

Figure 1. Pancreas sections, 10µm-thick, from Sprague-Dawley rats were incubated with 0.185 MBq/mL ¹⁸F-fallypride in incubation buffer (50 mM Tris HCI buffer, pH 7.4, containing 120 mM NaCl, 5 mM KCl, 2 mM CaCl₂, 1 mM MgCl₂, 1 mM NaEDTA, and 0.1 mM sodium ascorbate, pH 7.4) in the absence or presence of 100µM haloperidol for 1 hour in a 37°C. Sections were washed twice with equal volumes of cold incubation buffer, after which the slides were quickly air-dried then exposed to phosphor screens over night and activity detected by autoradiography. The total ¹⁸F-fallypride binding in sections measured in digital light units per mm² (DLU/mm²) (n=4) is shown. * p<0.05

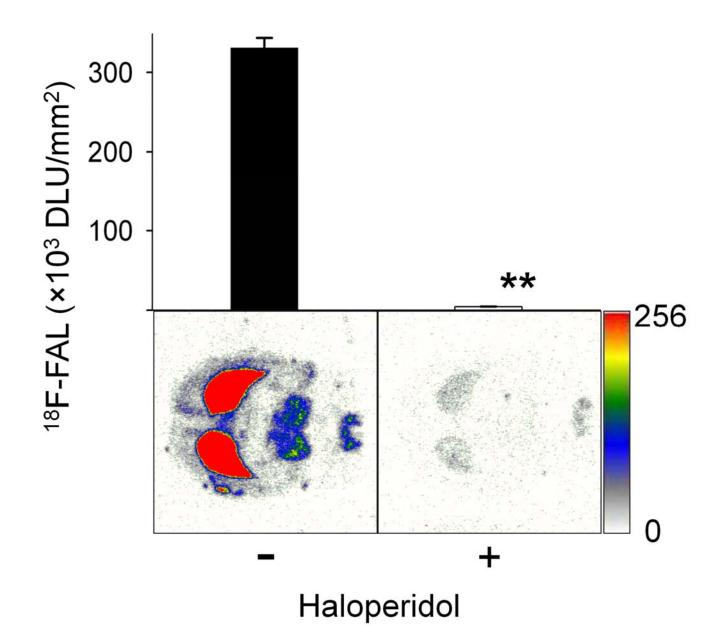


Figure 2. Autoradiographic images of fresh-frozen rat brain sections (10 μ m) incubated with 0.185 MBq/mL ¹⁸F-fallypride in the presence or absence of 100 μ M haloperidol. The total ¹⁸F-fallypride binding activity over striatum was measured in digital light units per mm² (DLU/mm²) (n=4) is shown. ** p<0.001

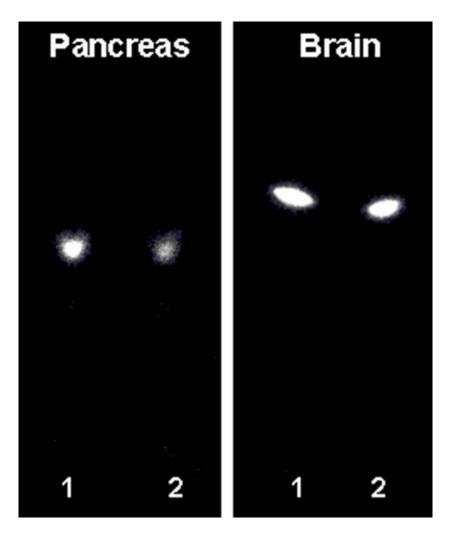


Figure 3. Autoradiographic images of thin-layer chromatography of ethyl acetate extracts of rat pancreatic and brain tissues 3 hrs post ¹⁸F-fallypride injection (28-37 MBq). Organs were procured after cardiac perfusion with 10 mL phosphate buffered saline. For characterization of ¹⁸F-fallypride in tissues, brain and pancreas were taken separately and homogenized in 2 mL of 1.15% KCl buffer by a Tekmar tissue homogenizer at half maximal speed. To each homogenate, 2 mL of a methanol solution containing 2% acetic acid was added and solutions were vortexed. Aliquots of 1.5 mL from each tube were removed and reference ¹⁸F-fallypride was added (~1-4 kBq). Solutions were then vortexed. Tissue homogenate aliquots with and without reference ¹⁸F-fallypride were centrifuged at 10,000xg for 10 min. Supernatants were removed, concentrated, and spotted on silica thin layer chromatographic (TLC) plates (Silica Gel IB2-F, 2.5x7.5 cm; J.T. Baker) and eluted with (9:1) dichloromethane:methanol. Plates were then exposed to phosphor screens and autoradiograms

THE JOURNAL OF NUCLEAR MEDICINE • Vol. 52 • No. 7 • July 2011 Garcia et al.

show: *lane 1*, Reference ¹⁸F-fallypride was added to tissue extracts; *lane 2*, Extracted ¹⁸F-fallypride in the same tissue from rats administered ¹⁸F-fallypride.

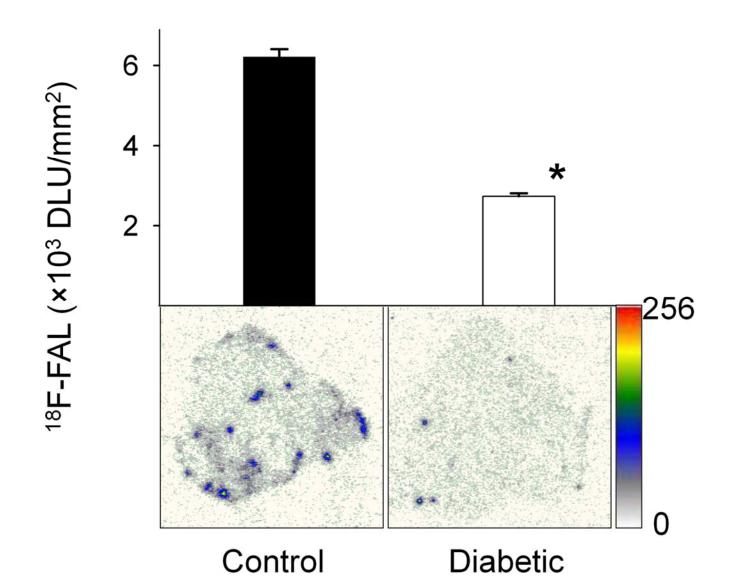


Figure 4. Autoradiographic images of fresh-frozen rat pancreatic sections (10 μ m) from control and diabetic rats incubated with 0.185 MBq ¹⁸F-fallypride (n=4 in each case) and activity over the sections were measured in digital light units per mm² (DLU/mm²). Chemical destruction of islets decreases ¹⁸F-fallypride binding to pancreas. * p<0.01

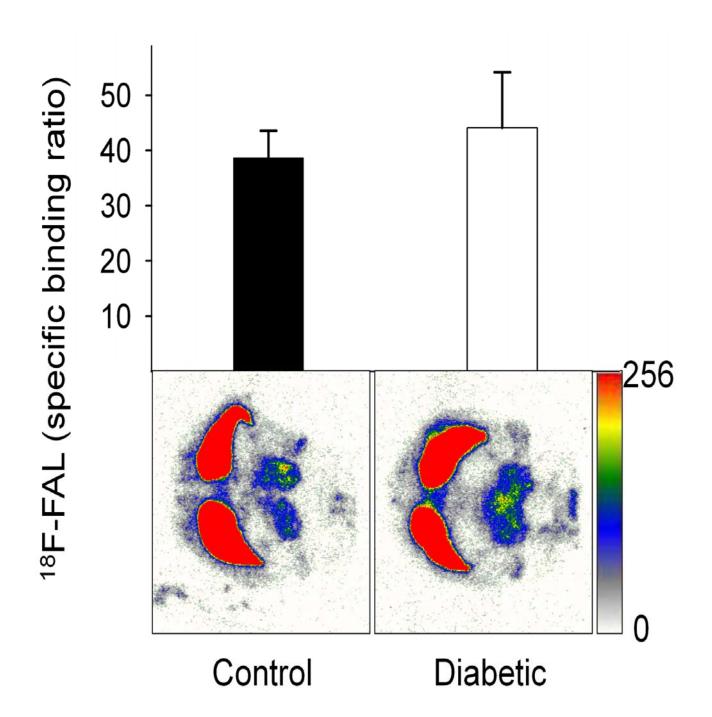


Figure 5. Autoradiographic images of fresh-frozen horizontal brain sections of control and diabetic rats. Specific binding ratio (striatum activity-cerebellum activity) / cerebellum activity) was computed for ¹⁸F-fallypride (n=4). Black and white bars indicate ¹⁸F-fallypride binding ratio in control and diabetic animals.

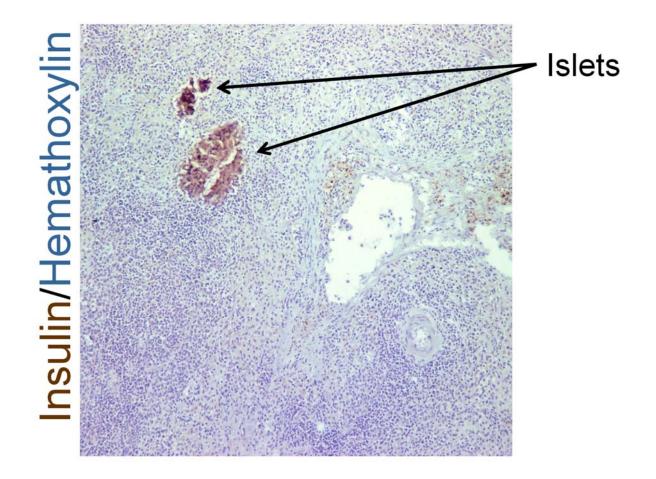


Figure 6. Immunostaining and hematoxylin staining: Frozen pancreas and spleen sections, 10µm thick were fixed in 4% paraformaldehyde in PBS for 10 min and endogenous peroxidase quenched in 1.5% hydrogen peroxide for 15 min. Insulin staining was accomplished using 1:50 of an HRP conjugated insulin affibody (Abcam cat. #: ab31907) for 45 min at room temperature. The HRP signal was detected with DAB Peroxidase Substrate Kit (Vector Labs, cat # sk-4100). Tissues were then counter stained with Gill's hematoxylin 2x per (as per manufacturers instructions) for 5 min, differentiated with 1-2 dips in acid alcohol (1% HCl in 70% EtOH), blued in 0.1% sodium bicarbonate solution and dehydrated to 100% ethanol. Slides were then mounted with permount and glass coverslips. Figure shows Insulin staining of spleen sections after completion of imaging study showing presence of grafted islets.

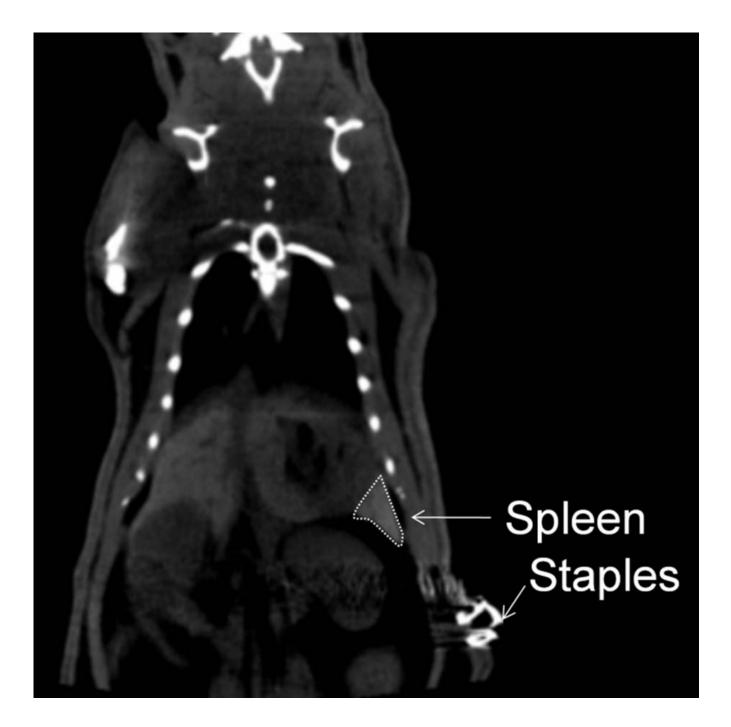


Figure 7. Contrast MicroCT imaging of the spleen in the islet-transplanted animal. EXIA 160-XL was injected i.v. 30 min prior to microCT imaging of abdomen. A 10 min scan revealed accumulation of contrast in the spleen and was detectable among other abdominal organs in all projections.