Odorgenetics with 2-pentanone: a novel cell manipulation technique.

Odorgenetics with 2-pentanone: a novel cell manipulation technique

Zhixiao Li[#], Yanqiong Wu[#], Anne Manyande, Duozhi Wu[*], Hongbing Xiang[*]

As a means of safely and efficiently managing brain functional diseases, neuromodulation technology has become one of the fastest developing fields in medicine,[1] with its type and scope of application constantly changing, and many patients with brain functional disorders worldwide have benefited from it. Despite the emergence of new technologies, the neuromodulation technique that can achieve high spatiotemporal precision and is easy to use clinically is still lacking. Therefore, developing fast, controllable, and easy-to-use innovative neuromodulation technology is highly important for both basic research in the brain neuroscience field and the clinical treatment of functional brain diseases.

Recently, Professor Ke first proposed a novel cell manipulation technique—odorgenetics.[2] This technology demonstrated a new paradigm of an odor molecule–receptor system that specifically expresses *Drosophila* odor receptor 35a[3,4] and the coreceptor odor receptor 83b dimer complex (DOR) of fruit flies on target cells. The odor molecule 2-pentanone can specifically bind to DORs expressed on the cell membrane and increase intracellular calcium levels, thereby regulating calcium-dependent and voltage-dependent cellular physiological processes such as neuronal firing, muscle contraction, and cell secretion.

Since 2-pentanone can be inhaled into the bloodstream and cerebrospinal fluid, it only takes 3–5 minutes to interact with DORs to regulate the activity of neurons or cells. The operation is very simple and provides great convenience for the clinical translation of odorgenetics. Moreover, 2-pentanone can be rapidly expelled from the body through exhalation, thereby quickly terminating the reaction, which also ensures the safety and controllability of odorgenetics in clinical use.

This technology, which first proposed the concept of "odorgenetics" (**Figure 1**), is also a cell manipulation technique with great potential for application in multiple clinical fields.

Development of the cell manipulation technique: As one of the earliest neuromodulation techniques, electrical stimulation can directly activate or inhibit specific brain regions. In clinical practice, electrical stimulation has been used for the treatment of diseases such as Parkinson's disease, major depressive disorder, and intractable pain and is currently the most commonly used neuromodulation method in clinical practice. Despite having enormous clinical potential, electrical stimulation still has several serious disadvantages. First, prolonged conduction of a high current can cause thermal damage to the tissue surrounding the electrode, leading to neuronal apoptosis. Second, electrical stimulation is a nonspecific neural regulatory tool that affects all types of neurons near the electrode. Finally, the use of electrical stimulation is relatively complex, as it requires specialized equipment, embedding electrodes via some methods, and repeatedly adjusting the stimulation parameters.

Accurately modulating neuronal activity has always been the dream of neurologists, and the emergence of optogenetics has brought this dream into reality. Optogenetics can control neuronal activity on a millisecond time scale,[5-7] and with this technology, scientists have made a qualitative improvement in their understanding of complex neural network connections. However, this method requires light to directly enter brain tissue. Due to the difficulty of blue light penetrating the entire tissue, expensive specialized equipment, such as customized blue light sources with fiber optics or two-photon illumination systems, must be used to deliver light. In addition, considering the need for invasive device implantation and sufficient power (energy consumption), it is difficult to apply ptogenetics to disease treatment in clinical practice.

Chemical genetics is another commonly used neuromodulation technique that has advantages such as a long duration of action and simple operation and is therefore widely used in basic research.[8] However, the real regulatory effect of chemogenetic designer receptors exclusively activated by designer drugs (DREADDs) is exerted by clozapine, a metabolite of clozapine-N-oxide.[9,10] As a broad-spectrum antipsychotic drug, clozapine has numerous targets and complex pharmacological properties in the body, which greatly reduces the specificity of DREADD.[11] In addition, the irreversible slow metabolic process of clozapine-N-oxide in the body limits its potential application in the emergency control of diseases such as epilepsy. Therefore, the clinical application of DREADD is also limited.

In addition, other neuromodulation techniques include local pharmacology, magnetic stimulation, and focused ultrasound.[12] However, due to the inherent limitations of these techniques, there are many barriers to their promotion. Therefore, the development of simple and specific controllable "cell manipulation technology" is important for disease treatment.

How was odorgenetics discovered? What can be done? Optogenetics has extremely high temporal–spatial resolution, and chemical genetics is simple and convenient to perform. However, is it possible to develop a neuromodulation technology that combines the advantages of both methods? Inspired by the commonly used gas anesthesia in clinical practice, Professor Ke's team of odorgenetics sought to develop a technology to manipulate neural activity through gas. In this way, precise and efficient regulation of neuronal activity could be achieved through gas inhalation and exhalation, and clinical applications would be extremely convenient. Based on this idea, the research team immediately began their investigation.

To be successful, the first step in the research process was to look for suitable odor molecules as mediators for triggering cellular processes or neuronal reactions. The odor molecules first need to be able to quickly enter the bloodstream through respiration and cross the blood–brain barrier to ensure efficient access to the targeted brain area. Second, odor molecules should not contribute substantially to metabolic processes in the body to minimize off-target effects as much as possible. Finally, odor molecules must be quickly eliminated from the body to ensure safe and controllable clinical application.

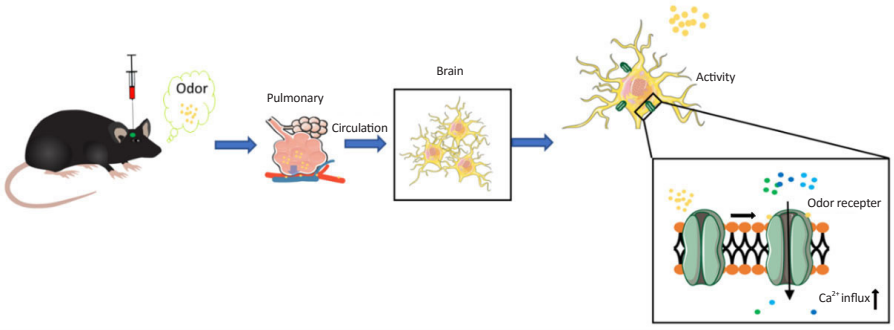

Figure 1 | **Pattern diagram of the odorgenetics.**
The odor molecule 2-pentanone is inhaled into the bloodstream and cerebrospinal fluid, where it can specifically bind to DORs expressed on the cell membrane and increase intracellular calcium levels, thereby regulating calcium-dependent and voltage-dependent cellular physiological processes such as neuronal firing, muscle contraction, and cell secretion. The figure was made with Adobe Photoshop 2024. DOR: *Drosophila* odor receptor 35a and coreceptor odor receptor 83b dimer complex.

After extensive screening, Professor Ke's team identified an odor molecule that met the above requirements: 2-pentanone. Through liquid chromatography–mass spectrometry analysis, it was confirmed that 2-pentanone can rapidly enter the blood and cerebrospinal fluid of mice through inhalation, and its effect can be exerted within 3–5 minutes. When inhalation is stopped, 2-pentanone can be exhaled in its original form within a few minutes. As a "cell manipulator," 2-pentanone can quickly exert its effects by simple inhaled administration during operation and application; it can also be terminated by controlling exhalation at any time. Thus, these findings lay the foundation for subsequent clinical use, which is simple, efficient, safe, and controllable.

After odor molecules that met the requirements were screened, the next step was to identify a matching receptor and confirm *in vitro* and *in vivo* that the odor receptor can be stably expressed in neurons and other cells and can be activated by 2-pentanone to regulate neuronal activity or cellular physiological functions (such as cell secretion).

After years of research and validation, Professor Ke's team designed and constructed DORs composed of odor receptor 35a and the coreceptor odor receptor 83b that can be stably expressed in cultured cell lines, neurons, or animals. The two receptors can assemble into hetero tetramers on the cell membrane, forming a typical ligand-gated cation channel.

Using techniques such as calcium imaging and patch clamp, the team confirmed that 2-pentanone can specifically bind to the designed DORs expressed on the cell membrane and increase intracellular calcium levels, thereby regulating calcium-dependent and voltage-dependent cellular physiological processes. This finding also indicates that odorgenetics can be applied in a wide range of basic research and disease treatment fields in the future.

In the functional validation experiment, DORs were selectively expressed in targeted organs, and Professor Ke's team reversibly manipulated mouse behavior and various physiological activities through the inhalation of 2-pentanone. Once GABAergic neurons in the central nucleus of the amygdala selectively express DORs, inhaling 2-pentanone can manipulate predatory biting behavior. If DORs are expressed in the pancreas, inhaling 2-pentanone can manipulate insulin release and lower blood sugar. In the case of skeletal muscle expressing DORs, inhaling 2-pentanone can induce skeletal muscle contraction. These results further confirm the effectiveness and wide range of odorgenetic applications of these materials.

There are several limitations in current odorgenetics. (1) Equipment for gas inhalation needs to be developed. Only by improving the relevant equipment and quantifying the concentration and capacity of inhaled gases can precise research be conducted. (2) To increase the infection efficiency of relevant cells/neurons, viral vectors need to be further optimized to better manipulate their cellular functions. (3) 2-Pentanone combines with DORs to induce calcium influx and exert excitatory effects. It is necessary to search for other odor ligands that exert inhibitory effects to better conduct neuroscience research and provide new avenues for future human disease treatment.

Conclusion: Odorgenetic technology is a new cellular manipulation technique that involves both optogenetics and chemogenetics. This technology combines the on/off properties and spatiotemporal resolution of optogenetics with the simplicity and ease of use of chemogenetics. In practical applications, odorgenetics is a safe, simple, specific and efficient "remote control system" that allows us to "freely" and reversibly regulate the physiological functions of various cells within minutes. It is a new cellular manipulation technique with great potential for clinical application.

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