June 1985

Effects of Capsaicin on the Innervation of Developing Molar Teeth in the Neonate Mouse

Laurence Arvis

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THE EFFECTS OF CAPSAICIN ON THE INNERVATION
OF DEVELOPING MOLAR TEETH IN THE NEONATE MOUSE

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D.D.S., University of PARIS, 1977
D.D.S., University of GENEVA, 1979

A Thesis
Submitted in Partial Fulfillment of the
Requirements for the degree of
Master of Dental Science
at
The University of Connecticut
1985
THE EFFECTS OF CAPSAICIN ON THE INNERVATION OF DEVELOPING MOLAR TEETH IN THE NEONATE MOUSE

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ACKNOWLEDGEMENTS

I would like to express my gratitude to the following individuals: Drs. KOLLAR, LANGELAND and YAEGER for their constructive criticism during the elaboration of this thesis.

Dr. SAFAVI, who provided a flexible clinical schedule, and whose quiet encouragement permitted me to work on this project.

Dr. FRANK for her expert advise in biostatistics.

Dr. PASCON for his introduction to many laboratory procedures.

Drs. FISHER, MINA, MONTE DE OCA, RICHMAN, and THOMAS for their friendship and support during the past years.

I would like to thank most particularly my major advisor, Dr. WHITEHEAD for his many hours of patient counsel, and for sharing his technical knowledge in a spirit of enthusiasm and freedom, which helped me to accomplish this work.
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LITERATURE REVIEW

1. DESCRIPTION OF MOLAR TOOTH DEVELOPMENT IN THE MOUSE

The development *in vivo* of the mouse molar tooth germ has been described previously in detail (Gaunt, 1955, 1956; Cohn 1957; Hay, 1961; Lumsden, 1979).

For comparison with the present experimental material, a brief description of the sequences of normal development is necessary. In the first part of this section the development of the mesenchymal and epithelial components of the tooth germ, together with associated structures of the lower jaw will be described. The second part will review in detail the innervation of the molar tooth germ proper.

A. PRENATAL DEVELOPMENT

The gestation period of the mouse is 20–21 days. For simplification, only the first molar (M1) will be described, considering that the details of the second molar (M2) development are similar but M2 remains 2 days behind M1 during all the stages of development. Starting at 10 days of embryogenesis (E10) the mandibular rudiments join in the mid-line to form an arch.

By E 12, mesenchymal cells condense to form Meckel's cartilage, and between E 12 and 13, the first sign of tooth formation is characterized by the condensation of a band of oral epithelial cells along the upper free margin of the jaw. Gradually, these epithelial bands grow deeper into the underlying mesoderm to form a terminal swelling representing the bud stage of M1.
The mandibular bone begins to calcify at E14-15 and the inner and outer enamel epithelium of the bud start to differentiate with the simultaneous appearance of the stellate reticulum.

The future dental papilla first appears as a concentration of mesenchymal cells and vascular elements inside the invaginating enamel organ; M1 is in the cap stage by the end of this period. By E 15, M1 is in bell stage and the dento-enamel junction indicates future cusp formation. The E 16 stage is characterized by an increased vascularity of the dental papilla with differentiation of the dental sac and progressive apposition of bone around the tooth germ. An increase in size of the tooth germ, due to the expansion of the stellate reticulum, and the appearance of the stratum intermedium can be seen on day E 17. By E 18, the first appearance of the pattern of the cusps, associated with the proliferation of the inner enamel organ can be seen. The final shape of the crown is completed as the first ameloblasts start to differentiate on day E 19-20. Dentinogenesis begins, with the first differentiation of mesenchymal cells into odontoblasts around time of birth (E 20).

B. POSTNATAL DEVELOPMENT

One or 2 days after birth (D 1-2), predentine starts to calcify concurrent with the initiation of amelogenesis and by D 3-4 dentinogenesis is complete.

The first sign of calcification of the enamel matrix occurs at 5 days; root formation and eruption begin around 10 days. Enamel maturation is complete and root formation, periodontal membrane
differentiation, and eruption are progressing during D11-15. Eruption occurs between the 16th and the 17th days, and is completed by 20 days. By D 21-25, M1 is in occlusion; cementogenesis starts at this time. The roots will reach their mature length by the end of this period.
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3. Cap Stage:

Fine nerve fibers form a plexus underneath the dental papilla. At this stage, the dental sac differentiates; it receives innervation 4 days earlier than the dental papilla (Mohamed and Atkinson, 1982). The papers of Weatherford (1939) on the dental development in newborn rats, and Kerebel (1964) on newborn kittens, as well as studies in human fetuses (Humphrey, 1965; Kubota, 1959), similarly confirmed the earlier innervation of the periodontal tissues than the dental pulp.

The nerve fibers remain outside of the developing tooth until the bell stage. This also contrasts with the innervation of the soft tissues of the jaws, and the face, which occurs a considerable time in advance (E 12-14) of the tooth pulp (Van Exan and Hardy, 1980). Likewise for human fetuses, Humphrey (1965) found no nerve fibers within the developing dental pulp before the 24th fetal week, however, at 14th weeks, he observed nerve fibers in the subepithelial tissue of the oral mucosa.

The reason for the time interval between the innervation of the dental sac and the surrounding tissues, and the innervation of the dental papilla is unknown. One explanation for relatively delayed dental innervation is that the nerve fibers may be deflected by some mechanical barrier (e.g. tightly packed mesenchymal cells) to avoid the developing tooth germ (Fearnhead, 1967). Humphrey (1965) suggested the possibility of a transient negative neurotrophic effect of the enamel organ on nerve fibers during the embryonic stage. It is also possible that entrance of the complete set of axons relates in some way to dentin formation (Johnsen and Karlsson, 1963), since the two developmental events coincide at a later time.
In any event, all previous studies indicate that timing of innervation of the dental papilla is correlated with the stage of development of the tooth germ, rather than with the chronological age of the animal.

It is presently speculative to attribute any identity to the immature nerves which approach the teeth since their neurotransmitters are not known. However, the fact that they are not present in the dental pulp at early stages implies that if they are involved in inductive influences, these influences may act at a distance.

In conclusion, the delayed in growth of nerve fibers into the developing tooth may be regulated by some local factors, and remains a field for future investigations.

4. Dentinogenesis and Amelogenesis:

Although the literature on the innervation of the tooth germ is controversial on the exact timing of nerve fiber ingrowth, and the nature, location, and number of these axons, most of the investigators agree that nerve fibers do not enter the dental papilla before dentinogenesis begins. In a LM study, Corpron and Avery (1973) reported that the dental papilla of the first molar was devoid of nerves on day 1, which corresponds to the initiation of dentinogenesis.

Mohamed and Atkinson (1982) showed that the first dental structure to receive Substance P-like immunoreactive nerve fibers was the dental follicle at day 1 after birth.

The first fibers entering the dental papilla do so when both ameloblasts and odontoblasts have differentiated and begin to secrete their respective tissues (Mohamed and Atkinson, 1983): these fibers are
directed toward the tip of the papilla, together with small arteries located in the central portion of the papilla; some fibers are also observed free from the blood vessels.

Wasserman (1939) confirmed this observation in a LM study on rats, and stated that nerves begin to enter the dental papilla only after birth. Weatherford (1939) described the presence of bundles of myelinated nerves in the molar pulp of neonatal rats at 5 days. Bernick (1959) using rat molars described only fine non-myelinated fibers entering the pulp at ages varying from 1 to 15 days. A study of the innervation in neonatal kittens (Kerebel, 1964) showed that, at birth, the dental papilla contained only a small population of unmyelinated fibers, but the tooth germ is surrounded by myelinated and unmyelinated fibers of larger diameter.

Fearnhead (1967), in a study of human embryos, described dental papillae in the advanced bell stage, which contained a few pioneer nerves; when dentinogenesis began, most of these tooth germs possessed nerve fibers, but the fibers had not reached the odontoblasts.

The fact that nerve fibers are the last major structures to appear in the developing pulp implies that the nervous system does not initiate hard tissue formation. Conceivably, there are subsequent neural influences on the quantity and/or quality of hard tissue secretion (Corpron and Avery, 1973).

5. Further Calcification of the Crown and Eruption:

The principal change of innervation occurring between the commencement of dentinogenesis and molar eruption consists of a general increase in the number and diameter of nerve fibers. By day 4,
substance P-like immunoreactive axons enter the dental papilla of M1, half of them consisting of single fibers, not associated with blood vessels (Mohamed and Atkinson, 1982). By day 5, the central pulp contains both myelinated and unmyelinated nerve fibers, associated with blood vessels (Corpron and Avery, 1973), but until 1 week, none of these fibers has reached the odontoblast layer (Mohamed and Atkinson, 1982). At day 6, substance-P fibers enter the dental papilla of M2, and reach the follicle of the third molar (Mohamed and Atkinson, 1982).

By 9 days, some unmyelinated fibers are observed in the odontoblastic layer of M1, and at day 15, numerous nerves extend into the basal portion of the odontoblastic layer (Corpron and Avery, 1973).

Each of the nerve fibers exhibits many branches which arborize as spherical, then spindle-shaped terminal fields during development. During the eruption of M1, the subodontoblastic neural plexus increases in density, and when occlusion occurs (day 25), some nerve fibers are seen looping in the predentin (Corpron and Avery, 1973).

From 25 to 70 days, the proportion of nerves in the subodontoblastic area appears to increase substantially, with both myelinated, and unmyelinated fibers present; the ratio of nerves to odontoblasts approximates 1 to 10 (Corpron and Avery, 1973). In general, the maturation of dental innervation is characterized by a progressive increase in number of myelinated axons; the significant changes in unmyelinated axons are increases in axonal size, a reduction in the number of axons per schwann cell units, but no reduction in axon number (Johnsen et al. 1983).
Compared with the mouse molar, which completes its sensory innervation a month after eruption, the human molar takes several years to reach the same stage of neural maturation (Fearnhead, 1967).

B. ULTRASTRUCTURAL IDENTIFICATION OF NERVE TYPES IN THE MOUSE MOLAR

Nerve resection studies have been used to clarify the identity of nerves in the pulp and dentinal tubules of the mouse molar (Corpron et al. 1972; Avery and Cox, 1977): Resection of the inferior dental nerve results in degeneration of all myelinated and most of the unmyelinated fibers, including massive disappearance of intradentinal nerve endings. Resection of the cervical sympathetic ganglion produces degeneration of only few nerve fibers, implying that the pulp contains only a small proportion of sympathetic fibers. However, after resection of both structures, a small population of nerve endings persists (Yax et al. 1977), indicating the presence of accessory nerves supplying the molar tooth.

Measurements of nerve fibers in the mouse molar have not been reported yet, but the size range of unmyelinated fibers is constant in other mammals, with a value of 0.2 to 1.7 um which is the range of C-fibers involved in pain transmission and autonomic innervation. The range of diameter of the myelinated fibers is 1 - 4 um which corresponds to the A-delta range involved in conduction of pain (Fried and Hildebrand, 1981 a,b).

The ultrastructural study of pulpal and dentinal innervation has been performed in normal adult mice by Corpron and Avery (1973). Three types of nerve fibers were identified: 1. fine diameter unmyelinated fibers, lying free in the ground substance, possibly related to pain
afferent transmittion. 2. unmyelinated, possibly autonomic fibers associated with blood vessels. 3. myelinated fibers which lose their myelin sheath when they reach the plexus of Raschkow.

Two types of vesiculated nerve endings have been described: one containing granular vesicles, possibly adrenergic in nature, and the other agranular, attributed to cholinergic function (Byers, 1984; Avery and Cox, 1977).

Small unmyelinated fibers were traced as far as 80 um into the dentinal tubules, but did not seem to reach the peripheral dentin; these intradental fibers contained neurotubules, mitochondria and small agranular vesicles, the majority of them were seen in close association (200 Angstroms) with the odontoblasts.

By using the false neurotransmitter 5-OH-DA in adult mice, Avery et al. (1980) showed that the highest percentage of adrenergic nerve endings resides in the pulp horn, majority of them being located in proximity of the blood vessels. However, no ultrastructural morphologic distinction has been made between unmyelinated autonomic and unmyelinated sensory nerve fibers.
III. NEUROTROPHISM AND DEVELOPMENT

A. EFFECTS OF DENERVATION ON TOOTH DEVELOPMENT

Many experiments have been conducted at different stages of tooth development, using nerve resection or neurotoxic substances, in order to elucidate neural influences on the growth or the maintenance of the tooth.

1. Nerve Resection:
   - IN THE MOUSE:

   The effects of Inferior alveolar nerve (IAN) resection on the growth, and eruption of the incisor of adult mice was investigated with LM (Chiego and Singh, 1974) and EM (Avery and Cox, 1977). The denervated tooth appeared less pigmented, thinner, with diminished rate of eruption 28 days after surgery, the dentin presented cellular inclusions, and the odontoblast layer was poorly defined. However, the tooth returned to its normal appearance by 60 days, possibly due to a compensatory mechanism present in continuously erupting teeth.

   The effect of IAN resection, and sympathectomy on the proliferation rate within the cellular components of adult mouse incisors was evaluated (Chiego et al. 1981; Klein et al. 1981). Autoradiographic analysis, following injection of 3H-thymidine showed a reduction of cellular proliferation within the enamel organ while SCG resection alone had only a minor influence.

   More recently, effects of denervation on collagen synthesis by the odontoblasts was investigated in the adult mouse incisor by autoradiographic analysis of dentinogenesis, following injection of 3H-fucose (Chiego et al. 1983). IAN and SCG resection results in
Increased Incorporation of 3H-fucose, which suggests a regulatory role of the nervous system in glycoprotein synthesis by the odontoblasts.

- IN OTHER SPECIES:

Nerve resection experiments give variable results depending upon whether the sensory, or autonomic innervation is interrupted, and on the technical procedure used.

Unilateral IAN resection in kittens (Edwards and Kitchin, 1938) resulted in an accelerated eruption rate, with no modification in size of the tooth while resection of SCG resulted in accelerated growth rate, as well as an increase in the size of the tooth germ. The authors concluded that sympathectomy may increase vascularity of the tooth germ, which in turn, furnishes more nourishment and stimulates growth. However, histological examination revealed no noticeable difference between control and denervated teeth.

In contrast, IAN resection in 10 day old rats (Brown et al. 1961) produced a chalky appearance of the incisor, characterized histologically by a marked thinning of enamel and dentin. These changes were attributed not to metabolic or vascular perturbation but to a loss of proprioception. This may increase abrasion which, in turn, accelerates the rate of eruption of the teeth. Such acceleration of eruption might not permit a normal pattern of deposition and calcification of the hard tissues.

The effects of IAN denervation on dentinogenesis was also investigated in adult rats (Rehak, 1963), and rabbits (Avery et al. 1971). Histologically, the dentine showed irregularities such as trapped odontoblasts, widened predentin and irregular calcification
patterns. The authors concluded that IAN may have some direct effect on dentinogenesis. The dramatic increase in dentin deposition in the denervated rabbit Incisor (Avery et al. 1974) suggests that an inhibitory relationship exists between nerves and dentin formation.

Loss of sympathetic function after SCG resection has provided contradictory results: An acceleration in growth rate, and size of the tooth germ (Edward and Kitchin, 1938), or no change (Avery et al. 1974).

None of the studies cited above presented ultrastructural evidence to clarify whether the surviving nerves were sensory, or sympathetic in nature, and possible influences of innervation on tooth development remain uncertain despite these efforts. Results obtained in nerve resection experiments may be equivocal for the following reasons:

a) Choice of the tooth type:

Most of these studies have used rodent incisors. These incisors exhibit a continuous eruption. This may not be a typical model for studying dental neurotrophism, since compensatory mechanisms (i.e. rapid renewal of the tissues), associated with constant eruption may mask the effects of denervation proper (Chiego et al. 1981). Moreover, continuously erupting teeth differ in many ways from mature posteruptive teeth such as molar or primate teeth. Generally, in continuously erupting teeth, there are fewer axons most of which are immature, umyelinated, and associated with blood vessels. Another characteristic feature, which may relate to neurotrophic effects, is the total absence of sympathetic nerve fibers (Bishop, 1981), and the fact that pulpal nerves do not have any relationship with the odontoblasts (Byers, 1984). Finally, most of the experiments were performed on adult animals, with
well differentiated teeth; it may be more informative to study the
effect of denervation at earlier stages.

b) Choice of the technique:

Most of the experiments described above, using nerve resection, are
subject to certain technical limitations. The proximity of major
vessels to the inferior alveolar foramen is such that the chance of
hemorrhage resulting in local ischaemia is high. Compromised vascular
supply may introduce artefacts in the uptake properties of affected
teeth in the autoradiographic experiments. Moreover, effects produced
by IAN resection may result from the unintentional severing of
vasoconstrictor fibers which are close to, or within, the sheath of the
sensory nerves (King, 1936).

The choice of the tooth in terms of its location in the jaw is an
important factor to consider for nerve resection experiments. Most of
the studies have used the incisor tooth. However, because of the
transmedian innervation which is unaffected by unilateral surgical
procedures (Fried and Hildebrand, 1978), the effect of denervation may
be partial. The same problem exists but to a lesser extent perhaps for
the molars which are innervated by accessory nerves.

When experiments are performed on neonate animals, the nursing
mother does not accept, and feed the operated animals as well as the
control, and the incapacity for the young animals to feed normally,
because of the surgical trauma, may introduce some degree of variation
in the final results (Brown et al. 1961). The loss of proprioception
following IAN resection renders the animals careless about their teeth,
producing a rapid attrition and occlusal trauma, which cannot be
controlled during a long-term experiment, but which may produce changes in tooth morphology which are difficult to discriminate from possible changes due to neurotrophic effects alone (Brown et al. 1961).

c) Limitation of the technique:

A major problem with nerve resection experiments is that many days are required following the surgical procedure, to allow nerve degeneration. This does not permit observations of changes which might have occurred immediately following denervation. Moreover, after long survival times, reinnervation of teeth by regenerating alveolar nerves may have occurred (Robinson, 1981). The use of a neurotoxin which results in an immediate nerve damage, might better permit study of the early events which occur after nerve degeneration. Long term observation following neurotoxic denervation is also possible, because nerve damage is usually irreversible, due to the direct cytotoxic effect which often results in the degeneration of the cell bodies, rendering reinnervation impossible. Moreover, neurotoxins, such as capsaicin, or 6-OH Dopamine, destroy relatively specific population of nerves, unlike nerve section which can be non-selective.

2. Use of Neurotoxins:

- IN THE MOUSE:

The effects of guanethidine-sulfate, a chemical sympathectomy agent, have been detailed previously (Evans et al. 1979): Neonatal chronic treatment with high doses of guanethidine-sulfate, resulted in a long-lasting degeneration of 95% of the peripheral adrenergic neurons, due to the loss of ganglionic cell bodies. Treatment of mice from birth until 14 days, followed by 3H-thymidine injection, showed no effect of
the resulting sympathectomy on cellular proliferation in the enamel organ, and pulpal compartment of the incisor tooth (Klein et al. 1981).

Holje et al. (1983) were the only investigators to study the possible effects of neonatal capsaicin treatment upon the microscopic anatomy of the trigeminal nerve branches related to rat molars. The pharmacological effects of capsaicin (which will be reviewed in detail in the next section) are characterized by a selective and massive destruction of peripheral C-fibers and their cell bodies, which occurs with exceptional rapidity (within a few hours). Six months after the treatment, the authors observed degenerative effects, at central and peripheral levels, with a 58% reduction of fibers in the IAN. Surprisingly, however, ultramicroscopic examination of the mandibular molar pulps did not show any reduction in the number of nerves. Qualitatively, control and experimental teeth were identical. However, the authors did not specify which type of molar was examined. This may be an important factor, considering that susceptibility of each molar type may vary because of the different stages of development. Finally, the long time period observation (6 months) might have allowed nerve sprouting from uninjured fibers, and did not permit observation of any early neurotoxic effects of the drug.

- IN OTHER SPECIES:

The LM technique widely used in the denervation studies has not permitted reliable recognition of unmyelinated fibers, or morphological differences between sensory, and adrenergic axons. In an attempt to differentiate between autonomic fibers and sensory fibers, which both contribute unmyelinated axons to the dental pulp, Fried and Hildebrand
(1978) administrated 6-OH Dopamine systemically to adult cats. The specific neurotoxic action of 6-OH Dopamine on sympathetic fibers has been previously described (Sachs and Jonsson, 1975). At the ultrastructural level, the degenerative signs are similar to those seen after surgical sympathectomy, but, since 6-OH DA has a direct action on the terminals, the degeneration appears faster. Adrenergic nerve terminals completely disappear after 48-72 hours. 48 hours after injection, marked alterations of only a few unmyelinated axons was observed at ultramicroscopic level; the non-pulpal tissue had a normal appearance. However, no quantitative analysis was performed.

B. EFFECTS OF DENERVATION ON OTHER SYSTEMS

In other systems, dramatic neurotrophic influences on developing structures have been demonstrated. For example, the taste bud requires an interaction with its nerve for normal development, maintenance, and regeneration in vivo. When tongue fragments from rat fetuses are placed in contact with IX, and X cranial ganglia in a culture chamber, the taste bud develops normally; but taste buds do not form in tongue explants that do not contain sensory ganglia (Farbman, 1972). In an ultrastructural study (Farbman, 1965), nerve fibers were present in the presumptive area of the taste papillae before the taste buds formed. This presents similarity with the early ingrowth of nerves during tooth development. Lingual epithelial cells which contact nerve fibers during development transform into taste bud cell types. It has also been reported that taste buds atrophy, or disappear after denervation, and, that buds become recognizable only after nerve regeneration (Zalewski, 1974). Only certain types of neurons influence taste bud formation.
Neurons from cranial, or spinal sensory ganglia were able to induce bud formation, but sympathetic neurons failed to produce this transformation (Zalewski, 1972). Another interesting finding is the capability of the epithelium of ventral tongue, which normally does not contain buds, to form taste buds, when combined with sensory ganglionic neurons which induce bud regeneration (Zalewski, 1972). But, apparently, epithelial specificity seems to be required since skin epithelium failed to form buds. It is clear that nerves play an important role in the development and regeneration of the taste bud. This neuronal (trophic) influence is thought to originate in the nerve cell body as a chemical substance that passes down the fiber to the tongue where it transforms epithelial cells of the papilla into taste cells (Zalewski, 1974; Guth, 1958; Sloan et al. 1983).
A. INTRODUCTION

Capsaicin is the irritant component of the capsicum plant (red pepper, chili pepper). For centuries, extracts of red peppers have been used as potent remedies for different types of pain, although until recently, there was no rationale to explain this effect.

The first study related to capsaicin was done by Hogyes in 1978 (cited by Jancso et al. 1970) who observed that topical application of the drug on the skin of dogs was accompanied by hyperemia, while systemical administration produced a fall in body temperature. Jancso et al. demonstrated the wide physiological effects of the drug, including a selective neurotoxic action on primary afferent neurons, associated with a long-lasting desensitization to chemical irritants. More recently, many studies have shown that capsaicin affects pain transmission, possibly by blockage of the axonal transport of the neurotransmitter substance P in primary afferent sensory neurons, leading to their degeneration.

B. PHYSICAL AND CHEMICAL PROPERTIES

Capsaicin consists of white platelets which melt at 63-65o C, and are soluble in organic solvents, such as alcohol, ether, or fatty oils. Its chemical formula is: C18 H27 O3N, and its molecular weight is 305.40 daltons. Capsaicin is weakly acid, and contains a phenol group; its structural formula, identified as methyl-vanillinic-acid, is represented in Fig. 1.
The capsicin molecule needs specific structural requirements to maintain its potency (Jancso and Kiraly, 1981; Szolcsanyi and Jancso, 1976): The presence of a free hydroxyl group on the aromatic ring is essential for pungency, a critical distance linking the acylamide and vanillyl moieties must be maintained (the substitution of the acylamide linkage by an esterlic group abolishes the desensitizing activity of the compound). Finally, the presence of the alkyl chain, with an optimal length of 10-12 atoms of carbon, also seems to be essential in order to produce a desensitizing effect.

These features suggest that capsicin interacts with a molecular recognition site, which is able to distinguish subtle structural changes among congeners (Nagy, 1982).

C. GENERAL PHARMACOLOGICAL PROPERTIES

A considerable number of studies indicate that capsicin has several important pharmacological effects in the cardiovascular and respiratory systems, in the thermoregulatory system, in the gastrointestinal tract, and finally, in the nervous system.

1. Cardiovascular and Respiratory Effects:

Triad of responses evoked in animals treated systemically with capsicin, consists of hypotension, bradycardia, and apnea: The severe pulmonary constriction is coupled with a reflex dilatation of the venous system, which leads to a decrease in cardiac output, resulting in decreased blood pressure (Jancso and Such, 1983). It has been suggested that capsicin acts by neural reflex mechanisms, originating from chemoreceptors of the vascular apparatus (Monsereenusalorn, 1982).
Capsaicin appears to activate selectively certain unmyelinated (type-C) primary afferent fibers, and its cardiovascular, and respiratory reflex action appears related to activation of a distinct population of nerves in the vagus nerve (Papka et al. 1984).

2. Effects on Thermoregulation:
Capsaicin given systemically at lower doses (1.4 micron g/g body weight), induces a transient fall in body temperature (Jancso et al. 1970). Higher doses (65 mg/kg) apparently desensitize the central hypothalamic warmth receptors: At this dose capsaicin can no longer lower body temperature, but produces a pronounced hyperthermia in response to high ambient temperature. At the ultramicroscopic level, the drug induces morphological changes in small type B cells of the spinal ganglia and in the preoptic area, visible 2 days after initial treatment (Szolcsanyi et al. 1971). This effect is proportional to the dose injected, and seems to be irreversible.

3. Gastrointestinal Effects:
Capsaicin given orally to animals, produces a copious flow of saliva, and a slight increase in gastric secretion (Feng, 1929). Pathological changes of the mucosa including oedema, and ulceration, has been reported in humans and experimental animals fed with diets supplemented by hot peppers (Makara et al. 1965). Capsaicin may exert its effect directly on both mucosal receptors and absorptive cells by increasing the gastrointestinal mobility and by reducing fat absorption, which can reduce the growth rate of the animals (Monsereenusorn, 1983).
4. **Effects of Capsaicin on the Nervous System:**

a) Anatomical effects on primary sensory neurons:

- **Systemic administration** of capsaicin to neonate rats results in degeneration of small type B cells of sensory ganglia, 30 minutes after injection. After 4 hours, degeneration of unmyelinated fibers in the dorsal root occurs and at 2-8 hours, axon terminal degeneration is apparent in the dorsal horn, in the superficial layers of the trigeminal nucleus caudalis, and in small areas of the trigeminal nucleus oralis, the solitary nucleus and in the area postrema. At 48 hours, no sign of terminal degeneration is apparent (Jancso, 1978; Jancso and Kiraly, 1980). This degenerative effect can be elicited in animals up to 14 days of age (Jancso and Kiraly, 1977; Jancso et al. 1981), but is not observed in adult animals (Jessel et al. 1978). Degeneration is not seen in other structures of the CNS (Nagy et al. 1980).

Controversy has arisen as to whether systemic administration of capsaicin leads exclusively to C fibers degeneration or whether some small myelinated A-delta fibers are also affected. Welk et al. (1984) found a 50% reduction in the number of unmyelinated type C fibers in the rat saphenous nerve, but no change in the number of the small type A-delta myelinated nerve fibers. Similarly, Scadding (1980) showed a 50% reduction on the number of unmyelinated axons in mouse sural nerve and a 64% reduction on the number of unmyelinated fibers in rat saphenous nerve while the myelinated fibers were unaffected by the drug. These results agree with Holje et al. (1983), who reported exclusively reduction of unmyelinated fibers in the inferior alveolar nerve of mice. In contrast, Nagy et al. (1983) reported a loss of both small myelinated
(40%), and unmyelinated fibers (94%) from dorsal roots of rats treated neonatally with capsaicin. Another study conducted by Jancso and Kiraly (1980), also reported a neurotoxic effect of the drug on both myelinated, and unmyelinated fibers of rats saphenous nerves, with 60% reduction of unmyelinated fibers, and only 10% loss of small myelinated A-delta fibers. These effects are permanent in neonate (Jancso and Kiraly, 1977), and dose dependent, with a threshold dose of 15 mg/kg and a saturating dose of 50 mg/kg (Jancso and Kiraly, 1981). The drug affects principally type-C fibers of all sizes, with less destruction of small type A-delta fibers (Nagy et al. 1983). However, at least 5% of type -C fibers remains after treatment (Nagy et al. 1980).

**Local application** of capsaicin to the sciatic nerve of adult rats causes modifications in pain perception to different stimuli, but its effect is reversible, and not accompanied by nerve degeneration (Fitzgerald and Woolf, 1982).

b) Neurochemical effects:

Recent observations by Nagy et al. (1981) have suggested that substance P, and other putative neurotransmitters, are involved in the pharmacological effects of capsaicin. The systemic effects of prenatal capsaicin treatment were investigated on 15-day foetuses mice by indirect immunofluorescence conducted one week after birth, and indicated an extensive depletion of substance P immunoreactive fibers from the dorsal horn and peripherally from cutaneous nerve endings in the skin of the forepaw and from cells of the dorsal root ganglion. The observed effects of capsaicin on 15-day foetal mice were identical to those described for neonatal animals (Atkinson and Chaggar, 1983).
Systemic administration of capsaicin, in both adult and neonate rats, results in depletion of substance P in small size sensory neurons, and their terminals in the CNS (Gamse, 1982; Nagy et al. 1980, 1981, 1983). The magnitude of depletion varies with the neurotransmitter (somatostatin, and the vasoactive intestinal peptide are much less sensitive to capsaicin than substance P, Nagy et al. 1981), and its specific anatomical location (the depletion of substance P is 55% in the dorsal horn, 95% in the dorsal root ganglia, 70% in the trigeminal ganglion, and 80%, peripherally in the saphenous and vagus nerves, Nagy et al. 1983). Finally, its action is time dependant (the best effect is seen between 2-5 days), and is irreversible for neonates (Nagy et al. 1981).

c) Effects on pain sensation:

The initial contact of capsaicin with the skin and the mucous membrane, following systemic or local treatment, produces a violent irritation, including hyperaesthesla, scratching, and contractures of the animals (Fitzgerald, 1983). These effects are followed by a long-lasting desensitization of both young and adults animals to further noxious stimull (Jancso and Kiraly et al. 1977; Jancso, 1981; Nagy et al. 1981). While desensitization of the animals to chemical pain has been reported unanimously, the effects of capsaicin on other types of nociceptive responses are less certain. It seems that higher doses are required for mechanical, or thermal analgesia, and the effects are variable depending the dosage used, the methodology adopted in the behavioral tests and the species (e.g. Gamse, 1982). Numerous studies have reported a diminished sensitivlty to noxious thermal stimull in
neonate (Gamse, 1982), and adult treated animals (e.g. Fitzgerald and Woolf, 1982). In contrast, Welk et al. (1984) found no modification in response to thermal stimuli. The same papers showed inconclusive results about intense mechanical stimuli, depending on the type of stimuli applied. However, it appears that the analgesic effect of the drug seen at 2-10 days postnatal, progressively disappears in older animals which would explain a negative response to noxious tests performed at 11 days vs. a positive response at 3 months after treatment. However, it appears that changes in substance P alone cannot explain all the effects observed after Capsalgin treatment. While a reduction in nociception is always found with depletion of substance P after neonatal treatment, the inverse is not always true.

In conclusion, the mechanism of true analgesia produced by capsalgin is not well understood, and will require additional investigation.

d) Effects on neurogenic oedema:

A role of sensory nerves in the inflammatory processes has been well demonstrated by the fact that oedema can be stimulated by antidromic stimulation of the nerve endings, causing a release of the neurotransmitter substance P (Jancso and Szolcsanyi, 1972). The inflammatory reaction, or neurogenic oedema, mediated by peripheral C-fibers, is abolished, either permanently in neonate, or reversibly in adult animals, after systemic (Jancso et al. 1967; Jancso and Szolcsanyi, 1972; Jancso and Kiraly, 1977; Jancso et al. 1980; Lundblad et al. 1983; Sarla et al. 1983 a,b), or local (Jancso et al. 1980; Lundblad et al. 1983) administration of the drug. Additional evidence
that nociceptive nerve endings play a role in neurogenic inflammation is suggested by the fact that, in all the above studies, the onset and threshold dose of capsaicin required for the abolition of neurogenic inflammation were the same as for chemical analgesia. However, capsaicin treatment may alter some non-neurogenic aspects of the inflammation as well, because its effects are accompanied by a stimulation of prostaglandin synthesis and a lower responsivity to histamine and bradykinins (Couture and Cuello, 1984). Interestingly, the reduction of type C containing substance P fibers which mediate inflammatory reaction, results in poor wound healing in the treated animals (Fitzgerald, 1983). The fact that substance P stimulates the proliferation of T lymphocytes (Payan et al. 1983) suggests a possible control of the immunologic response by peripheral nerves.

e) Effects on the physiology of sensory neurons:

The effect of local application of capsaicin has been investigated on the rat saphenous nerve (Welk et al. 1984); the polymodal nociceptors showed equally reduced responses to heat mechanical and chemical stimulation.

Following systemic administration of capsaicin in adult cats the evoked potential elicited by stimulating electrically the saphenous nerve shows a substantial reduction of the C2 component; however, the A-alpha, beta and gamma, and the C1 responses remained unchanged. Possibly, capsaicin modifies the nerve conduction by inducing an hyperpolarization of the cell membrane resulting in a prolonged duration of the action potential (Szolcsanyi, 1977). In contrast, other studies
report no effect in peripheral nerve physiology in adult animals (Welk et al. 1984; Salt et al. 1982).

D. MECHANISM OF ACTION OF CAPSAICIN

1. Specificity:

None of the effects of capsaicin such as its neurotoxic action has been observed in the autonomic system (Jancso and Kiraly, 1980). LM and EM examinations showed that the fibers containing synaptic vesicles characteristic of noradrenergic or cholinergic terminals were not affected, while the affected fibers containing large dense-cored vesicles characteristic of peptide-containing nerves, disappeared after treatment (Papka et al. 1984). The non-adrenergic nature of substance P fibers was also supported by the lack of effect of 6-OH-Dopamine pretreatment on levels of substance P (Sarla et al. 1983a), or the unaltered level of noradrenaline after capsaicin treatment (Jancso et al. 1980). Moreover, this suggests that substance P fibers seen in the sympathetic trunk and ganglia (Table II) are sensory in origin, and confirms the specificity of the drug for primary afferent sensory neurons. These observations together with the lack of effect on dorsal horn choline acetyltransferase, and glutamic acid decarboxylase activities (Nagy et al. 1981), confirmed the degree of specificity of the neurotoxic action of capsaicin on substance P neurons.

Concerning its neurotoxicity, capsaicin acts on type C2 fibers, while having no effect on type C1, A-alpha, beta, and large size A-delta fibers (Szolcsanyi, 1977, Nagy et al. 1980). This suggests that the specificity of capsaicin may involve molecular sites which may be unique to the cell membrane of certain types of primary afferent sensory
neurons. However, not all neurons containing substance P are sensitive to capsaicin, and in the neonatally treated animal, the unaltered level of substance P in the Substantia Nigra, striatum, and Hypothalamus, which are known to contain substance P, suggests that the neurotoxicity of capsaicin is restricted to peripheral substance P-containing neurons (Nagy et al. 1980). Apparently, capsaicin does not appear to act directly on CNS neurons, and the electron microscopic observation of degeneration and glial engulfment in the dorsal horn are secondary to degeneration of primary afferent fibers and terminals. The primary site of action of capsaicin administrated systemically, seems to be the cell body of the primary afferent neuron itself and consequently, the central and peripheral branches would be affected indirectly by centrifugal degeneration (Jancso and Kíraly, 1977, 1980; Jancso, 1978).

There is still some controversy concerning the specificity of action of capsaicin in the peripheral nervous system. Recent evidence has suggested that substance P fibers are not equally sensitive to capsaicin in all regions: The presence of different sub-types of substance P contained neurons has been shown by Immunocytochemistry (Brodin et al. 1981), and one possible reason for the variable effect of capsaicin on different types of nerve fibers is that certain neurons possess, and others lack capsaicin receptors. Possibly capsaicin receptors are present in a specific sub-population of substance P neurons. This hypothesis is supported by recent studies (Lundblad et al. 1983, Weil et al. 1984), which have reported capsaicin-resistant substance-P fibers in the trigeminal and saphenous nerves. However, the long period of observation of these experiments (3-4 months) may have allowed reinnervation by sprouting from unaffected fibers. The lack of
effect of the drug was also reported in the gut (Holzer et al. 1980, Atkinson and Chaggar, 1983) which is known to contain a high concentration of substance P produced by endocrine cells and intrinsic neurons. This result suggests that capsaicin acts selectively on primary afferent substance P neurons without affecting other types of substance P neurons such as substance P neurons intrinsic to the gut.

The developmental stage of the animal could also modify the specificity of capsaicin versus substance P nerve fibers, by the absence or reduction of binding sites, or by the minor synthesis of substance P due to the immaturity of the neuronal system. However, substance P fibers have been demonstrated at early stages of development (Mohamed and Atkinson, 1982; Atkinson and Chaggar, 1983). Moreover, undifferentiated neurons at certain stages of development have increased sensitivity to the drug, since a broader spectrum of changes and more intense effects have been widely reported after systemic administration of the drug in neonate, compared with adults animals (Jancso et al. 1980).

Finally, the effects of capsaicin treatment on sensory neurones must be interpreted with care, because the neurotoxic effect of the drug may not be limited to a simple peptide phenotype. The coexistence of several peptide transmitters in the same neuron has been shown previously (Johansson et al. 1981). Somatostatin (Kessler and Black, 1981), or the vasoactive intestinal polypeptide (Uddman et al. 1980), as well as other peptides could be present together with substance P in individual neurons, and could therefore be affected by capsaicin, as these cells degenerate.
2. **Mechanism of Action:**

Several mechanisms have been proposed to explain the neurotoxic action of capsaicin. Depletion of substance P immediately after capsaicin treatment, which results in impairment of these fibers, has been suggested (Lundblad et al. 1984). However, the rapid onset of anatomical effects observable ultramicroscopically within 5 minutes after systemical administration (Papka et al. 1984), seems to indicate that anatomical alterations of the sensory nerves precede detectable depletion of substance P, which occurs only 5 hours after treatment. Therefore, the functional impairment of the nerve fibers may not result directly from depletion of substance P.

Another alternative may be that the inhibition of axonal transport interrupts the transport of substance P, as well as other substances such as nerve growth factor (Kessler and Black, 1981), and other neurotransmitters which may exert a trophic influence on the nerve itself, resulting in irreversible damage to the neuron.

Also, the mode of administration of capsaicin seems to be a critical factor. When the drug is applied locally on peripheral nerves, the onset of analgesia tested by the application of noxious stimuli on the skin, occurred 4 days before any anatomical effect is detectable in the dorsal horn. Apparently, the peripheral effect of capsaicin occurs before the effect at the central level. The different axoplasmic flow between central and peripheral processes of the nerve fiber characterized by the fact that 50% less proteins transit centrally from the dorsal root ganglion (Ochoa, 1976), may relate to the differential effect of the drug.
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is able to deplete substance P from primary afferent neurons without an apparent loss of nociceptive transmission to nucleus caudalis and dorsal horn. Moreover, the 60% depletion of substance P observed in the trigeminal nucleus caudalis does not alter all the neurons responding to noxious and non-noxious stimuli of the face (Salt et al. 1982).

3. Site of Action:

The exceptional rapidity of action of capsaicin has suggested that the chemical may have access to the entire length of the neuron and damages the nerve by direct toxic action to the cell body. The central and peripheral branches of the primary afferent neurons being affected indirectly by centrifugal degeneration (Jancso and Kiraly, 1977, 1980; Jancso, 1978). The earlier effects seen in the central branches of the primary afferent neurons may be related to the size difference between central and peripheral branches, the former showing in general reduced diameters (Ochoa, 1976). A possible target of the toxic attack in the axon would be implicated enzymes concerned with energy transformation. Histochemical studies have shown a rise of ionic calcium in type B neurons of the sensory ganglion 20 minutes after administration of capsaicin which precedes ultrastructural changes occurring 30 minutes later (Jancso et al. 1984). The initial ballooning and then shrinkage of the nerve fibers visualized both in immunofluorescence and at EM levels are similar to the watery degeneration attributed by Papka et al. (1984) to an early breakdown of ion pumps resulting in the interruption of axoplasmic transport followed by the final anatomical alterations of the axons.
are similar to the watery degeneration which Papka et al. (1984) attributed to an early breakdown of ion pumps, resulting first in the interruption of axoplasmic transport, followed by the final anatomical alterations of the axons.

E. CAPSAICIN AS A TOOL TO STUDY THE ROLE OF SUBSTANCE P IN THE DENTAL PULP

1. Introduction:

The peptide substance P is one of 20 peptides known to be candidates as neurotransmitter in mammalian neurons (Pernow, 1983).

At the central level, substance P shows an unequal distribution the substantia nigra, hypothalamus, solitary nucleus and dorsal horn containing the highest amounts. Immunological studies in rats have demonstrated the presence of substance P at high concentration in the dorsal horn, particularly in the lamina I and II in the outer laminae of the nucleus caudalis of the trigeminal nucleus and in type B neurones of the spinal ganglia, where it is contained in 20% of the cells (Hokfelt et al. 1975). The concentration of substance P is much lower in the peripheral terminals, than at the central terminals of primary sensory neurons, which supports the idea that substance P may be a neurotransmitter at the level of the first central synapse (Ochoa, 1976).

Peripherally, substance P nerve fibers are present in most sensory organs (Bucsiics et al 1983 e.g. Table I), localized in two main systems: the peripheral and central branches of primary sensory neurones (mostly in unmyelinated and small diameter myelinated fibers), and the intrinsic neurons of the gastrointestinal tract (Brodin and Nilsson, 1981). It is
also present in sympathetic ganglia, where substance P is transported by sensory fibers which course near the sympathetic neurons (Matthews and Cuello, 1982).

A detailed mapping of the early ontogeny of substance P has shown that substance P fibers are first detectable around 14-16th day of gestation in the mouse spinal cord, and by 17th day, substance P-positive fibers are demonstrable in peripheral terminals, and sensory ganglia. Conceivably the elaboration of substance P in the sensory neurons occurs long before the nerves become responsive to stimuli (Atkinson and Atkinson, 1983).

2. Role of Substance P in the Dental Pulp:

a) Localization:

Substance P is present in relatively large amounts in the dental pulp. The tooth in the cat at least seems to be the substance P-richest organ outside the CNS (Brodin et al. 1981b, e.g. Table II). Immunochemical studies have shown that substance P-positive fibers are often associated with blood vessels but also apparent as free nerve endings (Mohamed and Atkinson, 1982; Olgar et al. 1977). Some fibers are also found in the odontoblast-predentin border, sometimes in close association with the odontoblast processes, ending about 60 um near the mineralized dentin (Wakisaka et al. 1984). These fibers disappear totally after transection of the inferior alveolar nerve, but sympathectomy does not affect substance P fibers, which supports a sensory role of these nerve fibers (Olgar et al. 1977a).

b) Substance P and neurogenic inflammation:

Experiments conducted on dental pulp have shown that antidromic
electrical stimulation of the inferior alveolar nerve produces a release of substance P (Brodin et al. 1981a,b; Olgar et al. 1977b; Kroeger, 1968), associated with an increase in pulpal blood flow (Rosell et al. 1981, Brodin et al. 1981b, Gazellus et al. 1981). This effect is selectively blocked by specific substance P antagonists (Rosell et al. 1981), which suggests the presence of several sub-populations of receptors. Chromatographic studies (Brodin et al. 1981b) have also indicated the coexistence of different sub-groups of substance P in the dental pulp. Also, possibly other transmitters agonists such as the vasoactive-intestinal-peptide (Udman et al. 1980), or antagonists, such as somatostatin (Gazellus et al. 1981) have a regulatory effect on substance P.

In contrast, other studies have shown a lack of effect of systemic administration of either substance P or capsaicin (Lundberg et al. 1984; Sarle et al. 1983a,b; Jancso and Szolcsanyi, 1972) in modifying the pulpal circulatory status. These results may be inconclusive because the marked systemic vasodilatation occurring simultaneously in the surrounding tissues may lead to a low vascular tone, accompanied by a regional decrease in perfusion pressure in the tooth.

At the present little is known about the exact nature of neural control of vasodilatation in the dental pulp. Cholinesterase positive nerve fibers, and monoaminergic terminals have been demonstrated in the dental pulp (Pohto and Antila. 1972). Also, histamine -like fluorescence was detected in the walls of blood vessels, and may constitute a counterbalance to nervous vasoconstriction, especially when the vasodilatative innervation is lacking (Pohto and Antila, 1972). Finally, the characteristics of substance P mediated vasodilatation seems
different from those of cholinergic-mediated vasodilatation (Gazellus et al. 1981). Cholinergic neurons may differ from substance P neurons, in that local application of capsaicin on peripheral nerves, such as sural or sciatic nerves, blocks the axoplasmic transport of substance P, and somatostatin without affecting the transport of noradrenaline and cholinesterase (Gamse et al. 1982). Therefore, substance P seems to exhibit most of the characteristics of a possible mediator of neurogenic inflammation in the dental pulp: It is present in sensory C fibers, many of which accompany blood vessels, it is released from peripheral terminals, and possesses a high potency as vasodilator. Finally, pulpal tissues contain enzymes capable of degrading substance P (Kroeger, 1968).

c) Substance P and nociception in the dental pulp:

- at the central level, substance P may function in nociceptive afferent transmission since the SPLI immunoreactivity distribution found in the superficial layers of the trigeminal nucleus caudalis correlates with the anatomical distribution of nociceptive nerve fibers in the dental pulp (Salt et al. 1983, Henry et al. 1980). Electrophysiological studies have shown that nucleus caudalis neurons responding to
electrical pulp (presumably noxious) stimulation, are also responsive to lontophoretically applied Substance P. The fact that neurons excited by noxious stimuli are also invariably excited by substance P has suggested that substance P acts at the central level, with an excitatory role in transmission of pain, possibly at the primary afferent synapse (Henry, 1980). However, substance P may have a modulatory rather than a neurotransmitter function because the excitatory effect of substance P has a slow onset, and is most pronounced on neurons showing ongoing firing (Salt et al. 1982, 1983).

Peripheral Substance P does not seem to have any direct excitatory effect on peripheral nerve endings, since local application of Substance P in the cat tooth failed to excite dental nerves (Gazellus et al. 1977). However a modulatory effect has been reported, and Gazellus et al. (1977) showed that local application of Substance P on dentin reduces the subsequent neuronal response to hypertonic NaCl solution application, for a period of 30 minutes. The author concluded that this modulatory effect may be produced either by direct action of Substance P on the sensory nerve endings or, possibly by an increased blood flow, which in turn modifies the response to further stimuli.

The effect of neural control on blood circulation by means of Substance P, and its possible modulatory action on sensory nerve response, has also been postulated by Edwall and Scott (1971) and Edwall (1982) who showed that a thermal stimulus applied to dentin, superimposed with induced sympathetic vasoconstriction, was inadequate to evoke any sensory nerve, or vasoactive substances, and the sensory mechanism of teeth has also been suggested by Kroeger (1968).
d) Possible trophic effect of Substance P:

Besides a vasoregulatory action and a possible modulatory role in nociception in the dental pulp, Substance P may act as a trophic factor. This possibility which is suggested by observation of the changes of dental growth pattern which occur following deveneration experiments, has not been investigated. The evidence of disturbance in calcium transport after Substance P depletion induced by Capsaicin (Jancso et al. 1984), and the dependance of hormone release on calcium in other systems (Karcsu et al. 1982) implies that Substance P could regulate the secretory activity of the odontoblasts (Kroeger, 1968).

3. CONCLUSIONS:

Because of the various pharmacological effects of Capsaicin on Substance P, it is difficult to relate the Substance P containing neurons to a specific function. Moreover, the multiple varieties of physiological functions of Substance P suggested by the effects of Capsaicin treatment seem inconsistent with only one type of Substance P, but, rather different several subpopulations of the peptide may be present in neurons distributed to different organs.

In conclusion, it appears that Capsaicin may be a useful probe of the distribution, and function of certain unmyelinated fibers in the primary sensory neurons, but the drug does not appear to be a highly specific neurotoxin for Substance P-containing fibers. Indeed the alteration of other peptide systems may also be involved in the effect of Capsaicin.
THE EFFECTS OF CAPSAICIN ON THE INNERVATION OF DEVELOPING MOLARS IN THE NEONATE MOUSE

1. INTRODUCTION


The administration of capsaicin to young animals produces a wide range of physiological effects including the loss of neurogenic inflammation (Jancso et al. 1967, 1980; Jancso and Szolcsanyi, 1972; Jancso and Kiraly, 1977; Lundblad et al. 1983; Sarla et al. 1983a,b) and an irreversibly elevated nociceptive threshold. Substance P-containing primary sensory neurons mediating chemogenic nociception are known to be affected by capsaicin (Jancso, 1978), although other sensory modalities have also been shown to be affected (Gamse, 1982; Gamse et al. 1982; Fitzgerald and Woolf, 1982).

At the ultrastructural level, the chemical produces a selective and near total degeneration of unmyelinated fibers of the sensory spinal nerves, with a special affinity for type C and small size A-delta fibers (Scadding, 1980; Szolcsanyi, 1977). It also kills small type-B cells of spinal ganglia (Jancso and Kiraly, 1977) and the central terminals of

In the trigeminal sensory complex, the administration of capsaicin produces degeneration of central terminals of primary afferent neurons and loss of substance P in the trigeminal nuclei (Salt et al. 1982; Jancso, 1978; Jancso and Kiraly, 1980). At the periphery, various trigeminal fields present a significant decrease in substance P content (Jancso and Szolcsanyi, 1972; Lundberg et al. 1984; Lundblad et al. 1983; Sarla et al. 1983a) associated with a loss of unmyelinated axons. The decrease is 85% in the dorsal root and 58% in the inferior alveolar nerve (Holje et al. 1983).

Teeth reportedly contain a preponderance of fibers mediating pain, including predominantly A-delta and C type fibers (Anderson et al. 1970). In addition, the dental pulp shows one of the highest tissue levels of substance P in the body (Brodin and Nilsson, 1981), and nerve fibers showing substance P-like immunoreactivity are numerous in the dental pulp (Olgar et al. 1977a; Mohamed and Atkinson, 1982). All functions of substance P-containing nerve fibers, and their possible role in the dental pulp have not been elucidated.

Recent studies using nerve resection have suggested that pulpal nerve fibers may exert a formative influence during dental development (Chiego et al. 1981; Klein et al. 1981; Chiego et al. 1983; Avery et al. 1974; Avery and Cox, 1977). Besides a modulatory role in nociception (Gazellus et al. 1977; Edwall and Scott. 1971; Matthews, 1976, Kroeger, 1968), substance P containing nerve fibers may exert some trophic influences on the secretory activity of the odontoblasts (Kroeger, 1968). This possibility, which is suggested by observation of the
changes of dental growth pattern occurring after denervation, has not been investigated.

In the present report, the effect of capsaicin was investigated to determine if the neurotoxin reduces dental innervation and if so, if this reduction affects the development of dentin and enamel. It was also possible to identify the type of nerve fibers, presumably those containing substance P which disappear after treatment. Finally, this study was an attempt to determine if capsaicin can be an useful probe of the distribution and function of unmyelinated nerves contained in the dental pulp. Such information would clarify the mechanism of pain transmission at the level of the tooth.
II. MATERIALS AND METHODS

A. TREATMENT OF THE ANIMALS

The solution of capsaicin was prepared as described by Jancso et al. (1967). Capsaicin is practically insoluble in water, so the 1% solution is prepared as follows: 0.1 gm of crystalline capsaicin is dissolved with a mortar and pestal in 2-3 drops of ethanol, to which 15 drops (about 0.6 mg) of Tween 80 are added; the final volume is made up to 10ml with physiological saline.

The drug was injected subcutaneously in the backs of 054/10 strain mice in a volume of 0.1 ml. All injections were started at the second day after birth because in a previous pilot study, earlier administration of the drug threatened the survival of the animals. Animals then received daily subcutaneous injections of either capsaicin at 50 mg/kg body weight (5 animals), or the vehicle control solution (5 animals). At 8 days, the animals were killed with ether 4 hours following the last injection.

In a pilot study, we also investigated whether capsaicin administered prenatally would influence the formation of tooth germs. Female mice of 8 days pregnancy were injected subcutaneously with incremental doses of capsaicin to a final amount of 50 mg/kg. At 15 days of pregnancy, the foetuses were removed by cesarean section.

B. PREPARATION OF THE TISSUES FOR ELECTRON MICROSCOPY

All animals were perfused through the left ventricle of the heart for a period of 10 minutes with fixative containing 1% paraformaldehyde-2.5% glutaraldehyde in phosphate buffer (pH 7.4). Right and left halves
of the mandible were excised under a stereoscopic microscope and tissue blocks containing the 3 molars removed from one half of each jaw and placed in fixative overnight at 4°C (this temperature was chosen to avoid depolymerization of microtubules, and to minimize autolysis). The specimens were rinsed 3 times, 10 minutes each, in 0.1 phosphate buffer containing sucrose and CaCl₂ and postfixed with 2% osmium tetroxide in phosphate buffer (pH 7.4) for 2 hours. The specimens were stained on bloc at 4°C with 2% uranyl acetate for 2 hours. After 6 rinses, 10 minutes each, in acid-sodium acetate buffer, the specimens were dehydrated in increasing graded ethanol, passed through propylene oxide twice 10 minutes each and placed in 1:1 propylene oxide: Epon 812 overnight, then in pure Epon under vacuum for 24 hours. Finally, the specimens were embedded in fresh Epon and cured in a 60°C oven for 48 hours.

For the pilot study, the foetuses were decapitated and their head immersed in the same fixative for 48 hours.

C. BLOCK SELECTION, SECTIONING and STAINING

Semi-thin (2 um) sections were cut with a glass knife in the transverse plane relative to the long axis of the teeth. Sections were stained with toluidine blue, and viewed with the light microscope to check the general condition and orientation of the specimen, and to select the area of interest. Blocks were further trimmed to include the entire apical cross section of the pulp of each molar, as illustrated in Fig. 2. For the first molar, which exhibits a larger size, the pulp area was split into 2 separate blocks at the level of the furcation area, in order to facilitate subsequent thin sectioning. The
orientation of the specimens was checked under the light microscope. In the mouse molar, the apical margin of the developing tooth is formed by the epithelial root sheath. For the specimens used in this study, sections displaying a complete circle of epithelial root sheath were selected. Thin sections of 600-1000 A (silver to light gold) were cut serially on a Reichert Om U3 microtome, using a diamond knife; the sections were placed on ultrathin bar grids, and stained for 15 minutes with 2% alcoholic uranyl acetate, and 30-60 seconds with 0.2% lead citrate. The grids were examined with a Zeiss EM 10.

D. ELECTRON MICROSCOPY- QUANTITATION

Sections were selected as suitable for examination when well fixed, unfolded, and lying entirely inside the border of the grid.

1. Counting of the Nerves:

Systematic examination was performed on an average of 6 sections per molar, with the entire pulpal area being scrutinized. The work was carried out at 4000X and 6300X magnification. Profiles of questionable axons were examined at high magnification (8000X) for definitive identification. For each tooth, the whole cross sections were examined for the presence of nerves and all fields containing axons were photographed with an average of 30 photographs for M1 and 15 photographs for M2. The negatives were enlarged 4 to 7 times to provide prints with a final magnification of 25200 to 56000X. Counts of all nerves in each tooth were obtained from the micrographs. Six sections of the same area were viewed to overcome the possibility that bars of the grid could obscure areas of the pulp containing nerves.
2. Measurement of Axonal Diameter:

- SAMPLING OF FIBERS: The sensitivity, reliability, and standard deviation of an estimate of the experimental group depends on the magnitude and distribution of the abnormality, and on the sampling procedure; usually, for clinical applications, a minimum of 100 fibers must be sampled to get representative results in detecting an abnormality (Dyck et al. 1984). In this experiment, 3 teeth of each control molar type, and 4 teeth of each experimental molar type were used. The higher number of specimens utilized from the experimental group compensated for the lower number of nerve fibers seen in this group, so that the number of nerves evaluated in control and experimental groups was almost equivalent. Teeth were selected based upon the technical quality of the overall specimen. Nerve measurement was performed on all axons contained in each cross section.

- PROCEDURE OF MEASUREMENT: The average diameter of all nerve fibers was calculated by adding long axis (the largest dimension) and short axis (being the shortest distance across the widest part of the fiber, measured at right angle to the long axis) and dividing by 2.

E. LIGHT MICROSCOPY

The other half-jaw of each animal was fixed for 48 hours in the same fixative solution used for the perfusion. After routine dehydration and infiltration the specimens were oriented, embedded in paraffin, and 5 μm serial sections of the mandible were cut in the transverse plane (longitudinal relative to the molars) and stained with hematoxylin and eosin. In the pilot study, the heads of six foetuses were prepared similarly for light microscopy. Longitudinal sections
through the bud of the first molar were stained with hematoxylin and eosin.

F. QUALITATIVE EVALUATION OF THE DATA

The evaluation of the data from the experimental group was dependent on the following methodologic considerations:

1. **Non Invasive Methods:**

   The temporal response of experimental animals to systemic intoxication was evaluated by comparing with controls the following parameters:
   
   a. body weight
   b. behavioral signs: such as irritability, hyperactivity, panting, vasodilatation, respiratory and circulatory changes were monitored for a period of 1 hour after each injection. We sought evidence of capsaicin-induced desensitization by comparing the behavioral effects of the first and last injections in the chronically treated animals.

2. **Invasive Methods: Postmortem Studies:**

   - **ELECTRON MICROSCOPIC STUDY:** For the morphological examination, the following criteria were considered as evidence of nerve degeneration: local condensation of microtubules, axoplasm with watery content and/or devoid of organelles, presence of axonal debris, clumped material impossible to identify, presence of sequestered debris, or dense membranous bodies within the cytoplasm of Schwann cells. In the present report, the terminology used to describe the neuronal components is consistent with the description of Aguayo and Bray (1975), and Ochoa
and Mair (1969a-1969b). The term Schwann cell unit refers to structures totally enclosed by the basal lamina of the Schwann cell. Early stages of myelination are consistent with the description of Peters et al. (1970): An axon is considered in the early stage of myelination when isolated from other axons by a distinct Schwann cell, and embedded within a short mesaxon.

LIGHT MICROSCOPIC STUDY: Many studies have reported effects of denervation on the rate of tooth eruption (Rehak, 1963; Edward and Kitchin, 1938), and the formation of hard tissues (Avery et al. 1971, 1974; Chiego and Singh, 1974; Chiego et al. 1981) during tooth development. In this study, the following morphological features have been evaluated in a search for changes following denervation:

a. General changes in shape and size of the tooth, or reduction in the volume of the pulp chamber.

b. Modification of the rate of eruption, evaluated by the distance between the tip of the cusps to the oral cavity.

c. Amount and general pattern of dentine and enamel versus the control group, evaluated by the presence of dentinal changes such as irregular dentine including the formation of osteodentine with entrapped odontoblasts, widened predentine, globular dentine, and irregular pattern of calcification. Changes in the ameloblast layer, associated with distortion of the inner enamel epithelium, stellate reticulum and enamel. The level of the section at which the hard tissues were examined corresponds to the cervical and central area of the crown, containing the most recently formed layer of dentin and enamel, as illustrated in Figure 2. Similar observations were carried-out at the ultrastructural level by an
examination of the ameloblasts, odontoblasts, and their secretory products in electron micrographs of transverse sections through the central part of the crown of 2 molars from each group.
III. RESULTS

A. GENERAL SYSTEMIC EFFECTS

All the animals treated with capsicin showed immediate and severe signs of circulatory and respiratory disturbances after the first injection, including bradycardia, apnea and an alteration in the frequency and amplitude of the respiration. This first excitatory phase, which was variable in magnitude from one animal to the next, lasted for about 10-20 minutes. The animals also exhibited marked behavioral changes, such as convulsions, irritability and hyperactivity during the first minutes after injection, followed by a prostration of the animals for the next hour. The mortality rate in the experimental group was approximately 20%, apparently caused by the anaphylactic shock created by the drug. During the first 2-3 days of injection, there was a decrease of approximately 20% in body weight for all animals; this perturbation in growth pattern persisted until the end of the experiment for most animals, while a few showed final weights comparable to the animals of the control group. By the end of the experiment, there was the complete absence of circulatory and respiratory effects of the drug. The behavioral component seemed to decrease progressively after the first days of injection, and the last injection produced little or no behavioral change. Injection of the solvent to the control animals did not produce any systemic effects.

B. NEUROGENIC INFLAMMATION

All animals injected with capsicin presented with an immediate flushing of the entire body. This effect, which most visible in the skin
of the legs and the face, disappeared progressively during the next
hours, and further injections of the drug did not elicit an inflammatory
response. In the control group, the injection of the solvent did not
elicit the general inflammatory reaction observed in the experimental
group. A mild inflammatory reaction was present at the locus of
injection. This reaction occurred after each control injection and
lasted for a period of 10 to 15 minutes.

C. PAIN SENSIBILITY - DESENSITIZATION EFFECT

In the experimental group, the manifestations of noxious mechanical
stimuli produced by the needle used for injection, and noxious chemical
stimuli elicited by capsaicin, declined over time and were absent at 8
days. This desensitization occurred rapidly: Chemical and mechanical
analgesia occurred at 24 and 72 hours, respectively, after the first
injection. In the control group, the manifestations of noxious
mechanical stimuli, and noxious chemical stimuli elicited by the
detergent contained in the solvent occurred after each injection, and the
intensity of the reaction was not modified during the length of the
experiment.

D. NEUROTOXIC EFFECT - NERVE DEGENERATION

1. Qualitative Electron Microscopy:

a) Control group:

In all the sections studied, most of the axons were seen centrally
in the pulp, in close association with blood vessels, often grouped into
large bundles enclosed by a common Schwann cell (Figs. 3-5). In the
second molar, most of the Schwann cell units contained groups of axons
unseparated by Schwann cell cytoplasm and many of these axons lay close
to the satellite cell's basal lamina (Figs. 6 & 7). However, in the first molar, which is in a more mature stage, there was a progressive separation of these axons by expansion of Schwann cell processes (Figs. 3 & 4). The axons contained microfilaments, microtubules, vesicles and electron-dense bodies. In general, large axons exhibited an increase of both size and number of mitochondria, and microtubules and microfilaments were less randomly disposed compared with small axons (Fig 3). The stage of immaturity of nerves in the second molar was characterized by the predominance of neurofilaments densely packed in the axoplasm (Fig. 6). In both tooth types, nearly all the nerve fibers were unmyelinated, except rare axons of large diameters (over 1 um) showing early stage of myelination in the first molar. There were no myelinated fibers in any of the apical cross sections of the second molar. In both first and second molars, unmyelinated axons showing the largest circumference (> 2 um) were frequently observed in Schwann cell units of large size (i.e. containing many axons) (Figs. 3 & 4).

b) Experimental group:

There was no sign of nerve degeneration in experimental first and second molars (Figs. 8-11). The Schwann cells appeared normal, and the surrounding pulpal structures did not show any abnormal features. Features indicating recent axon degeneration, such as presence of axonal debris, sequestered material (e.g. phagosomes) within the cytoplasm of Schwann cells, Schwann cells lacking an axonal relation or containing some axons as well as empty spaces, were absent from all micrographs examined. Some few axons were seen with electron-lucent cytoplasm, devoid of organelles (Fig. 9). However, the axolemma of these axons
were intact, and their associated Schwann cells appeared normal. These features were attributed to incomplete fixation, since a few axons in the normal nerves presented the same abnormality (Fig. 4). Some axons contained vacuoles resembling swollen mitochondria (Fig. 10) as seen in some nerves from control teeth (Fig. 1).

Signs of reinnervation characterized by the predominance of axons of smaller diameter or changes in the topographical arrangement of the axons and their respective Schwann cells was also investigated. In the present study, it was not possible to make a clear morphological distinction between small diameter axons which appear in normal conditions in the developing teeth, and possible sprouting from intact collateral nerves. Growth cones (nerve profiles containing many clear vesicles with different sizes and shapes) representing the leading edges of growing fibers were rarely seen.

2. QUANTITATIVE ELECTRON MICROSCOPY

a) Control group:

**Number of nerves:** In general, nerve fibers of the first molar were far more numerous than in the second molar, with an average number of 114 for the first molar, and 42 for the second molar. The numbers of axons counted in cross sections of individual teeth are shown in Table III. The quantitative analysis showed a wide range of variability in the number of nerves within each group (70-151 for the first molars, 25-50 for the second molars) (Fig. 14).

**Nerve diameter:** The axonal diameter distribution of the unmyelinated nerve fibers is shown in Fig. 15. A total of 358 axons for the first molar and 125 axons for the second molar were measured. The first molar
showed a nearly normal distribution with a range of 0.2-2.3 µm, and a mean diameter of 0.4 µm. The immature state of the second molar was demonstrated by the asymmetric shape of the curve showing a greater proportion of small and medium diameter fibers at this stage. Axons over 1 µm in diameter were rarely seen; the range of distribution was 0.1-1.6 µm with mean of 0.3 µm.

b) Experimental group:

**Number of nerves**: For the experimental first and second molar, the quantitative analysis showed, as in the control group, a wide range of variability within each molar type, with a larger number of nerve fibers for the first molar (80, average) than the second molar (30, average) (Fig. 14). However, compared with control teeth, a decrease in the number of unmyelinated fibers was evident (30% for the first molar, and 34% for the second molar). The number of nerves in each tooth for experimental molars as for control ones was quite variable (40-119 for the first molars, 15-50 for the second molars). Therefore, the result only approached statistical significance with a p value < 0.1 for a t-test of the raw data comparing experimental M1 with M1 control. Similarly, the difference between experimental and control second molar approached statistical significance with a p value < 0.1. These p values reflect the small sample size and variability in the data. Because the differences between individual tooth types both approached statistical significance, the data from all experimental teeth were pooled to increase the sample size, and compared with data from all control teeth. A second t-test was then performed, but not on the raw data, because of large differences in the number of nerves between molar types for both
experimental and control teeth. Rather, this t-test was performed by ranking separately the raw data of the first and second molars, and pooling the rank values of all experimental, versus all control teeth (Table III). This t-test with the larger N value indicated a significant difference between all experimental and all control teeth with a p value < 0.025. The difference suggests that the experimental treatment resulted in diminished innervation of molars.

**Nerve diameter:** A total of 343 axons for the first molar, and 121 axons for the second molar were measured. The distribution of axonal diameters for both groups is shown in Fig. 15. The histogram of the experimental first molar showed a nearly normal distribution with a range of 0.1-2.4 um, and a mean diameter of 0.3, 0.4 um. However, a slight left shifted distribution was observed for the experimental first molar compared to the control first molar. This reflects a relative increase in the number of small diameter axons in the experimental tooth. For the second molar, the size distribution had a right shifted distribution with a mean diameter larger than the control (0.4 um) and a range of 0.1-1.5 um.

E. EFFECTS ON TOOTH DEVELOPMENT

1. **Control Group:**

   a) LM observation:

   At this stage of postnatal development, the first molar exhibited its entire thickness of dentin and enamel (Fig. 18). The root formation was initiated for most of the teeth, characterized by the division of the root trunk into two roots. In the second molar, the layers of dentin and enamel had not reached their complete
thickness, and represented approximately 2/3 of the amount present in the first molar (Fig. 20).

b) EM observation:

In the central part of the crown, a fine network comprising small and large collagen fibrils, were observed in the predentin at the vicinity of the distal end of the odontoblasts. The gradual pattern of dentin mineralization was characterized by an increased density in contrast to the predentin, and followed the geometric distribution of the collagen fibrils, as previously reported (Orban, 1972). In the first and second molars, the outermost part of the dentin exhibited areas of globular mineralization (Fig. 16). The enamel matrix was composed of a fine network of collagen fibrils which were surrounded by finger-like ameloblastic projections. The apatite crystals were orientated parallelly to the long axis of the rods (Fig. 16). The rods followed a wavy pattern orientated obliquely relative to the dentin surface.

2. Experimental Group:

a) LM observation:

The light microscopic examination of the experimental first and second molars showed no change in the size and shape of the tooth (Figs. 19 & 21). The amount of hard tissues present at this time was virtually identical to the control teeth. The second molar which is in an earlier stage of development was not affected by the treatment and exhibited a normal pattern of dentin and enamel formation. Our pilot study confirms these results in that all tooth germs of first molars examined during the cap stage exhibited a normal pattern of development.
b) EM observation:

These results were confirmed in electron micrographs, showing that deposition of enamel, and dentin matrix, followed by their maturation were indistinguishable from the normal pattern (Fig. 17).
IV. DISCUSSION- CONCLUSIONS

1. General Systemic Effect:

The initial painful sensation engendered by the first injection of capsaicin, followed immediately by marked systemic reactions, are similar to previous studies (Jancso and Such, 1983; Jancso and Kiraly, 1977; Nagy et al. 1980), and constitute evidence that, in the present study, capsaicin has produced a systemic effect.

2. Neurogenic Inflammation:

The initial flushing observed after the first injection is probably a manifestation of systemic neurogenic inflammation, described in previous studies (Jancso et al. 1967; Jancso and Kiraly, 1977; Jancso and Szolcsanyi, 1972; Lundblad et al. 1983; Lundberg et al. 1984). This inflammatory response involves the stimulation of the sensory nerves by excitation of the pain receptors with chemical irritant substances such as capsaicin and results in the initial release of substance P from the peripheral processes of primary afferent sensory neurons (Jancso, 1964; Gamse, 1982; Nagy et al. 1980, 1981, 1983). The inability to elicit an inflammatory response 24 hours after the first injection corresponds to the depletion of substance P from sensory neurons mediating the neurogenic inflammation (Gamse, 1982; Nagy et al. 1980, 1981, 1983), and possibly constitutes the first manifestation of nerve damage (Fitzgerald, 1983; Jancso and Kiraly, 1977; Jancso, 1978; Jancso and Kiraly, 1980; Jancso et al. 1984; Nagy et al. 1980).
3. Pain Sensation-Desensitization Effect:

The progressive desensitization to chemical noxious stimuli subsequent to capsaicin treatment reported in the present study has been reported by all previous investigations and occurs after all routes of administration. The diminished response to painful mechanical stimulation by the needle used for the injection, observed in our experiment, correlates with previous studies (Szolcsanyi and Jancso, 1976; Szolcsanyi, 1977; Gamse, 1982; Nagy et al. 1980), but disagrees with other reports where neonatal administration of the drug did not reduce the response to noxious mechanical stimuli (Welk et al. 1984; Fitzgerald and Woolf, 1982). Variability between species in the sensitivity to the drug may explain this difference (Gamse, 1982). Mice may be an optimum species to study the action of capsaicin on neurons which subserve mechanical noxious stimuli. The specificity of capsaicin which is restricted to chemoreceptors in adults of certain species (Gamse, 1982) may have a broader spectrum of action in neonate animals. This age difference has been attributed to the immature stage of the neurons which seems to increase in their sensitivity to the drug (Jancso and Kiraly, 1981). The simultaneous disappearance of neurogenic inflammation, and the abolition of nociceptive responses to chemical stimuli observed in the present study, suggests that these two changes may be intimately connected, possibly mediated by the same substance P-containing neurons of the peripheral nervous system (Jancso et al. 1980).
4. Neurotoxic Effect - Nerve Degeneration:

a) Control group:

The wide range of variability in the number of nerves within each control molar type (70-151 for the first molar, 25-50 for the second molar) correlates with previous quantitative studies of normal teeth. Pulps of permanent incisors in 3 month old cats contained 40-61 axons (Fried, 1982) while in cats aged 7 months, the range of axonal number was 92-394 (Fried and Hildebrand, 1981b). In another study conducted by Johnsen and Karlsson (1974), the pulps of permanent incisors of cats aged 5-8 weeks contained 369-495 axons. These results indicate that the axonal number varies widely for a particular tooth, from one animal to another. The technical difficulty in obtaining a reproducible level of section, along with the fact that many axons branch in the first 1-2mm at the root foramen (Bueltmann et al. 1972) may introduce variability in the number of nerve profiles. In the present study, a careful selection of comparable specimens was undertaken, and the entire pulp area was evaluated to minimize errors inherent in sampling methods. It was assumed that the variation related to technical procedure, occurs in a randomized fashion, without favoring one group of specimens over the other.

Only a few studies counted nerve fibers in developing teeth. A quantitative study conducted by Fried and Hildebrand (1981a) showed no axons in the developing primary incisor of 1-5 day old kittens (exhibiting the same developmental stage as the permanent molar of 8 day old mice used in our study). This difference may be explained by the fact that, in general, permanent teeth are innervated much earlier than their primary counterparts (Fried and Hildebrand, 1981a,b). Also, the
higher number of axons observed in a permanent tooth, as opposed to a primary tooth, may be due to its larger size (Johnsen and Karlsson, 1977). In another study performed on developing permanent incisors in 2.5 month old kittens (similar to permanent first molars of 8 day old mice, in terms of hard tissue development), Fried and Hildebrand (1981b) were able to identify 20 unmyelinated axons, all contained in Schwann cell units. By 3 months, when half of the root was formed, 40-60 axons were localized along central pulpal blood vessels. About 80% of these axons were unmyelinated with a maximum diameter of 1 um and a peak at 0.1-0.2 um. The myelinated axons measured 1-3.5 um and number varied between 3-19. Both myelinated and unmyelinated axons reached maturity 4 months later. In our study, both first and second molars showed a high proportion of axons with a diameter less than 0.6 um and a peak diameter of 0.3-0.4 um reflecting the immaturity of the innervation at this age (Ochoa and Mair, 1969a,b).

The distinction between myelinated and unmyelinated axons was sometimes difficult in the present material, because at this early stage, large unmyelinated axons overlapped in size (over 1 um) with small myelinated axons which appear to be involved in the initial stages of myelination. However, the topographical criteria based on the fact that some larger axons appeared to be gradually isolated from other axons by a unique Schwann cell investment permitted us to distinguish them from unmyelinated axons which shared the same Schwann cell. While they were not counted separately, axons classified as myelinated were clearly in the minority.

Also, the relationship between axon diameter and initiation of myelinization was evident in the present study, and correlates with
other investigations showing that myelination commences when an axon reaches a diameter of 1-2 μm (Peters et al. 1970; Ochoa and Mair, 1969a). However, at this early stage of myelination, no distinct cytologic difference between early myelinated and clearly unmyelinated axons was evident. Neither was true myelin ever seen in any of the teeth examined.

The predominance of small sized unmyelinated nerve fibers, along with the fine structural features of most of these axons which contained mitochondria, clear vesicles, a few dense core vesicles and a fine network of microtubules and microfilaments, is consistent with a sensory role (Holland, 1980; Bishop, 1981). However, autonomic fibers may have also been included in the quantitative analysis. No particular features, notwithstanding their slight tendency to lie near blood vessels (Avery et al. 1980), permitted us to distinguish autonomic from other unmyelinated axons.

b) Experimental group:

The absence of histological signs of neural degeneration 6 days after the first injection may be due to the mechanism of action of the drug itself. The neurotoxic effect of capsaicin is an extremely rapid process acting directly on the cell bodies of the neurons (Jancso and Kiraly, 1977; Jancso, 1978; Jancso and Kiraly, 1980). Previous studies have shown that the neural debris resulting from the neurotoxic effect of the drug could not be demonstrated at spinal cord levels more than 48-72 hours after neonatal treatment (Jancso, 1978; Jancso and Kiraly, 1980), or at peripheral levels, after 24 hours (Papka et al. 1984). The lack of any degenerative signs in peripheral nerves at longer times
after capsaicin treatment (1-6 months) has been also reported (Scadding, 1980; Holje et al. 1983). The present study which focuses on the neural effects of the drug, suggests that even the short period of 6 days was sufficient to obtain a total disappearance of capsaicin-sensitive fibers contained in the dental pulp.

The small and variable effect of capsaicin in reducing the number of pulpal nerves in the present study was unexpected since neonatal treatment has been shown to produce a selective degeneration of a large proportion (58-90%) of nociceptive fibers (Gamse, 1982; Nagy et al. 1980; Jancso et al. 1967; Jancso and Kiraly, 1980). In the present study, there was only a 30% and 34% reduction of nerves for the first and second molars respectively. The tooth pulp, reportedly, contains almost exclusively nerve fibers related to pain transmission (Anderson et al. 1970; Matthews, 1976) and histochemical examinations of young dental pulps have shown a substantial amount of substance P (Brodin et al. 1981). Previous studies in dogs and cats have demonstrated that the amount of substance P found in the dental pulp was dramatically higher in mature animals (22x higher in cats, 7x in dogs), than in young animals (Brodin et al. 1981b). Variability also exists between species, possibly within the same species, from animal to animal, or tooth to tooth. Nevertheless, an immunofluorescence study conducted by Mohamed and Atkinson (1982) has shown that neonate mouse molars do contain substance P-positive fibers first detectable in 4 day old mice. But this experiment did not indicate which type of nerve contained substance P, or how many nerves were substance P-positive. Therefore, we were unable to predict the number of substance P fibers present at this age, and
consequently, how many fibers might have degenerated after capsalcin treatment.

The present result suggests that the many dental nerve fibers which survive capsalcin treatment either do not contain substance P, or they do contain substance P but are different in nature from such fibers seen in other areas of the body which degenerate after treatment.

If the nerves which survive capsalcin treatment do not contain substance P, it implies that substance P containing fibers constitute only a small percentage of the entire pulpal innervation and these are the ones eliminated after capsalcin treatment. Either the majority of pulpal nerves are not involved in pain transmission, or they utilize a different neurotransmitter for nociception. However, the desensitization to chemical and mechanical pain seen in other areas (e.g. dorsal skin) shows that in these areas neurons containing substance P and possibly other neurotransmitters are affected by the drug, and did take part in pain sensitivity. The total nerve supply of the dental pulp appears to be complex. Indeed, the tooth pulp may not contain exclusively nerve fibers related to pain transmission as generally assumed. Microscopic studies have suggested that pulpal fibers are almost exclusively related to pain transmission, because of the fine diameter of unmyelinated fibers (Johnsen and Karlsson, 1977; Fried and Hildebrand, 1981a), and the absence of perineurium which place the axons in an optimum situation to sense changes, both chemical and physical, within the extracellular space (Holland, 1980; Harris and Griffin, 1968). However, the use of retrograde transport of horseradish peroxidase demonstrated the presence of labelling in the trigeminal mesencephalic, spinal and main sensory nuclei, which suggests that
dental pulp contains specialized sensory nerve endings for modalities (e.g. touch, pressure, temperature and proprioceptive sensibilities) as well as pain (Byers, 1980; Byers and Kish, 1976; Chiego et al. 1979). Also, electrophysiological studies have observed that impulses from dental pulp are sometimes transmitted with high conduction velocity (Narhi et al. 1982a,b), implying that some intra pulpal A-delta, and C fibers may derive from large axons of the inferior alveolar nerve. If dental pulps are partially supplied by large diameter afferent fibers, these axons are not likely to be influenced by capsaicin treatment.

Whether the substance P found in peripheral nerves is similar to that found in fibers of the pulp is not known. Neither is it known whether substance P fibers are equally sensitive to capsaicin in all regions. Either the substance P present in the dental pulp may be localized in fibers some of which are resistant to capsaicin, or multiple sub-types of substance P containing neurons may be present in the dental pulp, only some of which are distinguishable by their susceptibility to capsaicin. This would explain the modest effect of the drug in the present study. This idea gains support from recent studies which have reported capsaicin-resistant substance P fibers in the trigeminal and saphenous nerves, and in the gut (Lundblad et al. 1983; Welk et al. 1984; Holzer et al. 1980).

Two additional possibilities must be considered to account for the modest effect of capsaicin in reducing the number of dental nerves. Either the structures responsive to capsaicin have not yet developed in neonate animals, or extensive neuronal sprouting has occurred immediately after treatment.
Most studies have shown that capsaicin exerts its maximum effect at early stages of development. However, the fact that nerve fibers are the last major structures to appear in the developing tooth implies that their maturation is delayed perhaps to a great extent relative to those in other body locations. Indeed, the maturation of the tooth, and maturation of its nerve supply appear to be 2 separate events, which occur at different times during development. Because of the immaturity of the nerves in 2-8 day old animals, the percentage of capsaicin-sensitive fibers may be very small compared with those present in later developmental stages when the sensory fibers begin functioning. Such a delayed effect of capsaicin has been observed in the preoptic region of neonate rats (Hajos et al. 1983).

Extensive reinnervation occurring immediately after treatment may have masked the actual neurotoxic effect of the drug. Nerve section experiments have demonstrated that 6 to 9 weeks are necessary to obtain complete reinnervation of the tooth (Robinson, 1981). However, in our experiment, partial denervation is expected, since capsaicin affects specifically substance P fibers. Therefore, collateral reinnervation (sprouting) from unaffected nerves, which can occur within 1-2 days after partial nerve section (Devor et al. 1979), may have largely compensated for lost fibers. In the present study, it was not possible to clearly distinguish the small diameter axons, which are numerous in normal conditions in the cervical region of the tooth, from sprouts arising from intact collateral nerves. Indeed, the early developmental stage of dental innervation in an 8 day molar, which exhibits a massive proliferation and arborization of nerve fibers may have masked a partial reinnervation. Interestingly, the histogram of axon diameter in the
experimental first molars exhibits a small left shifted distribution as compared to the control first molars, evidenced by a slightly increased number of small size axons. This modification in the size frequency may be the initial manifestation of reinnervation occurring in the first molar. During this process, there may be an overlap of sizes between axons sprouting after denervation and small size axons which survived capsicin treatment. Moreover, the possibility that reinnervation may have occurred earlier in the first molar is suggested by the marked difference in the number of nerves between first and second molars. Previous studies have demonstrated that the rate of reinnervation, observable 24-48h after denervation, is dependant upon the presence of adjacent normal nerves (Causey and Hoffman, 1955; Weddell, 1942). In this study, the possibility that rapid reinnervation may have occurred is also supported by the observation that most of the dental nerve fibers were associated with blood vessels and in general, fibers regenerate most rapidly in close proximity to blood vessels, presumably because of a ready supply of nutritional factors (Weddell, 1942).

Our results contradict a previous study done by Holje et al. 1983, in which the authors did not demonstrate any neurotoxic effect of capsicin on rat molars. However, they did report a massive loss of unmyelinated axons from the dorsal root (85%), a large effect on the inferior alveolar nerve (58%), and the mental nerve (37%). Paradoxically, the average number of unmyelinated axons in the experimental molars (63%) was greater than in the control teeth (59%). The authors concluded that the nerves supplying the dental pulp were not affected by capsicin treatment. However, the methods for sampling
nerves, as well as the type of tooth, and the histological criteria used to evaluate the electron micrographs were not described. Also, the lack of statistical evaluation of the results did not permit an accurate comparison with the control group. Moreover, the absence of systematic qualitative evaluation of both control and experimental nerves, could not permit the detection of subtle morphological changes which might have occurred after treatment. Perhaps the most significant difference between the present study and that of Holje et al., which might account for the different results, was the survival time of the animals. In Holje et al.'s study, long term survival of the animals (6 months) might have allowed a high degree of neuronal sprouting to occur. The fact that a higher number of unmyelinated nerves was found in the experimental molars is an interesting finding which was not discussed by the authors. Indeed, one of the most outstanding features of histological reinnervation following partial denervation, consists of nerve fibers being more numerous than normal (Causey and Hoffman, 1955). The invasion of only a few collaterals at first, in marked contrast to the massive proliferation and branchings of additional nerves may explain the difference between our results and those of the more long term study by Holje et al. (1983).

5. **Effect on Tooth Development:**

The normal appearance of dental structures in the experimental group, at light and electron microscopic levels, correlates with a previous study (Holje et al. 1983), and indicates that nerves sensitive to capsaicin treatment do not exert any obvious trophic influences, at least during this stage of development. None of the dramatic changes
which have been previously reported after whole nerve resection, including an alteration in the rate and pattern of dentinogenesis (Avery et al. 1971, 1974; Avery and Cox, 1977; Chiego et al. 1983) and amelogenesis (Chiego et al. 1981; Brown et al. 1961) have been observed in the present study. Since only relatively few nerve fibers may contain substance P in the mouse molar during the time period of the present study, a reduction of these fibers may not have been sufficient to produce an effect on tooth development. Whether nerves survive capsaicin treatment and induce tooth formation as previously suggested (Kollar and Lumsden, 1979; Lumsden, 1979; Pearson, 1977) is not known.

6. Conclusion:

The desensitization of the animals to the drug, associated with marked behavioral changes contrast with the modest effect of capsaicin in reducing the innervation of growing teeth. It demonstrates that dental nerves are not an homogeneous population, and only a small percentage of these fibers may be comparable to spinal nociceptive-C fibers in terms of their susceptibility to capsaicin.

The normal appearance of dental structures in the experimental group shows that the nerves (presumably containing substance P) which are eliminated after capsaicin treatment, represent a small proportion of the total pulpal innervation, and for this reason, do not have an obvious inductive influence during this stage of development.

More work is needed to define the precise action of capsaicin on dental innervation. Future research might well focus on anatomical evidence of nerve degeneration in the hours immediately following the first administration of the drug; immunocytochemical proof that
substance P-containing nerves effectively disappear after treatment; and electrophysiological evidence of changes in the responses of dental nerves to chemical, mechanical and thermal noxious stimuli after capsaicin treatment.
FIG. 1. The structural formula of Capsaicin showing the three moieties contributing to the toxic activity (from Nagy, 1982).
**FIG. 2.** Drawings of the buccal aspect and transverse (upper right) and longitudinal (lower right) sections through the cervical region of a mandibular second molar. At the cervical region, odontoblasts and ameloblasts are differentiating. Hertwig's sheath (HS) is at the bottom of the illustration. Dentin (D), predentin (PD), odontoblasts (O) and dental papilla (DP) are internal to the enamel organ. Short ameloblasts (SA) are facing newly formed enamel matrix, tall ameloblasts (TA) are adjacent to recently calcified enamel (E) (hematoxylin and eosin, X40 for upper figures).
**Fig. 3-5.** Electron micrographs of a mandibular first molar control. Transverse section through the cervical region of the pulp.

**Fig. 3 (top):**

Typical arrangement of unmyelinated fibers in the dental pulp. A large Schwann cell unit containing unmyelinated axons of the largest (*, 2 microns) and the smallest (open arrow, 0.1 micron) size, and a Schwann cell with nucleus (SCN), lying close to a blood vessel (BV). Many axons lie adjacent to one another, unseparated by Schwann cell cytoplasm, while some large individual axons are enclosed by Schwann cell cytoplasm. Some artefacts are represented at the level of the mitochondria which appear swollen (arrows) (X 16300).

**Fig. 4 (bottom left):**

This Schwann cell unit lies close to a blood vessel. One axon (arrow) displays a dense core and a few clear vesicles. Some axons show electron-lucent cytoplasm lacking microtubules and neurofilaments (open arrows) (X 18620).

**Fig. 5 (bottom right):**

A group of axons clustered in the invagination of a Schwann cell nucleus (SCN). The Schwann cell cytoplasm contains granular mitochondria (mi). Some axons contain a few clear vesicles (arrows) (X 25800).
**Fig. 6-7.** Electron micrograph of a mandibular second molar control. Transverse section through the cervical region of the pulp.

**Fig. 6:**
A group of axons lies close to a blood vessel. Axons are seen in clusters, unseparated by Schwann cell processes. The axons contain a predominance of microfilament (arrows) with some microtubules. Note the paucity of other organelles. Collagen fibers (C) are seen in the surrounding region (X 35800).

**Fig. 7:**
A group of unmyelinated axons enclosed in a Schwann cell sheath. Some axons lie close to one another (arrows) (X 23200).
**Fig. 8-11.** Electron micrograph of a mandibular first molar experimental. Transverse section through the cervical region of the pulp.

**Fig. 8 (top):**
A Schwann cell unit close to a blood vessel (BV). Some of the developing axons are in close contact with their neighbors; another (*) is held individually by Schwann cell tongues which define short mesaxons (arrow) (X 35000).

**Fig. 9 (bottom left):**
A group of unmyelinated axons from the central part of the pulp. The axons contain neurofilaments (arrows), microtubules (open arrows), and large mitochondria (mi) (X 35000).

**Fig. 10 (bottom right):**
A Schwann cell unit from the central part of the pulp with characteristic association of small and large unmyelinated fibers. Some axons contain vacuoles (arrows) resembling swollen mitochondria seen in some nerves from control teeth (Fig. 3) (X 27200).
**Fig. 11:**

One axon (Ax) is in the first stage of myelination, separated from the other axons by a Schwann cell (SC). The mesaxon has elongated to form one almost complete turn (arrow). The axon contains microfilaments and microtubules distributed homogeneously (X 52500).
**Fig. 12-13:**

Electron micrograph of a mandibular second molar experimental. Transverse section through the cervical region of the pulp.

**Fig. 12:**

A group of unmyelinated axons close to a large blood vessel (BV) surrounded by a pericyte (PC). The axons contain clusters of microfilaments (solid arrows), and microtubules (open arrows) disposed peripherally, and occasional mitochondria (mi) (X 33300).

**Fig. 13:**

A small bundle of nerve fibers, one of which contains a mitochondrion (mi) and another, vesicles (arrows) (X 46400).
FIG. 14:

The average number and standard deviation of myelinated and unmyelinated nerves from mice injected with solvante and mice treated with Capsaicin. Note the reduction in nerve fibers in experimental teeth.
THE AVERAGE NUMBER AND STANDARD DEVIATION OF MYELINATED AND UNMYELINATED NERVES FROM MICE INJECTED WITH SOLVENT AND MICE TREATED WITH CAPSAICIN
FIG. 15:

Histograms of axon diameter in control and Capsaicin-treated mouse first and second molars at 8 days.
HISTOGRAM OF AXON DIAMETER IN CONTROL AND CAPSAICIN-TREATED MOUSE FIRST MOLARS AT 8 DAYS

- CAPSAICIN TREATED, N=366
- CONTROL, N=366

HISTOGRAM OF AXON DIAMETER IN CONTROL AND CAPSAICIN-TREATED MOUSE SECOND MOLARS AT 8 DAYS

- CAPSAICIN TREATED, N=120
- CONTROL, N=116
Fig. 16:
mandibular first molar control. Transverse section through the central part of the crown. The dentin (D) is completely calcified and exhibits area of globular calcification (arrows). The front of mineralization between dentin and enamel (E) appears as a scalloped line, and is materialized by the difference in size and orientation of the crystallites of enamel and dentin (X 16930).

Fig. 17 (bottom):
Electron micrograph of dentin and enamel in a mandibular first molar experimental. Transverse section through the central part of the crown. The enamel crystals (small arrows) are parallel to the long axes of the rods (large open arrows). The surfaces of the rods are distinct by marked changes in crystal orientation from one rod to the other. Rods are orientated obliquely to the dentin surface (D) and follow a wavy pattern. Enamel and dentin appear indistinguishable from the control tooth (X 18880).
**Fig. 18 (top):**

Light micrograph of a mandibular first molar control. Longitudinal section. At 8 days of postnatal development, the entire crown is covered by a full thickness of dentin and enamel. The mineralization is almost achieved, and root formation starts at this time. Note the presence of artefacts with disruption of the odontoblastic and ameloblastic layers (hematoxylin and eosin, X 25).

**Fig. 19 (bottom):**

Light micrograph of a mandibular first molar experimental. Longitudinal section. The experimental tooth germ shows no change in size and shape as compared to the control tooth (hematoxylin and eosin, X 40).
**FIG. 20 (top):**

Light micrograph of a mandibular second molar control. Longitudinal section. At 8 days, the formation of the crown is almost achieved. Note the difference in thickness of dentin and enamel as compared to the first molar control (Fig. 18) (hematoxylin and eosin, X 40).

**FIG. 21 (bottom):**

Light micrograph of a mandibular second molar experimental. Longitudinal section. The aspect of the experimental tooth germ is indistinguishable from the control tooth. Note the normal appearance of the periodontal tissues (hematoxylin and eosin, X 40).
TABLE 1

TISSUE CONCENTRATIONS OF SUBSTANCE P-LIKE IMMUNOREACTIVITY
(pmol/g. of tissue wet weight) IN VARIOUS MAMMALS.

<table>
<thead>
<tr>
<th>ORGANS</th>
<th>CATS</th>
<th>RATS</th>
<th>MICE</th>
<th>REFERENCE</th>
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<tbody>
<tr>
<td>DENTAL PULP</td>
<td>32</td>
<td></td>
<td></td>
<td>Brodin.1981</td>
</tr>
<tr>
<td>SKIN(snout)</td>
<td>3</td>
<td>.8</td>
<td>.9</td>
<td>Bucsics.1983</td>
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<tr>
<td>EYES(cornea)</td>
<td>.6</td>
<td>3.4</td>
<td>2.6</td>
<td>&quot;</td>
</tr>
<tr>
<td>ORAL MUCOSA</td>
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<td>3.7</td>
<td></td>
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<tr>
<td>CAROTID</td>
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<td>.2</td>
<td>.9</td>
<td>Papka.1984</td>
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<tr>
<td>LUNGS</td>
<td>.8</td>
<td>.3</td>
<td>.5</td>
<td>Bucsics.1983</td>
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<tr>
<td>STOMACH(fundus)</td>
<td></td>
<td>24</td>
<td>14</td>
<td>Pernow.1983</td>
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<tr>
<td>TRIGEMINAL GG.</td>
<td>24</td>
<td>7.8</td>
<td></td>
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<td>SPHENOPALATINE GG.</td>
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<td>SUP.CER.GG.</td>
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<tr>
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<td>&quot;</td>
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<td>DORSAL ROOT GG.</td>
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<tr>
<td>DORSAL ROOT</td>
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<tr>
<td>DORSAL SPINAL CORD</td>
<td>345</td>
<td>330</td>
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<td>Gamse.1982</td>
</tr>
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TABLE II

Tissue concentrations of substance P-like immunoreactivity (pmol/g) in dental pulps at different ages in mammalian canine teeth, and human premolars (From Brodin et al. 1981)

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<th>Developmental Stage</th>
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<th>Human</th>
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<tr>
<td>Young</td>
<td>1.5</td>
<td>1.9</td>
<td>5.2</td>
</tr>
<tr>
<td>Medium</td>
<td>16</td>
<td></td>
<td></td>
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<tr>
<td>Mature</td>
<td>32</td>
<td>12</td>
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### TABLE III

RANKED NUMBER OF AXONS IN TRANSVERSE SECTIONS OF CONTROL AND EXPERIMENTAL FIRST AND SECOND MOLARS

\( p < 0.025 \)

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<th>FIRST MOLAR</th>
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<th>EXPERIMENTAL</th>
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<tr>
<td></td>
<td>Rank#</td>
<td>#Nerves</td>
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<td>4</td>
<td></td>
<td>70</td>
</tr>
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<td>10</td>
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<td>151</td>
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<table>
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<th>SECOND MOLAR</th>
<th>CONTROL</th>
<th>EXPERIMENTAL</th>
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</thead>
<tbody>
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<td></td>
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<td>#Nerves</td>
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<td>9</td>
<td></td>
<td>50</td>
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