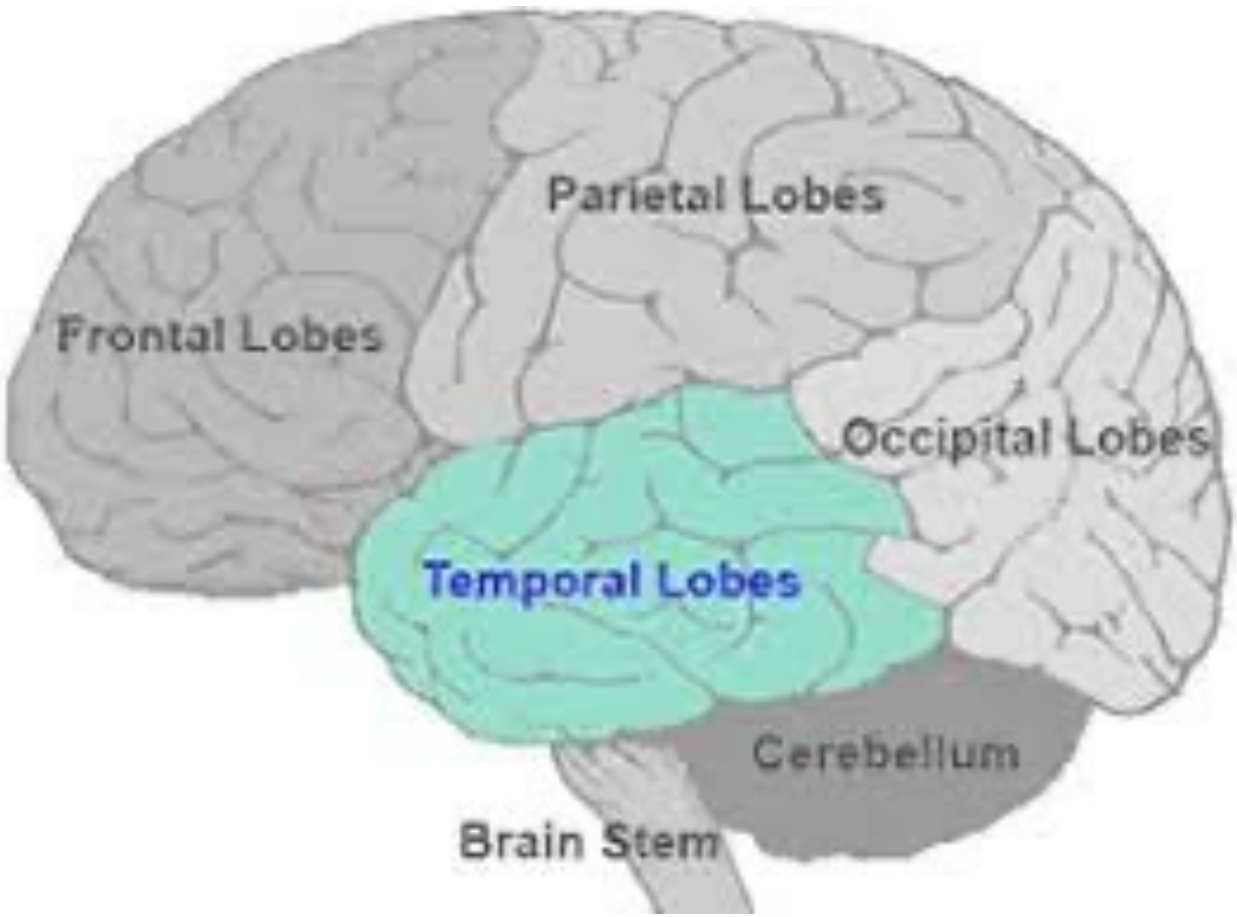
Neuroscience

- The **occipital cortex** is involved insight – it features many different layers, each of which processes a different component involved in perceiving the world visually.



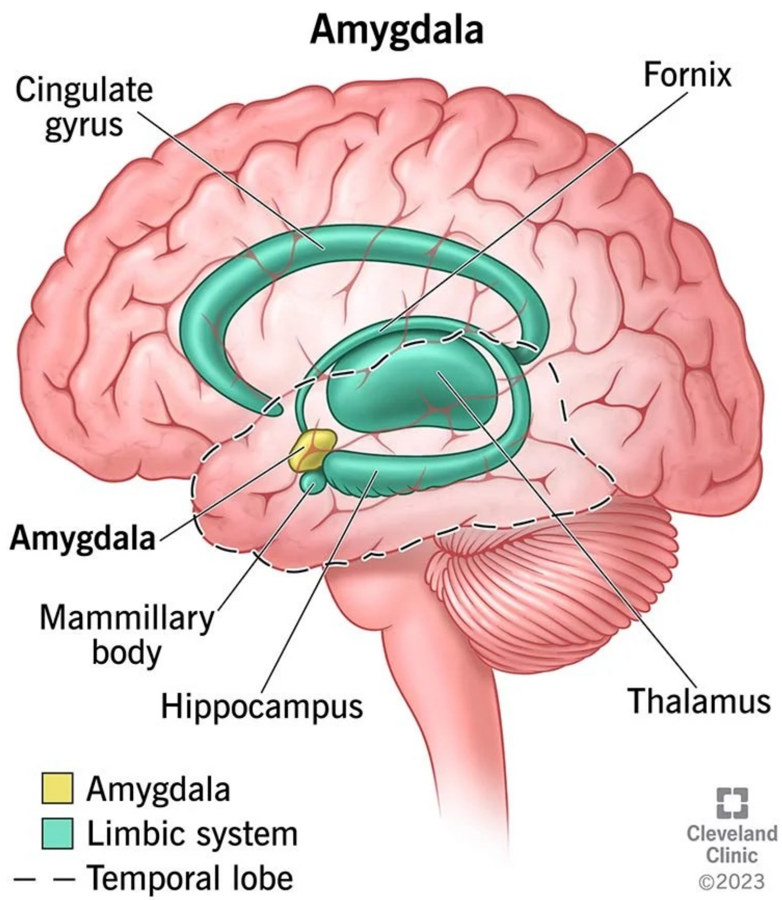
Neuroscience

- The **temporal lobes** are largely discussed for their relevance in language – the left side of the brain features Wernicke's and Broca's areas, involved in speech comprehension and speech production, respectively.



Neuroscience

Within the brain, there are several other regions that are of note, including the basal ganglia (a collection of regions involved in action selection), the hippocampi (involved in-memory processing), and the amygdalae (involved in fear processing).



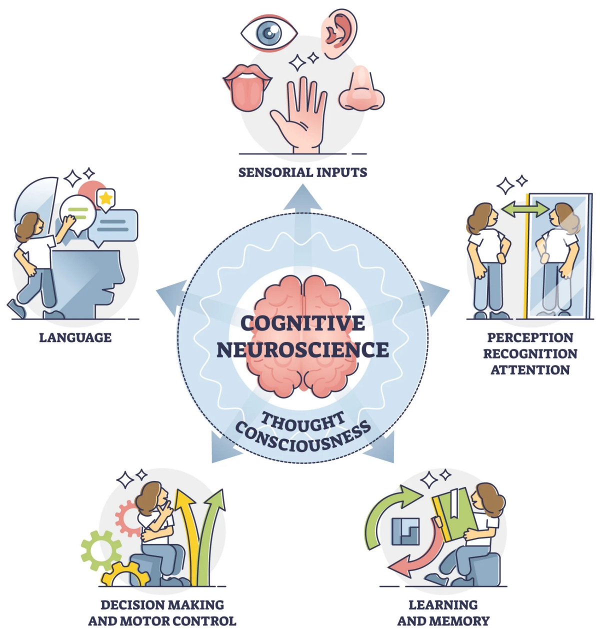
Neuroscience

There are many different branches of neuroscience – everything from computational, to pharmacological, to molecular neuroscience and well beyond. Hereafter we refer to two main ones only:

- Cognitive neuroscience
- Behavioral neuroscience

Cognitive neuroscience

- Cognitive neuroscience is concerned with the scientific study of biological substrates underlying cognition and mental processes and addresses questions such as how psychological/cognitive functions are reflected by neural activity in the brain.
- Typical data collection methods employed by cognitive neuroscientists are functional neuroimaging (fMRI, PET), electroencephalography (EEG), behavioral genetics, and lesion studies.



Behavioral neuroscience

- Behavioral neuroscience (also known as biopsychology), addresses the impact of the nervous system on attention, perception, motivation, performance, learning, and memory and their manifestations in human behavior.
- Studies in behavioral neuroscience focus on the interaction of brain and behavior in real or simulated environments.

Neuroscience

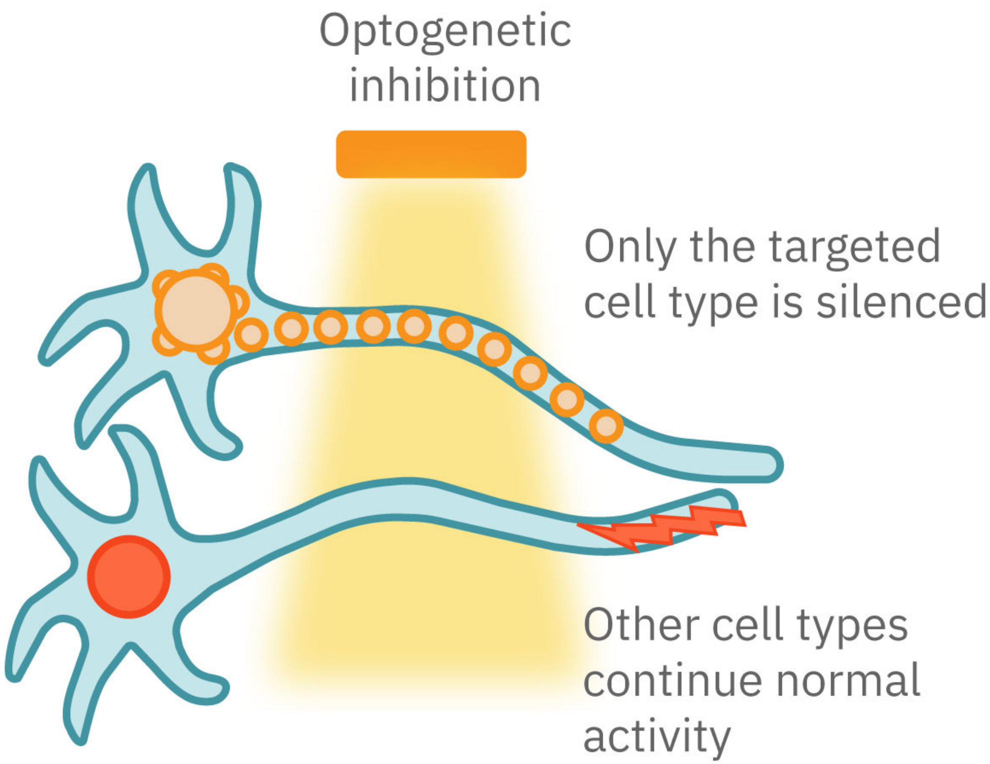
- Electrophysiological techniques have provided a collection of information about neurons and circuit function.
- These techniques have advantages in temporal resolution and sensitivity; however, they have limitations in examining neuronal circuit function. For example, with extracellular stimulation using electrodes, it is difficult to stimulate specific types and populations of neurons.
- Regarding intracellular electrical stimulation, only a limited number of neurons can be stimulated.
- In terms of electrophysiological recordings, despite their excellent temporal resolution and high sensitivity, they have limitations in providing spatial information and can only measure neuronal activity in a limited number of neurons.
- Considering the diversity of neurons in the brain, these limitations make it difficult to identify the function of each neuron type and neuronal circuits.

Optogenetics

Recent advances in optical techniques provide powerful tools to overcome these limitations in addressing the functions of neurons.

A prominent example is optogenetics, which enables the optical measurement and control of neuronal activity.

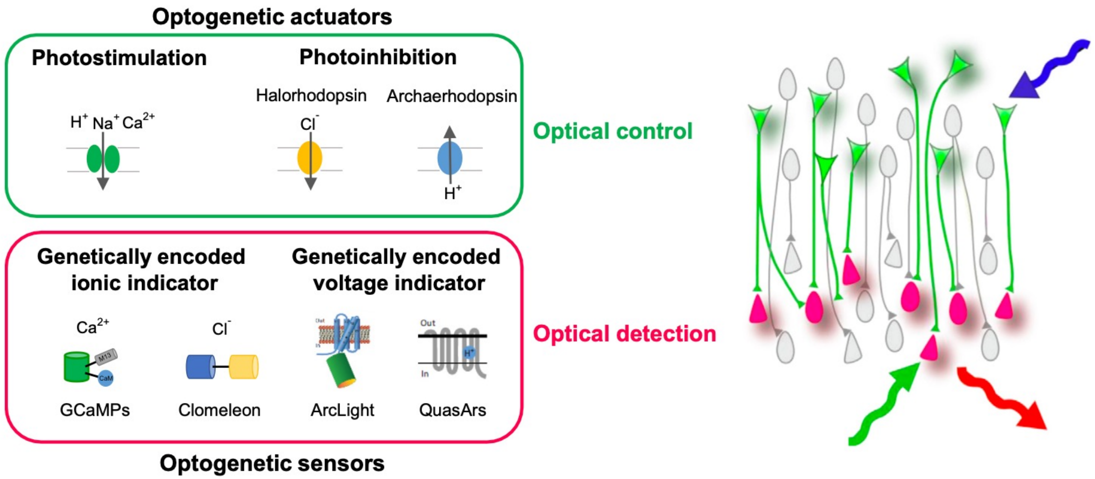
Optogenetics refers to the integration of optics and genetics, which enables the expression of light-sensitive proteins in genetically defined populations of neurons.



Optogenetic probes

There are two types of optogenetic probes: optogenetic actuators and optogenetic sensors.

Optogenetic actuators allow for controlling neuronal activity, whereas optogenetic sensors enable the detection of neuronal activity

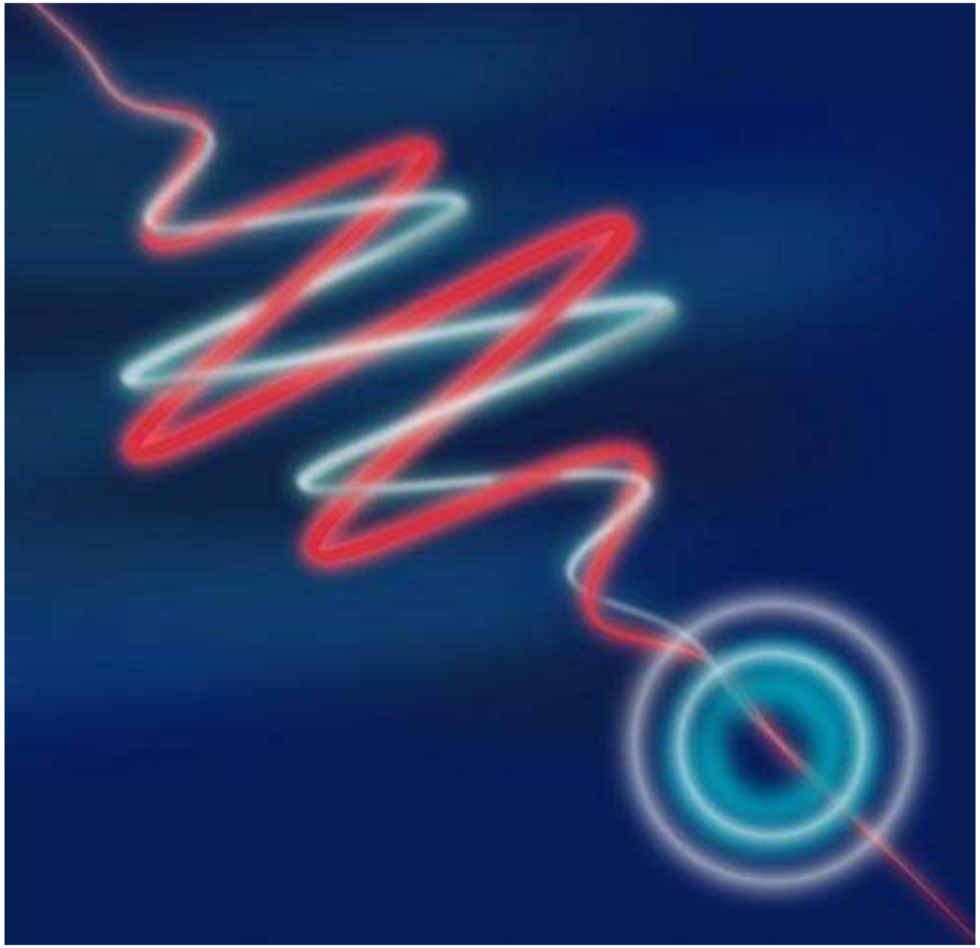


Electromagnetic background

- A photon is an elementary particle that is a quantum of the electromagnetic field, including electromagnetic radiation such as light and radio waves, and the force carrier for the electromagnetic force.
- Photons are massless, so they always move at the speed of light in vacuum, 299792458 m/s.
- The photon belongs to the class of boson particles.

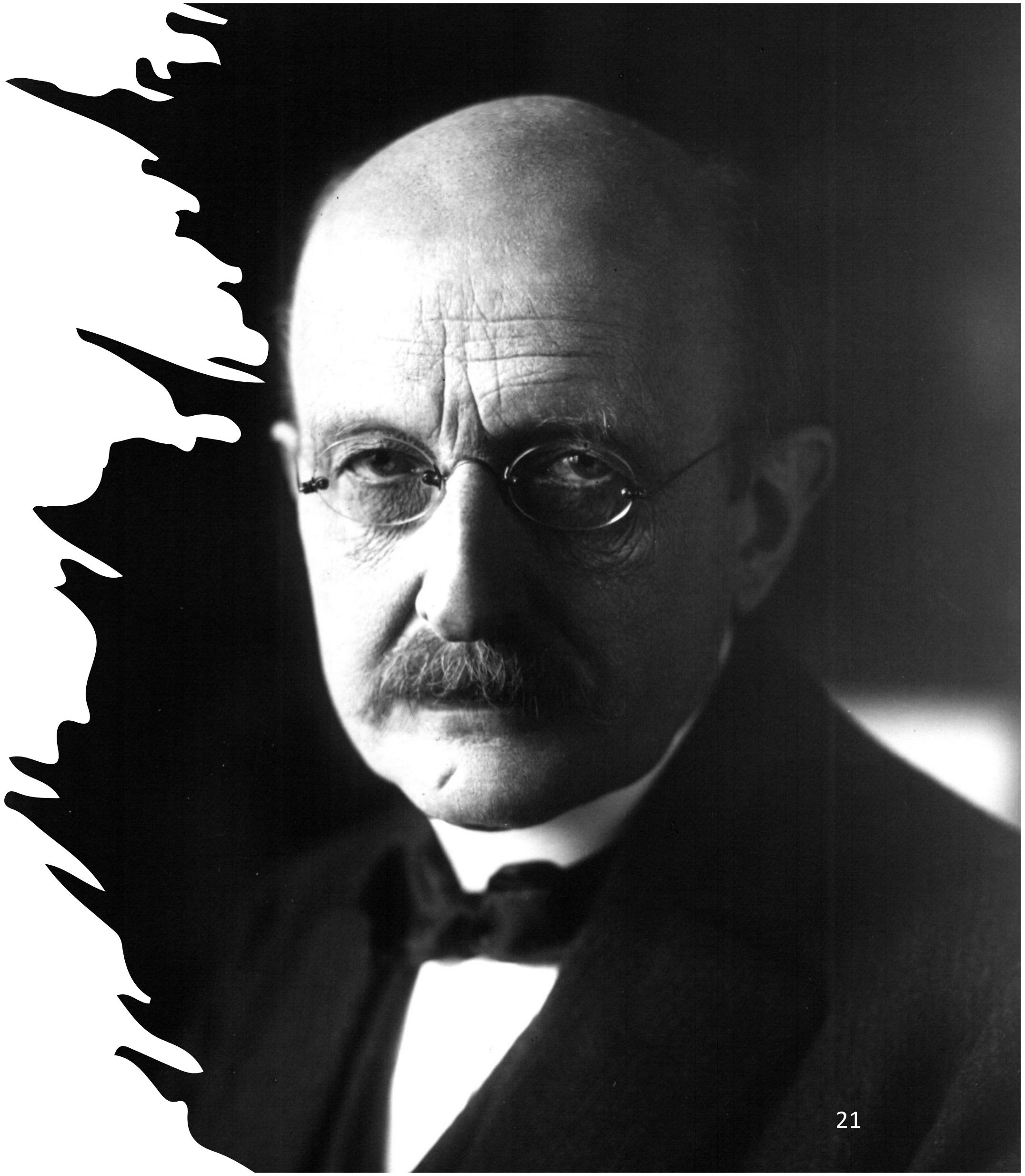
Electromagnetic background

- As with other elementary particles, photons are best explained by quantum mechanics and exhibit wave–particle duality, their behavior featuring properties of both waves and particles.
- The modern photon concept originated during the first two decades of the 20th century with the work of Albert Einstein, who built upon the research of Max Planck.



Max Planck

- Max Karl Ernst Ludwig Planck (23 April 1858 – 4 October 1947) was a German theoretical physicist whose discovery of energy quanta won him the *Nobel Prize in Physics in 1918*.
- Planck made many substantial contributions to theoretical physics, but his fame as a physicist rests primarily on his role as the originator of quantum theory, which revolutionized human understanding of atomic and subatomic processes.

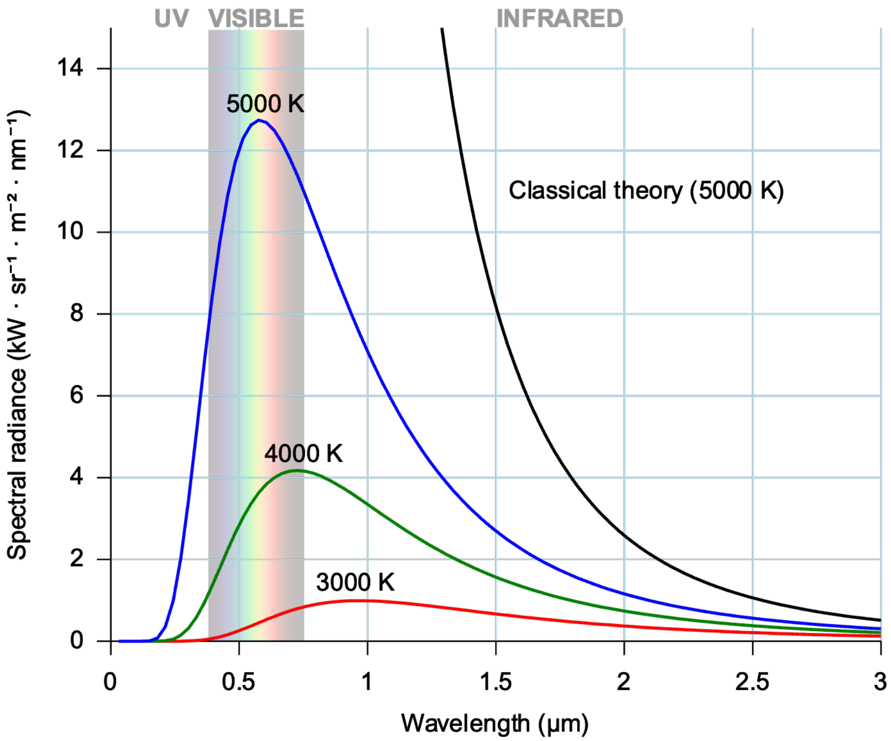


Max Planck

- On December 14, 1900 he published his first work on quantum theory, marking the beginning of modern physics.
- Quantum mechanics introduced the concept of energy constituted, like matter, by entities that could not be further divisible (quanta) and solved problems that could not be explained through Newton's mechanics, which is not applicable to very small objects such as atoms.
- Planck described the energy exchanges in the emission and absorption phenomena of electromagnetic radiation as phenomena that occur in a discrete form (and not in a continuous form as the classical electromagnetic theory).

Max Planck

- Planck's radiation law, a mathematical relationship formulated in 1900 by German physicist Max Planck to explain the spectral-energy distribution of radiation emitted by a blackbody.
- Blackbody: a hypothetical body that completely absorbs all radiant energy falling upon it, reaches some equilibrium temperature, and then reemits that energy as quickly as it absorbs it.



Max Planck

- In classical physics, the Rayleigh-Jeans law related the intensity of the radiation given off by a blackbody to the frequency at a specific temperature.
- At low frequencies, this equation matches experimental results, but at high frequencies the equation diverges sharply.
- These high frequencies are in the ultraviolet region of the electromagnetic spectrum, and so this difference was called the ultraviolet catastrophe.

Max Planck

- German theoretical physicist Max Planck explained the ultraviolet catastrophe using a new physics that was later called quantum mechanics.
- In this theory, Planck theorized that energy was absorbed and radiated in discrete packets of energy called quanta.
- This replaced the concept that energy, including light energy, moved only as waves of a particular wavelength and frequency and that these waves were continuous in their frequencies throughout the spectrum.
- Planck theorized that matter could not take on all energies of the spectrum but instead could absorb energy only at specific equally spaced energy levels.
- *If only specific frequencies could be absorbed, then only specific frequencies could be emitted*.

Max Planck

- Planck assumed that the sources of radiation are atoms in a state of oscillation and that the vibrational energy of each oscillator may have any of a series of discrete values but never any value between.
- Planck further assumed that when an oscillator changes from a state of energy E_1 to a state of lower energy E_2, the discrete amount of energy $E_1 - E_2$, or quantum of radiation, is equal to the product of the frequency of the radiation, symbolized by the Greek letter ν and a constant *h*, now called Planck's constant, that he determined from blackbody radiation data; i.e., $E_1 - E_2 = h\nu$.

Photon Energy

- **The energy of a photon is given by this equation:**

$$E = h\nu$$

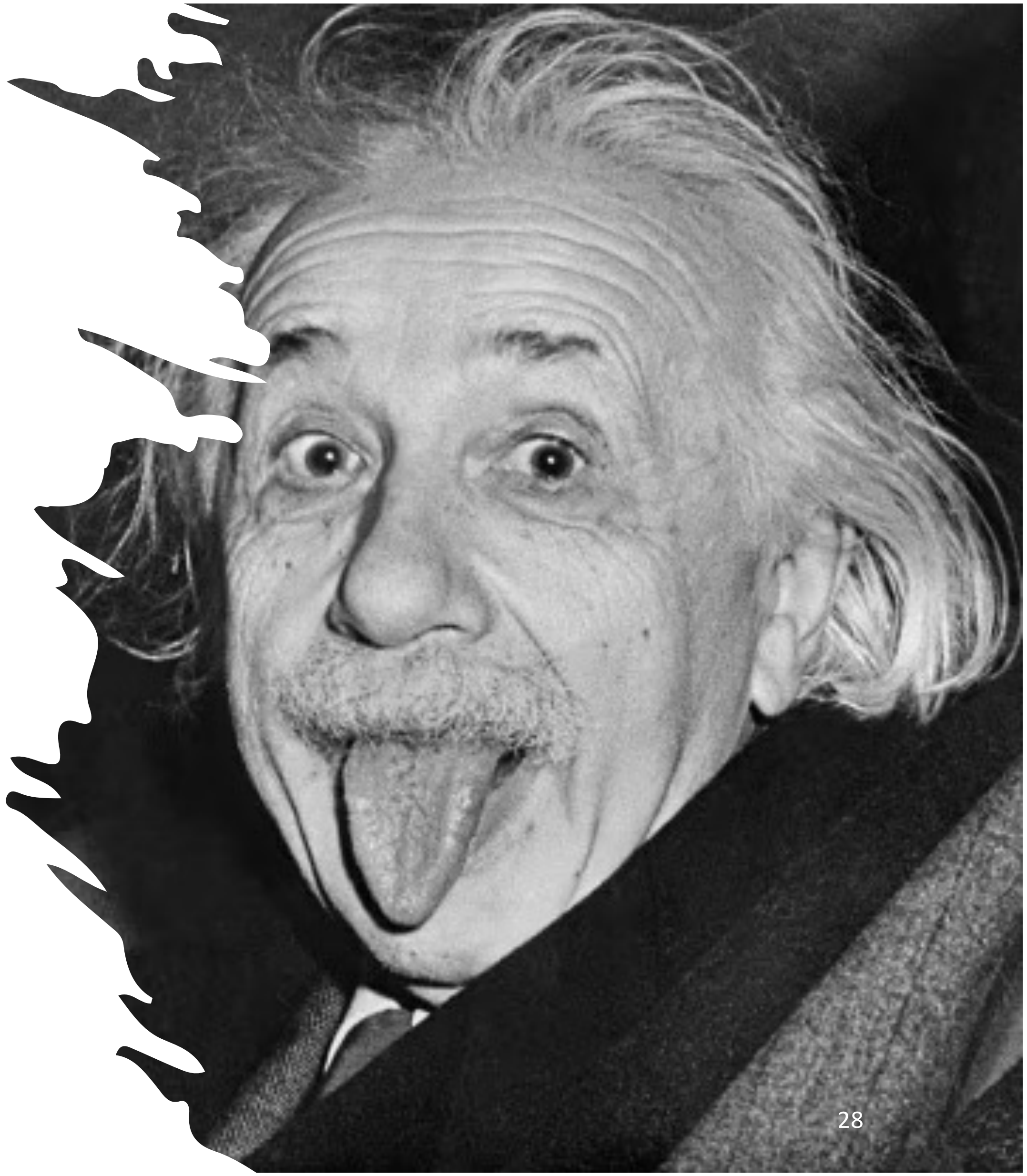
- **where** **h = 6.6262 x 10^{-34} J•s**
  ν = frequency (Hz)

Max Planck

- Planck did not offer a physical basis for his proposal; it was largely a mathematical construct needed to match the calculated blackbody spectrum to the observed spectrum.
- In 1905 Albert Einstein gave a ground-breaking physical interpretation to Planck's mathematics when he proposed that electromagnetic radiation itself is granular, consisting of quanta, each with an energy $h\nu$.

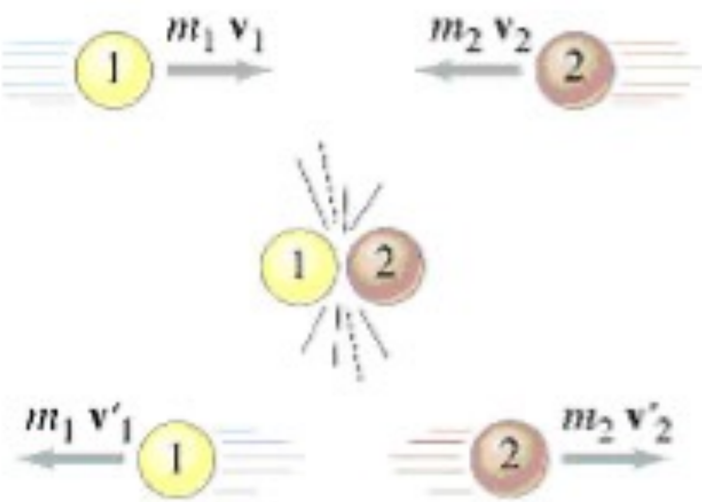
Albert Einstein

- Albert Einstein was a German-born theoretical physicist who is widely held to be one of the greatest and most influential scientists of all time.
- Best known for developing the theory of relativity, Einstein also made important contributions to quantum mechanics, and was thus a central figure in the revolutionary reshaping of the scientific understanding of nature that modern physics accomplished in the first decades of the twentieth century.
- His mass–energy equivalence formula $E = mc^2$, which arises from relativity theory, has been called "the world's most famous equation".
- He received the 1921 Nobel Prize in Physics "for his services to theoretical physics, and especially for his discovery of the law of the photoelectric effect", a pivotal step in the development of quantum theory.




VICTORIA
HER MAJESTY'S THEATRE
CUV 325C

Classical physics



$$\vec{p}_{prima} = \vec{p}_{dopo}$$

$$m_1\vec{v}_1 + m_2\vec{v}_2 = m_1\vec{v}_1{}' + m_2\vec{v}_2{}'$$

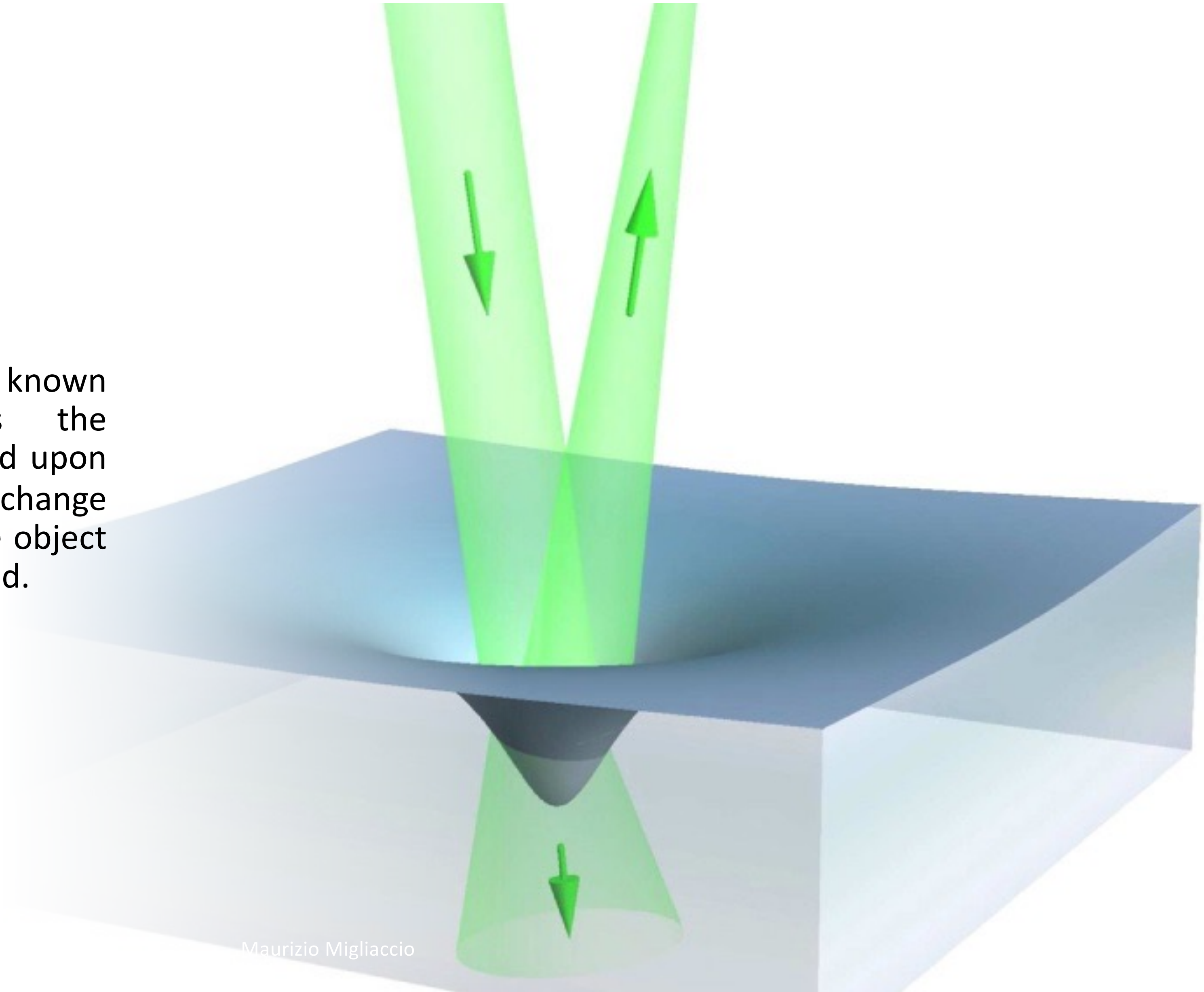
Momentum Conservation Law

- The law of momentum conservation can be stated as follows: For a collision occurring between object 1 and object 2 in an isolated system, the total momentum of the two objects before the collision is equal to the total momentum of the two objects after the collision.
- That is, the momentum lost by object 1 is equal to the momentum gained by object 2.

Move on

Light pressure

Radiation pressure (also known as light pressure) is the mechanical pressure exerted upon any surface due to the exchange of momentum between the object and the electromagnetic field.



Light pressure

- Johannes Kepler put forward the concept of radiation pressure in 1619 to explain the observation that a tail of a comet always points away from the Sun.
- Johannes Kepler (27.12.1571 – 15.11.1630) was a German astronomer, mathematician, astrologer, natural philosopher, etc.
- He is a key figure in the 17th-century Scientific Revolution, best known for his laws of planetary motion, and his books *Astronomia nova*, *Harmonice Mundi*, and *Epitome Astronomiae Copernicanae*.

Light pressure

- The assertion that light, as electromagnetic radiation, has the property of momentum and thus exerts a pressure upon any surface that is exposed to it was published by James Clerk Maxwell in 1862, and proven experimentally by Russian physicist Pyotr Lebedev in 1900.
- Pyotr Nikolaevich Lebedev- Пётр Никола́евич Ле́бедев – (24.2.1866 – 1.3.1912) was a Russian physicist. Lebedev was the creator of first scientific school in Russia.

Light pressure

- The pressure is very small, but can be detected by allowing the radiation to fall upon a delicately poised vane of reflective metal in a Nichols radiometer.
- A Nichols radiometer was the apparatus used by Ernest Fox Nichols and Gordon Ferrie Hull in 1901 for the measurement of radiation pressure.

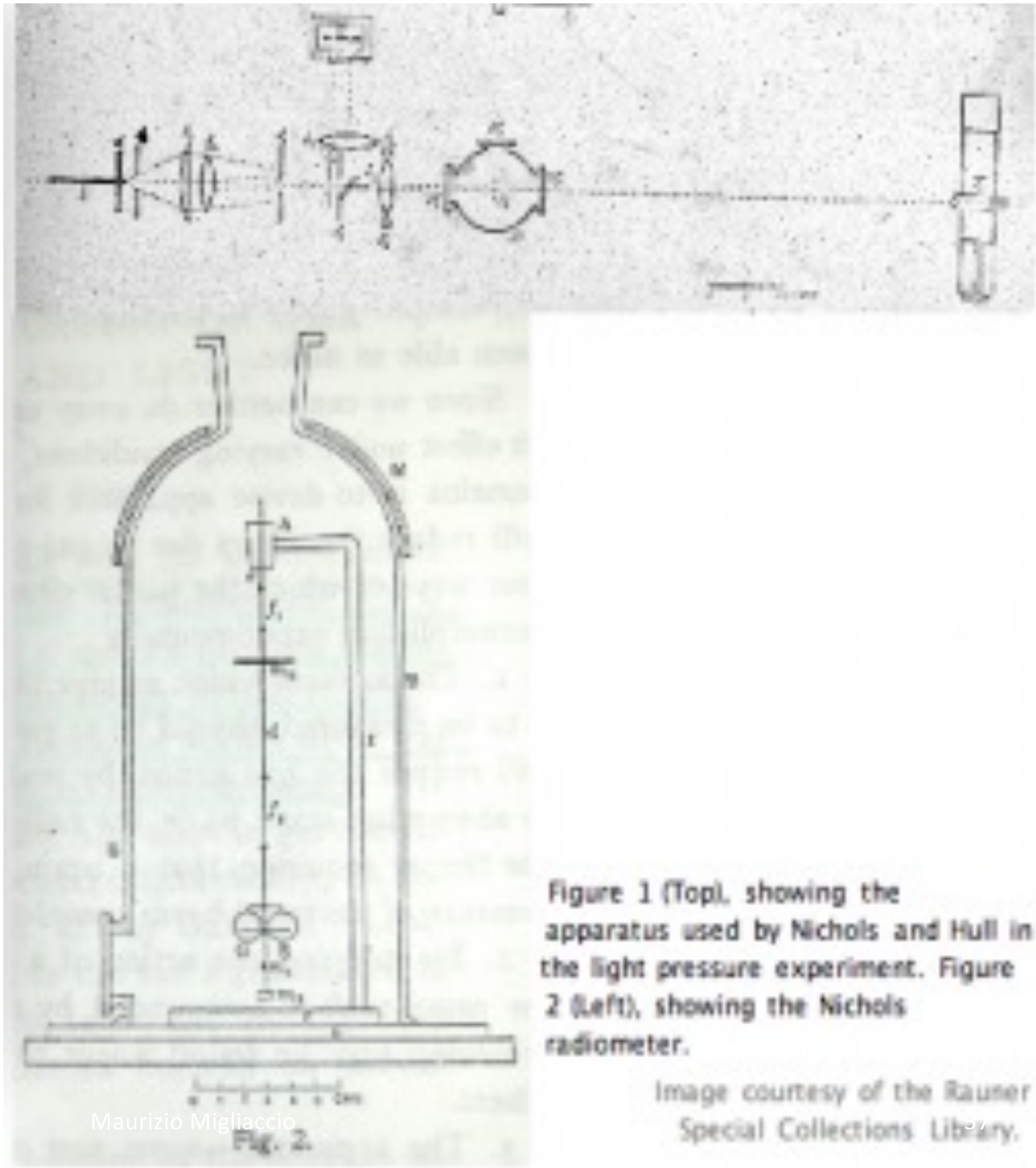


Figure 1 (Top), showing the apparatus used by Nichols and Hull in the light pressure experiment. Figure 2 (Left), showing the Nichols radiometer.

Image courtesy of the Rauner Special Collections Library.

Light pressure

Terrestrial applications of radiation pressure became feasible with the advent of lasers in the 1960s. In part because of the small diameters of their output beams and the excellent focusing properties of the beams, laser intensities are generally orders of magnitude larger than the intensities of natural light sources.

Light pressure

- Normally the momentum of a certain object is defined as the product of its mass and its velocity.
- The momentum general expression has a more complicated form given by the Einstein special relativity:

$$E^2 = (pc)^2 + (mc^2)^2$$

- This equation holds for a body or system, such as one or more particles, with total energy *E*, invariant mass *m*, and momentum of magnitude *p*; the constant *c* is the speed of light. It assumes the special relativity case of flat spacetime and that the particles are free. But a photon is massless !

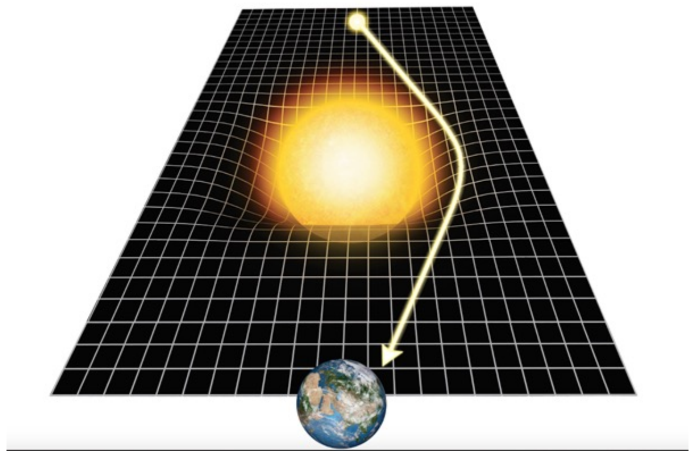
Photon

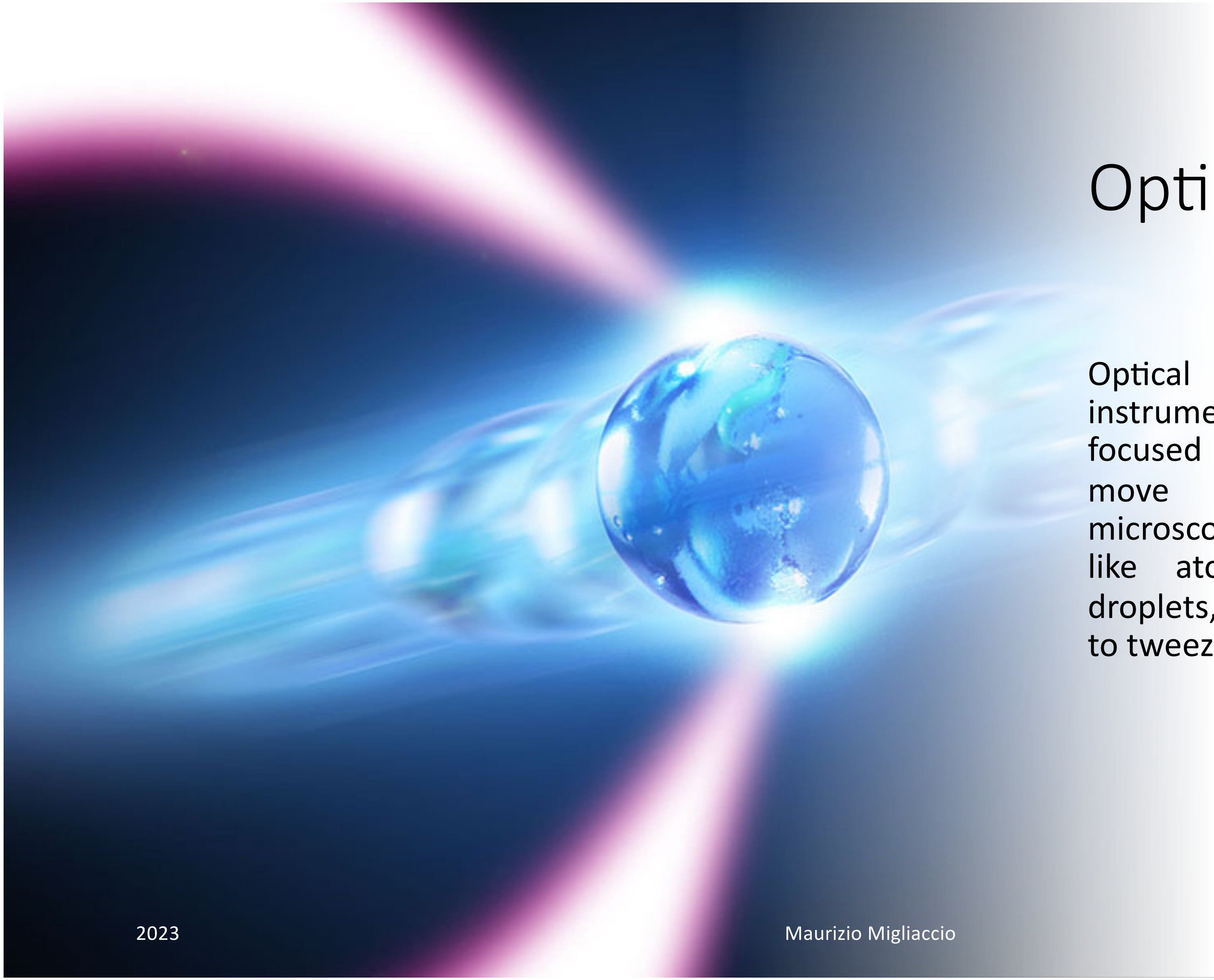
If the object is massless, as is the case for a photon, then the equation reduces to:

$$E=pc$$

It can be rewritten in other ways using the de Broglie relations:

$$E = \frac{hc}{\lambda} = \hbar c k$$





Optical tweezers

Optical tweezers are scientific instruments that use a highly focused laser beam to hold and move microscopic and sub-microscopic objects like atoms, nanoparticles and droplets, in a manner similar to tweezers.

Optical tweezers

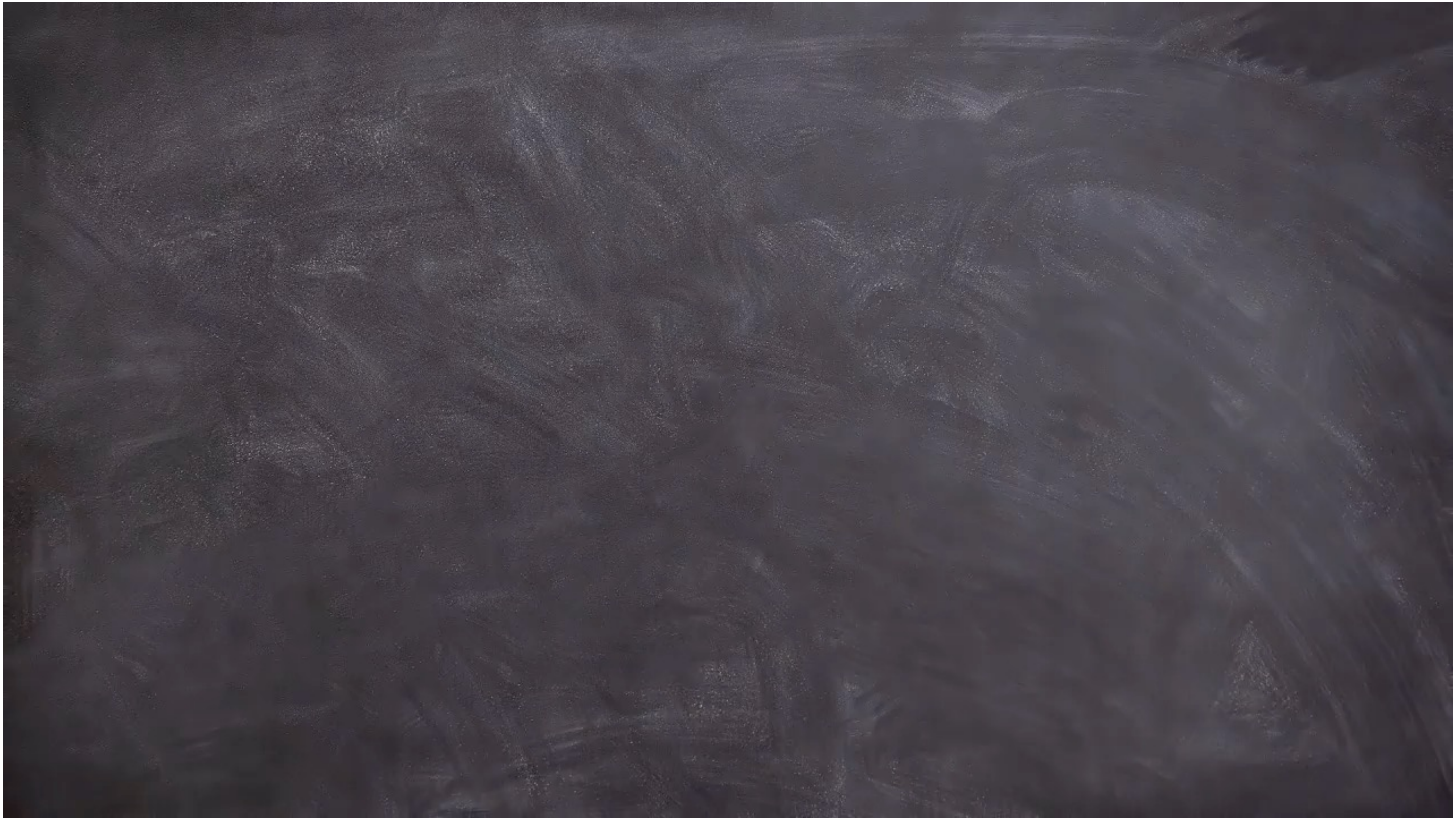
- Optical tweezers can be used to apply precise and very localized optical forces to microscopic particles.
- Using only light, Optical tweezers are able to influence the motion of objects in a non-contact way, as well as inside optically transparent cells or living organisms.
- Optical tweezers use light to trap and manipulate small particles.
- Optical tweezers are especially useful for studying neurons.

Optical tweezers

- The most common optical tweezers configuration involves using a highly focused laser beam, usually in the visible to near-infrared wavelength range (i.e., between 0.5 and 1 μm).
- At the beam focus, small particles can become trapped when the optical forces are large enough to overcome the other forces acting on them such as Brownian motion or fluid drag.
- High absorption is not desirable in biological systems as it leads to substantial heating and subsequent destruction of the system under study. The most common way to reduce absorption is to choose a laser wavelength in the near infrared (IR) region.

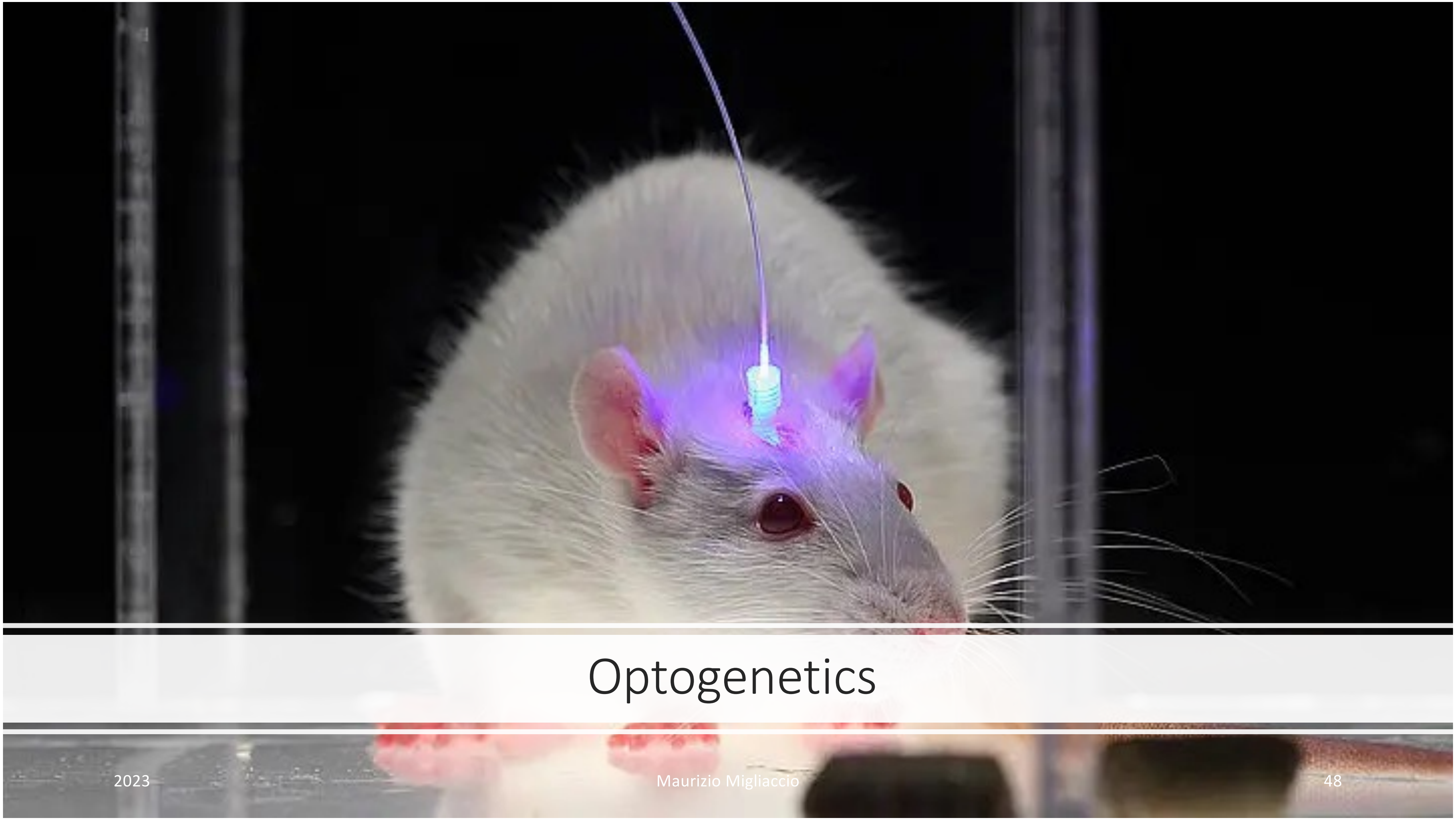
Optical tweezers

- Standard optical tweezers are able to manipulate particles in the wavelength to super-wavelength range (≥100 nm) which can often be manipulated directly using tightly focused beams.
- The diffraction limit puts a restriction on the minimum spot size achievable in conventional optical tweezers, this makes manipulating sub-wavelength sized particles (∼1–100 nm) more difficult.
- One solution is to use larger auxillary particles as handles.

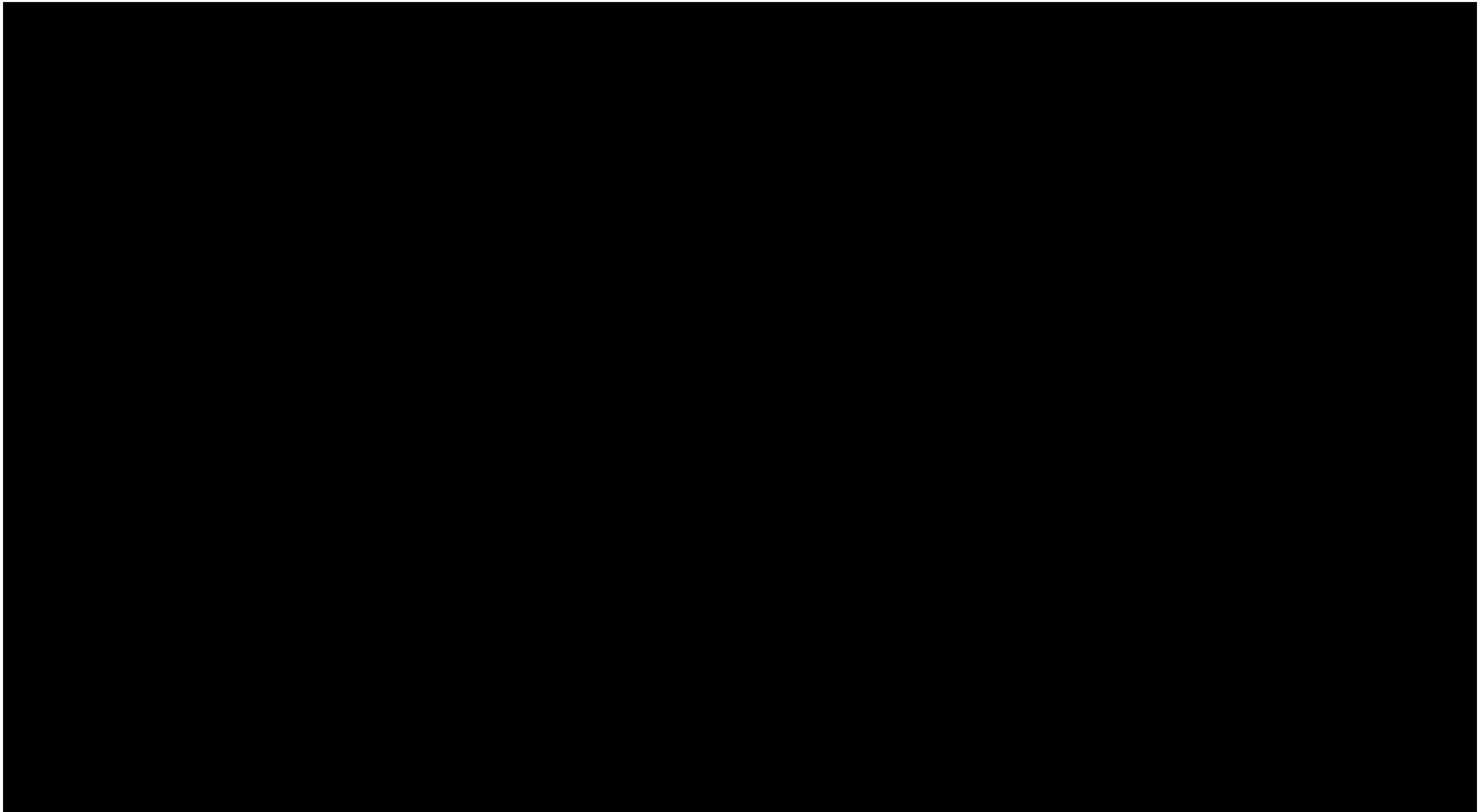


Arthur Ashkin

- Arthur Ashkin (September 2, 1922 – September 21, 2020) was an American scientist and Nobel laureate who worked at Bell Laboratories and Lucent Technologies.
- Ashkin has been considered by many as the father of optical tweezers, for which he was awarded the *Nobel Prize in Physics 2018* at age 96.



Optogenetics



First tests

- Treating anxiety no longer requires years of pills or psychotherapy. At least, not for a certain set of bioengineered mice.
- In a study recently published (2011) in the journal Nature, a team of neuroscientists turned these high-strung prey into bold explorers with the flip of a switch.
- The group, led by Dr. Karl Deisseroth, a psychiatrist and researcher at Stanford, employed an emerging technology called optogenetics to control electrical activity in a few carefully selected neurons.
- First, they engineered these neurons to be sensitive to light. Then, using implanted optical fibers, they flashed blue light on a specific neural pathway in the amygdala, a brain region involved in processing emotions.

First tests

- While such tools are very far from being used or even tested in humans, scientists say optogenetics research is exciting because it gives them extraordinary control over specific brain circuits — and with it, new insights into an array of disorders, among them anxiety and Parkinson’s disease.
- Mice are very different from humans but because “the mammalian brain has striking commonalities across species,” the findings might lead to a better understanding of the neural mechanisms of human anxiety.

Conclusions