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Commentary

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Seeing how we smell

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PET allows noninvasive imaging of a variety of events in the body, including the activity of neuronal circuits in the brain that are involved in cognition and behaviors, by using radiotracers that detect relevant biological reactions. A major impediment to expanding PET applications to study the brain has been the lack of radiotracers that can identify and measure specific types of neurons or glial cells. In this issue of the *JCI*, Van de Bittner and colleagues describe a promising step toward solving this problem by identifying and describing a radiotracer, [^{11}C]GV1-57, that appears to specifically label olfactory sensory neurons (OSNs), which are essential for olfaction (Figure 1). This tracer, if its specificity is confirmed, has the potential to become a prototype for future radiotracers that can identify other neuronal cell types and would allow visualization and in-depth characterization of these neurons and their genesis.

In vivo imaging with PET

The ultimate goal of medicine is to understand the human body well enough so that diagnosing and repairing a problem in patients becomes as simple as fixing a piece of complex equipment. A required step in this direction is the ability to visualize how the elements of our body function together. In the case of the brain, these elements are the interconnected neurons, the glial cells with which neurons interact, and the cerebrovascular system. One of the imaging technologies that allows us to noninvasively explore these functional components in vivo is PET.

To visualize the brain or other organs by PET, the patient or an animal is injected with a molecule, called a radiotracer, which contains a positron-emitting isotope, usually ^{15}O, ^{13}N, ^{11}C, or ^{18}F, that the PET camera can detect. The properties of a particular tracer determine the physiological, neurochemical, or pharmacological processes that are able to be captured. For example, 2-^{18}F-fluoro-2-deoxy-D-glucose (FDG), an analog of glucose, accumulates as a function of the cell's metabolic rate, thus enabling PET to visualize distinct processes such as metastasis and brown fat deposition in the body, both of which have very high metabolic rates. FDG also allows visualization of neuronal circuits in the brain, as metabolism is increased when these circuits are activated (1). Similarly, the concentrations of particular enzymes, receptors, transporters, or neurotransmitters can be measured if a suitable PET radiotracer is available.

PET imaging has provided a wealth of information on both the normal physiology of the brain, including cerebral metabolism, cerebral blood flow, blood-brain barrier permeability, synaptic and intracellular signaling, and neuroplasticity, and on pathological processes, such as amyloid (2) and tau (3) accumulation and gliosis (4), among others. Pharmacological studies have also used PET to measure drug target engagement in the brain and other organs, and drugs themselves can be labeled, thereby allowing in vivo characterization of drug pharmacokinetics and biodistribution. A major impediment to expanding PET applications to study the brain has been the lack of radiotracers that can identify and measure specific types of neurons or glial cells.

In this issue, Van de Bittner et al. describe a promising step toward solving this problem (5). Specifically, the authors discovered and characterized a new PET radiotracer, [^{11}C]GV1-57, which, they conclude, labels the mature olfactory sensory neurons (OSNs) of the olfactory epithelium, the tissue involved in recognizing smells (Figure 1). The olfactory epithelium consists of three cell types: OSNs, sensory-supporting cells, and germ cells (or undifferentiated basal cells) (6). The OSNs are true neurons (Golgi type I) that have the unique property of being continuously renewed by neurogenesis, a process involving the cycle of birth, maturation, and death (7). As a consequence of continuous neurogenesis, the olfactory epithelium contains OSNs at different stages of maturation, while the number of live OSNs at any given time is a result of the balance between the rates of their generation and death (7).

By studying the structure-function relationship of a class of radiotracers, Van de Bittner and colleagues determined that [^{11}C]GV1-57 is specifically taken up by the olfactory epithelium of rodents and nonhuman primates. The authors then identified OSNs as the cells targeted by [^{11}C]GV1-57 by surgically removing the olfactory bulbs in rats, a procedure that causes complete degeneration of mature OSNs but does not affect the other two cell types of the olfactory epithelium (8). [^{11}C]GV1-57 binding correlated with the number of mature OSNs (quantified via the OSN-specific olfactory marker protein) after surgery, indicating that the tracer targets mature OSNs. Other cell types, such as immature OSNs and sustentacular cells, were not associated with changes in [^{11}C]GV1-57 binding after bulbectomy. However, the surgery only partially decreased [^{11}C]GV1-57 binding, and future studies will therefore need to establish whether the remaining binding

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Figure 1. In this issue, Van de Bittner and colleagues describe a PET radiotracer that has the potential to allow visualization of OSN formation and turnover.

is nonspecific or due to a particular cell type other than mature OSNs.

There are two main implications of the discovery of this new radiotracer, should its specificity to mature OSNs be confirmed. First, it would provide proof of principle that a specific neuronal type can be visualized in the body. Second, this molecule would provide a tool for studying neurogenesis in the brain in animals and, potentially, in humans.

Detecting specific neuronal types

To our knowledge, [^{11}C]GV1-57 is the first PET radiotracer that can identify and quantify a specific neuronal subtype, irrespective of the neuron's functional state. Previously developed PET tracers have been considered to be "blind" to the cell type, because they bind receptors, transporters, or enzymes, the concentration and functions of which are associated with the activity of more than one type of neuron. For example, radiotracers for dopamine transporters were initially developed as biomarkers of dopamine neurons (9, 10), but subsequent studies revealed that the expression of these transporters in the membrane is dynamic and varies as a function of the extracellular dopamine levels (upregulating when dopamine levels are high and downregulating when levels are low) (11). Dopamine transporter expression also depends on circadian rhythms (12). Similarly, up- and downregulation of serotonin and norepinephrine transporters present in the membrane preclude their use as markers of noradrenergic and serotonergic neuronal populations. L-3,4-dihydroxyphenylalanine (L-DOPA), which labels tyrosine hydroxylase (an enzyme involved in dopamine synthesis), was proposed as a marker of dopamine neurons, but its concentration also varies depending on the neurotransmitter demand (13). Various other ligands, such as those that bind to dopamine D2 and D3 receptors, which can be labeled by[^{11}C]-raclopride and [^{18}F]-fallypride; μ opiate receptors, which are identified by [^{11}C]carfentanil (14); or monamine oxidase A, which is targeted by [^{11}C]-clorgyline (15, 16), are all expressed in multiple neuronal types and some also in glial cells.

The discovery that a radiotracer can target a specific type of neuron is particularly remarkable, because it provides a proof of principle that cell types in the brain can be noninvasively identified. This proof implies the possibility that tracers for other neuronal types can also be developed. Time will tell whether these tracers will be discovered by the approach used by Van de Bittner and colleagues or otherwise, but in any case, a pioneering finding like theirs often opens a floodgate of further discoveries and their practical applications. The resulting noninvasive imaging approaches would be very timely for the BRAIN (Brain Research through Advancing Innovative Neurotechnologies) initiative, which is aimed at "identifying the diversity of brain cells" (17), a goal that would be difficult to reach without new tools for classifying,

characterizing, and manipulating specific cell types in animal models and in humans.

Tracers like [^{11}C]GV1-57 and screening approaches to find them would be of great benefit, as the taxonomy of many neuronal types have yet to be defined, and current classifications based on anatomy, physiology, and even genetic expression patterns may still prove to be insufficient to classify the 1×10^{11} neurons in the human brain, of which 145 types have been recognized so far (18). How incomplete this list might be is suggested by the finding that the mere 302 neurons of the nematode *Caenorhabditis elegans* fall into 118 classes (19).

Visualizing neurogenesis

Because OSNs undergo neurogenesis, a poorly understood process by which new neurons are generated throughout life (7), [^{11}C]GV1-57 may serve as a prototype tool to study how new neurons are generated in the brain. Van de Bittner et al. tested this possibility by injuring the olfactory epithelium with zinc sulfate irrigation in mice and then used [^{11}C]GV1-57 to visualize OSN death two to three days after injury and OSN repopulation over the subsequent two months. These experiments showed that the net gain of mature OSNs after injury might reflect neurogenesis, neuron maturation, and/or changes in neuron death, as all of these processes can ultimately influence the total mature OSN population. These observations open the possibility of testing whether [^{11}C]GV1-57 or its successors can be used for monitoring and measuring neurogenesis in the human brain. Because the loss of smell is associated with or precedes several neurodegenerative diseases (20), radiotracers like [^{11}C]GV1-57 may be used diagnostically as early indicators of Alzheimer's disease and, consequently, for testing therapeutic interventions aimed at promoting neurogenesis.

Summary

[^{11}C]GV1-57 is the first reported PET radiotracer that can detect a specific neuronal type (Golgi type I); thus, the identification of this radiotracer provides hope that future targets for identifying cell diversity in the living brain can be found. As [^{11}C]CV1-57 happens to detect neurons continuously produced by neurogenesis in the brain, this tracer and, likely, its successors have the potential to serve as biomarkers for trauma, toxicity, and neurodegeneration, as well as reporters for therapeutic development.

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