Supplementary Information Online:  
Methods  

_Denervation of the_  
_Retroperitoneal Fat Pad_  

As part of:  
Tracing from fat tissue, liver and pancreas:  
A neuroanatomical framework for the role of the brain in type 2 diabetes  

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Fat denervation for tracing studies

The autonomic supply of the retroperitoneal fat pad in male Wistar rats is characterized by a. diffuse sympathetic nerve fibers, from lateral and dorsal directions, and b. one single vagal nerve bundle traveling along blood vessels from the diaphragm to the superior tip of the fat pad.

Sympathetic denervation

1. The abdominal cavity is opened in such a way that retroperitoneal fat pad, kidney and part of the diaphragm are visible (figure 1). The vagal fibers run along an artery and vein from the diaphragm to the tip of the fat pad (green). Multiple small sympathetic fibers arise from medial and dorsal directions, three large sympathetic fibers enter the fat pad medially (red).^1^  
2. The retroperitoneal fat pad is cleaved at the level of the kidney and carefully removed from the kidney, dorsal muscles, abdominal wall and connective tissues, working from caudal to rostral. For this step, microsurgery technique is obligatory to warrant the integrity of the sectioned fat pad, which is moved laterally to expose the vagal and superior sympathetic fibers (figure 2a, detail figure 2b).  
3. Finally, the superior sympathetic fibers are cut in a way that only a bundle of vagal fibers and adjacent blood vessels remain as a connection between the fat pad and the body of the animal (figure 3).  
4. The fat pad is lifted up and inspected for residual nerve bundles^2^ (figure 4).  
5. After the denervation the tracers are injected into the fat pad. This procedure demands an intact, undamaged fat pad, since placement of one bolus of tracer in the superior part of the fat pad without leakage is necessary to achieve successful tracing.

Parasympathetic denervation

The vagal denervation procedure is the opposite of the sympathetic denervation: here all adjacent fibers of the blood vessels entering the superior tip (figures 1-3) of the retroperitoneal fat pad are removed.
fig 2a

Vagal

Sympathetic

Retroperitoneal fat pad
fig 3

Vagal

Sympathetic (cut)

Retroperitoneal fat pad
Literature