

# Nerve regeneration in transplanted organs and tracer imaging studies: A review

Yan Huang<sup>1</sup>, Zhigang He<sup>1</sup>, Anne Manyande<sup>2</sup>, Maohui Feng<sup>3\*</sup>, Hong-bing Xiang<sup>4\*</sup>

<sup>1</sup>Tongji Hospital of Tongji Medical College, Huazhong University of Science and Technology, China, <sup>2</sup>School of Human and Social Sciences, University of West London, United Kingdom, <sup>3</sup>Zhongnan Hospital, Wuhan University, China, <sup>4</sup>Huazhong University of Science and Technology, China

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### *Keywords*

Organ Transplantation, nerve tracers, sympathetic nerve, parasympathetic nerve, visceral sensory plexus

### *Abstract*

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The technique of organ transplantation is well established and after transplantation the patient might be faced with the problem of nerve regeneration of the transplanted organ. Transplanted organs are innervated by the sympathetic, parasympathetic, and visceral sensory plexuses, but there is a lack of clarity regarding the neural influences on the heart, liver and kidneys and the mechanisms of their innervation. Although there has been considerable recent work exploring the potential mechanisms of nerve regeneration in organ transplantation, there remains much that is unknown about the heterogeneity and individual variability in the reinnervation of organ transplantation. The widespread availability of radioactive nerve tracers has also made a significant contribution to organ transplantation and has helped to investigate nerve recovery after transplantation, as well as providing a direction for future organ transplantation research. In this review we focused on neural tracer imaging techniques in humans and provide some conceptual insights into theories that can effectively support our choice of radionuclide tracers. This also facilitates the development of nuclear medicine techniques and promotes the development of modern medical technologies and computer tools. We described the knowledge of neural regeneration after heart transplantation, liver transplantation and kidney transplantation and apply them to various imaging techniques to quantify the uptake of radionuclide tracers to assess the prognosis of organ transplantation. We noted that the aim of this review is both to provide clinicians and nuclear medicine researchers with theories and insights into nerve regeneration in organ transplantation and to advance imaging techniques and radiotracers as a major step forward in clinical research. Moreover, we aimed to further promote the clinical and research applications of imaging techniques and provide clinicians and research technology developers with the theory and knowledge of the nerve.

### *Contribution to the field*

In this review, we focused on the application of nerve tracer imaging techniques, summarizing current knowledge and mechanisms of action regarding nerve regeneration in organs where transplantation techniques have been widely performed, such as the heart, liver, and kidney. Moreover, we aimed to further promote the clinical and research applications of imaging techniques and provide clinicians and research technology developers with the theory and knowledge of the nerve.

1 **Nerve regeneration in transplanted organs and tracer imaging studies: A review**

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3 Yan Huang<sup>1,2</sup>, Zhigang He<sup>1</sup>, Anne Manyande<sup>3</sup>, Maohui Feng<sup>4,#</sup>, Hongbing Xiang<sup>1,#</sup>

4  
5 <sup>1</sup> Tongji Hospital of Tongji Medical College, Huazhong University of Science and  
6 Technology, Wuhan 430030, Hubei, China;

7 <sup>2</sup> Department of Interventional Therapy, the First Affiliated Hospital of Dalian Medical  
8 University, Dalian 116000, Liaoning, China;

9 <sup>3</sup> School of Human and Social Sciences, University of West London, London, UK;

10 <sup>4</sup> Department of Gastrointestinal Surgery, Wuhan Peritoneal Cancer Clinical Medical  
11 Research Center, Zhongnan Hospital of Wuhan University, Hubei Key Laboratory of  
12 Tumor Biological Behaviors and Hubei Cancer Clinical Study Center, Wuhan 430071,  
13 Hubei, China.

14  
15  
16 #Address correspondence to: Hongbing Xiang, E-mail: xhbtj2004@163.com; Maohui  
17 Feng, E-mail: Fengmh@whu.edu.cn

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1 **Abstract**

2 The technique of organ transplantation is well established and after transplantation the  
3 patient might be faced with the problem of nerve regeneration of the transplanted organ.

4 Transplanted organs are innervated by the sympathetic, parasympathetic, and visceral  
5 sensory plexuses, but there is a lack of clarity regarding the neural influences on the  
6 heart, liver and kidneys and the mechanisms of their innervation. Although there has  
7 been considerable recent work exploring the potential mechanisms of nerve  
8 regeneration in organ transplantation, there remains much that is unknown about the  
9 heterogeneity and individual variability in the reinnervation of organ transplantation.

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21 provide clinicians and nuclear medicine researchers with theories and insights into  
22 nerve regeneration in organ transplantation and to advance imaging techniques and  
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26 nerve.

27 **Keywords:** Organ transplantation, nerve tracers, sympathetic nerve, parasympathetic  
28 nerve, visceral sensory plexus.

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## 1 **1. Introduction**

2 Human organ transplantation is a very significant development in modern medicine. By  
3 removing diseased and necrotic organs and replacing them with healthy and viable ones,  
4 patients with life-threatening conditions can be given a second chance at life. As of  
5 2019 epidemiological surveys have shown that more than 100,000 patients worldwide  
6 need organ transplants each year(Vanholder et al., 2021). Despite the important role of  
7 organ transplantation in extending the life of patients, transplanted organs are exposed  
8 to multiple risks in the recipient, including modification of the transplantation technique,  
9 immune rejection of the transplanted organ, autonomic innervation after denervation of  
10 the transplanted organ, and health monitoring of the transplanted organ(Poole et al.,  
11 2019). Current organ transplantation techniques include allogeneic, xenogeneic, and  
12 future organ transplantations that target highly differentiated cell and tissue  
13 transplantation techniques that can minimize immune rejection in the recipient(Sayegh  
14 and Carpenter, 2004; Timsit et al., 2019).

15 Successful organ transplantation is not only dependent on the histocompatibility  
16 between the donor and recipient, but also on the functional recovery of the transplanted  
17 organ in the recipient tissue, which is one of the criteria for successful  
18 transplantation(Patchell, 1994; Byersdorfer and Turnquist, 2021). Nerve regeneration  
19 in organ transplantation is the process by which a patient goes from a completely  
20 denervated donor organ to a progressive regeneration of autonomic nerves after organ  
21 transplantation(Grupper et al., 2018). The most critical aspect of the nerve regeneration  
22 process in transplanted organs is the interaction between the Schwann cells and axons.  
23 The process of organs transplantations is a process of immune response(Bolívar et al.,  
24 2020). Antigens are present in the Schwann cells of the peripheral nerves of the donor  
25 organ, which connect to the nerves of the recipient organ and produce a variety of  
26 immune factors, resulting in an immune rejection reaction. It is therefore crucial that  
27 sufficient immunosuppressive drugs are given during the organ transplantation process  
28 to provide a better environment for nerve regeneration in the organ transplant(Sarker et  
29 al., 2018).

30 Autonomic innervation plays a crucial role in maintaining organ function and

1 transplanted organs face transient denervation. By performing peripheral nerve  
2 anastomoses during transplantation and giving neurotrophic factor drugs  
3 postoperatively, the nerves of the transplanted organ have demonstrated that the repair  
4 process can be slow. Several studies have shown that denervated organs have a strong  
5 regenerative capacity, and this has led to the widespread use of organ transplantation  
6 and saving countless lives(White et al., 2015).

7 Scientists were able to further observe the neuroanatomical patterns and their normal  
8 physiological functions in vivo until the advent of conventional tracers in the 1970s,  
9 which completely broke this bottleneck and led to a rapid advancement in neuroscience  
10 and cognitive science(Kristensson, 1970). Scientists subsequently exploited the  
11 physiological properties of rabies virus transmission on nerve axons to develop viral  
12 tracers with specificity and high expression(Ugolini, 2010). In conclusion, both  
13 traditional neural tracers and viral tracers have been widely used in scientific  
14 investigations to reveal neuronal connections between brain regions, neurotransmission  
15 between skeletal muscles or visceral organs and the brain, and to further clarify the  
16 neural and functional localization of the cerebral cortex. With the development of the  
17 field of nuclear medicine, radionuclide testing gradually emerged in the 1990s as a  
18 clinical application to measure and monitor blood flow, urine, and neurotransmitter  
19 expression in patients(Schwaiger et al., 1990). Among other things, radionuclide tracers  
20 can be used to assess the functional status of organs and tissues and to determine the  
21 progression and prognosis of a patient's disease with the greatest precision and speed  
22 in the patient. These are the advantages of radiotracers, such as: ① the simplicity and  
23 sensitivity of the means of detection; ② the fact that they are in accordance with the  
24 normal physiological conditions of the organism and can be metabolized in vivo; and  
25 ③ the fact that they can be precisely localized and can provide detection at the  
26 molecular and atomic level. We have shown in Table 1 the advantages and  
27 disadvantages of conventional tracers, viral tracers, and radioactive tracers. In addition,  
28 we have depicted in Figure 1 a timeline for the development of radiotracers.

29 Currently, with the development of imaging technology, the use of neural tracer  
30 techniques to visualize the regenerative processes of autonomic nerves and to label

1 neuronal and neurotransmitter neurotransmission can reveal the sympathetic and  
2 parasympathetic reinnervation processes in animals and humans(Bravo et al., 2015;  
3 Wang et al., 2018). Positron emission tomography imaging (PET)(Brust et al., 2014),  
4 single photon emission computed tomography (SPECT) combined with magnetic  
5 resonance (MRI) and computed tomography (CT)(Srinivas et al., 2010), involving  
6 radiologically targeted molecules to label the chemical neurotransmitters between  
7 synapses, such as  $^{11}\text{C}$ -hydroxyephedrine ( $^{11}\text{C}$ -HED),  $^{11}\text{C}$ -epinephrine(Sasano et al.,  
8 2008),  $^{11}\text{C}$ -phenylephrine (PHEN)(Raffel et al., 1999),  $6\text{-}^{18}\text{F}$ -  
9 fluorodopamine(Goldstein et al., 1993),  $^{13}\text{N}$ -ammonia, and flubrobenguane  
10 (FBBG)(Zelt et al., 2021) has been found to be useful in presynaptic neuronal transport  
11 processes in the cardiovascular system. Our review will demonstrate the use of a  
12 radiotracer in the reinnervation of the heart, liver, and kidney after transplantation as  
13 shown in table 2. Numerous studies have illustrated the usefulness of radiotracer  
14 applications in probing the neuroanatomy of the cardiovascular system, organ  
15 transplantation and the nervous system(Bailey and Willowson, 2013). Therefore, we  
16 have summarized the current widespread use of nerve tracers in autonomic disorders in  
17 table 3. We showed the innervation of the heart, liver, and kidney in detail in Figure 2,  
18 with sympathetic regeneration of the heart transplant as an example, and we have  
19 labelled the commonly used sympathetic tracers in the diagram. The image was created  
20 using Biorender.com.

## 21 **2. Reinnervation of a transplanted heart**

### 22 **2.1 Reinnervation of the sympathetic plexus in the transplanted heart**

23 In 2022, 57-year-old David Bennett became the first patient in history to have a pig  
24 heart transplant, but passed away 2 months following the allogeneic cardiac  
25 transplantation(Burki, 2022). Back in 1947, Dr Christiaan Barnard set the record for  
26 the first in orthotopic heart transplant(Brink and Hassoulas, 2009). The first patient,  
27 Louis Washansky, passed away 18 days after the operation, but the second patient,  
28 Philip Blaiberg, survived for almost two years(Cooper, 2001). We are grateful to the  
29 pioneer of heart transplantation, the courageous Dr Christiaan Barnard! While  
30 orthotopic heart transplantation is now well established, patients who have undergone

1 cardiac transplantation continue to face a variety of post-operative complications and  
2 immune rejection reactions, the causes and mechanism of which deserve to be  
3 adequately explored.

4 Heart transplantation is the treatment of choice for patients with end-stage heart  
5 failure(Mehra, 2017). In both allogeneic and orthotopic heart transplantation, the donor  
6 heart faces denervation and is vulnerable to a variety of cardiovascular events in the  
7 absence of central innervation, such as arrhythmias, abnormal chest pain, sudden atrial  
8 fibrillation, sudden cardiac death, and stroke(Joglar et al., 2021; Firoz et al., 2022).

9 With the popularity of the heart transplantation approach and extensive research, it has  
10 been reported that the sympathetic nerves of the heart gradually regenerate over time,  
11 restoring sympathetic innervation to the heart and participating in the rhythm regulation  
12 of the heart and the perfusion of the heart muscle(Jakus et al., 2022). The state of cardiac  
13 transplant sympathetic reinnervation varies from one heart transplant patient to another,  
14 with some heterogeneity in various regions of the heart(Wilson et al., 1991). The  
15 phenomenon of reinnervation after heart transplantation is therefore an innovative point  
16 of clinical research!

17 With the technical support of medical imaging, nerve tracers are used to label the major  
18 transmitters of the post-sympathetic adrenergic nerves and thus determine the integrity  
19 of the sympathetic nerves(Pandit-Taskar and Modak, 2017). <sup>11</sup>C-HED functions as a  
20 catecholamine analogue with a neural tracer effect and is used in combination with a  
21 presynaptic norepinephrine transporter (NET, uptake-1) to assess the activity of  
22 presynaptic sympathetic neuronal transport in the myocardium(Zelt et al., 2021). When  
23 assessing the sympathetic integrity of the heart 1 year after heart transplantation using  
24 PET, 55% of patients (16) presented with <sup>11</sup>C-HED retention in the left anterior  
25 descending branch at a rate of 47%. Subsequently, after exercise stress stimulation of  
26 cardiac sympathetic fibers, patients with cardiac reinnervation present exhibited an 8%  
27 increase in left ventricular ejection fraction (LVEF) compared to denervated patients,  
28 with the greatest density of reinnervated sympathetic nerves found particularly in the  
29 left anterior interventricular region(Bengel et al., 2001). Thus, reuptake of  
30 catecholamine analogues by sympathetic nerve endings in the anterior interstitial region



1 of the heart could provide strong evidence of sympathetic reinnervation of the patient's  
2 heart.

3 Cardiac autonomic nerves are involved in the neuromodulation of the sinus node and  
4 heart rate variability (HRV) is a widely used non-invasive measure of the cardiac  
5 sympathetic and parasympathetic activity and can be used as a tool to assess the  
6 recovery of the autonomic nervous system after heart transplantation(Ernst, 2017). In  
7 one study, which assessed cardiopulmonary reinnervation in patients after heart  
8 transplantation between 2.5 months and 12 months, it was observed that low-frequency  
9 (LF) fluctuations in HRV of heart transplant patients in the supine position up to  $5.7\text{ms}^2$   
10 were observed as early as 6 months and was up to  $6.0\text{ms}^2$  when LF was tested again  
11 one year later, showing a significant increase in LF variability with sympathetic nerve  
12 regeneration(Christensen et al., 2021a). A positive correlation between exercise  
13 capacity and peak oxygen uptake and sympathetic reinnervation was observed in  
14 patients, with myocardial  $^{11}\text{C}$ -HED uptake being twice as high in the reinnervated  
15 group compared to the denervated group(Schwaiblmair et al., 1999).

16 Further labelling of sympathetic nerves using catecholamine analogue tracers revealed  
17 a positive correlation between the degree of presynaptic retention of  $^{11}\text{C}$ -HED in  
18 sympathetic nerves and the HRV produced by sinus node innervation. At 5 years'  
19 follow-up of post-heart transplant patients, 58% (22) of patients had a mean  $^{11}\text{C}$ -HED  
20 retention rate of 10.7% in the left ventricle, while LF in patients with sinus node  
21 reinnervation in HRV was up to  $5.9\text{ms}^2$ . This indicates that sympathetic regeneration is  
22 a continuous process, with an increase in HRV observed as early as 3.5 months, while  
23 5 years later patients showed a wide distribution of sympathetic reinnervation in the  
24 anterior and some lateral regions of the left ventricle(Uberfuhr et al., 2000).

25 Iodine-123-meta-iodobenzylguanidine ( $^{123}\text{I}$ -mIBG), a sympathetic blocker  
26 guanethidine(Zhang et al., 2014), is a pseudo neurotransmitter analogue released from  
27 the presynaptic membrane of sympathetic nerves, bound to uptake-1 at presynaptic  
28 terminals labeled and stored in vesicles, and used to assess presynaptic sympathetic  
29 neuronal vesicle storage activity(Mabuchi et al., 2005). In a study, the heart to  
30 mediastinal rate ratio (H/M) and the elution rate of the heart were determined using

1 SPECT assessment 4 hours after <sup>123</sup>I-mIBG into the body(Dilsizian and  
2 Chandrashekhar, 2022). In patients with allogeneic heart transplants, increased <sup>123</sup>I-  
3 mIBG uptake by cardiac sympathetic nerves occurred in 80% of patients 10 years after  
4 heart transplantation compared to patients 2 years after heart transplantation. Of these,  
5 sympathetic reinnervation occurred as early as 2 years after allogeneic heart  
6 transplantation, at which time cardiac sympathetic reinnervation of nerve fibers to <sup>123</sup>I-  
7 mIBG uptake can be as high as 18%(Estorch et al., 1999). Another study followed up  
8 patients after ectopic heart transplantation and found that <sup>123</sup>I-mIBG scintigraphy  
9 remained absent 6 years after ectopic heart transplantation under the detection of  
10 SPECT(Yap et al., 2006). It is hypothesized that non-anatomical heart transplantation  
11 may result in limited regeneration of cardiac sympathetic fibers. However, Jenkins et  
12 al. found that <sup>123</sup>I-mIBG scintigraphy assessed sympathetic regeneration in patients and  
13 that sympathetic reinnervation did not correlate with circadian blood pressure  
14 regulation in patients. This suggests that <sup>123</sup>I-mIBG scintigraphy only confirms the  
15 presence of nerve regeneration after cardiac transplantation and that cardiac  
16 sympathetic function had not been fully restored(Jenkins et al., 1997).

17 A recent study reported that quantitative tracers of cardiac sympathetic nerves, 4-<sup>18</sup>F-  
18 fluoro-meta-hydroxyphenethylguanidine (<sup>18</sup>F-4F-MHPG) and 3-<sup>18</sup>F-fluoro-para-  
19 hydroxyphenethylguanidine (<sup>18</sup>F-3F-PHPG), can be used to quantify nerve density in  
20 denervated regions of patients with ischemic myocardial infraction. It is thus,  
21 reasonable to speculate their future application in the exploration of full neurological  
22 recovery in patients after heart transplantation(Raffel et al., 2022).

## 23 **2.2 Reinnervation of the parasympathetic plexus in the transplanted heart**

24 In 1995 researchers focused their attention on parasympathetic imaging techniques,  
25 using the stimulating effect of meglumine diatrizoate contrast agents on  
26 cardiopulmonary chemoreceptors. The heart rate was seen to decrease in heart  
27 transplant patients after chemical tracer infusion, which could be inferred from the  
28 intact and regenerative parasympathetic innervation of the patients. This study revealed  
29 that 6 years after heart transplantation, patients did not only have the parasympathetic  
30 reinnervation that the investigators expected, but the rate of residual recipient sinus

1 nodes (RSN) in heart transplant patients showed an increasing trend. This probably  
2 occurred without remodeling of the parasympathetic efferent nerves after  
3 surgery(Arrowood et al., 1995). The researchers then injected norepinephrine into heart  
4 transplant patients to stimulate their pressure receptors to observe changes in cardiac  
5 vagal tone, and four years after transplantation the patients still did not show increased  
6 vagal tone(Arrowood et al., 1997).

7 Although the results of earlier clinical studies have been unsatisfactory, the latest  
8 findings show that cardiopulmonary receptors are reinnervated one year after heart  
9 transplantation. When Wyller et al. evaluated the autonomic activity of the heart, they  
10 found that patients showed an increase in LF in the supine position to 5.7ms<sup>2</sup> as early  
11 as 6 months after transplantation and a trend towards a decrease in heart rate (HR) at  
12 rest 12 months after transplantation. During the 20° head-up tilt test the patient showed  
13 a decrease in right atrial pressure and a decrease in cardiac output as HR increased.  
14 These findings support the idea that cardiopulmonary receptors are subject to cardiac  
15 parasympathetic reinnervation one year after transplantation(Wyller et al., 2021).

16 In physiological studies, autonomic transection of the heart transplant leads to vagal  
17 denervation of the heart, manifested by the resting tachycardia and variable hourly  
18 cardiac malfunction in patients after heart transplantation(Levy et al., 1981). In one  
19 study that followed up cardiopulmonary exercise tests in heart transplant patients,  
20 cardiac sinus node parasympathetic regeneration could be observed in year two. There  
21 was an increase of up to 6.0 ms<sup>2</sup> in high-frequency (HF) fluctuations in the HRV index,  
22 and an LF/HF ratio of up to 84% plus an increase in tachycardia response during  
23 Valsalva exercise in patients in the supine position. It is thus, evident that  
24 parasympathetic reinnervation begins to gradually strengthen two years after heart  
25 transplantation(Christensen et al., 2021b).

26 With the development of neural tracers, chemical tracers acting on cardiopulmonary  
27 receptors are no longer sought singularly but are combined with PET techniques to  
28 observe the release of acetylcholine (ACh) transmitters following parasympathetic  
29 activation(Khajehali et al., 2018). However, unlike neural tracers of catecholamine  
30 analogues, Ach is subject to specific degradation by acetylcholinesterase (AChE),

1 which results in more challenging imaging techniques for observing cardiac  
2 parasympathetic nerves. As a result, non-invasive HRV testing is predominantly used  
3 clinically for cardiac parasympathetic reinnervation. (-)-<sup>18</sup>F-  
4 fluoroethoxybenzovesamicol (<sup>18</sup>F-FEOBV) binds to acetylcholine transporter proteins  
5 and is prominently labelled at cholinergic nerve endings in the heart. Although <sup>18</sup>F-  
6 FEOBV has not been explored to date for postoperative parasympathetic regeneration  
7 in cardiac transplant patients, it is expected to be a tool for future use in cholinergic  
8 radiological studies(Petrou et al., 2014).

### 9 **2.3 Reinnervation of the sensory plexus in the transplanted heart**

10 As a result of heart transplantation, the heart is denervated by sensory fibers. Once a  
11 heart transplant recipient develops ischemic angina, the patient's presentation is highly  
12 atypical, making the diagnosis more difficult for the clinician(DeFilippis et al., 2017).  
13 Statistics suggest that during the 5-year period after heart transplantation, post-  
14 transplant cardiac allograft vasculopathy (CAV) was present in 50% and coronary  
15 arteriosclerosis in 10% of patients(García-Baizán et al., 2021).

16 Research has also shown that heart transplant patients present with pain in the anterior  
17 thoracic region after 3 years and stenosis occlusion of the coronary arteries is seen on  
18 the coronary computed tomography angiography (CTA). The patient with heterogeneity  
19 of coronary stenosis, which occurs mainly in the left anterior descending coronary  
20 artery results in complete obstruction of the right coronary vessel. At the same time,  
21 post-transplant in the anterior thoracic region has been associated with sensory nerve  
22 regeneration in some cases and not in others, and this regeneration often has a negative  
23 effect, hence, modulating and reconstructing nerve regeneration in the transplanted  
24 heart has great potential for clinical research(Stark et al., 1991)!

## 25 **3. Reinnervation of a transplanted liver**

### 26 **3.1 Reinnervation of the sympathetic plexus in the transplanted liver**

27 Liver transplantation is one of the treatments for chronic liver failure and hepatocellular  
28 carcinoma (HCC)(Tan et al., 2022). The liver has a unique ability to regenerate and the  
29 donor liver can return to normal liver morphology after 2 months, but research is still  
30 needed on the pattern and mechanism of regeneration and innervation of the liver(Haga

1 et al., 2008). The loss of autonomic innervation of the transplanted liver and the  
2 progressive decrease in catecholamine release from the hepatic denervation leads to an  
3 increase in hepatic blood flow (HBF) through a reduction in action alpha-adrenergic  
4 receptors. However, the change in HBF is not significant and therefore the restoration  
5 of HBF cannot be used as a decisive indicator of hepatic nerve regeneration(Kurosawa  
6 et al., 2002).

7 As the liver is denervated, the autonomic nervous system gets out of balance with  
8 respect to hepatic glucose uptake, and the net hepatic glucose uptake (NHGU) is no  
9 longer inhibited by sympathetic nerves. This process can result in an imbalance  
10 between the liver's food uptake function and the body's postprandial glucose regulation,  
11 hence the manifestation of the metabolic syndrome in liver transplant patients(Myers  
12 et al., 1991; Moore et al., 2012).

13 Clinical studies have reported that during the 3 to 5 years following liver transplantation,  
14 50% of patients are susceptible to metabolic syndrome (MetS), obesity and type 2  
15 diabetes as a result of immunosuppression and liver denervation(Becchetti et al., 2020).  
16 Thus, monitoring the computed tomography attenuation values (CT-AV) of liver  
17 transplant patients 6 months after surgery revealed that patients with values of CT-AV  
18 below 60% in the 1-week postoperative group showed signs of impaired liver function  
19 at 6 months after surgery due to steatosis or ballooning of the transplanted liver. It was  
20 therefore, hypothesized that CT-AV quantification at one week following liver  
21 transplantation could be used to assess the prognosis and regeneration of liver  
22 transplants(Iida et al., 2005). Liver transplant reinnervation is thus, an issue of scientific  
23 interest, both from the perspective of exploring the mechanisms of liver transplant  
24 nerve regeneration and improving the complications following liver transplant  
25 denervation.

26 Kjae et al. studied sympathetic nerve regeneration after liver transplantation and  
27 established two liver groups: a liver transplantation group (n=13, <30 months after  
28 transplantation) and a normal control group (n= 11, normal individuals without liver  
29 transplantation). When both groups were subjected to liver biopsy for catecholamine  
30 levels, the norepinephrine concentration in the liver transplantation group was found to

1 be only 0.022nmol/g, and the levels of catecholamines 99% lower compared to  
2 normal controls, showing that sympathetic regeneration of liver sympathetic nerves was  
3 still not present 2 to 3 years after live denervation(Kjaer et al., 1994).

4 In rodent studies, direct observation of liver sections from 3 to 6 months after liver  
5 transplantation in rats using immunohistochemistry revealed a positive trend for  
6 growth-associated protein 43 (GAP-43) as a marker of neuronal plasticity over time.  
7 However, nerve regeneration in the liver portal vein occurred only between 5 days and  
8 3 months after liver transplantation and had ceased in the liver by 6 months. Meanwhile,  
9 the ubiquitin hydrolase protein gene product 9.5 (PGP9.5) expression in nerve axons  
10 had returned to normal levels(Kandilis et al., 2014).

11 The results observed from rodents demonstrate that nerve regeneration in the liver is  
12 completed at 3 to 6 months, and in the available human studies reported, some  
13 investigators used the same immunohistochemical technique to determine the  
14 regeneration of liver nerves in humans by direct observation of nerve  
15 regeneration(Boon et al., 1992). Fifteen months after liver transplantation,  
16 immunostaining for PGP9.5 was found to be positive on liver sections from  
17 transplanted patients, but the expression of nerve regeneration appeared restricted, with  
18 positive expression only at portal nerve fibers(Dhillon et al., 1992).

### 19 **3.2 Reinnervation of the parasympathetic plexus in the transplanted liver**

20 Several studies have confirmed that regeneration of Schwann cells wraps around axons  
21 after peripheral nerve injury (PNI) and that vagus nerve regeneration is similarly  
22 repaired and remyelinated by Schwann cells distal to axons(Clements et al., 2017).

23 Animal studies have revealed that Netrin-1, a laminar adhesion-associated protein  
24 expressed in Schwann cells and axons of motor and sensory neurons, targets and  
25 activates the Netrin-1 signaling pathway in Schwann cells to promote the regeneration  
26 function of peripheral nerve cells after PNI(Taïb et al., 2022). Wang et al. demonstrated  
27 that exogenous supplementation of Netrin-1 in mice after liver transplantation  
28 accelerated regeneration of the hepatic vagus nerve. Netrin-1 expression was  
29 significantly reduced in the liver tissue of mice after liver resection ( $p < 0.05$ ).

30 Subsequently, exogenous supplementation of Netrin-1 was administered in the tail vein

1 of mice and positive expression of GAP-43 was observed in the liver tissue one week  
2 after surgery, as evidenced by the nerve regeneration in the liver one week after Netrin-  
3 1 administration. Subsequent detection of positive anti-choline acetyltransferase (ChAT)  
4 in the liver tissue further confirmed that Netrin-1 promotes vagal nerve regeneration in  
5 the liver(Wang et al., 2022). It is certainly a revelation to us that the research and  
6 development of exogenous targeted neurological drugs for post-liver transplant patients  
7 point to pharmaceutical innovation that will help in the rehabilitation of liver transplant  
8 patients with neurological regeneration.

9 It is well known that the central nervous system plays an important physiological  
10 regulatory role on the autonomic nerves of the liver(Liu et al., 2021). Neuro-humoral  
11 regulation of the liver is mediated through sympathetic afferents to the central nervous  
12 system, with projections in the lateral hypothalamus (LH) and ventral medial  
13 hypothalamus (VMH) to the dorsal motor nucleus of the vagus (DMV) and subsequent  
14 projections to primary neurons involved in hepatic vagal innervation(Berthoud and  
15 Neuhuber, 2019). An experiment involving rats showed that partial hepatectomy with  
16 concomitant destruction of VMH in a rat's brain resulted in an increase in DNA  
17 synthesis in the liver. This probably led to compensatory stimulation of hepatocyte  
18 regeneration by the hepatic vagus nerve as a result of disruption of the central nervous  
19 system(Kiba et al., 1994). The hepatic vagal branch has an independent innervation  
20 function on DNA synthesis in hepatocytes, and once liver denervation has occurred,  
21 this results in a delayed effect on DNA synthesis in hepatocytes(Kato and Shimazu,  
22 1983; Kiba et al., 1995). David et al. observed that the hepatic vagus nerve secretes  
23 ACh, which acts on muscarinic acetylcholine receptor 3 (mAChR3) in hepatic  
24 progenitor cells (HPCs) to promote hepatocyte regeneration. Rats with hepatic vagal  
25 branches removed showed impaired proliferation of hepatocytes and bile duct epithelial  
26 cells(Cassiman et al., 2002). It is clear that hepatic parasympathetic denervation leads  
27 to a decrease in the rate of DNA synthesis in hepatocytes and thus interferes with  
28 hepatic regeneration. The rate of DNA synthesis in hepatocytes is reduced by  
29 parasympathetic denervation of the liver, thereby interfering with liver regeneration.  
30 In the cytoplasm of nerve cells, presynaptic ChAT acts to synthesize ACh from choline

1 (Cho) and acetyl coenzyme A (CoA), which binds to muscarinic receptors and  
2 participates in cholinergic signalling(Sarter and Parikh, 2005). The use of the  
3 radiotracer  $^{14}\text{C}$ -Cho to label the synthesis of hepatocyte membranes and the process of  
4 acetylcholine transmitter synthesis has been found, and it is hypothesized that  $^{14}\text{C}$ -Cho  
5 could be used as a diagnostic for new-onset HCC and recurrence after liver  
6 transplantation(Kuang et al., 2010). In contrast to neural regeneration in heart  
7 transplantation, patients who have undergone liver transplantation for HCC are at a risk  
8 of HCC recurrence over a 5-year period(Agopian et al., 2015). Despite the temporary  
9 lack of studies on hepatic vagal reinnervation, it is suggested that studies using nerve  
10 tracers to monitor cancer recurrence in patients after transplantation are more clinically  
11 relevant and have a highly complementary diagnostic role for prognosis after liver  
12 transplantation(Kim et al., 2020).

#### 13 **4. Reinnervation of a transplanted kidney**

##### 14 **4.1 Reinnervation of the efferent nerves in the transplanted kidney**

15 According to literature, as of November 2021, surgeons have transplanted alpha-gal  
16 knockout porcine kidneys into brain-dead patients. The results indicate that after 54  
17 hours of in vivo renal filtration function of the allogeneic kidneys, the patients had yet  
18 to experience significant immune rejection, showing the great strides being made today  
19 with allogeneic kidney retransplantation(Cooper and Hara, 2021; Dolgin, 2021).

20 Kidney transplantation is the most effective treatment for end-stage renal disease  
21 (ESRD) and is effective in improving the survival of patients with renal failure, with an  
22 estimated survival of 19.2 years for kidney transplant patients(Poggio et al., 2021). In  
23 anatomical studies, the kidney is innervated by the sympathetic ventral plexus  
24 emanating from T12-L2 spine cord, and the nerves of the kidney are involved in  
25 innervating water and sodium metabolism and fluid regulation in the kidney(Sakakura  
26 et al., 2014). Interruption of renal sensory afferent and sympathetic efferent nerves after  
27 renal transplantation leads to inactivation of sympathetic efferent nerve effectors, which  
28 in turn activate the renal-renal reflex and inhibit the renin-angiotensin-aldosterone  
29 system. This process implies the development of reduced renin secretion, diuresis and  
30 pro-sodium excretion, and the lowering of blood pressure(Frame et al., 2016).



1 In animal experiments, renal denervation was shown to reduce blood pressure in rats  
2 with nephrogenic hypertension. Compared with rats with hypertension caused by renal  
3 artery ligation, renal denervation restored normal plasma renin secretion, reduced  
4 arterial blood pressure by  $44 \text{ mmHg} \pm 3 \text{ mmHg}$  and reduced HR by  $33 \pm 9$  to  $61 \pm 9$  beats  
5 per minute, showing that renal denervation improved the symptoms of nephrogenic  
6 hypertension(Oliveira et al., 1992). One study used myocardial uptake of  $^{123}\text{I}$ -mIBG to  
7 measure autonomic recovery after renal transplantation. They reported a reduced  
8 elution rate of  $^{123}\text{I}$ -mIBG for cardiac sympathetic nerves assessed 3 months after renal  
9 transplantation ( $p < 0.05$ ) and no differential change in the assessment of HRV in  
10 patients. It is proposed that sympathetic overexpression due to ESRD was reduced after  
11 renal transplantation and that the  $^{123}\text{I}$ -mIBG uptake rate could be used as a specific  
12 indicator of renal denervation(Kurata et al., 2004).

13 Given the involvement of renal sympathetic over-activation in the pathophysiology of  
14 hypertension, the implementation of renal sympathetic denervation (RDN) is suggested  
15 to be an effective treatment for intractable hypertension(Mahfoud et al., 2022; Sarathy  
16 and Salman, 2022). In the Global SYMPPLICITY Registry project, the Symplicity flex  
17 catheter was used to examine ambulatory blood pressure changes in 3,000 patients 3  
18 years after RDN. The results showed a decrease in systolic blood pressure (SBP)  
19 starting 6 months after RDN ( $-11.7 \pm 28.6 \text{ mmHg}$ ,  $p < 0.001$ ) which continued to  
20 decrease ( $-16.6 \pm 28.6 \text{ mmHg}$ ,  $p < 0.001$ )(Mahfoud et al., 2019). However, experiments  
21 in animals have revealed a regeneration of renal nerves after RDN. Findings at 5.5  
22 months after RDN in sheep, showed positive tyrosine hydroxylase (TH) staining in the  
23 perirenal tissue, while renal norepinephrine levels were detected reaching 88.9% and  
24 131.0% after 11 months of RDN. These results suggest that nerve regeneration in the  
25 perirenal artery 5.5 months after RDN and innervation could return to normal levels  
26 after 11 months. But this study is limited to sheep with ductal RDN and may serve as a  
27 partial reference for renal denervation in humans(Booth et al., 2015).

28 In a pathological study, Gazdar et al. used immunohistochemistry to detect renal nerve  
29 fibers by observing patients from 5 days to 3012 days after renal transplantation.  
30 Positive Bodian staining of regeneration axons were observed as early as 28 days after

1 renal transplantation, and substantial nerve regeneration around the axons after 8  
2 months(Gazdar and Dammin, 1970). Similarly, Rabelink et al. studied renal  
3 sympathetic regeneration in allogeneic transplanted kidneys 2 months later, and  
4 compared a healthy control group with a renal transplant group for head-out water  
5 immersion (HOWI). They found a marked increase in urinary sodium and potassium  
6 concentrations in renal transplant patients. It is speculated that 2 months after renal  
7 transplantation the kidney is still denervated and that the reduction in renal vascular  
8 resistance led to transplant patients exhibiting diuresis and natriuresis(Rabelink et al.,  
9 1993).

10 Mauriello et al. followed up patients for 5 months to 11 years after renal transplantation  
11 and found that nerve regeneration could be observed in patients at 5 months of  
12 transplantation, with positive staining for the neuronal regeneration and GAP-43 seen  
13 in the renal arteries. In contrast, 10 years after transplantation in patients with  
14 concomitant hypertension, TH was detected in renal tissue positively labelled in  
15 sympathetic nerve fibers. It is hypothesized that the patients experienced regeneration  
16 of renal sympathetic nerves in response to hypertension stimulation(Mauriello et al.,  
17 2017). Nevertheless, hypertension after renal transplantation is not associated with  
18 regenerative innervation of the renal arteries. Grisk et al. reported a remarkable increase  
19 in mean arterial pressure 3 weeks after renal transplantation, but TH mRNA levels in  
20 sympathetic nerve fibers were not yet significantly altered. This demonstrates that  
21 sympathetic nerves were not yet regenerated 3 weeks after renal transplantation and  
22 that post-transplant hypertension was not neurogenic(Grisk et al., 2000). In addition,  
23 Rasmussen et al. observed a 30% reduction in  $^{123}\text{I}$ -mIBG uptake by the transplanted  
24 kidney 1 month later compared to  $^{123}\text{I}$ -mIBG uptake by the donor kidney within 4 hours  
25 preoperatively ( $p < 0.005$ ). This illustrates a positive correlation between recovery of  
26 renal function in the transplanted kidney and renal reinnervation(Rasmussen et al.,  
27 2020).

#### 28 **4.2 Reinnervation of the afferent nerves in the transplanted kidney**

29 It has been reported that renal sensory afferent nerve cells originate from the dorsal root  
30 ganglion (DRG) of the ipsilateral T10-L2, mostly located in the cortical spinal cord area

1 and the renal pelvic wall(Marfurt and Echtenkamp, 1991; Ma et al., 2002). Increased  
2 ureteral pressure stimulates pressure receptors and chemoreceptors in the kidney and  
3 increases the release of calcitonin gene-related peptide (CGRP) and substance P (SP)  
4 in the renal pelvis tissue. This activates the afferent nerve in the ipsilateral kidney and  
5 the renal-renal reflex in the contralateral kidney for contralateral urination and  
6 natriuretic response(Kopp and Smith, 1989). As the sensory afferent nerves of the  
7 kidney are involved in the neuromodulation of renal chemoreceptors,  
8 mechanoreceptors and nociceptive receptors, once renal denervation has occurred,  
9 renal denervation of afferent nerves leads to impaired renorenal reflexes in the  
10 kidney(DiBona and Kopp, 1997; Barry and Johns, 2015). Based on the importance of  
11 renal afferent nerves in physiological studies, it is worth exploring the reinnervation of  
12 renal afferent nerves.

13 Rodionova et al. investigated the regeneration process of unilateral renal denervation  
14 by measuring the levels of the neural markers TH and CGRP in rat kidney tissue  
15 sections. Compared to one week after denervation, rats exhibited a marked increase in  
16 CGRP and TH levels 3 months after surgery, which showed some degree of  
17 regeneration of afferent and efferent nerves in the kidney after 3 months. Meanwhile,  
18 the fluorescent tracer 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine  
19 methanesulfonate (Dil) was injected into the ipsilateral DRG cells of the kidney. The  
20 results confirmed that ipsilateral renal denervated afferent nerve regeneration is not  
21 positively correlated with the innervation of the contralateral afferent nerve(Ai et al.,  
22 2007; Rodionova et al., 2016). The same study, which confirmed denervation in sheep,  
23 found that CGRP levels in renal tissues returned to normal 11 months after RDN(Booth  
24 et al., 2015). Interestingly, there is species heterogeneity in the reinnervation of renal  
25 afferent nerves(Mulder et al., 2013; Booth et al., 2015). While anatomical and  
26 biochemical studies currently provide support for renal afferent sensory reinnervation,  
27 no definitive human studies have been reported and further clinical trials are needed to  
28 address these questions.

## 29 **5. Conclusion**

30 Transplanted organs face reinnervation of autonomic nerves. This review on neural

1 regeneration in organ transplantation analyses the use of molecularly targeted imaging  
2 techniques and immunohistochemical methods that probe the autonomic nervous  
3 system of parenchymal organs such as the heart, liver, and kidney, further supporting  
4 the reinnervation of those autonomic nerves in transplanted organs.

5 In our review of the latest radiotracers for neural regeneration in organ transplantation,  
6 we found that the development of radiotracers for sympathetic catecholamines and  
7 catecholamine analogues is currently dominated by the development of  
8 acetylcholinergic tracers. In addition, the development and application of  
9 acetylcholinergic tracers cause a chemical reaction that make the molecules susceptible  
10 to decomposition and instability. This could be the reason why the current exploration  
11 of neural tracers for parasympathetic nerves is still in the early stages. Despite this  
12 negative impact, the use of radiotracers in neuroscience has greatly improved our  
13 neurological understanding of the central and peripheral nervous systems. In an  
14 environment where diagnostic molecular tracers are showing a spurt in development as  
15 the means of imaging technology continue to evolve, it is on the basis of the unique  
16 advantages of radioactive tracers themselves in accurately identifying nerve  
17 reinnervation that we need to be more refined and quantified in organ transplantation  
18 research. Here we find out that the *N*-[3-bromo-4-(3-<sup>18</sup>F-fluoro-propoxy)-benzyl]-  
19 *guanidine* (LMI1195) tracer is expected to be one of the most promising sympathetic  
20 tracers, and existing studies have successfully assessed the heterogeneity of cardiac  
21 innervation in patients by quantifying the myocardial uptake of norepinephrine  
22 transporter protein in specific regions(Sinusas et al., 2014; Higuchi et al., 2015).  
23 Another novel tracer, <sup>11</sup>C-GMO, has an excellent kinetic uptake rate and has been  
24 shown to have a half-life of 217 hours in myocardial sympathetic neurons of rats, 100  
25 times the half-life of <sup>123</sup>I-mIBG. It is the stable and prolonged metabolism of <sup>11</sup>C-GMO  
26 that can be used to finely measure early cardiac sympathetic reinnervation  
27 processes(Raffel et al., 2013). We therefore venture to hypothesize that future studies  
28 could focus on quantifying the density of regenerating nerves in transplanted organs,  
29 which could help predict the prognosis and diagnostic assessment of organ transplant  
30 patients. In conclusion, our review elucidates the reinnervation of heart, liver, and

1 kidney transplants with the support of imaging techniques, providing clinicians with  
2 diagnostic tools and guidelines in the field of nerve regeneration.

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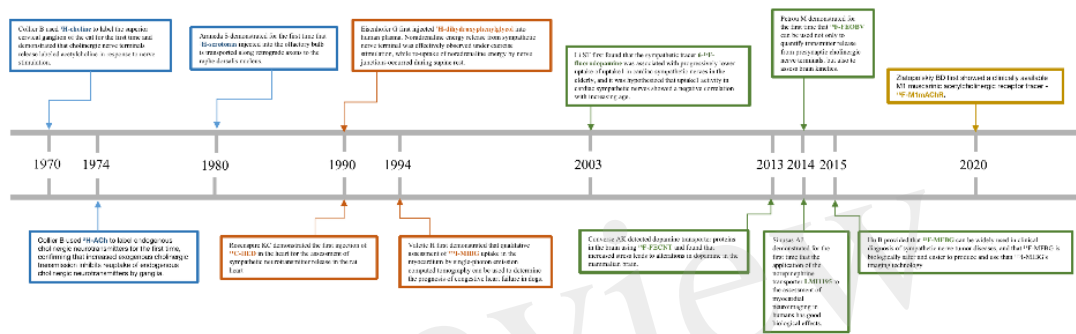
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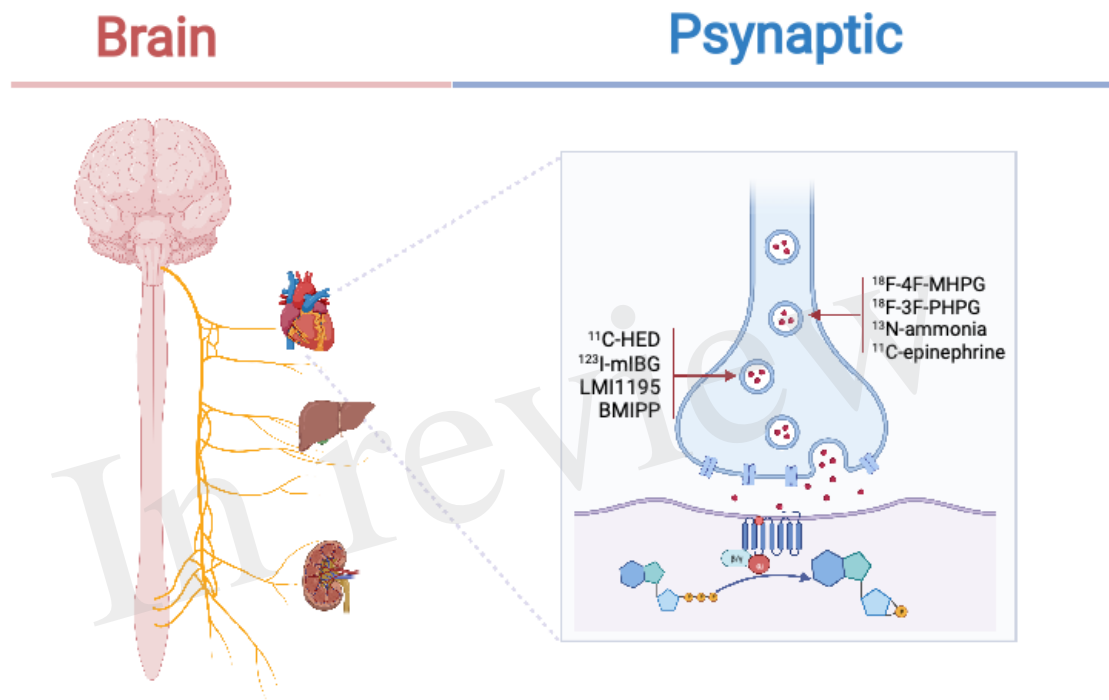
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Figure 1: Depicts a timeline of the development of radiotracers.

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9 **Figure2 Depicts the present state of sympathetic innervation of transplanted**  
10 **hearts.** Labeling of commonly used radioactive neurotransmitters in the presynaptic  
11 membrane, such as  $^{11}\text{C}$ -hydroxyephedrine( $^{11}\text{C}$ -HED), Iodine-123-meta-  
12 iodobenzylguanidine ( $^{123}\text{I}$ -mIBG), *N*-[3-bromo-4-(3- $^{18}\text{F}$ -fluoro-propoxy)-benzyl]-  
13 **guanidine (LMI1195)**,  $^{123}\text{I}$ -15-(*p*-iodophenyl)-3-*R,S*-menthyl pentadecanoic acid  
14 (BMIPP), 4- $^{18}\text{F}$ -fluoro-meta-hydroxyphenethylguanidine ( $^{18}\text{F}$ -4F-MHPG), 3- $^{18}\text{F}$ -  
15 fluoro-para-hydroxyphenethylguanidine ( $^{18}\text{F}$ -3F-PHPG),  $^{13}\text{N}$ -ammonia,  $^{11}\text{C}$ -  
16 epinephrine. This figure was created using the biorender application, (Biorender.com)





1 Table 1 Describes the advantages and disadvantages of traditional neural tracers, viral tracers, and radioactive tracers currently in use.

Species	Product	Advantages	Disadvantages
Traditional neural tracers	Horseradish peroxidase	<ul style="list-style-type: none"> <li>① more sensitive.</li> <li>② Only fluorescent microscopy and electron microscopy are required for observation.</li> </ul>	<ul style="list-style-type: none"> <li>① Involved in cellular metabolism.</li> <li>② Unstable and easily degraded.</li> <li>③ Restricted conditions of use.</li> </ul>
	Cholera toxin B		
	Fluoro-Gold		
	Wheat germ agglutinin		
Viral tracers	Adeno-associated viral vector	<ul style="list-style-type: none"> <li>① High sensitivity, directionality and selectivity compared to traditional neural tracers.</li> <li>② Highly infectious.</li> <li>③ No attenuation of the signal transmission of neural tracers</li> </ul>	<ul style="list-style-type: none"> <li>① High cytotoxicity.</li> <li>② For scientific investigation only.</li> </ul>
	Rabies virus		
	Herpes simplex virus		
Radioactive tracers	Commonly used radioisotopes are carbon-11, nitrogen-13, oxygen-15, iodine-131 and iodine-135	<ul style="list-style-type: none"> <li>① High sensitivity, safe and radiation-free.</li> <li>② Simple detection means, suitable for in vivo</li> </ul>	<ul style="list-style-type: none"> <li>① The need for a dedicated laboratory.</li> <li>② The need for specialist technicians.</li> </ul>

In review

		<p>experiments and clinical diagnosis.</p> <p>③ Non-cytotoxic.</p> <p>④ Conforms to the normal physiological conditions of the organism and is metabolizable.</p> <p>⑤ More precise localization, widely used for cardiovascular diseases and tissues and organs.</p>	<p>③ Protection is sometimes required.</p>
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1 Table 2 The use of clinically available radiotracers in heart and renal transplantation

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Organ transplantation	Nerve tracer	Technology	Nerve reinnervation	Nerve regeneration time	Reference
Heart transplantation	$^{11}\text{C}$ -hydroxyephedrine( $^{11}\text{C}$ -HED)	PET/CT	Sympathetic nerve	1 years	(Schwaiblmair et al., 1999; Bengel et al., 2001)
Heart transplantation	$^{11}\text{C}$ -hydroxyephedrine( $^{11}\text{C}$ -HED)	PET/CT	Sympathetic nerve	5 years	(Uberfuhr et al., 2000)
Heart transplantation	Iodine-123-meta-iodobenzylguanidine ( $^{123}\text{I}$ -mIBG)	SPECT	Sympathetic nerve	2 years	(Estorch et al., 1999)
Renal transplantation	Iodine-123-meta-iodobenzylguanidine ( $^{123}\text{I}$ -mIBG)	SPECT	Sympathetic nerve	6 months	(Rasmussen et al., 2020)

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4 In clinical studies, Iodine-123-meta-iodobenzylguanidine ( $^{123}\text{I}$ -mIBG) and  $^{11}\text{C}$ -hydroxyephedrine( $^{11}\text{C}$ -HED) are the most widely used radionuclide  
 5 tracers for sympathetic nerve regeneration in cardiac transplants.  $^{123}\text{I}$ -mIBG has also shown significant tracer effects in sympathetic nerve  
 6 regeneration in renal transplants. We found that cardiac sympathetic reinnervation was observed as early as 1 year after heart transplantation and  
 7 renal sympathetic reinnervation 6 months after renal transplantation.

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Table 3 The targeted molecular tracers currently used clinically in autonomic nervous system diseases

Application	Nerve tracer	Technology	Target	Reference
Cardiomyopathy	4- <sup>18</sup> F-fluoro-meta-hydroxyphenethylguanidine ( <sup>18</sup> F-4F-MHPG), 3- <sup>18</sup> F-fluoro-para-hydroxyphenethylguanidine ( <sup>18</sup> F-3F-PHPG)	PET/CT	Sympathetic nerve	(Raffel et al., 2018; Raffel et al., 2022)
Myocardial infarction	<sup>13</sup> N-ammonia, <sup>11</sup> C-epinephrine	PET	Sympathetic nerve	(Sasano et al., 2008)
Ischemic cardiomyopathy	Flubrobenguane (FBBG)	PET	Sympathetic nerve	(Zelt et al., 2021)
Heart failure	<i>N</i> -[3-bromo-4-(3- <sup>18</sup> F-fluoro-propoxy)-benzyl]-guanidine (LMI1195)	PET	Sympathetic nerve	(Sinusas et al., 2014; Higuchi et al., 2015; Chen et al., 2018)
Vasospastic angina	Iodine-123-meta-iodobenzylguanidine ( <sup>123</sup> I-mIBG), <sup>123</sup> I-	PET	Sympathetic nerve	(Watanabe et al., 2002)

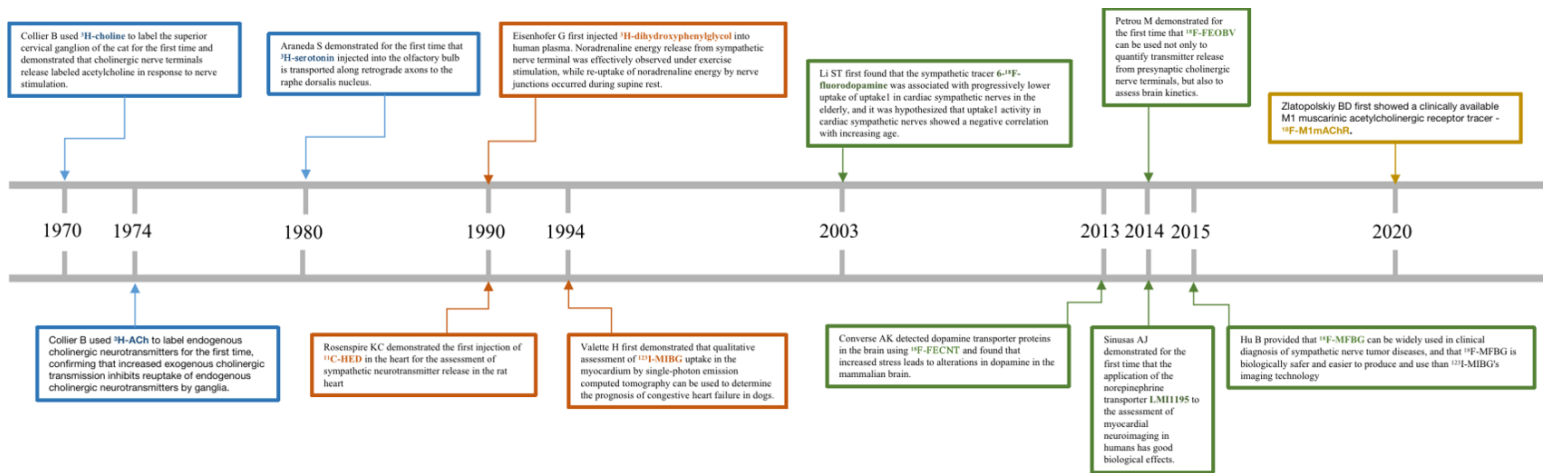
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	15-( <i>p</i> -iodophenyl)-3- <i>R,S</i> -menthyl pentadecanoic acid (BMIPP)			
Alzheimer's disease	(-)- <sup>18</sup> F-fluoroethoxybenzovesamicol ( <sup>18</sup> F-FEOBV)	PET	Presynaptic cholinergic	(Mulholland et al., 1998; Petrou et al., 2014)
Hepatocellular carcinoma	<sup>14</sup> C-Cho	PET	Choline	(Kuang et al., 2010)
Prostate carcinoma, Parkinson's disease	<sup>11</sup> C-donepezil	PET	Parasympathetic nerve	(Gjerløff et al., 2015; Nielsen et al., 2019)

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Figure 1.TIF

In review



## Brain

## Psynaptic

