Development of the innervation of the urogenital complex in the fetal mouse

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Summary. The developing urogenital complex of the fetal mouse was studied by means of silver impregnation and electron microscopy. These studies showed that: 1) the mesonephric field is innervated during prenatal stages (Wolffian nerve); 2) nerve penetration precedes the differentiation of the gonads and related ducts; and 3) the Wolffian nerve arises during the earliest stages from the first pair of abdominal rami communicantes. The identity between the fetal Wolffian nerve and the nerve of the suspensory ligament (higher pathway) of the adult is discussed.
Key words. Innervation; urogenital complex; fetal mouse.

The autonomic nerve supply to the gonads and the genital tract has been extensively studied in adult mammals of both sexes[1-5]. In the female, three groups of ovarian-oviductal nerves have been recognized[6]. These groups of nerves arise from the renal, mesenteric and hypogastric plexuses: however, the center of origin of these nerve fibers has not yet been identified[7,8].

The extensive literature on the adult pattern of innervation contrasts with the general lack of information on the growth of nerves to the developing urogenital complex (UGC). Only one reference on the prenatal innervation of the human genital tract (lower pathways) was found[9]. Recently published researches on the structural components of the UGC as well as on the influence of the mesonephros on gonadal differentiation do not refer to the existence of nerves in the developing UGC[10-14].

The present investigation was undertaken to provide information which may help to answer the following questions: 1) Can an embryological study help to elucidate the primary source of the higher nervous pathways supplying the adult gonadal complex (via the suspensory ligament)? 2) Is the UGC innervated at prenatal stages? 3) Do nerves reach the UGC before differentiation of the gonads and their annexa?

For this purpose mouse fetuses were studied by means of silver impregnation and electron microscopy to trace the origin of nerves and to establish their sequence of growth during development. Some of the results have been published as an abstract[15].

Materials and methods. White mice (virgin females) were induced to ovulate by treatment with 5 IU PMS and 5 IU HCG, i.p., and mated afterwards. Ovulation time (12 h after HCG injection) was taken as the zero point to determine the age of fetuses (day stage, dst). Pregnant mothers were sacrificed by cervical dislocation at defined stages of gestation (10–19½ dst).

36 fetuses (10–19½ dst) were silver impregnated using the Ramón y Cajal and De Castro method[16]. The samples were paraffin-embedded and serial sections cut (10–15 µm) following either transverse or sagittal planes.

17 urogenital complexes (13–18 dst) were fixed in 2.5% buffered glutaraldehyde, postfixed in 1% buffered OsO_4 and embedded in araldite. Thick sections stained with methylene blue (light microscope) and thin sections stained with uranyl acetate (TEM) were used to determine the state of gonadal and ductal differentiation.

Results. Timing of nerve growth. Serial sections of silver-impregnated specimens showed no nerve fibers in the developing UGC before day 13½ (the quality of the silver impregnation in the youngest specimens was checked in the neighboring fields, i.e. the digestive tract).

At the 13½ dst (fig. 1a) the first fibers arising from the first pair of subdiaphragmatic rami communicantes (RCs) enter the mesenchymal pedicle of the Wolffian body (Wb). At this stage as well as at the 14½ dst (see below) the prevertebral chains are not fully formed, and both RCs (right and left) are united like a collar at the preaortic space. Migrating neuroblasts appear, scattered along the RCs trajectory (see figs 1a, b).

At the 14½ dst (fig. 1b) both the number and length of the Wolffian nerve (Wn) fibers have increased considerably, and they reach the mesenchymal bridge uniting the Wolffian and the Müllerian blastemata. Within the Wb the nerve bundle makes contact successively with the mesonephric tubules and with the Wolffian duct (Wd). Branching off has not started yet.

At the 15½ dst the UGCs have shifted caudally. In the female they are located retrolateral to the kidney, in the male the UGCs are located at both sides of the urogenital sinus. The Wn is longer than in preceding stages and may be divided for the purpose of description into two portions. A proximal part extends from the 1st abdominal chain ganglion to the UGC. At this stage the prevertebral chain shows a more mature pattern and the Wn is no longer seen coming out from the rami communicantes. This first part of the nerve trajectory occurs within a mesenchymal ribbon which is attached to the outer face of the kidney. This ribbon is continuous with the stalk of the Wb.

Inside the Wolffian body (distal portion) the main bundle and its branches distributes among the tubular components of the region, maintaining a close relation to the basement membrane of the ducts (fig. 2). The main fascicle ends in the Müllerian field forming a loop around the Müllerian duct (fig. 3).

The Wn bundle and its branches seems to end as isolated fibers among the mesenchymal cells surrounding the ducts without

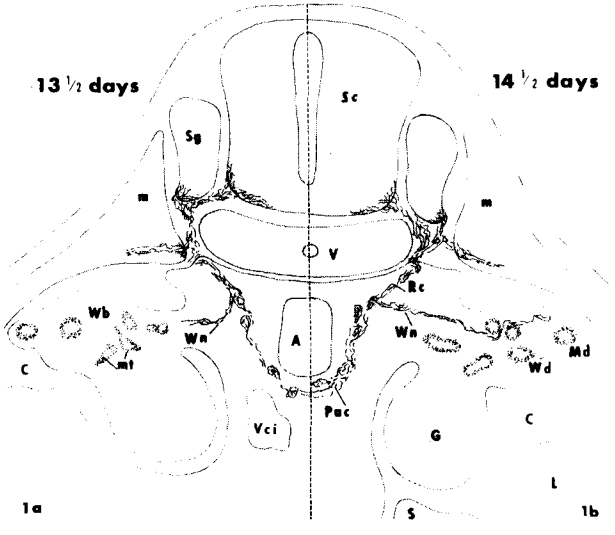


Figure 1. Schematic drawing from transverse silver-stained sections showing the trajectory of the growing Wolffian nerve (Wn) at two stages of the fetal development. In 1a (13½ dst) the nerve arising from the rami communicantes enters the proximal part of the Wolffian body. In 1b (14½ dst) the nerve has reached the mesenchymal bridge uniting Wolffian and Müllerian blastemata. A, aorta; C, celoma; G, gonad; L, liver; m, muscle mass; Md, Müllerian duct; mt, mesonephric tubules; Pac, preaortic space; RC, rami communicantes; S, stomach; Sc, spinal cord; Sg, spinal ganglion; SMr, sensory-motor root; V, vertebral body; VCi, cava inferior vein; Wb, Wolffian body; Wd, Wolffian duct; Wn, Wolffian nerve.

terminal specializations; however, in some cases end-boutons or growth cones were recognized.

At the stages referred to above and at the following prenatal stages (16–19 dst) a similar pattern of innervation was found. The Wn and its branches were not seen extending beyond the cephalic field of the Wolffian and Müllerian ducts or to penetrate into the gonads.

Microganglia (fig. 4) composed of small groups of neurones (4–15 cells) were observed interposed along the trajectory of the main bundle or its branches (16–19 dst). Axonal enlargements (boutons) were observed in the periphery of these ganglia. Their size and shape suggest that they are immature growing fibers. Most of the microganglia were found in the Wb of the male, whereas in the female they were less frequently observed.

Timing of differentiation of the urogenital complex. In the female gonads (14½ dst) the most noticeable change indicating the onset of differentiation in the oogonial nuclei involves the loss of the crusty appearance of the chromatin.

At the 15½ dst homologous chromosomes were in the process of pairing as demonstrated by the existence of single axial densities or already-organized synaptonemal complexes (TEM) in a significant number of nuclei. At the 16½ dst synaptonemal complexes were seen in most of the sex cells.

Semithin sections of male gonads revealed the organization of the testicular cords at the 14½ dst.

In the following days (16½ onwards) it is possible to observe the regression of the duct (Müllerian or Wolffian) according to the sex of the fetus and the development of the other duct.

Discussion. A recent histochemical and TEM report[8] showed that sectioning of the nerve running within the suspensory ligament resulted in a marked diminution of the adrenergic content in the ovarian tissue. It can therefore be accepted that at least part of the neural supply to the proximal part of the gonadal complex comes from this higher pathway.

The nerve within the suspensory ligament was easily traced by these authors[8] up to the celiac plexus but its center of origin could not be identified.

Bahr et al.[4] have suggested that in view of the embryological position of the gonads their nerve supply may come from sympathetic chain ganglia located in the lower thoracic region (T9–T11) but they did not offer any factual information supporting this idea.

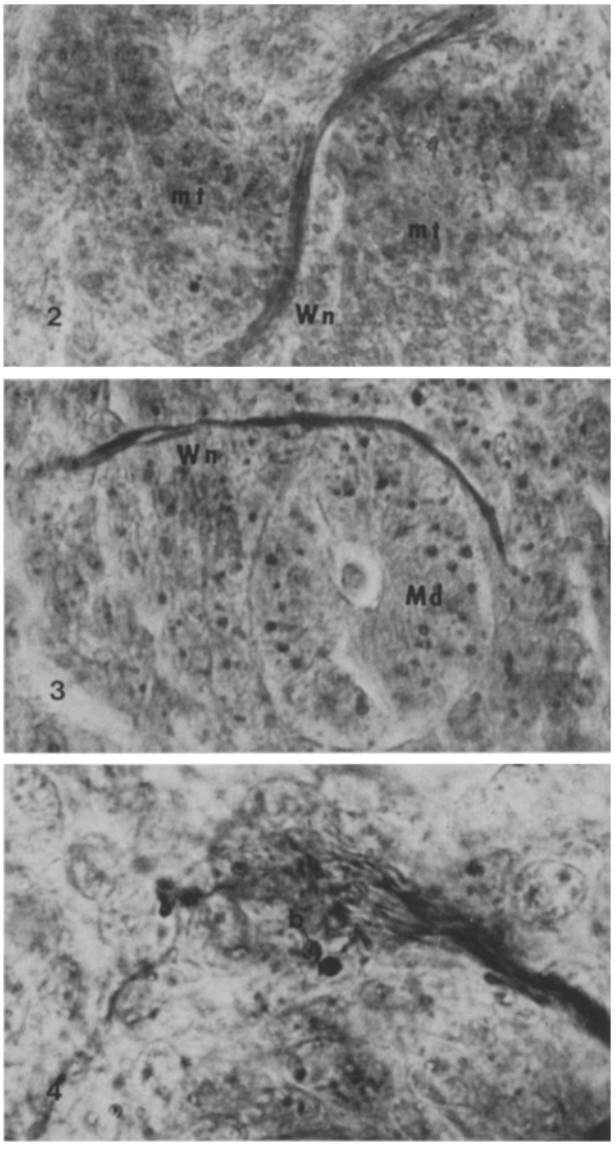
The embryological research reported here showed: 1) at earlier stages (13–14 dst) the nerve supplying the UGC (Wolffian nerve) arises from the 1st pair of subdiaphragmatic rami communicantes; 2) at more advanced stages (15 dst onwards) the Wn appears connected with the 1st abdominal sympathetic chain ganglia; 3) the pedicle of the Wolffian body changes during development into the suspensory ligament; and 4) at prenatal stages the Wn runs and branches in the Wolffian body and in the cephalic portion of the Wolffian and Müllerian ducts (mesonephric field).

From the observations made, it appears clear that the Wn is in the adult the nerve of the suspensory ligament (higher pathway). This contention is also supported by the presence of growth cones in the fibers of the Wn which may indicate that further advances to the neighbor fields (gonads and ducts) may occur.

In view of the precocious growth of the axons of the Wn (when the sympathetic chain ganglia are not fully formed) it can be proposed that the initial fiber contingent may come directly from spinal cord neurones (preganglionic fibers).

The significance of the profuse innervation as well as the presence of microganglia in the proximal part of the UGC requires further study because mesonephric tissue is known to play an important role in the differentiation and organization of the gonads[17].

It is interesting to remark that nerve penetration in the Wb starts at the 13½ dst whereas organization of testicular cords in the male and onset of meiosis in the female occur 24 and 48 h later respectively. Nerve penetration also precedes the beginning of the regression of the duct not related to the sex of the fetus and the further development of the other duct (i.e. differentiation of the ductal muscular layer).



Figures 2 and 3. Both figures illustrate the trajectory of the Wolffian nerve (Wn) in a female specimen at 16½ dst. *2* The main Wn bundle passes between two mesonephric tubules (mt) in apparent contact with their basal lamina. *3* illustrates the terminal portion of the main bundle flanking the Müllerian duct (Md).

Figure 4. A microganglion found in the Wolffian body of a male specimen at the 16½ dst. The figure illustrates the surface of the ganglion with nerve fibers located at its periphery. Highly impregnated boutons are seen (b).

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Sensory projections from dorsal and ventral appendages in *Drosophila* grafted to the same site are different

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Summary. Owing to a new transplantation technique, we have been able to study the sensory projections of homologous and heterologous appendages grafted to the same abdominal site in *D. melanogaster*. Axons from homologous transplants exhibit similar terminal patterns, whereas those from heterologous transplants do not. It is suggested that ectopic sensory axons specifically recognize central areas and pathways occupied by axons from homologous appendages.
Key words. Neuronal specificity; sensory projections; serial homology; ectopic transplantation; *Drosophila melanogaster*.

The sensory system of insects has been widely used to study neuronal specificity by analyzing the pattern of sensory projections in the central nervous system (CNS) under various experimental conditions. Homoeotic mutants of *D. melanogaster* can help to assess how displacement of sensory cells affects their central projection[1-5]. Sensory axons from homoeotically transformed antennae and probosces project successfully into centers of serially homologous appendages (or less frequently into their proper center) but do not extend into other regions of the CNS[6,7]. This suggests that these centers are recognized because they are serially homologous themselves[7]. One might therefore expect that the sensory projections from heterologous structures would differ if they were placed at an identical site on the body surface. Owing to a new technique, we were able to transplant dorsal and ventral appendages to the same abdominal site and to examine the sensory projections. As predicted, we found consistent differences in the projection patterns of heterologous tissues, but similarities in the patterns from homologous structures.

Materials and methods. Supernumerary dorsal appendages (wings and halteres) and ventral appendages (fore-, mid-, hindlegs and antennae) were produced in the pleural membrane of the fifth abdominal segment by a modified technique of 'surface transplantation'[8,9] (fig. 1). A small incision was made with a fine steel blade into the lateral abdominal cuticle of a light brown prepupa; then the desired imaginal disk from a white prepupa was injected with a glass capillary underneath the integument. Under optimal conditions, the disk is incorporated by the surrounding host's epidermis, allowing the evagination of the supernumerary appendage during metamorphosis. According to our experience, abdominal forelegs and halteres are everted at a frequency of about 70% of eclosed flies, mid-, hindlegs, antennae and wings at approx. 20%. The sensory projections were visualized by peroxidase diffusion. Peroxidase (HRP, Sigma Type VI, 10% w/v) was applied for 3 h following excision of abdominal bristles or cutting parts of grafted appendages. After another 4–14 h of survival, flies were fixed in 2.5% glutaraldehyde. The histochemical reaction[16] was performed on the dissected thoracic CNS.

Results. As a reference, we studied the central projections of two sensilla situated close to the transplantation site, and close to where imaginary dorsal and ventral appendages should develop in an abdominal segment. We chose the mechanosensory bristles located in the posterior corners of the fourth abdominal tergites and sternites (fig. 2A and B). Their axons reach the thoracico-abdominal ganglion through the main abdominal nerve and terminate in the fused abdominal neuromeres. The arborizations of the dorsal bristle remain strictly ipsilateral (fig. 2A), whereas those of the ventral bristle form ipsi- and contralateral branches (fig. 2B).

The sensory fibers from all types of supernumerary appendages enter the CNS via the main abdominal nerve as well. Most of the axons terminate in the abdominal neuromeres, but others extend into thoracic regions. In the abdominal ganglion, wing and haltere terminals occupy predominantly the ipsilateral side, as do the terminals of dorsal abdominal bristles (fig. 3C). In contrast, sensory projections from the first, second and third legs, or from antennae form both ipsi- and contralateral branches, like those of ventral abdominal bristles (fig. 3E).

In thoracic neuromeres, the differences between the projection patterns from dorsal and ventral transplants are even more accentuated. In the majority of HRP-fills from grafted wings or halteres, nerve fibers pass from the abdominal termination area in anterolateral direction into the ipsilateral normal wing center ('lateral pathway'). Comparison with fills from normal wings reveals that the fibers in this 'lateral pathway' follow the course of normal wing fibers extending back from the wing center into the metathoracic neuromere (cf. figs 3C with 3A). From the wing center, axons of wing or haltere transplants turn back towards the midline and bifurcate into an anterior and a posterior pathway (fig. 3C). The former extends into the neck connective; its course appears to correspond to the massive ascending tract followed by normal haltere axons (fig. 3B). Fibers from campaniform sensilla on the supernumerary haltere frequently branch off the 'lateral pathway' in the third thoracic neuromere and terminate in the center of normal haltere campaniform sensilla, called 'medial tuft' by Palka et al.[10] (cf. figs 3C and 3B). Sensory fibers from leg or antenna transplants are not observed in the 'lateral pathway', but instead, single axons projecting along the thoracic midline and into the neck connective may be present (fig. 3E). Scattered fibers extending along the midline into the head occur in normal leg projections as well[6] (fig. 3D).

In order to know whether afferents from supernumerary appendages are able to form functional connections in the CNS, we tested for an extension of the proboscis following gustatory stimulation of abdominal forelegs (for details see Stocker[11]). In 10 out of 33 flies, contact of the transplant with a 0.4 M sucrose solution yielded an extension almost as strong as in the normal