CHAPTER 4

INNERVATION OF THE PREGNANT RAT UTERUS

Uterine horns from 4 rats in the 20th day of a 21-22 day pregnancy were used in this portion of the project.

The average weight of the near-term pregnant uterine horns was 2.58 + 0.52 (mean + SD; n = 6). Their mean area, mean length and mean width were 82.07 + 12.13 cm^2, 24.3 + 4.62 cm and 3.37 + 0.15 cm, respectively. Two uterine horns were excluded from these measurements because there were portions of the horns in which there were no foetuses. Foetuses were present in both uterine horns in all 4 rats. The number of foetuses in each horn varied between 3 and 11. The average fetal weight was 4.00 + 0.17 g. In the two horns that were not completely filled with foetuses, the fertilized eggs had implanted at the cervical end of the uterus, leaving the ovarian ends empty.

Pregnant uteri stretched more than the non-pregnant uteri and were also larger in size. In whole mount preparations, it was clear that the pregnant uterine wall was much thinner than the non-pregnant, probably be due to the increased "stretchiness" of the pregnant uteri. In the two horns that were not full of foetuses, the portions of the horn without foetuses did not stretch as much as portions containing foetuses.

Unpaired student t-tests were done to compare the weights and areas of the non-pregnant and pregnant uteri. The difference in weight of uteri was not statistically significant ($P > 0.05$) but the difference in the area of the uteri was statistically highly significant ($P \leq 0.01$). This significant increase in area indicates that pregnant uteri stretch much more than the non-pregnant uteri. As a result, whole mounts of the pregnant uterine horns were thinner than the whole mounts of non-pregnant horns.

Although linea uteri were a prominent feature of the non-pregnant rat uterus, they were not apparent in the four 20-day pregnant rats in this study.

Sympathetic innervation of the pregnant rat uterus

TH- and NPY-immunoreactivity were used to mark sympathetic nerves in pregnant as well as non-pregnant uteri. Very substantial reductions in both TH- and NPY-immunoreactive axons were observed in the pregnant rat uterus at day 20 of a 21-22 day pregnancy.

Tyrosine Hydroxylase (TH)

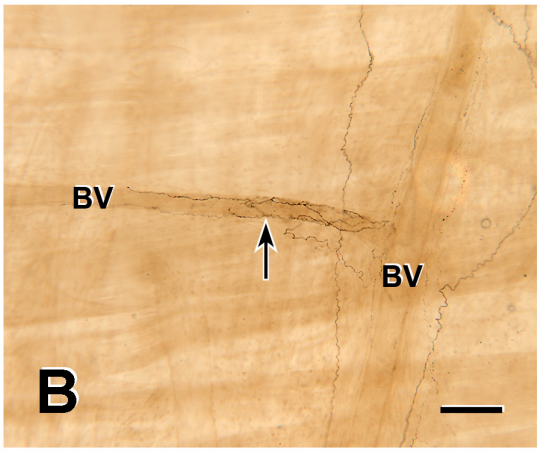
The number of TH-immunoreactive axons was very significantly reduced in the pregnant uterus. A few axons in bundles entered the uterus at the mesometrium along with blood vessels or, in some instances, alone (Figure 4.1 A). There were very few non-varicose TH axons associated with blood vessels and these gradually reduced in number so that there were none on the anti-mesometrial side in the ovarian region of the uterus (Figure 4.1 B & 4.1D). In the cervical region, however, rare TH axons were seen on the anti-mesometrial side. In this region, also, the density of TH-positive axons decreased from the mesometrial side to the anti-mesometrial side (Figure 4.1C). The very few nerve bundles with staining lay along blood vessels and contained mostly non-varicose TH-immunoreactive axons. The number of TH-positive axons in each of these bundles was very much reduced compared to the non-pregnant rat uterus. Rare non-varicose TH axons travelled from blood vessels to the endometrium.

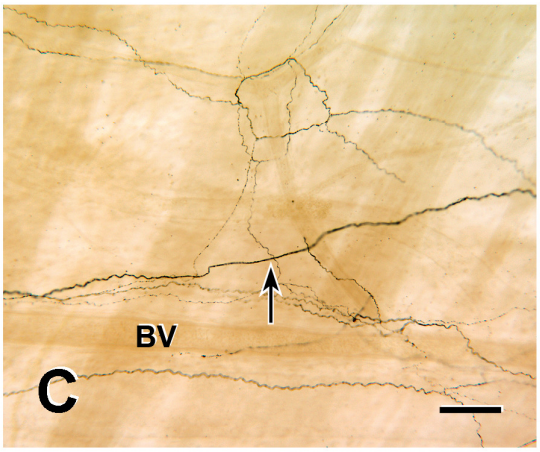
More TH-immunoreactive axons were present at the cervical end of the pregnant uterus than at the ovarian end. At the cervical end, TH-positive axons were found on both the mesometrial and anti-mesometrial sides. At the ovarian end, TH-containing axons only

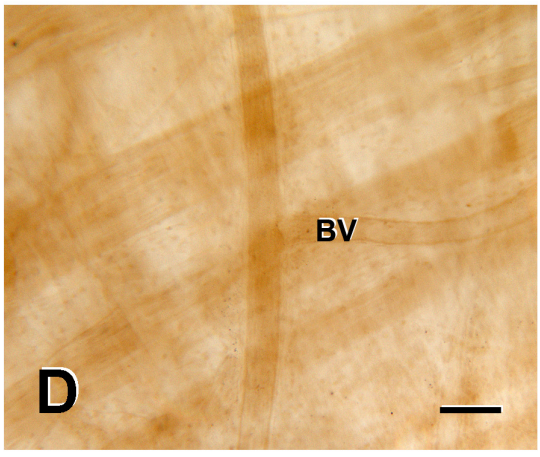
FIGURES 4.1A - 4.1D TH innervation in the pregnant rat uterus

A, Full thickness whole mount preparation (3 cm × 1.5 cm) from the ovarian end of pregnant uterine horn stained for TH. Letters indicate the location of the micrographs shown in 1B and 1D. Bar, 5 mm . **B**, TH-immunoreactive axons around blood vessels in the mesometrial region gradually disappear at the ovarian end of pregnant uterine horn. Bar, 100 µm. **C**, TH-positive axons are slightly higher in density at the cervical end of the pregnant uterine horn compared to the ovarian end. Bar, 100 µm. **D**, Anti-mesometrial region at the ovarian end of pregnant uterine horn with no TH-immunoreactive axons. BV= blood vessel. Bar, 100 µm.

TH : Pregnant







occurred on the mesometrial side and were completely absent on the anti-mesometrial side.

Neuropeptide Y (NPY)

NPY-immunoreactive axons were also very significantly reduced in the pregnant uterus (Figure 4.2A). Rare non-varicose NPY-positive axons entered the uterus along with blood vessels or travelled alone at the mesometrium. The density of NPY innervation was less than that of the TH innervation in the pregnant uterus. Compared to the dense plexus of NPY-immunoreactive axons surrounding blood vessels in the non-pregnant uterus, the perivascular plexus of axons immunoreactive for NPY in the pregnant uterus was very significantly reduced in density. In the pregnant uterus, the perivascular NPY axons were mostly non-varicose (Figure 4.2B & 4.2C). The non-pregnant uterus also contained a network of thick nerve bundles that contained NPY axons and were not associated with blood vessels. This plexus was absent in the non-pregnant uterus. Almost all blood vessels in the pregnant uterus also completely lacked innervation. (Figure 4.2D)

NPY axons were present in similarly very low density in the ovarian and cervical regions of the pregnant uterus. In the ovarian region NPY axons were found only at the mesometrial side but the anti-mesometrial side of the cervical region contained a few NPY axons.

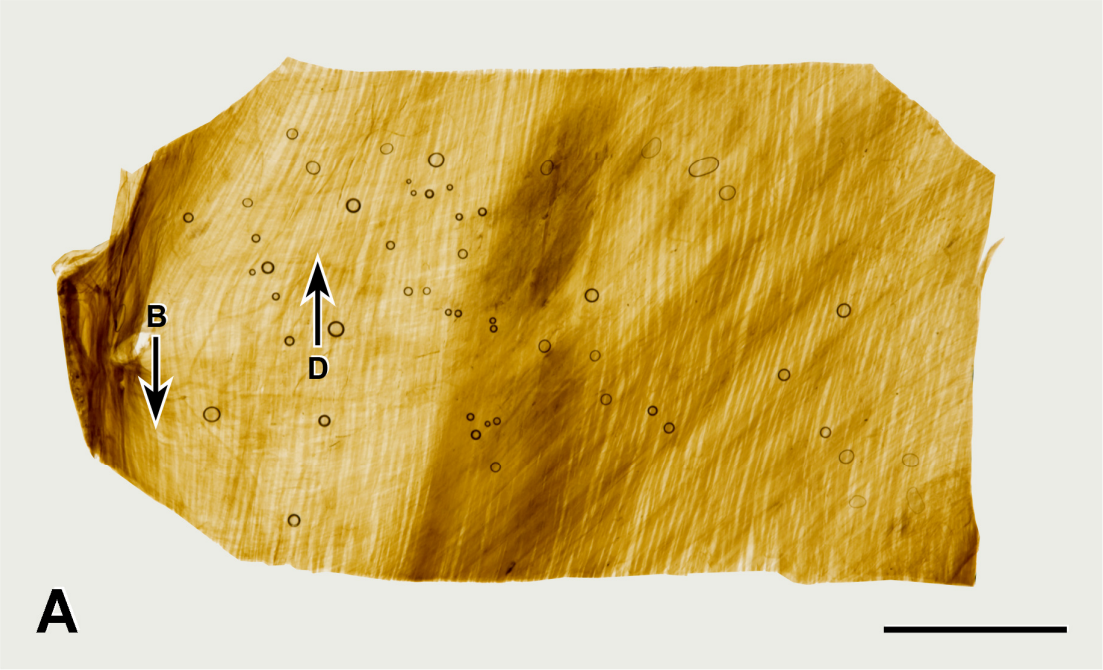
Sensory innervation of the pregnant rat uterus

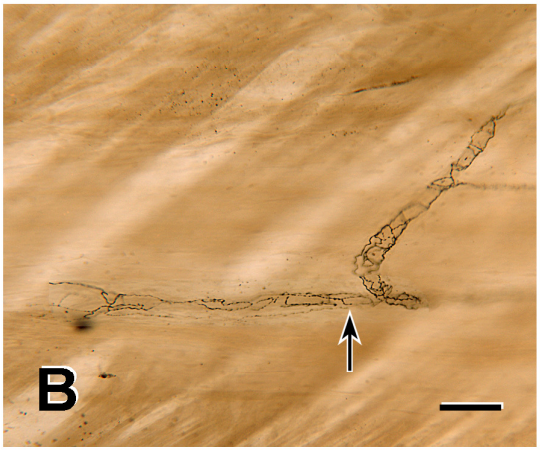
Immunoreactivity for calcitonin gene-related peptide (CGRP) and substance P (SP) were used to identify sensory nerves in pregnant uteri as well as in the non-pregnant uteri. A very significant reduction in both CGRP- and SP-immunoreactive axons was observed in the ovarian region of the pregnant rat uterus at day 20 of a 21-22 day pregnancy. The cervical region of the pregnant rat uterus was not studied.

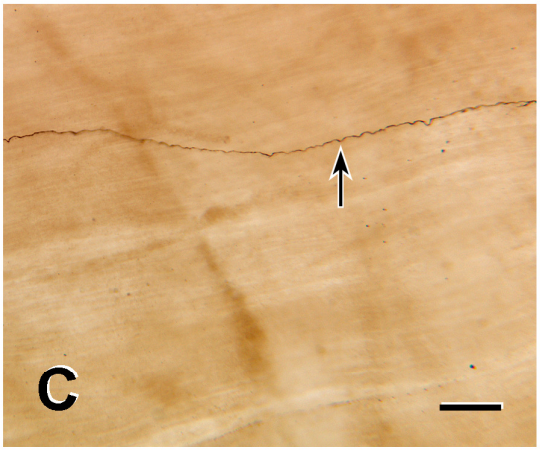
FIGURES 4.2A - 4.2D NPY innervation in the pregnant rat uterus

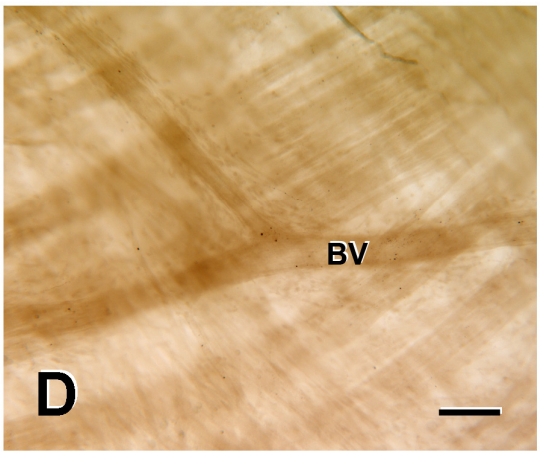
A, Full thickness whole mount preparation (3 cm × 1.5 cm) from the ovarian end of pregnant uterine horn stained for NPY. Letters indicate the location of the micrographs shown in2B and 2D. Bar, 5 mm. **B**, Montage of two different focal planes showing NPY-positive axons around blood vessels in the mesometrial region of the ovarian end of pregnant uterine horn. Bar, 100µm. **C,** NPY-positive axon at the cervical end of the pregnant uterine horn. Bar, 100 µm. **D**, Anti-mesometrial region of the ovarian end of pregnant uterine horn with no NPY-immunoreactive axons. BV= blood vessel. Bar, 100 µm.

NPY : Pregnant









Calcitonin Gene-Related Peptide (CGRP)

Very few CGRP-immunoreactive axons were found in the near-term pregnant uterus (Figure 4.3A). These rare non-varicose CGRP axons entered the uterus in bundles that were not associated with blood vessels. Rare nerves immunoreactive for CGRP were found on the mesometrial side of the pregnant uterus (Figure 4.3B) but no nerves were seen on the anti-mesometrial side (Figure 4.3C). CGRP axons were not encountered around blood vessels.

Substance P (SP)

A massive reduction in the number and density of SP-immunoreactive axons was seen in the pregnant rat uterus (Figure 4.4A). Rare SP-positive axons entered the uterus from the mesometrium in nerve bundles that did not appear to be associated with blood vessels. Very few non-varicose SP axons occurred on the mesometrial side (Figure 4.4B) and none were seen in the anti-mesometrial side (Figure 4.4C). SP-containing axons were not found around blood vessels in the pregnant uterus.

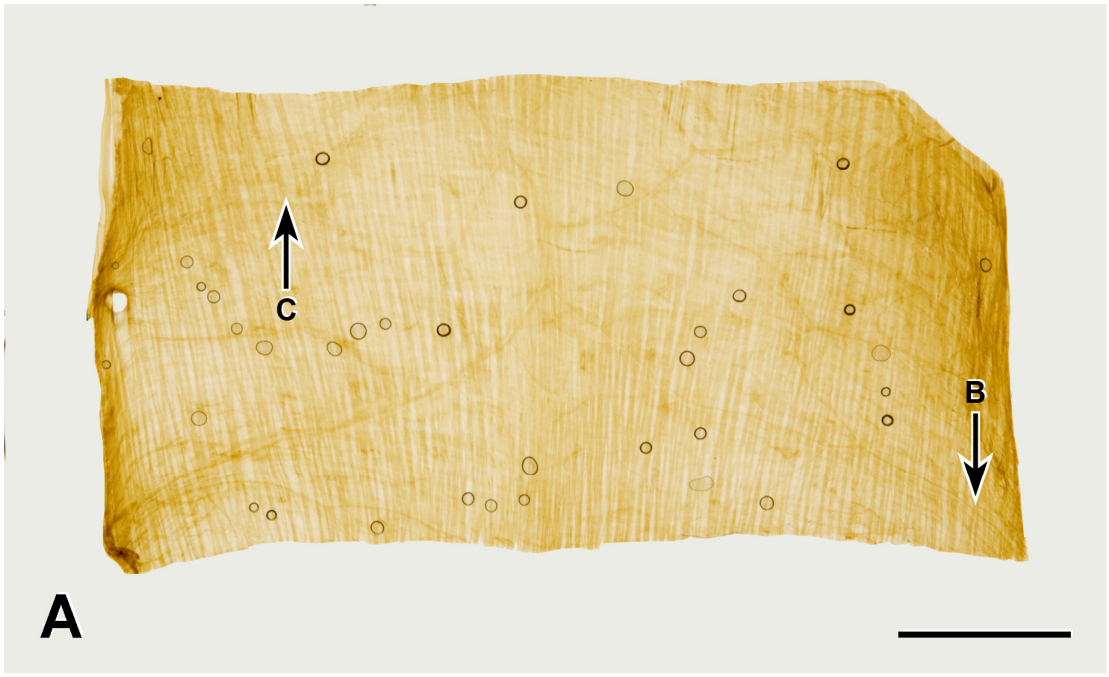
Parasympathetic innervation of the pregnant rat uterus

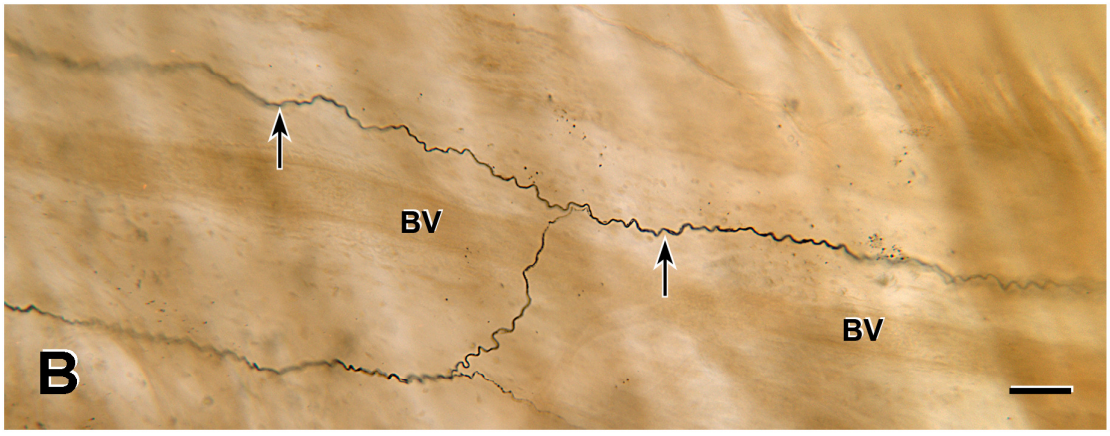
Parasympathetic nerves were examined in pregnant uteri immunohistochemically stained for nitric oxide synthase (NOS) and vesicular acetylcholine transporter (VAChT) immunoreactivity. A large reduction in both NOS- and VAChT-immunoreactive axons was observed in the ovarian region of the pregnant rat uterus at day 20 of a 21-22 day pregnancy. The cervical region of the pregnant rat uterus was not studied.

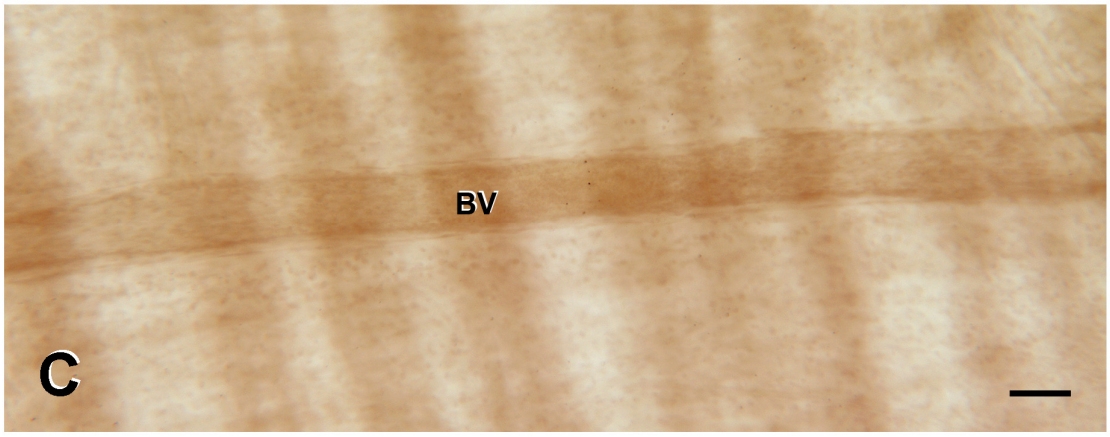
FIGURES 4.3A - 4.3D CGRP innervation in the pregnant rat uterus

A, Full thickness whole mount preparation (3 cm × 1.5 cm) from the ovarian end of pregnant uterine horn stained for CGRP. Letters indicate the location of the micrographs shown in 3B and3C. Bar, 5 mm. **B,** CGRP axons near a blood vessel but not associated with the blood vessel in the mesometrial region of the ovarian end of pregnant uterine horn. Bar, 100 µm. **C,** Anti-mesometrial region at the ovarian end of pregnant uterine horn with no CGRP-immunoreactive axons. Bar, 100 µm.

CGRP : Pregnant



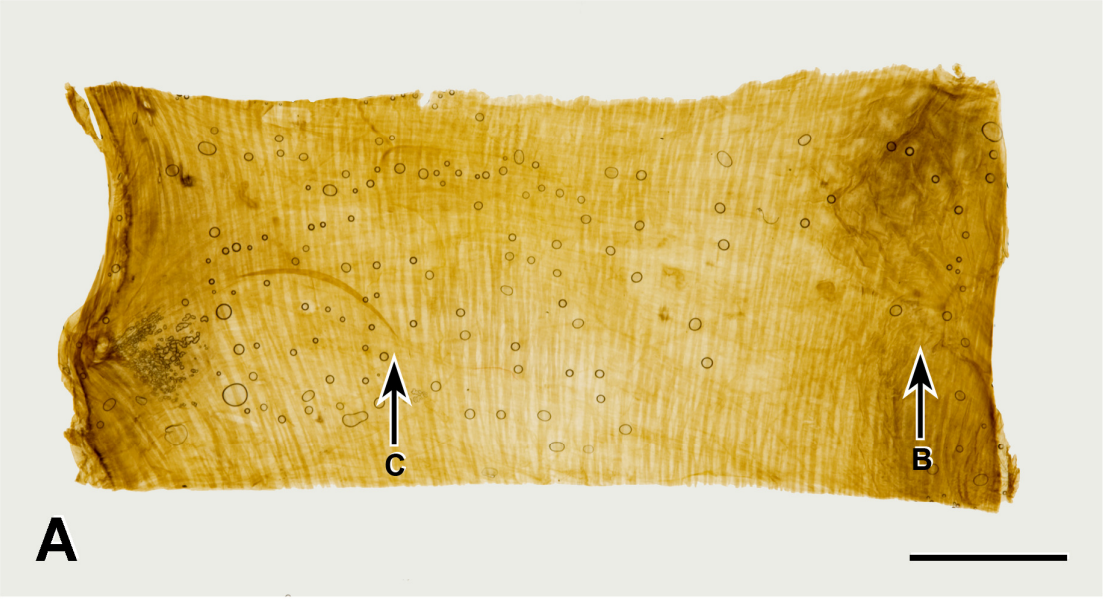


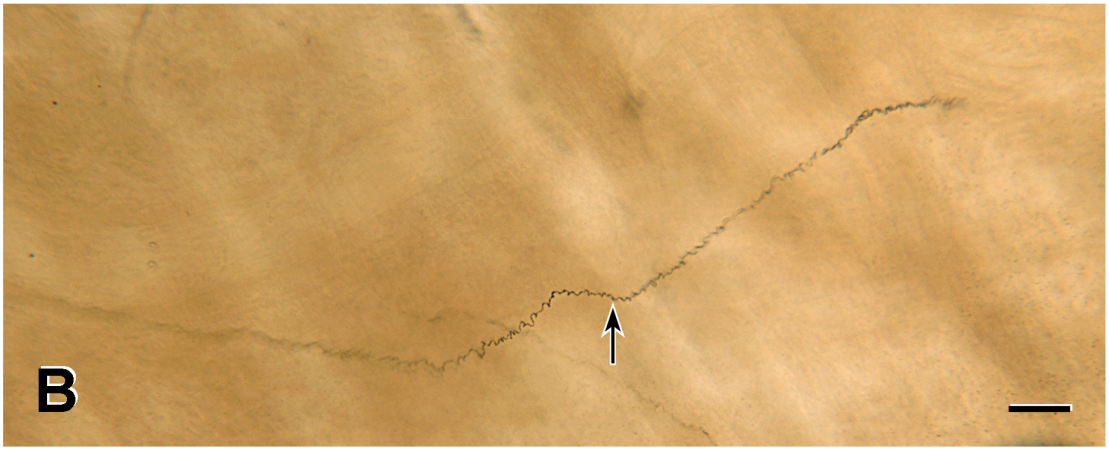


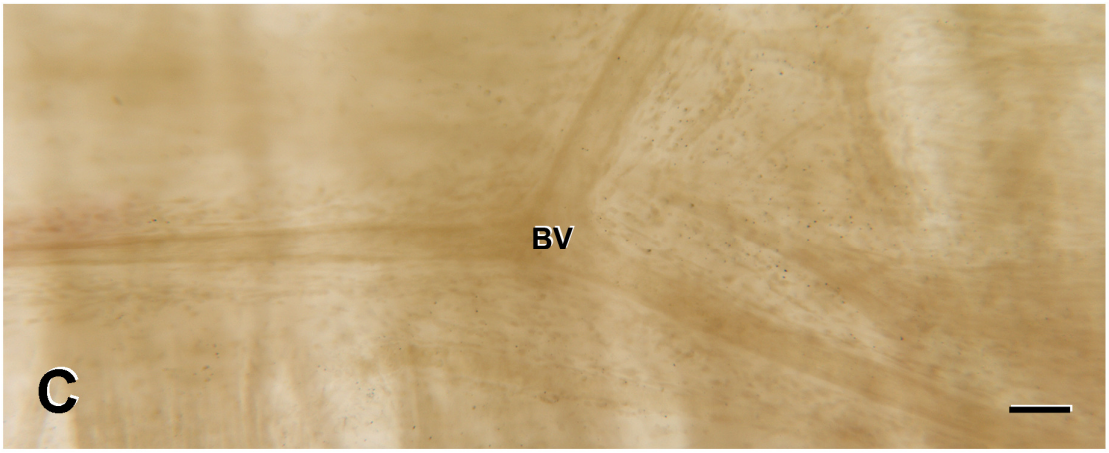
FIGURES 4.4A - 4.4C SP innervation in the pregnant rat uterus.

A, Full thickness whole mount preparation (3 cm × 1.5 cm) from the ovarian end of pregnant uterine horn stained for SP. Letters indicate the location of the micrographs shown in 4B and 4C. Bar, 5 mm. **B,** SP-positive axon in the mesometrial region of the ovarian end of pregnant uterine horn. Bar, 100 µm. **C,** Anti-mesometrial region at the ovarian end of pregnant uterine horn with no SP-immunoreactive axons. BV= blood vessel. Bar, 100 µm.

SP : Pregnant







Nitric Oxide Synthase (NOS)

Pregnancy significantly reduced the density of NOS-immunoreactive axons in the uterus (Figure 4.5A). A few non-varicose NOS-positive axons were seen on the mesometrial side (Figure 4.5B) but the anti-mesometrial side lacked NOS innervation (Figure 4.5C). There were fewer NOS-positive axons in the pregnant uterus than TH- and NPY-immunoreactive axons. A few NOS axons were seen near blood vessels but they did not form a perivascular plexus in the pregnant uterus as they did in the non-pregnant uterus. NOS-immunoreactive axons entered the pregnant uterus in bundles that did not appear to be associated with blood vessels.

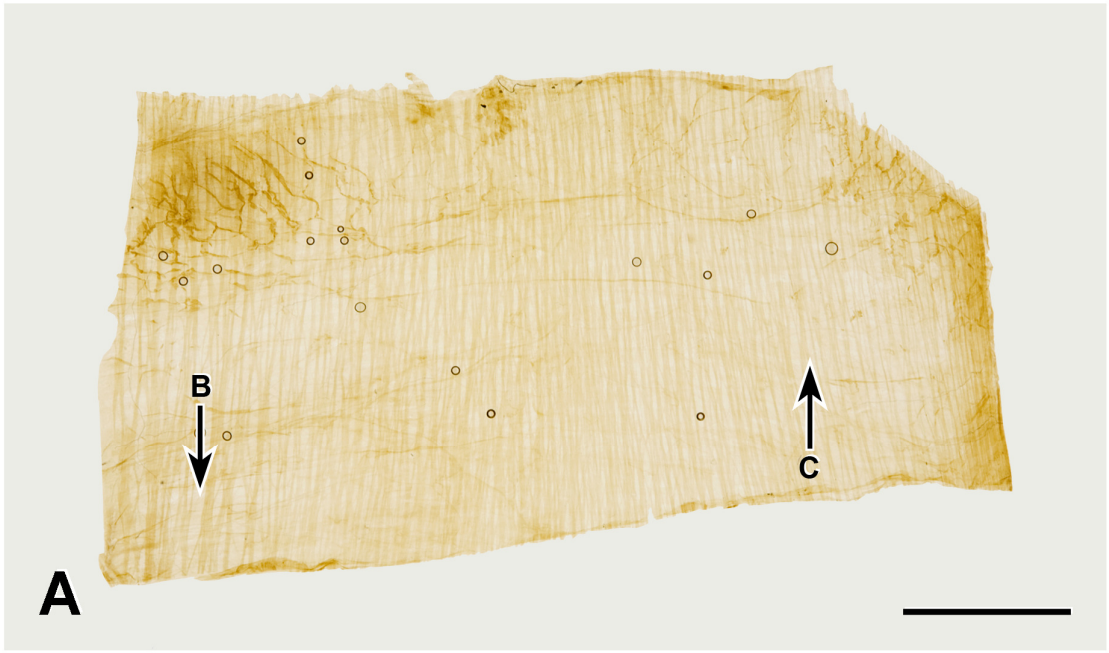
Vesicular Acetylcholine Transporter (VAChT)

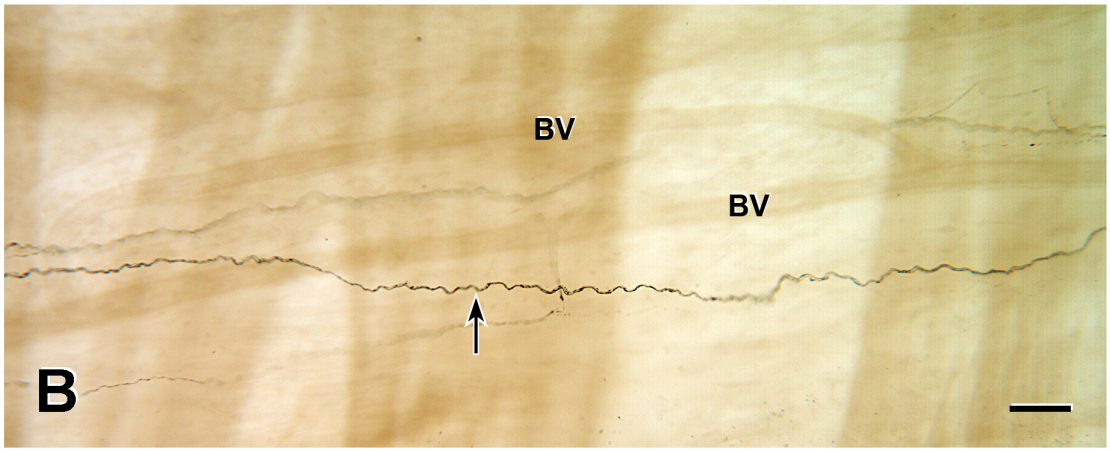
Pregnancy caused a large decrease in the density of VAChT innervation at the ovarian end of the uterus (Figure 4.6A). Very few non-varicose VAChT axons were seen in the pregnant uterus (Figure 4.6B). Of the six neurochemically-identified types of axons, the density of VAChT-containing axons in the pregnant uterus was lowest. A large proportion of the pregnant uterus did not show any immunoreactivity (Figure 4.6C). Like NOS-immunoreactive axons, VAChT-positive axons entered the pregnant uterus in bundles that did not appear to travel with blood vessels.

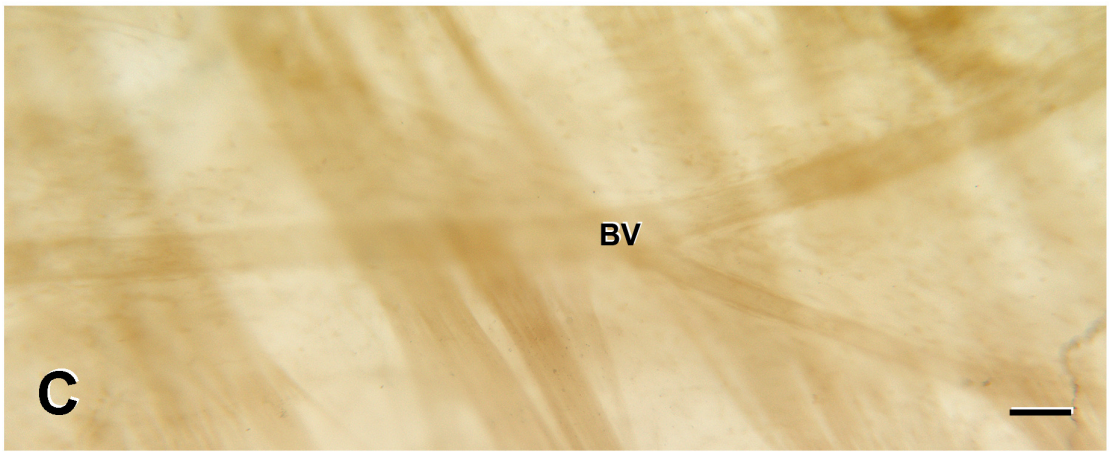
FIGURES 4.5A - 4.5C NOS innervation in the pregnant rat uterus

A, Full thickness whole mount preparation (3 cm × 1.5 cm) from the ovarian end of pregnant uterine horn stained for NOS. Letters indicate the location of the micrographs shown in Figures 5B and 5C. Bar, 5 mm. **B,** NOS-positive axons near blood vessel but not associated with the blood vessel in the mesometrial region of the ovarian end of pregnant uterine horn. Bar, 100 µm. **C,** Anti-mesometrial region at the ovarian end of pregnant uterine horn with no NOS-immunoreactive axons. BV= blood vessel. Bar, 100 µm.

NOS : Pregnant







FIGURES 4.6A - 4.6C VAChT innervation in the pregnant rat uterus.

A, Full thickness whole mount preparation (3 cm × 1.5 cm) from the ovarian end of a pregnant uterine horn stained for VAChT. Letters indicate the location of the micrographs shown in 6B and 6C. Bar, 5 mm. **B,** VAChT-positive axon in the mesometrial region at the ovarian end of a pregnant uterine horn. Bar, 100 µm. **C,** Anti-mesometrial region at the ovarian end of pregnant uterine horn with no VAChT-immunoreactive axons. BV= blood vessel. Bar, 100 µm.

VAChT : Pregnant