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PET reporter systems for the brain

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Abstract

Positron emission tomography (PET) can be used as a non-invasive method to longitudinally monitor and quantify the expression of proteins in the brain *in vivo*. It can be used to monitor changes in biomarkers of mental health disorders, and to assess therapeutic interventions, such as stem cell and molecular genetic therapies. The utility of PET monitoring depends on the availability of a radiotracer with good central nervous system (CNS) penetration and high selectivity for the target protein. This review evaluates existing methods for the visualization of reporter proteins and/or protein function using PET imaging, focusing on engineered systems, and discusses possible approaches for future success in the development of high-sensitivity and high-specificity PET reporter systems for the brain.

Keywords

PET tracer; PET imaging; reporter probes; central nervous system; gene therapies; stem cell therapies

The value of PET reporter systems to basic and clinical neuroscience

PET is a noninvasive imaging technique used to visualize physiological processes that occur at the molecular level in living subjects [1]. Among many applications, PET reporter systems (see Glossary) have demonstrated utility in the monitoring of disease progression and response to treatment in the periphery [2]. PET reporter systems allow quantitative

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assessment of transduced genes and cells *in vivo*, with a sensitivity of approximately 10^{-12} M, making it possible to monitor the expression of therapeutic genes or survival and trafficking of cells for gene therapy [3]. The ability to visualize stem cell/transgene expression without the need for invasive biopsies makes PET imaging well suited to the rapidly expanding field of neurotherapeutic interventions. A brain PET reporter gene may not confer a therapeutic effect directly, but it can be expressed alongside a therapeutic gene, thus providing an indirect measure of therapeutic gene expression [4]. Although there is a growing need for imaging and monitoring of the expression of reporter genes in the brain, there is currently no single PET reporter system that is suitable for the visualization of therapeutic transgene expression in the brain. This review summarizes the qualities of the PET reporter systems most commonly used in research and therapeutic contexts, comments on their suitability for PET imaging of the brain and provides considerations for the development of the next generation of brain PET reporter systems.

PET reporter systems

The main PET reporter systems currently available are listed in Table-1 and are discussed in more detail in this review. The characteristic features of a good PET reporter system for the mammalian brain include the following: the reporter gene should be small enough to be packaged in a viral vector and should not significantly disturb cell biology; the reporter probe should have high brain accessibility through passive diffusion across the blood-brain barrier without efflux transporter liability, low background binding (to maximize signal-to-noise), and absence of radiometabolites in brain (to maximize the accuracy of quantitative measures) [5, 6]. Usually, radiotracers are bioactive molecules labeled with a short-lived positron emitting radionuclide, carbon-11 ($t_{1/2} = 20.4$ min) or fluorine-18 ($t_{1/2} = 109.8$ min). Carbon-11 can be inserted into a wide array of bioactive compounds by with several accessible C-11 labeled synthons. Fluorine-18 is also a widely used radionuclide because of its longer half-life providing an option to transport the F-18 labeled radiotracers to nearby non-cyclotron centered medical or research facilities.

PET reporter systems: applications and design—Molecular genetic tools have revolutionized basic systems neuroscience research and have tremendous potential for applications in medical diagnosis and the treatment of human diseases. For example, several advances have been made in gene therapy in the past two decades; genetically engineered stem cells have been used to deliver proteins to neighboring cells, kill cancer cells, and reduce graft-host rejection [7]. PET reporter genes can be engineered into stem cells to allow *in vivo* tracking of cell delivery and migration in both the periphery and in the brain [8]. PET reporter probes can target the therapeutic gene of interest, a co-expressed reporter gene, or an endogenous protein that the therapeutic intervention is designed to modulate. In pre-clinical studies, it is common for a therapeutic gene and reporter gene to be cloned with a regulatory sequence, such as a promoter or enhancer, that targets expression to the cell type of interest [9]. A radiolabeled reporter probe can bind to the reporter protein with high affinity, and hence provide a quantitative measure of protein expression in cells of interest using PET imaging. The imaging of reporter gene expression has been used in several biological applications, including the monitoring of disease progression such as cancer and degenerative neurological conditions, monitoring of gene therapy, and tracking cell

migration [10]. PET reporter probes interact with gene products to image their expression via one of three different mechanisms (Fig. 1B):

- i. Intracellular entrapment of radiotracers after phosphorylation by enzymes encoded by reporter genes [11]. Enzyme-based reporter gene systems include herpes simplex virus 1 thymidine kinase (HSV1-tk) and its mutant forms such as human γ -mitochondrial thymidine kinase type 2 (h γ tk2), as well as a mutant of human deoxycytidine kinase (hdCK).
- ii. Radiotracers bind protein receptors encoded by reporter genes [12]. Many receptor-based reporter genes systems are available, including human somatostatin receptor type 2 (hSSTR2), dopamine D2 receptor (D2R), and optogenetic-based and chemogenetic-based reporters.
- iii. Accumulation of radiotracers into cells mediated by membrane transporters encoded by reporter genes [13]. Transporter-based reporter genes include the human sodium–iodide symporter (hNIS), and human norepinephrine transporter (hNET).

A reporter probe is associated with each reporter gene. The probe will concentrate at the site of reporter gene expression and/or function. Thus, the measured radioactivity concentration of the reporter probe in the region of interest reflects the level of reporter gene expression/function. Expression level can be influenced by several factors including level of transcription, regulation of translation, and post-translational regulation of gene product. Thus, PET monitoring can provide valuable information regarding the level and stability of protein expression.

PET radiotracers do not generally alter biological processes because they are injected at tracer mass doses. Some PET radiotracers are substrates for efflux transporters at the Blood-Brain-Barrier (BBB), therefore systemic injections do not result in good brain uptake. A direct intrathecal infusion can overcome this issue; however, this approach produces additional risks and technical challenges [14]. PET is widely used in medical diagnosis because it can provide quantitative measurements of receptor density, target engagement, occupancy, volume of distribution, and binding potential using different models such as the ‘2-tissue compartmental model’ or ‘reference tissue model’ [15]. Several PET radioligands and their potential applications for molecular brain imaging have been discussed in recent literature [12, 16].

Intracellular entrapment PET reporter systems

In these systems, the reporter probe concentration reflects both gene expression level and function i.e., enzyme activity. There are several enzyme-based PET reporter systems, including the ones discussed next.

Herpes Simplex Virus – thymidine kinase (HSV1-tk)—The HSV-1-TK reporter system [11] has been used extensively in the periphery over the past 20 years to investigate protein-protein interactions, assess the temporal dynamics of different promoters, monitor embryonic stem (ES) cells after transplantation, assess anticancer therapy paradigms, and

to assess gene and cell therapies. HSV1-tk causes phosphorylation of specific pyrimidine-based radiotracers, a mechanism like that of [^{18}F]FDG trapping inside the cell after phosphorylation [17]. The resulting phosphorylated compounds do not cross the cell membrane readily, and hence accumulate within enzyme-expressing cells. The accumulated radioactivity within the cell reflects the level of HSV1-tk enzyme activity [18]. The radiotracers initially developed to undergo phosphorylation and entrapment by this enzyme reporter gene system include [^{14}C]FIAU, [^{124}I]FIAU, [^{131}I]FIAU [8- ^3H]/[8- ^{14}C]ganciclovir, and [8- ^{18}F]fluoroganciclovir [19]. A mutant HSV1-tk reporter gene, named HSV1-sr39tk, was developed for applications in animal models and human gene therapy [20]. The mutant enzyme, combined with new radiolabeled bicyclic nucleoside analogues (BCNAs) such as 8- ^{18}F]fluoropenciclovir (FPCV) and 9-(4- ^{18}F]fluoro-3-(hydroxymethyl)butyl]guanine) ([^{18}F]FHBG), significantly improved sensitivity for imaging of reporter gene expression *in vivo* [21]. However, the low lipophilicity of the PET radiotracers for this system results in poor permeability across the blood brain barrier.

Other enzyme-based PET reporter systems—Several other enzyme-based reporter genes have been developed, including human mitochondrial thymidine kinase-2 (h tk2) [22] and its associated mutants such as hTK2-N93D/ L109F [23], human deoxycytidine kinase (hdCK) [24], and a human D-mitochondrial thymidine kinase type 2 (hDTK2) [25], using specific pyrimidine-based radiotracers [26]. The varicella zoster virus-thymidine kinase (VZV-tk) gene and its nucleoside reporter probes are an alternative neuro-PET reporter system with radiolabeled bicyclic nucleoside analogues as reporter probes. VZV-tk expression in xenografts produced up to 60% higher uptake than HSV1-tk *in vivo* [27]. However, these alternative enzyme-based PET reporter systems also use BCNA probes, thus the issue of poor brain accessibility is likely to limit their utility for PET imaging in the CNS.

Protein binding-based PET reporter genes

Somatostatin receptor (SSTR)—The somatostatin receptors (SSTR) belong to the G-protein coupled receptor family and cause inhibitory effects on cell proliferation as well as release of active hormones. Initially, several reporter probes were developed for SPECT imaging of hSSTR2 such as [^{111}In]diethylenetriamine pentaacetic acid–octreotide, [$^{99\text{m}}\text{Tc}$]P829, [$^{99\text{m}}\text{Tc}$]P2045, and [^{188}Re]P829, but these ligands failed to provide adequate selectivity. Subsequently, the first PET probes, [^{68}Ga]DOTATATE and [^{68}Ga]DOTATOC, have superseded the SPECT reporter systems on account of their improved specific uptake, low background activity, and rapid clearance. AAV-mediated gene transduction into muscle and liver cells has been visualized using the aforementioned PET probes [28]. In addition, these radiotracers are used clinically for imaging neuroendocrine tumors [29, 30]. However, high expression of endogenous SSTR2 in the neocortex, amygdala, claustrum, and several other regions [31] is expected to limit the sensitivity and specificity of this system in the brain.

Dopamine D2 receptor (D₂R)—In animal models, the expression of wild type and mutated dopamine type 2 receptors (D₂Rs) has been quantified with PET using the specific D₂R binding reporter probes [^{11}C]raclopride, [^{18}F]fallypride, and 3-(20- ^{18}F]fluoroethyl)-

spiperone ($[^{18}\text{F}]\text{FESP}$) [32, 33]. A major caveat in this system is the potential for activation of transgenic D_2Rs by endogenous dopamine ligands, perturbing transduced cells by changing cyclic AMP levels. To avoid such problems, the D_2R mutant gene, $\text{D}_2\text{R80A}$, was developed, with reduced affinity for the endogenous ligand, dopamine (DA). The $\text{D}_2\text{R194A}$ gene mutation further substantially reduced the effect of DA-mediated changes in cAMP levels and enhanced the affinity of $[^{18}\text{F}]\text{FESP}$ ligand binding [34]. The $\text{D}_2\text{R80A}$ receptor-based gene system employs radiotracers that can cross the BBB. However, high expression of endogenous D_2R in the striatum, substantia nigra, frontal neocortex, and several other regions [35] is likely to limit the sensitivity and specificity of this system in the brain.

Cannabinoid type 2 receptor—The human type 2 cannabinoid receptor (hCB_2) has been used as a reporter gene because of its low baseline expression in the brain. The reporter probe is a ^{11}C -labeled CB_2 ligand, $[^{11}\text{C}]\text{GW405833}$. Expression of $\text{hCB}_2(\text{D80N})$, a non-signaling form of CB_2 , permits visualization with $[^{11}\text{C}]\text{GW405833}$ PET imaging, without producing a physiological effect [36]. One concern regarding this system is that brain uptake of $[^{11}\text{C}]\text{GW405833}$ is relatively low. In rats, PET reporter imaging of hCB_2 demonstrated longitudinal reporter gene expression up to 96 days after transduction [36]. A major concern with this system is that CB_2 receptor expression is upregulated in activated microglia, as occurs in several CNS disorders associated with neuroinflammation [37], thus the utility of this system in monitoring the effect of treatments associated with such disorders is limited.

Optogenetic-chimeric receptors—Optogenetic systems allow targeted control of neuronal activity in animal models via administration of light. With recent advances in cell-type and pathway selectivity, these tools have widespread utility in preclinical animal models with potential to translate into human therapeutic applications [38, 39]. Several optical tools have been developed to activate or inhibit neuronal circuits, such as channelrhodopsin-2, halorhodopsin, and optoXRs [40, 41]. However, major challenges for use in large animals include monitoring transgenic receptor location, expression level, and function *in vivo*, noninvasively and longitudinally [12]. A PET reporter system for optogenetics allows the longitudinal visualization of opsin expression in mice and squirrel monkeys [42]. This reporter system, named ‘ChRERa’, consists of chimeric opsins tagged with a small human estrogen alpha protein epitope. The complementary reporter probe, $[^{18}\text{F}]\text{fluoro-estradiol}$ ($[^{18}\text{F}]\text{FES}$), permits longitudinal noninvasive detection of opsins in the brain. The ChRERa reporter gene system is currently undergoing evaluation in old-world NHP.

Chemogenetic receptors—Chemogenetics offers a minimally invasive approach that can be used to control neuronal activity in the mammalian brain via systemic injections of activator ligand [43]. Chemogenetic technology has been used in animals to modulate specific neuronal populations by targeting them to express an exogenous receptor that has been engineered to be activated only by a selective agonist. Because it requires no implanted device, and can manipulate large regions of the brain for long periods of time (hours~), chemogenetics holds promise for therapeutic treatment of human brain diseases [44–46], although extensive tests of safety and efficacy will be needed

prior to routine clinical use. Two classes of chemogenetic receptors have been widely adopted in the research community: Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) and Pharmacologically Selective Actuator Modules activated by Pharmacologically Selective Effector Molecules (PSAM/PSEM) [47]. The localization of muscarinic DREADDs using PET in rodent and nonhuman primate (NHP) brain was first established with [^{11}C]clozapine-*N*-oxide ([^{11}C]CNO) and [^{11}C]clozapine ([^{11}C]CLZ) [50, 51]. PET reporter imaging of DREADDs demonstrated longitudinal monitoring of neuronal grafts differentiated from induced pluripotent stem cells in mice [49] and virally expressed DREADDs in macaque monkeys [48]. Recently, a ligand with higher selectivity for DREADDs, [^{11}C]deschloroclozapine ([^{11}C]DCZ), was developed for NHP imaging [50, 51] (Fig. 2A–B). Additional PET radiotracers for DREADD visualization labeled with fluorine-18, [^{18}F]JHU37152 and [^{18}F]JHU37107, also exhibit high-affinity DREADD binding *in vivo*, and have a longer half-life, permitting use in research facilities that do not have a cyclotron on-site [52]. A follow up study demonstrated receptor quantification with [^{11}C]DCZ by establishing the radiometabolite-corrected input function, measured from concurrent blood sampling, which enabled an estimation of total volume of distribution (V_T) using a 2-tissue compartmental model [51]. The same study confirmed that reference tissue models provide a useful (albeit slightly underestimated) measure of DREADD expression.

PSAM expression has been visualized *in vivo* in rodents and monkeys via the binding of [^{18}F]ASEM, a radiolabeled $\alpha 7$ nAChR antagonist, as this ligand also binds with relatively high affinity to the PSAM⁴ - GlyR (Fig. 2C–D) [53, 54]. Competitive binding experiments with PET using [^{18}F]ASEM confirmed the brain penetrance and PSAM⁴ receptor occupancy, *in vivo*, of a new generation of activating ligands – the ultrapotent PSEMs (uPSEMs) [53].

The development of a PET radioligand for the DREADD chemogenetic receptors has facilitated the adoption of this technology in NHPs, a critical step towards clinical translation. Promising alternative chemogenetic systems, such as the PSAM/PSEM system have been designed with clinical applications in mind [55] and would likely benefit from improved probes (see Outstanding Questions).

Pyruvate kinase muscle isoenzyme 2 (hPKM₂) receptors—The human pyruvate kinase muscle isoenzyme 2 (hPKM₂) has been used as a PET reporter gene with [^{18}F]DASA-23 as a reporter probe for imaging transduced tissue in the CNS because of favorable reporter probe pharmacokinetics, including BBB permeability. The reporter gene was delivered into the brain of mice using adeno-associated virus 9 (AAV9). PET imaging studies, performed over 8 weeks to evaluate the time course of PKM₂ expression, revealed an increase in the brain uptake of [^{18}F]DASA-23 in the AAV transfected mice (Fig. 2E–F) compared to healthy controls [56]. This approach can be modified such that the functional gene is coupled with a PKM₂ reporter gene to allow indirect evaluation of functional gene expression using [^{18}F]DASA-23. The PKM₂ system has been tested in mice but does not yet have proven efficacy in larger mammals. PKM₂ plays a role in cell proliferation, which may limit the suitability of this system for clinical applications.

Bacterial dihydrofolate reductase (ecDHFR) receptors—Unlike the other enzyme-based reporter systems, the ecDHFR-based reporter system relies on the direct visualization of protein expression, rather than protein products, and can be fused with a protein of interest to permit detection via the reporter probe, [¹⁸F]FE-TMP (Fig. 2G–H). The ecDHFR/TMP reporter system enables non-invasive visualization of neuronal ensemble activity through ecDHFR expression under an activity-dependent promoter in mice and neuronal tracts in the deep brain regions of NHPs [57]. However, this system is still in its first generation and has areas that need to be improved in terms of reporter probe characteristics.

Transporter based PET reporter genes

Human sodium-iodide symporter (hNIS)—The hNIS gene encodes a transmembrane glycoprotein, belonging to the family of sodium-mediated symporters. It is widely recognized as a biomarker and therapeutic target for thyroid cancer due to its high expression in the thyroid gland. This reporter system, utilizing iodine-131 or iodine-124, has been translated into clinical gene therapy for thyroid cancer and has been used as a reporter gene in preclinical studies for monitoring transduced immune cells and pluripotent stem cells *in vivo*, imaging the transcriptional activity of several specific promoter systems, and muscular dystrophy [58, 59]. The hNIS has also been considered as a therapeutic gene in targeted radiotherapy using [¹³¹I]iodide [60]. New ¹⁸F-labeled derivatives have been developed as radiotracers for imaging hNIS, which include [¹⁸F]tetrafluoroborate, and [¹⁸F]fluorosulfate. [¹⁸F]Tetrafluoroborate has been tested in clinical trials and in thyroid cancer patients [61]. The hNIS reporter system is not well suited to brain imaging because quantification of gene expression is not possible with radiolabeled iodide because it is not metabolically trapped within transduced cells.

Human norepinephrine transporter (hNET)—The hNET gene produces a transmembrane protein that facilitates transportation of norepinephrine, epinephrine, and dopamine from the synapse to presynaptic neurons. Several PET radiotracers have been developed for hNET imaging, including [¹¹C]ephedrine, [¹²⁴I]meta-iodobenzylguanidine (MIGB), and [¹⁸F]meta-fluorobenzylguanidine (MFBG) [62]. PET imaging of hNET has been translated into the clinic for the visualization of neuroendocrine tumors and heart disease [63]. However, the blood–brain barrier permeability appears to be poor in practice due to the presence of highly polar guanidine moieties in their structures [64]. More recently developed hNET radiotracers, with better CNS penetration, have been used to demonstrate that noradrenergic projections undergo degeneration in PD - as quantified with [¹¹C]MeNER PET [65], and in NHP to assess the occupancy of norepinephrine transporters - as quantified with [¹¹C]MRB [66]. Both radiotracers have good brain penetration. However, hNET is distributed throughout the brain, with especially high levels of expression in the thalamus, midbrain, and amygdala, thus limiting the sensitivity and specificity of this system [67].

Human serotonin transporter (hSERT)—The hSERT gene encodes a transmembrane protein that regulates extracellular fluid serotonin concentrations. Several PET radiotracers have been developed for hSERT imaging, including [¹¹C]DASB, [¹¹C]McN 5652, [¹¹C]ADAM, [¹¹C]DAPA, and [¹¹C]AFM. Of these radiotracers, [¹¹C]DASB penetrates

the blood–brain barrier most quickly, and hence can be used to visualize hSERT using shorter scan times; [^{11}C]AFM has the highest signal-to-noise ratio, and hence provides the most spatially accurate report of hSERT expression. In human brain, hSERT expression is somewhat denser in medial temporal lobe structures and ventral pallidum but is generally lower throughout the brain than hDAT and hNET [68]. [^{11}C]DASB and [^{11}C]AFM display high signal-to-noise ratios in vivo, in part due to the relatively low endogenous expression levels of hSERT [69]. Thus, a non-functional hSERT mutant, combined with [^{11}C]DASB or [^{11}C]AFM might have potential for use as a PET brain reporter system.

Imaging therapeutic transgenes and stem cells in the brain

The translation of preclinical gene therapy studies into the clinic can be facilitated by the monitoring of therapeutic transgene expression in the brain using PET. For example, gene therapy has been used in the treatment of Parkinson's disease (PD) in clinical trials; the enzyme aromatic L-amino acid decarboxylase (AADC) improves the clinical symptoms of PD by synthesizing DA via the catalysis of systemically administered levodopa or carbidopa [70]. AADC transgene-based gene therapy for PD patients is currently in phase I/II clinical trials [70]. Many candidate gene therapies for PD patients are focused on increasing the expression of enzymes for the synthesis of DA such as AADC, tyrosine hydroxylase (TH), or GTP cyclohydroxylase 1 (GCH1) to increase DA bioavailability in the nigrostriatal synapses.

PET imaging of the DA transporter has been used to assess the survival and growth of ES cells genetically modified to develop into DA synthesizing neurons in a rat model of PD [71]. [^{18}F]FE-PE2I is used in the clinic to quantify presynaptic dopamine transporter (DAT) expression [72]. In a mouse model of PD, PET measurements revealed that ES cell treatment caused an increase in the expression of striatal presynaptic dopamine transporters and of postsynaptic DA D_2Rs [73] (Fig. 3A–D). PET imaging with [^{18}F]FECNT has shown partial recovery of DA transporter in the lesioned striatum after transplantation of ES cells. [^{18}F]FECNT shows good brain kinetics and has high selectivity and affinity to the DAT. It should be noted, however, that radiometabolites that enter the brain confound measurements based on reference tissue models [74]. In another PD model, PET imaging with [^{18}F]FBCTT-PET/CT revealed the restoration of the expression of pre-synaptic DAT, and [^{18}F]fallypride-PET/CT demonstrated functional DA release in the lesioned striatum after transplantation of ES cells [75]. In sum, longitudinal and noninvasive PET imaging of ES cells can be used to assess the regulation, survival, and function of DA neurons, providing valuable insights for the study of therapeutic applications of human ES cells.

PET has been used to monitor the metabolic changes that occur after gene delivery in clinical trials of gene therapy for PD [76]. One study demonstrated 30% higher uptake of L-[^{18}F]3-fluoro- α -methyl tyrosine ([^{18}F]FMT) in the putamen region after AAV-AADC gene transfer in patients with moderate to advanced PD [77]. Another study demonstrated increases in enzyme activity assessed by PET in gene therapy using [^{18}F]FDOPA [78]. An open label, phase 1 clinical trial of PD gene therapy using AAV-GAD1 (glutamate decarboxylase 1), suggested significant improvements in PD symptoms [79]. The presence

of endogenous DAT limits the utility of the corresponding reporter probes for use in a universal brain PET reporter system.

Concluding remarks and future perspectives

PET imaging has been long used for the monitoring of stem cells and transgenes in the periphery, in both basic and clinical settings. Progress in the deployment of stem cell and transgene expression in pre-clinical models has been made in parallel with the development of improved methods for PET imaging in the brain. For example, longitudinal PET imaging in rats made it possible to monitor the loss of DA receptors in a model of Parkinson's disease, and the recovery of dopaminergic function after treatment with stem cells [71]. PET imaging of a chemogenetic receptor in a nonhuman primate made it possible to determine that the absence of a behavioral effect resulted from a failed injection; a follow-up injection improved the expression and rescued the behavioral effect [44]. These examples make clear why brain PET imaging could be so valuable in clinical settings. Many new stem cell and transgene-based therapies are undergoing clinical trials for the treatment of neurological and neuropsychiatric disorders [80]. Brain PET reporter probes for monitoring reporter expression and/or inflammation in a longitudinal fashion would make it easier to evaluate both the efficacy of treatment and track potential complications associated with these novel treatments. The absence of suitable brain PET reporter probes for certain treatments hinders such monitoring. The development of a 'universal' brain PET reporter system – one that could be combined with any neurotherapeutic treatment, and would confer high sensitivity and selectivity – would facilitate the development and application of such treatments in both basic and clinical contexts. A universal PET reporter system could also expedite the translation of promising techniques in preclinical use, including optogenetics and chemogenetics.

When designing an 'ideal' neuro-PET system, one can seek to optimize the physiochemical properties of candidate radiotracers to maximize brain penetrance and select target proteins that are physiologically inert and suitably sized for packaging into viral vectors. One can also seek to design protein-radiotracer pairs with high on-target affinity and low background binding. However, the metabolic stability of new radiotracers is difficult to predict (see Outstanding Questions). Overcoming the challenges described above to produce universal brain PET reporter systems may expand the utility of PET monitoring to stem cell treatments and transgenic methods of neuromodulation, which will be of tremendous value to both researchers and clinicians.

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Declaration of interests

MM has received research funding from AstraZeneca, Redpin Therapeutics, Dompé farmaceutici, and Attune Neurosciences, and is named as an inventor on a patent describing novel DREADD ligands (WO2019/157083). TM is named as an inventor on a patent for the use of DCZ as a PET reporter probe (WO2019/245047). Other coauthors declare no conflict of interest.

Glossary

Biomarker

A substance the presence of which is tightly correlated with the progression of an underlying phenomenon, e.g. a neurological or neuropsychiatric illness.

Binding potential

The ratio of receptor density (B_{max}) to radioligand equilibrium dissociation constant (K_D).

 B_{max}

Total density (concentration) of receptors in a sample of tissue *in-vitro* or concentration of available (free) receptors *in-vivo*.

Cyclotron

An apparatus in which charged atomic and subatomic particles are accelerated by an alternating electric field while following an outward spiral or circular path in a magnetic field to generate isotopes.

Enzyme-based PET reporter system

Intracellular entrapment of radiotracers after phosphorylation by enzymes encoded by reporter genes.

 K_D

Radiotracer equilibrium dissociation constant.

PET reporter gene

Gene whose product can be directly or indirectly imaged with a PET reporter probe.

PET reporter probe

Radiotracer with selective binding to a PET reporter gene product.

PET reporter system

Combination of both PET reporter gene and PET reporter probe.

Positron emission tomography (PET)

Molecular imaging modality based on the use of chemical or biologic agents labeled with positron-emitting radionuclides.

Radiotracer/Radioligand

Short-lived positron-emitting compound used for PET imaging.

Receptor based PET reporter system

Radiotracers that bind protein receptors encoded by reporter genes.

Reference tissue model

Analysis model that allows quantification of receptor kinetics without measuring the arterial input function, thus avoiding arterial cannulation and time-consuming metabolite measurements.

Standardized uptake value (SUV)

The decay-corrected tissue concentration of a PET tracer in a specific region of interest on the PET image divided by the injected activity and then divided again by body weight—when all the tracer is distributed evenly throughout the body, the SUV would be 1 for every region.

Standardized uptake value ratio (SUVR)

A quantitative method used in PET imaging to make regional comparisons within a subject as well as between subjects and computed as the degree of radiotracer uptake in a target region of interest with respect to a reference region.

Synthon

A moiety of a chemical compound that can be formed by a known synthetic process.

Transgene

A gene that is artificially introduced into the genome of an organism.

Transporter based PET reporter system

Accumulation of radiotracers into cells mediated by membrane transporters encoded by reporter genes.

Volume of distribution (V_T)

Defined as the ratio of the radioligand concentration in tissue target region (C_T , $\text{kBq}\cdot\text{cm}^{-3}$) to that in plasma (C_P , $\text{kBq}\cdot\text{mL}^{-1}$) at equilibrium.

2-tissue compartmental model

Follows two compartments that are for the two distinct kinetic compartments in the tissue ($C_1(t)$ and $C_2(t)$), representing, for instance, the free and receptor-bound tracer concentrations.

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Text Box 1:**What is PET imaging?**

Positron emission tomography (PET) is a method that takes advantage of the selective uptake of a radioactive compound (radiotracer) to infer the distribution of a molecular target within a living subject. The radiotracer emits two 511 MeV γ -rays in opposite directions when the emitted positron from radioactive decay annihilates with a nearby electron (Fig. 1A). These γ -rays are detected by a ring of detectors in the PET camera surrounding the subject, and the timing and locations of detection can be used to reconstruct the time and origin of radionuclide decay. This provides an approximate spatial measure of where the radiotracer was distributed when it decayed. If many decay events are clustered within a given region, it can be inferred that this region is rich in target receptor expression. PET cameras typically provide relatively low spatial resolution (maximum of ~2 mm for monkeys and smaller animals using microPET, and ~4 mm for humans). Therefore, multimodal imaging, such as PET-CT and PET-MRI, are valuable because they combine the high spatial resolution of CT/MRI (as low as 1 mm) with the sensitivity of PET (10^{-12} M), which is much higher than that of MRI (10^{-4} M). Due to its high sensitivity, PET can measure very low concentration (10^{-5} - 10^{-10} g) of radiotracers at a specific region of interest. PET, therefore, remains an invaluable molecular imaging modality in biomedical and clinical practice [81, 82].

Outstanding questions

PET reporter systems for brain applications have the potential to rapidly advance several technologies for basic systems neuroscience and therapeutic applications. What is the best approach to engineering systems with high selectivity and specificity?

How can we maximize the translational potential of PET reporter systems developed in lower order mammals? Engineering proteins with known cross-species homology, and/or reporter probes with existing approval for use in humans may be a good starting point.

The bioavailability of PET reporter probes is critical to the utility of the system. How can we optimize methods to maximize blood brain barrier penetrance? Of particular value would be a theoretical grounding for better understanding probe susceptibility to efflux transporters.

Can a 'universal' brain PET reporter system be developed? Several attempts have been made to create such a system (e.g. PKM₂, ecDHFR, and the ligand binding domain of estrogen receptor α in ChRERa), but all require further optimization before they can be universally adopted.

Highlights

- Positron emission tomography (PET) is a non-invasive modality for *in vivo* assessment of pathophysiological processes in living subjects.
- PET imaging detects γ -rays produced by decay of a radioactive compound (radiotracer) to infer the location of the binding of that compound within the brain, thus revealing the location of the receptor to which it bound.
- PET reporter systems are used to monitor the progression of disease and the expression of therapeutic transgenes, for instance in the context of stem cell and molecular genetic therapies.
- Successful PET reporter systems for the brain are improving the treatment of neurological diseases, and hold promise for the advancement of novel treatments for neuropsychiatric disorders.

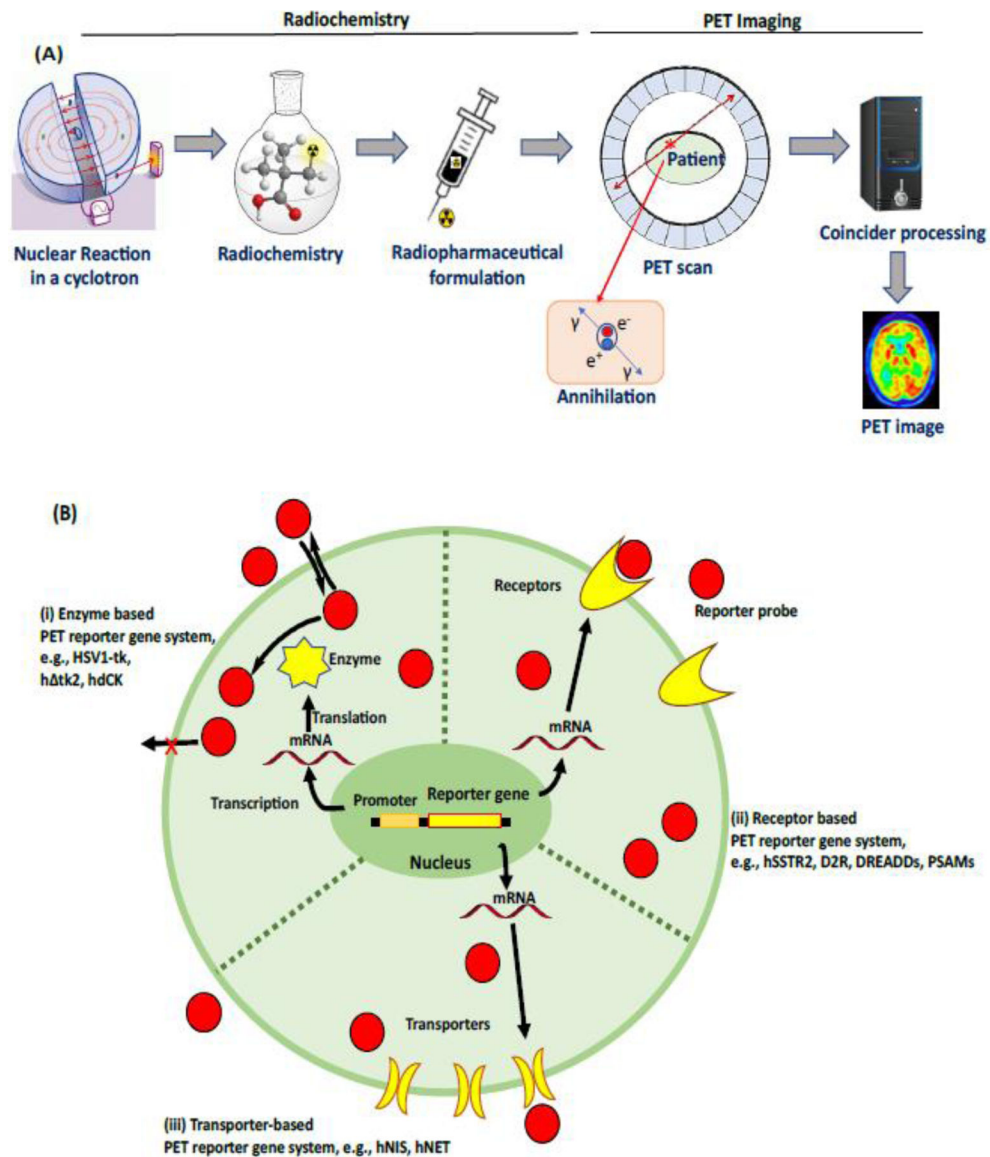


Figure 1: PET imaging: an overview.

A) Schematic representation of brain imaging with PET. A cyclotron generates radionuclides which are used for the production of PET reporter probes via one or more chemical reactions (radiochemistry). A final radiopharmaceutical formulation is prepared with a suitable vehicle after the isolation of the PET reporter probe using HPLC, and then injected into living subjects. PET imaging involves the reconstruction of the location of reporter probe within the body, via data acquisition and post-processing (see Text box 1 for details). B) Overview of the expression of reporter gene systems; three different mechanisms by which PET radiotracers image reporter gene expression based on (i) enzymes: intracellular entrapment of radiotracers after phosphorylation by enzymes encoded by reporter genes, e.g. HSV1-tk phosphorylates pyrimidine-based radiotracers, such as [^{18}F]FIAU, trapping the phosphorylated ligand inside the cell (ii) receptors: radiotracers bind protein receptors encoded by reporter genes, e.g. the human somatostatin receptor

type 2 (hSSTR2), dopamine D₂ receptor (D2R), and optogenetic-based receptors, encoded by a reporter gene (iii) transporters: accumulation of radiotracers into cells mediated by membrane transporters encoded by reporter genes, e.g. the human sodium–iodide symporter (hNIS), and human norepinephrine transporter (hNET) encoded by reporter gene.

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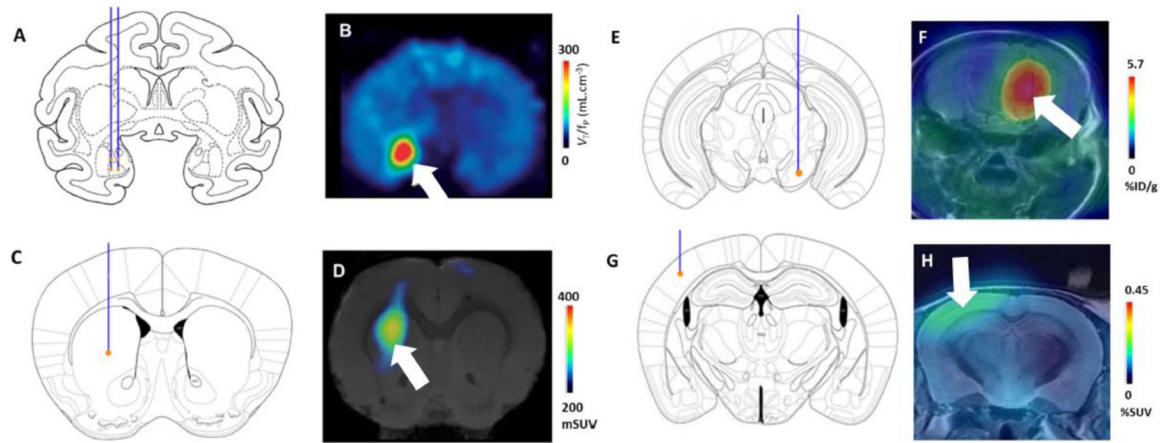


Figure 2. Brain PET reporter systems

(A, B) Chemogenetic - DREADD reporter system. Lenti-HA-hM4Di injections targeted to right amygdala in monkey. A: Injection location depicted on a coronal section. B: Baseline [^{11}C]DCZ binding (SUV) reveals contrast between the injection site (marked with an arrow) and the surrounding brain. (C, D) Chemogenetic - PSAM reporter system. AAV injection site in mouse left dorsal striatum (AAV-PSAM4-GlyR-EGFP). C: approximate injection location. D: PET image of mouse brain after [^{18}F]ASEM injection, showing striatal localization of PSAM4-GlyR. (E, F) PKM2 reporter system. AAV9-EF1A-PKM2 targeted to the ventral tegmental area / substantia nigra of mouse brain. E: injection location. F: [^{18}F]DASA-23 binding; arrow indicates region of radiotracer uptake. (G, H) ecDHFR reporter system. Recombinant ecDHFR-EGFP reporter protein was expressed in mouse somatosensory cortex after transduction with AAV-DJ. G: injection location. H: [^{18}F]FE-TMP binding; arrow indicates area of accumulation of the radiotracer. Figures B, D, F and H were adapted from [51], [53], [56] and [57] respectively.

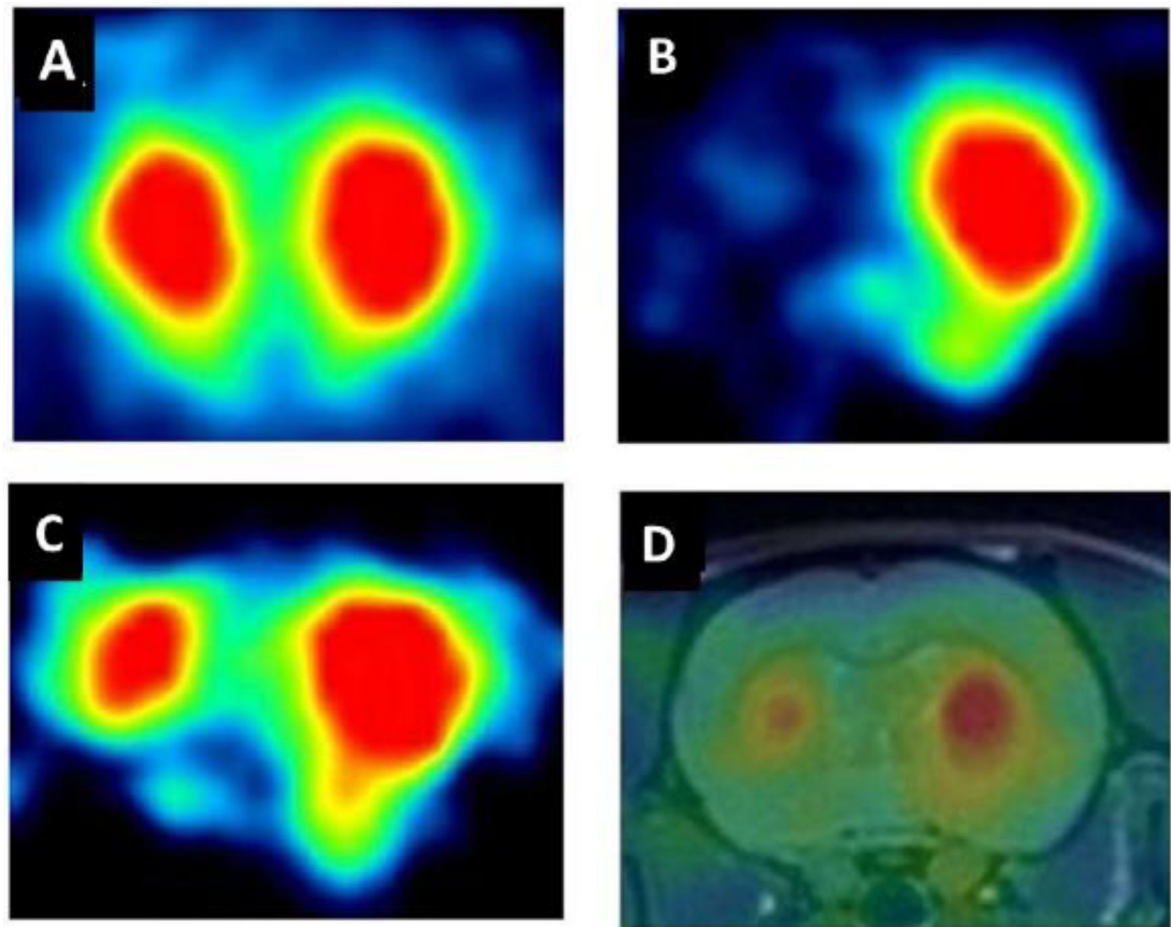


Figure 3: Recovery of dopamine transporter binding six months after DA stem cell transplantation in the rat striatum in a 6-hydroxy-dopamine model of PD.

(A) A naive rat showed bilaterally symmetrical uptake of $[^{18}\text{F}]$ FECNT, a radioligand for the dopamine transporter. (B) Unilateral injection of a dopaminergic toxin created a hemiparkinsonian rat, which displayed negligible $[^{18}\text{F}]$ FECNT uptake in the lesioned striatum. (C) ES cell transplantation partially restored uptake of $[^{18}\text{F}]$ FECNT in the lesioned striatum. (D) Coregistered PET and MR images after ES-cell transplantation. In this example, PET imaging of the dopamine transporter was used as a PET reporter system to monitor the survival and growth of transplanted cells. Figure adapted from [71].

Table-1:

List of PET reporter systems.

Reporter system	Reporter gene(s)	Reporter probe(s)
Intracellular entrapment:	HSV-1 TK HSV-1-sr39TK h TK2 hDTK2 hdCK VZV-tk Pyruvate Kinase M2 (PKM2) AADC TH/GAD1	[¹⁸ F]FIAU, [¹⁸ F]FMAU, [¹⁸ F]FEAU, [¹⁸ F]FHBG [¹⁸ F]DASA-23 6-[¹⁸ F]fluoro-L- <i>m</i> -tyrosine, [¹¹ C]raclopride
Protein binding:	hSSTR2 hD2R Cannabinoid (CB2) receptor ecDHFR Optogenetics - ChRERa Chemogenetics - DREADDs Chemogenetics - PSAMs	[⁶⁸ Ga]DOTATOC, [⁶⁸ Ga]DOTATATE [¹¹ C]raclopride, [¹⁸ F]fallypride, [¹⁸ F]spiperone derivative. [¹¹ C]GW405833 [¹⁸ F]FE-TMP [¹⁸ F]FES [¹¹ C]CLZ, [¹¹ C]DCZ, and [¹⁸ F]JHU37107 [¹⁸ F]ASEM
Transporter-based:	hNIS hNET hSERT	[¹⁸ F]tetrafluoroborate, [¹⁸ F]fluorosulfate. [¹¹ C]ephedrine, [¹²⁴ I]meta-iodobenzylguanidine (MIBG), and [¹⁸ F]meta-fluorobenzylguanidine (MFBG), [¹¹ C]MeNER, and [¹¹ C]MRB [¹¹ C]DASB, [¹¹ C]McN 5652, [¹¹ C]ADAM, [¹¹ C]DAPA, and [¹¹ C]AFM.