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[Original]

Innervation of mouse molars during the early stages of tooth germ development

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Abstract

The topography of nerves and first molar tooth germs in 11-14-day embryos was studied using silver-impregnated serial sections. Nerve fibers growing toward the developing tooth germ became visible on the 12th day, while the first sign of molar tooth differentiation was found as a thickening of the oral epithelium in 11-day embryos. From the 12th to 13th day the nerve fibers spread, forming a plexus close to the base of the tooth bud, and on the 14th day some entered into the dental follicle of the tooth germ at the early cap stage. However, no nerve fiber was found growing into the dental papilla during the observation period.

The observations showed that the earliest nerve fibers running toward the tooth forming area appeared after the histogenesis of the tooth germ started, and the timing and pattern of the innervation of the tooth germs revealed that tooth germs are a useful model for investigating the mechanism of nerve growth into developing peripheral organs.

Key words : Innervation, tooth development, mouse embryo

Introduction

There are two different aspects regarding the interaction between nerves and developing tooth germs; one is the participation of nerves in tooth development, and the other is the regulation of nerve growth by developing tooth germs. The idea of the tooth induction by nerves was first proposed by Pearson¹⁾, and is supported by the observation that the tooth-forming area is transiently innervated before the initiation of tooth development^{2,3)}. However, Lumsden *et al.*^{4,5)} clearly demonstrated that tooth induction can occur without trigeminal innervation. Thus it is improbable that the nerves play a role in the early development of tooth germs, although these results do not exclude the possibility of nerves participating in the later stages of tooth development. There is no study concerning the regulation of nerve growth by tooth germs, though histological observations have shown an intimate correlation between the

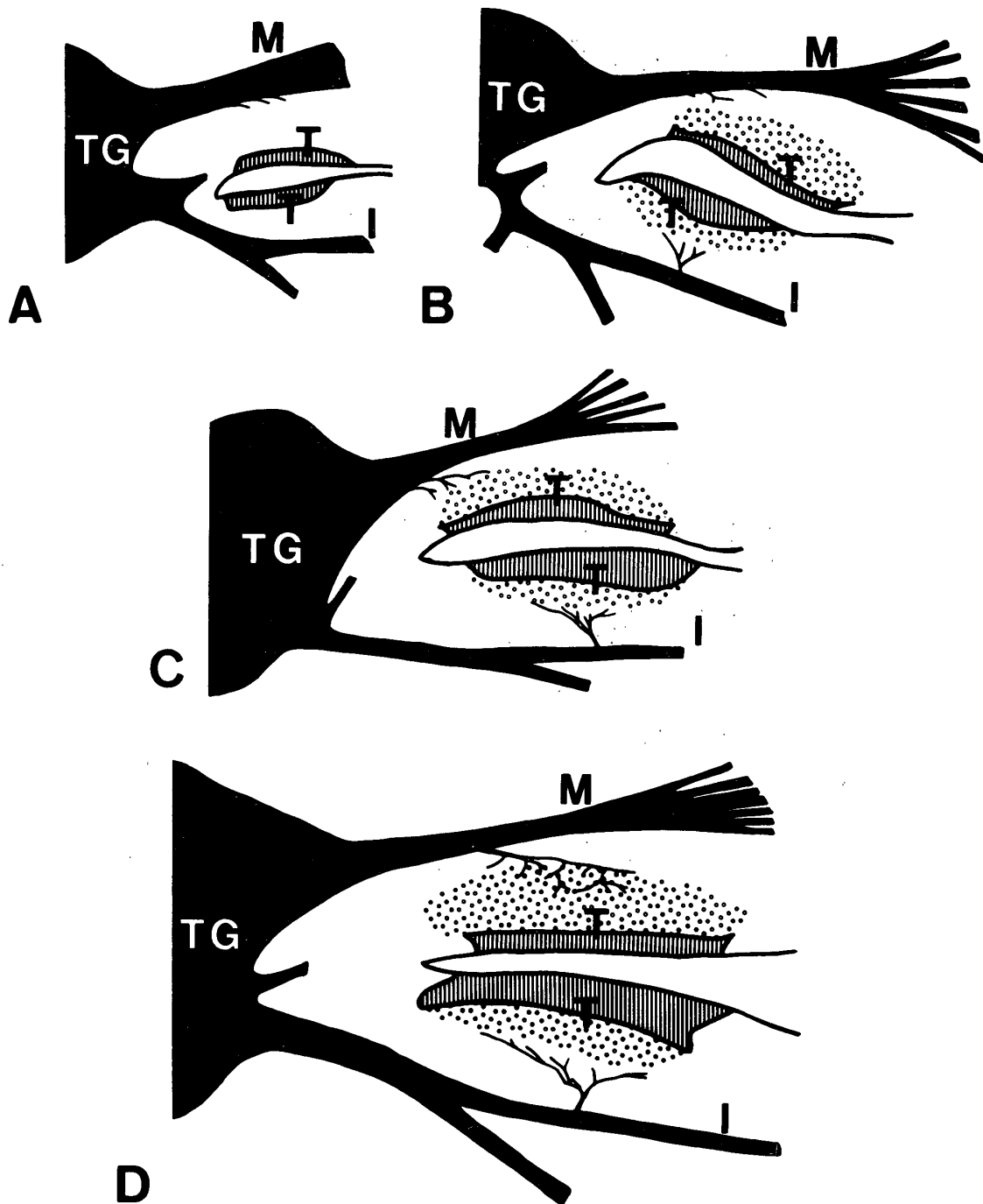


Fig. 1 . Lingual aspects of the developing nerves and tooth germs in 11-14 day embryos, drawn from several sagittal sections.

A. A drawing of an 11-day embryo. The main branches of the trigeminal nerve were already present (not all the branches are shown in the figure). The tooth germs (T) were just visible as epithelial thickenings. There were no identifiable nerve fibers running toward the tooth germs. I, inferior alveolar nerve; M, maxillary nerve; TG, trigeminal ganglion.

B. Drawing of early bud stage in a 12-day embryo. It was difficult to identify the nerve fibers approaching the maxillary molar tooth germ, while the nerve fibers growing toward the mandibular molar tooth germ were easily identifiable.

C. Drawing of late bud stage in a 13-day embryo. The nerve fibers had started to form a plexus at the base of the molar tooth germs.

D. Drawing of early cap stage in a 14-day embryo. The nerve plexus has become larger and more complex.

development of innervation and the developmental stage of individual teeth⁶⁾.

In the present study, the three-dimensional relationship between nerves and tooth germs in mouse embryos of 11-14 days was examined by reconstruction from camera lucida drawings of serial sections in order to determine both stages of tooth development and innervation.

Materials and Methods

Embryos of dd-Y mice were used. After overnight mating, females were examined for the presence of vaginal plugs on the following morning; that day was considered to be day 0 of embryonic development. The embryos were removed from females at 11-14 days of pregnancy after the mothers had been killed by cervical dislocation. The developmental stages were checked by referring to the data of Gruneberg⁷⁾. The heads of embryos were fixed in Bouin's fixative, embedded in paraffin, and serially sectioned at a 10- μ m thickness. After staining by Linder's silver method⁸⁾, both the frontal and sagittal sections were examined with light microscopy. The three-dimensional architecture of nerves and first molar tooth germs were reconstructed from camera lucida drawings of every fifth sagittal section.

Results

The developmental stages of the embryos differed among litters from females of the same pregnancy date, but it was possible to arrange the litters in a developmental series according to their external features. The extent of innervation invariably correlated with the developmental stage of the embryos as well as with that of the tooth germs.

Initial stage of tooth development

Epithelial thickening, which is the first indication of molar tooth differentiation, was observed at 11 days of embryonic development (Figs. 1-A and 2). The main branches of the trigeminal nerve were already present at this stage (Fig. 1-A). Although some fine nerve fibers from the maxillary nerve were observed running toward the oral cavity, they were not directed toward the thickened oral epithelium. There were no branches of the inferior alveolar nerve running toward the primordium of the mandibular molar tooth germ.

Nerve growth toward developing tooth germ

Inflection of the thickened epithelium started in the 12-day embryos, and here the earliest nerve branch was observed running in the direction of the developing tooth germ. This earliest branch growing toward the mandibular molar tooth germ was clearly identifiable, as it was the only branch of the inferior alveolar nerve in the area and as it ran perpendicularly to both the inferior alveolar nerve and the medio-distal axis of the tooth germ (Fig. 3). On the other hand, the nerve branches from the maxillary nerve arose obliquely and ran a shorter distance to the maxillary tooth germ in comparison with the mandibular case (cf. Figs. 1-B and 4). Therefore, it was relatively difficult to identify nerve fibers growing toward the maxillary molar tooth germ during the early stages.

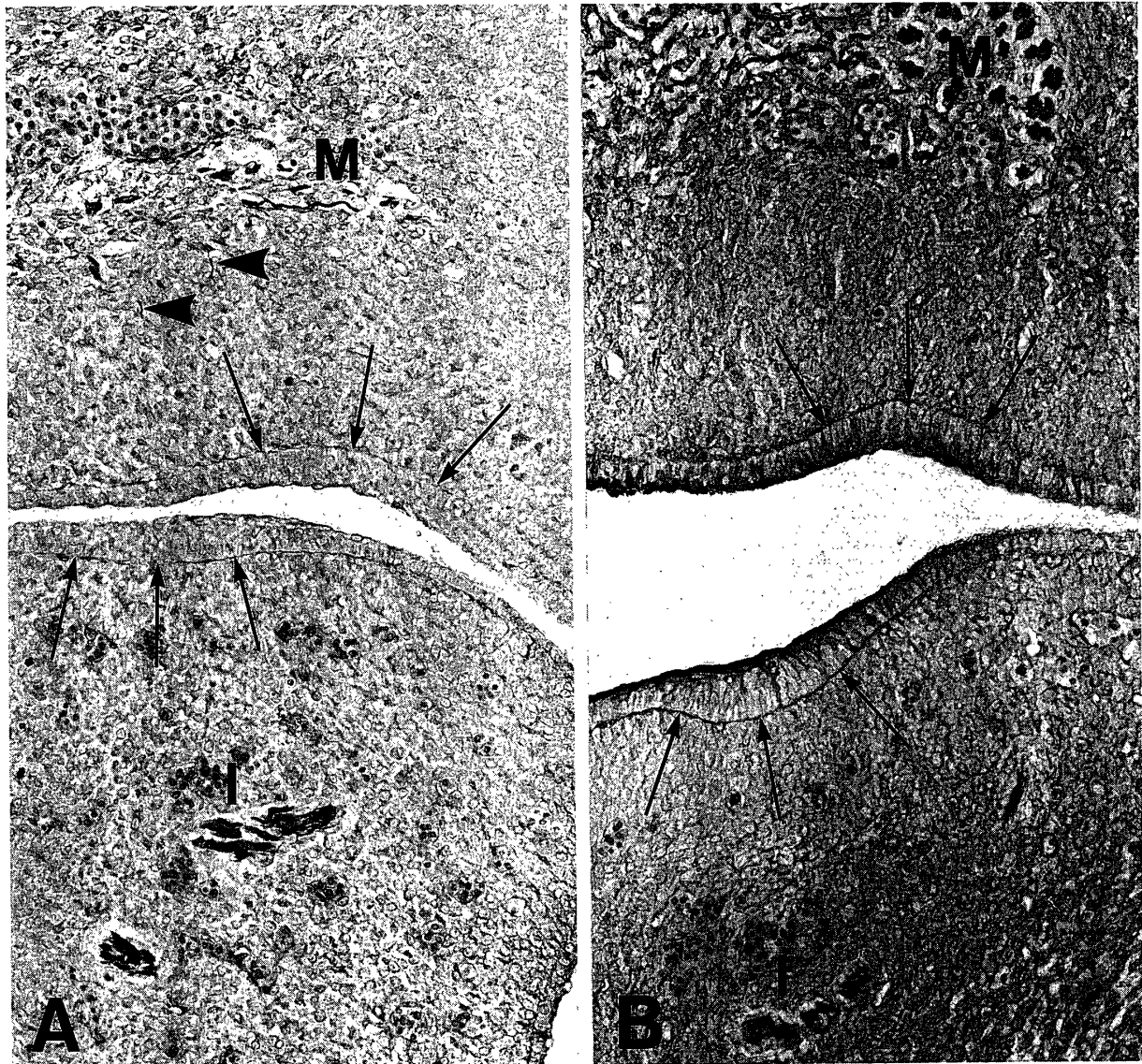


Fig. 2 . Sagittal (A) and frontal (B) sections of 11-day embryos showing the epithelial thickenings (arrows) which indicate the first sign of molar tooth germ development. As the section was cut in a slightly oblique plane, the maxillary and mandibular tooth germs appear to incompletely face each other in A. Although fine nerve fibers (arrowheads) arise from the maxillary nerve (M) toward the oral cavity, they are not considered to be running in the direction of the tooth germ (see text). X 200.

Nerve plexus formation close to the base of developing tooth germ

From the 12th to 13th day, the epithelial inflection became more prominent and mesenchymal cells condensed around it (bud stage). The nerve branch reached near the base of the tooth germ, divided into smaller branches, and spread out (Figs. 1-C and 4). The fibers were close to but never in the dental papillae (Fig. 4). As the tooth development progressed, the nerve fibers at the base of the tooth germ increased in both thickness and number, forming a plexus (Figs. 1-C and D). At the end point of our observation (14 days of embryonic development), some fibers were growing out from the plexus into the developing follicle of the tooth germ at the early cap stage (Figs. 1-D and 5).



Fig. 3 . Sagittal section of mandibular molar-bearing area in a 12-day embryo showing the earliest stage of nerve growth toward the developing tooth germ. A small branch (arrowhead) arising from the inferior alveolar nerve (I) is running in the direction of the thickened epithelium (arrows). X 200.

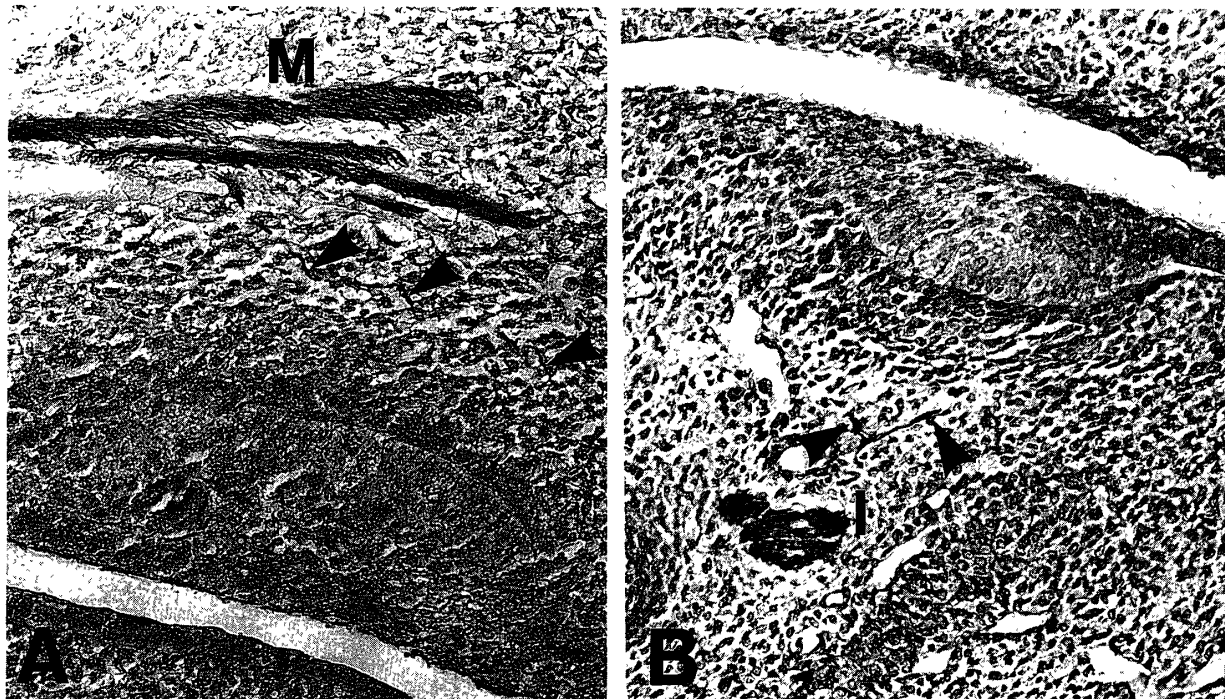


Fig. 4 . Sagittal sections of 12-day embryo, in which the tooth germs are at a more advanced stage than in Fig. 3 . Maxillary (A) and mandibular (B) molar-bearing areas show the spreading nerve fibers (arrowheads) under the base of the tooth germs. X 200.



Fig. 5. Sections of 14-day embryos. Frontal section of an maxillary molar-bearing area (A) showing the nerve fibers running along the developing follicle, and sagittal section of a mandibular molar-bearing area (B) showing the nerve forming a plexus close to the tooth germ. Arrowheads indicate nerve fibers. X 200.

Discussion

Studies concerning the early innervation of the tooth-forming area, which were carried out using silver-impregnated sections²⁾ and whole mounts of mouse embryos³⁾, showed transient innervation of the area before the tooth germ differentiation, and supported the idea of tooth induction by nerves that was proposed by Pearson¹⁾. However, tooth germ formation in the absence of trigeminal innervation was demonstrated by experimental growth in culture and homografts^{4,5)}, allowing the conclusion that innervation plays no part in the determination of tooth development. Furthermore, recent studies^{9,10)} have revealed that the epithelium of the mandibular arch of mouse embryos might have tooth-inductive potency as early as the 9th day of embryonic development. According to the observation by Lumsden³⁾, the presumptive tooth-forming area is transiently innervated at Theiler's stage 17 (10.5 days) when tooth morphogenesis has not started, and re-innervated at stage 20 (12 days). We did not use Theiler's criteria for the determination of developmental stage, and the gestation period of mice differs among different strains. However, the timing and pattern of the innervation we observed here is clearly consistent with those of the "re-innervation" phase mentioned by Lumsden³⁾, which may represent the primary interaction between nerves and tooth germs. There are many histological studies concerning early innervation of developing teeth^{1-3,6,11,12)}, but the precise stage at which the innervation starts has not been determined. Therefore, it is noteworthy that the nerve branch running toward the tooth germ first appeared on the 12th day when the tooth germ differentiation had already started. The innervation after tooth germ differentiation makes us suppose that some factors produced by the tooth germs guide the nerve fibers during the initial stage of tooth development.

The formation of the nerve plexus close to the tooth germs and the absence of a nerve supply for the dental papillae during the early stages have also been shown in several studies of normal tooth development^{1,6,11,12)}. In a sequential study of the innervation of developing murine dentition, Mohamed and Atkinson⁶⁾ noted that the development of innervation correlates with the developmental stage of the individual teeth rather than with the chronological age of the animal. Furthermore, Granholm¹³⁾ observed that the ophthalmic branch of the trigeminal nerve innervates the tooth germ grafted *in oculo* and forms a plexus resembling the normal sensory network from the alveolar branches of the trigeminal nerve. These results suggest that the tooth germs regulate the development of the innervation in some way. As regarding the plexus formation near the tooth bud, the process may be regulated by some neurotrophic factors produced by the cells at the base of the tooth germs. Similarly, the differential nerve supply for dental follicles and dental papillae at the cap stage may be explained by the production of neurotrophic factors by the dental follicle cells, and/or the nerve guidance by extracellular matrix components. Another explanation for the exclusive distribution of nerve fibers in the dental follicle is that there may be barriers that prevent the innervation of the

dental papilla. Several extracellular matrix components are known to change their distribution in tooth germs during development¹⁴⁻²⁰. Some may act as nerve adhesion molecules or barriers for innervation. The role of these components in the development of tooth innervation remains to be elucidated.

In the present study we observed the earliest nerve fibers arising from the inferior alveolar nerve toward the tooth germ on the 12th day of gestation. In mouse embryos the first visible primordia of the sympathetic chain appear on the 11th day²¹) and the superior cervical ganglion becomes visible under the dissecting microscope late during the 13th day²²), while the main branches of the trigeminal nerve are already present on the 11th day. This makes it unlikely that the earliest fibers which arise from the inferior alveolar nerve on the 12th day are sympathetic ones, even though the post-ganglionic sympathetic fibers join the trigeminal nerve and reach the developing tooth germs during the early stages of tooth development. According to the observations by Mohamed and Atkinson^{23,24}), neither cholinergic nor adrenergic nerve fibers appeared in the mouse tooth germs by the end of their observation period (1 week after birth), while substance P-containing fibers were found in the dental follicle and papilla 4 days later than the innervation shown by non-specific histological method. These observations also suggest that the innervation by the trigeminal nerve precedes the autonomic innervation. Consequently, the earliest nerve fibers are possibly trigeminal rather than autonomic fibers.

The aim of the present study was to delineate the topographical relationship between developing nerves and tooth germs during the early stages, and we found that it is relatively easy to identify the first nerve fibers growing toward the tooth germs, especially in the mandible. This finding, as well as the intimate correlation between tooth differentiation and innervation that is discussed above, suggests that the developing tooth germs are an excellent model for studying the factors regulating the early innervation of peripheral organs.

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発生初期のマウス臼歯歯胚へ神経侵入過程

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抄 録

胎生11日から14日のマウス胎仔における神経と臼歯歯胚の立体的位置関係を渡銀法で染色した連続切片の観察により明らかにした。胎生11日歯胚の分化の兆候は口腔上皮の僅かな肥厚として認められるが, その肥厚部分へ向かって伸びる神経線維はまだみられなかった。胎生12日目になると歯胚を形成する上皮はより厚さを増し, そこへ向かって伸びる神経線維が初めて観察された。胎生12日目から13日目にかけて歯胚にかけて歯胚の基底部に到達した神経は分枝して神経叢を形成し, 胎生14日目には一部が帽状期歯胚の歯小囊に侵入するが, 歯乳の侵入はまだ認められなかった。

以上の観察から, 歯胚へ向かう神経の伸長は歯胚形成開始後に始まることが明らかになった。歯胚への神経侵入の経過は発生過程の末梢器官への神経侵入の機構を研究する上で有用なモデルであるとおもわれる。